

SN36: Neutralization of *Phalaris arundinaceae* (Reed Canarygrass) Root Exudates

Abstract

Reed canarygrass (*Phalaris arundinaceae*, RCG) is an extremely aggressive plant invading wetlands in the Upper Midwest. Invasion by RCG ultimately leads to a profound diminution of native plant diversity and consequent loss of wetland functionality. Our ability to control invasion by RCG and reestablish native plant communities has been largely unsuccessful because of our limited understanding of the mechanisms that allow RCG to become invasive. Our initial studies show that RCG uses a 2-step plant-soil feedback mechanism to become invasive. In this scenario, this species of exotic plant is suspected of releasing unknown chemicals into the soil that shapes the microbial community associated with it, and it is this RCG-associated community of soil microbes that interferes with the growth of native wetland plants. Here we propose to conduct a series of experiments that will confirm these previous results and then isolate and identify the putative chemicals believed to be linked to the reduction of native plant diversity in Minnesota wetlands. The identification of these chemicals will enable us to develop management practices that will neutralize them or limit their production, and establish diverse native plant communities on wetland restoration sites.

Background

RCG is among the most aggressive plants invading wetlands in Minnesota. Where it has invaded, native plant diversity has been drastically reduced. Our ability to effectively manage and control its invasion and reestablish native plant communities have been unsuccessful because of our limited understanding of the mechanism that allows it to become invasive. Previous studies by others have implicated nitrogen enrichment increases the competitive ability of RCG (Studdy et al. 1995; Vetsch et al. 1999; Reisterer et al., 2000, as reviewed by Lavergne and Molofsky 2004). However, the effect of nutrient levels for the invasive success of RCG is ambiguous. At high nutrient levels, RCG suppresses the growth of native species by out-competing them for available sunlight (Green and Galatowitsch 2002). On the other hand, at low nutrient levels, RCG is able to out-compete the root growth of native species at low nitrate levels (Green and Galatowitsch 2001; Green and Galatowitsch 2002). It is therefore evident that more than one mechanism for the invasive success of RCG is at work.

Several hypotheses have been proposed to explain the invasive success of plants (reviewed by Heierro et al 2005), but one has specific relevance to our study of RCG. The Enemy Release Hypothesis states that introduced species become invasive in their introduced range because they are no longer kept in check by their natural enemies such as the predators, herbivores, and pathogens they coevolved with (Keane and Crawley 2002). Although it is well-established that escape from natural enemies might contribute to successful invasion, our most research has been limited to the effects of aboveground herbivory (Bever 2003). However, microbial interactions and plant-soil feedbacks are attracting increased attention from researchers (Crawley 2002; van der Putten and Peters 1997; Callaway et al. 2004). A plant-soil feedback involves a two step process. In step one plants change the soil community; in step two, this change in the soil community in turn affects the growth of the plants. These feedbacks include the negative effects of soil pathogens and the positive effects of beneficial soil mutualists on the plants' growth or can indirectly affect the neighboring plant species (Bever et al. 1997). Feedback between plants

and soil communities can strongly determine a plant's establishment, invasion, and persistence in local habitats (Bever et al. 1997). Feedbacks can vary among plant species; dissimilar species acquire pathogens at different rates. Some plant species acquire pathogens quickly once established and kept at relatively low densities. Whereas other plant species acquire pathogens more slowly and are not affected by negative feedbacks until their population densities reach high levels. Invasive plant species appear to slowly accumulate pathogens and can quickly reach high densities because they have escaped their natural enemies (Klironomos 2002).

