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Plant Pathogens at Work: Improving Weed Control Efficacy

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Research in the area of the use of plant pathogens as biological control agents for weeds is conducted using either the classical or the bioherbicidal approach. In the classical approach, a pathogen is typically imported from a foreign location to control an introduced weed target. The pathogen is applied or released into a small weed population relative to the total infestation. This is commonly a single application and if conditions are favorable, the pathogen spreads throughout the target weed population. In the inundative or bioherbicide strategy, an indigenous pathogen is cultured to produce large quantities of inoculum that are applied at high rates to the entire target weed population. There are hundreds of plant pathogens that have been tested for their potential as bioherbicide candidates. Research on the development of plant pathogens for biological control using the inundative or bioherbicide approach has moved from determining host range and demonstrating pathogenicity to investigating systems that enhance the efficacy of these agents.

Enhancing Bioherbicides

Formulation with water-retaining additives to reduce dew dependence. The requirement for long periods of dew or moisture is a major hurdle in the development of foliar fungal pathogens into bioherbicides, mainly because humidity and dew period duration can significantly limit disease initiation and disease progress. The utilization of formulations that minimize the influence of humidity is one approach to overcoming this restraint (5). The addition of an invert oil emulsion to conidial suspensions of *Colletotrichum truncatum*, a biocontrol agent for hemp sesbania (*Sesbania exaltata*), reportedly resulted in 100% control in the absence of dew in the greenhouse. In the field, the same formulation caused > 95% control, similar to the control achieved with the chemical herbicide acifluorfen (8) (Fig. 1). Improved bioherbicidal efficacy by the addition of oil emulsions, with no or little exposure to dew, has been reported for several other bioherbicide pathosystems (1,4,55). Different modes of action have been proposed for the activity seen with invert emulsions, including phytotoxicity (4,48) (Fig. 2).



Fig. 1. (A) Spores of *Colletotrichum truncatum*, a biological control agent, (B) giving excellent control of hemp sesbania (*Sesbania exaltata*) in the field. Photos courtesy of Doug Boyette.

