7 Bioherbicides for Weed Control

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7.1 Introduction

Management of weeds is a necessary but expensive challenge. Chemical weed control accounts for over $14 billion spent annually (Kiely *et al*., 2004), excluding immense indirect costs to producers, consumers and the environment, and resulting also in the development of resistant weed biotypes. While chemical herbicides effectively control unwanted vegetation, many herbicides are no longer available due to lack of re-registration, competition from other products, and development of numerous genetically modified crops with resistance to broad-spectrum herbicides, namely glyphosate and gluphosinate. The implementation of conservation tillage practices to promote soil quality, to minimize erosion, or to simplify crop management has increased reliance on 'burn-down' herbicides and placed additional selection pressure on weeds to develop resistance. After years of applying herbicides, often in the presence of high weed pressure, 180 species of herbicide-resistant weeds have been identified (WeedScience, 2006). The majority of herbicide usage is for agronomic areas or turf, but few herbicides are registered for, or are being developed for, smaller markets or niche weed problems, such as invasive weeds in non-cropland areas. Furthermore, chemical weed control is not an option in organic cropping systems and near to sensitive natural habitats. The high costs involved in developing and registering chemical herbicides, and recent trends in environmental awareness concerning pesticides in general, have prompted researchers to develop additional weed control tools, such as biological weed control using plant pathogens.

A review of pathogen-based weed control prospects by Charles Wilson (1969) noted that 'the idea of using plant pathogens to control weeds is almost as old as the science of plant pathology itself', but that the 'seeds of the idea … have lain dormant since their sowing'. Since that review, almost 40 years ago, numerous pathogens for weed control have been identified and a few have enjoyed limited commercial success (Hoagland, 1990, 2001).

Classical pathogen-mediated biocontrol of weeds generally employs an exotic pest to manage a weed population. This is an effective weed management strategy in many systems (Bedi *et al*., 2002; also see Blossey, Chapter 6, this volume). An alternative method is to overwhelm the target weed with direct pathogen application, or multiple applications of a pathogen. Because this tactic uses biological agents in an application similar to chemical herbicidal applications, it is often called the 'bioherbicidal' approach. When the plant pathogens are fungi, these bioherbicides are often called 'mycoherbicides'.

7.2 Discovery of Bioherbicides

A scientific strategy should be utilized in the discovery of classical biocontrol agents (Berner and Bruckart, 2005). Often the weed pest to be controlled is exotic, so a search is made near its region of origin to find potential biocontrol organisms that have co-evolved with the given weed host. These potential biocontrol agents are then screened for possible undesirable, non-target effects, and studied to evaluate environmental parameters for efficacy. High levels of host specificity are desirable and often unavoidable, as with the rust pathogens. An example of this ongoing work is the effort to control yellow starthistle (*Centaurea solstitialis*) by *Puccinia jaceae* var. *solstitialis* (Bruckart, 2006).

The bioherbicidal approach, in contrast with classical biocontrol, more commonly relies on indigenous pathogens. A common assumption is that highly virulent pathogens always make the most effective bioherbicides, but this concept has been effectively challenged (Hallett, 2005). Some host–pathogen interactions produce dramatic symptoms, but may not meaningfully reduce the weed population. In contrast, one of the most commercially successful bioherbicides to date, *Colletotrichum gloeosporioides* f. sp. *aeschynomene*, does not produce impressive symptoms or particularly rapid mortality of northern jointvetch (*Aeschynomene indica*), the target weed. Instead, its success has been due to (i) its low cost of production; (ii) its comparatively simple formulation requirements; and (iii) its rapid and efficient secondary spread in the field (Bowers, 1986; Smith, 1991; D.O. TeBeest, personal communication). This might not be the only model for success with a bioherbicide, but it provides a useful benchmark for comparing candidate bioherbicides.

Other practical considerations for the commercial success of a bioherbicide include the ease of obtaining both patent and product registration. Registration of a chemical herbicide is a lengthy and expensive process, but the US Environmental Protection Agency has recognized potential environmental benefits for biopesticides, simplifying the process, so that registration can be achieved in as little as 1 year (EPA, 2006; Slininger *et al*., 2003). Furthermore, some of the expense of bioherbicide registration can be reduced for 'low-risk' applications.

Finally, the discovery of candidate bioherbicides must consider economics, and several questions need to be answered.

- Can propagules be generated on low-cost substrates?
- Will they remain viable in storage after production?
- Will production require lengthy incubation in expensive bioreactors?
- Will the inoculum require extensive processing before application?
- What conventional herbicides will it compete with?
- Is the market large and stable enough to recoup development and registration costs?
- Will the product generate sufficient profit?

It would be beneficial if markets could be identified where low-cost chemical inputs are not available, such as in organic crop production or small-acreage, but potentially high-value, crops that have been neglected by the chemical industry. Invasive weeds in natural or low-input managed ecosystems, such as public lands, forests and conservation easements may also be attractive targets for bioherbicides, as they may be perceived as more compatible than chemical herbicides in these sensitive areas. Bedi *et al*. (2002) described a semi-quantitative means of measuring the commercial potential of a bioherbicide candidate, incorporating many of these traits.

7.3 Mycoherbicide Production Technology

For practical and economic reasons, the propagules of a bioherbicide must be rapidly and inexpensively produced. Asexually produced fungal spores, or conidia, are generally the most cost-effective and easiest to produce under laboratory conditions (Templeton *et al*., 1979). Since spores provide the most common method for natural dispersal and typically have longevity, they should logically serve as the best candidates as infective units of mycoherbicides. For fungi that that do not produce spores, or do not produce them readily or efficiently, the production and use of mycelial fragments may be possible (Boyette *et al*., 1991b). Mycelial formulations present challenges because they are

generally more difficult to quantify, less readily separated from the culture medium, and are less infective than conidia. Spores often have longer shelf-lives and are more tolerant of suboptimal storage conditions (Churchill, 1982). The recent development of *Mycoleptodiscus terrestris* for the control of hydrilla (*Hydrilla verticillata*) has identified microsclerotia as a readily produced, desiccation-tolerant inoculum (Shearer and Jackson, 2006).

Selection of culture medium

Defined media, vegetable juice or agar culture can be used in the production of inoculum for experimental bioherbicidal systems (Boyette, 2000; Gressel, 2002). Researchers must recognize the unique nutritional requirements of each candidate bioherbicide with regard to carbon and nitrogen sources, pH, inorganic and trace elements and, in some cases, vitamins, amino acids or essential oils. However, to mass-produce mycoherbicides on a large scale for pilot tests or industry evaluation, these requirements must be met within the context of economic constraints. These requirements are most often realized using crude agricultural or industrial products that are readily available at low cost. Nitrogen sources, such as soybean flour, corn steep liquor, distillers solubles, brewers yeast, autolysed yeast, milk solids, cottonseed flour and linseed meal, are some of the materials that have been used to produce mycoherbicides. Carbon sources commonly tested include cornstarch, cornflour, glucose, hydrolysed-corn-derived materials, glycerol and sucrose (Churchill, 1982). Additionally, some fungi require light for sporulation, which may add complexity and increase production costs.

Carbon sources that do not maximize vegetative growth may enhance sporulation. The carbon, nitrogen and mineral levels that lead to optimal growth and sporulation may require precise and empirical balancing (Jackson, 1997). In addition to the effect on growth and sporulation, the carbon : nitrogen (C:N) ratio may affect viability, longevity and virulence of the fungus. For example, the vegetative growth of *Fusarium solani* f. sp. *phaseoli* was increased by a high C:N ratio, while virulence of the fungus on its host plant, *Phaseolus vulgaris*, was decreased. Conversely, a low C:N growth medium resulted in decreased vegetative growth and increased virulence (Toussoun *et al*., 1960). In contrast, Phillips and Margosan (1987) found the spore volume, nuclear number and virulence of *Botrytis cinerea* against the hybrid rose (*Rosa* spp.) increased linearly in response to increasing glucose concentration.

Slininger *et al*. (2003) reviewed a systematic approach taken to evaluate both the commercial potential of biocontrol strains and media selection. Media selection involves producing large quantities of inoculum quickly and inexpensively while simultaneously maintaining pathogen virulence. In the case of *Colletotrichum truncatum* for control of hemp sesbania (*Sesbania exaltata*), it was discovered that the optimum C:N ratio for production of conidia did not yield the most virulent conidia. Therefore, a balance point was found to achieve these two goals (Jackson *et al*., 1996, 1997; Wraight *et al*., 2001)

Inoculum density may also affect fungal sporulation. Slade *et al*. (1987) found that a high inoculum density (2.5×10^6 spores/ml) of *Colletotrichum gloeosporioides* resulted in slimy masses of conidia, called 'slime spots', when grown on several commonly used growth media. Slime spots are associated with microcyclic conidiation, where sporulation occurs directly after spore germination in the absence of mycelial growth. Conversely, reduced inoculum concentrations or concentrated growth media resulted in dense, vegetative mycelial growth; and microcyclical conidiation did not occur (Hildebrand and McCain, 1978; Slade *et al*., 1987).

