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Microbial Weed Control and Microbial Herbicides

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1. Introduction

Microbial weed control represents an innovative means to manage troublesome weeds and utilize the naturally occurring biological herbicides produced by soil microorganisms. These compounds kill or hinder the growth of weeds so that beneficial plant species can gain a competitive advantage. The vast diversity of microorganisms in our environment is largely untapped, and the potential discovery and characterization of these microbial compounds represents an opportunity to complement chemical herbicides, or reduce the potential for erosion or soil degradation due to tillage for weed control. Invasive weeds continue to threaten the productivity of agricultural lands and natural areas; however, for many weeds adequate, cost-effective control measures presently are not available (Jones & Sforza, 2007). Discovery of biological controls for invasive plants represents an alternative way to slow the spread of these weeds using natural enemies (Jones & Sforza, 2007). Further advances in microbial genetics will continue to improve our understanding of the wealth of genetic diversity and potential in the soil and to better use plant-microbe interactions. The development of biocontrol agents would lessen the need for chemical herbicides and provide greater options for weed management. Microbes have a place in integrated, ecologically based weed management and their potential is only just being realized.

The concept of utilizing microbial herbicides has been explored for more than a quarter century, but there remain many challenges to overcome before they can be widely used in agricultural, range and forest lands, or waterways. Those challenges include improving the efficacy of the microbial activity, survival of microorganisms, persistence of the suppressive compound, delivery systems, determining host range, and avoiding injury to non-target organisms. Other considerations are interactions with chemical herbicides, regulations, commercialization and mass production, and economic feasibility.

Biological controls for weeds can generally be divided into one of three general types: classical, augmentative or inundative, and cultural. The classical approach involves the introduction of a control agent into an area where it did not previously exist, and where the agent eventually becomes able to sustain itself. An example of this is the release of *Puccinia chondrillina* to control *Chondrilla juncea* (rush skeletonweed; Barton, 2004). Augmentative or inundative biological control refers to repeated application of a foreign agent with the intent to reduce weed densities to a level where beneficial plant species can compete. An example

of an augmentative control is *Colletotrichum gloeosporioides* for control of sicklepod (*Senna obtusifolia* L.; Boyette, 2006). Cultural weed control might include crop rotation, fallow periods, sanitation to prevent the introduction and spread of weed seeds, and maintaining soil fertility to produce healthy crop plants. The successful history of insects in weed control and the ever increasing list of successful insect biological controls placed in the hands of land managers offers hope for microbial weed control. Use of biological herbicides requires a shift in thinking from the use of chemical herbicides, as biological controls will most likely not eliminate a weed problem as quickly or as thoroughly as some herbicides on an annual basis; the goal is to inhibit the weed pest below an economically damaging threshold over a long time period in order for beneficial species to gain a competitive advantage (Ghosheh, 2005). Among the criteria that Charudattan (2005) lists for determining which invasive plants make suitable candidates for microbial herbicides are those that have a number of available pathogens that might be suitable for biological control, and where the cost of using that control will be competitive with other control measures. There are a number of reasons for developing microbial herbicides, and those include the potential for herbicide resistant weeds; chemical herbicides may persist in soil for longer than one growing cycle, thus limiting options for crop rotations; there may be limitations on herbicide registrations for certain crops; there is the potential for fewer undesirable effects to the environment than from chemical herbicides; and finally there is the potential for injury to non-target organisms (Guske et al., 2004). Special considerations are needed for management of parasitic weeds (Sauerborn et al., 2007). Herbicide use to control parasitic weeds is difficult, because the greatest damage to the host plant has often occurred before the parasitic weed emerges above the soil surface. Another challenge is that parasitic weeds are closely related to their hosts, and selective control with herbicides is difficult (Sauerborn et al., 2007).

The various regions of the world may be plagued by specific invasive weed problems, while other weeds are problematic worldwide. No matter whether they are worldwide problems or regional challenges, many are elusive to management efforts. These weeds vary by region and ecosystem, and several microorganisms have been studied or are under development in greenhouse or field studies as potential sources for microbial herbicides (Table 1A and B).

Weed Pest	Ecosystem	Region	Biocontrol agent	Reference
<i>Abutilon theophrasti</i> (Velvetleaf)	Croplands	U.S., Canada	<i>Fusarium oxysporum</i> ; <i>Colletotrichum coccodes</i> ; <i>Pseudomonas putida</i> & <i>Acidovorax delafieldii</i>	Kremer & Schulte, 1989; Wymore & Watson, 1989; Owen & Zdor, 2001
<i>Ailanthus altissima</i> (Tree-of-heaven)	Native forests, urban areas	North America, Europe	<i>Aecidium ailanthi</i> J.Y. Zhuang sp. nov.; <i>Coleosporium</i> sp.; <i>Fusarium oxysporum</i> f. sp. <i>perniciosum</i> ; <i>Verticillium albo-atrum</i>	Review by Ding et al., 2006; Schall & Davis, 2009
<i>Alternanthera philoxeroides</i> (Alligatorweed)	Aquatic, upland sites	Worldwide	<i>Nimbya alternantherae</i>	Pomella et al., 2007
<i>Amaranthus</i> spp. (Amaranthus)	Croplands	Europe, North America	<i>Phomopsis amaranthicola</i>	Roskopf et al., 2006
<i>Amaranthus retroflexus</i> (Redroot pigweed)	Croplands	Europe, North America	<i>Alternaria alternata</i>	Lawrie et al., 2002a

Weed Pest	Ecosystem	Region	Biocontrol agent	Reference
<i>Amaranthus hybridus</i> (Pigweed), <i>Senna obtusifolia</i> (Sicklepod), <i>Crotalaria spectabilis</i> (Showy crotalaria)	Croplands	North America	<i>Phomopsis amaranthicola</i> , <i>Alternaria cassia</i> , <i>Colletotrichum dematium</i> , <i>Fusarium udum</i>	Chandramohan & Charudattan, 2003
<i>Arceuthobium tsugense</i> (Hemlock dwarf mistletoe)	Coniferous forests	Vancouver Island, Canada	<i>Neonectria neomacrospora</i>	Rietman et al., 2005
<i>Brunnichia ovata</i> (Redvine), <i>Campis radicans</i> (Trumpet creeper), <i>Pueraria lobata</i> (Kudzu)	Croplands, wastelands, natural areas	Southern U.S.	<i>Myrothecium verrucaria</i>	Boyette et al., 2006, 2008a
<i>Cannabis sativa</i> (Ditchweed)	Croplands, grasslands	Kazakhstan	<i>Fusarium oxysporum</i> f. sp. <i>cannabis</i>	Tiourebaev et al., 2001
<i>Carduus pycnocephalus</i> (Italian thistle)	Grasslands, woodlands	Tunisia	<i>Puccinia carduorum</i>	Mejri et al., 2010a
<i>Centaurea diffusa</i> (Diffuse knapweed), <i>Centaurea maculosa</i> (Spotted knapweed)	Rangelands	Western U.S., Canada	<i>Fusarium</i> spp.	Caesar et al., 2002
<i>Chenopodium album</i> (Common lambsquarters)	Croplands	Worldwide	<i>Ascochyta caulina</i>	Ghorbani et al., 2002; Vurro et al., 2001
<i>Chrysanthemoides monilifera</i> ssp. <i>monilifera</i> (Boneseed)	Natural areas	Southeastern Australia	<i>Endophyllum osteospermi</i>	Wood & Crous, 2005
<i>Cirsium arvense</i> (Canada thistle)	Croplands, rangelands, pastures, roadsides	Temperate regions of northern hemisphere	<i>Phyllosticta cirsii</i> ; <i>Stagonospora cirsii</i> ; <i>Alternaria cirsinoxia</i> ; <i>Pseudomonas syringae</i> pv. <i>tagetis</i> ; Mix of <i>Phoma destructiva</i> , <i>Phoma hedericola</i> , <i>Mycelia sterila</i> , <i>Phoma nebulosa</i> , <i>Phomopsis cirsii</i>	Evidente et al., 2008; Yuzikhin et al., 2007; Bailey, 2004; Gronwald et al., 2002; Tichich & Doll, 2006; Guske et al., 2004; Leth et al., 2008
<i>Cirsium arvense</i> (Canada thistle), <i>Ranunculus acris</i> (Tall buttercup)	Pasture	New Zealand	<i>Sclerotinia sclerotiorum</i>	Bourdot et al., 2006a
<i>Damasonium minus</i> (Starfruit)	Croplands (rice), aquatic	Australia	<i>Plectosporium alismatis</i>	Jahromi, 2007
<i>Eichhornia crassipes</i> (Water hyacinth)	Aquatic	Tropical, sub-tropical regions	<i>Alternaria eichhorniae</i> isolate 5; <i>Cercospora piaropi</i>	Shabana & Mohamed, 2005; Shabana, 2005; Tessman et al., 2008
<i>Euphorbia esula</i> /virgata (Leafy spurge)	Rangelands, natural areas	North America	<i>Uromyces scutellatus</i>	Caesar, 2006

