

Multistate Research Project Proposal

Project Number: S- 1001

Project Title: Development of Plant Pathogens as Bioherbicides for Weed Control

Requested Project Duration: October 1, 2001 through September 30, 2006.

Statement of the Issue(s) and Justification:

There is a clear need for the proposed research. Weed control is required to sustain and maximize agricultural productivity. Crop losses due to weeds in the United States are estimated to be nearly \$6 billion per annum, which is up from an estimate of \$ 4.1 billion about a decade ago (Bridges, 1992). Losses due to weeds in major crops in the United States, such as wheat, maize, rice, and soybean are estimated at 11% to 21% of the attainable yields (Oerke et al., 1994). In the Southern Region, losses due to weeds are said to exceed \$1.7 billion. In the absence of weed control, it is estimated that crop losses in the United States would amount to \$19.6 billion. These figures do not include the loss of productivity encountered in livestock production due to invasive weeds, the costs of weed control in natural areas (e.g., waterways and rangelands), recreational areas (e.g., golf courses and parkland), or urban and home landscapes.

Chemical herbicides and cultivation are used as the primary means of weed control in the United States. Based on pesticide sales figures, more herbicides are used than any other type of pesticide (Kearney, et al., 1988; USDA-ERS, 1988). Worldwide, chemical herbicides account for approximately 44% of the chemical pesticides used, compared with 29%, 21%, and 6% for insecticides, fungicides, and other pesticides (i.e., nematicides, plant growth regulators, etc.), respectively (Klassen, 1995). The pesticide production figures in the United States also generally support these figures since the proportion of the acreage treated with herbicides or insecticides in 1988 were, respectively, 96% and 40% for corn, 95% and 4% for soybean, and 94% and 63% for cotton (USDA-ERS, 1988). It is estimated that cessation of all pesticide use in the United States would reduce agricultural output by 30% (Fernandez-Cornejo et al., 1998). Furthermore, the economic benefit to the growers from the use of traditional chemical pesticides has steadily declined during the last four decades. For example, it is estimated that whereas in 1949 every \$ 1.00 spent on pesticides returned \$ 7.96 in benefits, the returns in 1991 amounted to only \$ 4.16 per \$ 1.00 spent (Teague and Brorsen, 1995).

Recently there has been a notable reduction in the amounts of chemical pesticides used, but the rate of decline for chemical herbicides use has been less than that for other chemical pesticides (Gianessi and Anderson, 1993). This decrease in pesticide use is likely to continue in the United States partly due to economic realities of global agricultural production and marketing as well as the national initiative for the reduction in the use of chemical pesticides. For instance, the passage of the Food Quality Protection Act (FQPA) in 1996 has mandated sweeping changes in the way pesticides are registered and re-registered by the EPA. When fully in force, the FQPA will initiate a progressive review of existing pesticides and in this process several older classes of

herbicides (including several important herbicide chemistries) are likely to be phased out either due to environmental and human health considerations or voluntary withdrawal from the markets by companies. The FQPA also requires that incentives be developed for nonchemical alternatives, reduced-risk pesticides, and for greater implementation of integrated pest management (IPM) (EPA-OPP, 2001), thus providing a powerful incentive to develop bioherbicides, for example.

The use of herbicide-tolerant transgenic crops is also expected to drastically change the way weeds are managed, in spite of the fact that this new technology has limitations. Among the major unknowns of this technology are the general uncertainties about acceptance of transgenic crops by the public and the high cost of transgenic seeds that small farmers may not be able or willing to afford. There is also the possibility of escape of the herbicide tolerance trait to desirable relatives of the weeds (e.g., canola and wild rape, among *Lolium* spp., and others.) Thus, the use of herbicide-tolerant transgenic crops is not a panacea.

Weed control becomes even more complicated when considering management of invasive weeds in natural and urban areas, where economic, environmental, or human risk concerns may entirely preclude the use of chemical herbicides.

Although chemical pesticides are an integral part of modern food production, there has been a significant cost to the society and the environment associated with the widespread use of these chemicals (Pimentel and Greiner, 1997). Thousands of cases of accidental poisonings and numerous accidental deaths from chemical pesticides, including some herbicides, are reported each year (Ecobichon, 1998). Extensive use of herbicides and other pesticides has resulted in groundwater contamination (Abdalla and Libby, 1987), and the use of certain herbicides has been linked to some types of cancer (Hoar et al., 1986). Other preventable problems, mainly from misuse of chemical herbicides can result, such as nontarget damage from spray drift and carry-over problems in soil and produce.

Finally, despite the extensive use of herbicides, many weed species continue to cause losses in agriculture, and the current strategies for control of these weeds are inadequate. One reason for this is the emergence of herbicide-resistant weeds (Holt and LeBaron, 1990). Many present-day herbicides have single-site modes of action and therefore are prone to promote rapid development of resistance among weeds. It took approximately 25 years for the first atrazine resistant weeds to be identified, but weeds resistant to sulfonylurea and imidazolinone appeared in less than 10 years after these herbicide chemistries were introduced and widely used. The intensive cultivation of herbicide-tolerant crops is likely to further accelerate the emergence of herbicide-resistant weeds consisting of mutant weed biotypes as well as naturally resistant weeds, the latter due to weed-shifting.

A different problem exists in some situations, such as vegetable crops (i.e., minor crops) and range lands, where the economic realities of the marketplace preclude the development and/or use of conventional chemical herbicides. Organic farming is gaining a foothold in the mainstream American agriculture, but this industry suffers from the general lack of biologically

based weed control options. Foods consumed by infants, children, and elderly and commodities that rely on a few classes of herbicide chemistries for weed control are particularly vulnerable in this regard. Problems related to the current restrictions in the use of certain chemical herbicides and the impending loss of methyl bromide as a broad-spectrum soil fumigant will further exacerbate weed problems in crops such as vegetables, strawberry, and many others. Development of herbicide-tolerant crops, along with the consolidation of major agrochemical companies and increasing globalization of agricultural production and marketing further limit the choices available to manage weeds on local and regional scales. Many of these developments disproportionately constrain small and mid-sized farmers, especially given the recent depressed prices for commodities.

Invasive weeds are emerging as another major threat to agricultural and natural areas and to the long-term health and biodiversity of our nation's land and water resources. Weed problems become more complicated in natural and urban areas, where economic, environmental, or human-health risks may entirely preclude the use of chemical herbicides. Lastly, weeds such as pigweeds (*Amaranthus* spp.), nutsedges (yellow nutsedge [*Cyperus esculentus*] and purple nutsedge [*C. rotundus*]), purslanes (*Portulaca* spp.), spurge (*Euphorbia* spp.), kudzu (*Pueraria lobata*), and various grasses and invasive weeds are not controlled effectively by available methods. Therefore, development of newer weed-management agents and technologies, including biologically based approaches, is of greater importance now than ever before.

Importance of the proposed multistate research and the consequences of not undertaking this cooperative endeavor are as follows. As explained above, the need for developing diversified weed management tools and strategies is more acute than ever. One of the alternatives to chemical weed control is biological control by using plant pathogens (Charudattan and Walker, 1982; TeBeest, 1991; Roskopf et al., 1999). "Biological control" is defined as "management of natural enemies (predators, parasites, and pathogens of pests) and selected beneficial organisms (antagonists, competitors, and allelopaths) and their products to reduce pest populations and their effects" (USDA-ARS, 1988). Plant pathogens used in an augmentative or inundative, biopesticide mode are referred to herein as bioherbicides.

In 1995, the S-268 Regional Research Project was initiated to evaluate and develop plant pathogens for biological control of weeds. S-268 succeeded two previous projects, S-136 and S-234, and collectively these cooperative projects served as the foundation for research and evaluation of nearly two dozens of pathogens as potential bioherbicides. These projects also helped to develop epidemiological and risk-analysis models to understand the performance and safety of two previously registered and commercialized bioherbicidal pathogens, *Colletotrichum gloeosporioides* f. sp. *aeschynomene* (Collego[®]), used for the control of northern jointvetch (*Aeschynomene virginica*), and *Phytophthora palmivora* (DeVine[®]) used for the control of stranglervine (*Morrenia odorata*) in Florida, understand some aspects of the genetics of these fungi, and develop systems to integrate their use in rice and citrus production (Bowers, 1986; Kenney, 1986; Ridings, 1986; Smith, 1986; TeBeest, 1982, 1988; TeBeest and Dickman, 1989; Cisar et al., 1996). It also provided a forum to develop scientific and technical concepts helpful

in the registration but not commercialization of *Puccinia canaliculata* as Dr. BioSedge[®] (Phatak, 1992).