Solid substrate fermentation

Solid substrate fermentation may be the only practical method for spore production if spores cannot be produced using submerged fermentation. Various cereal grains and vegetative residues have been used to produce simple, inexpensive inocula for a number of plant pathogenic fungi (Tuite, 1969). Hildebrand and McCain (1978) used wheat straw infested with *Fusarium oxysporum* f. sp. *cannabis*, to control marijuana (*Cannabis sativa*). Boyette *et al*. (1984) used oat seed infested with *F. solani* f. sp. *cucurbitae* to control Texas gourd (*Cucurbita*

texana). These types of bulky substrates are difficult to sterilize, inoculate and store until they are ready for use in the field. Some of these problems can be overcome by separation of spores from the substrate for subsequent drying, formulation and storage. These processes add cost and complexity to product development.

Combined solid substrate and submerged fermentations

Several mycoherbicides have been produced using combined solid and submerged fermentation techniques. *Alternaria macrospora*, a pathogen for control of spurred anoda (*Anoda cristata*), was first mass-produced by culturing fungal mycelium for 48 h in a vegetable-juice-based liquid medium. Fungal biomass was then collected, blended, mixed with vermiculite, spread into foil-lined pans, and exposed to either fluorescent light or direct sunlight to induce sporulation. After air-drying, the mixture was sieved, packaged, and stored at 4°C (Walker, 1981). This procedure has also been used to produce inoculum of *Colletotrichum malvarum* for control of prickly sida (*Sida spinosa*) and *F. lateritium* for control of spurred anoda, velvetleaf (*Abutilon theophrasti*) and prickly sida.

A modification of this technique was used to produce spores of *A. cassiae* for use as a mycoherbicide against sicklepod (*Senna obtusifolia*) (Walker and Riley, 1982). Fungal mycelium was grown in submerged culture for 24 h, collected, homogenized, poured into foil-lined trays, and then exposed to 10 min of ultraviolet light every 12 h for 5 days to induce sporulation. The mycelia sporulated prolifically as the medium dried. After 72 h, the spores were collected by vacuum, dried over calcium sulphate, and stored at 4°C. Approximately 8 g of spores were produced per litre of growth medium with this simple technique, to yield a product density of 10^8 spores/g (Walker and Riley, 1982). Sufficient quantities of *A. cassiae* spores were produced using this technique for a 2-year pilot study. This technique was also used to produce spores of *A. crassa* for jimsonweed (*Datura stramonium*) control (Quimby, 1989); *A. helianthi* for cocklebur (*Xanthium strumarium*) and wild sunflower (*Helianthus annuus*) control (Van Dyke and Winder, 1985); and *Bipolaris sorghicola* for johnsongrass (*Sorghum halepense*) control (Bowers, 1982).

Submerged culture fermentations

From both practical and economic perspectives, biocontrol fungi that sporulate in liquid culture are favoured over those that require additional steps to induce sporulation. This factor alone has proved to be advantageous for the commercial development of a fungus as a mycoherbicide (Bowers, 1982)

For early developmental studies using small-scale experiments, inoculum can be produced in shake-flasks. However, with shake-flasks it is difficult to maintain and adjust parameters that affect growth, such as the correct pH, temperature and aeration essential for optimal growth and sporulation. For larger quantities of inoculum or systems that require more precise control, laboratory-scale fermenters are essential. Some fermenters monitor and provide programmed control of environmental factors including temperature, agitation, dissolved oxygen and pH.

Slade *et al*. (1987) developed a simple method to assess inoculum production of *Colletotrichum gloeosporioides* in liquid culture using microplate assays of the fungus on various solid media. This system could possibly be used to provide an accurate, rapid and inexpensive means of screening various growth media for spore yield and virulence.

Systematic approaches to growth medium development can yield significant economic returns. Mitchell (2003) examined 47 carbon sources in an effort to maximize spore production of *Septoria polygonorum*, a pathogen of smartweed (*Polygonum* spp.). After identifying pea brine as the best carbon source, 38 factors (numerous inorganic amendments, fatty acids, complex nitrogen sources, surfactants, etc) were screened to find the best combination. The final composition yielded production of more than 10^8 spores/ml. A similar stepwise, surface-response modelling approach to medium selection was used by Mitchell *et al*. (2003) to maximize production of *Gloeocercospora sorghi*, a bioherbicide of johnsongrass (*Sorghum halepense*). By methodically testing numerous

components, at several concentrations, in many combinations, much higher production levels can be achieved, with concomitant economic returns.

Two registered mycoherbicides – Collego (Encore Technologies, Minnentoka, MN) and DeVine (Abbott Laboratories, North Chicago, IL) were both produced using submerged liquid culture techniques. The formulation of these bioherbicides is discussed later.

7.4 Bioherbicide Formulation

Bioherbicide formulations are engineered or strategically developed materials consisting of spores or other propagules of one or more microbial agent(s) previously identified as a weed pathogen. Various bioherbicide formulation types are possible and many have been explored on an experimental basis and are discussed throughout this chapter. Suspension concentrates, wettable powders, dry flowables, water-dispersible or wettable granules, and non-disintegrable granules are some of the possible formulations.

Formulations are developed for different reasons associated with manipulation (including handling and application), stabilization or shelf-life, and efficacy. The importance of formulation as it relates to each of these factors is discussed below.

Manipulation

Loose particles ranging in size from sub-micron up to several tens of microns (micrometres) are prone to aerosolization, i.e. becoming suspended in air for long periods of time (Griffin, 2004). Atmospheric aerosols that are microorganisms, plant material, and associated cell-wall materials and metabolites are specifically referred to as bioaerosols (Kuske, 2006; El-Morsy, 2006; Reoun An *et al*., 2006). Exposure to such aerosols can pose health risks (WHO, 2000) to developers and users of bioherbicides. Furthermore, other issues arising from aerosolized agents include loss of applied active ingredient and deposition to non-target or off-sites (Brown and Hovmøller, 2002; Griffin *et al*., 2003). This process of aerosolization and the associated hazards are similar to the risks with liquid spray application of synthetic pesticides (Tsai *et al*., 2005). Incorporation of bioherbicide propagules into macroscopic solids or other steps to minimize aerosolization may be an important technique to promote safe handling and application of agents.

Stabilization and storage

Stabilization and long-term storage of a mycoherbicide is dependent on formulation composition and the water content of the dried product. For long-term storage of agents, cellular metabolism can be controlled by lowering either the water activity (Aw) or the storage temperature of the product. Commercially, one would prefer to store the agent at ambient temperatures; therefore, the shelf-life of mycoherbicides should preferentially be extended by lowering the Aw. Reduced Aw implies the agent could be in either a solid-state formulation such as a granule or dispersed in oil. Oils can be phytotoxic to non-target plants (Tworkoski, 2002) and may undergo lipid oxidation upon extended storage. Thus, careful selection of oil type and inclusion of antioxidants as stabilizing agents may be necessary to prevent unwanted chemical changes to the formulation and to extend the shelf-life of oil-based mycoherbicides.

The science of choosing formulation ingredients to improve the stability of solid-state bioherbicides is not fully understood. However, the significance of formulation composition as it relates to storage stability of bioherbicide propagules has been demonstrated (Silman *et al*., 1993; Connick *et al*., 1996, 1997; Amsellem *et al*., 1999; Shabana *et al*., 2003; Müller-Stöver *et al*., 2004; Friesen *et al*., 2006). For example, in research on the shelf-life of either conidia or conidia plus mycelium of *Fusarium arthrosporioides* and *F. oxysporum*, 'Stabileze' (a mixture of starch, sucrose, corn oil and silica) was found to be superior to alginate bead formulations in preserving the viability of each weed control agent (Amsellem *et al*., 1999). Shelf-life of *C. truncatum* spores, formulated in a solid/perlite–cornmeal–agar mixture, at 15°C was longer than in a liquid formulation

or a solid/vermiculite mixture (Silman *et al*., 1993). In wheat flour–kaolin 'pesta' granules, *C. truncatum* spores germinated in 87% of granules that were stored for 1 year at 25°C (Connick *et al*., 1996). Viable pesta granules containing *C. truncatum* microsclerotia were observed after 10 years of storage at 4°C (Boyette *et al*., 2007b). In other studies, significantly different trends were observed in the viability of *F. oxysporum* f. sp. *orthoceras* microconidia in ten differently amended pesta formulations, each containing various adjuvants (Shabana *et al*., 2003). In all these pesta formulations, glycerol imparted a negative effect on shelf-life, but stillage (an alcohol manufacturing by-product) and Water Lock (a 'super-absorbent' polymer)-amended pesta formulations exhibited the worst shelf-life among those evaluated (Shabana *et al*., 2003).

In addition to formulation, storage stability can be improved further by maintaining the product at an optimal water content or water activity (Connick *et al*., 1996; Shabana *et al*., 2003). An example is the storage studies of pesta containing *C. truncatum* microsclerotia by Connick *et al*. (1997). These investigators were able to identify, for a single formulation, water activities ranging from 0.12 to 0.33 at 25°C that were conducive to long-term storage. At water activities above 0.33, the shelf-life of pesta with *C. truncatum* was inferior to granules stored in drier air environments.

Collectively, the above studies indicate that improvements in bioherbicide storage stability are possible through the choice of specific formulation ingredients and appropriate drying. Since shelf-life is dependent on optimal water activities, packaging is also an important parameter in improving the shelf-life of mycoherbicides.

