Environment and Natural Resources Trust Fund
2020 Request for Proposals (RFP)

Project Title: **ENRTF ID: 254-FH**

Safe Biopesticides for Protection of Minnesota Groundwater Resources

Category: H. Proposals seeking $200,000 or less in funding

Sub-Category: F. Methods to Protect, Restore, and Enhance Land, Water, and Habitat

Total Project Budget: $ 199,000

Proposed Project Time Period for the Funding Requested: June 30, 2022 (2 yrs)

Summary:

In order to protect Minnesota groundwater and human health, this project will identify bio-based chemical controls from fungi to replace highly toxic nematicides against the soybean cyst nematode.

Name: Kathryn Bushley

Sponsoring Organization: U of MN

Job Title: Dr.

Department: Plant and Microbial Biology

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Location:

Region: Statewide

County Name: Statewide

City / Township:

Alternate Text for Visual:

Shows distribution of soybean cyst nematode overlaid on groundwater resources of MN and project workflow for isolating and testing targeted compounds form fungi for control of this nematode pathogen.

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

PROJECT TITLE: Safe Biopesticides for Protection of Minnesota Groundwater Resources

I. PROJECT STATEMENT

This project aims to protect ground and surface waters from dangerous pesticides by developing safer, more targeted bio-based treatments for soybean diseases. Soybean is a major crop planted on 7 million acres of land across Minnesota. Plant parasitic nematodes such as the soybean cyst nematode (*Heterodera glycines*; SCN) contribute to the largest yield losses (>30% in some fields) among all pathogens of soybean, yet there are limited methods available for their control. For many years, nematicides were used to effectively control the SCN, but many of these chemicals were recently banned due to evidence of toxic effects in humans and wildlife. For example, the fumigant dibromochloropropane is a carcinogen that can persist for nearly a decade in groundwater, while the carbamate nematicide aldicarb causes significant risks to both humans and wildlife through consumption of contaminated water and food. Other approaches to managing SCN include crop rotation, which requires longer rotations that may not be economically sustainable, and the use of resistant soybean varieties that rely heavily on a single source of plant resistance. Recent research shows the nematode is rapidly overcoming this resistance and few additional sources of plant resistance have been identified or developed, especially for northern maturity regions in Minnesota where SCN is a recent arrival. Breeding new resistant varieties can take decades and in the immediate future, farmers may be left with few alternatives but to return to use of highly toxic nematicides as there are currently no safe and effective alternatives. In order to balance the needs of farmers and the health of Minnesota groundwater, there is great need for development of bio-based and highly targeted natural chemical controls that will effectively manage these agricultural pathogens without threatening the safety and health of water resources. The Bushley and Chen laboratories have isolated over 1,000 fungi from soybean fields in Minnesota and different life stages of the nematode. Fungi isolated from nematodes are likely to be nematode parasites, many of which produce compounds that show specificity to the SCN, thus avoiding non-target effects and toxicity towards wildlife and humans. This project will use these naturally occurring fungi isolated from fields in Minnesota for development of novel bio-nematicides against this major pest of soybean to prevent contamination of Minnesota's water resources.

II. PROJECT ACTIVITIES AND OUTCOMES

Activity 1: Identify compounds that inhibit nematodes from fungal isolates

Description: This activity will conduct chemical analysis on fungal cultures (~40) that have been previously screened and show bioactivity against the SCN by growing the fungus in a liquid broth medium, collecting the liquid filtrate, and measuring survival of SCN eggs or juvenile worms in bioassays. Fungi will be grown in two different media to produce secondary metabolite compounds. Crude extracts will be made from liquid culture filtrate and fungal tissue using both aqueous and solvent based extraction methods and the raw extract will be divided into fractions to separate out compounds. Fungal filtrates, extracts, and extract fractions will be tested for bioactivity using established laboratory bioassays to identify the active components. Analytical chemistry approaches, including HPLC, LC-MS, and nuclear magnetic resonance spectroscopy will be used to identify and structurally characterize the active chemical compounds responsible for bioactivity. Active compounds will also be tested against non-pathogenic nematodes (*Caenorhabditis elegans*) and mammalian cells to determine their target specificity and potential safety. This approach will isolate pure chemical compounds with high antagonism to the SCN that can be developed for deployment as safe and targeted nematicides. This information can also be used to develop some fungal strains as biocontrol agents in field settings.