Several other bioherbicide candidates were developed through regional trials, notably *Alternaria cassiae* (Charudattan et al., 1986), although registration and commercialization did not materialize. While in some cases this was simply be due to a lack of consistency of the bioherbicide agent or technical feasibility, the availability of efficacious chemical alternatives, coupled with the lack of coordinated efforts by researchers and commercial enterprises were also contributory factors. Nonetheless, it has been amply demonstrated that bioherbicide products are practical and economically sustainable in the marketplace. Moreover, their use over the past 25 years has not led to any risks to human health or the environment. The basic and applied research done through these cooperative projects have helped to develop and validate the concept of inundative biological control of weeds by the bioherbicide strategy (TeBeest, 1991; Rosskopf et al., 1999; Charudattan, 2000).

The success of the S-268 project and its predecessors can be measured also in terms of the scientific and technical knowledge gained. The members of this group have written two books, hundreds of refereed papers, reviews, and popular articles on the subject of biological control of weeds with plant pathogens. About twenty-five graduate students have been trained since the inception of the first cooperative project on this topic, S-136. Three bioherbicides have been registered and a classical biocontrol introduction of a rust fungus has resulted during this period. Several prospective bioherbicides have been patented.

Just during the last five years, under S-268, three pathogens have been cooperatively developed: *Colletotrichum truncatum* (COLTRU) for control of hemp sesbania (*Sesbania exaltata*), *Alternaria destruens* (Smolder) for dodders (*Cuscuta* spp.), and *Pseudomonas syringae* pv. *tagetis* for control of several weeds in the Asteraceae. Smolder and Mallet (*Colletotrichum gloeosporioides* f.sp. *malvae*) are currently under EPA review for registration. In addition, these regional projects have also helped to stimulate research on the use of novel materials for formulation, or as surfactants and spray adjuvants to improve the efficacy (Boyette, 1994) and host range of bioherbicide agents (Zidack et al., 1992; Boyette and Abbas, 1994), improve production methods (Chandramohan and Charudattan, 1998; Connick et al., 1991; Daigle and Cotty, 1994; Jackson, 1994; Jackson et al., 1994; Jackson and Schisler, 1994; Shabana et al., 1997; Quimby et al., 1994; Wyss et al., 1999; Yandoc and Charudattan, 1998), and develop effective delivery systems (Rosskopf et al., 1996; DeValerio et al., 2000). During this period, 97 refereed papers, 19 book chapters, 33 papers in conference proceedings, 51 abstracts, and 9 patents, and several miscellaneous publications were produced by the members and other participants of S-268 project. These contributions would not have been possible without the facility to engage in cooperative regional research under the auspices of S-268. In addition, the project has enabled the discovery of numerous new pathogens or pathogen records on weeds and clarification of weed and pathogen taxonomies, understanding of the epidemiology of several diseases, development of methods for microbial fermentation, formulation, and delivery, risk assessment, and molecular biology of host-pathogen relationships. Moreover, the development Smolder and Mallet as possible commercial products, methods development for COLTRU, and

cooperative testing of several other agents have been greatly facilitated by this cooperative research endeavor. Given this history, the consequences of not undertaking this proposed multistate research project include:

- 1) Further development of potentially useful bioherbicides will be abandoned because no single SAES has all the resources necessary to develop a pathogen from its discovery to the status of a commercial product.
- 2) The concept of regional research (i.e., multistate research project) will be key to the development and registration of bioherbicides in the next 5- to 10-year term.
- 3) There are many common weed problems throughout the continental United States, especially the Southern Region. Our ability to develop and register bioherbicides will be greatly aided by testing candidate pathogens in different states and regions, in North America. Also, strains of candidate pathogens may be distributed in several locations in the United States that differ in virulence, fitness, and other traits. Hence, bioherbicides can be developed more quickly more efficiently if scientists from several states cooperate toward common goals.
- 4) Although this new project is proposed for the Southern Region, participation by states and institutions outside this region will be beneficial for two reasons: A) Expertise available in the different institutions listed herein will be essential for the success of this project. B) Bioherbicide agents proposed to be developed under this project will need to be tested for efficacy against the target weeds in several regions within and outside the Southern Region, under different climatic and edaphic conditions.
- 5) A multistate research project organization, such as the one proposed, also offers a forum to develop new information and ideas. Recent annual meetings of S-268 have fostered participation by scientists from SAES, ARS, small colleges, and industry. Scientists from other countries (e.g., Canada, South Africa, and U.K.) have also frequently participated in the annual meetings.

The research proposed under this multistate project relates to national priorities established by the Experiment Station Committee on Organization and Policy (ESCOP), the Southern Region Strategic Plan (SAAESD, 2000), and the USDA-ARS. The Southern Region Strategic Plan has stressed the need to develop and integrate biological control with current pest control practices. Biologically based pest management has been recognized in this plan as the second most critical need for agriculture in the Southern Region. A similar emphasis on biological control has been also made by the National Research Council's Board on Agriculture (NRC, 1996) and the U.S. Congress' Office of Technology Assessment (OTA, 1995). Several SAES and the USDA-ARS are committed to biological control of weeds as a top research priority. In 1984, a Research Planning Conference on Biological Control attended by a broad group of stakeholders identified targets for research in biological control (USDA-ARS, 1984). Weed species identified for research in biological control included: velvetleaf (*Abutilon theophrasti*), cocklebur (*Xanthium strumarium*), the morningglories (*Ipomoea* spp.), the nutsedges (*Cyperus* spp.), sicklepod (*Cassia obtusifolia*), johnsongrass (*Sorghum halepense*), prickly sida (*Sida spinosa*), and spurred anoda (*Anoda cristata*). Areas of research priority for biological control for the USDA-ARS also have been determined (USDA-ARS, 1988). These include: mass

propagation, harvesting, packaging, storage, and distribution of biological agent populations, systematics, genetic improvement through biotype selection, conventional crosses, and genetic engineering, and exploration for new, more effective biological agents (USDA-ARS, 1988). A recent review of the USDA-ARS Weed Science program has further highlighted the need to increase efforts on biological control of weeds (USDA-ARS, 2001).

Related, Current, and Previous Work:

The first weed-control pathogens were discovered and deployed between 1969 and 1973 (Daniel et al., 1973; Smith, et al., 1973 a, b; TeBeest and Templeton, 1985; Ridings, 1986; Templeton et al., 1986). By 1982, 49 plant species were reported as targets for control by bacteria, fungi, nematodes, and viruses (Templeton, 1982). Recent analyses indicate that nearly 200 fungal pathogens have been screened as potential bioherbicides (Charudattan 1991, 2001). Of these, eight pathogens have been registered worldwide and about 50 are rated as having good to excellent potential for commercialization. About 46 different weeds have been identified as potential targets for bioherbicide research in different countries. About a third of these weeds are also the primary targets in the United States.

Several ongoing regional research projects address various aspects of biological control, including some in the Southern Region. However, only one other project, S-303, deals with biological control of weeds and complements this proposed multistate project on the development of plant pathogens as bioherbicides. The two projects are different in that the S-303 project deals with biocontrol of introduced insect pests and weeds through classical importation of biocontrol agents, while the proposed project is aimed at the use of indigenous plant pathogens in augmentative and inundative biocontrol strategies.

A CRIS search was done to see whether similar projects exist in other regions. Only one other multistate project related to weed biocontrol is in existence. This project, S-303, deals with classical biological control of arthropod pests and weeds through importation of natural enemies from abroad. This project has four objectives, including survey and importation of natural enemies of invasive pests; integration of exotic and indigenous natural enemies with other pest management approaches; evaluation of the effects of exotic natural enemies on nontarget organisms; and characterization of the role of indigenous natural enemies in suppressing pest and beneficial species. These objectives and the general aim and scope of this project do not conflict or duplicate the objectives proposed herein. The bioherbicide project proposed here deals exclusively with indigenous microbial plant pathogens used in the inundative biocontrol strategy. Accordingly, the proposed multistate project can compliment the S-303 project.

Objectives:

This proposal will have three main objectives, with bioherbicidal control of weeds as its overall goal.

Methods:

Objective 1: To evaluate and develop bioherbicide agents to control nutsedges, pigweeds, grasses, purslanes, sparges, kudzu, weeds in Asteraceae, and others.

The first objective will address the development of bioherbicide agents and a bioherbicide mixture to control several highly important weeds including nutsedges, pigweeds, grasses, purslanes, sparges, kudzu, and others. The biocontrol agents proposed here have all been previously shown to be effective in weed control and are critically in need of further research and development to realize their registration and utilization.

