



# Environment and Natural Resources Trust Fund

## 2021 Request for Proposal

### General Information

**Proposal ID:** 2021-019

**Proposal Title:** Does The Herbicide Paraquat Harm Insect Microbial Symbionts?

### Project Manager Information

**Name:** Ann Fallon

**Organization:** U of MN - College of Food, Agricultural and Natural Resource Sciences

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### Project Basic Information

**Project Summary:** We will investigate the herbicide, paraquat, for deleterious effects on microbial symbionts of arthropods. We will focus on Wolbachia bacteria, present in more than half of all insect species.

**Funds Requested:** \$157,000

**Proposed Project Completion:** 2024-06-30

**LCCMR Funding Category:** Small Projects (H)

**Secondary Category:** Foundational Natural Resource Data and Information (A)

### Project Location

**What is the best scale for describing where your work will take place?**

Statewide

**What is the best scale to describe the area impacted by your work?**

Statewide

**When will the work impact occur?**

In the Future

## Narrative

### **Describe the opportunity or problem your proposal seeks to address. Include any relevant background information.**

Roundup, or glyphosate, in combination with genetically engineered, Roundup-Ready crops, is extensively used in Minnesota. Not surprisingly, weeds are developing resistance to glyphosate, requiring higher levels of application and exacerbating health and environmental concerns. An alternative herbicide, paraquat, is used alone or in combination with glyphosate to manage resistant weeds. Unlike glyphosate, paraquat targets conserved oxidative pathways common to all organisms; since 2007, paraquat has been banned in the EU because of a possible link to Parkinson's disease.

Toxicity of paraquat to microbial symbionts may pose an unanticipated threat to non-target insects, including environmentally-friendly pollinators, predators and parasitoids, and species that participate in environmental recycling and contribute to soil health. Studies in my lab have uncovered an unexpected toxicity of paraquat to *Wolbachia*, a bacterium that resides in insect ovaries and testes. An estimated 50% of insect species naturally harbor *Wolbachia*, which is emerging as an environmentally-friendly tool for control of mosquito-borne diseases such as dengue fever in Brazil. In addition, paraquat is toxic to the soil arthropod *Folsomia candida*, a Collembolan that requires *Wolbachia* for reproduction. *Wolbachia* will serve as an indicator microbe for predicting herbicide effects on the diverse suite of symbionts that occurs in insects.

### **What is your proposed solution to the problem or opportunity discussed above? i.e. What are you seeking funding to do? You will be asked to expand on this in Activities and Milestones.**

*Wolbachia* has already been implemented to control mosquito-borne diseases in tropical countries, and has important potential as a gene drive agent to suppress reproduction of a wide range of agricultural pests including vectors of plant diseases. My goal is to explore whether the herbicide paraquat will affect *Wolbachia*, using the soil arthropod *Folsomia candida* (Collembola; springtail) and *Culex pipiens* mosquitoes as indicator species.

*Folsomia* is widely used as an environmental indicator of soil contamination. This Collembolan is a parthenogenetic all-female species whose reproduction depends on *Wolbachia*. When *Wolbachia* are inhibited, for example with antibiotics, *Folsomia* stops reproducing. We will determine whether the herbicide paraquat causes an antibiotic-like effect on reproduction and *Wolbachia* levels. *Folsomia* and other soil-dwelling arthropods are important in environmental turnover of soil nutrients.

Because paraquat is highly soluble, agricultural runoff is likely to contaminate mosquito breeding sites. To the extent that paraquat decreases *Wolbachia*, its presence in natural habitats will cause mating incompatibilities that can undermine control efforts. We will use a Minnesota mosquito, *Culex pipiens*, which has a natural infection with *Wolbachia*, to evaluate potential detrimental effects of paraquat on *Wolbachia* based mosquito control, a promising, environmentally-friendly method for suppressing vector-borne disease.

### **What are the specific project outcomes as they relate to the public purpose of protection, conservation, preservation, and enhancement of the state's natural resources?**

Since the 1950's, when DDT was in extensive use worldwide, the scientific community has steadily developed environmentally-friendly tools for advancing agricultural productivity and controlling insects. *Wolbachia* is one such microbial tool, whose use in control may be compromised by the herbicide paraquat. In vitro studies suggest that paraquat, a candidate for controlling glyphosate-resistant weeds, is toxic to *Wolbachia* at levels that do not affect the host cell. By disrupting symbionts, paraquat may pose an unanticipated threat to non-target pollinators, predators, parasitoids, and species that participate in environmental recycling and contribute to soil health. My goal is to evaluate this possibility.

