Title: Buffelgrass Biocontrol in the Sonoran Desert with Foliar Fungal Pathogens and Their Phytotoxic Metabolites

Principal Investigator: Susan E. Meyer, Research Ecologist, USFS RMRS Shrub Sciences Laboratory, 735 North 500 East, Provo, Utah 84606; 801-356-5122; smeyer@fs.fed.us.

BCIP Contact: Allen White, FS Region 3 Invasive Plants Program Manager

Cooperators:
Travis Bean, Research Specialist, University of Arizona
Bradley Geary, Department of Plant and Wildlife Sciences, Brigham Young University
Antonio Evidente, University of Naples

Amount Requested and Project Leveraging:
Total Amount Requested: $80,000 for two years (Year 1: $50,000, Year 2: $30,000)
Project Leveraging: federal match $43,800; nonfederal match $65,000 (see budget for details).

Project Goals and Supporting Objectives:

Buffelgrass (Pennisetum ciliare or Cenchrus ciliaris), a native of south and east Africa and southern Asia, is an important pasture grass in many semi-arid regions of the world (Bogdan 1977, t'Mannetje and Kersten 1992). It has become highly invasive in some parts of its introduced range, particularly in the Sonoran Desert of southern Arizona (Bowers et al. 2006, Burgess 1991, Rutman and Dickson 2002, Stevens and Falk 2009), where it has infested thousands of acres of public and private lands, including Saguaro National Park and the Coronado and Tonto National Forests. At present the only weapons available to deal with buffelgrass invasion into natural ecosystems are herbicides and physical removal with hand tools. Biocontrol has become an effective weapon to combat many weeds that invade natural systems. The biocontrol organisms used are most often insects, but fungal biocontrols have also been effective in some cases, for example, Puccinea chondrillina for biocontrol of Chondrilla juncea in Australia (Burdon et al. 1981). In a preliminary literature search for possible buffelgrass biocontrol agents, we learned that the foliar fungal pathogen Magnaporthe grisea (anamorph Pyricularia grisea) has been credited with causing major epidemics in buffelgrass pastures in southern Texas and adjacent northern Mexico (Rodriguez et al. 1999, Diaz-Franco et al. 2007). This suggested that it could potentially be used as a biocontrol in areas where buffelgrass is invasive.

Project Goal—We were provisionally funded through the BCIP program in FY2012 to initiate an investigation of the feasibility of using Magnaporthe grisea as a possible biocontrol for buffelgrass (first year funding only). In this follow-up proposal, we develop this line of investigation further by including an additional pathogen species (Cochliobolus australiensis, anamorph Bipolaris australiensis), and by adding a formal integrated pest management (IPM) component. Our goal for the current proposal is to determine whether foliar plant pathogens and/or their phytotoxic metabolites can be used as part of a successful IPM strategy in conjunction
with chemical herbicides for control of buffelgrass. We hypothesize that a host-specific pathogen and/or its phytotoxic metabolite could be used to weaken buffelgrass plants, so that they can be killed with lower application rates of the broad-spectrum herbicides currently used for control. This could ameliorate the problem of herbicide damage to non-target species in the natural desert communities where buffelgrass is invasive, and might also increase buffelgrass mortality by operating through a complementary physiological mechanism.

**Progress to Date**—We have made considerable progress in the six months since we received provisional BCIP funding. We have completed a survey of pathogens on buffelgrass in southern Arizona, and have identified the primary foliar fungal pathogen there as *Cochliobolus australiensis*. This organism is not currently reported to occur in the United States; it almost certainly arrived in infested buffelgrass seed from East Africa. This pathogen was found at endemic levels in all sampled Arizona populations in summer 2012, which was a dry summer with only sporadic monsoon activity. We have established a population of buffelgrass in the greenhouse in Provo for experimental purposes, and are in the process of establishing similar experimental populations of the non-target native grass hosts Arizona cottontop (*Digitaria californica*) and tanglehead (*Heteropogon contortus*), two important species with growth phenology that overlaps that of buffelgrass. We have demonstrated that *C. australiensis* is the causal agent of the observed leaf spot disease on buffelgrass. We have produced this organism in liquid culture and demonstrated that the culture filtrate contains one or more phytotoxins that can cause leaf lesions similar to those caused by the disease. We have extracted secondary metabolites from lyophilized filtrate samples; these are now being chemically characterized.

We have not yet detected *Magnaporthe grisea* in southern Arizona. Once we realized that it was apparently not present there, we initiated an effort to obtain samples of this pathogen from buffelgrass in Texas. We are on track to obtain these samples from our Texas collaborators in the next few weeks. The monsoons were sporadic and late in Texas this year as well, and buffelgrass green-up has been correspondingly late, delaying the appearance of the disease.

