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USE OF PLANT PATHOGENS AS BIOHERBICIDES TO MANAGE WEEDS IN HORTICULTURAL CROPS

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Abstract. **Certain fungal, bacterial, and viral pathogens can be mass-produced and used as biological herbicides to control weeds in crops. This approach, referred to as the "bioherbicide" or the "inundative" biological control strategy, is based on our ability to manipulate certain weed-pathogen systems to cause highly damaging levels of disease epidemics during critical periods of weed interference and by doing so minimize present and future weed impacts in crop fields. Worldwide about 10 bioherbicide products have been developed and used commercially to manage weeds in various crops, including several horticultural crops. In fact, one of the first bioherbicides registered by the EPA was developed for control of a weed in Florida citrus. Currently, we are developing bioherbicides to manage weeds in citrus, vegetables, pastures, and natural areas, targeting pigweeds (*Amaranthus* spp.), purple nutsedge (*Cyperus rotundus* L.), several invasive grasses, dodder (*Cuscuta* spp.), and tropical soda apple (*Solanum viarum* Dunal). The following is a brief overview of this topic.**

The U.S. Environmental Protection Agency (EPA) regards any microbial pathogen offered for public use as a biological weed control agent a biopesticide (i.e., a bioherbicide). Generally, pathogens that can be industrially mass-produced and host-pathogen systems that can be manipulated to create managed epidemics are suited for the bioherbicide approach. Bioherbicides can be integrated into intensively managed horticultural production systems because of their potential to yield quick and reliable levels of weed control. They can be used to manage invasive weeds in natural areas and in situations where nonchemical alternatives to weed control are needed. In addition, bioherbicides based on naturally occurring pathogens that are not genetically modified, should be acceptable in organic agriculture. Although bioherbicides can be used as the sole option for the management of certain weeds in particular situations, typically they have been used as a minor supplement to conventional chemical herbicides.

A list of pathogens that have been developed and registered or sanctioned for use as bioherbicides since the early 1980s is given in (Table 1). One of two early bioherbicides registered was DeVine (*Phytophthora palmivora*), which was developed to control strangler vine (aka mildewed vine, *Morrenia odorata*) in citrus in Florida. This was followed in the next quarter century by the development and use of several pathogenic fungi and a bacterium to control weeds in horticultural crops, turf, and forestry. These bioherbicides have fulfilled highly specialized, small market needs, and several of them are no longer in use due to economic reasons. Nonetheless, their development represents an important contribution to weed science, plant pathology, and horticultural sciences. I predict that several additional bioherbicides and new breakthroughs in bioherbicide technology are likely to follow.

Some Prospective Bioherbicides under Development in Florida

Bioherbicide research is being carried out at three locations in Florida: the University of Florida/IFAS, Gainesville; the USDA-ARS-U.S. Horticultural Research Laboratory, Fort Pierce; and to a limited extent at the USDA-ARS-Invasive Plants Research Laboratory, Fort Lauderdale (where the principal focus is on classical biological control agents). Working as a team, scientists at these locations are attempting to develop bioherbicides and application technologies for five major weeds or weed complexes, as described below.

Phomopsis amaranthicola Rosskopf et al., a broad-Spectrum Bioherbicide for Amaranthus spp.

A fungal pathogen, *Phomopsis amaranthicola,* was discovered in Gainesville and described as a new species pathogenic to *Amaranthus* spp. (Rosskopf, 1997; Rosskopf et al., 2000a, b) and was shown to have potential for use as a bioherbicide (Rosskopf et al., 2000a). The fungus is pathogenic only to plants in the genus *Amaranthus* (Rosskopf et al., 2005) and it could be grown on common laboratory media such as V8-juice agar, tomato-paste agar, potato-dextrose agar, and several inexpensive substrates (DeValerio et al., unpublished).

