Title: Preliminary Evaluation of *Pyricularia grisea* as a Potential Biocontrol for Invasive Buffelgrass (*Pennisetum ciliare*) in the Sonoran Desert of Arizona

**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.

**Cooperators:**

Allen White, FS Region 3 Pesticide Specialist and Invasive Plants Program Manager
Travis Bean, Research Specialist, University of Arizona
Bradley Geary, Department of Plant and Wildlife Sciences, Brigham Young University
Antonio Evidente, Department of Plants, Soils, Environmental, and Animal Production Science, University of Naples

**Amount Requested and Project Leveraging:**

Total Amount Requested: $75,000 for two years (Year 1: $40,000, Year 2: $35,000)
Project Leveraging: federal match $54,580; nonfederal match $32,000 (see budget for details).

**Project Goals and Supporting Objectives:**

Buffelgrass (*Pennisetum ciliare*), 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).

We propose to examine the feasibility of using a fungal grass pathogen, *Pyricularia grisea* (teleomorph *Magnaporthe grisea*) or its phytotoxic metabolites for inundative biocontrol of invasive buffelgrass. This foliar pathogen already occurs on buffelgrass in its North American range, and has been reported to reach epidemic levels where buffelgrass is cultivated as a pasture grass in southern Texas (Rodriguez *et al.* 1999) and northern Mexico (Díaz-Franco *et al.* 2007), as well as in Queensland, Australia (Perrott and Chakraborty 1999). It causes a foliar (leaf spot) disease known as a blight or blast that can vary in severity from small lesions on a few plants to mortality of whole pastures. Our goal is to determine the potential for the safe and effective use of this pathogen for biocontrol as part of an integrated pest management plan for buffelgrass eradication in its invaded range.

The *Magnaporthe grisea/M. oryzae* species complex is a very well-studied group of plant pathogens, primarily because one subgroup of host-specific strains of *M. oryzae* represents
the causal agent of rice blast disease, which is a major cause of yield loss in an extremely important food crop worldwide. This pathogen has also become a model organism for the study of pathogenesis (Valent 1990, Howard and Valent 1996, Talbot 2003, Ebbole 2007). This means that a great deal of information is already available on the genomics, genetics, physiology, and epidemiology of this group, as well as many well-developed tools for answering questions about its biology. This will make it relatively easy to examine the biology of pathotypes on buffelgrass. On the other hand, proposed use of an apparently close relative of an economically important crop pathogen for weed biocontrol raises questions of safety which must be satisfactorily addressed before any field implementation of this organism for buffelgrass biocontrol.

**Our study will have five principal objectives:** 1) Determine the current distribution of *Pyricularia grisea* on buffelgrass and on other grass hosts in the invaded range in southern Arizona, 2) Determine the environmental conditions necessary for successful germination, infection, and conidial production for pathogen strains on buffelgrass, 3) Compare environmental conditions in areas where the disease reaches epidemic levels in cultivated buffelgrass pastures with conditions in the invaded range, using climate envelope modeling, 4) Characterize the phytotoxins produced by strains of *Pyricularia grisea* from buffelgrass and examine their ability to cause disease-like damage on buffelgrass host plants, and 5) Determine the phylogenetic relationships of the *P. grisea* strains on buffelgrass using molecular genetic tools and determine potential effects on non-target hosts, including co-occurring native grasses and important grass crops, in cross-inoculation trials.

**Epidemiology**—*Pyricularia grisea* requires 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, which are triggered into forcible release by darkness, increasing their chances of encountering new host leaves wet with dew. 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 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). The pathogen can sometimes infect inflorescences and can be dispersed with host seeds, but there is no evidence for systemic infection or vertical transmission from plants to seeds (Long et al. 2001).

**Host Range, Host Specialization, and Population Genetic Structure**—The *Magnaporthe grisea* species complex has a very wide host range among the grasses and has even been reported from a few other monocot families (Takan et al. 2011). At the strain or pathotype level, however, this group is characterized by a high degree of host specificity and molecular-genetic differentiation on different hosts (Zellerhoff et al. 2006, Faivre-Rampant et al. 2008). The two major clades in this complex are not interfertile, are strongly differentiated genetically, and have non-overlapping host ranges, and have thus recently been re-classified as distinct
species (Couch and Kohn 2002). The strains on buffelgrass have never been characterized, but strains on other Pennisetum species clearly belong to the clade now designated as *M. grisea* (Wilson and Hanna 1992), while strains on most economically important grass crops, including rice, wheat, barley, and finger millet (*Eleusine coracana*), belong to the *M. oryzae* clade (Couch and Kohn 2002). Interestingly, the strains on rice worldwide are more closely related to each other than to other members of the complex, suggesting that the host shift onto rice happened a single time, most likely early in the domestication process (Couch et al. 2005). This means that it is highly unlikely that strains from buffelgrass would ever be able to make such a host shift. Even species within a single genus or cultivars of a single crop often have host-specific pathotypes of this pathogen that are avirulent on plants of close congeners (Yoshida et al. 2009).

