

Final Abstract

Final Report Approved on January 15, 2026

M.L. 2022 Project Abstract

For the Period Ending June 30, 2025

Project Title: Forever Green

Project Manager: Mitchell Hunter

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

Mailing Address: 411 Borlaug Hall 1991 Upper Buford Circle

City/State/Zip: Saint Paul, MN 55108

Phone: (651) 675-7380

E-mail: mhunter@umn.edu

Website: <https://cfans.umn.edu/>

Funding Source:

Fiscal Year:

Legal Citation: M.L. 2022, Chp. 94, Sec. 2, Subd. 10i

Appropriation Amount: \$763,000

Amount Spent: \$763,000

Amount Remaining: -

Sound bite of Project Outcomes and Results

This project advanced six novel crops that have the potential to protect Minnesota's water, soil, and wildlife by keeping the soil covered all year round. Researchers made progress on improving crop germplasm, understanding pest interactions, and developing higher-value end uses.

Overall Project Outcome and Results

During the period of this award, the research sub-projects supported by this project advanced the development of perennial and winter-annual crops and cropping systems. Due to funding under LCCMR, our researchers: (1) better understand the relationship between pennycress and a common soybean pathogen, which can help increase adoption of pennycress in corn-soybean rotations and investigate pennycress lines may have greater resistance to SCN, (2) identified hardy lines of perennial cereal rye and initiated variety development for varieties suitable for MN, (3) evaluated elite hybrid hazelnut lines and identified the best-performing commercial variety for Minnesota conditions that displayed high winter survival and vigor, (4) demonstrated that fungal fermentation improves the nutritional and functional value of winter camelina and pennycress meals as animal feed and may increase the value of this crop to farmers by providing additional markets for oilseed byproducts, (5) completed three cycles of selection for seedling vigor

in silflower and began developing genomic data resources for silflower which will enable more efficient breeding, and (6) enhanced perennial sunflower breeding through improving yield and fertility. All of these outcomes, when implemented within Minnesota's farming community, have enormous potential to improve water quality, decrease pollution, and increase farmer income.

Project Results Use and Dissemination

The project disseminated results through: (1) the Forever Green Forum, a two-day event drew over 281 farmers, industry, policymakers, NGOs, and more, (2) the weekly Forever Green Seminar Series, (3) Government and Legislator information sessions and tours on CLC crops, (4) presentations and field tours for many groups, including the Minnesota Agricultural Water Quality Certification Program, the CWC, and the Friends of the Mississippi River, (5) presentations at local and national conferences, including the American Phytopathological Society, Minnesota Organic Conference, and ASA, CSSA, and SSSA International Annual Meeting and (6) scientific studies in Fermentation and the Journal of ASABE.



Environment and Natural Resources Trust Fund

M.L. 2022 Approved Final Report

General Information

Date: January 30, 2026

ID Number: 2022-303

Staff Lead: Lisa Bigaouette

Project Title: Forever Green

Project Budget: \$763,000

Project Manager Information

Name: Mitchell Hunter

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

Office Telephone: (651) 675-7380

Email: mhunter@umn.edu

Web Address: <https://cfans.umn.edu/>

Project Reporting

Final Report Approved: January 15, 2026

Reporting Status: Project Completed

Date of Last Action: January 15, 2026

Project Completion: June 30, 2025

Legal Information

Legal Citation: M.L. 2022, Chp. 94, Sec. 2, Subd. 10i

Appropriation Language: \$763,000 the second year is from the trust fund to the commissioner of agriculture for grants to the Board of Regents of the University of Minnesota to fund the Forever Green Agriculture Initiative and protect the state's natural resources while increasing the efficiency, profitability, and productivity of Minnesota farmers by incorporating perennial and winter-annual crops into existing agricultural practices.

Appropriation End Date: June 30, 2025

Narrative

Project Summary: The Forever Green Initiative will fund up to eight research projects focused on developing perennial and winter-annual crops that protect soil, water, and other natural resources.

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

Protection and enhancement of Minnesota's natural resources is complicated by agricultural production systems that primarily produce summer-annual crops. These crops only cover the soil during the warm season; at other times, soil is vulnerable to erosion and the loss of nutrients to surface water or groundwater. Increasing vegetative cover on agricultural land can help protect soil health and water quality while improving wildlife habitat, pollinator resources, and carbon sequestration potential. Traditionally in the U.S., such conservation has been undertaken through land retirement, which is costly and limits opportunities for rural economic development.