We conducted an experiment in which we compared the growth of RCG and Tussock sedge (*Carex stricta*, a native wetland sedge) from reciprocal plantings in soils collected from RCG and sedge rhizospheres. Sedge grown in RCG soil exhibited significant reductions in biomass relative to sedge grown in sedge soil. In contrast, soil type had no effect on RCG biomass. In addition, microbial community-level carbon substrate utilization (CLSU) analysis revealed two interesting observations. First, sedge had little influence on the CLSU patterns of RCG soil microbes, whereas RCG had a considerable effect on those of sedge soil microbes. Second, CLSU patterns for soil microbes associated with the rhizosphere of RCG growing in RCG soil were obviously different from those of microbes distant from the rhizosphere in the same soil, but very little difference was observed between CLSU patterns yielded by microbes within and distant from the rhizosphere of sedge. These results suggest that RCG displaces sedge via a soil-microbe feedback, such that 1) organisms that are uniquely beneficial to sedge are excluded as a result of competition with microbes favored by RCG, or 2) RCG creates an environment that favors the growth of organisms pathogenic to sedge.

As such, our observations are entirely consistent with the Enemy Release Hypothesis and its application to plant-soil feedbacks. Moreover, our results are quite clearly proof of principle: RCG roots influence the soil microbial community to the extent that it is not able to support the growth and success of native wetland plants as represented by Tussock sedge. RCG releases unknown chemicals into the soil, producing a microbial community that reduces the growth of native wetland plants. We propose to conduct a series of experiments that will confirm this by applying soil extracts of RCG root exudates to the rhizospheres of both RCG and Tussock sedge to identify the compounds that result in the formation of a microbial community that inhibits sedge. Extracts identified as inhibitory will be fractionated into individual components. Chemical analogues of these components will be added, along with RCG soil extracts, to Tussock sedge rhizospheres in order to identify analogues that mitigate the inhibitory effects of RCG root exudates. The identification of competing analogues is the necessary first step toward the development of innovative and effective management practices which prevent the degradation of native wetland communities.

Research Hypothesis and Objectives

Our overall objective is to facilitate desirable soil conditions for the establishment of diverse native plant communities on restored/created wetlands. Our initial results have provided evidence that, consistent with the Enemy Release Hypothesis, RCG roots exude one or more compounds that influence the composition of soil microbes, leading to a negative impact on the growth and success of native wetland plants. To effectively manage RCG invasions and reduce the loss of native plant diversity, we need a greater understanding of the nature of these root

exudates and we must begin to develop an understanding of the biotic and abiotic conditions that influence their production and effect their inactivation or neutralization. Accordingly, the **driving hypothesis** behind the proposed research is that **the effect of the compound released by RCG roots that shapes the formation of soil microbial communities favorable to RCG and hostile to native wetland plant species is subject to interference by steric competition.**

To this end, the objectives of the proposed research are:

- 1) To confirm that root exudates are responsible for the invasive success of RCG
- 2) To characterize products in RCG root exudates that drive the establishment of soil microbial communities that are inhibitory to native wetland plants.

The potential impacts of this research are:

- 1) By verifying the mechanisms for the invasive success of RCG we will be better able to develop strategies to restore/create wetlands with diverse native plant communities.
- 2) By characterizing the products in RCG root exudates we hope to develop a means to neutralize them, limit their secretion, and facilitate the establishment and success of native plant communities.

Description of Results/Products

Upon successful completion of the proposed research, we will have identified a potential means by which RCG modeling of wetland soil microbial communities can be prevented, thereby permitting the establishment of native wetland plant species and enabling the development of wetlands with high biodiversity and functionality. To be sure, additional research will be needed not only to determine the safety and efficacy of the identified compounds, but how frequently they must be used and at what concentrations. Nevertheless, it is anticipated that these results will significantly narrow efforts toward RCG control to those that will have the highest probability for success while being the simplest and least expensive to reduce to practice.

Expected Benefits and End Users

The principle benefit of this project will be a comprehensive understanding of the mechanisms for the invasive success of RCG. With this information, we will be able to provide better wetland restoration and management techniques to develop and maintain native plant communities and subsequent habitat and food for wildlife supported by Minnesota's wetlands. This information will be available and used by all governmental wetland management agencies.

Brief Research Methodology

Overview

Topsoil from RCG and sedge communities will be collected, sterilized, and portioned into 8 large tanks. Four tanks will be seeded with RCG, two with sedge, and two will remain unseeded and kept in the greenhouse (fallow) as background controls, and the remainder will be stored frozen for later use (see below). The seeded and fallow treatments will be maintained for a four month growing period for the training phase of our experiments.