Efficacy

Finally, the efficacy of weed pathogens may be enhanced through formulation. Formulations amended with particular adjuvants and nutrients can (i) stimulate biological activity while reducing biological competition from pre-existing microorganisms, (ii) protect the agent from environmental factors such as UV light, wind, and rainwater removal from the target plant surface, (iii) facilitate and sustain propagule germination, growth and infection, and (iv) improve coverage and agent–target interaction of the formulated spray droplets on plant tissue.

The first mycoherbicide registered and marketed in the USA was based on the phytopathogenic fungus *Phytophthora palmivora*. The product, DeVine, was used to control strangler vine or milkweed vine (*Morrenia odorata*), a pest of Florida citrus orchards. The product had a shelf-life limited to a few weeks and required refrigeration during storage and shipment to preserve the live chlamydospores. Nevertheless the need to effectively control strangler vine assured commercial success for several years. The product is no longer commercially available because the market niche is too small to sustain commercial interest (Ridings, 1986). This, however, is an example of the potential for agents with short shelf-lives that might be found for target weeds that are of regional importance. For example, of the estimated 3 million hectares covered by kudzu (Forseth and Innis, 2004), several hundred thousand hectares of kudzu could be within a 500 km distribution radius of a mid-south USA bioherbicide production site.

The second US bioherbicide product was registered in 1982. It was based on *Colletotrichum gloeosporioides* f. sp. *aeschynomene* (CGA). The Upjohn Company, in collaboration with researchers at the University of Arkansas and the US Department of Agriculture, was able to mass-produce CGA and market a formulated biological control agent under the trade name Collego. It was developed for northern jointvetch control in soybean and rice fields, and approved for use in Arkansas and Louisiana. The Collego product was delivered in two packages – one of dried CGA spores in an inert carrier material, the other an inert rehydrating osmoticum for reviving spores before spraying. The components of both packages were added to the desired volume of water immediately before application (reviewed in Smith, 1986). While this product has not been available commercially for several years, changing agronomic practices have led to renewed interest in biological control of northern jointvetch. An effort is under way to bring this product back to the marketplace under the name LockDown (K. Cartwright, Agricultural Research Initiatives, personal communication).

BioMal (Philom Bios, Saskatoon, Canada) is another registered mycoherbicide based on the hydrophilic fungus *Colletotrichum gloeosporioides* f. sp. *malvae*. It was delivered as a wettable silica gel powder for control of round-leaved mallow (*Malva pusilla*). The spores, as formulated, dispersed readily in water for application, and routinely provided more than 90% control in the field (Ridings, 1986).

Granular formulations

With some exceptions, liquid mycoherbicide formulations are generally best suited for use as post-emergence sprays and are used primarily to incite leaf and stem diseases. Conversely, pathogens that infect below the soil surface are best delivered in a solid or granular formulation. Granular formulations are better suited for use as pre-planting or pre-emergence mycoherbicides than are liquid spray formulations because: (i) granules provide a buffer from environmental extremes, (ii) granules can provide a food-base for the fungus, prolonging persistence, and (iii) granules are less likely to be washed away from the treated areas (Mitchell, 1986; Wymore *et al*, 1988).

A cornmeal–sand formulation of *Fusarium solani* f. sp. *cucurbitae* was used to produce a mixture of mycelium, microconidia, macroconidia and chlamydospores. The ratio of these spore types can be altered by the addition of various nutrients to the basal medium. Excellent control (96%) of Texas gourd was achieved using pre-planting and pre-emergence applications with granular formulations of this fungus (reviewed in Boyette, 2000)

Vermiculite has also been used effectively to prepare solid substrate mycoherbicide formulations. Walker (1981) produced mycelia of *Alternaria macrospora* in liquid shake culture and mixed the mycelium with vermiculite. The fungus sporulated profusely in the mixture and, after air-drying, applications were made both pre-emergence and post-emergence to spurred anoda. Pre-emergent application of fungus-infested vermiculite resulted in control rates equivalent to those achieved with post-emergence foliar sprays.

Granular formulations of several biocontrol fungi have also been made using sodium alginate (Walker and Connick, 1983; Weidemann and Templeton, 1988); a procedure adapted from research with time-released herbicide formulations (Connick, 1982). Fungal mycelium is mixed into a sodium alginate solution with various fillers, such as kaolin clay, and the mixture is dripped into 0.25 M calcium chloride. The Ca^{2+} ions react with the sodium alginate to form gel beads. The beads are allowed to harden briefly in the calcium chloride solution and then they are collected, rinsed and air-dried. Granules are relatively uniform in size and shape and can be used in a manner similar to pre-planting or pre-emergence herbicides, or rehydrated and exposed to UV light to induce spore production for other applications.

A pasta-like process is another approach to producing granules of several different fungi, such as *C. truncatum* for hemp sesbania control, *F. lateritium* for velvetleaf control, and *F. oxysporum* for sicklepod control. Granules are produced by mixing semolina wheat flour and kaolin clay with fungal propagules contained in a liquid component; either water or residual liquid growth medium. The mixture is kneaded into dough, rolled into thin sheets with a pasta press, and air-dried for 48 h. Sheets are then milled and sieved to obtain uniform-sized granules which are stored at 4°C. These granules, called 'Pesta', provided 90–100% weed control in glasshouse tests. In field tests, 'Pesta' granules containing *C. truncatum* provided 80–85% control of hemp sesbania in 3 years of tests (Connick *et al*., 1993; Boyette *et al*., 2007b). Various mycoherbicide formulations are listed in Table 7.1.

Adjuvants for liquid formulations

The simplest mycoherbicide delivery system is suspension of the agent in water for spray application. However, many weeds possess a waxy cuticle that inhibits the even spreading of droplets across the leaf surface, thus preventing uniform distribution of the agent. Surfactants facilitate distribution of an agent across the phylloplane by reducing surface tension caused by the waxy cuticle. Various non-ionic surfactants have been used in mycoherbicide research. Some surfactants may affect the growth or germination of fungal propagules.

Table 7.1. Experimental and commercial bioherbicide formulations.

Weed host	Pathogen	Formulation[a]
Liquid suspension formulations		
Spurred anoda (*Abutilon theophrasti*)	*Fusarium lateritium*	Water + Tween-20 surfactant (0.02%)
Spurred anoda (*Abutilon theophrasti*)	*Colletotrichum coccodes*	Water + sorbitol (0.75%)
Round-leaved mallow	*Colletotrichum gloeosporioides* f. sp. *malvae*	**BioMal**
Annual bluegrass (*Poa annua*)	*Xanthamonas campestris*	**Camperico**
Spurred anoda (*Anoda cristata*)	*Alternaria macrospora*	Water + nonoxynol surfactant (0.02%) + sucrose (5% w/v)
Giant ragweed (*Ambrosia trifida*)	*Protomyces gravidus*	Water
Field bindweed (*Convolvulus arvensis*)	*Phomopsis convolvulus*	Water + gelatin (0.1%)
Jimsonweed (*Datura stramonium*)	*Alternaria crassa*	Water + nonoxynol surfactant (0.4%)
Florida beggarweed (*Desmonium tortuosum*)	*Colletotrichum truncatum*	Water + nonoxynol surfactant (0.4%)
Sicklepod (*Cassia occidentalis*)	*Alternaria cassiae*	Water + nonoxynol surfactant (0.4%)
Common purslane (*Portulaca oleracea*)	*Dichotomophthora portulacaceae*	Water + Tween-20 (0.02%)
Horse purslane (*Trianthema portulacastrum*)	*Gibbago trianthemae*	Water + Tween-20 (0.02%)
Hemp sesbania (*Sesbania exaltata*)	*Colletotrichum truncatum*	Water + nonoxynol surfactant (0.2%); paraffin wax, mineral oil, soybean oil lecithin; unrefined corn oil
Eastern black nightshade (*Solanum ptycanthum*)	*Colletotrichum coccodes*	Water + Tween-80 surfactant (0.02%)
Stranglervine (*Morrenia odorata*)	*Phytophthora palmivora*	**DeVine** Chlamydospores in water
Solid formulations		
Velvetleaf (*Abutilon theophrasti*)	*Fusarium lateritium*	Sodium alginate, kaolin granules
Northern jointvetch (*Aeschynomene virginica*)	*Colletotrichum gloeosporioides* f. sp. *aeschynomene*	**Collego** Component A: dried spores Component B: rehydrating agent + surfactant
Spurred anoda (*Anoda cristata*)	*Alternaria macrospora*	Vermiculite
Texas gourd (*Cucurbita texana*)	*Fusarium solani* f. sp. *cucurbitae*	Cornmeal/sand; sodium alginate–kaolin granules
Marijuana (*Cannabis sativa*)	*Fusarium oxysporum var. cannabis*	Fungus-infested wheat straw
Hemp sesbania (*Sesbania exaltata*)	*Colletotrichum truncatum*	Fungus-infested wheat gluten/kaolin clay (Pesta)
Sicklepod (*Cassia occidentalis*)	*Fusarium oxysporum*	Fungus-infested wheat gluten/kaolin clay (Pesta)
Sicklepod (*Cassia occidentalis*) and others	*Alternaria cassiae*	**CASST**

[a] Names in bold are commercial formulations.
Adapted from Boyette (2000).