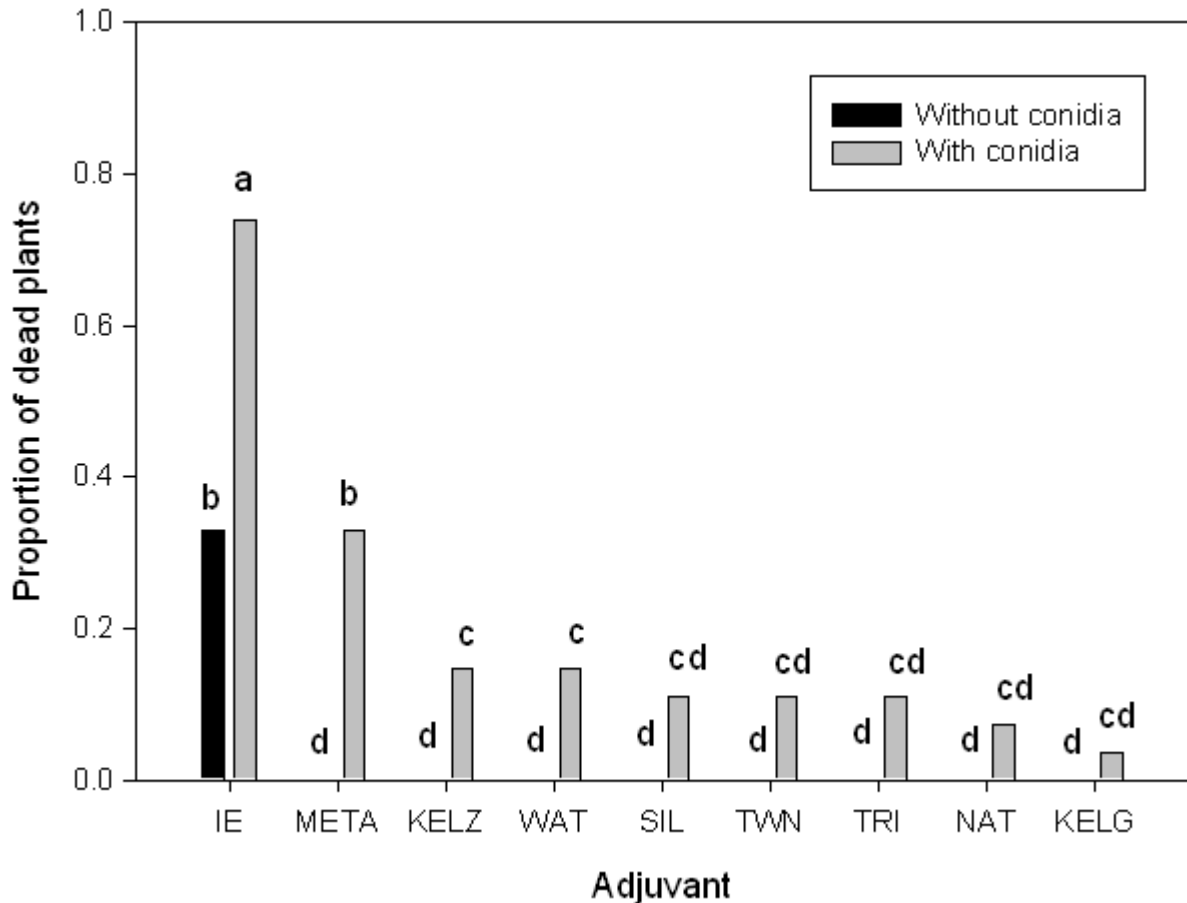


Fig. 2. Effect of various adjuvants on the bioherbicidal efficacy of *Phomopsis amaranthicola*. The adjuvants tested were: IE = invert emulsion, META = Metamucil, KEL = Kelzan, WAT = water, SIL = Silwet L-77, TWN = Tween 20, TRI = Triton X-100, NAT = Natrasol, and KELG = Kelgin. Efficacy was measured in terms of proportion of dead *Amaranthus hybridus* plants. Graph shows data from three trials (gray bars show the effect of conidia + adjuvant and black bars show the effect of the adjuvant alone). Bars with the same letters are not significantly different from each other at $P = 0.05$, as determined by Duncan's Multiple Range test. From Rosskopf et al. (48).

Broadening the spectrum of bioherbicides. While host specificity is desirable in some situations, particularly in areas where crop and weed species are closely related, there are situations where several species of problematic weeds occur and broad-spectrum activity is required. This has been often cited as a major limitation to the bioherbicide approach in cropping systems (28).

Formulation with fruit pectin and plant filtrates allowed *Alternaria crassa*, a biocontrol agent specific to jimsonweed (*Datura stramonium*), to infect hemp sesbania, showy croton, and eastern black nightshade (7). The selectivity of *A. crassa* and *A. cassiae* was also suppressed by formulation with an invert emulsion, resulting in the successful infection of eight other plant species other

than their target hosts (3). In addition, *C. gloeosporioides*, isolated from coffee senna (*Senna occidentalis*), controls sicklepod (*S. obtusifolia*) when formulated as invert emulsion or corn oil/Silwet L-77 emulsion (Doug Boyette, *personal communication*) (Fig. 3).

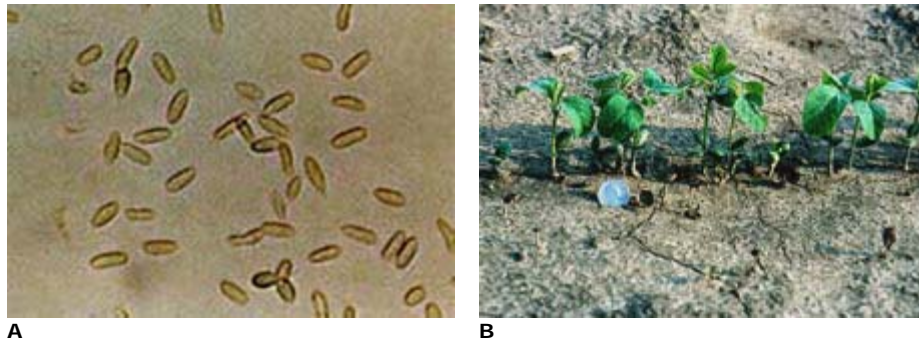


Fig. 3. (A) Spores of the biological control agent, *Colletotrichum gloeosporioides*, which was isolated from coffee senna (*Senna occidentalis*). (B) When combined with an oil-based formulation, the pathogen will also control sicklepod (*S. obtusifolia*). Photos courtesy of Doug Boyette.

