Environment and Natural Resources Trust Fund
2017 Request for Proposals (RFP)

Project Title: **ENRTF ID: 120-D**

Microbial Associates of the Emerald Ash Borer

Category: D. Aquatic and Terrestrial Invasive Species

Total Project Budget: $ 400,000

Proposed Project Time Period for the Funding Requested: 3 years, July 2017 – June 2020

Summary:

This project will investigate microbes associated with the invasive Emerald Ash Borer with the goal of identifying strain or chemical compounds that can be used for biological control.

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Location

Region: Statewide

County Name: Statewide

City / Township:

Alternate Text for Visual:

Project Workflow

_____ Funding Priorities	_____ Multiple Benefits	_____ Outcomes	_____ Knowledge Base
_____ Extent of Impact	_____ Innovation	_____ Scientific/Tech Basis	_____ Urgency
_____ Capacity Readiness	_____ Leverage	_____ TOTAL	_____%

PROJECT TITLE: Microbial Associates of the Emerald Ash Borer

I. PROJECT STATEMENT

This project will investigate microbes associated with the EAB and closely related Bupestrid beetles in Minnesota with the goals of 1) identifying microbial strains associated with the EAB that can be used as biocontrol agents, and 2) identifying chemical signaling compounds produced by either beetle or microbes involved in the invasive symbiosis that can also serve as targets for biocontrol. Since its introduction to North America in 2002, the Emerald Ash Borer (EAB) has spread rapidly across Eastern North America and into the Midwest and now poses a severe threat to the many natural forest resources of Minnesota. Microbes associated with insects and are increasingly believed to be key factors contributing to the success of invasive insect species. While research is investigating biocontrol with parasitoids of the EAB, current control strategies rely on standard quarantine and removal approaches and there is great need to identify novel strategies to control this invasive pest.

Microbial symbionts play important roles in mediating tree-insect interactions. For example, Bark and Ambrosia beetles (Family Curculionidae), wood boring beetles that carry fungal and bacterial symbionts in specialized structures called mycangia, introduce their microbial associates directly into tree hosts. Symbionts perform many functions that increase beetle survival and tree damage, from serving as food for larvae to producing enzymes that enhance wood decay or detoxify plant defense compounds to producing compounds that mimic beetle pheromones that attract additional adult beetles to the infection site. The vast majority of pheromones belong to a class of volatile chemicals known as terpenes. Changes in the taxonomic composition and/or biochemical capacity of microbial symbionts have been shown to contribute to invasiveness by allowing a greater range of responses of all organisms involved in the symbiosis. For example, the red-turpentine beetle (RTB), introduced into China from North America in the 1970s, acquired a novel Chinese strain of its primary fungal symbiont (2) that enabled it to expand into a major pest of pine forests responsible for killing over 10 million pine trees. Conversely, the Emerald Ash Borer (EAB) (*Agrilus planipennis,* Family Buprestidae) which was introduced from Asia and may have also experienced changes in microbial symbionts that allow it to be highly pathogenic in North America. **By characterizing the hidden taxonomic and chemical diversity of microbes associated with the Emerald Ash Borer and closely related Buprestid beetles native to Minnesota, we will 1) identify symbionts that contribute to invasiveness of the EAB and 2) identify chemical signaling compounds involved in invasion that can be targeted for biocontrol.**

II. PROJECT ACTIVITIES AND OUTCOMES

Activity 1: Identify and characterize microbial communities associated with the EAB and closely related Buprestrid beetles. **Budget: $250,000**

External and gut-inhabiting microbial symbionts will be characterized by both traditional culturing methods to create a strain depository and high-throughput sequencing approaches to identify shifts in microbial assemblages associated with EAB and related native Buprestid beetles. Bacterial symbionts will be characterized by 16S-based marker metagenomics. Fungi and yeast will be identified using the ITS1 and LSU regions of the fungal ribosomal RNA. **Expected Outcomes:** Metagenomic datasets generated for this project will allow comparison of microbial assemblages associated with the EAB with those of related native beetles to identify the key microbial players in EAB. Isolation and sequencing of strains will facilitate experimental investigation of mechanisms (Activity) that contribute to invasiveness and allow future development of biocontrol strains.