## Activities and Milestones

**Activity 1: Year 1: Review available literature; learn lab skills; maintain colonies; complete half of course credits; deliver thesis proposal seminar**

**Activity Budget:** \$55,000

### Activity Description:

This project is designed for completion by a graduate student in Entomology over a 3 year period. A student with a strong academic background and motivation will be selected, with priority given to an applicant identified as a "self-starter" in recommendation letters. Training and supervision will be provided in all methodologies needed to accomplish goals of the project. In Activity 1, to be accomplished during the first year, the student will review the literature, prepare a thesis proposal seminar to be delivered to the department, and master techniques for extracting DNA and measuring Wolbachia abundance under experimental conditions. Coursework will include statistical analysis. Baseline Wolbachia abundance will be established in in control and paraquat treated insects, and in dissected tissues expected to contain Wolbachia. In addition the dose of paraquat that kills 50% of host insects (LC-50) will be determined using appropriate statistics and independent biological replicates. The goal is to establish baseline information needed for completion of research goals.

### Activity Milestones:

Description	Completion Date
Complete literature review and present department seminar on project goals and planned procedures	2021-12-31
Learn how to maintain mosquito and collembola colonies; establish LC-50 values with paraquat	2022-02-28
Master DNA extraction and PCR-based measurement of Wolbachia genome copies in control insects	2022-06-30

**Activity 2: Collect and analyze mosquito data; establish dose response curves, conduct simulated field studies; determine whether paraquat effects are reversible**

**Activity Budget:** \$30,000

### Activity Description:

In year 2, numbers of Wolbachia relative to paraquat dose will be established, comparing mosquitoes, which survive without Wolbachia, and parthenogenetic Collembola, which go extinct without Wolbachia. This difference relates to the precise way in which Wolbachia manipulates reproduction in these species.

We will document paraquat concentrations that affect Wolbachia in adult mosquitoes fed on paraquat-laced sucrose, and establish whether Wolbachia loss persists into subsequent generations. We will further evaluate how levels of Wolbachia in larvae exposed to paraquat from agricultural run-off change during maturation to adults. Simulated field levels of paraquat may decrease, but not eliminate Wolbachia in adult mosquitoes reared from treated larvae. Offspring of these adults will be reared in treated and untreated water, to determine whether recovery occurs in the absence of paraquat, whether treatment over several generations results in elimination of the microbe, and whether Wolbachia develops resistance to paraquat.

This baseline information will predict whether ongoing release of Wolbachia-infected mosquitoes for disease control in developing countries, and potential future use of Wolbachia in Minnesota, is likely to be impacted by environmental levels of paraquat. Wolbachia-based insect control provides an environmentally friendly option in the context of global warming and emergence of invasive species.

### Activity Milestones:

5/22/2020

Description	Completion Date
Document whether paraquat effects are reversible in mosquitoes	2022-07-31
Perform simulated field tests with paraquat-treated water for larval rearing	2022-09-30
Establish dose response curve with mosquitoes and for Wolbachia within mosquitoes	2022-12-31

### Activity 3: Analyze paraquat effects on Collembola as an independent test species that requires Wolbachia for reproduction

**Activity Budget:** \$31,000

**Activity Description:**

Activity 3 will investigate effects of paraquat on the soil arthropod, *F. candida*, a parthenogenetic species of Collembola. No males occur in *F. candida*; Wolbachia is an essential symbiont that causes eggs to develop as if they had been fertilized by sperm. Similarly, Wolbachia induces parthenogenesis in parasitoid wasps used for biological control of insect pests. In contrast, mosquitoes can reproduce without Wolbachia as long as the microbe is absent from both sexes.

Consistent with an effect resembling fertilization, Wolbachia cannot be eliminated from *F. candida*. The Collembolan stops reproducing, but remains viable when Wolbachia levels are suppressed and resumes laying eggs when suppression is removed, even after weeks or months of treatment. Wolbachia from *F. candida* appear to enter a reversible quiescent state when survival is threatened by antibiotics. This capability is consistent with the Wolbachia genome, which encodes genes that reversibly inhibit replication of better-studied bacteria, such as the microbe that causes tuberculosis. We will determine whether paraquat causes a reversible reproductive quiescence and evaluate whether quiescence occurs at levels expected in soil, after paraquat is used as a herbicide. Collembola feed on fungi and yeast, and effects of paraquat on these food sources will also be evaluated.