Phomopsis amaranthicola initially causes leaf spots that promote extensive leaf abscission. Subsequently, stem lesions form that ring the stem and cause shoot death (Fig. 1). Symptoms appear in about one week after the fungus is sprayed over susceptible *Amaranthus* plants and the disease progresses steadily in the following weeks. The fungus sporulates profusely on infected plants, which promotes the development rapid secondary disease cycles and intra-field disease spread. Given its wide host range to several weedy *Amaranthus* spp., *P. amaranthicola* could be developed as broad-spectrum bioherbicide for pigweeds of which many are problematic weeds.

Morales-Payan et al. (2002, 2003b) have shown that one or two early applications of *P. amaranthicola* made 10 and 20 d after weed emergence (DAE) can significantly reduce interference of *Amaranthus lividus* and *A. dubius* with bell pepper, Caribbean-bonnet pepper, and eggplant and significantly improve crop yields. Wyss et al. (2004) and Morales-Payan et al. (2003c, 2005) have screened chemical pesticides, surfactants, and adjuvants for their inhibitory activity with *P. amaranthicola* and determined that it is possible to integrate the use of this bioherbicide with crop-protection chemicals. Collectively

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Table 1. A list of pathogens registered or approved worldwide as bioherbicides.

Pathogen	Weed	Product name
Alternaria destruens E.G., Simmons	*Cuscuta* spp.	Smolder
Chondrostereum purpureum (Pers.:Fr.) Pouzar	Broad-leaved trees	Chontrol MycoTech BioChon
Colletotrichum gloeosporioides	*Malva pusilla* Sm.	BioMal
Colletotrichum gloeosporioides (Penz.) Penz. & Sacc. in Penz. f.sp. *aeschynomene*	*Aeschynomene virginica* (L.) B.S.P.	Collego
Cylindrobasidium laeve (Pers.:Fr.) Chamuris	*Acacia* spp.	Stumpout
Phytophthora palmivora (E.J. Butler) E.J. Butler	*Morrenia odorata* (Hook. & Arn.) Lindl.	DeVine
Puccinia canaliculata (Schwein.) Legerh.	*Cyperus esculentus* L.	Dr. BioSedge
Puccinia thlaspeos C. Schub.	*Isatis tinctoria* L.	Dyer's woad rust strain Woad 006489
Xanthomonas campestris Migula pv. *poae*	*Poa annua* L.	Camperico

these studies have helped to establish the feasibility of further development of *P. amaranthicola* as a bioherbicide.

Dactylaria higginsii (E.S. Luttrell) M.B. Ellis, a Bioherbicide Agent for Purple Nutsedge

Dactylaria higginsii is a fungal pathogen of purple nutsedge (*Cyperus rotundus*) and other *Cyperus* spp. in the southeastern USA, the Caribbean, Brazil, South Africa, and several other regions worldwide. It causes leaf spots, foliar blight, and premature withering of leaves. Under high disease severity, nutsedge growth rate, as determined from plant dry weight, tuber number, and tuber weight, is affected (Kadir and Charudattan, 1999). We have tested an isolate of this pathogen found in Gainesville and demonstrated its potential as a bioherbicide agent for purple nutsedge (Kadir and Charudattan, 1999; Kadir et al., 1999, 2000b). Kadir et al. (2000a) have also determined the environmental conditions necessary for disease and epidemic development and shown that the pathogen is highly specific to plants in the sedge family, Cyperaceae (Kadir and Charudattan, 1999). Furthermore, Kadir et al. (2000b) have demonstrated the efficacy of this pathogen as a bioherbicide agent for purple nutsedge in field trials. They have also shown that *D. higginsii* disease could help reduce interference from purple nutsedge and improve yield in greenhouse-grown tomato (Kadir et al., 1999).

Fig. 1. Leaf spots and stem lesions caused by *Phomopsis amaranthicola* on redroot pigweed, *Amaranthus retroflexus*.

