

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

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Cooperators:

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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 (Diaz-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 (Gutierrez-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 pyricuol (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, Tsurushima 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, 30C) 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 25C. 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 25C), 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.

Research Timetable (March 2012 - March 2014)

Research Activity	Dates	PI's Responsible
Obtain <i>P. grisea</i> isolates from buffelgrass leaf tissue and produce in pure culture.	March 2012 - September 2012	Bean, Meyer, Geary
Obtain climate data from areas affected by <i>P. grisea</i> in cultivated buffelgrass pastures.	March 2012 - June 2012	Meyer
Carry out blight disease surveys; obtain seeds of buffelgrass and potential nontarget hosts.	June 2012 - October 2012	Bean
Complete climate envelope modeling and prepare manuscript on potential vs. actual distribution of pathogen in invaded range.	June 2012 - February 2013	Meyer, Bean
Place <i>P. grisea</i> 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.	September 2012 - December 2012	Geary
Carry out genetic characterization and phylogenetic analysis, prepare manuscript on relationships of <i>P. grisea</i> on buffelgrass to <i>P. grisea</i> on other hosts.	January 2013 - June 2013	Geary, Meyer
Carry out phytotoxin characterization and host plant leaf bioassays with crude filtrates and purified phytotoxins.	January 2013 - August 2013	Evidente, Meyer
Carry out growth chamber/greenhouse inoculation trials - host range study.	January 2013 - March 2013	Meyer
Carry out growth chamber/greenhouse inoculation trials - window of infection study.	April 2013 - June 2013	Meyer
Analyze data and prepare manuscripts on host range and window of infection studies.	July 2012 - March 2014	Meyer, Geary
Analyze data and prepare manuscript on phytotoxin characterization.	August 2013 - March 2014	Evidente, Meyer

Literature Cited

- Bogdan AV. 1977. Tropical Pasture and Fodder Plants (Grasses and Legumes). pp. 66-74. Longman: London and New York.
- Bowers JE, Bean TM, and Turner RM. 2006. Two decades of change in distribution of exotic plants at the Desert Laboratory, Tucson, Arizona. *Madroño* 53:254-265.
- Brooks ML. 1999. Alien annual grasses and fire in the Mojave Desert. *Madroño* 46:13–19.
- Burdon JJ, Groves RH, and Cullen JM. 1981. The impact of biocontrol on the distribution and abundance of *Chondrilla juncea* in southeastern Australia. *Journal of Applied Ecology* 18:957-966.
- Burgess TL. 1991. Exotic plants at the Desert Laboratory. *Madroño* 53:254-265.
- Burquez-Montijo AM, Miller ME, Yrizar AM, and Tellman B. 2002. Mexican grasslands, thornscrub, and the transformation of the Sonoran Desert by invasive exotic buffelgrass (*Pennisetum ciliare*). Invasive exotic species in the Sonoran region (B Tellman, ed.), pp. 126–146, The University of Arizona Press, The Arizona-Sonora Desert Museum, Tucson, AZ.
- Caltagirone LR. 1981. Landmark examples in classical biological control. *Annual Review of Entomology* 26: 213-232
- Clarke PJ, Latz PK, and Albrecht DE. 2005. Long-term changes in semi-arid vegetation: invasion of an exotic perennial grass has larger effects than rainfall variability. *Journal of Vegetation Science* 16:237–248.
- Couch BC, Fudal I, LeBrun MH, Tharreau D, Valent B, Kim PV, Notteghem JL, and Kohn LM. 2005. Origins of host-specific populations of the blast pathogen *Magnaporthe oryzae* in crop domestication with subsequent expansion of pandemic clones on rice and weeds of rice. *Genetics* 170:613–630.
- Couch BC, and Kohn LM. 2002. A multilocus gene genealogy concordant with host preference indicates segregation of a new species, *Magnaporthe oryzae*, from *M. grisea*. *Mycologia* 94:683–693.
- Daehler CC, and Goergen EM. 2005. Experimental restoration of an indigenous Hawaiian grassland after invasion by Buffel grass (*Cenchrus ciliaris*). *Restoration Ecology* 13: 380 –389.
- Diaz-Franco A, Mendez-Rodríguez A, and Garza-Cedillo R. 2007. Tizón foliar del pasto buffel: Su presencia in Tamaulipas, Mexico. 2007. *Agricultura Técnica en México* 33 (3): 285-295.
- Ebbole DJ. 2007. *Magnaporthe* as a model for understanding host-pathogen interactions. *Annual Review of Phytopathology* 45:437-456.