Simultaneous control of northern jointvetch (*Aeschynomene indica*) and winged waterprimrose (*Jussiaea decurrens*) was accomplished by applying a combination of two host-specific pathogens, *C. gloeosporioides* f. sp. *aeschynomene* and *C. gloeosporioides* f. sp. *jussiae* (11). A mixture of three host-specific pathogens was used for simultaneous and efficacious control of pigweed, sicklepod, and showy croton (16). Excellent control of seven grass species was also achieved when conidia of three pathogens (*Dreschlera gigantea*, *Exserohilum rostratum*, and *E. longirostratum*) were applied together (Fig. 4) (17).



Fig. 4. The multiple-pathogen strategy proposed by Chandramohan et al. (17) allows for the control of a broader spectrum of weeds than a single pathogen used alone and may address long-term concerns about the potential for resistance.

Another approach is to utilize pathogens that have a host-range that is not as restricted. *Myrothecium verrucaria* was first evaluated for sicklepod (53) and kudzu control (12), but has since been evaluated for multiple weed targets (Fig. 5). However, the use of *M. verrucaria* is likely to be limited due to its production of trichothecenes, although these toxins have not been detected in treated plants (2). Additional research into the necessity of trichothecenes for pathogenesis and their potential for mammalian toxicity when the pathogen is used in a bioherbicide system is warranted.



Fig. 5. Impact of *Myrothecium verrucaria* on a mixed stand of spurge and purslane species. The broad-spectrum pathogen had no effect on tomatoes later transplanted into the plots (Doug Boyette, *personal communication*). Photo courtesy of D. Boyette.

Enhancing bioherbicidal efficacy through delivery or application systems. Application of high volumes of propagules has been done to ensure complete coverage of infection courts and to provide ample moisture needed for spore germination for maximum infection (38). The requirement for high application volumes for bioherbicides can deter their use because this entails transport of a heavier load to the application site and longer application times. It has been shown that high volumes do not necessarily ensure high levels of disease. According to Lawrie et al. (40), the number of lesions produced or the infection density is influenced not by the application volume alone but by the spray droplet size, droplet retention and distribution, inoculum concentration, and spray application volume. The type of spray equipment employed, as well as the formulation and the inoculum concentration influences droplet size, droplet retention, and distribution.

Chapple and Bateman (18) reported significant differences in the number of deposited droplets that did not contain any spores in a spray equipment comparison. The percentage of droplets without any spores was 67.3% for the hydraulic flat fan, 95% for the air blast sprayer, and 6.5% for the spinning disc. Yandoc (55) employed an air assisted sprayer versus a hand-held ultra low volume sprayer (ULVA) to apply *Bipolaris sacchari* conidia (formulated in an oil emulsion) to cogongrass (*Imperata cylindrica*) in the field and found that application with the ULVA resulted in greater damage (> 50%) than with the air-assisted sprayer (< 35%).

Another interesting approach to application technology involves the use of the virus *Tobacco mild green mosaic virus* (TMGMV) for control of tropical soda apple (*Solanum viarum*) (22). This weed is a problem in cattle pastures, and control with a biological control agent requires applications over large areas. The virus has a dramatic impact, causing complete plant death (Fig. 6). Novel application methods have been effective and could easily be implemented by ranchers. These include low pressure application (20 psi) of the virus combined with plant abrasion using either a section of chain-link fence or carpet, and high pressure application (400 psi) directly to plants (Fig. 7).