Weed Pest	Ecosystem	Region	Biocontrol agent	Reference
<i>Euphorbia heterophylla</i> (Wild poinsettia)	Croplands (soybean)	Brazil	<i>Sphaceloma poinsettiae</i>	Nechet et al., 2004
<i>Galium spurium</i> (False cleavers)	Croplands	Canada	<i>Plectosporium tabacinum</i>	Zhang et al., 2002
<i>Gaultheria shallon</i> (Salal)	Native forests	Canadian and American Pacific Coast	<i>Phoma exigua</i> ; <i>Valdensinia heterodoxa</i>	Zhao & Shamoun, 2006; Vogelgsang & Shamoun, 2004; Wilkin et al., 2005
<i>Hydrilla verticillata</i> (Hydrilla)	Aquatic	U.S. and worldwide	<i>Fusarium culmorum</i> ; <i>Mycoleptodiscus terrestris</i>	Shabana et al., 2003; Shearer & Jackson, 2006
<i>Isatis tinctoria</i> (Dyer’s woad)	Natural areas	Western North America	<i>Puccinia thlaspeos</i>	Kropp et al., 2002; Kropp & Darrow, 2006
<i>Lantana camara</i> (Lantana)	Natural and cultivated lands	South Africa	<i>Mycovellosiella lantanae</i> var. <i>lantanae</i> ; <i>Corynespora cassiicola</i> f. sp. <i>lantanae</i>	Den Breeyen & Morris, 2003; Pereira et al., 2003
<i>Matricaria perforata</i> (Scentless chamomile)	Croplands	Canada	<i>Colletotrichum truncatum</i>	Graham et al., 2006, 2007; Peng et al., 2005a
<i>Miconia calvescens</i> (Velvet tree; miconia)	Natural areas	Hawaii and French Polynesia	<i>Ditylenchus drepanocercus</i>	Seixas et al., 2004
<i>Nasella neesiana</i> (Chilean needle grass)	Pastures, grasslands	Australia, New Zealand	<i>Uromyces pencanus</i>	Anderson et al., 2010
<i>Orobanche cumana</i> (Sunflower broomrape)	Croplands	Mediterranean region, southeast Europe	<i>Fusarium oxysporum</i> f. sp. <i>orthoceras</i>	Müller-Stöver & Sauerborn, 2007
<i>Orobanche ramosa</i> (Broomrape; branched broomrape)	Croplands	Central & western Europe	<i>Fusarium oxysporum</i> (FOG); <i>Fusarium</i> spp.	Müller-Stöver et al., 2009; Kohlschmid et al., 2009; Boari & Vurro, 2004
<i>Orobanche aegyptiaca</i> (Egyptian broomrape)	Croplands	India, Israel	<i>Fusarium solani</i>	Sharma et al., 2011; Dor & Hershenhorn, 2009
<i>Orobanche crenata</i> , <i>Orobanche foetida</i> (Broomrape)	Croplands	Northern Tunisia	<i>Pseudomonas fluorescens</i> Bf7-9	Zermane et al., 2007
<i>Papaver somniferum</i> (Opium poppy)	Illicit plants		<i>Pleospora papaveracea</i>	Bailey et al., 2004
<i>Portulaca oleracea</i> (Common purslane), <i>Trianthema portulacastrum</i> (Horse purslane), <i>Euphorbia maculata</i> (Spotted spurge), <i>Euphorbia supina</i> (Prostrate spurge)	Tomato fields	Southeastern U.S.	<i>Myrothecium verrucaria</i>	Boyette et al., 2007a
<i>Raphanus raphanistrum</i> (Wild radish)	Croplands, vineyards	Australia	<i>Hyaloperonospora parasitica</i> ; <i>Pseudomonas fluorescens</i>	Maxwell & Scott, 2008; Flores-Vargas & O’Hara, 2006

Weed Pest	Ecosystem	Region	Biocontrol agent	Reference
<i>Salsola kali</i> (Russian thistle)	Croplands, pastures, rangelands	Western U.S., Eurasia	<i>Uromyces salsolae</i>	Hasan et al., 2001
<i>Schinus terebinthifolius</i> (Brazilian peppertree)	Forests	Florida, U.S.	<i>Neofusicoccum batangarum</i>	Shetty et al., 2011
<i>Senecio vulgaris</i> (Common groundsel)	Croplands (carrots)	Switzerland	<i>Puccinia lagenophorae</i>	Frantzen & Müller-Scharer, 2006
<i>Senna obtusifolia</i> (Sicklepod)	Croplands	Southeastern U.S.	<i>Colletotrichum gloeosporioides</i>	Boyette et al., 2007b
<i>Sesbania exaltata</i> (Hemp sesbania)	Croplands	Southern U.S.	<i>Colletotrichum truncatum</i>	Boyette et al., 2007c, 2008b
<i>Sonchus arvensis</i> (Perennial sowthistle)	Croplands	North America, Europe	<i>Alternaria sonchi</i>	Evidente et al., 2009a
<i>Striga hermonthica</i> (Striga, Witchweed)	Croplands	Africa	<i>Fusarium oxysporum</i> (Foxy 2; PSM 197); <i>F. oxysporum</i> (4-3-B); <i>F. nygamai</i> ; <i>Pseudomonas fluorescens</i> ; <i>P. putida</i>	Venne et al., 2009; Yonli et al., 2004; Ahonsi et al., 2002
<i>Taraxacum officinale</i> (Dandelion)	Turfgrass	Worldwide	<i>Phoma herbarum</i> ; <i>Phoma macrostoma</i> ; <i>Sclerotinia minor</i>	Stewart-Wade & Boland, 2004; Zhou et al., 2004; Abu-Dieyeh & Watson, 2006, 2007
<i>Ulex europaeus</i> (Gorse)	Native areas, pastures, forests	New Zealand	<i>Chondrostereum purpureum</i> ; <i>Fusarium tumidum</i>	Bourdot et al., 2006b; Yamoah et al., 2008
<i>Xanthium strumarium</i> (Common cocklebur)	Croplands	Southern U.S.	<i>Alternaria helianthi</i>	Abbas et al., 2004
<i>Xanthium occidentale</i> (Noogoora burr)	Croplands, rangelands	Australia	<i>Puccinia xanthii</i>	VanKlinken & Julien, 2003

Table 1A. List of current biological control research projects on dicotyledonous weed species that have shown promise in greenhouse and/or field trials, including the invasive weed species, ecosystem, region of importance, biological control agent and reference.