Evidente A. 2006. Chemical and biological characterization of toxins produced by weed pathogenic fungi as potential natural herbicides. *In: Natural Products for Pest Management*, ACS Symposium Series 927, Eds. Rimando AM, and Duke SO, ACS Division of Agricultural and Food Chemistry, Inc., Washington, USA, pp. 62-75.

Evidente A. 2010. New fungal metabolites, as antifungals, herbicides and insecticides for agrarian plants pests. *In: Comprehensive Bioactive Natural Products* (Ed. Gupta VK), Studium Press, Vol 1, Chapter 15, pp. 333-389.

Evidente A, and Abouzeid MA. 2006. Characterization of phytotoxins from phytopathogenic fungi and their potential use as herbicides in integrated crop management. *In: Handbook of Sustainable Weed Management*, Eds. Singh PH, Batish DR, and Kohli RK. The Harworth Press Inc., New York, pp. 507-532.

Evidente A, Andolfi A, Vurro M, Zono MC, and Motta A. 2002. Cytochalasins Z1, Z2 and Z3, three 24-oxa[14]cytochalasins produced by *Pyrenophora semeniperda*. *Phytochemistry* 60:45-53.

Evidente A, Andolfi A, Vurro M, Fracchiolla M, Zonna MC, and Motta A. 2005. Drazepinone, a trisubstituted tetrahydronaphthofuroazepinone with herbicidal activity produced by *Drechslera siccans*. *Phytochemistry* 66:715-721.

Fairfax RJ, and Fensham RJ. 2000. The effect of exotic pasture development on floristic diversity in central Queensland, Australia. *Biological Conservation* 94:11-21.

Faivre-Rampant O, Thomas J, Allègre M, Morel JB, Tharreau D, Nottéghem JL, Lebrun MH, Schaffrath U, and Piffanelli P. 2008. Characterization of the model system rice–*Magnaporthe* for the study of nonhost resistance in cereals. *New Phytologist* 180:899-910.

Greer CA, and Webster RK. 2001. Occurrence, distribution, epidemiology, cultivar reaction, and management of rice blast disease in California. *Plant Disease* 85:1096-1102.

Gutierrez-Ozuna R, Eguiarte LE, Molina-Freaner F. 2009. Genotypic diversity among pasture and roadside populations of the invasive buffelgrass (*Pennisetum ciliare* L. Link) in north-western Mexico. *Journal of Arid Environments* 73:26-32.

Howard RJ, and Valent B. 1996. Breaking and entering: Host penetration by the fungal rice blast pathogen *Magnaporthe grisea*. *Annual Review of Microbiology* 50: 491-512.

Iwasaki S, Muro H, Nozoe S, Okuda S, and Sato Z. 1972. Isolation of 3,4-dihydro,3,4,8-trihydroxy-1(2H)-naphthalene and tenuazonic acid from *Pyricularia oryzae* Cavara. *Tetrahedron Letters* 1:13-16.

Iwasaki S, Nozoe S, Okuda S, Sato Z, and Kozaka T. 1969. Isolation and structural elucidation of a phytotoxic substance from *Pyricularia oryzae* Cavara. *Tetrahedron Letters* 45:3977-3980.