However, a new approach is emerging, based on augmenting current crop production systems with economically viable perennial and winter-annual crops. Such crops enable farmers to move toward continuous vegetative cover on their land—protecting soil, water, and other natural resources—while also producing marketable agricultural commodities. To realize the potential of this new approach, it is essential to develop and maintain active research programs that can breed economically viable varieties of these crops and determine how to manage them within cropping systems.

Likewise, robust end uses, markets, and supply chains are critically needed to enable scaling and maximize the benefits for Minnesota's natural resources.

What is your proposed solution to the problem or opportunity discussed above? Introduce us to the work you are seeking funding to do. You will be asked to expand on this proposed solution in Activities & Milestones.

The University of Minnesota's Forever Green Initiative (FGI) is the national leader in this new approach to protecting and enhancing natural resources while driving new economic opportunities for growers, industry, and communities. The FGI focuses on development of new perennial and winter-annual crops, new agricultural production systems that include these crops, and new supply and value chains that provide profitable markets for these crops. For example, FGI is taking this approach to advancing winter-hardy "cash cover crops" such as pennycress, camelina, and winter barley, and perennial crops such as intermediate wheatgrass (Kernza®) and hybrid hazelnuts.

There are sixteen FGI crop teams spanning disciplines including genomics, breeding, agronomy, natural resource sciences, food science, sociology, economics, and commercialization. This interdisciplinary approach allows for rapid scientific advancement, leading to crop varieties with attractive characteristics for farmers coupled with information on agronomic management and ecological benefits. Food science research and engagement with potential end-users paves the way for rapid commercialization, which is critical to scaling up production and thus realizing the potential for water quality improvement.

The requested funding will support continued advancement of these crops, cropping systems, and supply chains. Funds will be awarded via a competitive process to ensure maximum impact.

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?

Research sub-projects supported by this project will help advance development of perennial and winter-annual crops and cropping systems that help protect soil and water resources while also producing marketable commodities. Specific outcomes could include: improved understanding of crop genomes that enable more efficient breeding; development of enhanced breeding programs that accelerate progress toward release of commercial varieties; improved knowledge of management practices that enhance yield, profitability, and ecosystem services; enhanced understanding of natural resource outcomes; advances in food science that show the nutritional and functional characteristics of the crops; and development of new potential end uses and supply chains.

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

Activities and Milestones

Activity 1: Selecting sub-projects

Activity Budget: \$1

Activity Description:

Funds will be awarded to up to eight sub-projects, with a maximum award amount of \$150,000, following a request for proposals (RFP) and peer review process. There will be a single RFP in the fall of 2022 with funding awards made by early 2023. Proposals will be reviewed by a panel including FGI faculty and staff and department heads from across the College of Food, Agricultural, and Natural Resources Sciences (CFANS). The RFP process will be overseen by the CFANS Associate Dean for Research and Graduate Programs. The Associate Dean's office and FGI staff will work together to develop the RFP and evaluation criteria based on academic best practices, experience with past FGI RFPs, and the FGI strategy and mission. Evaluation criteria will include: 1) alignment with the FGI strategy for protecting natural resources, 2) technical and scientific merit, 3) qualifications of the research team, and 4) timeliness and necessity of the project. All peer reviewers will score proposals and the panel will meet to discuss and select proposals for funding and set funding amounts. The Associate Dean and FGI leadership will meet to review the panel's recommendations and ensure alignment with the FGI strategy and mission.

Activity Milestones:

Description	Approximate Completion Date
Develop RFP and evaluation criteria	September 30, 2022
Release RFP to Forever Green Initiative researchers	September 30, 2022
Receive proposals	October 31, 2022
Peer review panel completes review and meets to discuss proposals	January 31, 2023
Final funding awards determined and announced	February 28, 2023

Activity 2: Awarding and administering sub-projects

Activity Budget: \$762,993

Activity Description:

Each sub-project recommended for funding will be added as an activity in an amended work plan for LCCMR review and approval. Proposals will be developed using the LCCMR work plan and budget structures during the RFP process, so that the materials can be readily uploaded into the LCCMR dashboard. Leaders of each sub-project will be given access to the dashboard to upload their information. The project manager, Mitch Hunter, will assist as needed and ensure that all materials are complete for all new sub-projects before submitting the work plan amendment request. The project manager will also liaise with Margaret Wagner at the Minnesota Department of Agriculture (MDA) to ensure that the budget categories used align with MDA categories, since the funds will be supplied as a contract between MDA and UMN. When the work plan is amended, funds will be shifted from the budget line "to be awarded to sub-projects" to the budget line "awarded to sub-projects" in Professional and Technical Contracts. The project manager will ensure timely project updates and submission of the final report and findings.