1-A. Development of *Dactylaria higginsii* as a bioherbicide for nutsedges (*Cyperus* spp.):

Dactylaria higginsii, recently isolated from purple nutsedge in Florida, has been shown to have good potential as a bioherbicide for this weed. When applied as a postemergent foliar spray, it causes high levels of shoot mortality in a number of nutsedge species. (Kadir and Charudattan, 2000). The host range of the fungus is restricted to *Cyperus* spp. and *Kyllinga brevifolia* (= *Cyperus brevifolius*), and it has been shown to be capable of suppressing purple nutsedge competition with tomato and pepper (Kadir and Charudattan, 2000; Kadir et al., 1999, 2000a, b). The bioherbicidal use of this fungus has been patented (Kadir and Charudattan, 1997), and industrial collaboration has been set up by the University of Florida to develop and register this bioherbicide.

Roles and responsibilities: the following stations will participate in this objective: USDA-ARS-SWSRU, MS; Sylvan, PA; FL SAES; IN SAES; CA SAES, Riverside; NC SAES; MT SAES; USDA-ARS-USHRL, FL; and PR SAES. Individuals from these stations (See Appendix-Projected Participation) will follow the common protocols described below.

Experimental and commercial prototype inoculum preparations of *D. higginsii* will be provided to cooperators. The fungus will be field-tested in different crops chosen by the cooperators according to regional needs. The potential to use *D. higginsii* as an alternative to methyl bromide for nutsedge control will be determined in some states. The primary target will be purple nutsedge. Yellow nutsedge, green kyllinga, rice flat sedge (*Cyperus iria*), and globe sedge (*C. globulosus*) may also be targeted. Tests will be done using common experimental protocols and experiments will be replicated and repeated. Data gathered will include percent weed control, aboveground biomass, tuber number, and tuber weight. Effects of *D. higginsii* on the competitiveness of nutsedges and on the growth and yield of selected crops will be studied. *Dactylaria higginsii* will be applied as a post-emergence over-spray in one half of the treated plots. Experiments will be repeated. Data taken will include number of nutsedge plants, percent nutsedge control, tuber number, above ground biomass, tuber weight, and crop yields (where applicable).

1-B. Development of *Microsphaeropsis amaranthi* and *Phomopsis amaranthicola* as broad-spectrum bioherbicides to control pigweeds:

Two fungal pathogens, *Microsphaeropsis amaranthi* (= *Aposphaeria amaranthi*) (Mintz et al., 1992) and *Phomopsis amaranthicola* (Roskopf, 1997; Roskopf et al., 2000) have been shown to be potential bioherbicide agents. These pathogens have overlapping and complementary host ranges, each being capable of controlling some, but not all weedy species of *Amaranthus*. The epidemiological conditions conducive for disease development and weed control by these fungi have been determined (Mintz et al., 1992; Roskopf, 1997; Roskopf et al., 2000). The nontarget safety of these fungi has also been confirmed: all pigweed species screened were susceptible, whereas the crop plants tested were immune (Mintz, 1991; Roskopf, 1997). The bioherbicidal use of *P. amaranthicola* has been patented (Charudattan et al., 1995, 1996).

Roles and responsibilities: the following stations will participate in this objective: USDA-ARS-SWSRU, MS; FL SAES; IN SAES; NC SAES; MT SAES; USDA-ARS-USHRL, FL; and PR SAES. Individuals from these stations (See Appendix-Projected Participation) will follow the common protocols described below.

Preliminary unpublished data on the complementary interactions between *M. amaranthi* and *P. amaranthicola* indicate that the two fungi may be used in combination to obtain broad-spectrum control of several species of *Amaranthus* and increase the level of weed control through synergism between the pathogens. Further multistate research will be key to the next phase of technology transfer with both fungi. Accordingly, we propose to develop methods for large-scale production and bioherbicide formulations of these fungi, evaluate their efficacy in commercial fields, and establish systems to integrate the bioherbicides in crop production.

Large-scale production of these fungi will be developed in the participants' laboratories and transferred to an industrial partner. Various materials and culturing methods will be evaluated for large-scale production. Solid-state and liquid-fermentation methods will be evaluated, by varying factors such as carbon:nitrogen (C:N) ratios, types of C and N, temperature, light, aeration, and the addition of solid material such as wheat husks, corn-cob grits, and others. Suitable prototype commercial preparations of these fungi will be produced and tested in multistate field trials using different crops. Testing will be done using common experimental protocols and the studies will be replicated and repeated. Percent weed control and crop yield will be the criteria used to determine the efficacy of these bioherbicide agents.

1-C. A multiple-pathogen approach to control several weedy grasses:

Weedy grasses are difficult to control. Depending on the location and crops, different grass species cause problems. Chemical herbicides and mowing are commonly used in appropriate situations, but these methods are costly and may have adverse environmental impacts (e.g., pesticide runoff from lawns and golf courses). Furthermore, grass herbicides, such as atrazine, metribuzin, and simazine, are at risk of being phased out as a result of the FQPA (Anonymous, 2001). Biological control offers an alternative, and Chandramohan et al., (2000) have shown that *Drechslera gigantea* isolated from large crabgrass (*Digitaria sanguinalis*) and *Exserohilum*

rostratum and *E. longirostratum*, respectively from johnsongrass (*Sorghum halepense*) and crowfootgrass (*Eleusine indica*) can be used as a cocktail to control seven or eight different weedy grasses. In a field trial conducted in Lake Alfred, FL, crowfootgrass, guineagrass (*Panicum maximum*), johnsongrass, large crabgrass, southern sandbur (*Cenchrus echinatus*), Texas panicum (*Panicum texanum*), and yellow foxtail (*Setaria glauca*) were controlled with the mixture of these fungi. In another field trial conducted in Ft. Pierce, FL, the fungal mixture was effective in controlling a natural stand of guineagrass. When applied in an invert emulsion, two applications of these pathogens gave 85% control of all seven grasses in two weeks after the second application, and the control lasted for 10 to 14 weeks without regrowth of the grasses (Chandramohan et al., 2000). The use of a mixture of two pathogens for simultaneous control of two weeds has been demonstrated previously (Boyette et al., 1979), but this system has not been commercially developed and subjected to the EPA registration process. Therefore, a multistate effort will be undertaken to test the pathogen cocktail under different climatic conditions, against different grass weeds, and in different crop and noncrop situations. The results of these studies will be used to further develop the multiple-pathogen system for registration and commercialization.

Roles and responsibilities: the following stations will participate in this objective: USDA-ARS-SWSRU, MS; Agric. & Agri-Food Canada, SK; FL SAES; IN SAES; MT SAES; and USDA-ARS-USHRL, FL. Individuals from these stations (See Appendix-Projected Participation) will follow the common protocols described below.

Under this multistate project, methods will be developed for large-scale production of inoculum of *D. gigantea*, *E. longirostratum*, and *E. rostratum*. Nutrient requirements (C- and N-sources, minerals, growth factors) and physical conditions of culture (temperature, pH, agitation-aeration) will be studied. Efforts will be made to replace commercial laboratory media with less expensive C- and N-sources (e.g., molasses, grain mash, fish meal, soybean meal, whey, and others). Production will be attempted in stationary liquid cultures, shake cultures and small- (500 ml) and large-scale (>25 liters) liquid-fermenter cultures.

Efficacy determinations will be made in field plots. Different grasses will be targeted for control, depending on regional needs. Sugarcane, citrus, and other tree crops, turf, home gardens, and other sites where grasses are a problem will be targeted. Common experimental protocols will be developed and used among the cooperating states. Efficacy data from the above trials will be used to further develop these fungi as a bioherbicide cocktail. An industrial partner will be identified for commercial development and registration.

1-D. Myrothecium verrucaria as a broad-spectrum bioherbicide for purslanes, spurges, kudzu, and other weeds:

Common purslane (*Portulaca oleracea*), horse purslane (*P. portulacastrum*), spotted spurge (*Euphorbia maculata*), prostrate spurge (*E. prostrata*), and kudzu (*Pueraria lobata*) are serious weed pests in many areas of the United States, including the Southern Region. Purslanes and spurges cause problems in vegetables such as tomato and pepper. Kudzu is a notorious weed

along roadsides, natural areas, right-of-ways, etc. in the South. These weeds often form dense, complex, difficult-to-control populations that cannot be easily managed with chemical herbicides. Methyl bromide has long been used as a fumigant to control purslanes and sparges, and the loss of this broad-spectrum fumigant will cause serious problems to the growers. A plant pathogen, *Myrothecium verrucaria*, shows promise as a bioherbicide for controlling several of weeds including sicklepod (*Senna obtusifolia*) and hemp sesbania (*Sesbania exaltata*) in addition to those named above (Walker and Tilley, 1997; Boyette et al., 1999).