In preparation for the first of two proposed experiments, soil from two RCG tanks will be removed, and soluble, non-particulate material will be extracted into water by dividing the soil into several parcels and resuspending each parcel in 3 volumes of water, followed by

autoclaving, and coarse filtration. This procedure will also be used with one sedge tank and one fallow tank. The water extracts from one RCG tank as well as those from the sedge and fallow tanks will be fractionated by ion-exchange chromatography using DEAE-cellulose in a 250 ml bed volume in a continuous gradient of 0.25M – 5M KCl. 20-25 ml fractions will be collected from each extract, and identical fraction numbers from each tank will be pooled. The extracts from the second RCG tank will likewise be fractionated, but each fraction will be stored at -80°C after pooling. Each RCG fraction will be applied daily to dedicated groups of 10 replicates each of sedge and RCG, beginning at 10 days after emergence. As controls, each sedge or fallow fraction will also be applied to dedicated replicates of sedge and RCG at the same time. At monthly intervals, soil microbial communities will be examined by community-level *substrate* utilization (CLSU) and terminal restriction fragment length analysis (T-RFLP). After 4 months, plants will be collected for total biomass measurements.

RCG soil extract fractions identified from the above experiment as having inhibitory activity will be further fractionated (from the retained RCG fractions described above) and identified by High Performance Liquid Chromatography (HPLC) with UV/Vis light and/or Mass Spectrometer (MS) detectors. Competing analogues will be selected from commercial sources (e.g., Aldrich, Acros) where possible, based on structural similarities. The experiments described above will be repeated, with one set of RCG and sedge receiving each inhibitory component of RCG soil extract (or crude fraction as a positive control), and a second set receiving each inhibitory component and an equimolar dose of the selected analogue. Analytical methods will be identical to those mentioned above, described in detail below.

Analytical Approaches

Fifteen 1 g soil samples will be randomly collected from each plant-soil treatment at monthly intervals (beginning with time 0 and ending with the harvest date the beginning of each phase) and combined into 3-5 g pools for each treatment. DNA will be extracted for terminal restriction fragment length polymorphism (T-RFLP) analysis from a portion of each pool (described below), and the remainder of each pool will be examined for community-level carbon substrate utilization (CLSU, described below). Plant height and biomass will be measured at the end of each phase. Soil samples will be collected at the beginning and end of each phase and frozen for extraction and HPLC analysis. Samples will also be collected from each plant-soil treatment to measure total microbial biomass. Mean height and biomass data for each plant-soil treatment will be compared using one way and two way Analysis of Variance (ANOVA) and Tukey's *post-hoc* statistical tests for significant differences in both the growth of RCG and sedge between treatments. CLSU and T-RFLP results will be analyzed and compared by principle components analysis (Widmer, et. al. 2001) and hierarchical cluster analysis. Total microbial biomass will be determined for each soil sample collected as described elsewhere (Turner, et al., 2001).

Soil collection – Soils will be collected from the rhizospheres of RCG and Tussock sedge communities locally. Each soil type will be sieved with a 2 cm sieve to remove coarse organic matter. Half of both RCG and sedge soils will be triple autoclaved for 20 minutes at 121°C over the course of 3 successive days to kill all biotic organisms. Newly purchased and washed 40/70 grit silica sand will be autoclaved at 110°C for 12 hours so that it can be used as a neutral

substrate for microbial inoculation. The sterile and non-sterile RCG and sedge soils will then be mixed with the sterile sand in a 9:1 (sand: soil) volumetric proportion to produce four soil treatments, RCG soil; autoclaved RCG soil; sedge soil; and autoclaved sedge soil.

CLSU analysis – Difference in the composition of microbial communities can be revealed by comparing the sources of carbon that can be used by each community. BIOLOG plates contain 95 different carbon substrates. Microbial catabolism of each substrate is detected by a redox indicator, and the use of substrates can be quantitatively evaluated through the use of a microplate photometer. Each inoculated plate will be read twice daily for up to 7 days. Normalization procedures described by others will be used to control for differences in microbial numbers among samples (Garland and Mills, 1991).