Activity Milestones:

Description	Approximate Completion Date
Submit work plan revision with new subprojects	March 31, 2023
Provide ongoing grant support and ensure timely submission of project updates	June 30, 2025
Submit final report and findings	June 30, 2025

Activity 3: SP1 - Anderson - Develop Soybean Cyst Nematode Management Strategies for Pennycress as an Oilseed Cover Crop in Corn-Soybean Production

Activity Budget: \$1

Activity Description:

Pennycress is a harvestable winter oilseed crop intended for inclusion in Midwest corn and soybean rotations. However, incorporating pennycress into the soybean-corn production system is challenged by the widespread devastating pest, the soybean cyst nematode (SCN), which can also parasitize pennycress. Therefore, inclusion of pennycress in the system may increase SCN populations and hinder soybean productivity in the system. The impact of SCN in pennycress must be investigated before it can be commercialized as an oilseed cover crop in soybean-corn production systems. The goal of this project is to develop knowledge needed to manage this risk. Specifically, the objectives of this project are to (1) refine our understanding of SCN overwinter survival in pennycress, and 2) identify pennycress accessions with the greatest and least resistance to SCN, to enable future work identifying resistance genes. This research will both improve our mechanistic understanding and reduce risk for growers. The ultimate outcome will be improvement of ecosystem services, clean water and reduced soil erosion, increased crop productivity, and economic benefits for growers.

Activity Milestones:

Description	Approximate Completion Date
Establish experiment to study SCN survival on pennycress under field conditions	September 30, 2023
Evaluate at least 30 pennycress lines for SCN resistance in inoculated conetainers	May 31, 2024
Repeat field study (Milestone 1) and analyze results of two studies	June 30, 2025
Prepare and deliver project Outreach, including presentations and one or more publications	June 30, 2025

Activity 4: SP2 - Bajgain - Initiating A Perennial Cereal Rye Breeding Program for MN Environments Using Phenotypic and Genomic-based Selection Methods

Activity Budget: \$1

Activity Description:

We will initiate a breeding and genetic improvement program for perennial cereal rye (PC-Rye), a grain crop with dual use potential. PC-Rye has high biomass, vigorous growth, and produces grain that can be used for feed and food. We have assembled a diverse panel of 2160 PC-Rye accessions that were obtained by open-pollinating 620 accessions that were evaluated in St. Paul, MN during 2020-2022. We will combine traditional breeding principles and methodologies with high-throughput DNA fingerprinting and robust statistical modeling methods to improve the PC-Rye germplasm. Important traits such as winter survivability, perenniability, grain yield, seed size, threshability, plant height, lodging, and disease resistance will be evaluated in St. Paul fields. The diversity panel will be genotyped on a high-throughput sequencing platform to discover DNA markers that will be used in genetic mapping of aforementioned important traits. The use of DNA markers in genomic selection, a predictive method of selection that combines information obtained from DNA markers and phenotypic data, will also be explored. This project will evaluate a diverse PC-Rye panel in MN conditions for the first time, and use genetic tools to improve the germplasm. Best performing plants will be selected for their potential use in variety development.

Activity Milestones:

Description	Approximate Completion Date
Assess a diverse perennial cereal rye germplasm for important agronomic traits	July 31, 2024
Discovery of genome-wide DNA markers using high-throughput sequencing approach	August 31, 2024
Identify best-performing lines to be used as parents of synthetic variety populations	September 30, 2024
Use DNA markers in genetic mapping and genomic selection	November 30, 2024

Activity 5: SP3 - Braun - Evaluating 2nd Generation Hybrid Hazelnut Germplasm for Minnesota, Phase Two: Establishing Multi-Location Replicated Yield Trials

Activity Budget: \$1

Activity Description:

We have been working since 2008 to improve the nut quality of hybrid hazelnuts while retaining their winter hardiness, disease resistance and yield potential. These are hybrids between domesticated large-seeded European hazelnuts (*Corylus avellana*), which are not hardy in Minnesota, and wild smaller-seeded American hazelnuts (*Corylus americana*), which are adapted to our climate and diseases. We will soon release our first generation of selections to growers. This proposal pertains to our second generation, progeny from controlled crosses between our top first-generation and European hazelnut selections from Oregon. Based on four years of nut data about the 2014-2015 cohort of these 8,000 F1 seedlings, we will select the best to move into multi-location replicated yield trials. We will propagate the top 30, primarily by mound layering, to establish in six replicated yield trials, alongside our top first-generation selections and top selections from other breeding programs. We will also continue to evaluate the 2016 cohort of F1 seedlings, for later addition to the replicated trials. This project will also contribute plant material to collaborators developing propagation protocols and support small scale trials of our own with seed graft layering and stem cuttings.