- Kim JC, Min JY, Kim HT, Cho KY, and Yu SH. 1998. Pyricuol, a new phytotoxin from *Magnaporthe grisea*. *Bioscience, Biotechnology, and Biochemistry* 62:173-174.
- Long DH, Correll JC, Lee FN, and teBeest DO. 2001 Rice blast epidemics initiated by infested rice grain on the soil surface. *Plant Disease* 85: 612–616.
- Olsson AD, Betancourt JL, McClaran MP, and Marsh SE. 2011. Sonoran Desert Ecosystem transformation by a C4 grass without the grass/fire cycle. *Diversity and Distributions* in press.
- Perrott RF and Chakraborty S. 1999. *Pyricularia grisea* causes blight of buffel grass (*Cenchrus ciliaris*) in Queensland, Australia. *Tropical Grasslands* 33:201-206.
- Rehfeldt GE, Crookston NL, Warwell MV, and Evans JS. 2006. Empirical analyses of plant-climate relationships for the western United States. *International Journal of Plant Science* 167:1123-1150.
- Rodriguez O, Gonzalez-Dominguez J, Krausz JP, Odvody GN, Wilson JP, Hanna WW, and Levy M. 1999. First report and epidemics of buffelgrass blight caused by *Pyricularia grisea* in South Texas. *Plant Disease* 83:398.
- Rogstad A (ed.). 2008. The Buffelgrass Strategic Plan. Arizona-Sonora Desert Museum, Tucson, AZ.
- Rutman S, and Dickson L. 2002. Management of buffelgrass on Organ Pipe Cactus National Monument, Arizona. In: Tellman B (ed), *Invasive exotic species in the Sonoran region*. University of Arizona Press, Tucson, pp. 311-330.
- Stevens J, and Falk DA. 2009. Can buffelgrass invasions be controlled in the American Southwest? Using invasion ecology theory to understand buffelgrass success and develop comprehensive restoration and management. *Ecological Restoration* 27: 417-427.
- Takan JP, Chipili J, Muthumeenakshi S, Talbot NJ, Manyasa EO, Bandyopadhyay R, Sere Y, Nutsugah SK, Talhinas P, Hossain M, Brown AE, and Sreenivasaprasad S. 2011. *Magnaporthe oryzae* populations adapted to finger millet and rice exhibit distinctive patterns of genetic diversity, sexuality and host interaction. *Molecular Biotechnology* DOI 10.1007/s12033-011-9429-z.
- Talbot NJ. 2003. On the trail of a cereal killer: Exploring the biology of *Magnaporthe grisea*. *Annual Review of Microbiology* 57:177–202.
- 't Mannetje L, and Kersten SMM. 1992. *Cenchrus ciliaris* L. In: 't Mannetje, L. and Jones, R.M. (eds) *Plant Resources of South-East Asia No. 4. Forages*. pp. 77-79. Pudoc Scientific Publishers, Wageningen, the Netherlands.

- Tsurushima T, LeDinh D, Kawashima K, Murakama J, Nakayashiki H, Tosa Y, and Mayama S. 2005. Pyrichalasin H production and pathogenicity of *Digitaria*-specific isolates of *Pyricularia grisea*. *Molecular Plant Pathology* 6: 605–613.
- Tsurushima T, Nakayashiki H, Tosa Y, and Mayama S. 2009. Pathogenicity related compounds produced by blast fungus. In: *Advances in Genetics, Genomics and Control of Rice Blast Disease Part III*, Wang GL, and Valent B, Eds. Springer. p. 247-255.
- Valent B. 1990. Rice blast as a model system for plant pathology. *Phytopathology* 93: 1378–1385.
- Valent B, and Chumley FG. 1991. Molecular genetic analysis of the rice blast fungus *Magnaporthe grisea*. *Annual Review of Phytopathology* 29:443–467.
- Whisenant SG. 1990. Changing fire frequencies on Idaho's Snake River Plains: ecological and management implications. Symposium on cheatgrass invasion, shrub die-off, and other aspects of shrub biology and management. McArthur ED, Romney EM, Smith SD and Tueller PT, eds., pp. 4–10, U.S. Department of Agriculture, Forest Service, Intermountain Research Station, Ogden UT.
- Wilson WP, and Hanna WW. 1992. Disease resistance in wild *Pennisetum* species. *Plant Disease* 96: 1171-1175.
- Yang XB, and teBeest DO. 1993. Epidemiological mechanisms of mycoherbicide effectiveness. *Phytopathology* 83:891-893.
- Yoshida K, Saitoh H, Fujisawa S, Kanzaki H, Matsumura H, Yoshida K, Tosa Y, Chuma I, Takano Y, Win J, Kamoun S, and Terauchia R. 2009. Association genetics reveals three novel avirulence genes from the rice blast fungal pathogen *Magnaporthe oryzae*. *The Plant Cell* 21: 1573-1591.
- Zellerhoff N, Jarosch BL, Groenewald JZ, Crous PW, and Schaffrath U. 2006. Nonhost resistance of barley is successfully manifested against *Magnaporthe grisea* and a closely related *Pennisetum*-infecting lineage but is overcome by *Magnaporthe oryzae*. *Molecular Plant Microbe Interactions* 19:1014–1022.

