

Project title:

Identification and Characterization of the Bacteria that Form Root Nodules with Pea Plants at the SAP.

Submitted by

Jennifer Winther (Biology) and Gwynne Powell (undergraduate)

Section 1: Project Description and Abstract

Nitrogen is a major limiting nutrient for plant growth. Plants with root nodules make their own chemical nitrogen (ammonia) from nitrogen gas and do not require the addition of nitrogen rich fertilizers for growth. Root nodules are plant structures that house special bacteria that convert atmospheric nitrogen (N_2) into ammonia (NH_3). In fact, nodule forming plants (e.g. legumes including soybeans and peas) increase the soil concentration of available nitrogen and have long been used in sustainable agricultural practices. The purpose of this research project is to identify and cultivate the species of bacteria that form symbiotic root nodules on pea plants grown at the SAP. Recent studies have demonstrated that there are more species of bacteria that form root nodules than previously thought and that some bacteria species provide more benefits to certain legumes than others. Once the bacterial symbionts have been identified and cultivated, we plan to quantify the benefit of the different bacterial species to the pea plants in a controlled greenhouse setting. At the completion of this project we hope to have identified those bacteria that are the best nodule symbionts with the peas grown at the SAP and to be able to provide the SAP with beneficial bacteria inoculum.

Section 2: Engagement of Undergraduate Students

This research is being developed with, and will be carried out by, the undergraduate student Gwynne Powell. There is the hope and expectation that other interested undergraduate students will take part. It is expected that this research will lead to future projects based around plant symbionts and sustainable agriculture practices.

Section 3: Budget Justification

The proposed research project has three major areas of cost: 1. Isolation and cultivation of nodule bacteria species; 2. Identification of bacteria species based on sequencing; and 3. Assessing the benefit of isolated bacteria species on the pea plants.

Isolation and cultivation: Yeast mannitol agar with Congo red is needed to isolate and culture bacteria (\$120). Petri dishes and culture tubes must be purchased to store and isolate bacteria (\$100). In order to assess the growing conditions of the pea plant, detailed soils analyses (including nitrogen measures) are required from plants grown at the farm and those grow in the greenhouses. In total 4 soil samples will be sent to MSU for analysis (\$100). Total: \$320

Identification of Bacteria Species: The only way to definitively identify bacteria species is to use sequence data. We will use the well-established bar coding region of the small subunit of the ribosomal DNA (16S). The 16S region is directly amplified from isolated bacteria via Polymerase Chain Reaction (PCR) using specific primers. dNTPs (\$150) must be purchased to supplement PCR chemicals and materials already in possession. The 16S region amplified via PCR must be treated with exo-sap before sequencing (\$200). Sequencing is \$8 per sample at Annis Water Research Institute (this includes sequencing using the forward and reverse primers for each PCR product). In the initial screening we plan to isolate bacteria from up to 70 different nodules. We also will need to confirm the identity of the bacteria from the nodules of the inoculated greenhouse grown plants, up to 30 more samples. In total, we plan to sequence 100 different bacteria PCR samples for a total sequencing cost of 800 dollars. Disposables and other chemicals are already available. Total: \$1150

Assessing benefit of different bacteria species: Up to ten different isolated bacteria species will be chosen to inoculate peas and grow in the greenhouse. There will be at least ten replicates for each treatment and control for a total of 110 plants. We will need to purchase pots for the greenhouse (60 dollars). In addition to measurements based on mass, length, and # of nodules, we want to quantify the benefit of the bacteria to plant nutrition by analyzing the nutrient components of the plant tissue. We will send plant tissue samples from the SAP and from the greenhouse experiment for nutrient analysis at MSU, for a total of 15 samples (15X24=360 dollars). Total: \$420

The total cost excluding stipend is \$1890

The research will have direct benefits to the SAP and will lead to the development of future projects and research directions in Dr. Winther's lab and classes (see faculty research statement). Gwynne Powell is a dedicated student and involved at the SAP. During Fall 2017 Gwynne mastered the techniques required to complete the proposed project in a timely manner. She is dedicated to completing the project and disseminating the results. We estimate that Gwynne will spend upwards of 200 hours on this project this spring and summer and spend 50+ hours towards preparing and presenting the study to various audiences including the Sustainability Showcase at the end of the Fall 2018 semester. Although this project will be pursued if the grant is funded without a stipend, we are requesting a stipend in the amount of \$2000 for Gwynne Powell.