Biological herbicides represent a means to reduce dependence on synthetic herbicides; focus on ecologically grounded methods of management; reduce weed seed bank populations through environmentally friendly practices; and potentially reduce costs of weed control in crop production, rangeland restoration, forestry and aquatic systems (Bailey et al., 2010; Kennedy & Stubbs, 2007).

2. Challenges for weed control with microbial herbicides

The list of challenges in developing a successful microbial herbicide is long. Once a potential biological control agent is identified, the first challenge lies in reproducing lab and/or greenhouse results successfully in the field. The potential biological control agent and the toxin responsible for weed inhibition must be able to survive the harsh, and often unpredictable, environmental conditions that exist in the field. Li & Kremer (2006) showed inhibition of several weed species using rhizobacteria isolated from various weed hosts in

Weed Pest	Ecosystem	Region	Biocontrol agent	Reference
<i>Aegilops cylindrica</i> (Jointed goatgrass)	Croplands	Western U.S.	<i>Pseudomonas</i> spp.; <i>Xanthomonas</i> spp.	Kennedy & Stubbs, 2007
<i>Avena fatua</i> (Wild oat)	Cereal crops, croplands, native areas	North America, Europe, Australia	<i>Fusarium avenaceum</i> ; <i>F. culmorum</i> ; <i>Drechslera avenacea</i> (conidial state of <i>Pyrenophora chaetomioides</i>); <i>Puccinia coronata</i> f. sp. <i>avenae</i>	deLuna et al., 2011; Ghajar et al., 2006; Carsten et al., 2001
<i>Bromus diandrus</i> (Great brome)	Croplands	Tunisia	<i>Pseudomonas trivialis</i> strain X33D	Mejri et al., 2010b
<i>Bromus tectorum</i> (Downy brome; cheatgrass)	Croplands, rangelands, natural areas	Western North America	<i>Pseudomonas fluorescens</i> strain D7; <i>Pyrenophora semeniperda</i> ; <i>Ustilago bullata</i>	Kennedy et al., 1991, 2001; Meyer et al., 2001, 2007
<i>Digitaria sanguinalis</i> (Giant crabgrass; large crabgrass)	Croplands	China	<i>Curvularia eragrostidis</i> QZ-2000; <i>C. intermedia</i>	Zhu & Qiang, 2004; Jiang et al., 2008; Tilley & Walker, 2002
<i>Elytrigia repens</i> (Quackgrass)	Croplands	Temperate regions of northern & southern hemisphere	<i>Ascochyta agropyrina</i> var. <i>nana</i>	Evidente et al., 2009b
(Grasses)	Croplands	Australia	<i>Pyrenophora semeniperda</i>	Medd & Campbell, 2005
<i>Microstegium vimineum</i> (Japanese stiltgrass)	Forests	Eastern U.S.	<i>Bipolaris</i> sp.	Kleczewski & Flory, 2010
<i>Poa annua</i> (Annual bluegrass)	Turfgrass	Japan	<i>Xanthomonas campestris</i> pv. <i>poae</i> (JT-P482)	Imaizumi et al., 1999
<i>Setaria viridis</i> (Green foxtail)	Croplands	Worldwide	<i>Drechslera gigantea</i> ; <i>Exserohilum rostratum</i> ; <i>E. longirostratum</i> ; <i>Pyricularia setariae</i>	Casella et al., 2010; Green et al., 2004

Table 1B. List of current biological control research projects on monocotyledonous weed species that have shown promise in greenhouse and/or field trials, including the invasive weed species, ecosystem, region of importance, biological control agent and reference.

greenhouse studies. They noted the importance of greenhouse studies as a step toward identifying isolates that may be suitable for testing under the more variable conditions of the field, where there is greater competition from indigenous organisms and unpredictable environmental factors. In any case, the conditions under which a microorganism best survives depend on the microorganism itself and it cannot be assumed to be the same for all biocontrol agents. Cool, moist conditions are required for survival of the deleterious

rhizobacteria (DRB) used to control jointed goatgrass (*Aegilops cylindrica*) and downy brome (*Bromus tectorum*) in the field (Kennedy & Stubbs, 2007). For greatest biocontrol success, field application of organisms should be timed when rains are expected. On the other hand, Boguena et al. (2007) found that extremely cold temperatures reduced the ability of *Ustilago bullata* to infect downy brome and may limit its use as a biological control. One challenge to the development of fungal bioherbicides is the inability to infect the weed pest without a period of free water retention (Chittick & Auld, 2001). Favorable moisture and temperature conditions are critical to the efficacy of many mycoherbicides. Tichich et al. (2006) found that for optimum population growth of the pathogen *Pseudomonas syringae* pv. *tagetis* targeting Canada thistle (*Cirsium arvense*), periods of wet weather were required. This moisture requirement often limits candidates for biocontrol. After investigating the dew period requirements of three fungal pathogens that infected green foxtail (*Setaria viridis*), Peng & Boyetchko (2006) found that *Drechslera gigantea* was the best choice for control of green foxtail because this pathogen was the most virulent and it did not require a specific dew temperature for efficacy.

For successful biocontrol, the microorganism's growth stage needs to be matched with the period of greatest vulnerability of the weed. Studies are needed to ensure that the most virulent stage of biocontrol agent growth also coincides with the susceptible host habit and an active growth period of the host. The efficacy of *Plectosporium alismatis* to reduce starfruit (*Damasonium minus* (R. Br.) Buch) in rice was increased by using conidia and chlamydospores (Cliquet & Zeeshan, 2008) and applying the biocontrol agent on juvenile rather than older starfruit (Jahromi, 2007). Qiang et al. (2006) found that the mycelia of *Alternaria alternata* strain 501 were able to infect the host plant, *Eupatorium adenophorum*, in a much shorter time than with conidia. The *Pseudomonas fluorescens* strain D7 has the greatest efficacy on downy brome when the bacterium is applied in the fall. In addition, populations of *P. fluorescens* D7 in soil are greatest in the fall and spring, which coincides with active root growth of downy brome (Kennedy et al., 1991).

Another challenge to successful microbial herbicides is developing strains that are effective against weed populations with high genetic diversity. Diversity within a species can lead to inconsistent results with biocontrol measures (Bailey, 2004; Ward et al., 2008). Biological control isolates may be specific to the region where they were first isolated, for example Ash et al. (2008) studied fungal pathogens from Korea and Australia, and found that Korean isolates showed less pathogenicity on Australian weeds than the Australian isolates. The efficacy of *Sclerotinia minor* on dandelion (*Taraxacum officinale*) was dependent upon weed accession, age and plant competition (Abu-Dieyeh & Watson, 2007). Both physiological and ecological considerations need to be examined for successful biocontrol interactions.

Prior to commercialization of a biological herbicide, extensive host-range studies must be completed to determine effects of the agent on non-target organisms in order to minimize any harmful effects. Potential microbial herbicides must be virulent on the target species, while non-target plants remain disease-free (Bailey, 2004). Wapshere's (1974) concept of concentric spheres of related plant species is a starting point for investigations of non-target plant species. In host-range studies of *Alternaria alternata*, a potential biological control of water hyacinth (*Eichhornia crassipes*), the biocontrol agent also inhibited the weed water lettuce (*Pistia stratiotes* L.; Mohan Babu et al., 2002). They evaluated 29 economically and environmentally significant species that encompassed more than 18 families. After finding that only two plant species were

inhibited, they concluded that *A. alternata* would not be harmful to any economically important plants. Kennedy et al. (2001) found that *Pseudomonas fluorescens* strain D7 inhibited the target weed, downy brome, a few other *Bromus* species, jointed goatgrass and medusahead (*Taeniatherum caput-medusae*) in bioassays, greenhouse and field studies. They investigated representatives from a wide array of families that included native, crop and weed species, while concentrating on families, tribes, subtribes and accessions closely related to *Bromus* species. No dicots and only a few monocots were negatively affected by the bacterium. While a few other grasses were suppressed slightly by *P. fluorescens* D7 in bioassays, they were not suppressed in greenhouse studies using nonsterile soil. These two examples illustrate the importance of extensive testing of other plant species. The lack of host-range testing often leads to the early demise of a potential biocontrol agent.