When *M. verrucaria* spores were applied with 0.05% surfactant (Silwet L-77), the fungus caused foliar necrosis and biomass reduction in several crop, ornamental, and weed species. Plant responses ranged from no reduction in dry weight to 100% mortality. When the fungus was applied by directed spray to mixed plantings of sicklepod and soybean seedlings, with the latter mostly protected from the spray, only sicklepod plants were killed (Walker and Tilley, 1997). This capacity of *M. verrucaria* to cause necrotic damage on several plant species, including many weeds, provides an opportunity to develop this fungus as a broad-spectrum bioherbicide.

Roles and responsibilities: the following stations will participate in this objective: USDA-ARS-SWSRU, MS; USDA-ARS-FDWSU, MD; USDA-Forrest Service, GA; FL SAES; IN SAES; MT SAES; USDA-ARS-EBCL, France; Forestry Canada, BC; and PR SAES. Individuals from these stations (See Appendix-Projected Participation) will follow the common protocols described below.

Myrothecium verrucaria has a number of desirable characteristics that favor its use as a bioherbicide. As reported by Yang and Jong (1995), *M. verrucaria* is usually a weak pathogen, but severe plant damage can result when high numbers of spores are applied with a suitable surfactant. Secondary spread of the pathogen to uninoculated plants is not a problem since this pathogen does not readily reproduce on the infected host (Walker and Tilley, 1997). Therefore, additional research is justified to assess the potential of *M. verrucaria* as a bioherbicide.

The aim of this sub-objective is to develop *M. verrucaria* as a broad-spectrum bioherbicide for species of purslane, sparges, kudzu, and other difficult-to-control weeds. Under this sub-objective, the effectiveness of *M. verrucaria* as a bioherbicide will be determined in greenhouse and field trials. Systems will be developed for mass production of inoculum for cooperative multistate trials that will be conducted by using common protocols. Suitable experimental designs, replications, and repetitions will be included. Different crops (purslanes, sparges, and other weeds in row crops) and weed-infested sites (kudzu) will be used for field trials.

Using optimum conditions for disease development, the effect of *M. verrucaria* will be determined on the basis of weed kill, weed biomass, reproduction, and survival of weed propagules. Disease/damage incidence, disease/damage severity and weed control will be rated at suitable intervals. Plants will be harvested, also after suitable periods, for biomass and productivity measurements. A rating system will be used in choosing the most promising biocontrol agents.

To confirm efficacy of *M. verrucaria* under field conditions, field plots will be established in different states. Results from these studies will be used to further develop and register *M. verrucaria* as a broad-spectrum bioherbicide. Appropriate industrial collaboration will be established for commercial development and registration of this agent.

The potential to control invasive vines, such as old-world climbing fern (*Lygodium japonicum*), skunkvine (*Paederia foetida*), and others with *M. verrucaria* will be determined in FL.

The types and nature of toxic metabolites produced by *M. verrucaria* and their safety to human and environmental health will be determined.

1-E. Development of Pseudomonas syringae pv. tagetis as a bioherbicide for weeds in the Asteraceae:

The bacterial pathogen *Pseudomonas syringae* pv. *tagetis* (PST) is the causal agent of a disease characterized by apical chlorosis on several members of Asteraceae. Johnson et al. (1996) have demonstrated that this bacterium can be used to control various weeds in and outside the Asteraceae family. Spray application of this bacterium, at 5×10^8 cells per ml, in an aqueous buffer containing the surfactants Silwet L-77 (0.1%) or Silwet 408 (0.2%) resulted in 100% disease incidence and a higher level of disease severity on Canada thistle (*Cirsium arvense*) than observed under natural infections. In addition to *C. arvense*, the following plants were severely diseased when sprayed with the bacterial cells: *Ambrosia artemisiifolia*, *Helianthus annuus*, *H. tuberosus*, and *Tagetes erecta*. Under field conditions, high levels of plant mortality (57-100%) were seen in the case of *A. artemisiifolia*, *C. arvense*, *Conyza canadensis*, *Lactuca serriola*, and *Xanthium strumarium*. In addition, severe injury was seen on infected *Setaria viridis* and *Abutilon theophrasti*. Symptoms appear in some species within 3 to 4 days and populations of *A. artemisiifolia*, *C. canadensis*, *L. serriola*, and *X. strumarium* were virtually eliminated, while populations of *C. arvense* were significantly reduced compared to controls. Tissue formed before the bacterium was applied, such as mature leaves, was not affected. However, once apical chlorosis was induced, seed production appeared to be inhibited in the case of *C. arvense* (Johnson et al., 1996).

The PST strains currently in use come from Minnesota or bordering states. Strains of this bacterium from other regions have not been compared for their ability to induce disease. However, white chlorosis is a symptom commonly observed in Canada thistle and a few other native composite plants across the United States. Although it has not been definitely determined, because of the symptoms and the plant family affected, it is generally assumed that the causal agent is PST. There are several compelling reasons that the correct identification of this chlorosis-inducing organism be determined. First, demonstrating that PST is already endemic to the areas where the biological control agent is intended for release would facilitate getting permits to release the bacterium as a biological control agent. Second, current studies with PST have been limited to one or a few strains from Minnesota and bordering states, while white

chlorosis in Canada thistle has been reported from as far east as Maryland. The virulence of strains from other areas should be compared to insure that the best possible candidate is used when developing the final product. Lastly, genetic differences between the current test strains and endemic strains of PST should be documented so that studies on the stability and spread of the biological control agent are not confounded by observations of the endemic strains of PST.

PST is a weak pathogen of many Asteraceae plants, such as marigold. If inadvertently applied to nontarget plants, those plants could become diseased. Therefore, host range of this bacterium may be tested in greenhouses in different states, according to local needs. Test plants will be screened at various developmental stages. Preliminary greenhouse screening of PST pathogenicity on several aster weeds of turf and nursery crops is already underway. Species tested and found to be susceptible are: *T. officinale*, *Artemisia vulgaris* (mugwort), *Bellis perennis* (English daisy), *Chrysanthemum leucanthemum* (oxeye daisy), *Achillia millefolium* (yarrow), *Ambrosia artemisiifolia* (common ragweed), *A. trifida* (giant ragweed), *Cirsium vulgare* (bull thistle), *Conyza Canadensis* (horseweed), *Galinsoga ciliata* (galinsoga), *Hieracium* spp. (hawkweed), *Senecio vulgaris* (common groundsel), *Solidago* spp. (goldenrod), and *Youngia japonica* (Asiatic hawkbeard).

Field evaluations of this bacterial pathogen in Minnesota have indicated that it can effectively control Canada thistle in soybean and corn without causing crop damage. Although Canada thistle does not exist in much of the Southern Region, several other members of the Asteraceae family that cause problems in this region. Propagules of weeds of interest will be collected locally in cooperating states. Tests will be done in crop and noncrop situations.

Roles and responsibilities: the following stations will participate in this objective: USDA-ARS-SWSRU, MS; Agric & Agri-Food Canada, SK; FL SAES; MA SAES; IN SAES; USDA-ARS-SASL, MD; NC SAES; and MT SAES. Individuals from these stations (See Appendix-Projected Participation) will follow the common protocols described below.

Under this sub-objective, it is proposed to develop and register this bacterium as a bioherbicide for several weeds in the Asteraceae and a few other families. The bacterium will be provided for multistate trials. Weeds in turf, roadside, right-of-way, and natural areas will be targeted, depending on the cooperating states. The exotic invasive weed, Asiatic hawkbeard (*Youngia japonica*, Asteraceae) as well as *Taraxacum officinale* (dandelion, Asteraceae), *Gnaphalium purpureum* (purple cudweed, Asteraceae), *Asperugo procumbens* (catchweed, Boraginaceae), *Galium* spp. (bedstraw, Rubiaceae), *Stellaria media* (common chickweed, Caryophyllaceae), *Ambrosia artemisiifolia* (common ragweed, Asteraceae), and *Bidens* spp. (beggartick, Asteraceae) will also be targeted.

The causal agent of chlorosis in Composite weeds and other native plants in the various states where this type of symptom has been observed will be isolated, identified, and tested. Sites where chlorotic Composite weed and native species are present will be recorded and plant samples collected. Using standard laboratory procedures, bacteria will be isolated from the plant material showing lesions and/or chlorosis. In some cases, such as with Canada thistle, isolation

of the organism may be complicated because tagetitoxin is readily transported within plants to meristematic tissues and Canada thistle plants are often interconnected through a lateral root system. Consequently, the infection may be localized in one plant while chlorosis develops in other connected plants. In such situations, it may be necessary to isolate and observe the plants before attempting to isolate the bacterium. Isolates will be bioassayed for toxin production using sunflower seedlings and seedlings of the plant species from which the organism was isolated. Using current genomic techniques, such as amplified restriction length polymorphism (AFLP), isolates will be genetically characterized and compared with other known strains of PST. For those instances where a chlorosis-inducing organism is not isolated from the plants expressing the PST-like disease, a simple technique for determining the presence of tagetitoxin in white-chlorotic tissue will be developed to determine if PST is the likely causal agent.