The bioherbicidal efficacy of *D. higginsii* has been evaluated in several field studies in Florida and Puerto Rico. Morales-Payan et al. (2003a) have shown that application of *D. higginsii* twice (8 + 18 DAE) or thrice (8 + 18 + 25 DAE) reduced yield loss to 31% and 24%, respectively, as compared to weed-free pepper (Fig. 2). Semidey et al. (2003) have determined that onion yield was higher in plots sprayed three times as compared to the yield from one and two applications of *D. higginsii*.

Rosskopf et al. (2003) are examining the potential of *D. higginsii* as an alternative to methyl bromide fumigation in an integrated approach to purple nutsedge management in a tomato production system. Application of *D. higginsii* during fallow as a way to reduce tuber production by the weed is also being examined in this study.

A constraint facing *D. higginsii* as a bioherbicide agent is the need for suitable methods for its large-scale production. Accordingly, Wyss et al. (2001) have identified several media and methods for mass production of this fungus, and Morales-Payan et al. (2004) have identified several nutrients and adjuvants that improve spore yields and virulence. Yandoc et al. (2003) and Morales-Payan et al. (2003a) have screened pesticides and surfactants that are commonly used in vegetable production in Florida and identified chemicals that could be used with the bioherbicide through proper integration such as timing and application sequence.

Further development of *D. higginsii* for public use will depend on our ability to mass-produce a highly efficacious and economical bioherbicide formulation. For this reason, we are currently testing several low-cost production methods for their commercial viability. We are also exploring the feasibility of applying this fungus in organic mulch.

Control of Weedy Grasses in Crops and Natural Areas

A diverse group of native and exotic grass species is considered problematic, including some that are desirable and economically valuable in some situations and weedy in others. Several invasive grasses such as cogongrass (*Imperata cylindrica* (L.) Beauv.), guineagrass (*P. maximum* Jacq.), torpedograss (*Panicum repens* L.), and tropical signalgrass (aka alexandergrass, *Urochloa subquadripara* (Trin.) R. D. Webster) cause problems in natural areas, forestry, waterways, turf, and some horticultural crops. It is difficult and impractical to develop bioherbicides tailored to each of the many grass weeds; an alternative would be to develop a broad-spectrum bioherbicide for

Fig. 2. Effect of *Dactylaria higginsii* on purple nutsedge. Top left: leaf spots on purple nutsedge leaves caused by *D. higginsii*. Top right: Effect of application of conidia of *D. higginsii* in water (D) or three different carriers: A, 0.05% N-gel; B, 0.02% Silwet L-77; and E, 0.05% Metamucil. C, untreated control. Bottom row, from left to right: effect of one, two, or three applications of *D. higginsii* conidia and the corresponding increase in leaf mortality.

several, if not all, weedy grasses. We have designed such a bioherbicide with a cocktail of three fungi in an approach we call "the multiple-pathogen strategy" (Chandramohan and Charudattan, 2001, 2003) (Fig. 3). Essential components of the cocktail are the fungi, an emulsion that acts as an adjuvant, and phytotoxic metabolites produced by the pathogens (Charudattan et al., unpublished). The fungal component consists of three fungi, *Drechslera gigantea* Heald & Wolf, *Exserohilum longirostratum* (Subram.) Sivan., and *E. rostratum* (Drechsler) Leonard & Suggs, which were isolated from three different grass species collected in different locations in Florida. Each of these fungi is pathogenic to a primary host and several alternative hosts. This overlapping host range enables these fungi to be used in a bioherbicide cocktail with activity against several grasses. In addition to the broad-spectrum activity, the level of weed control could be improved by using more than one pathogen in the cocktail (Chandramohan and Charudattan, 2001).

These fungi cause leaf spots, leaf lesions, and leaf blights, and kill the photosynthetic tissues. Symptoms appear in about one week after the fungus is sprayed on the foliage and the disease progresses steadily over the following two to three weeks. The treated grass foliage is killed and the control lasts for 10 weeks or more. Rhizomes are not killed and hence the grasses will regrow after a period of bioherbicide-caused suppression. Evaluations over the past five years have confirmed that these fungi are effective for grass management under field conditions (Chandramohan and Charudattan, 2001; Chandramohan et al., 2000, 2002a, b, 2003, 2004).