Total cost of funding the grant with a stipend is \$3890.

Project Description and Research Methodology

Nitrogen is a major limiting nutrient in plant growth because it is a chief component of all proteins and genetic material in every plant cell. Although 78% of the Earth's atmosphere is comprised of nitrogen gas (N₂), that nitrogen is not available for most life to use as building blocks. Atmospheric Nitrogen must be reduced to ammonia either through biological nitrogen fixation, through human activity via the Haber-Bosch process, or through lightening. Most atmospheric nitrogen is converted into ammonia by a relatively small subset of Bacteria and Archaea that have the nitrogenase enzyme. In conventional agriculture systems, the nitrogen demand of crop plants is often met with the addition of human generated nitrogen which is monetarily costly, resource intensive, and inefficient. In sustainable agriculture systems, crop rotation, ground cover, intercropping, and optimization of organic fertilizer use have been developed to maximize nitrogen use efficiency and decrease inputs (Spiertz, 2010). Many of these sustainable practices exploit a mutualistic symbiosis found between Rhizobia bacteria and the roots of legumes (e.g. clover, soybeans, peas, beans). In these Rhizobia-legume symbioses, the legumes provide food and housing to the bacteria in root nodules and, in exchange, the bacteria fix atmospheric nitrogen into biologically available ammonia. This allows plants to thrive in low nitrogen environments and the plant biomass eventually contributes to the available nitrogen content in the soil.

In consultation with Youseff, we have decided to study the rhizobia-legume symbiosis found in peas grown at the SAP. Peas are one of the first crops planted in the hoop houses in February for Spring Harvest. As such, peas play an important role in establishing the soil conditions for the rest of the growing season. In addition, Youseff is interested in exploring the benefits of adding a soil probiotic (EM-1) on crop production. This proposed study allows him to gain information on another dimension of plant health (state of root symbioses) in order for him to further assess the impact of that treatment. The main goal of this research project is to identify, cultivate and characterize the species of bacteria that form symbiotic root nodules on Peas grown at the SAP. We will isolate and compare nodule forming bacteria between peas grown in soil treated with EM-1 and to those without, and then assess the impact of some of those bacteria on peas in a greenhouse experiment.

Currently there are 14 genera of bacteria that are known to form Rhizobia-legume associations (Lindstrom and Mousavi, 2010). Based on previous work in my lab on the role of intercropping clover on microbial diversity, we know that all 14 genera are found in the soil at the SAP. What is unknown is what is the identity of the bacterial symbionts with SAP crop legumes and if the identity matters? Recent studies have demonstrated that different species of rhizobia bacteria have different impacts on growth and nutrient quantity in the legume crop (Hildago et al., 2017; Zou et al., 2016; Bianco et al., 2013; Laranjo et al., 2013). At the completion of this project we hope to have identified those bacteria that are the best nodule symbionts with the legumes grown at the SAP and to be able to provide the SAP with bacteria inoculum that could help support the economic and ecological sustainability goals of the SAP.

The project has two major phases: 1. Isolation and identification of Rhizobia species and 2. Experimentally testing the effectiveness of the different Rhizobia species in a greenhouse setting. Isolation and identification of bacteria symbionts will commence at the end of April and should

be completed by mid-May. The greenhouse experiment will start mid-May and continue through the beginning of August. Data collection, interpretation and dissemination will continue through Fall 2018 semester. We will follow the established protocol of isolating and cultivating rhizobia from crushed root nodules (Somasegaran & Heinz, 1994). Rhizobia species are all gram negative and capable of being cultured on Yeast Mannitol agar with Congo Red where the bacteria colonies will be white, raised, and have an entire margin (Somasegaran & Heinz, 1994). Isolated bacteria will be identified using sequence data. We will PCR amplify the small subunit of the ribosomal DNA barcoding region (~700 bp) using established primers and protocols (Zou et al., 2016). PCR products will be screened, cleaned and sent for sequencing at AWRI. Sequences will be analyzed using standard phylogenetic methods. Up to ten different bacterial species will be chosen to inoculate the legume of choice and grown in the greenhouse. After at least 8 weeks of growth, various measures of productivity and symbiont effectiveness will be measured including total biomass, number of nodules, and plant nutrient composition. There will be at least ten replicates for each treatment and control for a total of 110 plants. Plants will be grown in the KHS greenhouse in Allendale in soil sterilized from the SAP. Identity of bacterial symbionts will be verified via sequencing of select nodules.