T-RFLP analysis – DNA will be extracted from soil, and ribosomal RNA genes (rDNA) will be amplified using fluorescently-labeled primers in the Polymerase Chain Reaction (PCR). The PCR products will be digested with restriction enzyme *MspI*, and fragments will be separated and visualized using a LiCor 4300 DNA Analyzer. Because each taxon has a unique rDNA sequence, each may generate different banding patterns following digestion. Soils containing similar communities will have similar banding patterns; where the community compositions are significantly different, the banding patterns will also differ.

HPLC-UV/VIS and/or MS – The original soil extracts and extracts prepared from soils collected periodically from the tanks and from the RCG rhizosphere will be analyzed by Reverse Phase HPLC-UV/VIS. A mixture of solvents (methanol, acetonitrile) and columns (C-18 pre-column, ODS, C-18) coupled with several different wavelengths (280 nm, universal for phenolics, 260 nm catechins, etc) will be used to separate and screen components. Those fractions that show biological activity will be analyzed by HPLC-MS. The MS fingerprint will be used to search existing libraries of compounds to identify the compounds.

RCG rhizomes will also be collected. After collection the rhizomes will be rinsed with deionized water and divided into the root section and stem/leaf section. Both sections will be weighed for biomass determination. Aliquots of roots (approximately 150 grams) will be crushed using a ball grinder. The crushed roots will be extracted with water and methanol. These extracts will be analyzed for compounds using HPLC-UV/Vis. Components will be separated and collected by HPLC. Those fractions showing biological activity will be further analyzed by HPLC-MS. The MS will give a fingerprint of the compound.

Timetable for Completing Proposed Research Tasks

- | | |
|--------------------------------|----------------------------|
| 1. Soil prep. | 5. T-RFLP testing |
| 2. Soil training | 6. HPLC analysis |
| 3. CLSU testing | 7. Draft final report |
| 4. Plant harvest/data analysis | 8. Finalize project report |

Task	Month																								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
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Budget Requirements

Staff or Contract Services: Bradley Cook (10% effort) - \$15134
 Timothy Secott (10% effort) - \$24202
 Bertha Proctor (5% effort) - \$12480
 2 Graduate Students (75% effort) - \$48000

Supplies: \$14669

Travel: \$515

TOTAL BUDGET: \$115000

B. Other Funds being Spent during the Project Period

\$13140 (direct costs) in the form of Graduate Teaching Assistantships as partial support for the graduate students working on the project.

C. Past Spending (from other sources)

\$13140 (direct costs - Graduate Teaching Assistantship)
 \$6000 Supplies

This study will be laboratory intensive for two years. There will be 3 faculty and 2 graduate students. Both graduate students will be under the direct supervision of the PI and one Co-PI (Secott). The PI and one Co-PI (Secott) will receive 25 days of support for each of the two years. The second Co-PI (Proctor) key person will receive 14 days support over the duration of the project. The two graduate students will receive approximately one-third of their support for two years through teaching assistantships within the Department of Biological Sciences at MSUM (approximately \$13,140 in direct matching funds from MSUM) and the remaining two-thirds of

their support (stipend and tuition for one semester and summer for each year) through this grant. Supplies include HPLC columns, solvents, sand, DNA extraction reagents, PCR primers, BIOLOG plates, electrophoresis reagents (buffers, agarose, polyacrylamide, etc.) and laboratory consumables (pipet tips, gloves, etc.).

Matching Funds

In addition to providing matching funds in the form of Graduate Teaching Assistantships and tuition waivers for one semester each year for each graduate student assigned to this project, Minnesota State University, Mankato will provide in-kind matching funds including office space and computer facilities for key personnel and graduate students for the duration of the project. Support services including greenhouse/laboratory, library access and services, statistical expertise, accounting services, copying costs, publication costs, some travel, and office/laboratory/greenhouse maintenance and power will be also be provided by MSUM for the duration of the project.