Objective 2: To develop and evaluate formulations to improve performance and standardization of selected bioherbicides.

The second objective will focus on the development and evaluation of suitable formulations for the delivery of the microbial weed-management agents selected for multistate trials. Successful application of microbial weed-management agents requires the development of suitable formulations. Considerable research has been done with various weed-pathogen systems to develop suitable formulations, adjuvants, and carriers. Research on this aspect is essential to move the microbial agent forward to the marketplace. Since each weed-pathogen system is influenced by specific biotic and epidemiological interactions, formulation research, by necessity, requires empirical approaches to the development of methods and materials. Few laboratories in the nation have the expertise to develop microbial formulations, but the USDA-ARS laboratory in New Orleans, LA is one of the premier institutions in this field, and we have enlisted two leading experts from this laboratory (Drs. Connick and Daigle) to participate in this project.

Proper formulations, adjuvants, and carriers should be developed to ensure effectiveness and feasibility of the bioherbicides selected for this project. Formulations enable spreading of very small amounts of active ingredients (e.g., microbial propagules) over a wide area, prolong the shelf-life, improve efficacy, and facilitate transportation, all without adversely affecting the effectiveness of the agent. In some cases, an adjuvant or a carrier may serve as the formulation. This is usually the case with dry pellet or powder formulations. In addition to the basic formulation, materials that act as surfactants may be added to the formulated product to further enhance the activity or usability of the product. Formulation of biological agents is complicated by the need for the organism to remain alive and virulent through processing. Therefore, usually the emphasis is on improving the agent's longevity and efficacy. In addition, formulation of a microbial agent may enable combinations of bioherbicides as well as bioherbicide agents and chemical pesticides to be used simultaneously without affecting performance.

The most commonly used additive in bioherbicide formulations is a surfactant, such as Silwet L-77, an organosilicone compound that reduces the surface tension and enables small spores, cells, and mycelia to enter natural openings in the foliage (Zidack and Backman, 1996).

Materials capable of forming aqueous gels when added to spore suspensions have also been explored. Such materials help to stick the spores to the leaf surface, while providing a hydrophilic matrix in which the spores may germinate more readily in the absence of an adequate dew period (Shabana et al., 1997). In the case of the bioherbicide Collego (*Colletotrichum gloeosporioides* f.sp. *aeschynomene*; Encore Technologies), a dried spore preparation is resuspended in water and a prepackaged amount of sugar, as an osmoticum and nutrient source, is added to allow for the gradual rehydration and germination of spores.

Formulations that encapsulate the microbial propagules in starch granules and other materials have been developed and tested. Encapsulation allows the biocontrol agent to be delivered in a conveniently applied form; however, adherence to the leaf surface requires that the surface be prewetted, which is not realistic for large-scale commercial use (McGuire and Shasha, 1992). Two additional formulations that have become increasingly popular for microbiological control agents of weeds and for diseases are alginate pellets and one termed Pesta[®]. These solid formulations can be easily adapted to a number of different fungi (Daigle and Cotty, 1992; DeLucca et al., 1990). Organisms that have been formulated in this manner have excellent shelf life compared to unformulated organisms, and the formulation itself can actually act as a spore-production medium.

Invert emulsions have shown considerable promise as carriers for bioherbicides (Womack et al., 1996; Yang and Schaad, 1996). The knowledge gained from invert-emulsion formulation has led to the extensive use of vegetable oils, with the oils apparently causing no alteration to the cuticular layer of the leaf surfaces (Egley and Boyette, 1995). Work with *Alternaria* spp. has indicated that invert emulsions can cancel the need for prolonged free moisture on plant foliage (Amsellam et al., 1990). Control of hemp sesbania was dramatically improved by the use of invert emulsion in the absence of dew, again indicating that high levels of weed control may be achieved by providing a prolonged period of water availability (Boyette, 1994). Recently, the use of sunscreens has been shown to be useful in protecting spores that are not melanized (Prasad, 1993). For instance, inoculum of *Chondrostereum purpureum* formulated with an ultraviolet protectant was able to withstand a wider range of environmental conditions than unprotected inoculum.

Thus, the aim of this objective is to discover and develop appropriate solid and liquid materials for the delivery of bioherbicide agents chosen for this project. Materials and methods will be evaluated cooperatively to develop appropriate formulations for pathogens undergoing testing under this multistate project. Suitable solid matrices and liquid formulations will be tested and developed for stabilizing bacterial and fungal weed pathogens during processing, storage, and application.

Roles and responsibilities: the following stations will participate in this objective: USDA-ARS-SWSRU, MS; Sylvan, FL SAES; USDA-ARS-SRRC, LA; IN SAES; USDA-ARS-EBCL, France; USDA-ARS-USHRL, FL; and Macdonald College, PQ. Individuals from these stations (See Appendix-Projected Participation) will follow the common protocols described below.

Various granular matrices consisting of cereal grain flours, clays, and adjuvants will be evaluated. Inoculum of candidate pathogens will be prepared by liquid and solid-state fermentation. The inoculum will be incorporated into formulations tailored to the particular requirements of each pathogen. Water-dispersible granules for spray application (for aerial pathogens) and granules that remain intact in the presence of water (soilborne pathogens) will be developed and tested. Commercial granulation equipment such as twin-screw and low-pressure screen extruders will be used, as needed. Studies to optimize drying, levels of water activity for necessary for propagule survival, and stability at various temperatures will be done. Several liquid formulations will also be tested and developed. Ionic and nonionic surfactants, with special emphasis on organosilicones, oils, emulsions, and gels will be evaluated.

Objective 3: To evaluate bioherbicides in multistate field trials in different crops and as alternatives to methyl bromide:

The third objective will involve multistate trials to field test experimental and pre-commercial bioherbicide formulations in various crops (e.g., vegetables, strawberry, floriculture, turf, rangelands, etc.) and as methyl bromide alternatives.

Methyl bromide is a low molecular weight, volatile, organic compound that is highly toxic to most living organisms. One of the principal uses of this compound is in agriculture as a fumigant to disinfest soil prior to the planting of high value crops (EPA, 1997). Methyl bromide is a Class 1 ozone depleting substance (ozone depletion potential > 0.2). Recent legislative actions taken by the U.S. Congress in October 1998 have made specific changes to the Clean Air Act, essentially phasing out the pest-control use of methyl bromide, in accordance to the Montreal Protocol phase-out schedule for developed countries. The final methyl bromide phase-out date in the United States is now set as 2005. In the United States, producers of fresh-market vegetables and fruits, including tomato, pepper, and strawberry utilize production systems that are highly dependent upon methyl bromide for soil disinfestation. Soilborne pathogens and weeds are the primary pests controlled with the use of methyl bromide and several regions in the United States depend on methyl bromide for cost-effective production of several crops. Florida alone accounts for 25% of the total methyl bromide consumption in the United States (EPA, 1997). Without methyl bromide, production of these crops is projected to decline by 61.4% to 63.5%, resulting in an annual loss of \$ 388 million to Florida growers alone (Spren et al., 1995).

Generally, very few registered herbicides are available for use in minor crops. While some new chemistries are being introduced for minor-crop use, the sensitivity of certain crops to these chemicals is a constraint. Therefore, growers are urgently seeking effective replacements that will have no or minimal nontarget effects and can be used in an integrated pest-management approach.

Roles and responsibilities: the following stations will participate in this objective: Sylvan; FL SAES; CA SAES, Riverside; and USDA-ARS-USHRL, FL. Individuals from these stations (See Appendix-Projected Participation) will follow the common protocols described below.

The bioherbicide agents identified under Objective 1 and developed according to Objective 2 will be evaluated in different cooperating states as possible alternatives to methyl bromide. The choice of crops, cropping systems, weed targets, and bioherbicide candidates will be made according to the local needs of participating states and institutions.

Objective 4: To safely enhance the virulence of bioherbicides by selection of variants of the plant pathogen that overproduce a target amino acid.