Activity Milestones:

Description	Approximate Completion Date
Finalize selections of second-generation germplasm for propagation and further evaluation	May 31, 2023
Secured first plants for replicated trials through propagation efforts (seed grafting, stem cutting, mound layering)	November 30, 2023
Clones of second generation germplasm that was propagated in 2023 established in field trials.	May 31, 2024
Complete bulletin with grower recommendations about 1st-generation UMHDI germplasm and germplasm from Rutgers and Grimo.	June 30, 2025

Activity 6: SP4 - Hu - Advancing Biorefinery of Camelina and Pennycress Meal for Valuable Products

Activity Budget: \$1

Activity Description:

This activity aims to develop a biorefinery process using naturally available fungi to improve the nutritional values and edibility of pennycress (PM) and camelina (CAM) oilseed meals, and therefore increase the applications and profitability of these cover crops. Fungal strains used in this project are *Trametes versicolor* and *Aspergillus oryzae*, which are generally considered as safe and have great capacity of degrading incalcitrant components in lignocellulosic biomass. The fungal processed meal will be extracted with buffer to obtain the biocatalysts produced with remaining solids dried and served as high-value products potentially for human food and different types of animal feeds. Valuable products from the proposed biorefinery process include high-protein and low-fiber feeding ingredients for monogastrics, high-digestible fiber and low-lignin feeding ingredients for ruminants, fermented flavor paste as human food ingredients, as well as lignocellulolytic biocatalysts including but not limited to cellulase, xylanase, laccase, and peroxidases. Besides developing the biorefinery process, this project will conduct detailed chemometrics analysis on the chemical and nutrient composition of fermented PM and CAM. A mouse feeding trial will also be carried out to examine the nutritional value and safety profile *in vivo*. The results will facilitate future development of commercial products from these oilseed meals.

Activity Milestones:

Description	Approximate Completion Date
Understand composition of fermented feeds through chemometric analysis	June 30, 2023
Understand nutritional value and safety of fermented feed	September 30, 2024
Evaluate fermented oilseed meal for potential use in food and feed products	June 30, 2025
Understand the economic feasibility of oilseed processing for valuable bioproducts	June 30, 2025

Activity 7: SP5 - Smith - Improving Silflower Stand Establishment

Activity Budget: \$1

Activity Description:

Silphium integrifolium Michx. (silflower) is a native perennial related to sunflower that has potential as a perennial oilseed crop that could deliver important ecosystem services. One obstacle to the production of silflower in Minnesota is establishing a vigorous stand in the first year of production. We propose to complete three cycles of recurrent selection for vigorous seedling growth and establishment to generate improved germplasm for this trait that can be introgressed into our breeding program. We will also use the base population and individuals from cycles of selection to understand the genetics of seedling vigor using association mapping. We will conduct a genome wide association study (GWAS) with an established panel to characterize and map other traits associated with vigorous seedling growth and establishment. This project will generate advanced breeding germplasm that is enhanced for seedling establishment, new information about the genetic architecture for seedling establishment traits, and marker tools that will be integrated into the silflower breeding program to improve these traits.

Activity Milestones:

Description	Approximate Completion Date
Initiate selection of cycle 2 individuals	August 31, 2023
Complete GWAS analysis of the <i>Silphium</i> Domestication Panel	December 31, 2023
Initiate selection of cycle 3 individuals	June 30, 2024
Complete genetic gain experiment	June 30, 2025

Activity 8: SP6 - Stupar - Development of germplasm resources for creating a perennial sunflower for food production and wildlife services

Activity Budget: \$1

Activity Description:

This project aims to generate a novel perennial sunflower crop that will help to preserve water and soil resources while enhancing economic opportunities for farmers and rural communities. Using intermated progeny from hybrids of perennial *Helianthus tuberosus* and the domesticated annual *H. annuus*, we will continue to advance the already nine generations of selection on this material. To date, we have made great progress in uniting desirable perennial traits from *H. tuberosus* (e.g. over winter survival, perennial structures) with desirable domestication traits from *H. annuus* (e.g. apical dominance, large heads). Recent developments demonstrate progress in overcoming the plant fertility issues that have challenged this breeding program in the past. We have recently altered our approach to emphasize repeated rounds of self-pollination, which has resulted in restored fertility in several lines. Through this effort, we have developed both sub-populations of highly fertile inbred lines, and less fertile lines with strong perenniality traits. We plan to mate the fertile inbred lines with the lines exhibiting strong perenniality, resulting in an expanded pool of useful breeding lines. This genetically diverse breeding pool with elevated fertility will greatly enhance progress in the development of this promising crop.