This project has been developed with input from Youssef and the undergraduate Gwynne Powell. We have also consulted with the greenhouse manager Christina Hipshier. We do not anticipate that this project will have any negative impact on the SAP as plants for study will be collected after harvest (or during routine thinning). In planning this project, we have considered a variety of ways to involve and inform the diverse SAP community. These include setting up an informational booth at the farmer's market and/or farm stand; presenting our research at the Sustainability Showcase at the end of the Fall 2018 semester; attending Farm Club meetings, Biology Club meetings, and SAP volunteer hours to discuss the project and seek feedback from GVSU students; and presenting at the Van Andel West Michigan Regional Undergraduate Science Research Conference. This project seeks to reflect the SAP's mission and values by exploring the ecological components of sustainable food systems, including SAP community members from multiple disciplines in its development, and focusing on student-led investigation.

References

Bianco L, J Angelini, A Fabra, and R Malpassi. 2013. Diversity and Symbiotic Effectiveness of Indigenous Rhizobia-Nodulating *Adensmia bicolor* in soils from Central Argentina. *Current Microbiology* 66: 174-184. doi: 10.1007/s00284-012-0260-y.

Hildago A, FJ Lopez-Baena, JS Ruiz-Sainz, and JM Vinardell. 2017. Studies of rhizobial competitiveness for nodulation in soybean using a non-destructive split-root system. *AIMS Microbiology* 3(2): 323-334. doi: [10.3934/microbiol.2017.2.323](https://doi.org/10.3934/microbiol.2017.2.323).

Laranjo M, A Alexandre, and S Oliveira. 2013. Legume growth -promoting rhizobia: An overview on the *Mesorhizobium* genus. *Microbiological research* 169: 2-17. doi: <https://doi.org/10.1016/j.micres.2013.09.012>

Lindstrom K and SA Mousavi. 2010. Rhizobium and other N-fixing Symbiosis. In: *Encyclopedia of Life Science (ELS)*. John Wiley & Sons, Ltd: Chichester.

doi:10.1002/9780470015902.a0021157

Somasegaran PH and H Heinz. 1994. Handbook for Rhizobia: Methods in legume-Rhizobium technology. xvi, 450. doi: 10.1007/978-1-4613-8375-8.

Spiertz, JHJ. 2010. Nitrogen, Sustainable Agriculture and food security: a review. *Agronomy for Sustainable Development* 30:43-55 doi: <https://doi.org/10.1051/agro:2008064>

Zou L, YX Chen, P Penttinen, Q Lan, K Wang, M Liu, D Peng, X Zhang, Q Chen, K Zhao, X Zeng, and KW Xu. 2016. Genetic Diversity and Symbiotic efficiency of nodulating Rhizobia isolated from root nodules of Faba bean in one field. *Plos One* doi: <https://doi.org/10.1371/journal.pone.0167804>

2. Updated Faculty CV

Jennifer Winther

Department of Biology
Grand Valley State University
Henry Hall 224
Allendale, MI 49401
e-mail: jwinthe@gvsu.edu
616-331-5060

CURRICULUM VITAE

Education

University of Colorado, Boulder

PhD: Ecology & Evolutionary Biology, 2008

Dissertation Title: Arbuscular Mycorrhizal Associations in Ferns and Lycopods

Advisor: Dr. William Friedman

University of Chicago

Bachelor of Arts: Biology, 2000

Professional Experience

Assistant Professor, Grand Valley State University, Fall 2011-current

Visiting Assistant Professor, Colby College, Fall 2010- Spring 2011

Teaching Fellow, Harvard University, Spring Semester 2010

Postdoctoral Research Fellow, Harvard University, Cambridge, January 2008-January 2010

Research Assistant, University of Colorado, Boulder, Summer 2000, Summer 2001, 2002-Spring 2004, Spring 2005 (NASA Astrobiology Grant to William Friedman)

Teaching Assistant, University of Colorado, Boulder, Academic year 2000-2002, Fall 2004, Spring 2006, Spring 2007

Teaching Assistant, University of Chicago, 1999-2000

Peer Reviewed Publications

Winther, Jennifer L. and William E. Friedman. 2009. Phylogenetic affinity of arbuscular

mycorrhizal symbionts in *Psilotum nudum*. *Journal of Plant Research* 122(5): 485-496

Winther, Jennifer L. and William E. Friedman. 2008. Arbuscular mycorrhizal associations in Lycopodiaceae. *New Phytologist* 177 (3): 790-801.