**Supporting Objectives**—For *C. australiensis* strains from southern Arizona, we will: (1) determine the environmental conditions necessary for successful germination, infection, and conidial production, (2) determine potential disease effects on non-target hosts, including co-occurring native grasses, in greenhouse trials, and (3) develop technology to produce conidial inoculum in sufficient quantity for common garden and field trials. For both *C. australiensis* and *M. grisea*, we will: (4) characterize the phytotoxins produced in liquid culture and examine their ability to cause disease-like damage on buffelgrass host plants as well as on co-occurring native grasses, and (5) develop technology to produce phytotoxic filtrates in sufficient quantity for common garden and field trials. We will then: (6) conduct common garden experiments with buffelgrass and two co-occurring native grasses to determine the effects of *Cochliobolus* inoculum, *Cochliobolus* phytotoxins, and *Magnaporthe* phytotoxins when applied singly and in combination at different application rates. Lastly, we will: (7) take the most promising treatments from these common garden trials, apply them alone and in combination with selected chemical herbicide treatments to buffelgrass plants and non-target native grass host plants in the field, and measure their impact on plant survival and vigor.
We have opted for now not to pursue the possibility of using *M. grisea* directly as a biocontrol agent for buffelgrass in Arizona. It apparently does not occur there naturally, and its use would require a problematic new introduction. In addition, because of its impacts on buffelgrass pastures in other parts of the introduced range, the notion of using it for biocontrol is politically sensitive. *Cochliobolus australiensis*, on the other hand, occurs naturally on buffelgrass in Arizona already and would be used in an augmentative biocontrol strategy rather than as a new introduction. It is currently not known to occur in buffelgrass forage pastures.

**Literature Review**—*Cochliobolus australiensis* has a fairly wide host range, primarily among warm season grasses (Manamgoda et al. 2011). It is not known whether it forms host-specific races, but this is a common phenomenon in the genus. Foliar pathogens on warm season grasses generally require warm temperatures and a period of high humidity and accompanying leaf wetness for conidia to germinate and infect host leaves (Greer and Webster 2001). Successful infections result in lesions that then produce a new generation of conidia. Under extended favorable conditions, this polycyclic life cycle can result in exponential increases in numbers of infected plants and infection severity, the scenario for epidemic disease. Several consecutive nights with ambient relative humidities >75% are reported to be conducive to epidemic disease development of *M. grisea* on buffelgrass (Rodriguez et al. 1999, Diaz-Franco 2007). Under less favorable conditions the pathogen may be present at endemic levels and hardly noticeable. It survives the period of inactive plant growth as mycelium or conidia in or on senesced host leaves (Greer and Webster 2001). A similar life cycle probably describes *C. australiensis*, though the epidemiology of this species has not been specifically investigated. These pathogens rarely cause mortality in perennial hosts when acting alone, but the plants can be severely weakened and subject to increased mortality from compounding causes.

The pathogen *Magnaporthe grisea* is known to produce a wide array of phytotoxic compounds, including polyketides such as pyriculol, epipyriculol, dehydroxypyriculol, and pyriculol (Kim et al. 1998), pyricularin, tenuazonic acid, and cytochalasins (Talbot 2003). The cytochalasin pyrichalasin H is a host-specific phytotoxin produced only by pathotypes on *Digitaria*; concurrent application of this phytotoxin can render otherwise nonvirulent strains from other hosts pathogenic on *Digitaria* (Tsurushima et al. 2005). Application of some phytotoxins produced by the fungus, including pyriculol and tenuazonic acid, can cause typical brown leaf necrosis (Iwasaki et al. 1969, 1972, Tshurushima et al. 2009); these could potentially be used as biologically based herbicides (Strobel et al. 1991, Evidente 2006, Evidente and Abouzeid 2006, Evidente 2010).

Similarly, members of the genus *Cochliobolus* are known to produce a diverse suite of phytotoxic compounds, including bipolaroxin, sorokinianin, carboxotaxine, HC toxin, victorin, ophiobolin, and curvularin (Sugawara et al. 1985, Nakajima et al. 1994, Sivanesan 1987, Anthony et al. 2009). Some of these, for example, victorin from *C. victoriae*, causal agent of Victoria blight of oats, are highly host-specific (Wolpert et al. 1988). Phytotoxins produced by *Cochliobolus* and *Magnaporthe* species or pathotypes from buffelgrass have not been characterized, but novel metabolites with strong herbicidal properties could potentially be discovered, and these could possess some degree of host specificity that would reduce their impact on non-target hosts.
Project Justification/Urgency:

Invasion of natural ecosystems by fire-prone non-native grasses has been documented to result in wholesale ecosystem conversion from high diversity native woodland or shrubland to exotic grass monoculture dysclimax over large areas in a number of cases worldwide (Burquez-Montijo et al. 2002, Clarke et al. 2005, Daehler and Goergen 2005, Fairfax and Fensham 2000). The massive ecosystem conversions resulting from invasion of cheatgrass (*Bromus tectorum*) in the Great Basin and red brome (*B. rubens*) in the Mojave Desert indicate that this process can readily take place in North American deserts (Whisenant 1990, Brooks 1999). Buffelgrass poses this kind of threat to the Sonoran Desert, particularly to the iconic shrub-succulent plant communities such as saguaro woodland that support high biological diversity (Olsson et al. 2011). Buffelgrass is in a process of rapid range expansion in southern Arizona, and any realistic effort to stop its spread and protect high-value native ecosystems from destruction must happen quickly. There is tremendous public support for this effort in the Tucson area, as evidenced by the well-organized infrastructure in place for tackling this problem and the involvement of many hundreds of citizen volunteers in control projects (http://buffelgrass.org).

Project Approach:

**Objective 1**—To investigate environmental conditions necessary for infection and conidial production, five strains of *C. australiensis* from buffelgrass in Arizona will be chosen for conidial increase, and known densities of conidia will be spray-inoculated onto buffelgrass host seedlings (ten replicates per treatment per strain). Treatments will include a factorial combination of temperature (15, 20, 25, 30°C) and relative humidity period >80% in plastic tents (4, 8, 16 hours). After incubation, tents will be removed and plants will be placed in a low-humidity (30%) environment at 25°C. Disease development will be evaluated using established criteria (Valent and Chumley 1991).

**Objective 2**—Greenhouse inoculation trials to determine susceptibility of Arizona cottontop and tanglehead to *C. australiensis* from buffelgrass will follow the above protocol, using temperature and wetness period conditions found to be optimal for pathogen infection.

**Objective 3**—Submerged fermentation technology (Dokken 2007) will be used to produce conidial inoculum for common garden and field trials. This method has been used successfully in development of commercial mycoherbicides (Charudattan 1991). We already have the fermentation equipment needed to optimize and carry out inoculum production.

**Objective 4**—In order to determine the feasibility of using phytotoxins from *M. grisea* as herbicides on buffelgrass, we will follow the protocol we have already largely completed for *C. australiensis*. We will produce five pathogen strains in liquid culture (potato dextrose broth at 25°C), quantify the phytotoxicity of the culture filtrates using host leaf puncture bioassays, extract the secondary metabolites from each culture filtrate, purify the secondary metabolites present, and determine their structure using chemical and spectroscopic techniques (Evidente et al. 2002, 2005). We will then carry out bioassays with the purified compounds to determine phytotoxicity on buffelgrass, Arizona cottontop and tanglehead.
**Objective 5**—We will also use submerged fermentation technology followed by filtering to remove living mycelium to produce phytotoxic filtrates of each pathogen in sufficient quantity for common garden and field trials. As mentioned above, we already have the needed equipment.

**Objective 6**—Common garden experiments to determine the effects of *Cochliobolus* inoculum, *Cochliobolus* filtrates, and *Magnaporthe* filtrates on buffelgrass and two native grasses will utilize established plants in pots and will take place under summer conditions in Provo, Utah. The design will include each treatment singly and in all combinations for seven treatment combinations plus a control. These eight treatment combinations will be applied to ten individuals of each host species in a randomized block design, and impacts on host plants will be quantified.

**Objective 7**—Monitoring of *C. australiensis* disease levels in the field in southern Arizona as a function of phenological stage and weather scenario during the summer previous to experimental installation, as well as examination of long-term records of monsoonal weather patterns in the area, will provide a basis for selection of the timing of field treatment applications. We will take the best treatment that includes pathogen inoculum and the best treatment that does not include pathogen inoculum from the common garden trials and apply them alone and in combination with two selected chemical herbicide treatments to plants of buffelgrass and two native grasses in the field in southern Arizona in a completely randomized design. Each treatment combination will be applied to ten individuals of each species for a total of 150 experimental units (plants), including untreated controls of each species. Treatments will be applied at the time of peak monsoonal weather and after green-up, and impacts on host plants will be quantified. The chemical herbicide treatments will be chosen based on work by Bean (2012). If resources and time permit, we will expand this component of the study to include parallel treatment applications at different rates, at different phenological stages and under different weather scenarios to determine optimal application rates and timing. Treated plants will be monitored for six months following treatment application to determine longer-term effects.