Other challenges are large-scale production and storage, survival of the organism, requirement of the agent for formulation, shelf-life of the organism, and delivery system of the biocontrol agent to the host plant. The ability to produce large quantities of microbial products, and maintain survival of the organisms, are the major obstacles to field-scale application of biological control agents (Amsellem et al., 1999). Teshler et al. (2007) examined shelf life of the *Sclerotinia minor* bioherbicide and found that storage temperatures less than 11°C increased *S. minor* shelf life, but CO₂ did not affect shelf life. Cooler storage temperatures helped to prolong the shelf life of *Fusarium oxysporum* (Foxy2 and PSM197) for control of *Striga*; however, vacuum packing was not helpful. The fungicide Apron XL enhanced shelf life, but another fungicide, Ridomil Gold (both fungicides Metalaxyl-M, Syngenta, Basle, Switzerland & Germany), did not (Elzein et al., 2009). The authors attributed this difference to the higher recommended application rate for Ridomil Gold, or possibly that Apron XL is ineffective against ascomycetes such as *Fusarium oxysporum*. While most of the microbial herbicides developed to-date have been mycoherbicides, bacterial herbicides are becoming available and have different challenges and advantages. These include simpler fermentation processes, ease of upscaling and mass-production, production of secondary metabolites and lack of spore formation that may require specific growth conditions (Li et al., 2003).

Application technology and cultural practices also affect the efficacy of microbial herbicides applied in field situations. Aerosol applications in the field may need different inoculum levels than what are found to be successful in greenhouse studies. In addition, droplet size, and spray direction may need to be readjusted to reach the weed or soil for greatest weed reduction (Lawrie et al., 2002b). Byer et al. (2006) found that finer droplet size led to greater spray retention of *Colletotrichum truncatum* on scentless chamomile and *Colletotrichum gloeosporioides* f. sp. *malvae* on round-leaved mallow. Peng et al. (2005b) looked at spray retention for efficacy of the biological control agent *Pyricularia setariae* on green foxtail. They looked at sprayer type and nozzle size for varying application rates and droplet sizes. In general, finer droplet size was more advantageous, and the authors suggested further study with other factors such as use of adjuvants and formulations. Boyette et al. (2007b) found that *Colletotrichum gloeosporioides* was an effective biocontrol agent of sicklepod in soybean; however, the wider row spacings required repeat applications of the agent for adequate weed suppression. The challenges to successful biocontrol are many, and continued research and development are needed to offer the alternative of microbial weed management.

3. Successful microbial herbicides

The majority of biological herbicides developed to-date are mycoherbicides; however, several bacterial herbicides are under development as well. A screening procedure for a bacterial biological control agent has been developed by the authors (Figure 1). Our screening procedure takes advantage of the soil microbial community and the diversity within. This screening includes sampling the soil and plant material at the peak of population growth and when the suppression naturally occurs. An initial assay can separate the weed-suppressive microorganisms from the bulk of the native population. Further multiple screenings on various plants are suggested to get rid of those isolates that may also inhibit beneficial plants. While inhibition of the target weed is critical, non-host range must be determined early in the process to ensure the product progresses through the registration process and eventually becomes marketable. Both steps need to be thorough to ensure that no lesser known, but economically important, host is detected later in the process. Souissi and Kremer (1998) utilized a multiple-well plate procedure with leafy spurge (*Euphorbia esula* L.) callus to rapidly determine phytotoxicity of rhizobacterial isolates, and Vidal et al. (2004) have developed a successful method to produce yellow starthistle (*Centaurea solstitialis* L.) calli for bioassay screening of biocontrol pathogens such as *Phoma exigua*. A quick substitute bioassay will reduce the length of time needed for the initial screenings. In many cases, a quick screen is not possible (Kennedy et al., 1991), but automating data recording can often make data collection easier (Doty et al., 1994).

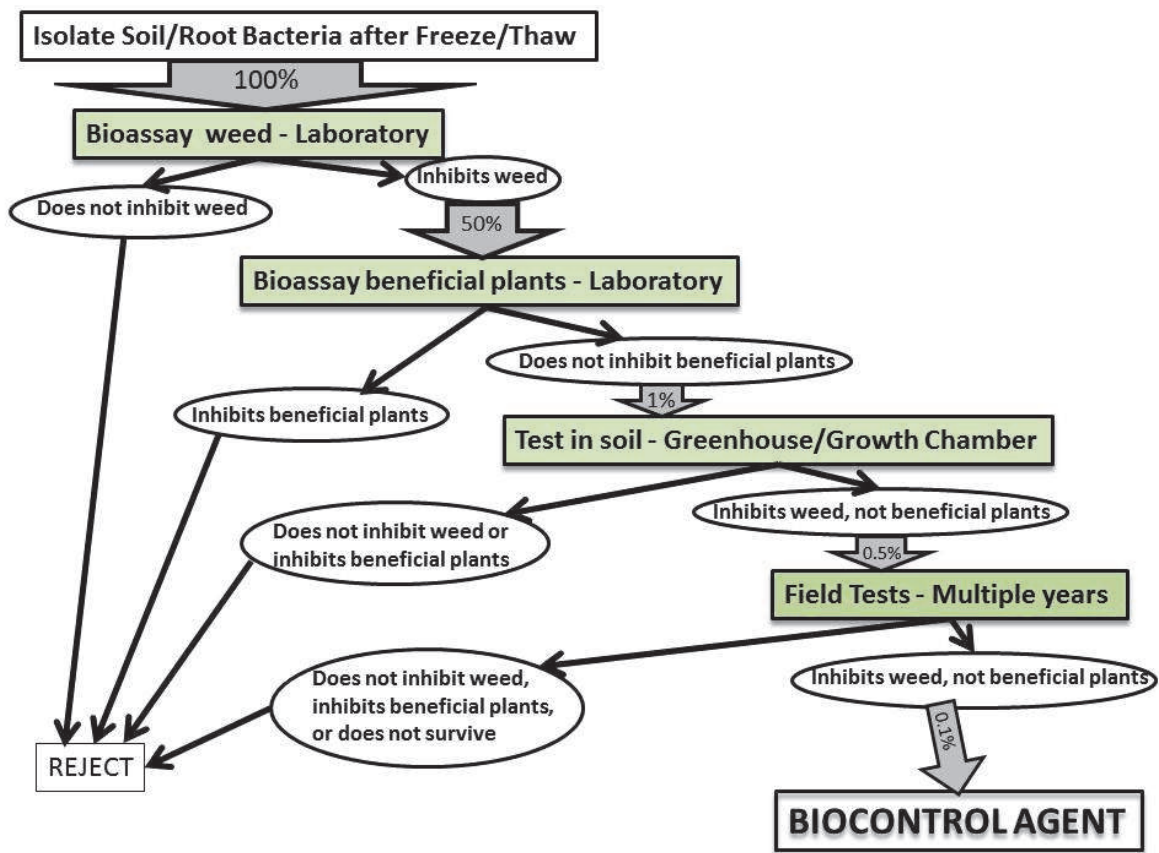


Fig. 1. Flow of assays to obtain a biological control organism from soil that successfully suppresses the growth of target organisms but has a limited host range and does not inhibit beneficial plant species.