Other ways of delivering bioherbicides, specifically solid-based formulations, have been tested for pre-emergence bioherbicides that are meant to attack weeds at or below the soil surface (9). Among the substrates used are Pesta (wheat-gluten matrix) for *C. truncatum*, *A. crassa*, and *Fusarium lateritium* (23) and *F. oxysporum* f.sp. *orthoceras* (49), and cornmeal-sand for *F. solani* f. sp. *cucurbitae*, a bioherbicide for Texas gourd (10). Formulation with solid substrates allows for improved shelf life and also acts as a buffer when extreme conditions occur in the field (6).

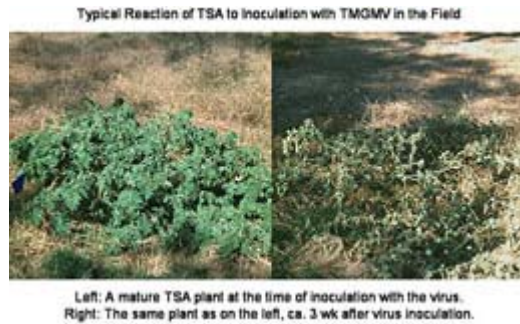


Fig. 6. Impact of the application of the biological control agent *Tobacco mild green mosaic virus* (TMGMV) on tropical soda apple (*Solanum viarum*).



Fig. 7. Tropical soda apple is a problem weed on cattle ranches and its control requires coverage of large acreages. The use of the biological control agent *Tobacco mild green mosaic virus* (TMGMV) is feasible through the use of novel application approaches, including abrasion of plants while spraying the virus and high-pressure spraying but not with the mow-and-spray application.

In another study, composted chicken manure supported the growth of *Trichoderma virens* as well as its production of viridiol, a compound that inhibits weed seed germination and emergence (34). In greenhouse tests, the application of *T. virens* infested manure reduced emergence by 77% and dry weight by 68% of naturally occurring miscellaneous weed species (34). Similarly, the combination of an allelopathic cover crop, rye, with *Trichoderma*-inoculated compost was used to control weeds in transplanted vegetables (29).

Enhancing biocontrol efficacy through selection and use of amino acid excreting strains. Tiourebaev et al. (51) have attempted a novel approach to enhancing virulence of weed biological control agents by selecting for strains that are capable of excreting high levels of amino acids. Improved efficacy of *F. oxysporum* f.sp. *cannabis*, a potential biocontrol agent for *Cannabis sativa* was achieved with valine-excreting mutants. The mutants failed to infect or cause damage to other plant species tested, indicating no change in the host-range. Other weed-pathosystems that might employ this approach are under investigation (52,58)

Combination of biocontrol agents with herbicides and other chemical agents that predispose weeds to infection. One factor that can influence the level of weed suppression through biological means is the ability of a target weed to resist infection and colonization by the biological control agent. The application of the biocontrol agent *A. cassiae* to sicklepod resulted in increased activity of phenylalanine ammonia-lyase (PAL) (31), an enzyme responsible for the synthesis of phenolic compounds that have been shown to protect plants from pathogen attack. Hoagland (32) presented several approaches for improving biocontrol efficacy by disrupting the target weed's

defense mechanisms, including the use of herbicides or other compounds that affect key enzymes, blocking the synthesis of secondary plant metabolites, or breaking down physical barriers to pathogen attack, all of which had encouraging results.

Enhanced bioherbicidal efficacy of *E. monoceras* on *Echinochloa crus-galli* was observed when the pathogen was applied with δ -aminolevulinic acid, a precursor of tetrapyrroles, which are involved in the bleaching and killing of plant tissue (30). Gressel et al. (26) demonstrated that the efficacy of a weakly pathogenic agent, *C. coccodes* on velvetleaf (*Abutilon theophrasti*), can be improved by applying chemical agents that repress host plant defenses. A low dose of fungal inoculum applied with calcium chelators resulted in increased infectivity by *C. coccodes* and reduced callose formation. Hodgson et al. (33) reported 75 to 90% velvetleaf mortality from the application of tank-mix combinations of *C. coccodes* and lower rates of thidiazuron, a plant growth regulator. The mixes were almost twice as effective as the thidiazuron applied alone (33).