**Expected Products and Outcomes**

At the end of two additional years of investigation of the potential for use of these fungal pathogens and their phytotoxic metabolites as components of an IPM program for buffelgrass control in the Sonoran Desert, we will have learned several important things about the feasibility of this approach. First, we will know whether the pathogens or their phytotoxic metabolites have sufficient negative impact on buffelgrass to be worth pursuing as biocontrols. We will know whether treatments involving the use of these fungal pathogens or their products can work synergistically with chemical herbicide treatments in an IPM approach to increase buffelgrass mortality. And we will know whether this new approach can reduce damage to non-target native grass hosts in areas treated to control buffelgrass. In a broader context, this project, if successful, could open the way for greatly increased use of fungal biocontrol organisms and biologically based herbicides in conjunction with existing chemical herbicides for a more environmentally benign and possibly more effective approach to invasive plant species control.
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<tr>
<th>Research Activity</th>
<th>Dates</th>
<th>PI’s</th>
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<tr>
<td>Obtain <em>M. grisea</em> isolates from buffelgrass leaf tissue and produce in pure culture. (Obj. 4)</td>
<td>By March 2013</td>
<td>Meyer, Geary</td>
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<td>Carry out <em>M. grisea</em> phytotoxin characterization and host plant leaf bioassays with crude filtrates and purified phytotoxins. (Obj. 4)</td>
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<td>Establish experimental greenhouse populations of native grass species Arizona cottontop and tanglehead (Obj. 1, 2, 4, 6)</td>
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<td>Carry out <em>C. australiensis</em> growth chamber/greenhouse inoculation trials - window of infection study. (Obj. 1)</td>
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<td>Carry out <em>C. australiensis</em> growth chamber/greenhouse inoculation trials - host range study. (Obj. 2)</td>
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<td>Scale up inoculum/filtrate production in submerged fermentation culture for use in common garden experiment (Obj. 3, 5)</td>
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<td>Produce host test plants and carry out common garden experiment (Obj. 6)</td>
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<td>Monitor <em>C. australiensis</em> disease levels in the field and examine long-term monsoonal weather pattern records (Obj. 7)</td>
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<td>Produce inoculum and filtrates for field IPM trials</td>
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<td>Carry out field IPM trials in southern Arizona, analyze results (Obj. 7)</td>
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<td>Prepare final report, submit manuscripts, and produce technology transfer materials</td>
<td>December 2014-March 2015</td>
<td>Bean, Meyer, Geary, Evidente</td>
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Literature Cited


Sivanesan A. 1987 Graminicolous species of *Bipolaris, Curvularia, Drechslera, Exserohilum* and their teleomorphs. Mycol 158:1–261


## Budget

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Budget Explanation:

The Rocky Mountain Research Station will be the lead institution. We are requesting $5,000 for in-house use in support of the inoculum and filtrate production work and the common garden study. Permanent RMRS microbiology technician Suzette Clement will carry out this work as part of the RMRS contribution. We are also requesting travel funds ($3,000) for coordination meetings with collaborators at cooperating institutions. Contributed RMRS indirect costs are also indicated at 8% of in-house funds and 3% of pass-through funds.

Brigham Young University is requesting $24,000 on a subcontract to support the laboratory culture work, including obtaining pure cultures of both pathogens, placing these in long-term storage to preserve genetic characteristics, producing mycelial material for genetic characterization, producing conidial inoculum for greenhouse trials, and producing and extracting culture filtrates for phytotoxin characterization and bioassay. Brigham Young University will also carry out the greenhouse inoculation trials examining host range and window of infection. The personnel budget includes a one-year assistantship to support a graduate student to carry out this work under the supervision of PI Brad Geary. Brigham Young University will contribute an additional year of support for this graduate student and will also provide a mentored learning undergraduate student to help with the technical work. The supplies budget includes funds for culture work and greenhouse inoculation trials, while the travel budget with university matching funds will fund professional meeting attendance for the PI and graduate student.

Funds supplied on subcontract to the University of Naples Federico II will be used to support a Master's level student for one additional year under the direction of PI Antonio Evidente, and to purchase supplies, pay for analyses needed for phytotoxin characterization, and cover the costs of shipping samples. The University of Naples is contributing 50% of net in waived overhead as well as providing $6,000 in supplies.