Outcome	Completion Date
1. Culture and preserve strains of fungi and bacteria associated with the EAB	*June 2018*
2. Metagenomic analyses of fungal and bacterial communities associated with the EAB	*June 2019*
3. Analysis of data and identification of key strains associated with the EAB	*June 2020*

Activity 2: Characterize terpene metabolism of microbial symbionts using both chemical and functional metagenomic approaches **Budget: $150,000**

Terpene metabolism of microbial associates will be characterized by both chemical and genomic/bioinformatics approaches: 1) GC/MS headspace analyses of selected microbial strains will be used to identify production of specific beetle pheromones, 2) Terpene anabolic and catabolic pathways will also be characterized computationally from metagenomic and genomic data of microbial symbionts. **Expected Outcomes:** The identification of terpenes that function as either insect hormones or in the degradation of plant defense compounds will identify chemical mechanisms that can be targeted as attractants for biocontrol.

Outcome	Completion Date
1. GC-MS headspace screening of fungal and bacterial strains	*June 2019*
2. Metagenomic or genomic analysis of terpene pathways in key strains associated with EAB	*June 2019*
3. Chemical fractionation and isolation of compounds from strains with high activity	*June 2020*

III. PROJECT STRATEGY

A. Project Team/Partners

The Bushley laboratory has extensive experience in working with fungi and other microbes associates with insects and in genomic and metagenomic technologies. The PI has collaborated this past year with the EAB monitoring program at the Minnesota Department of Natural Resources (DNR) for outreach activities to the general public through workshops led by the DNR on the EAB and will continue to do so for this project. We will also investigate collaborating with the EAB monitoring program at the USDA to assist in collection of beetles.

B. Project Impact and Long-Term Strategy

Characterizing the microbial communities associated with the EAB has great potential to identify novel targets for biocontrol approaches that are badly needed to protect natural resources in Minnesota. This project will provide a critical mass of data and biological resources (microbial strains) that will provide a material for future translational research to employ biological control strategies identified through this project. Data and results will be disseminated both to the scientific community through peer-reviewed publications, to the natural resources community in Minnesota, and to the general public through outreach workshops to involve citizens in collection and isolation of microbes from the EAB. Dr. Bushley also has ongoing collaborations with several researchers in China and funding is being sought through the NSF to enable work to compare microbes associated with various invasive beetles, including the Emerald Ash Borer, in both their native habitat in China and in the United States that would provide synergistic support to this project.

C. Timeline Requirements

This project is scheduled for completion in three years. **Year1:** The first year will focus on collection of EAB and other Buprestid beetle specimens from diverse locations around the state of Minnesota. Collection will occur both during the summer flight season employing flight traps to collect adults and during the winter to collect larvae during removal of infected trees. We also plan to complete isolation of microbial strains during year 1. **Year 2:** the second year will focus on DNA isolation and metagenomics analyses of EAB microbial communities and in beginning screening chemical profiles of living culture strains. **Year 3:** Analysis of metagenomics community data and isolation of novel chemical compounds identified in screening of strains during year 2.