Molecular and physiological mechanisms underlying host-pathogen interactions are complex and each pathosystem needs to be studied in detail to identify genetic and physiological mechanisms that could be used to improve pathogens' virulence, environmental fitness, host specificity / host range, and other desired attributes. A few studies have been done to improve the efficacy of certain bioherbicide agents, including *Sclerotinia sclerotiorum*, *Colletotrichum gloeosporioides* f.sp. *aeschynomene*, and *C. gloeosporioides* f.sp. *malvae* (Sands et al., 1990; and reviewed in TeBeest et al., 1992; Goodwin, 2001). Some ideas, highly speculative at this time, have been proposed (Gressel, 2001) that aim to improve fungal bioherbicides by specific transgenic means. Goodwin and coworkers (reviewed in Goodwin, 2001) are examining host and pathogen genes expressed during infection of round-leaved mallow (*Malva pusilla*) by *C. gloeosporioides* f.sp. *malvae*. Pathogen genes coding for pectinase, arylsulfatase, and catalase (involved pathogenesis), and protein kinase (involved in the regulation of fungal metabolism), as well as plant genes encoding actin (involved in cellular processes such as cell division and cytoplasmic streaming) and glutathione *S*-transferase (induced in the host plant during the pathogen's transition from the biotrophic to necrotrophic phases) are being studied (Goodwin, 2001). While these studies represent a hopeful start, methods based on conventional strain improvement techniques, which can be used without costly regulatory delays, should also be examined.

Researchers at Montana State University have developed a promising strain-improvement technology to greatly enhance the virulence and efficacy of bioherbicides by selecting variants of pathogens that overproduced and excreted amino acids that were inhibitory to the respective target weed (Tiourebaev, 1999; Sands et al., 1999). This approach was modeled after "Frenching disease," a naturally occurring disease of tobacco (Steinberg, 1946). Steinberg et al. (1950) discovered that saprophytic bacteria growing on the roots of symptomatic plants were overproducing a single amino acid, isoleucine. Isoleucine is synthesized in plants via the branched chain amino acid pathway. The end-products of the pathway (valine, leucine and isoleucine) allosterically regulate the activity of acetolactate synthase (ALS). In "Frenching disease," overproduction of isoleucine in the rhizosphere by the saprophytic bacteria inhibited the activity of ALS in tobacco, shutting down synthesis of valine and leucine which disrupted essential protein metabolism.

Interestingly enough, several modern chemical herbicides also inhibit single biosynthetic enzymes in plants, rendering treated plants incapable of producing a metabolite essential for plant growth. For example, acetolactate synthase (ALS), the key enzyme in the branched chain

amino acid biosynthesis, is inhibited by the sulfonylurea herbicides. The herbicide glyphosate inhibits 5-enolpyruvylshikimate 3-phosphate synthase (EPSP), the key enzyme in the shikimic acid pathway (Amrhein, 1986).

Given this knowledge, scientists exposed *Fusarium oxysporum* f. sp. *cannabis*, a host-specific pathogen of marijuana plants (*Cannabis sativa*) (Tiourebaev et al., in press) to the valine analogs, norvaline and penicillamine, and selected spontaneous mutants that overproduced the amino acid valine (Tiourebaev, 1999). Valine is a product of the branched chain amino acid pathway and by feedback regulates ALS. Host plants were inoculated with the valine-overproducing mutants in growth-chamber studies. The virulence and rate of mortality were greater than that caused by the wild type strain of the pathogen. The host range of the valine-producing strain was unaffected.

Amino acid overproduction also enhances the virulence of *Fusarium oxysporum* f. sp. *papaveris* for control of *Papaver* sp. and *Pseudomonas syringae* pv. *tagetis* for control of houndstongue (*C. officinale*). Thus, this method is a novel, yet simple method, for increasing virulence of plant pathogens for weed biocontrol. The method can be easily adapted to any host-specific weed pathogen. Since this technique does not require recombinant genetics and is based simply on overproduction of amino acids, biosafety considerations are significantly reduced. It simply needs to be applied to obtain efficacious bioherbicides.

Roles and responsibilities: the following stations will participate in this objective: FL SAES; IN SAES; MT SAES; and USDA-ARS-USHRL, FL. Individuals from these stations (See Appendix-Projected Participation) will follow the common protocols described below.

Under this objective, amino acids or combinations of amino acids that are most inhibitory to the growth and development of the target weeds listed under Objective 1 will be determined. Spontaneous variants of plant pathogenic fungi or bacteria that are resistant to amino acid analogs and screen for overproduction of the selected amino acid(s) will be identified and selected. The virulence and host range of the amino acid overproducing bioherbicides will be confirmed in growth-chamber studies. Finally, the amino acid overproducing bioherbicide agents will be tested in multistate field trials.

Feasibility of the Proposed Work:

The technical feasibility of the bioherbicide approach to weed control has been well established, as evidenced by the continued use of two registered bioherbicide products, Collego and DeVine, since the early 1980s, albeit intermittently and on a relatively small scale. A wealth of scientific and technical information is available (e.g., Charudattan and Walker, 1982; TeBeest, 1991; and dozens of review articles and book chapters) that can guide and enable the proposed project. Expertise in plant pathology, weed science, microbial fermentation, formulation, and delivery systems, which are needed for this project, will be provided by the participants named. Participation by commercial industries will be crucial to the success of the project, and the recent incentives for the development of reduced risk pesticides (under the FQPA) have prompted

companies to reinvest the bioherbicide technology. Some small biotech companies and longstanding agrochemical companies have agreed to (or will be invited to) participate in this proposed multistate project.

The project is realistic and not ambitious. Since the bioherbicide prospects of the agents proposed herein for development have been already established, the project can focus fully on the technology development aspects.

Measurement of Progress and Results:

Outputs: One to five new, registered bioherbicides are expected to result from this multistate project. Even if the output results in one new registration, it will be a good achievement and the project will have a notable impact on the available weed control options. The project will target several highly important and difficult-to-control weeds such as purple nutsedge, pigweeds, grasses, kudzu, composite weeds, and others. Development and registration of commercial bioherbicides for these weeds will fill an important market niche for weed control products. This work will also provide for new biologically based, reduced risk pesticides.

Projected Impact: Development and registration of bioherbicides for economically important weeds, such as nutsedges, pigweeds, grasses, purslanes, and others named in this project will benefit growers in the Southern Regions and beyond. The scientific output from this study will stimulate further research investment in nonchemical weed-control alternatives. Reduction in the use of chemicals and development of an alternative weed control technology are two additional direct benefits that are likely for this project. The outcomes from this project will have a direct bearing on some of the mandates of FQPA, such as development of reduced-risk pesticides and implementation of IPM.

Milestones: Most of the background information and materials necessary to begin this project are already in place. The project is expected to meet its stated objectives by the year 2006. Tentative milestones are as follows: Years 1-3: Development of common protocols and completion of multistate field trials of the agents selected. Years 1-3: Development of formulations and delivery systems to facilitate field trials and to establish prototype commercial formulations. Years 2-5: Development of efficacy and label data for possible registration of promising agents. Years 4-5: Registration of promising bioherbicide products. Given the need to develop methods and materials for each agent-weed system, these milestones are realistic and achievable.

Appendix - Projected Participation:

Project or Activity Designation and Number: _____

Project or Activity Title: Development of Plant Pathogens for Biological Control of Weeds

Administrative Advisor: Dr. Gregory J. Weidemann, Arkansas Agricultural Experiment Station, Fayetteville, AR

| Participant Name and E-mail address | Institution and Department | Research | | | | | | Extension | | Objectives | | | | | | | |
|--|---|------------|--------------|------|-----------|----|-----|-----------|---------|------------|----|----|----|----|---|---|---|
| | | CRIS Codes | | | Personnel | | | FTE | Program | 1A | 1B | 1C | 1D | 1E | 2 | 3 | 4 |
| | | RPA | SOI | FOS | SY | PY | TY | | | | | | | | | | |
| Abbas, H. habbas@ars.usda.gov | USDA-ARS, SWSRU, Stoneville, MS | 712 | 4010 4020 | 1000 | 0.1 | | | | | X | X | X | X | X | | | |
| Bailey, K.L. baileyk@EM.AGR.CA | Agric. & AgriFood Canada, Saskatoon, SK, Canada | 213 | 2300 | 1140 | 0.1 | | | | | | | | | | | | |
| Berner, D.K. dana.berner@det.amedd.army.mil | USDA-ARS, FDWSU, Ft. Detrick, MD | 216 | 4020 | 1160 | 0.1 | | 0.1 | | | | | | X | | | | |
| Boyetchko, S.M. boyetchkos@EM.AGR.CA | Agric. & AgriFood Canada, Saskatoon, SK, Canada | 213 | 2300 | 1140 | 0.1 | | | | | | | X | | X | | | |
| Boyette, C.D. dboyette@ars.usda.gov | USDA-ARS, SWSRU, Stoneville, MS | 215 | 4010 4020 | 1140 | 0.1 | | | | | X | X | X | X | X | X | | |