ENRTF BUDGET: $115,000

Outcome	Completion Date
1. Extraction of fungal filtrates and tissue into fractions: 40 strains x 4 extracts per sample	*November 2020*
2. Bioassay testing to identify active fractions: Nematode bioassays	*February 2021*
3. Isolation of pure compounds: Chromatography and purification of compounds, combined with iterative bioassay testing against nematodes.	*July 2021*
4. Specificity testing: Testing of the most bioactive compounds (~top 10-20) against mammalian cells and non-pathogenic nematodes	*September 2021*

Activity 2: Test isolated chemical compounds for activity against nematodes in plant bioassays.
Description: This activity will test whether the most active compounds and extracts isolated in Activity 1 are effective when applied to a soybean plant grown in soil. Different methods of application of the compound, including drip irrigation, soil drench, and seed coating treatment, will be used in greenhouse potted plant assays to assess efficacy under more realistic plant growth conditions. Soybean seeds will first be sterilized and then planted into a sterilized sand/soil (70%/30%) mix. One week after emergence, each plant will be inoculated with ~1000 SCN juvenile nematodes. Treatments will consist of a control and the three different application methods which will each be tested at three concentrations (low, intermediate, and high) based on specific toxicity in nematode bioassays. Five replicate pots, each with 4 soybean plants, will be used for each treatment. Plants will be grown for 45 days with daily watering in the greenhouse. At harvest, the number of nematode cysts and eggs will be quantified as a measure of nematode control and plant health measurements such as chlorophyll conten and shoot height and biomass will also be recorded to assess any impacts on plant health. Greenhouse assays will allow us to efficiently screen a larger number of compounds to identify those that are most effective in controlling the nematode in soybean plants in preparation for field trials.

ENRTF BUDGET: $84,000

Outcome	Completion Date
1. Optimize method of application of best inhibitory compounds/extracts: drip irrigation, soil drench, seed treatment	*September 2021*
2. Conduct greenhouse trials using all methods and top performing compounds	*April 2022*
3. Repeat greenhouse trial with best performing compounds (top 2-3) and additional safety testing against non-target native species in preparation for field trials	*June 2022*

III. PROJECT PARTNERS AND COLLABORATORS: N/A

IV. LONG-TERM IMPLEMENTATION AND FUNDING:

This project will help protect Minnesota's water resources from toxic pesticides by the development of more environmentally responsible and sustainable pesticides for managing important agricultural pathogens such as the SCN in Minnesota. The outcomes will be identification of active compounds, development of optimal methods for their application, and testing their efficacy in plants and safety towards other non-target organisms. This work will lay the groundwork for field trials, which we envision for Phase II of the project. Both PI Bushley and Co-PI Chen have received synergistic funding from the Minnesota Soybean Research and Production Council to screen fungal isolates for bioactivity and test biocontrol agents. The team also plans to seek additional funding from the USDA-NIFA program to sustain longer-term funding for this research.