References

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- Bever, J.D. 2003. Soil Community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. *New Phytologist* 157:465-473.
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- Widmer, F., A. Fliessbach, E. Laczko, J. Schulze-Aurich, J., and J. Zeyer. 2001. Assessing soil biological characteristics: a comparison of bulk soil community DNA-, PLFA-, and Biolog-analyses. *Soil Biology and Biochemistry* 33:1029-1036.

Identification of Principal Investigators (resumes)

BRADLEY J. COOK

Education

Ph.D. The University of Montana, Div. of Bio. Sciences, Missoula, MT May 2001
M.S. The University of Montana, School of Forestry, Missoula, MT May 1994
B.S. The University of Montana, School of Forestry, Missoula, MT May 1989

Employment and Professional Experiences

2003-present Assistant Professor of Aquatic Ecology: Department of Biological Sciences, Minnesota State University-Mankato
2002-2003 Adjunct Assistant Professor: Division of Biological Sciences, The University of Montana.
2002-2003 Senior Researcher: Division of Biological Sciences, The University of Montana.
2001- 2002 Post-doctoral Research Associate: Flathead Lake Biological Station, The University of Montana.
1996-2001 Research Assistant and Ph.D. student: Flathead Lake Biological Station, The University of Montana.
1995-1996 Research Specialist II: Flathead Lake Biological Station, The University of Montana.
1992-1995 Research Specialist II: Riparian and Wetland Research Program, The University of Montana.
1989-present Private Wetland Consultant

Research Interests

To extend basic research into the arena of applied science. To determine the mechanisms for the establishment and expansion of invasive aquatic macrophytes. To determine the effects geomorphology, hydrographic regime, and disturbance have on wetland structure and function. To develop and apply functional assessments to plant communities, wetland, and watershed levels for restoration and management purposes.

Research Grants

Primary author

Using isotope tracers to determine hydrologic interactions between pothole wetlands and ground water. Minnesota State University, College of Science, Engineering and Technology Faculty Research Grant. 2004. \$4,000.
Production of sago pond weed and factors limiting growth in the Heron Lake system. Heron Lake Watershed District. 2004-2005. \$9000.
Developing a watershed and wetland monitoring and assessment program: landuse, invasive species and bioassessment criteria for vegetation. EPA Competitive Wetland Grant Program, 2002. \$55,200
National guidebook for the application of hydrogeomorphic assessments to depressional wetlands. US Army Corps of Engineers, Waterways Experiment Station. 2002. \$10,000
Field-testing the hydrogeomorphic approach to assessing wetland function: models and analysis of reference data. EPA Competitive Wetland Grant Program, 1998. \$68,500

Developing a hydrogeomorphic approach to assessing wetland function for herbaceous depressional wetlands. EPA Competitive Wetland Grant Program, 1997. \$70,000
Investigating the relationship between shallow groundwater hydrology and selected riparian habitat types. EPA Competitive Wetland Grant Program, 1995. \$43,000
The hydrogeomorphic approach to the functional assessment of wetlands: Field calibration for riverine wetlands. Consortium Funding (BOR-Boise, BOR-Billings, Crown Butte Mines Inc., Fed. Hwy. Adm., MDFWP, MDOT, MT Power, NPS, NRCS, USFWS) 3/96 - 9/98. \$105,000

Secondary author

Wildlife value of canary reedgrass in invaded wetlands of Minnesota. MN Dept. of Transportation, 9/05 - 9/07. \$99,132, with B.R. McMillan.
Five additional proposals totaling over \$255k.