The population genetic structure of the pathogen on rice in North America and other places far from the center of rice origin is characterized by a strong mating type bias, low sexual fertility, and the predominance of a few clonally reproducing lineages. Populations in south China are more diverse and show more evidence of outcrossing. Populations of *M. oryzae* on finger millet in Africa, on the other hand, have high genetic diversity, high sexual reproduction, and high outcrossing rates, probably because finger millet originated in East Africa (Takan et al. 2011). Co-occurring pathotypes on the rice and finger millet in Africa are totally unrelated, even though they both represent *M. oryzae*. If pathotypes on buffelgrass in North America follow this pattern, we expect low relationship with strains on other crops, low diversity and a predominance of a few clonal lineages, as buffelgrass in North America is far from its center of origin. This pattern of low diversity is expected to be even more extreme because buffelgrass is much less genetically diverse in its introduced range than any cereal crop species. It appears likely that the entire population of buffelgrass in the invaded range in southern Arizona is made up of a single apomictically reproducing cultivar, known as Common, American, or T-4464 (Guttierez-Ozuna et al. 2009). This may make the task of genetically characterizing pathogen strains quite straightforward. On the other hand, *P. grisea* has been reported from several native warm season grass species in Tamaulipas, Mexico, where buffelgrass is grown extensively for forage and has suffered serious blight disease epidemics (Diaz-Franco et al. 2007). The relationship of strains on buffelgrass to strains on co-occurring native grasses requires further study, both phylogenetically and in terms of pathotype specialization.

**Phytotoxin Production**—The pathogen *Pyricularia 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 other 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 (Evidente 2006, Evidente and Abouzeid 2006, Evidente 2010). Phytotoxins produced by pathotypes from *Pennisetum* have not been characterized, but novel metabolites with herbicidal properties could
potentially be discovered. Such herbicidal metabolites could be useful in the event that the pathogen itself cannot be used safely or effectively on buffelgrass infestations in Arizona.

**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 conversion 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).

The possibility of using biocontrol, including *P. grisea*, as part of the IPM strategy has been suggested several times, but no one has taken on the preliminary work to determine if this approach is feasible (Rogstad 2008). The fact that relatives of *P. grisea* are important crop pathogens has put a damper on the idea of buffelgrass biocontrol with this pathogen, as well as the fact that buffelgrass itself is considered a crop in other parts of its introduced North American range. In fact, considerable effort has apparently been devoted to developing blight-resistant buffelgrass cultivars (e.g., http://www.pogueagri.com/Buffelgrass_Laredo_Brand.aspx). However, in view of the urgency of finding a more effective IPM strategy for buffelgrass control in Arizona, the biocontrol potential of *P. grisea* in this context deserves critical examination.

**Approach**

**Objective 1**--We will initiate our study by determining to what extent the *P. grisea* already occurs on buffelgrass and on co-occurring grasses in the invaded range in southern Arizona. We will carry out disease surveys in at least 20 discrete buffelgrass invasion areas during the period of active growth, using a transect method with collection of diseased leaf tissue for culturing and confirmation of pathogen identity (Greer and Webster 2001). We will include estimates of disease severity in each area. Pathogen strains obtained from the field will be used in subsequent studies. We may encounter additional pathogens in this process; these will be cultured and placed in long-term storage for potential future investigations.

**Objective 2**--Pathogen strains obtained in the field survey, as well as strains obtained from areas where the disease impacts pasture plantings, will be used to determine the window of infection required by the pathogen. Ten strains 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). Care will be taken to preserve virulence through the culture process.

Objective 3--We will use climate envelope modeling procedures (Rehfeldt et al. 2006) to characterize climate across the known range of *P. grisea* on buffelgrass in North America, and compare that climate with the climate in the invaded range in southern Arizona, to determine how likely it is that this pathogen could cause disease at levels effective for biocontrol in the invaded range, either in an inundative strategy where massive amounts of inoculum are applied when weather conditions reach a critical threshold (Yang and teBeest 1993), or in a classical strategy where the disease is introduced to an area and expected to persist naturally and provide a level of long-term control (Caltigarone 1981).

Objective 4--In order to determine the feasibility of using phytotoxins from this pathogen as herbicides on buffelgrass, we will produce ten pathogen strains in liquid culture (potato dextrose broth at 25°C), quantify the phytotoxicity of the culture filtrates using host leaf bioassays, extract the secondary metabolites from each culture filtrate, purify the secondary metabolites present, determine their structure using chemical and spectroscopic techniques, and carry out bioassays with the purified compounds to determine phytotoxicity on the target host (Evidente et al. 2002, 2005).

Objective 5--We will genetically characterize *P. grisea* strains obtained from buffelgrass by obtaining molecular fingerprints for several genes commonly used for identification and characterization of fungi, including *Magnaporthe*. These include the ITS region of ribosomal RNA, and the actin, beta-tubulin, and calmodulin genes (Couch et al. 2005). DNA will be extracted from each strain and amplified using PCR with primers specific to these genes, and the resulting amplification products will be sequenced. Sequences will be compared with sequences in GENE BANK using BLAST technology, and phylogenetic software (e.g., PHYLIP) will be used to determine relationships with known strains. Cross-inoculation trials to determine susceptibility of non-target hosts to strains from buffelgrass and susceptibility of buffelgrass to locally obtained strains from other hosts will follow a similar protocol to that described earlier for determining the infection window, using conditions optimal for pathogen infection.