Budget

Budget Line Item	Funds Requested	Funds Contributed	Total Funds
USFS Rocky Mtn. Research Station (Lead)			
Personnel:			
PI (GS-15) 10%	0	30,000	30,000
Technician (GS-7) 20%	0	22,000	22,000
STEP technician 1200 hours @12/hr	14,400	0	14,400
Total Personnel	14,400	52,000	66,400
Supplies	4,600	0	4,600
Subcontracts:			
Brigham Young University	20,000	0	20,000
University of Naples Federico II	24,000	0	24,000
University of Arizona	12,000	0	12,000
Total Net Direct	75,000	52,000	97,000
Indirect (7.1% in house, 2.2% pass thru)	0	2,580	2,580
Total	75,000	54,580	129,580
Brigham Young University Subcontract			
Undergraduate techs (1780 hours @ \$9/hr)	12,000	4,000	16,000
Supplies	6,000	0	6,000
Travel (professional meeting)	2,000	2,000	4,000
Total Net Direct	20,000	6,000	26,000
Indirect (50% of net requested)	0	10,000	10,000
Total	20,000	16,000	36,000
University of Naples Federico II Subcontract			
MS student assistantship -one semester	7,500	0	7,500
Supplies	16,500	0	16,500
Total Net Direct	24,000	0	24,000
Indirect (50% of Net)	0	12,000	12,000
Total	24,000	12,000	36,000
University of Arizona Subcontract			
PI salary support (3 months @ \$4000/month)	12,000	0	12,000
Total Net Direct	12,000	0	12,000
Indirect (50% of Net Requested)	0	6,000	6,000
Total	12,000	6,000	18,000

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.

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

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

Beckstead J, Meyer SE, Molder C, and Smith DC. 2007. A race for survival: Can *Bromus tectorum* seeds escape *Pyrenophora semeniperda*-caused mortality by germinating quickly? *Annals of Botany* 99: 907–914.

Boguena T, Meyer SE, and Nelson DL. 2007. Low temperature during infection limits *Ustilago bullata* (Ustilaginaceae, Ustilaginales) disease incidence on *Bromus tectorum* (Poaceae, Cyperales). *Biocontrol Science and Technology* 17:33-52.

Meyer SE, Beckstead J, Allen PS, and Smith DC. 2008. A seed bank pathogen causes seedborne disease: *Pyrenophora semeniperda* on undispersed grass seeds in western North America. *Can. J. Plant Path.* 30:525-533.

Meyer SE, Nelson DL, and Clement S. 2010. The ecological genetics of the *Ustilago bullata*-*Bromus tectorum* pathosystem: A role for frequency-dependent selection? *American Journal of Botany* 97:1304-1312.

Meyer SE, Stewart TE, and Clement S. 2010. The quick and the deadly: growth versus virulence in a seed bank pathogen. *New Phytologist* 187:207-216.

Meyer SE, Quinney D, Nelson DL, and Weaver J. 2007. Impact of the pathogen *Pyrenophora semeniperda* on *Bromus tectorum* seedbank dynamics in North American cold deserts. *Weed Research* 47:54–62.

CURRICULUM VITAE: Travis M. Bean

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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.
- 2000-2001, Soil Conservation Intern, US Dept of Agriculture Natural Resources Conservation Service, Tucson, Arizona.
- 1999-2000, Plant Materials Intern, US Dept of Agriculture Natural Resources Conservation Service Plant Materials Center, Tucson, Arizona.