Funds supplied to the University of Arizona on subcontract will be used for 3 months of salary for PI Travis Bean each summer for two additional summers. Mr. Bean will continue to survey for pathogens, monitor disease levels, and examine patterns of monsoonal activity during summer 2013, and will be responsible for the IPM field trials during summer 2014. The University of Arizona is contributing 50% of net in waived overhead.
CURRICULUM VITAE: Susan E. Meyer, Research Ecologist

USFS Rocky Mountain Research Station Shrub Sciences Laboratory
735 North 500 East, Provo, UT 84606

Email: smeyer@fs.fed.us Phone: 801-356-5122 Fax: 801-375-6968

Education

B.S. University of Utah, 1969, Environmental Biology.
M.S. University of Nevada-Las Vegas, 1976, Biological Sciences.
Ph.D. Claremont Graduate School, 1980, Botany.

Professional Experience

1987-present. Research Ecologist, USDA Forest Service, Rocky Mountain Research Station, Shrub Sciences Laboratory, Provo, Utah.

1985-1987. Research Associate, Utah Division of Wildlife Resources, USDA Forest Service Shrub Sciences Laboratory, Provo, Utah.

1982-1985. Professor Investigador (Associate Research Professor), Centro Regional para Estudios de Zonas Aridas y Semiaridas, Colegio de Postgraduados, Salinas de Hgo., San Luis Potosí, Mexico.

Some Relevant Peer-reviewed Publications - Last Five Years


CURRICULUM VITAE: Travis Bean

Research Specialist, School of Natural Resources & Environment, University of Arizona. 1955 E. 6th St., Ste. 210
Tucson, AZ 85719
voice: 520 621.8589 fax: 520 621.3816

Education

• PhD, currently enrolled in Rangeland Ecology and Management program at University of Arizona (minor in Arid Lands Resource Sciences), expected graduation Spring 2013
• MS, Range Management, University of Arizona, 2002
• BS, Plant Science, University of Arizona, 2000

Work Experience

• 2004-present, Research Specialist, University of Arizona, Tucson, Arizona.
• 2000-2004, Research Assistant, Office of Arid Lands Studies, University of Arizona.
• 2003, Teaching Assistant, Native Plant Taxonomy Course, School of Natural Resources, University of Arizona.

Publications


Relevant Manuscripts in Preparation

• Bean, T.M., W.B. McCloskey, and G.M. Casady. Efficacy of imazapic and glyphosate for pre- and post-emergence control of buffelgrass.
• Casady, G.M., and T.M. Bean. Efficacy of monocot-selective herbicides for control on buffelgrass and effects on native vegetation.
CURRICULUM VITAE: ANTONIO EVIDENTE

University of Naples “Federico II” Department of Soil, Plant, Environmental and Animal Production Science, University of Naples “Federico II”, Department of Chemistry Science, Complesso Universitario Monte Sant’Angelo, Via Cinthia 4, 80126, Napoli. Email: evidente@unina.it

EDUCATION

1975. M.S. Institute of Organic and Biological Chemistry, University of Naples “Federico II”.

PROFESSIONAL EXPERIENCE

2000-present Full Professor of Organic Chemistry, University of Naples
1989-2000 Associate Professor of Organic Chemistry, University of Naples
1987-1989 Associate Professor of Organic Chemistry, University of Basilicata, Potenza.
1980-1987 Senior Research Associate, Organic Chemistry, University of Naples
1975-1980 Research Fellow at the Institute of Organic and Biological Chemistry

RESEARCH INTERESTS

Organic chemistry, biosynthesis, biochemistry, spectroscopy and synthesis of bioactive metabolites (phytotoxins, plant growth regulators, antibiotics, mycotoxins, fungicides, phytoalexins, elicitors, herbicides, proteins and polysaccharides) produced by phytopathogenic fungi, bacteria and plants. Structure and stereostructural determination of bioactive metabolites, structure-activity relationships and on their action mode.

REPRESENTATIVE PUBLICATIONS (68 publications last 5 years)


CURRICULUM VITAE: BRADLEY D. GEARY

Associate Professor of Plant Science, Pest Management Specialist, Department of Plant and Wildlife Sciences, 263 WIDB, Brigham Young University, Provo, UT 84602; (801)-422-2369; brad_geary@byu.edu

Educational History:
Ph.D., Plant Pathology, 1999, Washington State University, Pullman, WA
M.S., Plant Pathology, 1997, Washington State University, Pullman, WA
B.S., Agronomy, Crops and Soils, 1995, Brigham Young University, Provo, UT

Professional Positions:
2009 - present, Associate Professor, Plant Pest Management Specialist, Brigham Young University, Provo, UT
2003 – 2009, Assistant Professor, Plant Pest Management Specialist, Brigham Young University, Provo, UT
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1995 – 1999, Graduate Research Assistant, Washington State University, Pullman, WA
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