Since the early 1980's, there have been several successful biological herbicides released to the market. Biomal® (ATCC 20767) *Colletotrichum gloeosporioides* f. sp. *malvae*, was developed as a weed biocontrol agent against round-leaved mallow (*Malva neglecta*) and registered in 1992. DeVine was marketed as a control for stranglervine (*Morrenia odorata*) in citrus, and Collego was used to control northern jointvetch (*Aeschynomene virginica*) in rice. Velgo is a potential mycoherbicide for control of velvetleaf in the U.S. and Canada (Mortensen, 1998; Owen & Zdor, 2001). DeVine and Collego were registered for use in the United States, and each controls a single weed species. Four bioherbicides released in Canada included two strains of *Chondrostereum perpureum* (HQ1 (Myco-Tech Paste) and PFC 2139 (Chontrol Paste)) for control of trees and shrubs (Becker et al., 2005), *Colletotrichum gloeosporioides* f. sp. *malvae* to control round-leaved mallow (Cross and Polonenko, 1996; no longer available due to small market size), and *Sclerotinia minor* (IMI 3144141) for control of dandelions (Abu-Dieyeh & Watson, 2007; Bailey et al., 2010). CAMPERICO (*Xanthomonas campestris* pv. *poae* isolate JT-P482) was registered in Japan for control of annual bluegrass (*Poa annua* L.) in 1997 (Imaizumi et al., 1999). The fungal pathogen *Alternaria destruens* strain 059 (Smolder G and Smolder WP) was registered in the U.S. in 2005 for control of dodder (*Cuscuta* sp.) in field crops and ornamental plants (USEPA, 2005).

The mode of action of each biocontrol agent is as varied as the microorganisms themselves (de Luna et al., 2011). They range from simple but effective compounds like cyanide (Kremer & Souissi, 2001; Owen & Zdor, 2001) and organic acids to complex molecules with tertiary structure (Bouizgarne et al., 2006; Gurusiddaiah et al., 1994), and from secondary metabolites (Kroschel & Elzein, 2004) to plant growth regulators, such as auxins and ethylene (de Luna et al., 2005). Pedras et al. (2003) isolated toxins from *Pseudomonas fluorescens* strain BRG100. One of the toxins, pseudophomin A, showed greater inhibition of green foxtail than did the other, pseudophomin B, which showed greater activity against several plant pathogens. There are sufficient successful products out in the market or in development that indicate continued efforts will provide more microbial herbicides and better weed management options.

4. Formulations to improve success of microbial herbicides

In order to overcome the obstacles associated with development of a microbial herbicide, and dramatically improve the chances for success of microbial herbicides, numerous researchers have investigated combining the biological control organism with a formulation designed to improve application, survivability, and efficacy. Several research projects have studied the use of a formulation or carrier in combination with a biological control organism (Table 2).

As mentioned earlier, appropriate temperatures and length of dew period are critical to the success of fungal bioherbicides. Formulations can extend the period of time before dew is required, and adjuvants such as unrefined corn oil and Silwet L-77 can improve chances for success of mycoherbicides (Abbas et al., 2004; Boyette, 2006; Boyette et al., 2007a). Elzein et al. (2004) found that *Fusarium oxysporum* 'Foxy 2' could be encapsulated in a pesta formulation to improve shelf life. Amsellem et al. (1999) utilized the 'Stabileze' formulation (containing starch, sucrose, corn oil and silica) to enhance preservation of mycelia from two *Fusarium* spp. and found that they remained viable for over one year. The 'Stabileze' method has also been utilized to enhance survival of bacteria. Zidack & Quimby (2002) used this

Biological Control Agent	Weed Pest	Formulation / Carrier	Reference
<i>Alternaria eichhorniae</i> (Ae5)	Water hyacinth (<i>Eichhornia crassipes</i>)	Nine oil emulsions	Shabana, 2005
<i>Alternaria helianthi</i>	Common cocklebur (<i>Xanthium strumarium</i>)	Unrefined corn oil; Silwet L-77	Abbas et al., 2004
<i>Bipolaris</i> sp.	Japanese stiltgrass (<i>Microstegium vimineum</i>)	Tween 20	Kleczewski & Flory, 2010
<i>Colletotrichum gloeosporioides</i>	Sicklepod (<i>Senna obtusifolia</i>)	Adjuvants (unrefined corn oil; invert emulsion-MSG 8.25; Silwet L-77)	Boyette, 2006
<i>Colletotrichum truncatum</i>	Hemp sesbania (<i>Sesbania exaltata</i>)	Unrefined corn oil; Silwet L-77	Boyette et al., 2007c
<i>Curvularia eragrostidis</i> (QZ-2000)	Large crabgrass (<i>Digitaria sanguinalis</i>)	Tween 80; rapeseed oil	Zhu & Qiang, 2004
<i>Curvularia intermedia</i>	Large crabgrass (<i>Digitaria sanguinalis</i>)	Silwet L-77	Tilley & Walker, 2002
<i>Fusarium oxysporum</i> f. sp. <i>cannabis</i>	Ditchweed (<i>Cannibis sativa</i>)	Birch sawdust; wheat seeds; oat seeds	Tiourebaev et al., 2001
<i>Fusarium oxysporum</i> f. sp. <i>orthoceras</i>	Sunflower broomrape (<i>Orobanche cumana</i>)	Pesta granules; commercial iron fertilizers	Müller-Stöver & Sauerborn, 2007
<i>Fusarium oxysporum</i> (FOG)	Branched broomrape (<i>Orobanche ramosa</i>)	Pesta granules; alginate pellets	Kohlschmid et al., 2009
<i>Fusarium oxysporum</i> ; <i>F. arthrosporioides</i>	Broomrapes (<i>Orobanche</i> spp.)	Alginate beads; ‘Stabileze’ (starch, sucrose, corn oil, silica)	Amsellem et al., 1999
<i>Fusarium oxysporum</i> f. sp. <i>strigae</i> (Foxy 2)	<i>Striga</i> (<i>Striga hermonthica</i>)	Film-coat on sorghum seeds (gum arabic, 40%); Pesta granules; seed treatment (gum arabic, SUET binder)	Elzein et al., 2004, 2006, 2009
<i>Fusarium tumidum</i>	Gorse (<i>Ulex europaeus</i>)	Tween 80; 5% Triton X-100	Yamoah et al., 2008
<i>Helminthosporium gramineum</i> subsp. <i>echinochloae</i> ; <i>Curvularia lunata</i>	Barnyardgrass (<i>Echinochloa crus-galli</i>)	Tween 20	Zhang et al., 2007
<i>Mycoleptodiscus terrestris</i>	Hydrilla (<i>Hydrilla verticillata</i>)	Diatomaceous earth	Shearer & Jackson, 2006
<i>Myrothecium verrucaria</i>	<i>Portulaca</i> spp., <i>Euphorbia</i> spp.	Silwet L-77	Boyette et al., 2007a
<i>Myrothecium verrucaria</i>	Kudzu (<i>Pueraria lobata</i>)	Silwet L-77; unrefined corn oil	Hoagland et al., 2007
<i>Neonectria neomacrospora</i>	Hemlock dwarf mistletoe (<i>Arceuthobium tsugense</i>)	‘Stabileze’	Rietman et al., 2005
<i>Phomopsis amaranthicola</i>	<i>Amaranthus</i> spp.	16 adjuvants	Wyss et al., 2004
<i>Pseudomonas fluorescens</i> strain G2-11	Green foxtail (<i>Setaria viridis</i>); velvetleaf (<i>Abutilon theophrasti</i>)	Corn gluten meal; semolina flour	Zdor et al., 2005

Biological Control Agent	Weed Pest	Formulation / Carrier	Reference
<i>Pseudomonas syringae</i> pv. <i>tabaci</i> ; <i>P. syringae</i> pv. <i>tagetis</i>	Multiple weeds	'Stabileze' method (bacteria, oil, sucrose; silica)	Zidack & Quimby, 2002
<i>Pseudomonas syringae</i> pv. <i>tagetis</i>	Canada thistle (<i>Cirsium arvense</i>)	Silwet L-77; Canada thistle sap	Gronwald et al., 2002; Tichich et al., 2006; Tichich & Doll, 2006
<i>Puccinia thlaspeos</i>	Dyer's woad (<i>Isatis tinctoria</i>)	Surfactants Sylgard, IFA-S90, Regulaid	Kropp & Darrow, 2006
<i>Sclerotinia minor</i>	Dandelion (<i>Taraxacum officinale</i>)	Barley (<i>Hordeum vulgare</i>) grits	Teshler et al., 2007

Table 2. Research projects examining formulations or carriers to improve survival and efficacy of microbial herbicides.

method with two *Pseudomonas* spp., and showed that populations were still high after one year, and that components of the formulation could be varied depending on bacterial species response.