Publications

- Rogstad, A, T.M. Bean, A.D. Olsson, and G.M. Casady. 2009. Fire and invasive species management in hot deserts: resources, strategies, tactics, and response. *Rangelands* 31:6-13.
- Bean, T.M., and C.A. Hannum. 2009. Invasive grasses: a cause for concern. *Backyards and Beyond* 3(3): 4-5, Arizona Cooperative Extension, Tucson.
- Bowers, J.E., T.M. Bean, and R.M. Turner. 2006. Two decades of change in the distribution of exotic plants at the Desert Laboratory, Tucson, Arizona. *Madroño* 53:254-265.
- Bean, T.M., S.E. Smith, and M.M. Karpiscak. 2004. Intensive revegetation in Arizona's hot desert: the advantages of container stock. *Native Plants Journal* 5: 173-180.
- Bean, T.M., M.M. Karpiscak, and S.E. Smith. 2003. A prescription for restoring native vegetation on former agricultural land (Arizona). *Ecological Restoration* 21:214-215.

Relevant Manuscripts in Preparation

- Bean, T.M., J.K. Leary, and W.B. McCloskey. Evaluation of herbicide ballistic technology for control of buffelgrass and common range shrubs.
- Bean, T.M., W.B. McCloskey, and G.M. Casady. Efficacy of imazapic and glyphosate for pre- and post-emergence control of buffelgrass.
- Frid, F., T. Holcombe, J. Morisette, L. Brigham, T. Bean, and J. Betancourt. Using state and transition modeling to account for imperfect knowledge in invasive species management.
- Morisette, J., G. Newman, L. Frid, T. Holcombe, T. Kern, L. Brigham, T. Bean, and J. Betancourt. Integrating decision support and data management tools to control buffelgrass.
- Casady, G.M., and T.M. Bean. Efficacy of monocot-selective herbicides for control on buffelgrass and effects on native vegetation.
- Bean, T.M., J.E. Bowers, D. Backer, T.E. Esque, and C. Schwalbe. Differences in seedbank density and species richness between buffelgrass-infested and uninfested areas.

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)

- A. Evidente, A. Berestetskiy, A. Andolfi, M.C. Zonno, A. Cimmino, M. Vurro; “Relation between *in vitro* production of ascosonchine and virulence of strains of the potential mycoherbicide *Ascochyta sonchi*: a method for its quantification in complex samples”, *Phytochemical Analysis*, 17, 357-364, (2006).
- A. Berestetskiy, A. Dmitriev, G. Mitina, I. Lisker, A. Andolfi, A. Evidente: “Nonenolides and cytochalasins with phytotoxic activity against *Cirsium arvense* and *Sonchus arvensis*: a structure-activity relationships study” *Phytochemistry*, 69, 953-960, (2008)
- A. Evidente, A. Cimmino, A. Andolfi, M. Vurro, M. C. Zonno, A. Motta: “Phyllostoxin and phyllostin, bioactive metabolites produced by *Phyllosticta cirsii*, a potential mycoherbicide for *Cirsium arvense* biocontrol”, *Journal of Agricultural and Food Chemistry*, 56, 884-888, (2008).
- A. Cimmino, A. Andolfi, A. Berestetskiy, A. Evidente: “Production of phytotoxins by *Phoma exigua* var. *exigua*, a potential mycoherbicide against perennial thistles”, *Journal of Agricultural and Food Chemistry*, 56, 6304-6309, (2008).

CURRICULUM VITAE: BRADLEY D. GEARY

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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:

- Lee SJ, Strobel GA, Eisenman K, Geary B, Vargas PN, and Strobel SA. 2009. *Aurospheeria*, a novel coelomycetous genus. *Mycotaxon*. 107:463-472.
- Benson JH, Geary B, Miller JS, Hopkins BG, Jolley VD, and Stevens MR. 2009. *Phytophthora erythroseptica* (Pink Rot) development in Russet Norkotah potato grown in buffered hydroponic solutions II. pH Effects. *Amer. J. Potato Res.* DOI 10.1007/s12230-009-9102-2.
- Geary B, Ransom C, Brown B, Atkinson D, and Hafez S. 2008. Weed, disease, and nematode management in onion with biofumigants and metam sodium. *Hort. Tech.* 18:569-574.
- Gordillo LF, Stevens MR, Millard MA, and Geary B. 2008. Screening two *Lycopersicon peruvianum* collections for resistance to tomato spotted wilt virus. *Plant Dis.* 92:694-704.
- Geary, B. and D. A. Johnson. 2006. Relationship between silver scurf levels on seed and progeny tubers from successive generations of potato seed. *American J. Potato Research* 83(6):447-453.