

**Environment and Natural Resources Trust Fund  
2016 Request for Proposals (RFP)**

---

**Project Title:**

**ENRTF ID: 172-F**

Genetic Analysis of Minnesotas Most Endangered Tree Species

---

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

---

**Total Project Budget:** \$ 277,922

**Proposed Project Time Period for the Funding Requested:** 3 years, July 2016 to June 2019

**Summary:**

Seedlings derived from mature native Minnesota Eastern hemlock will be evaluated for genetic diversity, inbreeding and growth rates in three seasons. Information gathered will inform management of this endangered species.

---

**Name:** Stan C Hokanson

**Sponsoring Organization:** U of MN

**Address:** 258 Alderman Hall, 1970 Folwell Avenue  
St. Paul MN 55108

**Telephone Number:** (612) 624-1203

**Email** hokan017@umn.edu

**Web Address** \_\_\_\_\_

---

**Location**

**Region:** NE

**County Name:** Carlton, St. Louis

**City / Township:** Duluth, Esko

---

**Alternate Text for Visual:**

Map depicts the N.A. range of Eastern hemlock (in green) with the insert map depicting locations of MN native trees in Carlton and St. Louis county, MN

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

**Project Title:** Genetic Analysis of Minnesota's Most Endangered Tree Species

**LCCMR 2016 Funding Priority:** F

**Region:** Northeast **Counties Impacted:** Carlton, St. Louis **City/Township:** Duluth, Esko

**Project Summary**

Seedlings derived from mature native Minnesota Eastern hemlock will be evaluated for genetic diversity, inbreeding and growth rates in three seasons. Information gathered will inform management of this endangered species.

**Main Proposal:**

**PROJECT TITLE:** Genetic Analysis of Minnesota's Most Endangered Tree Species

**I. PROJECT STATEMENT**

Eastern hemlock (*Tsuga canadensis* (L.) Carrière) is Minnesota's most endangered native tree species. The species has never been common in Minnesota (see Appendix 1). Currently less than 50 native, mature trees exist within Minnesota. The species numbers have been severely reduced by fire, lumbering, and weather related events such as the 2012 flooding event in the Duluth region that resulted in the loss of three of the eight remaining native trees in the Hemlock Ravine Scientific and Natural Area. With so few trees remaining, the species is precariously close to being extirpated from Minnesota. In the research proposed herein, we will utilize microsatellite (SSR) markers to determine the genetic variation in seedlings produced by Minnesota's native trees. We will also characterize seedlings from trees at the Minnesota Landscape Arboretum and Eloise Butler Wildflower garden that are thought to be derived from native sources. These trees could serve as future seed orchards. The study conducted over three seasons will allow us to determine if there is significant variation for genetic diversity amongst seedlings originating from different parent trees and whether temporal variation in levels of genetic variation exists. Seed lots will also be evaluated for percent germination, growth rate, and survivorship, which will serve as indicators of vitality. The resulting information will inform future management decisions.

**II. PROJECT ACTIVITIES AND OUTCOMES**

**Activity 1:** Harvesting cones for seed production

**Budget:** \$38,704

<b>Outcome</b> (Each activity completed yearly 2016-2018)	<b>Completion Date</b>
1. Cones collected and seed extracted from all MN native trees	October
2. Cones obtained, seeds extracted from large WI and NC populations for genetic diversity comparisons (See Appendix 2)	"

**Activity 2:** SSR Marker Diversity Analysis

**Budget:** \$180,764

<b>Outcome</b> (Each activity completed yearly 2016-2019)	<b>Completion Date</b>
1. Harvest needles and send to lab (TBD) for DNA extraction/amplification	June
2. DNA results returned	October (est.)
3. Analyze lab results	December

**Activity 3:** Evaluating seedling germination, growth, survival    **Budget:** \$58,454

<b>Outcome</b> (Each activity completed yearly 2016-2019)	<b>Completion Date</b>
1. Seed sown, stratified	November
2. Seed removed from cooler for germination	February
3. Germination rates determined	May
4. Growth rates, survival	October

**III. PROJECT STRATEGY**

**A. Project Team/Partners (not requiring funding)**

Stan C. Hokanson, Professor, Department of Horticultural Science, UMN will manage all project activities. Hokanson is a co-advisor for Emily Ellingson, the primary researcher on the project.