Selected Publications

- Callaway, R.M., R.W. Brooker, P. Choler, Z. Kikvidze, C.J. Lortie, R. Michalet, L. Paolini, F.I. Pugnaire, B. Newingham, E.T. Aschehoug, C. Armas, D. Kikodze and **B.J. Cook**. 2002. Interdependence among alpine plants increases with stress: a global experiment. *Nature* 417: 844-848.
- Cook, B.J.**, and T.J. Nimlos. 1998. A field apparatus for measuring unconfined compressive strength. *Soil Sci. Soc. Am. J.* 62: 1234-1236.
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- Cook, B.J.** and F.R. Hauer. *In review*. Temporary connections make “Isolated” wetlands function at the landscape scale. *Wetlands*.
- Jarchow, M.E. and **B.J. Cook**. *In review*. Allelopathy as a novel weapon for the invasion of *Typha angustifolia*. *Oecologia*.
- Hauer, F. R., **B. J. Cook**, M. C. Gilbert, E. C. Clairain, Jr., and R. D. Smith. 2002. A Regional Guidebook for Applying the Hydrogeomorphic Approach to Assessing Wetland Functions of Intermontane Prairie Pothole Wetlands in the Northern Rocky Mountains, [ERDC/EL TR-02-7](#), U.S. Army Engineer Research and Development Center, Vicksburg, MS. p. 118. <http://www.wes.army.mil/el/wetlands/pdfs/trel02-7.pdf>
- Hauer, F. R., **B. J. Cook**, M. C. Gilbert, E. C. Clairain, Jr., and R. D. Smith. 2002. A Regional Guidebook for Applying the Hydrogeomorphic Approach to Assessing Wetland Functions of Intermontane Prairie Pothole Wetlands in the Northern Rocky Mountains, ERDC/EL TR-02-21, U.S. Army Engineer Research and Development Center, Vicksburg, MS. p. 159. <http://www.wes.army.mil/el/wetlands/pdfs/trel02-21.pdf>
- Hauer, F.R., **B. J. Cook**, M. Miller, C. Noble, and T. Gonser. 2001. Upper Yellowstone River hydrogeomorphic functional assessment for temporal and synoptic cumulative impact analyses. WRAP Technical Notes Collection, ERDCTN-WRAP-01-03, U.S. Army Engineer Research and Development Center, Vicksburg, MS. p. 16.

TIMOTHY E. SECOTT

Education

- 1977 – 1981 BS, Biology, Millersville University, Millersville, PA
- 1998 – 2002 PhD, Microbiology, Purdue University, West Lafayette, IN

Professional Experience

- 2003 – Present Minnesota State University, Mankato, Department of Biological Sciences
Mankato, MN, 56001. Assistant Professor
- 2002 – 2003 Purdue University, Department of Veterinary Pathobiology, West
Lafayette, IN 47907. Post-Doctoral Research Associate

Professional Society Memberships

American Society for Microbiology
International Association for Paratuberculosis
International Society for Infectious Diseases

Research Interests

Microbial community structure and function in vascular plant ecosystems, physiology of microbial dormancy and resuscitation, and mycobacterial attachment and invasion of mammalian epithelial cells. Current projects include 1) plant-soil feedbacks as a mechanism of invasion of wetlands by reed canarygrass, 2) succession of microbial communities in Antarctic glacial forelands, 3) detection and expression of autocrine growth factors that promote the resuscitation of dormant mycobacteria.

Selected Publications

Secott, T.E., T.L. Lin, and C.C. Wu. 2004. Mycobacterium avium subsp. paratuberculosis fibronectin-attachment protein facilitates M cell targeting and invasion through a fibronectin bridge with host integrins. *Infect Immun.* 72: 3724-3732.

Secott, T.E., T.L. Lin, and C.C. Wu. 2002. Fibronectin attachment protein is necessary for efficient attachment and invasion of epithelial cells by *Mycobacterium avium* subsp. *paratuberculosis*. *Infect Immun.* 70: 2670-2675.

Secott, T.E., T.L. Lin, and C.C. Wu. 2001. Fibronectin attachment protein homologue mediates fibronectin binding by *Mycobacterium avium* subsp. *paratuberculosis*. *Infect Immun.* 69:2075-2082.

Recent Poster Presentations

Daddacha, W., C. Ruhland, M. Krna, S. Strauss, T. A. Day and **T. Secott**. 2006. Comparison of microbial plant litter degradation potential in transitional and established Antarctic Peninsula

vascular plant ecosystems. 66th Annual Meeting, North Central Branch, American Society for Microbiology. Brookings, SD. October 20-21.