In host-range studies, each fungus was found to be pathogenic at different levels to one or more grass species (Chandramohan and Charudattan, 2001). Some graminaceous crops develop mild damage when sprayed with these fungi, singly or as a cocktail. Due to this reason, this bioherbicide system may not be used for over-the-top application in crops.

Yandoc et al. (2005) have tested *D. gigantea* with or without another fungal pathogen of grasses, *Bipolaris sacchari*, and concluded that these fungi, singly or in combination, can effectively suppress cogongrass. Yandoc et al. (2004) have also shown that these fungi could be used to suppress cogongrass interference in mixed plantings of cogongrass and bahiagrass. Chandramohan et al. (2002b) have determined that tropical signalgrass is susceptible to the bioherbicide mixture and that desirable turf grasses tested are either immune or resistant to each of the pathogens and the pathogen mixture. Bermudagrass sustained some injury from the pathogen/emulsion mixture, but recovered. Bahiagrass, bentgrass,

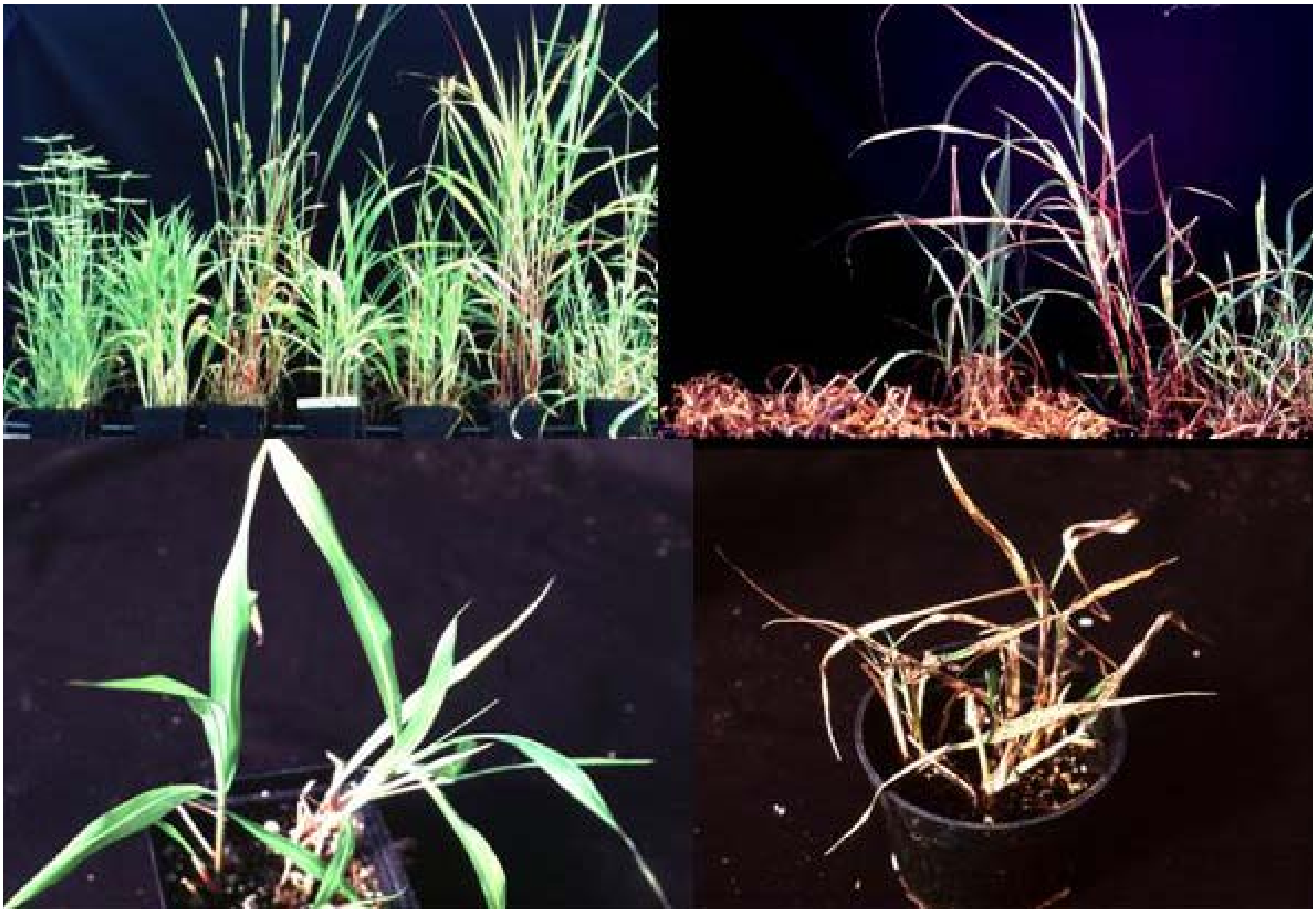

Fig. 3. Effect of a pathogen cocktail consisting of conidia of three fungi, *Drechslera gigantea, Exserohilum longirostratum,* and *E. rostratum* on weedy grasses. Top left: Control plants of seven different grasses targeted for control by the bioherbicide cocktail. From left to right: crowfootgrass, Texas Panicum, yellow foxtail, guineagrass, southern sandbur, johnsongrass, and large crabgrass. Top right: The grasses sprayed with the bioherbicide cocktail. Bottom row: guineagrass treated with the bioherbicide cocktail (right) and a control.

centipedegrass, and seashore paspalum were immune. St. Augustinegrass and zoysiagrass were resistant. Because St. Augustinegrass is resistant, the bioherbicide could be used as an over-the-top application in St. Augustine turf. In bermudagrass turf, spot treatments of the tropical signalgrass may be necessary because of the potential for injury from the pathogen-emulsion mixture (Chandramohan et al., 2002b). Additionally, Chandramohan and others have demonstrated the potential applicability of this grass bioherbicide system in sugarcane and natural areas (Lake Okeechobee and in north-central Florida) (Chandramohan et al., 2003, 2004).