For example, *Alternaria cassiae* spores do not germinate consistently in Tween-20 or Tween-80 non-ionic surfactants but readily germinate in 0.02–0.04% non-ionic, nonoxynol surfactants (Walker and Riley, 1982). Tests should be conducted to measure any effect of a given surfactant on the candidate mycoherbicide. Other liquid formulations are listed in Table 7.1.

Various adjuvants and amendments have been used either to improve or to modify spore germination, increase pathogen virulence, minimize environmental constraints, or alter host preference, each of which may greatly influence the mycoherbicidal performance of a candidate bioherbicide. The addition of sucrose to aqueous suspensions of *A. macrospora* resulted in greater control of spurred anoda (Walker, 1981). Also, increased spore germination and disease severity occurred on Florida beggarweed (*Desmodium tortuosum*) when small quantities of sucrose and xanthan gum were added to aqueous spore suspensions of *C. truncatum* (Cardina *et al*., 1988).

Disease severity on johnsongrass infected by *Bipolaris sorghicola* was significantly increased by adding 1% Soy-Dox to the fungal spray mixture. Similarly, the addition of sorbitol yielded a 20-fold increase in the number of viable spores of *C. coccodes* re-isolated from inoculated velvetleaf. When this amendment was added to *C. coccodes* for velvetleaf control, three 9-h dew periods on consecutive nights were as effective as a single 18-h dew treatment (Wymore and Watson, 1986).

Most pathogens being evaluated as mycoherbicides require high water activities (i.e. humidity greater than 80%, or dew) over a period of time in order to germinate, penetrate, infect and kill the target weed. This period of time ranges from 6 h to more than 24 h, depending upon the pathogen and the weed host (reviewed in Boyette, 2000). Invert (water-in-oil) emulsions can retard evaporation, thereby decreasing the length of time that additional free moisture is required for spore germination and for infection (Quimby *et al*., 1988; Daigle *et al*., 1990). In these studies, lecithin was used as an emulsifying agent, and paraffin oil and wax were used to further retard evaporation and help retain droplet size. Specialized spraying equipment was developed to deliver this viscous material (McWhorter *et al*., 1988; Quimby *et al*., 1988). Glasshouse and field results indicated that excellent control (>95%) of sicklepod with *A. cassiae* could be achieved with little or no dew (Quimby *et al*., 1988). This system was used to enhance hemp sesbania control in the field with *C. truncatum*. The control (95%) achieved was comparable to that achieved with the synthetic herbicide, acifluorfen. Less than 10% control of hemp sesbania occurred in plots treated with the fungus applied with a water-only carrier (Boyette *et al*., 1993).

Protection against ultraviolet radiation

Solar radiation is one reason for mycoherbicides that perform well in glasshouse trials to fail in the field or exhibit sporadic field efficacy (Yang and TeBeest, 1993; Walker and Tilley, 1997; Charudattan, 2000). The transmitted solar spectrum is attenuated by the windows of glasshouses and spectrally altered by cover materials that are typically treated with ultraviolet (UV) inhibitors to prolong their lifespan. Like some synthetic herbicide formulations, UV protection may be crucial in preserving the applied active ingredient, particularly for formulations that deposit agents onto leaf surfaces, where they remain exposed to solar radiation.

Recently, the effects of sunlight on mortality, germination rate, and germ tube length for different phytopathogenic species of *Colletotrichum* were explored (Ghajar *et al*., 2006a). Exposure to sunlight decreased germination rate and germ tube length of *C. gloeosporioides* conidia isolated from *Polystigma rubrum* subsp. *rubrum* (Stojanović *et al*., 1999). More recently, UV-A (320–400 nm) photons were found to stimulate appressorium formation, while UV-B (280–320 nm) photons delayed conidium germination in *C. orbiculare* and *Plectosporium alismatis*. As the dose of UV-B increased, these photons deactivated conidia and also delayed germination of the survivors (Ghajar *et al*., 2006a).

Studies on UV protection of fungal entomopathogens have indicated that protectants can prolong the viability of conidia (Burges, 1998; Burges and Jones, 1998; Leland and Behle, 2005). A calcium cross-linked lignin UV

barrier produced a tenfold increase in *Beauveria bassiana* germination response time (RT_{50}) from 2.8 to 28.3 h after incubation for 48 h (Leland and Behle, 2005). In a follow-up study by Ghajar *et al*. (2006b), formulations containing water- and oil-soluble compounds that protect against UV damage were explored as formulation additives and post-UV-B-exposed germination rates increased to levels similar to the unexposed or so-called 'dark' control. In addition, these authors reported a significant increase in disease development over control levels when *C. orbiculare* was applied with particular UV protectants in leaf disc bioassays (Ghajar *et al*., 2006b).

Formulation can alter or expand host selectivity of a bioherbicide. For example, the host selectivity of *A. crassa*, a mycoherbicide for jimsonweed, can be altered by the addition of water-soluble filtrates of jimsonweed or dilute pectin suspensions (Boyette and Abbas, 1994). Several plant species that were either resistant or which exhibited a hypersensitive reaction to the fungus alone, exhibited various degrees of susceptibility following these amendments. Among the important weed species that were highly susceptible to infection following addition of these amendments were hemp sesbania, eastern black nightshade (*Solanum ptycanthum*), cocklebur and showy crotalaria (*Crotalaria spectabilis*). Several solanaceous crop species, including tomato (*Lycopersicon esculentum*), aubergine (*Solanum melonegra*), potato (*S. tuberosum*) and tobacco (*Nicotiana tabacum*), also became susceptible to infection when these amendments were used. With proper timing of application, it is possible that these amendments could enhance the weed control spectrum of *A. crassa* (Boyette and Abbas, 1994).

Amsellem *et al*. (1991) found that the host specificities of *A. cassiae* and *A. crassa* were greatly expanded, and that a saprophytic *Cephalosporium* species became pathogenic when these fungi were formulated in an invert emulsion. Similarly, the host ranges of *C. truncatum* and *C. gloeosporioides* f. sp. *aeschynomene* (Collego) were also expanded when spores of either pathogen were formulated in an invert emulsion. In rice field plots, over 90% of hemp sesbania plants were controlled by Collego/invert emulsion treatments, while aqueous suspensions of Collego had no effect upon hemp sesbania (Boyette *et al*., 1991a, 1992). A similar response occurred with *C. truncatum*. Aqueous inundative or wound inoculations with aqueous spore suspensions of *C. truncatum* had no effect on northern jointvetch, but susceptibility to infection was induced when the fungus was formulated in the invert emulsion.

Most mycoherbicides have a limited host range. For the purposes of safety and registration, this is an advantage. However, from an economic standpoint, this could preclude the practical use of a candidate mycoherbicide, since a single weed species rarely predominates in row crop situations (McWhorter and Chandler, 1982). One solution to this limitation is to apply mixtures of pathogens to mixed weed populations. For example, northern jointvetch and winged waterprimrose (*Jussiae decurrens*), two troublesome weeds in rice, were simultaneously controlled with a single application of CGA and *C. gloeosporioides* f. sp. *jussiae* (Boyette *et al*., 1979). A mixture of these two pathogens with the addition of *C. malvarum* also effectively controlled northern jointvetch, winged waterprimrose and prickly sida (TeBeest and Templeton, 1985). Various weed pathogens may not be compatible with each other. Thus, mixtures of pathogens need to be screened prior to formulation.

7.5 Application Technology

In a recent review on the state of the art in bioherbicides, Hallett noted that application technology, especially liquid spray application, had lagged (Hallett, 2005). Citing published reports by Egley and Boyette (1993), Bateman (1998), Chapple and Bateman (1997) and his own unpublished results, he highlighted the role of droplet size and deposition patterns in weed control.

Bioherbicidal weed control research is often conducted with very high inoculum rates and unrealistically high application volumes (e.g. 'spray to runoff'). Ground-based herbicide application rates are generally less than 200 l/ha and aerial application rates are much lower. When application rates are expressed as CFU/ml, the actual number of infective units to treat an area is

obfuscated. Without a clear understanding of the true application rate, any assessment of a pathogen's potential as a biocontrol agent should be considered to be preliminary.

In using native pathogens to manage indigenous weed species, the weed scientist is attempting to alter the natural balance. This is hardly an anathema – altering this balance is intrinsic to most agronomic practices – but the ecological forces at work warrant consideration. The dose–response relationship should not be assumed to be linear over a broad range of inoculum concentrations and, as reviewed (Hallett, 2005), application rates are often well beyond the linear range. Density-dependent pathogen mortality, hyperparasitism and competition for infection sites conspire to reduce efficiency at these levels (Newton *et al*., 1997, 1998; Horn, 2006). Consequently, if inadequate control is provided at a given inoculum rate, it may be more cost-effective to reconsider adjuvants, formulation and delivery systems than to simply increase the dose.