James Bradeen, Professor, Department Head Plant Pathology, UMN will oversee molecular marker analysis. Bradeen is co-advisor for Emily Ellingson.

Minnesota Department of Natural Resources, is providing permits for collecting needle samples and cones for the project.

Minneapolis Park Board, Eloise Butler Wildflower Gardens is providing access to mature Eastern hemlock thought to be derived from native Minnesota trees.

**(Requesting funding)**

Emily Ellingson, M.S. candidate, Applied Plant Sciences, UMN is conducting this research in pursuit of her M.S. degree. We are requesting funding for Ellingson’s salary, fringe and benefits.

Minnesota Landscape Arboretum (MLA), is providing access to Eastern hemlock trees thought to be derived from extirpated Minnesota native trees. They are providing arborist services for cone harvests at the MLA and greenhouse space. We are requesting funding for plant growing materials to be used at the MLA.

**B. Project Impact and Long-Term Strategy**

We will develop a compilation of genetic information on the remaining native Eastern hemlock trees in Minnesota. The information will be shared with the MNDNR and through peer-reviewed publications and presentations at local, regional, and national meetings. We expect to know what trees are unequivocally native to Minnesota, which are genetically diverse, and which produce the most genetically diverse, robust seed crops. This information will allow agencies to make informed decisions regarding extant trees and potential restoration of Eastern hemlock. We began this research in May 2014 utilizing University funding. We will have completed a molecular characterization of all native and purportedly native Eastern hemlocks in Minnesota by March 2016. We will have completed two seed collections by October 2015. We will be requesting an additional two years of funding, 2019-2020 to complete the analysis of seedlings collected in fall 2017 and 2018.

**C. Timeline Requirements**

The LCCMR funded portion of this project will be three years in duration. The first seedlings we evaluate in the summer of 2016 will be those collected in October of 2014. The seedlings need to grow two years in order to ensure a non-lethal needle harvest. Subsequently, in the summers 2017-19 we will evaluate seedlings collected in 2015-17.

## 2016 Detailed Project Budget

**Project Title:** Genetic Analysis of Minnesota's Most Endangered Tree Species

*INSTRUCTIONS AND TEMPLATE (1 PAGE LIMIT)*

*Attach budget, in MS-EXCEL format, to your "2016 LCCMR Proposal Submission Form".*

*(1-page limit, single-sided, 10 pt. font minimum. Retain bold text and DELETE all instructions typed in italics. ADD OR DELETE ROWS AS NECESSARY. If budget item row is not applicable put "N/A" or delete it. All of "Other Funds" section must be filled out.)*