The strategy of applying sub-lethal rates of glyphosate with conidia of *A. cassiae* to improve the level of weed control has been tested. Sharon et al. (50) demonstrated that the ability of glyphosate to inhibit the accumulation of phytoalexin rendered sicklepod seedlings more susceptible to infection by *A. cassiae* even at low inoculum concentration levels. Peng and Byer (44) reported greater suppression of green foxtail (*Setaria viridis*) with a mixture of low rates of sethoxydim and *Pyricularia setariae* as compared to the level of control achieved with herbicide or pathogen alone. Several other pathogens have been tested for their compatibility with crop protection chemicals, including herbicides with which they might be tank-mixed (54,57) (Fig. 8).



Fig. 8. Fungi with potential for use as biological weed control agents in agroecosystems should be tested for compatibility with other pesticides that may be used in the system. Pictured here is a Petri plate assay of *Dactylaria higginsii*, a potential biological control agent for nutsedges, grown on media amended with pesticides that might be used in a conventional tomato production system (57).

Techniques for the Improvement of Weed Control Efficacy with Pathogens

Weed control through pathogen application and plant competition. The impact of bioherbicides on the target weed's ability to compete has been well demonstrated. An early example of this phenomenon is

Puccinia chondrillina, a rust specific to skeletonweed (*Chondrilla juncea*), which inhibited that plant's ability to compete with subterranean clover (*Trifolium subteraneum*) (27). Velvetleaf growth was stunted and seed production was reduced by the presence of *C. coccodes*, rendering it less competitive with soybean (25). *Dactylaria higginsii* applied to mixed plantings of tomato and purple nutsedge reduced interference from this weed and increased the yield of tomato (36).

Application of *Bipolaris sacchari* to mixed plantings of cogongrass and bahiagrass (*Paspalum notatum*) resulted in reduced cogongrass growth and allowed bahiagrass to flourish (56). This strategy involves the specific suppression of a weedy grass species while allowing for a beneficial grass species to establish and take over the niche vacated by the weed and prevent its re-establishment (Fig. 9).

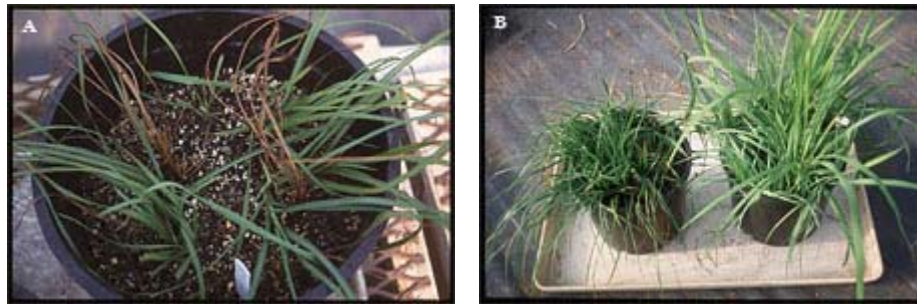


Fig. 9. (A) Selective control of cogongrass (*Imperata cylindrica*) by *Bipolaris sacchari*, formulated in an invert emulsion, in a mixed planting of the weed in bahiagrass (*Paspalum notatum*). (B) Control of cogongrass, as seen in the pot on the left side, allows bahiagrass to flourish when compared to the uninoculated control on the right.

Although it would seem logical that weed interference could be reduced by competition from increased crop plant density coupled with bioherbicide applications, Pitelli et al. (45) did not find evidence to support this hypothesis. In their study, soybean planting density did not impact the mortality or the dry weight of sicklepod sprayed with *A. cassiae* and/or *Pseudocercospora nigricans*. While similar studies of crop-weed-bioherbicide combinations are not common, the need for this type of competition study is necessary to characterize possible avenues for enhanced control using integrated weed management systems that include bioherbicides.