The method of production of mycoherbicides may directly determine the method of application. Mycoherbicides are applied in much the same manner as chemical herbicides and often with the same equipment. Tanks, lines and nozzles on the spraying system must be void of chemical residues that may be detrimental to mycoherbicides. A slurry of activated charcoal and liquid detergent can be used for cleansing spray equipment (Quimby and Boyette, 1987). Similarly, pesticides, especially fungicides, applied to mycoherbicide-treated areas may reduce mycoherbicide effectiveness. For example, the fungicide benomyl and the herbicide propiconazol, applied sequentially 7 and 14 days after Collego application, suppressed disease development in northern jointvetch (Khodayari and Smith, 1988). Similarly, the efficacy of DeVine was reduced if the fungicides Aliette and Ridomil were used within 45 days following mycoherbicide application (Kenney, 1986). This result might be expected, as these fungicides are active against *Phytophthora* spp., but it highlights the need to evaluate bioherbicides for compatibility with agronomic practices and agrochemical programmes.

7.6 Compatibility of Bioherbicides with other Management Practices

While classical biocontrol is most often practised in natural or low-input ecosystems, bioherbicides generally aim to control weeds in more highly managed systems. It is unrealistic to expect major changes in cropping systems to accommodate bioherbicides, so formulation and application technology must work with, or least not interfere with, accepted agronomic practices.

Wyss *et al*. (2004) recognized that agrochemicals can interfere with biocontrol agents in distinct phases. They measured the compatibility of synthetic herbicides, fungicides, insecticides and adjuvants with the *Amaranthus* spp. biocontrol agent, *Phomopsis amaranthicola*. Spore germination was tolerant of very high rates (over 2× maximum labelled rates) of some agrochemicals, such as benomyl, atrazine, imazethapyr and pendimethalin, but was completely inhibited by low rates (0.25× maximum labelled rates) of chlorothalonil, iprodione and diuron. Similar differences in compatibility were observed regarding vegetative growth and sporulation. The authors noted that this type of *in vitro* screening is useful, but that ultimately the agrochemical effects need to be assessed in the field so that effects on pathogenicity and weed control can be quantified. The purple nutsedge (*Cyperus rotundus*) pathogen, *Dactylaria higginsii*, was also evaluated for tolerance to several pesticides (Yandoc *et al*., 2006). The herbicide imazapyr was well tolerated by the fungus, but all other evaluated pesticides inhibited conidial germination and/or mycelial growth.

One study examined in-field interactions between a synthetic herbicide and a bioherbicide, *C. truncatum*, for hemp sesbania control (Boyette *et al*., 2007a). This weed is problematic in soybean and cotton fields in the southern USA, where glyphosate-based weed control management is common. In the context of that system, the authors evaluated *C. truncatum* for bioherbicidal efficacy in the field when applied before, simultaneously with, and after glyphosate applications. In these studies, the bioherbicide provided control of the weed target when applied after glyphosate, but not when applied simultaneously or before the herbicide (Fig. 7.1).

Yandoc *et al*. (2006), citing unpublished observations of Smith and Hallett, stated that glyphosate itself was not toxic to *Aposphaeria amaranthi* but the commercially formulated products were. We have observed that various commercial formulations of glyphosate have very different effects on *Myrothecium verrucaria* spore viability (unpublished data).

The addition of sublethal rates of the herbicides linuron, imazaquin and lactofen to *A. cassiae* spores significantly increased control of sicklepod when applied in an invert formulation (Quimby and Boyette, 1986). Control of velvetleaf was significantly improved by sequential applications of the herbicide 2,4 DB and spores of *F. lateritium*. However, spore germination and disease severity were greatly reduced when the fungus and herbicide were tank-mixed (reviewed in Boyette, 2000). Biocontrol of velvetleaf was also improved significantly by the addition of thiadiazuron, a cotton defoliant, to an aqueous spray mixture of *C. coccodes* (Wymore *et al*., 1988).

The rust fungus, *Puccinia canaliculata*, does not provide consistently high control of its host weed yellow nutsedge (*Cyperus esculentus*) when uredospores are applied alone, even under optimal environmental conditions (Bruckart *et al*., 1988). However, sequential applications of the herbicide paraquat followed by *P. canaliculata* spores resulted in a synergistic disease interaction, with almost complete yellow nutsedge control, compared to only 10% and 60% control, respectively, for paraquat or the fungus alone (Callaway *et al*., 1987).

Khodayari *et al*. (1987) demonstrated that the weed control spectrum of *Colletotrichum gloeosporioides* f. sp. *aeschynomene* can be extended by mixing with acifluorfen, a herbicide that is effective in controlling hemp sesbania, but is ineffective in controlling northern jointvetch. In these tests, both weeds were effectively controlled in soybeans by a single application of the mixture, providing that the microenvironment was favourable for infection.

Erwinia carotovora, a candidate bacterial bioherbicide for tropical soda apple (*Solanum viarum*) was evaluated for compatibility with commercial formulations of dicamba and triclopyr (Roberts *et al*., 2002). In this unique pathosystem, the biocontrol agent alone did not cause any leaf injury, but it prevented the

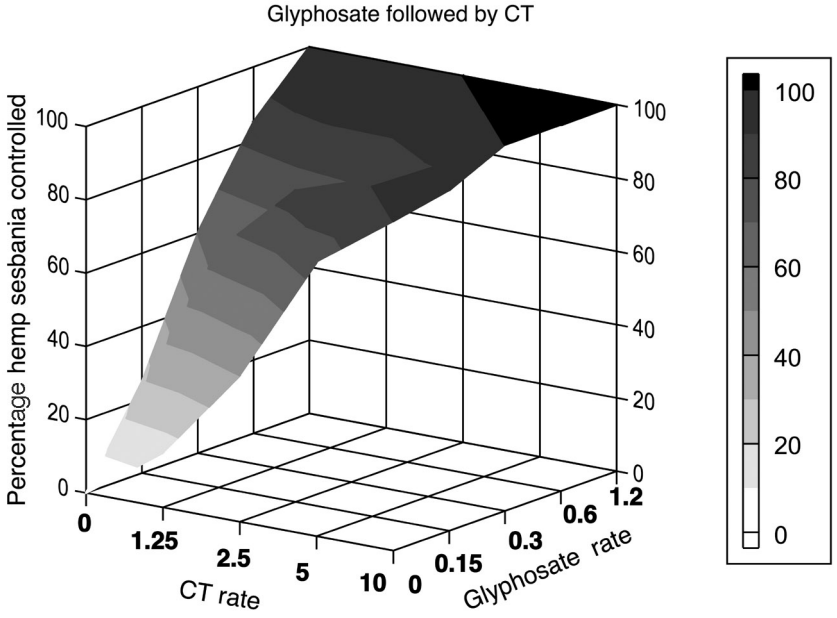

Fig. 7.1. Hemp sesbania control by *Colletotrichum truncatum* (CT) preceded by glyphosate.

regeneration of plants injured in the herbicide treatments.

In the previous examples, bioherbicides were used in concert with chemical herbicides to improve weed control. Shearer and Nelson (2002) evaluated co-application of *M. terrestris* with the herbicide endothall to improve control of hydrilla (*Hydrilla verticillata*) and also to minimize injury to non-target vegetation. At high treatment levels, both the pathogen and the herbicide have been reported to cause disease or injury to other plants (Shearer and Nelson, 2002; Shearer and Jackson, 2006). When used together, however, greater than 90% control was achieved in mesocosm studies, even with reduced rates of endothall and *M. terrestris*. Non-target plant injury still occurred, but the researchers suggested that this integrated control offered a means of effective hydrilla control while altering the dosage of bioherbicide and chemical herbicide to protect desirable vegetation (Shearer and Nelson, 2002).

7.7 Risk analysis and Mitigation for Bioherbicides

Beyond the aspects of efficacy and economic viability, there may be other risks associated with bioherbicides. Some of the identified hazards include toxicity, infection, or allergenic responses to producers or applicators of the biocontrol agent. After application there are risks of damage to non-target plants, direct impacts on wildlife or other microorganisms, and indirect species- or community-level effects by perturbations in trophic networks (Vurro *et al*., 2001; Delfosse 2005). While not trivializing the magnitude of these hazards, the likelihood of some of these events is generally low. Risk mitigation can be realized through lowering the intrinsic hazard or by reducing the probability of exposure. For example, *M. verrucaria* produces secondary metabolites; the trichothecene mycotoxins (Abbas *et al*., 2001, 2002). It might be possible to make this a more acceptable bioherbicide by altering of the conditions of inoculum growth and formulation to reduce or exclude these toxins from the final product (Hoagland *et al*., 2007a,b). Mutation, strain selection, strain improvement or genetic engineering could yield an atoxigenic isolate. It has been reported that infected plants do not accumulate detectable levels of trichothecenes (Abbas *et al*., 2001). Another risk factor associated with *M. verrucaria* is its extensive host range, which includes several important crop species (Walker and Tilley, 1997). Clarke *et al*. (2007) demonstrated the efficacy of *M. verrucaria* against old world climbing fern (*Lygodium microphyllum*) without significant disease symptoms on co-occurring native plant species. The potential threat posed by bioherbicides to non-target plants, which is actually less than that of many widely used herbicides, might be mitigated by restrictions on application sites and times. Furthermore, while *M. verrucaria* is an aggressive pathogen when formulated, it does not form infections when applied without surfactant, which effectively halts any secondary disease cycles or off-target movement.