Studies on large-scale inoculum production and formulation are needed to develop and register this bioherbicide system. Efficacy trials under commercial conditions (e.g., growers' fields, natural areas, home lawns, and gardens, etc.) and economic feasibility studies can then be undertaken with commercial prototype formulations.

Integrated Management of Dodder with a Bioherbicide, Ammonium Sulfate, and Low Rates of Glyphosate

Alternaria destruens E.G. Simmons, a fungal pathogen of dodders (*Cuscuta* spp.) was discovered and developed as a bioherbicide by Thomas A. Bewick, a former member of the University of Florida/IFAS Horticultural Sciences faculty (Bewick et al., 1986, 1987). It causes a blight disease on several dodder species, and the disease reduces the parasite's spread and host damage (Fig. 4). A commercial bioherbicide product of this fungus, Smolder, has been developed and registered by Loveland Products, Inc., Greeley, Colorado. Smolder is the latest bioherbicide registered in the United States.

Alternaria destruens is highly effective in mitigating the damage inflicted by dodder under field conditions. Field trials have been conducted in carrot, cranberry, sunn hemp, and others (T. A. Bewick, USDA-CSREES-NPS, Washington, D.C., pers. comm.). We are attempting to develop an integrated management system for lespedeza dodder, *C. pentagona,* by combining Smolder with ammonium sulfate and low rates of glyphosate (Cook et al., 2005). In a field study, dodder naturally parasitizing sunn hemp (*Crotalaria juncea*) could be killed five weeks after applications of *A. destruens.* Our aim is to develop a best management practice for dodder using Smolder as the key component.