Biocontrol and host plant resistance. A novel method of utilizing non-target plant competition was tested for management of striga (*Striga hermonthica*) (43). *F. oxysporum* was applied in each planting hole before sowing seeds of the resistant sorghum cultivar Samsorg 40 and the tolerant landrace Yar'ruruka. Plots treated with *F. oxysporum* and planted to each of the cultivars had significantly higher crop yield and lower striga infestation compared to plots that were planted with the same cultivars but not treated with *F. oxysporum*.

Deleterious rhizobacteria and cover crops for improved weed control. The potential of deleterious rhizobacteria (DRB) to serve as control agents of weeds relies on their ability to reduce weed emergence and to delay growth and development, giving the crop a competitive advantage (6). Reduced downy brome (*Bromus tectorum*) population and 18 to 35% yield increase in winter wheat through the application of the DRB *Pseudomonas fluorescens* has been reported (35,37). However, there are also reports of inconsistent performance by DRB, due to poor survival or low activity once applied in the field. An integrated approach utilizing DRB and cover crops for improved weed control was also tested (41). The cover crops support the proliferation and activity of the DRB before weed seed germination occurs, which enhances the level of weed suppression by the cover crops. The DRB-cover crop combination caused greater weed biomass reduction compared to cover crops alone. The characterization of cropping systems that support microbial populations that are weed-suppressive is an emerging area of research that shows great promise (24).

Combining pathogens and insects. Management of leafy spurge (*Euphorbia esula-virgata*) in natural areas has been problematic because chemical herbicides have been ineffective or inappropriate and leafy spurge can easily overcome damage from mechanical weed control measures and insect biocontrol agents (42). While the release of root-feeding flea beetles (*Aphthona* spp.) resulted in successful insect establishment, the insects did not significantly reduce the weed population (13). The frequent presence of soilborne pathogens at release sites where leafy spurge is in decline has spurred interest in combining soilborne pathogens isolated from leafy spurge roots and flea beetles for a more effective control (Fig. 10) (14). This approach may be possible for the control of white top (*Cardaria draba*), as plants with insect damage also harbor pathogens that contribute to the decline of plant stands (Anthony Caesar, *personal communication*) (Fig. 11). Interestingly, these interactions are even more complex than they initially appear. Pathogens associated with insect-infested weeds have been found to be more highly virulent than the same species of fungi isolated from diseased weeds that are not infested with the insects (15,39). A three-way interaction has also been found among deleterious rhizobacteria, fungal plant pathogens and insects (39).



Fig. 10. (A) Field location with a significant leafy spurge (*Euphorbia esula*) population prior to the release of the flea beetle *Aphthona flava* and (B) four years after the release. (Photo credit Norman Rees, ARS, courtesy of Tony Caesar). (C) At several sites where the weed stand was reduced, soilborne pathogens were found to be associated with the affected plants. Photo courtesy of Tony Caesar.

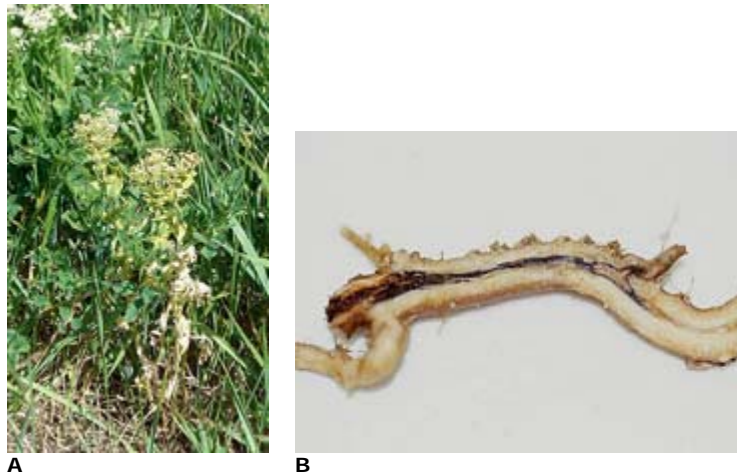


Fig. 11. (A) White top (*Cardaria draba*) with insect damage on foliage has (B) necrotic root systems associated with soilborne plant pathogenic fungi. Photos courtesy of Tony Caesar.