Elzein et al. (2006, 2010) studied seed coatings containing *Fusarium oxysporum* isolates to control *Striga*, and found a 40% gum arabic seed coating combined with dried chlamydospores to be the most effective combination of seed coating and inoculum type for causing disease in *Striga* (Elzein et al., 2006). Zhao & Shamoun (2005) tested combinations of gelatin and potato dextrose broth concentrations for optimum efficacy of *Phoma exigua* to control salal (*Gaultheria shallon*), a perennial evergreen shrub. *Fusarium oxysporum* f. sp. *orthoceras* (FOO) is known to suppress the root parasitic weed broomrape (*Orobancha cumana*) in sunflower. In addition, Benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH) induces sunflower resistance to *Orobancha cumana*. Using FOO and BTH, Müller-Stöver et al. (2005) improved the efficacy of *Fusarium oxysporum* f. sp. *orthoceras* (FOO) to reduce broomrape infection in greenhouse studies. They also found FOO incorporated into a wheat-kaolin and iron mix further improved efficacy by increasing FOO survival (Müller-Stöver & Sauerborn, 2007).

Chittick & Auld (2001) examined the use of hydrophilic polymers as a formulation for a mycoherbicide to improve efficacy of *Colletotrichum orbiculare* on *Xanthium spinosum* (Bathurst burr) in Australia. Hoagland et al. (2007) studied formulation, application method and growth media for control of kudzu (*Pueraria lobata*) using *Myrothecium verrucaria* fungi. Shabana (2005) found that the efficacy of *Alternaria eichhorniae* isolate #5 could be improved, and the requirement for a dew-period avoided, by applying the fungi using an oil emulsion. With that formulation, complete control of water hyacinth under field conditions in Egypt was achieved. Hurrell et al. (2001) found a granular mycelium-wheat formulation of *Sclerotinia sclerotiorum* controlled *Cirsium arvense* best in pasture lands of New Zealand when the formulation was a water-miscible powder applied as a slurry rather than a dry product. They also found that spring and early summer applications, when some moisture was present, were more effective at reducing the weed than late summer or early autumn. While some moisture was needed for efficacy, too much rain was thought to wash the agent off the leaf. Kohlschmid et al. (2009) found that a combination of alginate pellets and pesta granules formulated with the *Fusarium oxysporum* isolate FOG was more efficacious and reliable for

controlling the parasitic weed branched broomrape (*Phelipanche ramosa*) than the untreated control under field conditions. Boyette (2006) examined several adjuvants in combination with the mycoherbicide *Colletotrichum gloeosporioides* for control of sicklepod. These compounds reduced length of the dew period requirement, and improved the performance of this organism in controlling sicklepod. Boyette et al. (2007c) showed that *Colletotrichum truncatum* formulated with unrefined corn oil and the surfactant Silwet L-77 was able to effectively control hemp sesbania in the greenhouse and field by reducing the dew period requirement. Zhang et al. (2007) utilized protoplast fusion as a means to improve the biocontrol efficacy of *Helminthosporium gramineum* subsp. *echinochloae* strain HM1 against barnyardgrass (*Echinochloa crus-galli*) in rice.

Most of the research conducted to develop formulations that improve survival and efficacy of microbial herbicides has been directed at fungal herbicides; however, there have been a few studies aimed at improving delivery of bacterial herbicides. Zdor et al. (2005) studied effects of DRB (*Pseudomonas fluorescens* strain G2-11) in combination with corn gluten meal and semolina flour in soil assays using weed and crop species. Zhang et al. (2010) studied the stability of pyoluteorin, a polyketide metabolite produced by fluorescent pseudomonads that has shown potential to control weeds, among other pests. Tichich and Doll (2006) examined a novel application approach for the pathogen *Pseudomonas syringae* pv. *tagetis* where the sap of infected Canada thistle is extracted, combined with water and Silwet-77, and sprayed. While there was disease expression in the treated plants, it was not enough to control the Canada thistle and further work is needed on this approach.

Various technologies have been used and will continue to be used to enhance biological weed control (Cohen et al., 2002). The protoplast fusion technique was used to create new strains using *Helminthosporium gramineum* subsp. *echinochloae* strain HM1 (high pathogenicity, low spore formation) and *Curvularia lunata* (low pathogenicity, high spore formation) to create strains that effectively control barnyardgrass and other weeds in rice production (Zhang et al., 2007). Hypervirulence selection or manipulation may improve efficacy of biological control agents. Cohen et al. (2002) transformed genes of the indole-3-acetamide (IAM) pathway to cause an auxin imbalance that increased the virulence of *Fusarium oxysporum* and *F. arthrosporioides*, pathogenic on broomrape (*Orobancha aegyptiaca*). Sands and Pilgeram (2009) outline the steps to enhance virulence of the biocontrol agent using amino acid overproduction. They discuss control of the parasitic weeds *Orobancha* and *Striga*, which are especially challenging to control due to the close relationship they develop with their hosts. Economic formulations and genetic manipulations to alter phenotype will assist in the understanding and development of microbial herbicides.

5. Integrating microbial herbicides with other control measures

In many cases, microbial herbicides alone will not be enough to remedy invasive weed problems. Researchers worldwide have shown that an integrated approach utilizing microbial weed management in a synergistic or additive manner with chemical herbicides or in combination with cultural practices or biological controls with insects is more successful than any of the control measures alone. Innovative approaches for microbial management of several different weed species in various regions of the world have been employed to sustainably manage some of the world's worst weed problems in diverse systems.

Denoth et al. (2002) reviewed biological control projects on weeds and insects, and determined that for weeds, those projects where a number of agents were released showed greater success. Wandeler et al. (2008) used weevils as an insect vector to apply *Puccinia punctiformis* to creeping thistles (*Cirsium arvense*) in the field, causing systemic rust infection. Another integrated approach to biological control with microorganisms utilizes mixtures of organisms, rather than a single pathogen to control weed growth. Chandramohan & Charudattan (2003) propose the use of a mixture of four fungal plant pathogens (*Phomopsis amaranthicola*, *Alternaria cassia*, *Colletotrichum dematium* f.sp. *crotalariae*, *Fusarium udum* f.sp. *crotalariae*) to control pigweed (*Amaranthus hybridus* L.), sicklepod and showy croton (*Crotalaria spectabilis* Roth.). In their greenhouse study, they showed that it was possible to control these three weeds together using several fungal strains without losing efficacy or host-specificity. Some potential biological control agents work best as individual applications, and do not exhibit synergy when applied together. Dooley & Beckstead (2010) found no improvement in downy brome inhibition using *Pseudomonas fluorescens* strain D7 in combination with the fungal pathogen *Pyrenophora semeniperda*. These two microorganisms suppress downy brome at different growth stages. Another example of this is *Chondrostereum purpureum*, which is applied in spring and *Fusarium tumidum*, which is applied in early winter to reduce gorse (*Ulex europaeus*) regrowth in New Zealand forests (Bourdote et al., 2006b). These mycoherbicides inhibit gorse at different growth stages, but together they did not further reduce the regrowth of stumps. Further studies are needed to understand the weed reduction and ecological implications of consortia in biocontrol efforts.