| | | | | | | | | | | | | | | | | | |
|--|---|-------------------|----------------------|------------------------------|-----|-----|-----|-----|--|---|---|---|---|---|---|---|---|
| Britton, K.O. kbritton01@fs.fed.us | USDA-Forest Service, Athens, GA | 123 213 | 0613 4020 | 1140 | 0.1 | | | | | | | | X | | | | |
| Caruso, F.L. fcarus@umext.umass.edu | Cranberry Exp. Sta., Univ. Mass., Wareham, MA | 215 | 1121 | 1102 1160 | 0.1 | 0.1 | | 0.1 | | | | | | X | | | |
| Cascino, J. jcascino@sylvaninc.com | Sylvan America, Inc., Cabot, PA | 511 | 4020 | 1102 | 0.1 | | | | | X | | | | | X | | |
| Charudattan, R. rc@gnv.ifas.ufl.edu | Univ. Florida, Gainesville, FL | 213 215 | 2300 4020 4099 | 1140 | 0.1 | | 0.2 | | | X | X | X | | | X | X | X |
| Collins, R. rcollins@asrr.arsusda.gov | USDA-ARS, ACSL, MD | 213 215 | 2300 4020 4099 | 1100 1102 1140 2000 | 0.1 | | | | | | | | | | X | | |
| Fennimore, S.A. safennimore@ucdavis.edu | University of California-Davis, Salinas, CA | 213 | 2300 | 1140 | | | | 0.1 | | | | | | | | X | |
| Gronwald, J.W. gronw001@maroon.tc.umn.edu | USDA-ARS, Univ. Minn., St. Paul, MN | 215 | 2300 | 1140 | 0.1 | | | | | | | | | X | | | |
| Hallett, S.D. hallett@btpny.purdue.edu | Purdue Univ., West Lafayette, IN | 205 213 212 | 2410 2420 | 1140 | 0.1 | | | | | X | X | X | X | X | X | | X |

| | | | | | | | | | | | | | | | | | | |
|--|---|------------|------|------|-----|--|--|-----|--|---|---|---|---|---|---|--|---|---|
| Kremer, R.J. kremerr@missouri.edu | USDA – ARS Univ. of Missouri, Columbia, MO | 213 | 2300 | 1140 | 0.1 | | | | | | | | | | X | | | |
| Lueth, P. plueth@prophyta.com | PROPHYTA GmbH Inselstrasse 12 23999 Malchow Germany | 511 | 4020 | 1102 | 0.1 | | | | | | X | | | | | | | |
| Lydon, J. lydonj@ba.ars.usda.gov | USDA-ARS, SASL, Beltsville, MD | 213 | 2300 | 1140 | 0.1 | | | | | | | | | | X | | | |
| McGiffen, M.E. milt@ucr.ac1.ucr.edu | University of California, Riverside, CA | 213 | 1499 | 1140 | .01 | | | | | X | | | | | | | X | |
| Neal, J.C. joe_neal@ncsu.edu | North Carolina State University, Raleigh, NC | 213 215 | 4099 | 1140 | | | | 0.1 | | X | X | | | X | | | | |
| Peng, G. pengg@em.agr.ca | Agric & AgriFood Canada, Saskatoon, SK, Canada | 213 | 2300 | 1140 | 0.1 | | | | | | | X | | | | | | |
| Pilgeram, A.L. uplap@montana.edu | Montana State University, Bozeman, MT | 215 | 2300 | 1102 | 0.2 | | | | | X | X | X | X | X | | | | X |

| | | | | | | | | | | | | | | | | | |
|--|---|------------|------------------------------|------|-----|--|--|-----|--|---|---|---|---|---|---|---|---|
| Quimby, Jr., P.C. quimby@cirad.fr | USDA-ARS, EBCL, Montpellier, France | 213 | 2300 | 1140 | 0.1 | | | | | | | | X | | X | | |
| Rathinasabapathi, B. brath@gnv.ifas.ufl.edu | Univ. Fla., Gainesville, FL | 215 | 2300 | 1020 | 0.1 | | | | | | | | | | X | | X |
| Rayachhetry, M.B. raya@gnv.ifas.ufl.edu | Univ. Fla., Ft. Lauderdale Res. & Educ. Center, FL | 213 | 2300 | 1140 | 0.1 | | | | | | | | X | | | | |
| Roskopf, E.N. eroskopf@saa.ars.usda.gov | USDA-ARS- USHRL, Ft. Pierce, FL | 213 215 | 2300 4099 1460 1461 | 1140 | | | | | | X | X | X | | | X | X | X |
| Sands, D.C. uplds@montana.edu | Montana State University, Bozeman, MT | 215 | 2300 | 1102 | 0.1 | | | | | X | X | X | X | X | | | X |
| Semidey, N. n_semidey@rumac.uprm.edu | University of Puerto Rico, Mayaguez, PR | 215 | 1410 1451 | 1140 | 0.1 | | | | | X | X | | | | | | |
| Shamoun, S. SSHAMOUN@PFC.Forestry.CA | Pacific Forestry Centre, Victoria, BC, Canada | 123 213 | 0612 4020 | 1140 | 0.1 | | | | | | | | X | | X | | |
| Stall, W.M. wms@gnv.ifas.ufl.edu | Univ. Florida, Gainesville, FL | 213 | 1469 | 1140 | | | | 0.1 | | X | X | | | | | X | |
| Valencia, E. elideval@uprm.edu | Univ. of Puerto Rico, Mayaguez, PR | 215 | 1410 1451 | 1140 | 0.1 | | | | | X | X | | | | | | |

| | | | | | | | | | | | | | | | | | | |
|--|---|-----|--------------|------|-----|-----|-----|--|--|---|---|--|---|---|---|---|--|---|
| Watson, A.K. alan.watson@mcgill.ca | Macdonald College, McGill Univ., Quebec, Canada | 215 | 2300 | 1140 | 0.1 | 0.1 | | | | | | | | | | X | | |
| Widmer, T.L. twidmer@ars-ebcl.org | USDA-ARS, EBCL, Montpellier, France | 213 | 2300 | 1140 | 0.1 | | | | | | | | X | | | X | | |
| Zidack, N.K. uplnz@montana.edu | Montana State University, Bozeman, MT | 215 | 2300 | 1160 | 0.1 | | 0.1 | | | | | | | X | X | | | X |
| Zimmermann, T. tzimmer@uvi.edu | Univ. Virgin Islands, St. Croix, USVI | 215 | 1410 1451 | 1140 | 0.1 | | | | | X | X | | | | | | | |
| Total SY, PY, TY, and FTE | | | | | | | | | | | | | | | | | | |

Extension and Outreach Plan:

Information derived from this project will be made available to scientific audiences and to stakeholders through refereed journal articles, meeting abstracts, conference proceedings, extension bulletins, popular articles, and field demonstrations. A web page will be developed and maintained by the Chairman of the Technical Committee. Patentable materials and technologies developed through this project will be submitted for patenting through the appropriate institutions of the lead scientists/inventors.

Successful bioherbicide agents (i.e.: registered) will be made available through cooperating state and federal agencies and scientists. News releases, field demonstrations, and user-education workshops will be used to publicize and distribute these agents. Extension personnel at State Cooperative Extension Services will be trained in the use of registered agents through educational and demonstration material.

The success of this project will be determined in terms of the number of successful multistate field demonstrations, registered agents, and users trained in this technology.

Organization and Governance:

The organization and conduct of this project will be in accordance with the rules and procedures set forth in the "Format for Multistate Research Proposals," September 26, 2000 (<http://msstate.edu/org/saaesd>). This project will use the standard form of governance. A technical committee composed of one voting member from each SAES, one voting member from each USDA-ARS site, and as many participating members as approved from each SAES, USDA-ARS, and other institutional units. The Technical Committee will be responsible for planning the research project, coordinating the research activities of the participants, developing appropriate research methods and procedures for cooperative field trials, reporting of results, and for reviewing of research progress through annual meetings. The Executive Committee will consist of a Project Chair, a Secretary, 3 Objective Chairs, an Annual Meeting Chair, and the Administrative Advisor. The Project Chair, the Secretary, and the Objective Chairs will serve five-year terms. The Annual Meeting Chair will serve a one-year term during which he/she will serve as the local host for the annual meeting of the Technical Committee.