7.8 Conclusions

As noted earlier, Charles Wilson (1969) addressed the potential of bioherbicides almost 40 years ago. Since his writing, a few of those seeds have flourished but, in the words of a more recent review (Hallett, 2005), the field 'has languished in recent years'.

In the early years of bioherbicide discovery and development, the conventional thinking was that a good agent was one that could be grown cheaply and quickly, was an aggressive pathogen, was patentable, could be easily applied, had single-weed specificity, and that if satisfactory control was not realized, then inundate the weed with the pathogen (Templeton *et al*., 1979; TeBeest, 1991; Zidak and Quimby, 1998). Some of these principles are still valid; the marketplace has little use for slow-growing, fastidious bioherbicidal microorganisms. Other parts of the dogma have been effectively challenged. While a highly specific pathogen is desirable and intrinsically safe, economic realities may prevent the commercialization of such a pathogen. This premium on safety from specific agents may be unwarranted. Even before the advent of transgenic cropping systems, numerous successful synthetic herbicides with very broad activities were deployed. It may be possible to use broad-spectrum

pathogens without intolerable non-target effects (De Jong *et al*., 1999; Pilgeram and Sands, 1999). Instead of replacing herbicides, synergy between pathogens and chemical herbicides may be a more successful approach to weed management (Boyette *et al*., 2007a).

A portion of the bioherbicide canon rejected by many is the reliance on very high inoculum levels. While mycoherbicides offer natural weed control, there is nothing 'natural' about applying mycoherbicides in extraordinarily high titres, artificially deposited onto weed foliage, in anticipation of an epidemic. Gressel's review found published application rates from 200 to 500,000 times higher than necessary based on his assumptions (Gressel, 2002). This should be considered encouraging, since this suggests there is an opportunity to profoundly lower the doses and maintain weed control through improved formulation and application techniques.

The future of bioherbicides may lie with agents that were once considered too risky. Broad-spectrum bioherbicides such as *M. verrucaria*, *Sclerotinia sclerotiorum* and others might be made 'safe' by means of appropriate deployment strategies or development of ecologically impaired strains, respectively. Other pathogens with low virulence, but with desirable specificity or epidemiology, might be made more virulent through genetic modification (Gressel, 2002).

Through a pragmatic understanding of economic constraints and safety, the intelligent use of formulation and deployment methods, and with genetic engineering, the pathogen discoveries of the past might be harnessed as the bioherbicides of the future.

7.9 References

Abbas, H.K., Tak, H., Boyette, C.D., Shier, W.T. and Jarvis, B.B. (2001) Macrocyclic trichothecenes are undetectable in kudzu (*Pueraria montana*) plants treated with a high-producing isolate of *Myrothecium verrucaria*. *Phytochemistry* 58, 269–276.

Abbas, H.K., Johnson, B.B., Shier, W.T., Tak, H., Jarvis, B.B. and Boyette, C.D. (2002) Phytotoxicity and mammalian cytotoxicity of macrocyclic trichothecenes from *Myrothecium verrucaria*. *Phytochemistry* 59, 309–313.

Amsellem, Z., Sharon, A. and Gressel, J. (1991) Abolition of selectivity of two mycoherbicidal organisms and enhanced virulence of a virulent by an invert emulsion. *Phytopathology* 81, 985–988.

Amsellem, Z., Zidack, N.K., Quimby, P., Jr and Gressel, J. (1999) Long-term dry preservation of viable mycelia of two mycoherbicidal organisms. *Crop Protection* 18, 643–649.

Bateman, R.P. (1998) Delivery systems and protocols for biopesticides. In: Hall, F.R. and Menn, J.J. (eds) *Biopesticides: Use and Delivery*. Humana Press, Totowa, NJ, USA, pp. 509–528.

Bedi, J.S., Muller-Stover, D. and Sauerborn, J. (2002) Status of plant pathogens for the biological control of weeds. In: Koul, O., Dhaliwal, G.S., Marwaha, S.S. and Arora, J.K. (eds) *Biopesticides and Pest Management.* Campus Books International, New Delhi, India, pp. 316–331.

Berner, D.K. and Bruckart, W.L. (2005) A decision tree for evaluation of exotic plant pathogens for biological control of introduced invasive weeds. *Biological Control* 34, 222–232.

Bowers, R.C. (1982) Commercialization of microbial biological control agents. In: Charudattan, R. and Walker, H.L. (eds) *Biological Control of Weeds with Plant Pathogens.* John Wiley and Sons, New York, pp. 157–173.

Bowers, R.C. (1986) Commercialization of Collego: an industrialist's view. *Weed Science* 34 (Suppl. 1), 24–25.

Boyette, C.D. (2000) The bioherbicide approach: using phytopathogens to control weeds. In: Cobb, A.H. and Kirkwood, R.C. (eds) *Herbicides and Their Mechanisms of Action.* Sheffield Academic Press, Sheffield, UK, pp. 134–152.

Boyette, C.D. and Abbas, H.K. (1994) Host range alteration of the bioherbicidal fungus *Alternaria crassa* with fruit pectin and plant filtrates. *Weed Science* 42, 487–491.

Boyette, C.D., Templeton, G.D. and Smith, R.J., Jr (1979) Control of winged waterprimrose (*Jussiaea decurrens*) and northern jointvetch (*Aeschynomene virginica*) with fungal pathogens. *Weed Science* 27, 497–501.

Boyette, C.D., Templeton, G.E. and Oliver, L.R. (1984) Texas gourd (*Cucurbita texana*) control with *Fusarium solani* f. sp. *cucurbitae*. *Weed Science* 32, 497–501.

Boyette, C.D., Abbas, H.K. and Smith, R.J., Jr (1991a) Invert emulsions alter host specificity of biocontrol fungi. *Phytopathology* 71, 126.

Boyette, C.D., Weidemann, G.J., TeBeest, D.O. and Quimby, P.C., Jr (1991b) Biological control of jimsonweed (*Datura stramonium*) with *Alternaria crassa*. *Weed Science* 39, 673–667.

Boyette, C.D., Smith, R.J., Jr, Abbas, H.K. and McAlpine, J.R. (1992) Bioherbicidal control of hemp sesbania (*Sesbania exaltata*) and northern jointvetch (*Aeschynomene virginica*) in rice with anthracnose pathogen-invert formulations. *Proceedings of the Southern Weed Science Society* 45, 293.

Boyette, C.D., Quimby, P.C., Jr, Bryson, C.T., Egley, G.H. and Fulgham, R.E. (1993) Biological control of hemp sesbania (*Sesbania exaltata*) under field conditions with *Colletotrichum truncatum* formulated in an invert emulsion. *Weed Science* 41, 497–500.

Boyette, C.D., Hoagland, R.E. and Weaver, M.A. (2007a) Interaction of a bioherbicide and glyphosate for controlling hemp sesbania in glyphosate-resistant soybean. *Weed Biology and Management* (in press).

Boyette, C.D., Jackson, M.A., Bryson, C.T., Hoagland, R.E., Connick, W.J. and Daigle, D.J. (2007b) *Sesbania exaltata* biocontrol with *Colletotrichum truncatum* microsclerotia formulated in 'Pesta' granules. *BioControl* 52, 413–426.

Brown, J.K.M. and Hovmøller, M.S. (2002) Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. *Science* 297, 537–541.

Burges, H.D. (1998) Formulation of mycoinsecticides. In: Burges, H.D. (ed.) *Formulations of Microbial Biopesticides, Beneficial Microorganisms, Nematodes and Seed Treatments,* Kluwer Academic, London, pp. 132–185.

Burges, H.D. and Jones, K.A. (1998) Formulation of bacterial, viruses, and protozoa to control insects. In: Burges, H.D. (ed.) *Formulations of Microbial Biopesticides, Beneficial Microorganisms, Nematodes and Seed Treatments*, Kluwer Academic, London, pp. 33–127.

Bruckart, W.L. (2006) Supplemental risk evaluations of *Puccinia jaceae* var. *solstitialis* for biological control of yellow starthistle. *Biological Control* 37, 359–366.

Bruckart, W.L., Johnson, D.R. and Frank, J.R. (1988) Bentazon reduces rust-induced disease in yellow nutsedge, *Cyperus esculentus. Weed Science* 2, 299–303.

Callaway, M.B., Phatak, S.C. and Wells, H.D. (1987) Interactions of *Puccinia canaliculata* (Schw.) Lagerh. with herbicides on tuber production and growth of *Cyperus esculentus* L. *Tropical Pest Management* 33, 22–26.

Cardina, J., Littrell, R.H. and Hanlin, R.T. (1988) Anthracnose of Florida beggarweed (*Desmodium tortuosum*) caused by *Colletotrichum truncatum. Weed Science* 36, 329–334.

Chapple, A.C. and Bateman, R.P. (1997) Application systems for microbial pesticides: necessity not novelty. *British Crop Protection Council Mongraphs* 89, 181–190.

Charudattan, R. (2000) Current status of biological control of weeds. In: Kennedy, G.G. and Sutton, T.B. (eds) *Emerging Technologies for Integrated Pest Management: Concepts, Research and Implementation*. APS Press, St. Paul, MN, USA, pp. 269–288.

