Rec 9/30/19

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MTSU Clean Energy Initiative Project Funding Request

There are five (5) sections of the request to complete before submitting. See http://www.mtsu.edu/sga/cleanenergy.shtml for funding guidelines. Save completed form and email to cee@mtsu.edu or mail to MTSU Box 57.

1. General Information	
Name of Person Submitting Request Anthony Farone	
Department/Office Biology 2083 SCI	Phone # (Office) 898-5343
MTSU Box # 60	Phone # (Cell) 615-653-6537
E-mail Anthony.farone@mtsu.edu	Submittal Date 9/29/2019

2. Project Categories (Select One)			
Select the category that best describes the project.			
x	Energy Conservation/Efficiency	x	Sustainable Design
	Alternative Fuels		Other
	Renewable Energy		

3. Project Information
a. Please provide a brief descriptive title for the project. **b.** The project cost estimate is the expected cost of the project to be considered by the committee for approval, which may differ from the total project cost in the case of matching funding opportunities. **Any funding request is a 'not-to-exceed' amount. Any proposed expenditure above the requested amount will require a resubmission.** c. List the source of project cost estimates. d. Provide a brief explanation in response to question regarding previous funding.
3a. Project Title A Novel Biological Control for Mosquito Larvae
3b. Project Cost Estimate $7500

Total Request	$7500.00
Sequencing supplies and reagents	$2000.00
PCR supplies and reagents	$1500.00
Millipore syringe microfilters	$1000.00
Disposable micropipette tips	$1000.00
Protozoal growth reagents and supplies	$1000.00
Pipettes	$1000.00

3c. Source of Estimate

Fishersci.com

3d. If previous funding from this source was awarded, explain how this request differs?

An MTSU URECA summer grant was awarded to collect environmental water samples and teach the undergraduates how to identify the Lambornella protozoa we are interested using microscopes. This project will seek to isolate and then identify Lambornella organism by PCR and DNA sequencing.

4. Project Description

(Completed in as much detail as possible.)

a. The scope of the work to be accomplished is a detailed description of project activities.
b. The benefit statement describes the advantages of the project as relates to the selected project category.
c. The location of the project includes the name of the building, department, and/or specific location of where the project will be conducted on campus.
d. List any departments you anticipate to be involved. Were any departments consulted in preparation of this request? Who? A listing may be attached to this form when submitted.
e. Provide specific information on anticipated student involvement or benefit.
f. Provide information for anticipated future operating and/or maintenance requirements occurring as a result of the proposed project.
g. Provide any additional comments or information that may be pertinent to approval of the project funding request.

4a. Scope: Work to be accomplished

Protozoa are single-cell, animal-like organisms, that are prevalent in water and moist soils. They swim through these environments, typically engulfing bacteria and other microbes as their food source. Ciliated protozoa are surrounded by hair-like structures that help to propel them through their environments and to gather their food. Some ciliated protozoa will parasitize the exoskeletons of invertebrates, including insects. Much like a lamprey on a shark, they feed on the microbes on the insect surface, but rarely invade the insect tissue.

The ciliated protozoan *Lambornella clarki,* and other *Lambornella* species, are known to target mosquitoes, specifically mosquito larvae. They parasitize the larvae by attaching to the exterior of the larva cuticle (outer covering), then form a hole in the cuticle, and enter the hemocoel (blood-like fluid of the tissues) of the host, invading the tissue and killing the larva. *Lambornella*'s specificity to target mosquitoes and its high infectivity rate for the larvae (it takes only one ciliate to kill a larva) have led to the suggestion that the organism be used for the biological control of mosquito populations. The fact that *Lambornella* can also form a desiccation-resistant structure known as a cyst, also makes it an attractive biocontrol agent in that it would survive on surfaces long-term, even in the absence of water. *Lambornella* species have been found in habitats, such as in tree holes and on plants (water held by leaves or flowers) where mosquito larvae are also present. The cysts of the organisms could be part of dried discs/cakes added to standing water or even sprayed onto plants and foliage.

Decades ago, researchers at U.C. Berkeley proposed to mass-culture the ciliates; however, to our knowledge, there is no evidence that cultivation was attempted or successful, and the only paper available shows that *Lambornella* grew better when a bacterial contaminant was present in the growth medium. To date, there are no *Lambornella* cultures available from culture collections, and several of the few researchers who studied the ciliate have either retired or passed away.

With the current concern over mosquito-borne viral infections (Zika, Dengue fever, Chikungunya), it is worth revisiting the possibility of growing *Lambornella* for biological control applications. The overall goal of the summer URECA grant will be to find and culture *Lambornella* species, axenically (in pure culture), or with native bacteria as a food source. If it is successful, the mentoring team plan to submit a longer-term proposal to the USDA or NIH for further study of the use of *Lambornella* as a biocontrol agent. The project may have the additional benefit of identifying new protozoan species.