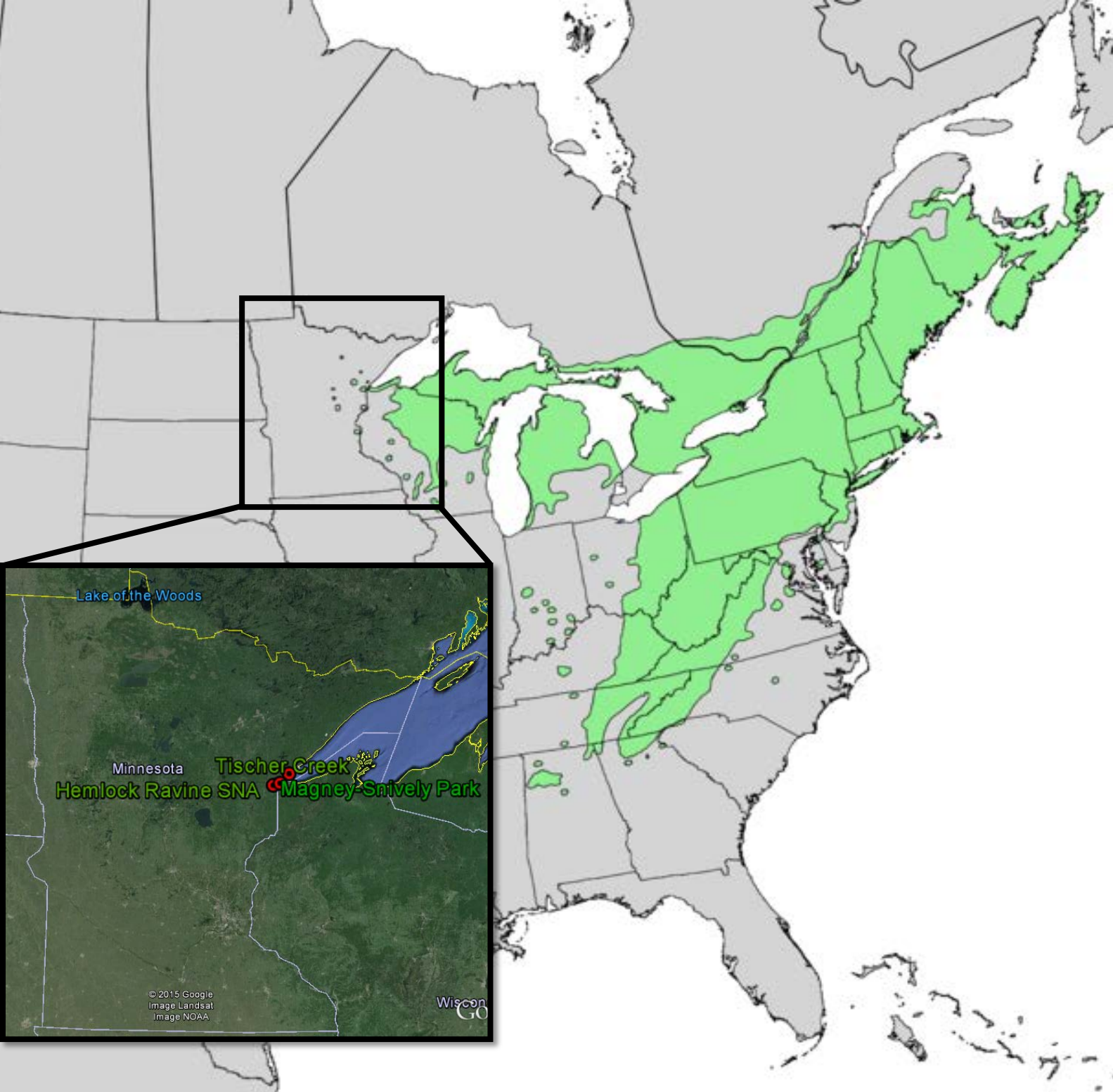
### IV. TOTAL ENRTF REQUEST BUDGET [ 3 ] years

<b>BUDGET ITEM</b> (See "Guidance on Allowable Expenses", p. 13)	<b>AMOUNT</b>
<b>Personnel:</b> Emily Ellingson, Graduate Student, (100% salary and fringe) Yr. 1 \$43,132, Yr. 2 \$44,353, Yr. 3 \$45,609	\$133,094
<b>Professional/Technical/Service Contracts:</b> Arborist for seed cone collection (climbing) Levy Tree Care \$80/hour for 10 days/yr. for three years	\$19,200
<b>Equipment/Tools/Supplies:</b> Microsatellite (SSR) analysis of seedlings @ \$16.00 per seedling, 2,000 seedlings/yr. for 3 years	\$96,000
<b>Equipment/Tools/Supplies:</b> Lab supplies reagents for DNA extraction, quantification, prep \$6,000/yr. for 3 years	\$18,000
<b>Equipment/Tools/Supplies:</b> Germination trays, seedling trays, tree pots, soil mix, fertilizer for greenhouse growing	\$3,500
<b>Acquisition (Fee Title or Permanent Easements):</b> N.A.	N.A.
<b>Travel:</b> Truck rental UMN Fleet Services, \$48/day, 12 days/yr. for 3 years	\$1,728
<b>Travel:</b> Meals, lodging, incidentals for travel to Duluth sites, \$150, 12 days/yr. for 3 years	\$5,400
<b>Additional Budget Items:</b> Fees to publish results in peer-reviewed journal, \$100/pg.	\$1,000
<b>TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND \$ REQUEST =</b>	<b>\$ 277,922</b>