Winther, Jennifer L. and William E. Friedman. 2007. Arbuscular mycorrhizal symbionts in *Botrychium* (Ophioglossaceae). *American Journal of Botany* 94(7): 1248-1255.

Manuscripts in preparation

Winther, Jennifer L. and Elena M. Kramer. An expanded analysis of gymnosperm type II MADS box genes reveals new complexity in gene lineage evolution. *PLOS ONE*.

Winther, Jennifer L. and Kennedy Aldrich. Diversity and distribution of arbuscular mycorrhizal fungi colonizing fruit trees in a sustainable small market garden. *Mycoscience*

Professional Fellowships and Grants

S3," Sponsored by Office of Undergraduate Research and Scholarship (OURS), Grand Valley State University, \$6,000.00. CO-PI
Spring 2016
“The Effect of Interseeded White Clover on Soil Microbial Diversity in Small-Scale Market Gardens”

S3," Sponsored by Office of Undergraduate Research and Scholarship (OURS), Grand Valley State University, \$6,000.00.
Spring 2014
“Fungal associations in Fruit trees”

"S3," Sponsored by Office of Undergraduate Research and Scholarship (OURS), Grand Valley State University, \$6,000.00.
Spring 2012
“MADS-box genes in gymnosperm cones”

Putnam Postdoctoral Fellowship, Harvard University (\$84,000.00) Jan 2008-Jan 2010

Invited Presentations

Winther, Jennifer. Hillsdale College, November 2, 2015. Evolution and of AM fungal associations. Hillsdale College, Hillsdale, MI

Winther, Jennifer. Hillsdale College, November 5, 2015. Ecology and Evolution and plant-AM fungal associations. Hillsdale College, Hillsdale, MI.

Winther, Jennifer. Hope College Seminar Series, February, 2014. Fungal associations in ferns and farms. Hope College, Holland, MI.

Winther, Jennifer L Arnold Arboretum Seminar Series. July 2011. New insights in the evolution and development of gymnosperm cones. Arnold Arboretum, Boston, MA.

- Winther, Jennifer L Harvard Herbarium Seminar. April 2010. New insights on type II MADS-box evolution in seed plants. Harvard University, Cambridge, MA.
- Winther, Jennifer L Harvard Herbarium Seminar. March 2008. Mycorrhizal symbioses in subterranean ferns and lycopsids. Harvard University, Cambridge, MA.
- Winther, Jennifer L XVII International Botanical Congress. July 2005. The ecology and evolution of endomycorrhizal associations in early lineages of land plants. Vienna, Austria.
- Winther, Jennifer L Ferns for the 21st century. Royal Botanic Gardens Edinburgh, 2004. The early evolution of mycorrhizal associations in land plants: a phylogenetic and evolutionary developmental perspective. Royal Botanic Gardens Edinburgh, Scotland

Oral Presentations

- Winther, Jennifer L. 2013 Fall Science Update. Tomatoes are berries? GVSU, Grand Rapids, MI.
- Winther, Jennifer L. Nov 2011. CLAS faculty research colloquium, "How is a pine cone like a flower? Understanding the evolution and development of gymnosperm cones," GVSU, Allendale.
- Winther, Jennifer L. 2011 Fall Science Update. Using Metamorphosis to Address Classification and Adaptation. GVSU, Grand Rapids, MI.
- Winther, Jennifer L. and Elena Kramer. July 2008. B and C class MADS-box gene diversity in gymnosperms. Annual meeting of the Botanical Society of America. British Colombia, Canada.
- Winther, Jennifer L. and William Friedman E. July, 2007. Mycoheterotrophic arbuscular mycorrhizal symbioses in ferns and lycopods. Joint Botanical Congress. Chicago, IL.
- Winther, Jennifer L. and William Friedman E. July, 2005. Evolution of arbuscular mycorrhizal symbioses in early lineages of land plants. Annual meeting of the Botanical Society of America, Austin, TX.