Churchill, B.W. (1982) Mass production of microorganisms for biological control. In: Charudattan, R. and Walker, H.L. (eds) *Biological Control of Weeds with Plant Pathogens*. John Wiley and Sons, New York, pp. 139–156.

Clarke, T.C., Shetty, K.G., Jayachandran, K. and Norland, M.R. (2007) *Myrothecium verrucaria*: a potential biological control agent for the invasive 'Old World climbing fern' (*Lygodium microphyllum*). *BioControl* [doi:10.1007/s10526–006–9035–3].

Connick, W.J., Jr (1982) Controlled release of the herbicides 2,4-D and dichlobenil from alginate gels. *Journal of Applied Polymer Science* 27, 3341–3348.

Connick, W.J., Jr, Nickle, W.R. and Boyette, C.D. (1993) Wheat flour granules containing mycoherbicides and entomogenous nematodes In: Lumsden, R.D. and Vaughn, J.L. (eds) *Pest Management: Biologically Based Technologies: Proceedings of the 28th BARC Symposium Series*. Beltsville, MD, USA, pp. 238–240.

Connick, W.J., Daigle, D.J., Boyette, C.D., Williams, K.S., Vinyard, B.T. and Quimby, P.C., Jr (1996) Water activity and other factors that affect the viability of *Colletotrichum truncatum* conidia in wheat flour–kaolin granules ("Pesta"). *Biocontrol Science and Technology* 6, 277–284.

Connick, W.J., Jr, Jackson, M.A., Williams K.S. and Boyette, C.D. (1997) Stability of microsclerotial inoculum of *Colletotrichum truncatum* encapsulated in wheat flour–kaolin granules, *World Journal of Microbiology and Biotechnology* 13, 549–554.

Daigle, D.J., Connick, W.J., Jr, Quimby, P.C., Jr, Evans, J.P., Trask-Merrell, B, and Fulgham, F.E. (1990) Invert emulsions: carrier and water source for the mycoherbicide, *Alternaria cassiae. Weed Technology* 4, 327–331.

De Jong, M.D., Aylor, D.E. and Bourdot, G.W. (1999) A methodology for risk analysis of pluvivorous fungi in biological control: *Sclerotina sclerotiorum* as a model. *BioControl* 43, 397–419

Delfosse, E.S. (2005) Risk and ethics in biological control. *Biological Control* 35, 319–329.
Egley, G.H. and Boyette, C.D. (1993) Invert emulsion droplet size and mycoherbicidal activity of *Colletotrichum truncatum*. *Weed Technology* 7, 417–424.
El-Morsy, E.-S.M (2006) Preliminary survey of indoor and outdoor airborne microfungi at coastal buildings in Egypt. *Aerobiologia* 22, 197–210.
EPA [Environmental Protection Agency] (2006) *Pesticides: Regulating Pesticides* [see http://www.epa.gov/pesticides/regulating/index.htm Accessed 26 May 2006].
Forseth, I.N., Jr and Innis, A.F. (2004) Kudzu (*Pueraria montana*): history, physiology, and ecology combine to make a major ecosystem threat. *Critical Reviews in Plant Sciences* 23, 401–413.
Friesen, T.J., Holloway, G., Hill, G.A. and Pugsley, T.S. (2006) Effect of conditions and protectants on the survival of *Penicillium bilaiae* during storgage. *Biocontrol Science and Technology* 16, 89–98.
Ghajar, F., Holford, P., Cother, E. and Beattie, A. (2006a) Effects of ultraviolet radiation, simulated or as natural sunlight, on conidium germination and appressorium formation by fungi with potential as mycoherbistats. *Biocontrol Science and Technology* 16, 451–469.
Ghajar, F., Holford, P., Cother, E. and Beattie, A. (2006b) Enhancing survival and subsequent infectivity of conidia of potential mycoherbistats using UV protectants. *Biocontrol Science and Technology* 16, 451–469.
Gressel, J. (2002) *Molecular Biology of Weed Control.* Taylor and Francis, New York.
Griffin, D.W. (2004) Terrestrial microorganisms at an altitude of 20,000 m in Earth's atmosphere. *Aerobiologia* 20, 135–140.
Griffin, D.W., Kellogg, C.A., Garrison, V.H., Lisle, J.T., Borden, T.C. and Shinn, E.A. (2003) African dust in the Caribbean atmosphere. *Aerobiologia* 19, 143–157.
Hallett, S.G. (2005) Where are the bioherbicides? *Weed Science* 53, 404–415.
Hildebrand, D.C. and McCain, A.H. (1978) The use of various substrates for large scale production of *Fusarium oxysporum* f. sp. *cannabis* inoculum *Phytopathology*, 68, 1099–1101.
Hoagland, R.E. (1990) *Microbes and Microbial Products as Herbicides: ACS Symposium Series 439*. ACS Books, Washington, DC, USA.
Hoagland, R.E. (2001) Microbial allelochemicals and pathogens as bioherbicidal agents. *Weed Technology* 15, 835–857.
Hoagland, R.E., Boyette, C.D., Weaver, M.A. and Abbas, H.K. (2007a) Bioherbicides: research and risks. *Toxin Reviews* (in press).
Hoagland, R.E., Weaver, M.A. and Boyette, C.D. (2007b) M*yrothecium verrucaria* as a bioherbicide, and strategies to reduce its non-target risks. *Allelopathy Journal* 19, 179–192.
Horn, B.W. (2006) Relationship between soil densities of *Aspergillus* species and colonization of wounded peanut seeds. *Canadian Journal of Microbiology* 52, 951–960.
Jackson, M.A. (1997) Optimizing nutritional conditions for the liquid culture production of effective fungal biological control agents. *Journal of Industrial Microbiology and Biotechnology* 19, 180–187.
Jackson, M.A., Shasha, B.S. and Schisler, D.A. (1996) Formulation of *Colletotrichum truncatum* microsclerotia for improved biocontrol of the weed hemp sesbania (*Sesbania exaltata*). *Biological Control* 7, 107–113.
Kenney, D.S. (1986) DeVine – the way it was developed: an industrialist's view. *Weed Science* 34 (Suppl. 1), 15–16.
Khodayari, K. and Smith, R.J., Jr (1988) A mycoherbicide integrated with fungicides in rice, *Oryza sativa. Weed Technology* 2, 282–285.
Khodayari, K., Smith, R.J., Jr, Walker, J.T. and TeBeest, D.O. (1987) Applicators for a weed pathogen plus acifluorfen in soybean. *Weed Technology* 1, 37–40.
Kiely, T., Donaldson, D. and Grube, A. (2004) *Pesticide Industry Sales and Usage: 2000 and 2001 Market Estimates*. Environmental Protection Agency (EPA), Washington, DC, USA.
Kuske, C.R. (2006) Current and emerging technologies for the study of bacteria in the outdoor air. *Current Opinion in Biotechnology* 17, 291–296.
Leland, J.E. and Behle, R.W. (2005) Coating *Beauveria bassiana* with lignin for protection from solar radiation and effects on pathogenicity to *Lygus lineolaris* (*Heteroptera*: Miridae). *Biocontrol Science and Technology* 15, 309–320.
McWhorter, C.G. and Chandler, J.M. (1982) Conventional weed control technology. In: Charudattan, R. and Walker, H.L. (eds) *Biological Control of Weeds with Plant Pathogens*. John Wiley and Sons, New York, pp. 5–27.
McWhorter, C.G., Fulgham, F.E. and Barrentine, W.L. (1988) An air-assist spray nozzle for applying herbicides in ultra-low volume. *Weed Science* 36, 118–121.
Mitchell, J.K. (1986) *Dichotomopthora portulacae* causing black stem rot on common purslane in Texas. *Plant Disease* 70, 603.

Mitchell, J.K. (2003) Development of a submerged-liquid sporulation medium for the potential smartweed bioherbicide *Septoria polygonourm*. *Biological Control* 27, 293–299.

Mitchell, J.K., Njalamimba-Bertsch, M., Bradford, N.R. and Birdsong, J.A. (2003) Development of a submerged-liquid sporulation medium for the johnsongrass bioherbicide *Gloeocercospora sorghi*. *Journal of Industrial Microbiology and Biotechnology* 30, 599–605.

Müller-Stöver, D., Thomas, H., Sauerborn, J. and Kroschel, J. (2004) Two granular formulations of *Fusarium oxysporum* f.sp. *orthoceras* to mitigate sunflower broomrape *Orobanche cumana*. *BioControl* 49, 595–602.

Newton, M.R., Kinkel, L.L. and Leonard, K.J. (1997) Competition and density-dependent fitness in a plant parasitic fungus. *Ecology* 78, 1774–1784.

Newton, M.R., Kinkel, L.L. and Leonard, K.J. (1998) Determinants of density- and frequency-dependent fitness in competing plant pathogens. *Phytopathology* 88, 45–51.

Phillips, D.J. and Margosan, D.A. (1987) Size, nuclear number, and aggressiveness of *Botrytis cinerea* spores produced on media of varied glucose concentrations. *Phytopathology* 77, 1606–1612.