Activity Milestones:

Description	Approximate Completion Date
Mate fertile inbred lines with lines exhibiting strong perenniability	July 31, 2023
Evaluate hybrid progeny in the new pool of breeding lines	September 30, 2024
Test elite lines of perennial sunflower developed by this project	June 30, 2025

Project Partners and Collaborators

Name	Organization	Role	Receiving Funds
TBD	TBD	The Forever Green Initiative collaborates with many partners to conduct research and develop supply chains. Some current partners include the Agricultural Utilization Research Institute, USDA Agricultural Research Service, The Land Institute, and a number of Minnesota-based food and agriculture businesses.	No
Margaret Wagner	Minnesota Department of Agriculture	Administrative partner and project collaborator	No

Dissemination

Describe your plans for dissemination, presentation, documentation, or sharing of data, results, samples, physical collections, and other products and how they will follow ENRTF Acknowledgement Requirements and Guidelines. Findings, data, and other research products will be disseminated through multiple channels. Updates on progress and research results will be disseminated by CFANS via websites, social media, publications, and media releases. Findings will be presented at local and national conferences and via peer-reviewed publications and student theses. Findings will also be shared with growers and industry partners at field days and meetings. The Forever Green Partnership, which unites members from private, public, and advocacy sectors around a common interest in increasing Continuous Living Cover in agriculture, provides another avenue to share outcomes with NGOs and companies, as does the MBOLD coalition, which includes many of the leading food and agriculture companies based in Minnesota. Crop varieties developed as a result of this research will be released to farmers in partnership with private seed companies. Funding from the Minnesota Environment and Natural Resources Trust Fund (ENRTF) will be acknowledged through use of the trust fund logo or attribution language on project print and electronic media, publications, signage, and other communications and outreach.

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 work be funded?

This project will enable FGI crop teams to continue the process of developing crops and moving them toward commercialization. Depending on the stage of crop development, implementation will proceed through ongoing scientific research and partnerships with seed companies, supply chain actors, and end users. The FGI has been supported by a wide range of funding sources over many years, including the Minnesota Legislature, LCCMR, other state funding, and a range of federal and private funding. The FGI and crop teams will continue to pursue funding from this diverse mix of sources to advance our research and lead to on-the-ground implementation.

Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Bene fits	# FTE	Class ified Staff?	\$ Amount	\$ Amount Spent	\$ Amount Remaining
Personnel										
							Sub Total	-	-	-
Contracts and Services										
To be awarded to sub-projects	Subaward	Budget reserve for awarding to subprojects upon approval by LCCMR				11		-	-	-
Awarded to sub-projects	Subaward	Total amount awarded to subprojects				0		\$763,000	\$763,000	-
							Sub Total	\$763,000	\$763,000	-
Equipment, Tools, and Supplies										
							Sub Total	-	-	-
Capital Expenditures										
							Sub Total	-	-	-
Acquisitions and Stewardship										
							Sub Total	-	-	-
Travel In Minnesota										
							Sub Total	-	-	-
Travel Outside Minnesota										
							Sub Total	-	-	-
Printing and Publication										

							Sub Total	-	-	-
Other Expenses							Sub Total	-	-	-
							Grand Total	\$763,000	\$763,000	-

Classified Staff or Generally Ineligible Expenses

Category/Name	Subcategory or Type	Description	Justification Ineligible Expense or Classified Staff Request
---------------	---------------------	-------------	--

Non ENRTF Funds

Category	Specific Source	Use	Status	\$ Amount	\$ Amount Spent	\$ Amount Remaining
State						
			State Sub Total	-	-	-
Non-State						
In-Kind	McKnight Foundation funding for Forever Green Initiative	Staff time administering the internal RFP process and completing progress reports	Secured	\$7,500	\$5,000	\$2,500
			Non State Sub Total	\$7,500	\$5,000	\$2,500
			Funds Total	\$7,500	\$5,000	\$2,500

Attachments

Required Attachments

Visual Component

File: [e6455fc4-0e4.pdf](#)

Alternate Text for Visual Component

A 42-page document providing an overview of the Forever Green Initiative, including the challenge we are addressing, our logic model, an overview of our proposed solution, and detailed information on our 17 crops and crop groups....