4b. Scope: Benefit Statement

The ciliated protozoan *Lambornella clarki,* and other *Lambornella* species, are known to target mosquitoes, specifically mosquito larvae. They parasitize the larvae by attaching to the exterior of the larva cuticle (outer covering), then form a hole in the cuticle, and enter the hemocoel (blood-like fluid of the tissues) of the host, invading the tissue and killing the larva. *Lambornella*'s specificity to target mosquitoes and its high infectivity rate for the larvae (it takes only one ciliate to kill a larva) have led to the suggestion that the organism be used for the biological control of mosquito populations. The fact that *Lambornella* can also form a desiccation-resistant structure known as a cyst, also makes it an attractive biocontrol agent in that it would survive on surfaces long-term, even in the absence of water. *Lambornella* species have been found in habitats, such as in tree holes and on plants (water held by leaves or flowers) where mosquito larvae are also present. The cysts of the organisms could be part of dried discs/cakes added to standing water or even sprayed onto plants and foliage.

4. Project Description (continued)

4c. Location of Project (Building, etc.)

2080 SCI Laboratory

4d. Participants and Roles

I will be the mentor for this project. I have been involved with protozoa-bacterial interaction studies for twenty years. I have mentored over 50 students and graduate students and have much experience with leading teams of students. I will be part of weekly laboratory meetings with students. I will also lead the water sample collection. I will also be instructing students on the DNA purification, polymerase chain reaction (PCR), and DNA sequencing techniques. I will be on-hand for questions and will check student notebooks.

4e. Student participation and/or student benefit

Students will learn identification of protozoa in samples, enrichment for protozoa, isolation techniques, culturing, molecular identification of ciliates through the polymerase chain reaction and DNA sequencing. Students will also learn microscopy techniques, including inverted phase-contrast microscopes and fluorescence microscopy. Student will also learn the isolation and identification of bacterial isolates from the water in which the protozoa were found, which may be used as food sources for the protozoa. The students will also maintain laboratory notebooks and learn procedures for data and statistical analyses.

4f. Future Operating and/or Maintenance Requirements

none

4g. Additional Comments or Information Pertinent to the Proposed Project

We appreciate the CEE funding of our recent ginseng proposal that resulted in two recent publications with 8 MTSU student authors* with the lead author being my PhD student Raj Ghosh who has graduated and has a research position at the University of Missouri:

Ghosh R*, Smith SA*, Nwangwa EE*, Arivett BA*, Bryant DL*, Fuller ML*, Hayes D*, Bowling JL*, Nelson DE, DuBois JD, Altman E, Kline PC, Farone AL.. Panax quinquefolius (North American ginseng) cell suspension culture as a source of bioactive polysaccharides: Immunostimulatory activity and characterization of a neutral polysaccharide AGC1. Int J Biol Macromol. 2019;139:221-232.

Ghosh R*, Kline P. HPLC with charged aerosol detector (CAD) as a quality control platform for analysis of carbohydrate polymers. BMC Res Notes. 2019;12(1):268.

We have utilized ginseng byproducts from the above research for biofuel studies and have recently begun utilizing hemp byproducts, as well.

5. Project Performance Information

Provide information if applicable.

a. Provide information on estimated annual energy savings stated in units such as kW, kWh, Btu, gallons, etc.
b. Provide information on estimated annual energy cost savings in monetary terms.
c. Provide information on any annual operating or other cost savings in monetary terms. Be specific.
d. Provide information about any matching or supplementary funding opportunities that are available. Identify all sources and explain.

5a. Estimated Annual Energy Savings (Estimated in kW, kWh, Btu, etc.) Longterm cost saving would be substantial if biological control could replace the petroleum used for pesticide production. Almost all pesticides are produced from hydrocarbons from petroleum. Mosquito control currently uses predominantly liquid pesticides that have traditionally used kerosene or some other petroleum distillate as a carrier State and local agencies commonly use the organophosphate insecticides malathion and naled that are applied by aircraft or on the ground by truck-mounted sprayers.
http://www.madehow.com/Volume-1/Pesticide.html#ixzz60w16GTr1

https://www.epa.gov/mosquitocontrol/controlling-adult-mosquitoes

5b. Annual Energy COST Savings ($)

Because this is a preliminary research project the annual savings are not calculated. However, the energy to produce Malathion listed in 5a above is:

Pesticide	**BTUs/lb**	**Application Rate**	**BTUs/Area**
	(x 1000)	**(lbs/Area)**	**(x 1000)**
Malathion	98.5	1.25	123.1

https://farm-energy.extension.org/energy-use-and-efficiency-in-pest-control-including-pesticide-production-use-and-management-options/

5c. Annual Operating or Other Cost Savings. Specify. ($) Because this is a preliminary research project the annual operating or other cost savings are not calculated.
5d.Matching or Supplementary Funding (Identify and Explain) The Biology Department has beena strong supporter of student research (Biol 4280 independent research) and Honors student research projects through the department supply budget.