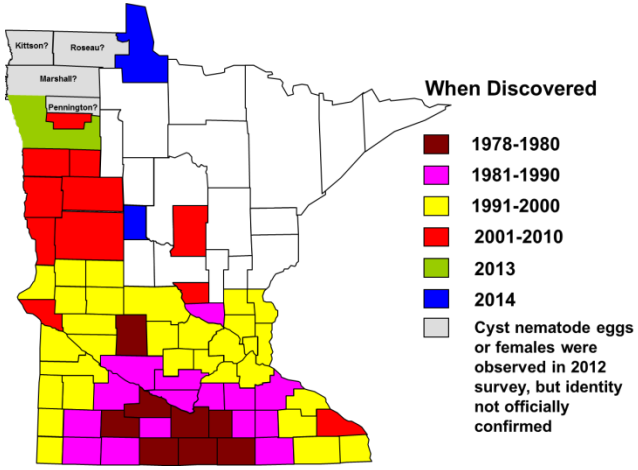
Attachment A: Project Budget Spreadsheet
Environment and Natural Resources Trust Fund
M.L. 2020 Budget Spreadsheet
Legal Citation:
Project Manager: Kathryn Bushley, Co-PI: Christine Salomon
Project Title: Safe biopesticides for protection of Minnesota groundwater resources
Organization: University of Minnesota
Project Budget: $199,000
Project Length and Completion Date: 2 years, July 2022
Today's Date: 04/08/2019

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Budget	Amount Spent	Balance
BUDGET ITEM			
Personnel (Wages and Benefits)	$ 175,000	$ -	$ 175,000
Faculty salary, $14,000 Total (.05 FTE each, 74% Salary, 36% fringe, for 2 years)			
Postdoctoral researcher, $122,000 total (1 FTE, 75% Salary, 25% fringe for 2 years)			
Technician, $32,000 Total (.5 FTE, 70% Salary, 30% fringe, for 2 years)			
Undergraduate researcher, $7,000 Total (.2 FTE, 100% Salary, no fringe, for 2 years)			
Professional/Technical/Service Contracts			
Growth chamber and greenhouse charges for plant assays	$ 2,000		$ 2,000
Equipment/Tools/Supplies	$ 22,000	$ -	$ 22,000
Media and consumables for growing fungi and performing bioassays			
Media, solvents, standards for chemical analysis			
Capital Expenditures Over $5,000			
	$ -	$ -	$ -
Fee Title Acquisition			
	$ -	$ -	$ -
Easement Acquisition			
	$ -	$ -	$ -
Professional Services for Acquisition			
	$ -	$ -	$ -
Printing			
	$ -	$ -	$ -
Travel expenses in Minnesota			
	$ -	$ -	$ -
Other			
	$ -	$ -	$ -
COLUMN TOTAL	$ 199,000	$ -	$ 199,000

SOURCE AND USE OF OTHER FUNDS CONTRIBUTED TO THE PROJECT	Status (secured or pending)	Budget	Spent	Balance
Non-State:		$ -	$ -	$ -
State:		$ -	$ -	$ -
In kind:		$ 107,000	$ -	$ 107,000
Unrecoverable University Indirect Costs @ 54% MTDC				
Other ENRTF APPROPRIATIONS AWARDED IN THE LAST SIX YEARS	**Amount legally obligated but not yet spent**	**Budget**	**Spent**	**Balance**
		$ -	$ -	$ -

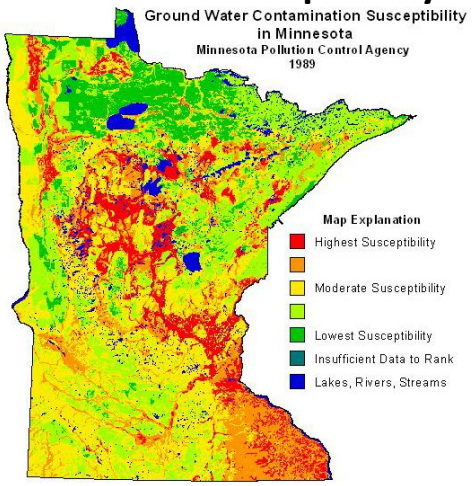
Safe Biopesticides for Protection of Minnesota Groundwater Resources

Distribution of Soybean Cyst Nematode (SCN)



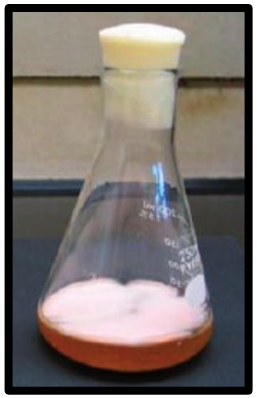
Source: Senyu Chen, UM. unpublished.

