SRF RESEARCH AWARD
PROPOSAL COVER SHEET

APPLICANT #1			
Last Name [redacted]	First [redacted]	M.I. [redacted]	Major Biology
Street Address [redacted]		Student ID # [redacted]	
City [redacted]	State [redacted]	ZIP [redacted]	
Phone [redacted]	E-mail Address [redacted]		
Student Rank Junior			

APPLICANT #2			
Last Name	First	M.I.	Major
Street Address		Student ID #	
City	State	ZIP	
Phone	E-mail Address		
Student Rank			

ADDITIONAL APPLICANTS (Please list all additional applicants below. A signature below is required for all applicants)

[redacted]

PROJECT INFORMATION

Project Title: Evaluation of species in the genus Acer for toxicity to equine erythrocytes

Project Start Date: April 2013	Project End Date: May 2015
Research Advisor: Jeffrey Lehman	Advisor Department: Biology
Have you requested SRF funding for this project in the past? Yes ☐ No ☑	In what year?
Has this project been funded by the SRF previously? Yes ☐ No ☑	If yes, what amount? $_____ In what year?

What is the total budgeted expense of your project? (This is the total cost, which may not be the amount you are requesting)

$ 773.00

[redacted]

AWARD REQUEST

Please indicate the amount you are requesting from the SRF: $ 400.00

[redacted]

OTHER INFORMATION

Does your project involve the use of human subjects? If so, what is the status of your IRB application? Approved ☐ Pending ☐

Does your project involve the use of animal subjects? (If so what is the status of your animal use and care application?) Approved ☑ Pending ☐

SIGNATURE (All applicants must sign)

Signature [redacted]	Date 3-26-13
Signature	Date

Evaluation of species in the genus *Acer* for toxicity to equine erythrocytes

1. *Goals and Objectives:* Red maple toxicosis is a life threatening syndrome that occurs in equids when dried or wilted red maple, *Acer rubrum*, leaves are consumed. It causes hemolysis of erythrocytes, Heinz body development, and methemoglobinemia. Symptoms include brown discoloration of mucous membranes, urine, and blood (Vin et al., 2002) along with depression, dehydration, anorexia, weakness, lethargy, cyanosis, colic, and ataxia (Divers et al., 1982). Clinical symptoms are usually observed after 48 hours and mortality is 60-65% within 3 to 6 days of ingestion in both experimentally and naturally occurring cases (Corriher et al., 1999).

The red maple toxin has yet to be identified but is thought to be a strong oxidizing agent (Mcconnico et al., 1992) that causes damage to red blood cell membranes and hemoglobin molecules. Oxidation of the cell membrane impairs the active and passive transport of ions causing hyperpermeability and the ultimate hemolysis of red blood cells (Corriher et al., 1999). Erythrocyte cell fragments are released into the blood stream causing vasoconstriction and activation of platelets inducing intravascular coagulation and renal failure (Vin et al., 2002). Methemoglobin is formed when the ferrous iron of hemoglobin is oxidized to its ferric form. This defective hemoglobin is unable to carry oxygen and is responsible for the characteristic brown discoloration of the blood and mucus membranes (Corriher et al., 1999). Fatality is common when equids are left untreated due to tissue hypoxia, caused from insufficient oxygen transport to vital organs (Vin et al., 2002).

This phenomenon has been observed consistently in equids that have consumed red maple leaves, but the effects on equids after the consumption of related species is relatively unknown. Therefore, the objective of this research is to evaluate the toxicity of extracts of dried leaves of *Acer rubrum*, as well as fourteen other related species, on equine erythrocytes. Specifically, I will quantify levels of toxicity across the leaves and plant parts of each of the fifteen species. In addition, I will test the level of toxicity of various purified fractions of red maple compounds separated via NMR by Dr J. Weidenheimer at Ashland University. The goal of the later work is to identify the potential oxidizing agent. The current thought of our lab is that the compound is a phytoalexin.

2. Significance: Little is known about the toxicity of different maple species, or about the identity of the chief compound responsible for causing toxicosis in *Acer rubrum*. Even though toxicosis is not a widespread problem in nature, it is a possible occurrence for animals in captivity that become bored or are poorly feed (Weber et al., 1997). This study will provide a clearer understanding of the interaction between different maple species and equids, as well as attempt to elucidate the active compound responsible for causing toxicosis. Personally, as well as professionally, this study will help me grow as a scientist by teaching me scientific processes and basic scientific techniques. It will help to prepare me for post-baccalaureate studies.

3. Methods: To address the objectives above, plant tissue (leaf blades and petioles) will be collected and evaluated for levels of toxicity on equine erythrocytes. Toxicity will be quantified based on percentage hemolysis and methemoglobin production.

Species collections and leaf preparation: A set of maple species taken from the major clades within the *Acer* genus will be evaluated in a replicated design including 15 species, five replication, and two experiments sites—Otterbein research greenhouse and Otterbein Community gardens. The accessions will be 3-year-old plants from clonally propagated field selections and from segregating sibling populations. Fresh leaves of each species will be collected from each sample tree, dried, and stored (-20 C). One gram of leaf tissue will ground in 10 ml of distilled water and filtered through miracloth and a 45μm syringe filter.

Blood collection and preparation: Equine blood will be collected in 5ml EDTA tubes from the jugular vein (animal use form submitted and accepted). Erythrocytes will be separated and washed

twice with a 0.9% NaCl solution and suspended in an equal part of saline buffer (pH 7.4, 110mM NaCl, 20mM Na_2HPO_2, 4mM KH_2PO_4).