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

<b>SOURCE OF FUNDS</b>	<b>AMOUNT</b>	<b>Status</b>
<b>Other Non-State \$ To Be Applied To Project During Project Period:</b> N.A.	N.A.	<i>Indicate: Secured or Pending</i>
<b>Other State \$ To Be Applied To Project During Project Period:</b> N.A.	N.A.	<i>Indicate: Secured or Pending</i>
<b>In-kind Services To Be Applied To Project During Project Period:</b> Minnesota Landscape Arboretum arborist services for seed cone harvest, \$80/hr. for 3 days/yr. for three yrs.	\$5,760	Secured
<b>Funding History:</b> Minnesota Ag. Experiment Station funds have been used to pay Grad Student salary from May 2014-June 2016	\$52,158	
<b>Funding History:</b> MN Ag. Experiment Station funds have been used to pay fees for arborist services, 2014, 2015	\$1,200	
<b>Remaining \$ From Current ENRTF Appropriation:</b> N.A.	N.A.	<i>Indicate: Unspent? Legally Obligated? Other?</i>

Appendix 1. North American range and remnant Minnesota native populations (insert) of Eastern hemlock (*Tsuga canadensis* (L.) Carrière).



## **Appendix 2. Project Manager Qualifications & Organization Description**

Dr. Stan C. Hokanson is a Professor in the Department of Horticultural Science where he directs the Woody Landscape Plant Breeding and Genetics program. Hokanson has studied gene flow and hybridization in both natural and agricultural systems and utilized a variety of molecular marker systems to study genetic diversity in several plant species including an ongoing effort in collaboration with Dr. James Bradeen to evaluate native deciduous azalea germplasm. Dr. Bradeen has a completely equipped molecular biology laboratory where all lab work will be conducted. Hokanson has access to greenhouse and field space for germinating and growing hemlock seedlings.

Hokanson and Bradeen have worked on a number of collaborative research projects that have resulted in successful graduate students and peer-reviewed manuscripts. Their labs meet on a bi-monthly basis to discuss research projects and findings. Hokanson will serve as the overall director of this project and will refer to Dr. Bradeen's molecular expertise in carrying forward those aspects of the project.

We have been working in conjunction with the Minnesota Department of Natural Resources, specifically individuals within the Endangered Species Program, Scientific and Natural Area Program, Division of Parks and Trails and Minnesota Biological Survey as we have developed this project. They have provided permits for collecting needle samples and cones for the project in Magney-Snively Park and Tischer Creek in Duluth, Hemlock Ravine Scientific and Natural Area, Jay Cooke State Park, Gooseberry Falls State Park and McCarthy Beach State Park. The later locations have Eastern hemlocks that are questionable Minnesota natives.

We have consulted with Susan Wilkins, Garden Curator at the Eloise Butler Wildflower Gardens (Minneapolis Park Board) regarding 38 mature Eastern hemlock thought to be derived from native Minnesota trees that are growing in the garden. They have provided us access to the trees. We will be evaluating tissue samples from the trees to determine whether they are Minnesota native in origin. If the trees do prove to be of Minnesota origin, they could provide an additional source of seed for any restoration efforts the DNR might undertake in the future.

We have been working with the Minnesota Landscape Arboretum (MLA), which is part of the University of Minnesota system and the home for Hokanson's field research efforts. The MLA is providing access to 17 Eastern hemlock trees thought to be derived from extirpated Minnesota native trees from the Mille Lacs Lake region. As mentioned previously, if these trees do prove to be of Minnesota origin, they could serve as a seed orchard and an additional source of seed for any restoration efforts the DNR might pursue in the future. The MLA is providing arborist services for cone harvests at the arboretum and greenhouse space for growing the plant material. We are requesting funding for germination flats, seedling trays, tree pots, potting soil, and fertilizer to be used for growing the trees at the MLA.

We are also in the process of identifying collaborators in Wisconsin and North Carolina who can collect Eastern hemlock cones from trees growing in more extensive populations in the northern part of its natural range (Wisconsin) and in the heart of its natural range (North Carolina). Due to their size, these populations are presumably characterized by trees with higher levels of genetic diversity. These individuals will provide good reference comparisons for our study.