Posters

- Michalski, Michael, Sheila Blackman, and Jennifer Winther. April 2017. The Effect of Interseeded White Clover on Soil Microbial Diversity in Small-Scale Market Gardens. Student Scholars Day, GVSU, Allendale, MI
- Michalski, Michael, Sheila Blackman, and Jennifer Winther. April 2017. The Effect of Interseeded White Clover on Soil Microbial Diversity in Small-Scale Market Gardens. MEEC, University of Illinois Urbana-Champaign ,IL

- Michalski, Michael, Sheila Blackman, and Jennifer Winther. November 2016. The Effect of Interseeded White Clover on Aspects of Soil Ecosystem Function in Small-Scale Market Gardens. Michigan Consortium of Botanist, Allendale, MI
- Michalski, Michael, Sheila Blackman, and Jennifer Winther. July 2016. The Effect of Interseeded White Clover on Soil Ecosystem Function in Small-Scale Market Gardens. Student Scholars Showcase, GVSU, Allendale, MI
- Aldrich, Kennedy and Jennifer Winther. April 2015. Symbiotic Relationships Between Arbuscular Mycorrhizal Fungi and the Roots of Fruit Trees. Student Scholars Day, GVSU, Allendale, MI
- Aldrich, Kennedy and Jennifer Winther. April 2014. Symbiotic Relationships Between Arbuscular Mycorrhizal Fungi and the Roots of Fruit Trees. Student Scholars Showcase, GVSU, Allendale, MI
- Aldrich, Kennedy and Jennifer Winther. July 2014. Symbiotic Relationships Between Arbuscular Mycorrhizal Fungi and the Roots of Fruit Trees. Student Scholars Showcase, GVSU, Allendale, MI.
- Winther, Jennifer L., Yanoschik, Michael, Gardner, Levi and Students of BIO 380. July 2013. Identity of arbuscular mycorrhizal fungi in sustainably managed agricultural crops. Botanical Society of America annual meeting, New Orleans, LA.
- Maryanski, Danielle and Jennifer L. Winther. July 2013. "Type II MADS-box Genes in Juniper Male and Female Cones. Botanical Society of America annual meeting, New Orleans, LA
- Maryanski, Danielle and Jennifer L. Winther. Nov. 2012. Type II MADS-box genes isolated from Juniper and Ephedra. Western Michigan Undergraduate Showcase, Van Andel Institute, Grand Rapids. MI.
- Maryanski, Danielle and Jennifer L. Winther. August 2012. Novel MADS-Box genes isolated from the gymnosperms Juniper and Ephedra. Student Summer Scholar exhibition, GVSU, Grand Rapids, MI.
- Greer, G., Winther, J. L., Evans, T., Toogood, J. June 2012. Community Assembly of Lower-Trunk Epiphytes At Mid-Elevation in El Yunque National Forest. Annual Meeting of Luquillo Long-Term Ecological Research Station. Luquillo, LTER and University of Puerto Rico, Rio Peidras, San Juan, Puerto Rico.
- Winther, Jennifer L. and Elena Kramer. April 2010. Major lineages of Seed Plant Type II MAD-box genes: new insights from diverse gymnosperms. 5th Annual Plant Biology Symposium. Harvard University, Cambridge, MA.
- Winther, Jennifer L. and Elena Kramer. April 2008. MADS-box gene diversity in

gymnosperms. 4th Annual Plant Biology Symposium. Harvard University, Cambridge, MA.

Winther, Jennifer L. and William Friedman E. March, 2005. Catastrophic asteroid impacts and the prevalence of non-photosynthetic underground plants. NASA Astrobiology Annual Meeting. Boulder, CO.

Professional Review Experience

Symbiosis
American Fern Journal
Mycorrhiza
Molecular Phylogenetics and Evolution
Genetica

Department Service

Personnel Committee, Committee Member	Fall 2014 - Present
Biology Advisory Committee, Committee Chair,	Fall 2013 - Present
Biology Curriculum Committee, Committee Member,	Fall 2012 – Present
Visiting, Affiliate, and Adjunct Committee, Committee Member	Fall 2011-Winter 2014