Supplemental Attachments

Capital Project Questionnaire, Budget Supplements, Support Letter, Photos, Media, Other

Title	File
Background Check Certification Form	25b5a5f8-069.pdf
Sub-Project Budget Template	735cf03c-e37.xlsx
Sub-Project Budget Template 1.17.25	861c799b-554.xlsx
Sub-Project Budget Template 6.30.25	63edcbf7-b54.xlsx
Activity 4. Stoll, H., Bajgain, P., & Anderson, J. A. (2025, November). Perennial cereal rye at the University of Minnesota: Breeding updates and genome constitution [Oral presentation]. ASA, CSSA, and SSSA International Annual Meeting, Salt Lake City, UT, United States.	900157a0-9a2.pdf
Activity 3. Chen, S., Heydari, F., Zhang, J., and Anderson, J. 2024. FGI Research Update - Soybean Cyst Nematode Associated with Pennycress. Forever Green Initiative Laboratory Meeting. Oct 4, 2024, University of Minnesota. (Presentation)	872da8bd-b4f.pdf
Activity 3. Chen, S., Hoerning, C., Heydari, F., Zhang, J. X., Anderson, J. A., Hunter, M., Wells, S., and Wyse, D. 2025. Minimal risk of pennycress as a winter oilseed cover crop for soybean cyst nematode management in soybean rotation systems. University of Minnesota Forever Green Initiative Forum. (Poster)	83008013-af3.pdf
Activity 7. Smith, K.P. Overview of research to develop Silflower crop production. 2025 Annual Silphium Meeting, Salina, KS, Aug. 26, 2025. (Presentation)	a9c7168f-47c.pdf
Activity 7. Krug, C.J., Recurrent Selection of Silphium integrifolium for Improved Seedling Vigor [Oral presentation]. Perennial Grains Workshop, Salina, KS, Sept. 24, 2024.	c4edf476-c7f.pdf
Activity 7. Smith, K.P., Schiffner, S., Krug, C., Beaubien, K., Nesser, S., Jungers, J. 2024. Silflower development: Genomics, breeding, and agronomics. Forever Green Initiative Laboratory Meeting. Nov 10, 2023, University of Minnesota.	d4f38689-ccb.pdf
Activity 7. Smith, K.P. University of Minnesota Silflower Breeding. 2023 Annual Silphium Meeting, St. Louis, MO, Sept 10-12, 2023.	ba17728c-9a0.pdf
Activity 7. Krug, C.J., Seedling Vigor Recurrent Selection Experiment [Oral presentation]. 2025 Annual Silphium Meeting, Salina, KS, Aug. 25, 2025.	0eeecf84-f63.pdf
Activity 8. Public Presentations to the Forever Green Seminar Series and Forum	444b9fbb-aa2.pdf
Activity 5. 2025 Hazelnut Cultivar Launch Poster for Forever Green Forum - May 2025	f65bd7d3-dae.pdf
Activity 5. Minnesota Hazelnut Week Webinar	9946c7ec-fc5.pdf

Activity 5. Forever Green Forum Poster: Delorean, E., Braun, L., Hamann, M. Enabling the Next Generation of Hazelnuts: Advanced Breeding Tools for a Resilient Midwest Crop.	34dbb733-0bc.pdf
Activity 5. Forever Green Initiative Seminar Series Presentations, University of Minnesota, St. Paul.	f82fc1a5-78c.pdf
Activity 5. Brainard, S., Delorean, E., Braun, L. A Plan for Breeding Better Hazelnuts for the Upper Midwest. (Presentation) UMHDI conference, Wisconsin Dells. March 2025.	a6931bfc-7f5.pdf