Experimentation: Extract (0, 25, 75, 100, or 200 µl) will be added to 1.0 ml of prepared equine erythrocytes and incubated at 37 C for 2 hours for hemolysis and 3 hours for methemoglobin. 25 µl of each incubated sample will be added to 1.0 ml of each of the following NaCl concentrations: 0%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, and 0.9% and immediately centrifuged at 2,800 × g for 15 min. Absorbencies of the supernatants will be recorded at 560, 576, and 630 nm to determine percentage hemolysis (%H) and percent methemoglobin (%M). Percentage hemolysis will be calculated according to Boyer et al (2002), and values will be plotted against the NaCl concentrations. Area under the hemolytic curve (AUHC) will be calculated from percentage hemolysis data for each level of leaf extract 20µl of each erythrocyte sample will be added to 2ml Triton X buffer (20mM KH_2PO_4, 16mM Na2HPO4, 0.05%Triton-X detergent) and centrifuged for 15 m at 2800 rmp. Absorbencies will be read at 560, 576, and 630 nm and percent methemoglobin will be determined from equations adapted from Wells et al., (1997). Also, intact and lysed blood cells will be microscopically examined with differential interference contrast.

Timeline: The animal care and use form was submitted and accepted by the committee in fall 2012, (see attached memo). Experiments will be set up this spring, and leaf tissue will be harvested and dried from the time of leaf emergence to leaf drop. From June to October 2013, percentage hemolysis and percent methemoglobin will be determined. In addition, cells structure and form will be documented microscopically. Data will be analyzed in fall 2013, and writing and thesis preparation will start in spring 2014

1. Boyer J, Breeden D, Brown D. *Am J Vet Res* 2002;63(4):604-610.
2. Corriher C, Gibbons D, Parviainen A, et al. *Compendium on Continuing Education for the Practicing Veterinarian* 1999;21(1):74-80.
3. Divers T, Cummings J, de Lahunta A, et al. *Am J Vet Res* 2006;67(1):120-126.
4. McConnico R, Brownie C. *Cornell Vet* 1992;82(3):293-300.
5. Vin, R., et.al. *Journal of Veterinary Emergency and Critical Care*. 12.3 (2002): 169-175.
6. Weber, M. and R.E. Miller. *Journal of Zoo and Wildlife Medicine* 28.2 (1997): 105-108.
7. Wells C., J. Baldwin, and R. Seymour. 1997. Marine and Freshwater Research 48:303-309.

4. Project Evaluation/Assessment: The success of the project will be measured by the new information gained about the toxicity of plant tissue of each of the different species. Also, the project will be evaluated by successful completion of a written honors thesis and presentations to scientific meetings (OAS and regional/national meetings).

5. Total Project Budget: The project will require financial support for supplies (cuvettes, microscopy supplies, and blood draw supplies) and plant material required by the experimental design. I will apply for funding from the SRF at Otterbein and seek money from the Biology department. If the total amount of money is not secured, then the number of samples will be reduced or we will seek additional outside funding or donations.

Expenses	Calculation (cost/unit * no. of units)	SRF Request	Additional Funding	Total
1. Cuvettes	$30 x 10 boxes	$90	$210	$300.00
2. Needles	$17 x 3 boxes	$0	$51	$51.00
3. Serum Separator Syringe kit (tubes)	$36 * 2 boxes	$60	$12	$72.00
4. Potted Plant material	$5 * 20	$0	$100	$100.00
5. Microscope filter(s)	$250 * 1	$250		$250
Totals		$400	$373	773.00

Official Memo: Protocol Approval Confirmation **Date:** 08/23/12

Protocol Approval Number: 2012-01-01-01

Participant: [redacted]

Faculty: Jeff Lehman

Project Title: Toxicity of Leaf Extract of Acer Species on Equine Erythrocytes

Approved By: Animal Care and Use Committee 2012 – 2013

Committee Chair: Dr. Sheri Birmingham

OTTERBEIN
UNIVERSITY

DEPARTMENT OF BIOLOGY
AND EARTH SCIENCE

1 South Grove Street
Westerville, OH 43081-2006
TEL (614) 823-1517
FAX (614) 823-3042
www.otterbein.edu

March 28, 2013

Student Research Fund Committee

Dear Committee Members,

It is my pleasure to support [redacted]'s proposal submitted to the Otterbein University Student Research Fund. For the last year, [redacted] has been working in my lab on a project that addresses the toxicity of species of the genus *Acer* when consumed by horses. During this time, she has assimilated a set of 3-year-old potted plants of 15 species of *Acer* to be used in greenhouse and field experiments. The 15 species represent all major clades in the genus *Acer*. The goal of [redacted]'s work is to quantify levels of toxicity for leaves and plant parts of each of the fifteen species. [redacted]'s project will require her to grow plants in an appropriate replicated design (2 locations x 5 replications x 15 species), extract plant products from leaf tissue, process equine red blood cells, and complete a bioassay that measures cell hemolysis and methemoglobin production. Results from [redacted]'s project will contribute to our understanding of the distribution of toxicity in the genus *Acer*. [redacted] is an excellent student (GPA 3.9). I strongly support her work and am committed to providing guidance and encouragement.

Sincerely,

Jeffrey S. Lehman

Jeffrey S. Lehman, Ph.D.
Department of Life and Earth Sciences