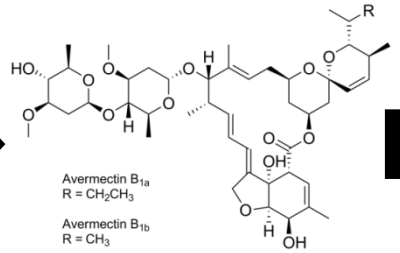
Groundwater Susceptibility



https://i.pinimg.com/736x/a9/c1/06/a9c106b9bfee1f87626427f62e21c8bc.jpg

Activity 1: Identify compounds that inhibit nematodes from fungal isolates





i) Fungal growth in liquid broth to produce bioactive compounds

ii) Extract and fractionate compounds for screening in nematode bioassays

iii) Chemical analysis to identify active compounds

Activity 2: Greenhouse testing and seed coats

VI. PROJECT MANAGER QUALIFICATIONS AND ORGANIZATION DESCRIPTION

Project Manager Qualifications:

Dr. Kathryn Bushley (PI) will serve as the project manager and coordinate the research project. She is a fungal biologist specializing in secondary metabolite compounds produced by nematode and insect pathogenic fungi and an Assistant Professor in the Department of Plant and Microbial Biology at the University of Minnesota. She earned a master's degree in Environmental Management from Duke University and a Ph.D. from Cornell University in Plant-Pathology. Her research program at the University of Minnesota focuses on characterizing the diversity and metabolic potential of fungi associated with nematodes and insects and their potential uses as biocontrol agents or sources of biopesticides against agricultural pathogens. She has received funding from both federal (United States Department of Agriculture, National Science Foundation) and local (Minnesota Soybean Research and Production Council, Minnesota Invasive Terrestrial Plants and Pests Center, and USDA-HATCH). Her laboratory is equipped for fungal growth and bioassay testing, and has access to greenhouse space.

Dr. Christine Salomon (Co-PI) is an Associate Professor at the Center for Drug Design and a faculty member in the Biotechnology Institute at the University of Minnesota. Dr. Salomon earned her Ph.D. at the Scripps Institution of Oceanography, UCSD, in the area of natural products chemistry from invertebrates and microbes. Dr. Salomon's current research program is focused on the discovery and utilization of novel microbes that can be used for biological control of agricultural pathogens and production of unique compounds for biomedical and biotechnological applications. She has secured both internal (Academic Health Center, Masonic Cancer Center, Healthy Foods Healthy Lives Institute) and external (United States Department of Agriculture and US Fish and Wildlife) support for her research program. Her laboratory is well-equipped for standard chemical analyses (HPLC) and she has access to more specialized chemistry equipment (NMR spectroscopy, LC-MS) through the Center for Drug Design.

Dr. Senyu Chen (Co-PI) is a Professor in the Department of Plant Pathology at the University of Minnesota and a researcher at the University's Southern Outreach and Research Station in Waseca, MN. He is a nematologist with over thirty years experience in both field and laboratory based studies of nematodes and characterization of microbial biocontrol agents of nematodes. He leads a nematology laboratory at the Waseca Experimental Station and maintains experimental plots for field trials testing microbial biocontrol agents.

Organizational Description:

The Department of Plant and Microbial Biology and Departments of Plant Pathology at University of Minnesota have strengths in studying the plant associated microorganisms. Both Dr. Bushley and Dr. Chen are members of the Stakeman-Borlaug Center, an institute focused on developing sustainable cutting-edge solutions to plant disease problems. Dr. Chen works at the Southern Outreach and Extension Center, which is an exceptional resource for agricultural research. Dr. Salomon is at the Center for Drug Design at the University of Minnesota and a member of the Biotechnology Institute, a research center decidated to finding practical solutions to challenging problems in environment, agriculture, and health sciences.