A Bioherbicide for Tropical Soda Apple

Tropical soda apple (*Solanum viarum*) is a highly invasive weed in cattle pastures in Florida and neighboring states. It is

Fig. 4. Effect of *Alternaria destruens* on dodder, *Cuscuta pentagona*. Healthy (left) and diseased and dying dodder parasitizing citrus.

slowly moving into crops and has the potential to affect sugarcane, citrus, and perennial crops in Florida (Charudattan, unpublished surveys). While screening for pathogens of this weed, we discovered that *Tobacco mild green mosaic tobamovirus* (common name: tobacco mild green mosaic virus; TMGMV), a plant virus that occurs worldwide, kills tropical soda apple (TSA) plants by triggering a lethal hypersensitive response from the plant (Pettersen et al., 2001; Charudattan et al., 2003a, b). Typically, two to three weeks after virus inoculation, TSA plants of all ages wilt suddenly and die quickly and completely without regrowth (Fig. 5). TSA is also susceptible to several other plant viruses in the begomovirus, potyvirus, and tobamovirus groups (McGovern et al., 1994), but unlike TMGMV, these viruses do not kill TSA. In fact, the TSA-killing ability of TMGMV is rather specific to this plant and virus combination (Charudattan et al., 2003b).

TMGMV has been successfully field tested in several cattle ranches in Florida. Up to 99% TSA control (weed-kill) has been obtained in some trials. Inoculation of 3 to 5 leaves per plant is sufficient to infect and kill the entire plant. As a plant virus, TMGMV does not pose any risks to nontarget fauna. To understand the extent TMGMV's host range and to assess the potential risks to nontarget plants from its use as a bioherbicide, we have conducted an extensive screening with 400 plants belonging to 174 genera in 58 families (Charudattan et al., 2003a). Included in this list were agronomic, ornamental, and vegetable crop plants, native plants, threatened and endangered plants, and weeds. Plants in the Solanaceae received extra scrutiny, with 171 plants representing 12 genera and 78 species tested. Five important crop plants, tomato, pepper, eggplant, potato, and tobacco, were screened, using several cultivars in each case. Based on this comprehensive host-range study on TMGMV, we have determined that only some (not all) cultivars of peppers and tobacco would be at risk but only if these plants were exposed to the virus by direct sprays, physical contact with treated TSA plants, or injury by virus-contaminated equipment.

The potential risk to pepper, tobacco, and a few other susceptible plants that develop mild mosaic symptoms, is negligi-

Fig. 5. Typical reaction of TSA plant to inoculation with TMGMV in the field, before (left) and 3 weeks after (right) inoculation.

ble and manageable for the following reasons: (a) TMGMV is not an insect- or nematode-transmitted virus and it is not seed-borne. Our tests with the South American beetle *Gratiana boliviana,* as well as field observations of Colorado potato beetle (*Leptinotarsa decemlineata*), confirm that insect dispersal of TMGMV in the field is not a valid concern. (b) To prevent spread of TMGMV between infected and healthy plants through contaminated agricultural tools, the tools can be easily disinfested with dilute detergent or bleach. (c) Infected TSA plants die quickly and completely without any regrowth and consequently the virus does not buildup to high levels in dying TSA plants. (d) Not all pepper and tobacco cultivars are at risk; several resistant and immune cultivars of these plants exist. (e) TMGMV is naturally present in Florida, the Southeast, and many parts of the world and therefore we will not be introducing anything new to Florida or the United States. (f) TMGMV is not reported to cause significant economic damage to susceptible crops anywhere in the world.

To facilitate registration and commercial development of this virus as a bioherbicide, we have initiated discussions with the EPA and assembled a data package for EPA's evaluation. If registered, TMGMV will be the first viral bioherbicide in the world and it will be used in cattle (beef and dairy) pastures, sod farms, and natural areas affected by TSA infestation.

Conclusion

Plant pathogens have been used safely and effectively as registered/approved bioherbicides to manage several weeds. Efforts are continuing in Florida to develop and register four new bioherbicides targeting pigweeds, purple nutsedge, invasive grasses, and tropical soda apple. In addition, research is underway to develop an integrated management system for dodder using the registered bioherbicide Smolder.

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