Media Links

Title	Link
Activity 4: University of Minnesota Extension Site: Perennial cereal rye grain: A dual-use crop to diversify agriculture.	https://blog-crop-news.extension.umn.edu/2024/12/perennial-cereal-rye-grain-dual-use.html
Activity 6: Kristin Boardman, Xiao Sun, Dana Yao, Chi Chen, Bo Hu, 2025. Fungal bioprocessing of camelina meal for improved nutritional profile, Journal of ASABE, Vol. 68(2): 217-224	https://elibrary.asabe.org/abstract.asp?AID=55243&t=3&dabs=Y&redir=&redirType=
Activity 6: Kristin Boardman, Xiao Sun, Dana Yao, Chi Chen, Leif van Lierop, Bo Hu, 2025. Increasing the Nutritional Value of Camelina Meal Via Trametes versicolor Solid-State Fermentation with Various Co-Substrates, Fermentation, 11(2), 77	https://www.mdpi.com/2311-5637/11/2/77
Activity 6: Sun, X., Boardman, K., Marks, D., Wyse, D., Hu, B., 2023. Fungal bioprocessing to improve quality of pennycress meal as potential feeding ingredient for monogastric animals. Fermentation 9(8): 732	https://www.mdpi.com/2311-5637/9/8/732
YouTube Recordings of 2025 Forever Green Forum	https://www.youtube.com/playlist?list=PLVS8VXGDu-B-pCjI9zI5gfoBq4tk-WdPp
Activity 3. Chen, S., Hoerning, C., Heydari, F., Zhang, J. X., Anderson, J. A., Hunter, M., Wells, S., and Wyse, D. 2025. Minimal risk and potential benefits of pennycress as a winter oilseed crop for managing soybean cyst nematode in soybean rotation systems in the northern climates. Journal of Nematology. 57:e2025-1. Page 28. DOI:	https://reference-global.com/article/10.2478/jofnem-2025-0049

10.2478/jofnem-2025-0049. (Abstract and oral presentation)	
Activity 3. Heydari, F., Hoerning, C., Zhang, J. X., Anderson, J. A., Hunter, M., Wyse, D., and Chen, S. 2025. Assessing winter pennycress (<i>Thlaspi arvense</i>) as a potential trap crop for soybean cyst nematode (<i>Heterodera glycines</i>) management. American Phytopathological Society 77th North Central Division Meeting, June 9-11 2025, South Dakota. (Abstract and poster)	https://planhealth2025.eventscribe.net/fsPopup.asp?PresentationID=1664417&mode=presInfo
Activity 3. Heydari, F., Hoerning, C., Zhang, J. X., Anderson, J. A., Hunter, M., Wyse, D., and Chen, S. 2025. Overwinter survival of soybean cyst nematode (<i>Heterodera glycines</i>) in pennycress and soil under cold conditions. <i>J. Nematol.</i> e2025-1. Page 63. DOI: 10.2478/jofnem-2025-0049. (Abstract and poster)	https://reference-global.com/article/10.2478/jofnem-2025-0049

Difference between Proposal and Work Plan

Describe changes from Proposal to Work Plan Stage

N/A

Additional Acknowledgements and Conditions:

The following are acknowledgements and conditions beyond those already included in the above workplan:

Do you understand and acknowledge the ENRTF repayment requirements if the use of capital equipment changes?
N/A

Do you understand that travel expenses are only approved if they follow the "Commissioner's Plan" promulgated by the Commissioner of Management of Budget or, for University of Minnesota projects, the University of Minnesota plan?

N/A

Does your project have potential for royalties, copyrights, patents, sale of products and assets, or revenue generation?

Yes

Do you understand and acknowledge IP and revenue-return and sharing requirements in 116P.10?

Yes

Do you wish to request reinvestment of any revenues into your project instead of returning revenue to the ENRTF?

No

Does your project include original, hypothesis-driven research?

Yes

Does the organization have a fiscal agent for this project?

Yes, Sponsored Projects Administration

Do you understand that a named service contract does not constitute a funder-designated subrecipient or approval of a sole-source contract? In other words, a service contract entity is only approved if it has been selected according to the contracting rules identified in state law and policy for organizations that receive ENRTF funds through direct appropriations, or in the DNR's reimbursement manual for non-state organizations. These rules may include competitive bidding and prevailing wage requirements

N/A

Work Plan Amendments

Amendment ID	Request Type	Changes made on the following pages	Explanation & justification for Amendment Request (word limit 75)	Date Submitted	Approved	Date of LCCMR Action
1	Amendment Request	<ul style="list-style-type: none"> • Activities and Milestones 	The Activity 1 description and milestone number 4 were updated to reflect the fact that the process used to select sub-projects is a peer review process. The review committee will be largely comprised of CFANS department heads.	September 12, 2022	Yes	September 13, 2022
2	Amendment Request	<ul style="list-style-type: none"> • Activities and Milestones • Budget - Professional / Technical Contracts • Attachments 	Sub-projects have been added as individual activities following selection in the RFP process. Funding has been shifted from "to be awarded to sub-projects" to "awarded to subprojects." The sub-project budgets have been uploaded in the provided template.	March 20, 2023	Yes	March 23, 2023