2017 Detailed Project Budget

Project Title: Microbial Associates of the Emerald Ash Borer

IV. TOTAL ENRTF REQUEST BUDGET 3 years

BUDGET ITEM *(See "Guidance on Allowable Expenses", p. 13)*	**AMOUNT**
Personnel: Postdoctoral researcher (1) - 3 years at 100% FTE, base salary of $41,000 annually with standard fringe of 22.4%. [total $151,000, with 81.5% salary ($123,000) and 18.5% fringe ($28,000)]; Technician (1) - 2 years at 100% FTE, base salary of $36,000 with standard 27.4% fringe [total $92,000, with 78.3% salary ($72,000) and 21.4% fringe ($20,000)]; Undergraduate researchers (2-4) - funding for one undergraduate researcher during academic year ($5,000) and during summer ($5,000) for years 1 and 2 (total $20,000, 100% salary, no fringe)	$264,000
Professional/Technical/Service Contracts: UM BMGC sequencing center for both metagenomic and sanger sequencing of fungal isolates (Sanger sequencing $10,000, metagenomics - total $40,000); Chemical analysis at Center for Proteomics and Mass Spectrometry ($20,000);	$90,000
Equipment/Tools/Supplies: Extraction kits for genomic DNA ($6,000), Consumables (culturing media, petri plates, tubes and tips, PCR mix - $10,000/year - total $30,000), chemical standards ($4,000)	$40,000
Travel: Travel for research team to travel to EAB infested sites around state. Budgeted for up to 1500 mi/.54 (U of MN mileage rate) = $810, 9 nights accomodation at $75/night = $675 for fieldwork during years 1-2.	$3,000
Additional Budget Items: Publication costs	$3,000
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST	=$ 400,000

V. OTHER FUNDS *(This entire section must be filled out. Do not delete rows. Indicate "N/A" if row is not applicable.)*

SOURCE OF FUNDS	**AMOUNT**	**Status**
Other Non-State $ To Be Applied To Project During Project Period: *Funding is being sought but has not been received for NSF Dimensions of Biodiversity grant to investigate microbial symbionts of insects*	$500,000	*pending*
Other State $ To Be Applied To Project During Project Period: *None*	$ -	
In-kind Services To Be Applied To Project During Project Period: *Waived indirect costs (Facilities and Administrative costs) for University of Minnesota (53%)*	$212,000	*secured*
Funding History: *None*	$ -	
Remaining $ From Current ENRTF Appropriation: *None*	$ -	

Timeline

Activity 1: Years 1-3

1. Culture strains from ~200 beetles from diverse sampling locations (Years 1-2).

2. DNA isolations and sanger and metagenomic sequencing of microbial communitnies from exterior insect surface and guts (Year 2).

3. Complete and analyze metagenomic data using unsupervised clustering and ANOSIM across species and sampling locations (Year 3).

Activity 2: Years 2-3

1. Screen isolated microbes for headspace volatiles (Year 2)

2. Bioinformatic analysis of terpene pathways in key EAB associated strains (Year 3)

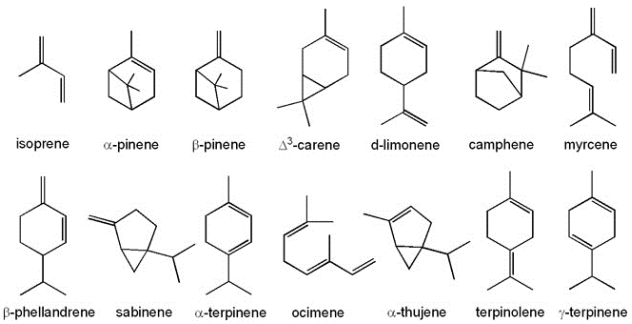
3. Chemical fractionation and isolation of compounds from high activity strains (Year 3)