Fig. 12. (A) Early symptoms of rust (*Puccinia psidii*) and melaleuca psyllid (*Boreioglycaspis melaleucae*) damage on young melaleuca (*Melaleuca quinquenervia*) twigs. (B) Melaleuca twig defoliation and dieback caused by the rust, psyllids, and weevil (*Oxyops vitiosa*). Photos courtesy of Min Rayamajhi.

The strategy for controlling weeds using the combination of insects and plant pathogens is supported by reports of population regulation resulting from damage from natural enemies (19,21). The rust *Puccinia psidii* is currently under investigation as a biological control agent for Australian melaleuca (*Melaleuca quinquenervia*) (46). Researchers in this area (Min Rayamajhi, personal communication) reported significant reduction in the absolute density and diameter based density of the Australian melaleuca in the presence of and during an increase in the population of natural enemies (i.e., weevils, rust, lobate lac scales, psyllids, and sooty molds) (Fig. 12). The impact of these pests is dramatic and allows for the regeneration of the understory (Fig. 13). The elucidation of the interactions between these natural enemies is the current area of research (47).



Fig. 13. (A) Crown thinning of *Melaleuca quinquenervia* resulting from multiple biological control agents, including rust, psyllids, and weevils. (B) and (C) *Melaleuca* crown thinning and tree mortality allowed development of understory vegetation. Photos courtesy of Min Rayamajhi.

The Future

The effort to develop plant pathogens as commercially available bioherbicides has not yielded the large number of products that the research would indicate. However, research in this area has nonetheless contributed significantly to the science and technology of biological control. The development of this weed-control technology, albeit used to a very limited extent, has resulted in several commercial products, including four pathogens registered within the past five years. Furthermore, as Hallett (28) points out, there are opportunities for the development of bioherbicides for some specialized niches, such as parasitic, urban, and allergenic weeds. For example, Smolder (*A. destruens*), has been registered recently for the control of several *Cuscuta* (dodder) species.

It is true that with the current state of the art, it is difficult for companies to sustain business with a single or few registered bioherbicide products. However, it is not a lack of proven efficacy that has limited the availability of these materials; it is the market-driven return on investment that is the constraint. For example, both DeVine and Collego are extremely effective materials, but according to Dave Goulet of Encore Technologies (*personal communication*) the market was too small and sporadic to maintain the economic viability of the products for his company. Fortunately, a new registrant, ARI Incorporated, has taken on the marketing of *C. gloeosporioides* f. sp. *aeschynomene* (the Collego pathogen), as the commercial product LockDown for use in rice in Arkansas, Louisiana, and Mississippi (Dave TeBeest, *personal communication*). In the developed world, the goal of most single-pathogen/single-weed bioherbicide research projects is a saleable product. Therefore, the long-term success of bioherbicides may depend on wholesale changes in consumerism and production systems that demand non-chemical approaches. Differences in regulatory requirements among countries may allow for some systems to gain greater acceptability in places where local production of inoculum for immediate use is realistic. In short, there are still stakeholders that would benefit from the bioherbicide technology (20).

Research on weed-pathogen systems has greatly contributed to knowledge in plant disease epidemiology, plant-microbe interactions, and biodiversity. This knowledge now provides the basis to understand and study multi-trophic interactions in weed-pathogen systems. Many of the problematic weeds are often found in monocultured crops or they exist in crops where low-cost weed control is critical. Although most biological control agents are too host-specific to individually address mixed weed populations in agronomic field crops, they can be targeted to manage those weeds that have the most impact on crop yield in high-value crops where control options are limited. Some cropping systems will inherently favor a single dominant weed species, leading essentially to a monoculture in which a host-specific biological control agent is ideally suited. Organic and conventional vegetable and herb production systems are particularly well suited to this approach and are in dire need of weed control options. Continuing to place emphasis on the development of integrated weed control systems that employ plant pathogens as components can greatly increase the number of successful biological weed control programs.

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