Strauss, S., C. T. Ruhland, **T. E. Secott**, and T. A. Day. 2006. Soil development and nutrient cycling during early succession of glacial forelands along the Antarctic Peninsula. Annual Meeting, Ecological Society of America, Memphis TN, July.

Rock, K.E., and **T. E. Secott**. 2006. Effect of oxygen depletion and conditioned media on the recovery in culture of dormant *Mycobacterium avium* subsp. *paratuberculosis*. 106th General Meeting of the American Society for Microbiology. American Society for Microbiology, Orlando, FL May 21-26.

Day, T.A., D.M. Bryant, S. Strauss, M. Thomey, **T.E. Secott**, and C.T. Ruhland. 2005. Effects of climate manipulation on vascular plant-dominated tundra along the Antarctic Peninsula. Synthesis of Biodiversity and Ecosystem Functioning in Victoria Land, Antarctica: an NSF-Sponsored Workshop, Jekyll Island, GA, April 21-23.

Selected Grants/Contracts

USDA NRI-CSREES, Spring 2007. \$230,000 (Pending). Water Quality Impairment and Fecal Indicator Bacteria Mobility in Southeastern Prairie Pothole Watersheds. Co-PI

MSUM Faculty Research Grant, Fall 2005. \$4500. Comparison of the structural and functional diversities of microbial communities in aged and recently emerged tundra ecosystems on the Antarctic Peninsula. PI.

American Society for Microbiology Undergraduate Research Fellowship, Summer 2005. \$5000. Effect of oxygen depletion and conditioned medium on the recovery in culture of dormant *Mycobacterium avium* subsp. *paratuberculosis*. PI.

Southern MN Water Resources Center, Summer-Fall 2005. \$5000. Use of fecal anaerobe DNA markers to indicate point sources of fecal contamination of water. Contract.

Li-Cor Genomics Education Matching Funds Program Grant, Summer 2004. \$40,000 Co-PI.

MSUM Faculty Research Grant, Fall 2003. \$4000. Elucidation of the Role of the Mycobacterial Cell Entry Gene in Attachment and Invasion of Epithelial Cells by *Mycobacterium avium* subsp. *paratuberculosis*. PI.

Bertha L. Proctor, Ph.D.

Education: SUNY Buffalo, Buffalo NY
1978 Ph.D. Biology (Limnology)
1975 M.S. and M.A. Natural Sciences and Biology (Ecology)
1972 B.A. Biology and Chemistry

Professional Experience:

1987-Present Professor, Program Director Environmental Sciences Program,
Department of Biological Sciences; Director Environmental Quality
Laboratory, Associate Director Water Resources Center (1990-2000) at
MN State University Mankato. 1987-1992 Associate Professor, Program
Coordinator Environmental Sciences Program,

1980-1987 Assistant Professor, Director GC-MS Laboratory, Graduate Program in
Environmental Sciences, University Texas-Dallas, Richardson, TX

Select External Funding:

Urban Stream Assessment-Thompson Ravine (\$100,434) with Robert Finley (2001-2003)
Rush River Assessment (\$27,500) with Robert Finley (2003-2005)
High Island Creek (\$33,633) with Robert Finley (2000-2002)
Conservation Partnership Grant (\$8,000) 2000-2001
NSF Ecology Workshop (\$506,000) with Dr. John Frey (1992-1995)
Minnesota River Assessment Project, Biological Assessment (\$75,000) 1989-1993

Select Publications and Reports:

Quade, H. and B. Proctor. 2004. The Rapidan Dam Research Project: Environmental Impacts of
Converting a Run-of-the-River Low Head Hydroelectric Dam to a Peaking Operation. Report to
Blue Earth County Board of Commissioners and MN Pollution Control Agency.

Proctor, B. 2004. Macroinvertebrates as an Indicator of Water Quality in the Rush River.
CleanWater Partnership Project on the Rush River, Report to the MN Pollution Control Agency.

Proctor, B. 2003. High Island Lake Water Quality Report, Report to Sibley County and the MN
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