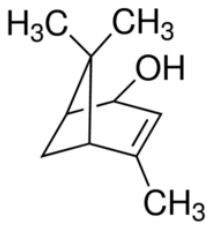
Project Workflow

Buprestid Beetles

bacterial → **16S rDNA**

fungal → **ITS/LSU**





(*S*)-*cis*-Verbenol 9

Project Manager Qualifications: The project manager, Dr. Kathryn Bushley, is an assistant professor in the Department of Plant Biology at the University of Minnesota. She is trained as a mycologist and plant pathologist (PhD, Molecular Plant Pathology, Cornell University, 2009). Since beginning her postdoctoral position at Oregon State University and the Institute of Microbiology of the Chinese Academy of Sciences (2009-2012), her research has focused on fungal symbionts and pathogens of insects. Her current research program at the University of Minnesota utilizes genomic and metagenomics techniques as well as chemical approaches to characterize fungi associated with insects and invertebrates and to investigate chemical compounds they produce for use in agriculture, natural resources, and medicine. She has experience managing projects involving large-scale isolation and metagenomics analyses of microbes. She is currently funded by the USDA to isolate and analyze fungal strains from the soybean cyst nematode (SCN) in Minnesota to identify potential biocontrol agents and novel nematicides produced by these fungi to control the SCN.

Selected publications: 1) Bajaj, R., Hu, W., Huang, Y., Chen, S., Prasad, R., Varma, A., and **Bushley, K.** 2015. The beneficial root endophyte *Piriformospora indica* reduces egg density of the soybean cyst nematode. Biological Control 90: 193-199**, 2) Kathryn E. Bushley**, Rajani Raja, Pankaj Jaiswal, Jason S. Cumbie, Mariko Nonogaki, Alexander E. Boyd, C. Alisha Owensby, Brian J. Knaus, Justin Elser, Daniel Miller, Yanming Di, Kerry L. McPhail, Joseph W. Spatafora. 2013a. The Genome of *Tolypocladium inflatum*: Evolution, Organization and Expression of the Cyclosporin Biosynthetic Gene Cluster. PLoS Genetics 9(6).

Organizational Description: The University of Minnesota is a public academic institution devoted to both research and teaching activities serving the state of Minnesota. **Laboratory space:** The project manager maintains a laboratory in Plant Biology in Bioscience 898 (898,890,890A, ~1200 sq. ft.) on the 8th floor of the Biological Sciences Building on the Saint Paul Campus. The laboratory contains two rooms for culturing fungi and molecular biological analysis. The first is equipped with a laminar flow hood dedicated to isolation of fungi from field material, a biosafety cabinet, full size incubators, shakers, and other equipment necessary for culturing fungi and bacteria. The other contains PCR machines, centrifuges, and other equipment for DNA extraction and preparation of metagenomics samples. The laboratory is designated as Biosafety level 2 to accommodate culturing of both insect and plant pathogenic fungi and has appropriate PP526 APHIS permits for both fungi and insects**. Computational resources:** All researchers in the Bushley laboratory are provided a desktop computer and software programs for sequence analysis (BioEdit, Sequencher), phylogenetic analysis (Mr.Bayes, RAxML, PHYML, etc.), word processing, electronic mail, and Internet access. Dr. Bushley is an active member of the Minnesota Supercomputing Institute (MSI) and all lab members have access to a >1000-node parallel Linux cluster with software packages for bioinformatics and metagenomic sequence analysis (e.g. BLAST, HMMR, alignment, mothur, Qiime etc.), genome analysis and assembly (e.g., ALLPATHS, Velvet, etc.). **Metagenomic Sequencing:** The University of Minnesota Genomics Center Core Facility (http://www.bmgc.umn.edu/) provides the following laboratory services relevant to this project: High Throughput DNA sequencing (Illumina HiSeq2000; Illumina MiSeq), Sanger DNA sequencing (ABI 3730xl), Bioanalyzer (Agilent Bioanalyzer 2100), and oligonucleotide synthesis. **Chemistry facilities:** The Bushley lab utilizes the University of Minnesota Mass Spectrometry Facility for chemical analyses (http://www.cbs.umn.edu/research/resources/center-mass-spectrometry-and-proteomics). The activities of the MS facility include core research, collaborative research, sample processing, and training of investigators to use equipment at an hourly charge. The center harbors ten distinct mass spectrometry machines, including a GCxGC TOF MS (Agilent 6890N GC, 7683B Autosampler) and a Saturn 3 Valarian/Agilent for Ion Trap MS or GC/MS with EI or CI Ionization.