Authorization:

Administrative Advisor Date

Chairman, Regional Multistate Research Committee Date

Administrator, CSREES

Date

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Appendix

Project Leaders:

Specialty:

| | | |
|---|---------------------|--------------------------------|
| California Division of Agriculture and Natural Resources (Calif. DANR) | S. Fennimore | Weed Science |
| | M. McGiffen | Weed Science |
| Florida Agricultural Experiment Station (FAES) | R. Charudattan* | Plant Pathology |
| | M.B. Rayachhetry | Plant Pathology |
| | B. Rathinasabapathi | Horticultural Sciences |
| | W. M. Stall | Horticultural Sciences |
| Indiana Agricultural Experiment Station (IAES) | S. G. Hallett | Plant Pathology |
| Massachusetts Agricultural Experiment Station (Mass. AES) | F. L. Caruso | Plant Pathology |
| Montana Agricultural Experiment Station (Mont. AES) | A.L. Pilgeram | Plant Pathology |
| | D.C. Sands | Plant Pathology |
| | N.K. Zidack | Plant Pathology |
| North Carolina Agricultural Experiment Station (NCAES) | J. C. Neal | Weed Science |
| Puerto Rico Agricultural Experiment Station (PRAES) | N. Semidey | Weed Science |
| Other Cooperators: | | |
| Agriculture and Agri Food Canada, Saskatoon, SK, Canada | K.L. Bailey | Plant Pathology |
| | S.M. Boyetchko | Microbiology |
| | G. Peng | Plant Pathology |
| Forestry Canada, Pacific Forestry Centre, Victoria, BC | S.F. Shamoun | Plant Pathology |
| Macdonald College, McGill University, Quebec, Canada | A.K. Watson | Plant Pathology & Weed Science |
| Sylvan America, Inc., Cabot, PA | J. Cascino | Plant Pathology |
| PROPHYTA GmbH, Malchow, Germany | P. Lueth | Plant Pathology |
| USDA-ARS European Biological Control Laboratory, Montpellier, France | P.C. Quimby, Jr. | Weed Science |
| | T.L. Widmer | Plant Pathology |
| USDA-ARS Foreign Disease and Weed Science Res. Unit, Ft. Detrick, MD | Dana Berner | Plant Pathology |
| USDA-ARS Horticultural Research Laboratory (USDA-ARS-USHRL), Ft. Pierce, FL | E.N. Roskopf* | Plant Pathol. /Microbiol. |
| USDA-ARS Southern Regional Research Center, New Orleans, LA | D.J. Daigle | Chemistry/Formulation |

| | | |
|--|--------------|---------------------------|
| USDA-ARS Southern Weed Science Research Unit, Stoneville, MS | H.K. Abbas | Microbiol./Biochem. |
| | C.D. Boyette | Plant Pathology |
| USDA-ARS University of Missouri, Columbia, MO | R.J. Kremer | Weed Science/Microbiology |
| USDA-ARS Weed Science Lab, Beltsville, MD | J. Lydon | Weed Science |
| USDA Forest Service, Athens, GA | K.O. Britton | Plant Pathology |

* Voting member of the state or unit.

Appendix

Critical Review and Publications:

The S-268 Cooperative Regional Research Project S-268 had four objectives which were mostly fulfilled during this project. The following is a critical review of accomplishments.

Objective 1: Evaluate selected pathogens as bioherbicides. Three pathogens were chosen for cooperative research and development: *Colletotrichum truncatum* (COLTRU project), *Alternaria destruens* (dodder project), and *Pseudomonas syringae* pv. *tagetis* (PST project). The potential for commercial development of all three pathogens as bioherbicides were established through cooperative research and testing of various inoculum preparations, formulations, and application methods. *Colletotrichum truncatum*, a pathogen of hemp sesbania (*Sesbania exaltata*), was shown to be an effective control for this weed in rice, soybean, and cotton. *Alternaria destruens*, a pathogen of *Cuscuta* spp. (dodders), was developed and tested as a control for dodder (*Cuscuta* spp.) in carrot, cranberry, safflower, and tomato. A data package was developed and submitted to the EPA for possible registration of this bioherbicide, under the trade name SMOLDER, in 2001. *Pseudomonas syringae* pv. *tagetis* (PST), a bacterial pathogen, when applied in an aqueous suspension containing an organosilicone surfactant such as Silwet L-77, causes severe apical chlorosis in its natural hosts, such as Canada thistle, common ragweed, giant ragweed, Jerusalem artichoke, and wild and volunteer sunflower. It also causes similar effects in several weeds that are not among its natural hosts, such as cocklebur, horseweed, and prickly lettuce. Results from the field trials conducted in several locations in the USA and Canada were mixed: some target weeds were controlled satisfactorily by PST in some locations and in certain crops but not in others. Therefore, further research will be needed to develop PST as a commercial bioherbicide.

Objective 2: Enhance efficacy of bioherbicide candidates. Under this objective, the effects of various oils, emulsions, surfactants, and solid substrates were tested to improve the efficacy, handling properties, and survival of *C. truncatum*, *A. destruens*, PST, and other pathogens. These materials were also evaluated as a means to overcome environmental constraints to infection and disease development. Effect of cultural conditions on the efficacy and survival of these pathogens was also evaluated in several studies. The potential for improving the bioherbicidal efficacy of some pathogens through enhanced toxin production was also studied. In general, plant oils and some oil emulsions improved the efficacy of both mucoid (e.g., *Colletotrichum*) and dry-spored (e.g., *Drechslera*) fungi with concomitant decrease in the length of the dew period required for infection. The surfactant Silwet L-77, in general, was found to improve the efficacy of the bacterial pathogen PST and certain small-spored fungi like *Myrothecium*. Emulsions were effective in enhancing the efficacy of small- (*Myrothecium*) and large-spored fungi (*Drechslera*, *Exserohilum*). Finally, the feasibility of controlling two or more weeds while also improving the level of weed control was demonstrated by using a mixture of two or three bioherbicidal pathogens (e.g., *Bipolaris sacchari*, *Drechslera gigantea*, *Exserohilum longirostratum*, *E. rostratum*).

Objective 3: Develop systems for mass-production of stable bioherbicide formulations. Several granular formulations of the abovementioned fungal pathogens were developed and tested. These included starch encapsulation, Pesta, peat prills, vermiculite, sodium alginate, dilute gelatin, and methyl cellulose, natural substrates such as crop residues, and seeds/grains. Water-

dispersible granule formulations containing a variety of mycoherbicide agents were also developed and tested. Survival of the pathogen propagules in the field and shelf-life of the formulations were evaluated. A technique for mass production and multiple-harvesting of bioherbicide fungi by a biphasic culturing system was developed and shown to be a feasible method for mass-production of two bioherbicide agents, *Drechslera gigantea* and *Exserohilum rostratum*.

Objective 4: Develop genetic characterization and transformation of bioherbicide candidates as a means of enhancing efficacy and assessing environmental risk. Molecular probes were developed to identify and characterize various isolates and species of pathogens studied in this project. The RAPD technique was used to assess the relatedness of several *Colletotrichum* species. Using molecular markers and cultural mating studies, seven *Colletotrichum* isolates from distantly related plant hosts (crops and weeds) were found to mate with each other in culture under controlled laboratory conditions and in the field. Progeny from crosses between isolates of different host origins showed normal Mendelian segregation. In other experiments, heterothallic isolates from the southern United States were unable to mate with strains from New Zealand indicating that geographically isolated inter-sterility groups exist in *C. gloeosporioides*. In field trials of inter-strain competition, a benomyl-resistant *C. gloeosporioides* strain was competitive with the wild-type isolate when coinoculated with the wild-type on northern jointvetch (*Aeschynomene virginica*). A nitrate non-utilizing mutant was not competitive in similar tests. When all three strains were inoculated in three-way competition tests, mutant strains were not competitive with the wild-type strain, decreasing to less than 5% of the total population of the fungus within 60 days. Collectively, these results indicate not only the possibility for gene exchanges between some bioherbicide pathogens and related pathogens in nature but also the existence of some inherent safety mechanisms controlled by fitness traits.

Molecular techniques were also used to understand the phylogenetic relationship among isolates of *Cercospora* species pathogenic to waterhyacinth. Isolates collected from several geographic regions of the world were compared by using partial DNA sequences from three protein-coding genes: elongation factor-1 α , β -tubulin, and histone H3. The isolates studied were grouped into a major group (clade-grouping) composed of isolates from Brazil, Venezuela, Mexico, Florida (United States), South Africa, and Zambia and a minor clade-grouping of isolates from Texas (United States). Conventional taxonomic characteristics such as the shape and dimensions of conidia were unreliable criteria for taxonomic/phylogenetic differentiation of isolates that composed the two clade-groupings. Therefore, it was concluded that the separation of the *Cercospora* species pathogenic to waterhyacinth solely by morphological and virulence characteristics was invalid. The description of the species *C. piaropi* was emended to include *C. rodmanii* as a synonym.

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