Pilgeram, A.L. and Sands, D.C. (1999) Mycoherbicides. In: Hall, F.R. and Menn, J.J. (eds) *Biopesticides: Use and Delivery*. Humana Press, Totowa, NJ, USA.

Quimby, P.C., Jr (1989) Response of common cocklebur (*Xanthium strumarium*) to *Alternaria helianthi*. *Weed Technology* 3, 177–181.

Quimby, P.C., Jr and Boyette, C.D. (1986) Pathogenic control of prickly sida and velvetleaf: an alternate technique for producing and testing *Fusarium lateritium*. *Proceedings of the Annual Meeting of the Southern Weed Science Society* 38, 365–371.

Quimby, P.C., Jr and Boyette, C.D. (1987) Production and application of biocontrol agents. In: McWhorter, C.G. and Gebhardt, M.R. (eds) *Methods of Applying Herbicides*. Monograph Series, No. 4, Weed Science Society, Champaign, IL, USA, pp. 265–280.

Quimby, P.C., Jr, Fulgham, F.E., Boyette, C.D. and Connick, W.J., Jr (1988) An invert emulsion replaces dew in biocontrol of sicklepod: a preliminary study. In: Hovde, D.A. and Beestman, G.B. (eds) *Pesticide Formulations and Application Systems*. ASTM-STP 980, American Society for Testing and Materials, Philadelphia, PA, USA, pp. 264–270.

Reoun An, H., Mainelis, G. and White, L. (2006) Development and calibration of real-time PCR for quantification of airborne microorganisms in air samples. *Atmospheric Environment* 40, 7924–7939.

Ridings, W.H. (1986) Biological control of stranglervine in citrus: a researcher's view. *Weed Science* 34 (Suppl. 1), 31–32.

Roberts, P.D., Urs, R.R., Wiersma, H.I. and Mullahey, J.J. (2002) Effect of bacterium–herbicide combinations on tropical soda apple. *Biological Control* 24, 238–244.

Shabana, M.S., Müller-Stöver, D. and Sauerborn, J. (2003) Granular Pesta formulation of *Fusarium oxysporum* f. sp. *orthoceras* for biological control of sunflower broomrape: efficacy and shelf-life. *Biological Control* 26, 189–201.

Shearer, J.F. and Jackson, M.A. (2006) Liquid culturing of microsclerotia of *Mycoleptodiscus terrestris*, a potential biological control agent for the management of hydrilla. *Biological Control* 38, 298–306.

Shearer, J.F. and Nelson, L.S. (2002) Integrated use of endothall and a fungal pathogen for management of the submersed aquatic macrophyte *Hydrilla verticillata*. *Weed Technology* 16, 224–230.

Silman R.W., Bothast, R.J. and Schisler, D.A. (1993) Production of *Colletotrichum truncatum* for use as a mycoherbicide: effects of culture, drying and storage on recovery and efficacy. *Biotechnology Advances* 11, 561–575.

Slade, S.J. Harris, R.F. Smith, C.S. and Andrews, J.H. (1987) Microcycle conidiation and spore-carrying capacity of *Colletotrichum gloeosporioides* on solid media. *Applied and Environmental Microbiology* 53, 2106–2110.

Slininger, P.J., Behle, R.W., Jackson, M.A. and Schisler, D.A. (2003) Discovery and development of biological agents to control crop pests. *Neotropical Entomology* 32, 183–195.

Smith, J.R., Jr (1991) Integration of biological control agents with chemical pesticides. In: TeBeest, D.O. (ed.) *Microbial Control of Weeds*. Chapman and Hall, New York, pp. 189–208.

Smith, R.J. (1986) Biological control of northern jointvetch (*Aeschynomene virginica*) in rice (*Oryza sativa*) and soybeans (*Glycine max*): a researcher's view. *Weed Science* 34 (Suppl. 1), 17–23.

StojanoviÊ, S., StaroviÊ, M. and MatijeviÊ, D. (1999) Factors affecting conidial germination of *Colletotrichum gloeosporioides* isolated from *Polystigma rubrum* subsp. *rubrum* stromata. *Acta Phytopathologica et Entomologica Hungarica* 34, 63–73.

TeBeest, D.O. (1991) *Microbial Control of Weeds*. Chapman and Hall, New York.

TeBeest, D.O. and Templeton, G.E. (1985) Mycoherbicides: progress in the biological control of weeds. *Plant Disease* 69, 6–10.

Templeton, G.E., TeBeest, D.O. and Smith, R.J. (1979) Biological weed control with mycoherbicides. *Annual Review of Phytopathology* 17, 301–310.

Toussoun, T.A., Nash, S.N. and Snyder, W.C. (1960) The effect of nitrogen sources and glucose on the pathogenesis of *Fusarium solani* f. *phaseoli*. *Phytopathology* 50, 137–140.

Tsai, M.-Y., Elgethun, K., Ramaprasad, J., Yost, M.G., Felsot, A.S., Herbert, V.R., and Fenske, R.A. (2005) The Washington aerial spray drift study: modeling pesticide spray drift deposition from an aerial application. *Atmospheric Environment* 39, 6194–6203.

Tuite, J. (1969) *Plant Pathological Methods: Fungi and Bacteria.* Burgess Publications, Minneapolis, MN, USA.

Tworkoski, T. (2002) Herbicide effects of essential oils, *Weed Science* 50, 425–431.

Van Dyke, C.G. and Winder, R.S. (1985) *Cercospora dubia* (Reiss) Wint. on *Chenopodium album* L.: greenhouse efficacy and biocontrol potential. *Proceedings of the Annual Meeting of the Southern Weed Society* 38, 373.

Vurro, M., Gressel, J., Butt, T., Harman, G.E., Pilgeram, A., St. Leger, R.J. and Nuss, D.L. (2001) *Enhancing Biocontrol Agents and Handling Risks*. IOS Press, Amsterdam, The Netherlands.

Walker, H.L. (1981) *Fusarium lateritium*: a pathogen of spurred anoda (*Anoda cristata*), prickly sida (*Sida spinosa*) and velvetleaf (*Abutilon theophrasti*). *Weed Science* 29, 629–631.

Walker, H.L. and Connick, W.J., Jr (1983) Sodium alginate for production and formulation of mycoherbicides. *Weed Science* 33, 333–338.

Walker, H.L. and Riley, J.A. (1982) Evaluation of *Alternaria cassiae* for the biocontrol of sicklepod (*Cassia obtusifolia*). *Weed Science* 30, 651–654.

Walker, H.L. and Tilley, A.M. (1997) Evaluation of an isolate of *Myrothecium verrucaria* from sicklepod (*Senna obtusifolia*) as a potential mycoherbicide agent. *Biological Control* 10, 104–112.

WeedScience (2006) *International Survey of Herbicide Resistant Weeds* [see http://www.weedscience.org/in.asp Accessed 26 May 2006].

Weidemann, G.J. and Templeton, G.E. (1988) Control of Texas gourd, *Curcurbitia texana*, with *Fusarium solani* f.sp. *cucurbitae. Weed Technology* 2, 271–274.

WHO [World Health Organization] (2000) *Guidelines for Concentration and Exposure-Response Measurements of Fine and Ultra-fine Particulate Matter for Use in Epidemiological Studies.* World Health Organization, Geneva, Switzerland.

Wilson, C.L. (1969) Use of plant pathogens in weed control. *Annual Review of Phytopathology* 7, 411–433.

Wraight, S.P., Jackson, M.A. and DeKock, S.L. (2001) Production, stabilization and formulation of fungal biocontrol agents. In: Butt, T., Jackson, C. and Magan, N. (eds) *Fungal Biocontrol Agents: Progress, Problems, and Potential*. CAB International, Wallingford, UK.

Wymore, L.A. and Watson, A.K. (1986) An adjuvant increases survival and efficacy of *Colletotrichum coccodes*: a mycoherbicide for velvetleaf (*Abutilon theophrasti*). *Phytopathology* 76, 1115–1116.

Wymore, L.A., Poirier, C., Watson, A.K. and Gotlieb, A.R. (1988) *Colletotrichum coccodes*, a potential bioherbicide for control of velvetleaf (*Abutilon theophrasti*). *Plant Disease* 72, 534–538.

Wyss, G.S., Charudattan, R., Rosskopf, E.N. and Littell, R.C. (2004) Effects of selected pesticides and adjuvants on germination and vegetative growth of *Phompsis amaranthicola*, a biocontrol agent for *Amaranthus* spp. *Weed Research* 44, 469–492.

Yandoc, C.B., Rosskopf, E.N., Pitelli, R.L.C.M. and Charudattan, R. (2006) Effect of selected pesticides on conidial germination and mycelial growth of *Dactylaria higginsii*, a potential bioherbicide for purple nutsedge (*Cyperus rotundus*). *Weed Technology* 20, 255–260.

Yang, X.B. and TeBeest, D.O. (1993) Epidemiological mechanisms of mycoherbicides effectiveness. *Phytopathology* 83, 891–893.

Zidak, N.K. and Quimby, P.C. (1998) Formulation and application of plant pathogens for biological weed control. In: Hall, F.R. and Menn, J.J. (eds) *Biopesticides: Use and Delivery*. Humana Press, Totowa, NJ, USA, pp. 371–382.