**Expected Products and Outcomes**

At the end of this two-year project, we should be able to make an informed decision to either abandon the idea of buffelgrass biocontrol with *P. grisea* based on lack of potential for safe and effective use, or to tackle the next stage of biocontrol development, namely a pilot inoculum and/or phytotoxin production system and small-scale experimental field application.
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<tr>
<th>Research Activity</th>
<th>Dates</th>
<th>PI's Responsible</th>
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<tbody>
<tr>
<td>Obtain <em>P. grisea</em> isolates from buffelgrass leaf tissue and produce in pure culture.</td>
<td>March 2012 - September 2012</td>
<td>Bean, Meyer, Geary</td>
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<td>Obtain climate data from areas affected by <em>P. grisea</em> in cultivated buffelgrass pastures.</td>
<td>March 2012 - June 2012</td>
<td>Meyer</td>
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<td>Carry out blight disease surveys; obtain seeds of buffelgrass and potential nontarget hosts.</td>
<td>June 2012 - October 2012</td>
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<td>Complete climate envelope modeling and <strong>prepare manuscript</strong> on potential vs. actual distribution of pathogen in invaded range.</td>
<td>June 2012 - February 2013</td>
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<td>Place <em>P. grisea</em> cultures in long-term storage to preserve genetic characteristics, produce mycelial material for genetic characterization, produce conidial inoculum for greenhouse trials, and produce culture filtrates for phytotoxin characterization and bioassays.</td>
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<td>Geary</td>
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<td>Carry out genetic characterization and phylogenetic analysis, <strong>prepare manuscript</strong> on relationships of <em>P. grisea</em> on buffelgrass to <em>P. grisea</em> on other hosts.</td>
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<td>Carry out phytotoxin characterization and host plant leaf bioassays with crude filtrates and purified phytotoxins.</td>
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<td>Carry out growth chamber/greenhouse inoculation trials - host range study.</td>
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<td>Carry out growth chamber/greenhouse inoculation trials - window of infection study.</td>
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<td>Analyze data and <strong>prepare manuscripts</strong> on host range and window of infection studies.</td>
<td>July 2012 - March 2014</td>
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<td>Analyze data and <strong>prepare manuscript</strong> on phytotoxin characterization.</td>
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Literature Cited


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Budget Explanation:

The Rocky Mountain Research Station will be the lead institution. We are requesting $19,000 for in-house use in support of the growth chamber and greenhouse inoculation experiments and ancillary laboratory work. The funds will be used to hire a STEP student to assist microbiology technician Suzette Clement with the experimental work and to purchase supplies for these experiments. Contributed indirect costs are also indicated at 7.1% of in-house funds and 2.2% of pass-through funds.

Brigham Young University is requesting $20,000 on a subcontract to support the laboratory culture work, including obtaining pure cultures, 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. The personnel budget includes wages for student technicians to carry out this work under the supervision of PI Brad Geary. The supplies budget includes funds for this culture work, and also for molecular genetic characterization of pathogen strains, which will take place at the BYU sequencing facility. BYU is contributing student wages in the form of mentored undergraduate learning projects for academic credit, as well as 50% of net in waived overhead.

Funds supplied on subcontract to the University of Naples Federico II will be used to support a Master's level student for one semester under the direction of PI Antonio Evidente, and to purchase supplies and pay for analyses needed for phytotoxin characterization. The University of Naples is contributing 50% of net in waived overhead.

Funds supplied to the University of Arizona on subcontract will be used for 3 months of salary for PI Travis Bean, who will perform the field blight disease surveys, obtain seeds for the host range and window of infection studies and infected tissue for obtaining pathogen isolates, as well as participating in the climate envelope modeling study. 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.


Some Relevant Peer-reviewed Publications - Last Five Years (19 total)


CURRICULUM VITAE: Travis M. Bean

Research Specialist, School of Natural Resources & Environment, University of Arizona. bean@email.arizona.edu

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.
- 2003, Teaching Assistant, Native Plant Taxonomy Course, School of Natural Resources, University of Arizona.

Publications


Relevant Manuscripts in Preparation

CURRICULUM VITAE: ANTONIO EVIDENTE

University of Naples “Federico II” Department of Soil, Plant, Environmental and Animal Production Science, Via Università, 100 Portici (NA) 80055. 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 and studies on 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
- 2000 – 2003, Assistant Professor and Extension Potato and Onion Specialist, University of Idaho, Parma, ID
- 1995 – 1999, Graduate Research Assistant, Washington State University, Pullman, WA
- 1998 – Graduate Teaching Assistant, Washington State University, Pullman, WA
- 1994-95 – Lab assistant, Soil and Plant Analysis Laboratory, Brigham Young University, Provo, UT

**Representative Refereed Journal Publications:**