In greenhouse studies, Caesar (2003) found that the combined effects of *Fusarium oxysporum* and *Rhizoctonia solani* with flea beetle adults and larvae resulted in greater inhibition of *Euphorbia esula/virgata* than any of the biological control agents alone. In their survey of *Lepidium draba* throughout Europe, Caesar et al. (2010) showed that plants sustaining both insect damage and disease were being colonized by the root pathogen *Rhizoctonia solani*. They concluded that when determining potential biological control agents, the synergistic relationships between plant pathogens and insects should be considered. Likewise, Kremer et al. (2006) found that the most effective biocontrol of leafy spurge occurred with the synergistic effect of “plant-associated microorganisms and root-damaging insects”. There was a higher incidence of *Fusarium* and *Rhizoctonia* isolates in *Euphorbia* plants that had injury caused by insect feeding. Rayamajhi et al. (2010) noted the additive effects of using combinations of a weevil, psyllid, and rust fungus (*Puccinia psidii*) to reduce regrowth of tree stumps of *Melaleuca quinquenervia* in southern Florida. The use of multiple enemies against invasive weeds is another combination that could be successful in biocontrol programs.

Babalola et al. (2007) examined the use of trap crops such as cowpea (*Vigna unguiculata*) combined with application of bacteria (*Enterobacter sakazakii* and *Pseudomonas* spp.) to stimulate germination and cause the subsequent death of the parasitic weed *Striga hermonthica*. Similarly, Ahonsi et al. (2003) used ethylene-producing *Pseudomonas syringae* pv. *glycinea* in combination with nitrogen-fixing *Bradyrhizobia japonicum* strains to induce germination and death in *Striga hermonthica* seeds in the presence of cowpea or soybean. No matter how successful a biocontrol agent may be, the control is never considered to be 100% and additional practices and management efforts need to be integrated with these microorganisms or insects to attain weed management or control.

Two methods have been employed to determine whether a synergistic relationship exists between a chemical herbicide and a microbial herbicide (Gressel, 2010): 1) random testing of herbicides, and 2) screening chemical herbicides with the intent to alter the target plant's defenses, leaving it more vulnerable to attack by pathogens. Better control of *Chenopodium album* was achieved by using low rates of herbicides in combination with toxins from *Ascochyta caulina* (Vurro et al., 2001). Jahromi et al. (2006) studied interactions between herbicides and *Plectosporium alismatis* to control starfruit in rice. Kropp & Darrow (2006) found that herbicides and surfactants did not negatively affect teliospore viability of *Puccinia thlaspeos* when it was sprayed on to *Isatis tinctoria* (Dyer's woad) plants in the field. Abu-Dieyeh & Watson (2006) examined the relationship between the fungal pathogen *Sclerotinia minor* Jagger applied with different turfgrass mowing heights compared to herbicide alone to control dandelion. In the greenhouse, *S. minor* caused greater damage to dandelions than the herbicide at all mowing heights; however, under field conditions, close mowing had an unfavorable effect on *Sclerotinia minor* for dandelion control. Magani et al. (2009) demonstrated that the fungal mycoherbicide *Fusarium oxysporum*, when applied in granular form followed by a post-emergence herbicide treatment, was successful in controlling the parasitic plant *Striga* in Nigeria.

Peng and Byer (2005) tested seven herbicides at reduced rates to determine whether there might be synergistic effects with *Pyricularia setariae* for control of green foxtail. Responses were variable and depended on weed growth stage, herbicide and application rates. However, each of the herbicides at a one-quarter rate applied with a sub-lethal dose of the biocontrol organism succeeded in controlling green foxtail in the greenhouse (Peng & Byer, 2005). Jahromi et al. (2006) studied interactions between the fungal pathogen *Plectosporium alismatis* and herbicides for control of starfruit in Australian rice production. In glasshouse experiments, there was no synergistic effect with the pathogen and 2-methyl-4-chlorophenoxyacetic acid (MCPA); however, there was a synergistic effect when the pathogen was applied after the sublethal dose of the herbicide Londax® (bensulfuron methyl, DuPont, Wilmington, DE).

Gressel (2010) notes that glyphosate is most commonly utilized in synergistic relationships with plant pathogens, and hypothesizes that this is due to the capability of glyphosate to affect multiple weed defense mechanisms. Boyette et al. (2008a) in a field study controlled redvine (*Brunnichia ovata*) and trumpetcreeper (*Campis radicans*) using the synergistic relationship between glyphosate and the fungus *Myrothecium verrucaria*. The combination of the chemical herbicide and the microbial herbicide was able to control the weeds better than either treatment alone. Boyette et al. (2008b) concluded that hemp sesbania (*Sesbania exaltata*) control might be augmented by utilizing the biological control agent *Colletotrichum truncatum* in combination with reduced rates of the herbicide glyphosate when the fungus is applied after the herbicide. In a previous experiment under controlled conditions, Boyette et al. (2006) tested *Myrothecium verrucaria* in combination with glyphosate on kudzu (*Pueraria lobata*), redvine and trumpetcreeper at various temperatures. Greatest disease development was achieved at higher temperatures, and weed inhibition was greatest when the fungus was applied after the herbicide rather than prior to or with the glyphosate. Cook et al. (2009) found that dodder (*Cuscuta pentagona*) was more effectively controlled in greenhouse studies using a mixture of the pathogen *Alternaria destruens*, glyphosate, oil and an ammonium sulfate surfactant than when using any of the treatments alone, while not harming the host citrus (*Citrus* spp.) plant.

Care must be taken when integrating biological controls with herbicides so that the herbicide does not affect microbial survival and efficacy. Ray et al. (2008) found that dose and herbicide type may be detrimental to the water hyacinth pathogen *Alternaria alternata*. The effects of four herbicides at recommended and reduced rates were tested on the rust fungus *Puccinia lagenophorae* to be used against *Senecio vulgaris* (common groundsel). Wyss & Müller-Scharer (2001) found that the herbicides they tested were either too toxic to the fungus, or did not increase plant susceptibility to the fungus, and so the combination of the rust fungus and herbicide was not an option for control of *Senecio vulgaris*. Shabana & Mohamed (2005) combined *Alternaria eichhorniae* isolate 5 with 3,6-dichloro-2-pyridinecarboxylic acid (MDCA) to weaken water hyacinth defenses, which increased disease severity and has the potential for greater biocontrol.

The soil possesses a wealth of genetic potential waiting to be discovered and used in weed management systems. Soil quality or the chemical, physical and biological properties of soil can influence plant-microbe interactions and the success of any biocontrol agent (Kennedy & Papendick, 1995). In addition, soil quality investigations often include weed populations as an indicator of soil quality, in part because a healthy soil and healthy desirable plant is the best weed control (Magdoff, 2001; Ryan et al., 2011). Soil investigations are needed in determining the impact of the soil environment on weed populations. The structure and function of soil microbial communities develop depending on the soil, location, climate, slope, aspect and vegetation. Management practices can influence the soil microbial community and plant-microbe interactions (Wander et al., 1995; Lupwayi et al., 1998; Kennedy & Schillinger, 2006). Cultural practices and application of amendments may also play a part in weed-suppressive soils, although the modes of action may be different for each practice. Cropping systems also influence weed-suppressive bacteria (Kremer & Li, 2003; Ryan et al., 2011). Kremer & Li (2003) found that high enzyme activity and greater volume of water stable aggregates correlated with more weed-suppressive bacteria. They found that uncultivated prairie and no-tillage systems contained the highest populations of deleterious rhizobacteria compared to other land use and soil quality indicators, and may be useful in selecting for weed-suppressive practices. In a similar study in Washington state, Kennedy & Stubbs (2007) could not find relationships among management systems and the prevalence of weed-suppressive bacteria.