**Activity Milestones:**

Description	Completion Date
Determine dose of paraquat that inhibits <i>F. candida</i> reproduction.	2023-01-31
Determine whether the effect on reproduction is reversible	2023-03-31
Determine changes in Wolbachia number in treated collembola	2023-06-30
Correlate Collembola reproduction and Wolbachia abundance environmental levels of paraquat	2023-07-31

### Activity 4: Write manuscripts and defend thesis

**Activity Budget:** \$41,000

**Activity Description:**

In this final phase of the project, experimental discrepancies will be resolved, appropriate statistical analyses will be applied, and manuscripts will be submitted for peer review and publication. The thesis will be defended.

**Activity Milestones:**

Description	Completion Date
First draft of thesis	2023-11-30
Preliminary review by advisory committee	2024-01-31
Oral seminar and thesis defense	2024-06-30
Write and submit manuscripts; finalize thesis	2024-06-30



## Long-Term Implementation and Funding

**Describe how the results will be implemented and how any ongoing effort will be funded. If not already addressed as part of the project, how will findings, results, and products developed be implemented after project completion? If additional work is needed, how will this be funded?**

This project is designed to be completed over a three year period by a MS-level student in Entomology. Support is requested for the student's stipend, tuition, laboratory supplies and travel to a domestic scientific meeting. The student may apply to continue for PhD studies and will be encouraged and aided in the preparation of fellowship applications for subsequent support.

Timeline:

Year 1: review published research; maintain insect colonies; conduct pilot experiments

Year 2: Revise and refine experimental protocols, focus on bring research data to publication

Year 3: Finalize results and submit manuscripts for peer-review; defend thesis

## Project Manager and Organization Qualifications

**Project Manager Name:** Ann Fallon

**Job Title:** Professor

**Provide description of the project manager's qualifications to manage the proposed project.**

Dr. Fallon's work focuses on reproduction in mosquitoes, with emphasis on the microorganism Wolbachia. Over the years, she has been funded by about \$10 million, mainly from the National Institutes of Health and US Department of Agriculture. She is a distinguished McKnight University Professor. In 2019 Ann received a lifetime achievement award for contributions to her department and was invited to membership in Sigma Xi, a Scientific Research Honor Society. She has trained 16 PhD, 13 MS and 30 undergraduate students.

Fallon has published more than 125 papers in peer-reviewed journals. Recent notable achievements include a major breakthrough in identifying the molecular basis for Wolbachia genes that contribute to reduced egg hatch in mosquitoes by PhD student John Beckmann. Beckmann furthered his discovery as a postdoc at Yale, and now holds a faculty position at Auburn University. Undergrad Leland Graber, who investigated the role of Wolbachia in springtails, is now a PhD student at Cornell. Garrett Chen is currently investigating Wolbachia in insect ovaries with support from the Undergraduate Research Opportunities Program over two years.

Wolbachia is an invertebrate-specific bacterium that reduces hatching of mosquito eggs. Long considered unique to mosquitoes, Wolbachia is now known to infect about half of all insect species, and has been implemented to reduce transmission of dengue virus by mosquitoes in Brazil. Short term effects on insect reproduction and longer term effects on insect speciation provide ecologically friendly approaches for control of medical and agricultural insect pests using Wolbachia. This proposal is based on the observation that paraquat is directly detrimental to Wolbachia in insect cell lines, and reduces reproduction of springtails, an important indicator of toxins in soil. Effects on insect microbial symbionts will be evaluated relative to levels of paraquat used as herbicide treatments and expected to accumulate in soil and aqueous runoff.

**Organization:** U of MN - College of Food, Agricultural and Natural Resource Sciences

**Organization Description:**

The College of Food, Agricultural and Natural Resources Sciences (CFANS) at the University of Minnesota encompasses 13 academic departments, and 10 research and outreach centers. CFANS seeks to provide global leadership in

agricultural sciences that will feed a growing world population while sustaining the environment and natural resources. Its mission includes extraordinary education, innovative science-based solutions, and dynamic public engagement. CFANS enrolls approximately 2000 undergraduates and 700 graduate students. The Department of Entomology has 20 faculty, 32 graduate students and 21 undergraduate minors.

## Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	\$ Amount
<b>Personnel</b>								
Graduate research assistant with tuition benefits		With supervision and advice from Dr. Fallon, the graduate student will carry out the experimental protocols, read and evaluate relevant literature, and prepare results for publication.			20%	300		\$135,000
							<b>Sub Total</b>	<b>\$135,000</b>
<b>Contracts and Services</b>								
							<b>Sub Total</b>	-
<b>Equipment, Tools, and Supplies</b>								
	Tools and Supplies	Plastic tissue culture materials, petri dishes, tubes, pipets, tips; chemicals and reagents	Disposables for routing experimental protocols needed to accomplish work over 3 years					\$11,000
							<b>Sub Total</b>	<b>\$11,000</b>
<b>Capital Expenditures</b>								
							<b>Sub Total</b>	-
<b>Acquisitions and Stewardship</b>								
							<b>Sub Total</b>	-
<b>Travel In Minnesota</b>								
							<b>Sub Total</b>	-
<b>Travel Outside Minnesota</b>								



	Conference Registration Miles/ Meals/ Lodging	Annual meeting of the ESA for student	Student will present results and gain professional experience	X				\$5,000
							<b>Sub Total</b>	<b>\$5,000</b>
<b>Printing and Publication</b>								
	Publication	two peer reviewed publications in open-access journals	fee of \$3000/article makes results available immediately without 1-year embargo					\$6,000
							<b>Sub Total</b>	<b>\$6,000</b>
<b>Other Expenses</b>								
							<b>Sub Total</b>	<b>-</b>
							<b>Grand Total</b>	<b>\$157,000</b>

## Classified Staff or Generally Ineligible Expenses

Category/Name	Subcategory or Type	Description	Justification Ineligible Expense or Classified Staff Request
<b>Travel Outside Minnesota</b>	Conference Registration Miles/Meals/Lodging	Annual meeting of the ESA for student	Attendance at an annual (domestic) meeting of the Entomological Society of America or equivalent is an important aspect of graduate education and provides an opportunity for a student to present results and align performance with scientific standards of the profession

Non ENRTF Funds

Category	Specific Source	Use	Status	Amount
<b>State</b>				
			<b>State Sub Total</b>	-
<b>Non-State</b>				
			<b>Non State Sub Total</b>	-
			<b>Funds Total</b>	-

## Attachments

### Required Attachments

#### *Visual Component*

File: [798b6aa4-8c5.pdf](#)

#### *Alternate Text for Visual Component*

Schematic showing weed control with potential effects on internal symbionts of insects

### Optional Attachments

#### *Support Letter or Other*

Title	File
Preliminary published studies	<a href="#">6648f2fd-353.docx</a>
Visual component	<a href="#">37c581fd-ac9.pdf</a>
Proposal approval UM	<a href="#">5db3a2e5-3f5.pdf</a>

## Administrative Use

**Does your project include restoration or acquisition of land rights?**

No

**Does your project have patent, royalties, or revenue potential?**

No

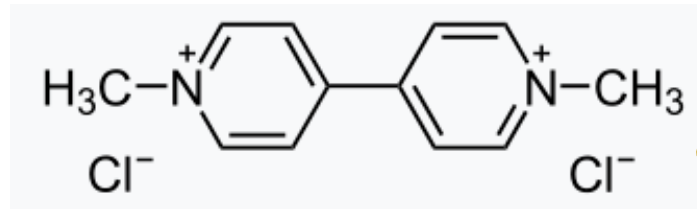
**Does your project include research?**

Yes

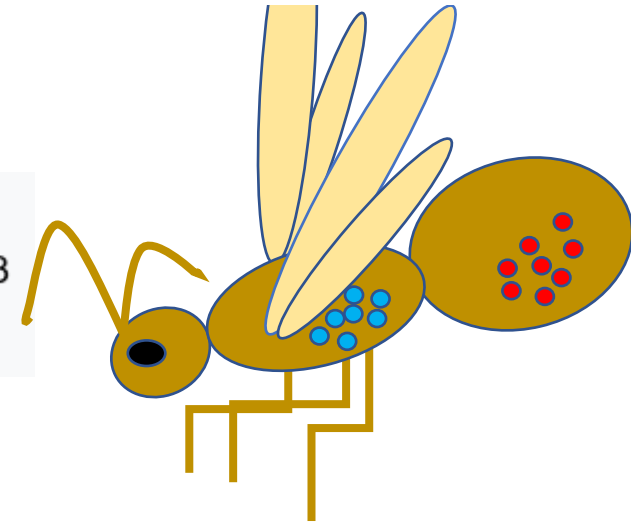
**Does the organization have a fiscal agent for this project?**

Yes, Sponsored Projects Administration

SAVE OUR SYMBIONTS



PARAQUAT



Herbicides and Resistant Weeds