College and University Service

CLAS Advising Mock MMI interviews	Fall 2017
University Academic Senate	Fall 2016-present
CLAS Curriculum Committee, Sabbatical Replacement,	Winter 2015
Majors Fair.	Fall 2015, Fall 2017
Awards of Distinction Scholarship Competition.	Winter 2015, Winter 2017.
Open Advising Session	Winter 2014, Winter 2017
Midwest Association of Graduate Schools Thesis Award, Committee Member,	Fall 2012, Fall 2013
Grand Valley Laker Experience Days, Biology Representative	Winter 2013

Research Space Committee, Committee Member,(Replacement) Winter 2012

Community Service

Science Olympiad, Session Chair, Invasive Species B Winter 2016, 2017

Science Olympiad, Score keeper, Winter, 2014

Science Olympiad, Session Chair, Forestry C Winter 2013, 2012

Fall Science Update Fall 2013, Fall 2011

Professional Service

National Science Foundation, Pre-proposal Reviewer March 24-26th 2014

Recent Teaching Experience

Evolution, BIO 210

Biology Capstone Bio 495

Plant Symbiosis BIO 380-10 (Now BIO 383 plant-fungal interactions)

Biology BIO120 (GVSU)

Genetics BIO 375 (GVSU)

Genetics Lab Bio 376

Membership in a Professional Society

Botanical Society of America

3. Faculty Research/Scholarship Statement: Demonstrate how this project fits with your larger scholarly/creative agenda

I am broadly interested in plant root symbionts and have been studying them since my PhD on fungal symbionts in the underground structures of ferns. This proposed research represents my fourth-time pursuing scholarship with students at the SAP. My interest in the SAP started while I was developing my plant symbiosis class (now called plant-fungal interactions, BIO 383). During that time Levi Gardner was spear heading the growth and development of the SAP. I approached him to allow my students to identify the fungal root symbionts in various crops. The students spent all semester on this project and presented their results to Levi during formal presentations and the results were also presented at a national conference as a way to showcase student research at student run farms. A couple students from the class even became involved in the SAP after learning about it in class. I have been a part of two S3 grants that conducted research at the SAP. Kennedy Aldrich (currently attending College of Veterinary Medicine at MSU) conducted research on the diversity and distribution of AM fungi on fruit trees at the farm (sadly those trees died years ago) and we are currently in the process of submitting that research for publication in a peer reviewed journal. In collaboration with Sheila Blackman, we co-advised Michael Michalski (expected to graduate Winter 2018) on his work on the role of intercropping clover on small market gardens in collaboration with Dr. Blackman's BIO 319 sustainable agriculture class. We are still in the process of data analysis with plans to prepare a manuscript for submission. I am committed to continuing to explore the role of plant symbionts in agriculture and incorporating this topic into my classes. In the near future I would like to teach BIO 319. I am excited about this project because it represents the first time I am moving beyond a description of the diversity to testing the impact of that diversity on plants. I am looking forward to seeing how this project develops and the directions it will take my scholarship and classroom teaching.

4. Letter of Support from Department Chair, or Unit Head



College of Liberal Arts and Sciences
Biology Department

January 25, 2018

Office of Undergraduate Research and Scholarship
230 Mary Idema Pew Library
1 Campus Drive
Allendale, MI 49401

Dear Members of the SAP Grant Proposal Review Committee:

Dr. Jennifer Winther has requested that I submit a letter in support of her application for a Sustainable Agriculture Place-Based Project Grant, and I am pleased to do so. Dr. Winther and Gwynne Powell, the undergraduate student collaborator, propose to identify the bacterial symbionts of legumes in soils of the SAP hoop house using genetic sequencing, isolate them by species, and test the effects of each bacterial species on the response of inoculated pea plants in the greenhouse. The proposed project will provide a high-impact research experience for one of our undergraduate students, while advancing our knowledge of the soil microbiota, a growing area of research that has been facilitated through the use of genetic sequencing. The results of the proposed project will provide detailed information that will benefit the management of hoop-house soils at the SAP by identifying which bacterial species represent the best symbionts for legumes being grown there, and will be able to provide a customized bacterial inoculant that is optimally suited to the soil/plant system present at the SAP. The proposed research is synergistic with Dr. Winther's teaching responsibilities and research expertise, and is strongly aligned with the Department's strategic plan goal of providing our undergraduate students with high-impact experiences during their academic careers at GVSU. For all of the reasons summarized above, I am happy to provide my strongest recommendation in support of this proposal.

Sincerely,

Neil W. MacDonald, PhD
Professor of Biology and Natural Resources Management
Chair, Biology Department

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