The concept of weed-suppressive soils can be defined as soils, amendments, or management practices that have the capacity to reduce or limit specific weeds. Management for weed-suppressive soils is the ultimate goal for biological control efforts. The dynamics of weed-suppressive soils may be similar to what is seen with disease-suppressive soils (Mazzola, 2004). The biology, chemistry and physical properties of soil that comprise a weed-suppressive soil need to be characterized in order to manage for suppressive soils. As with all soil quality determinations, no one indicator will explain the complexity of weed suppression.

6. Regulatory issues

Rigorous testing is required prior to the release of a biological herbicide to ensure the safety of humans, animals and the environment. Host-range studies are needed to reduce potential risk and ensure that beneficial, non-target plant species are unaffected by the biocontrol agent. However, the length of time needed to complete assessments of new biological

herbicides adds to the costs and the length of time required before an agent can be released (Ghosheh, 2005). Non-host testing is important and the ranges of plant species tested depend on the areas of release, ecosystem variability and potential for dissemination of the biocontrol agent by wind or water. Testing should cover all economically important plant species of the area, and those plants known to be involved in ecosystem maintenance. In agronomic ecosystems, the major crop species are of interest. The U.S. Environmental Protection Agency (EPA) published a list of the top 25 major agricultural crops. Plants were placed on this list because of their economic importance, ecosystem activity or total production values (EPA, 2011). In aquatic systems, several aquatic plants are suggested that include algae, aquatic bacteria, marine and freshwater diatoms. In rangeland ecosystems the non-target species would include native or near native plant species. It is recommended to test six species covering at least four families in the Dicotyledonae, and at least four species of at least two families in the Monocotyledonae. Testing must be performed on all plants of economic importance in agriculture, horticulture or rangeland systems or known to be beneficial to maintenance of the ecosystem that have any reasonable likelihood of serving as hosts. This selection of additional plant species should be based upon a survey of plants closely related (same genus or, if not available, same family) to the target plant and a survey of known hosts of pathogens closely related to the microbial herbicide (EPA, 2011; Wapshire, 1974).

With thorough host-range testing, very few, if any, detrimental effects occur from the release of fungal herbicides to control weeds (Barton, 2004). In a review of pre- and post-release records from 26 projects, Barton (2004) found that there were no reports of a fungal biological control agent striking an unintended plant species. Additional animal, avian, fish and daphnia testing are also required in many countries before bioherbicides can be registered. In addition, as with all research and new products where there are safety concerns, buffer zones are often required to protect animal pastures and other non-target areas (Bourdote et al., 2006a). The risk of applying a microbial herbicide to the environment needs to be considered at the beginning and throughout the development of biocontrol agents.

7. Future prospects for microbial herbicides in sustainable ecosystems

The future of biocontrol is bright and full of possibilities with the many novel, successful biocontrol agents being studied. The advancements in microbial genetics, microbial community analyses and understanding plant-microbe interactions continue to accumulate and will be instrumental in helping microbial biocontrol of weeds move forward.

The area of biological control using soil microorganisms needs further investigations to discover additional isolate-host pairs that are a biocontrol match consisting of a biocontrol agent of highest virulence in contact with the host at its greatest susceptibility. Formulations are needed to increase shelf life of the living organisms to improve survival and efficacy. Research and development of each biocontrol agent are needed so that stakeholders and industry buy in to the marketing, economics and time investments of this approach to weed management. An understanding of microbial community, weed, and soil quality characteristics, and management practices is needed for the development of weed suppressive soils.

Investigations of the structure and function of soil microbial communities are needed to advance the area of biological control. Traditional techniques of microbial analyses to describe the composition and diversity of microbial populations in soils has commonly relied on phenotypic characteristics alone, and molecular investigations add to the information on structure and function of the soils (Mazzola, 2004). Profiling or fingerprinting of soil and soil microbial community structure using substrate utilization and fatty acid methyl ester analyses may be the first step in targeting weed-suppressive potential. There are several nucleic acid-based methods that can be used to probe soil and identify those microbes that produce similar compounds to those already known. Probes will assist field studies of known agents to follow survival in soils and explore soil for additional weed-suppressive factors. Nucleic acid technologies provide greater information on genetics, and possibly function of a given organism. Array, pyrosequencing and metagenomic investigations can provide information on the microbial community and the biological agent within that community. Selection for hypervirulence; construction of molecular probes; understanding the genetic material of the agent, weed-suppressive compounds, and host-microbe interactions can be investigated more thoroughly with these methods. The continual development of novel molecular methods to investigate genetics of a system will provide key information to better understanding of the plant-microbe phenomena. These methods are forever changing and improving to allow us to have increased knowledge of the microbial portion of the ecosystem and the various interactions that can occur.

Soil microbial ecology and the soil microbial community will affect weed ecosystem dynamics, diversity, function, and populations. As with soil quality, the compilation of indicators has been attempted often to hone in on a few indicators of importance. No one approach or method can be used to characterize and follow biocontrol agents, or to isolate and research additional novel plant-microbe interactions. The future is bright for continued development of microbial herbicides to reduce herbicide reliance and provide multiple options in weed management.

8. Conclusions

The wealth of genetic potential of microorganisms on this earth is boundless. There have been many investigations of potential products for weed management. Some have been successful at suppressing weeds in the field and a select few are marketed products that now reduce weed infestations. Further studies are needed to continue to search for additional tools to combat weeds. Increasing our understanding of plant-microbe interactions will assist in this effort. Biocontrol agents need to be specific, competitive and well-matched with the weed of interest. The search for biocontrol agents from the environment entails not only finding microorganisms that inhibit a weed, but that are specific for the weed or related plant species and have an economically viable market. Host-range testing and non-target species testing are needed early in the process. In addition, the development of formulations and delivery systems is necessary to prolong the shelf-life and efficacy of the biocontrol agents in a variety of environments. Biocontrol should not be considered a stand alone option, but may be best if integrated with other methods of control, especially with those that are ecologically sound. Biocontrol agents to reduce or complement chemical herbicides expand options in weed management and tend toward the

use of ecologically based systems. They add additional tools in the arsenal of weed management efforts. There is a wealth of genetic potential in the soil and the environment to be explored, screened and tested for weed suppression.

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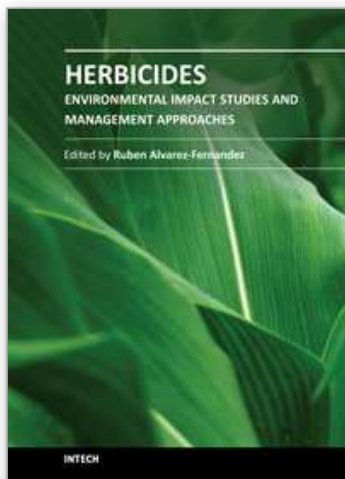
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Weeds severely affect crop quality and yield. Therefore, successful farming relies on their control by coordinated management approaches. Among these, chemical herbicides are of key importance. Their development and commercialization began in the 1940's and they allowed for a qualitative increase in crop yield and quality when it was most needed. This book blends review chapters with scientific studies, creating an overview of some the current trends in the field of herbicides. Included are environmental studies on their toxicity and impact on natural populations, methods to reduce herbicide inputs and therefore overall non-target toxicity, and the use of bioherbicides as natural alternatives.

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