Status Update Reporting

Final Status Update August 14, 2025

Date Submitted: December 19, 2025

Date Approved: January 15, 2026

Overall Update

All subprojects made progress toward their goals and are considered complete at the time of the submission of this report. During the period of this award, the research sub-projects supported by this project advanced the development of perennial and winter-annual crops and cropping systems. Due to funding under LCCMR, our researchers: (1) better understand the relationship between pennycress and a common soybean pathogen, which can help increase adoption of pennycress in corn-soybean rotations, (2) identified hardy lines of perennial cereal rye and initiated variety development, (3) evaluated elite hybrid hazelnut lines and identified the best-performing commercial variety for Minnesota conditions, (4) demonstrated that fungal fermentation improves the nutritional and functional value of winter camelina and pennycress meals as animal feed, (5) completed three cycles of selection for seedling vigor in silflower and began developing genomic data resources for silflower which will enable more efficient breeding, and (6) enhanced perennial sunflower breeding through improving yield and fertility. All of these outcomes, when implemented within Minnesota's farming community, have enormous potential to improve water quality, decrease pollution, and increase farmer income.

Activity 1

This activity was previously marked complete.

(This activity marked as complete as of this status update)

Activity 2

Forever Green worked with all subproject leaders to ensure that they supply timely progress reports. We have consistently reminded them of LCCMR acknowledgement requirements. Ongoing support was provided to sub-grantees to administer their sub-projects. We have now supported the submission of the final report, so this activity is complete.

(This activity marked as complete as of this status update)

Activity 3

Pennycress is a winter oilseed crop intended for inclusion in Midwest corn and soybean rotations. However, pennycress is a host of the soybean cyst nematode (SCN), a significant pathogen of soybean. Consequently, incorporating pennycress into these rotations may increase the risk to soybean productivity. The goal of this project was to generate the knowledge necessary to manage this risk. Specifically, we investigated the genotype interaction between pennycress and SCN to determine resistance levels. Fourteen pennycress lines, selected for their low and high levels of SCN reproduction, were evaluated for resistance against 16 inbred lines and 4 field populations of SCN. These inbred lines represent various virulence types in the state. Female Indices (FI, a measure of SCN reproduction, with FI = 100 corresponding to a susceptible soybean control) for individual SCN–pennycress combinations ranged from 2.5 to 191.8, with an average of 42.9. These results indicate that certain SCN–pennycress genotype combinations supported high levels of SCN reproduction, raising concerns about potential SCN virulence in pennycress, but also indicate that some pennycress lines may have greater resistance to SCN. The SCN overwinter survival project is continuing with funding from a new MDA FGI grant.

(This activity marked as complete as of this status update)

Activity 4

One of the major goals of this objective was to evaluate a large perennial cereal rye (PC-Rye) population for perenniality and other agronomic traits. We observed true perenniality in 786 plants which were transplanted to a field in St. Paul in

fall 2023 and evaluated in summer 2024. Grain yield, plant height, resistance to lodging and diseases, seed size, thousand grain weight, and survival were phenotyped. Plants were also genotyped and genomic selection was applied to select the next cycle of individuals. Moreover, four synthetic varieties were initiated in Fall 2024 based upon the parents' performance in this trial. While this activity is officially complete, the rye breeding project will continue to improve populations and develop varieties suitable for MN and neighboring regions.

(This activity marked as complete as of this status update)

Activity 5

1) In Spring 2025, the project finished planting the replicated trials of controlled cross progeny. Survival and vigor of these plants was 100% at St. Paul and Waseca, 98% at Staples and 86% at Lamberton. These young plants are taller and narrower than the Phase One selections, reflecting the high level of European germplasm in them. Relatively little winter damage was observed in Spring 2025, and several even bore a few nuts.

2) In 2025 we did not add any new selections to the replicated trials because only one was found that was as good as those previously selected. This one selection will be propagated in 2026 and added to new replicated trials alongside any outstanding selections identified from 2025 data.

3) We found a clear winner from the cohort of selections planted in 2017 in our Joint Performance Trials: Northern Blais, from Grimo Nut Nursery in Ontario. Gertens Nursery in Inver Grove Heights has already secured the license to propagate it in the United States. None of the other 2017 selections are winter hardy enough for Minnesota, but several of the Midwest selections bore outstanding yields in 2025. A publication reporting these results is in progress.

(This activity marked as complete as of this status update)

Activity 6

Camelina Meal (CaM) fermentation was carried out by inoculating three industrial fungi: *Aspergillus oryzae* (AO), *Trametes versicolor* (TV), and *Pleurotus ostreatus* (PO). The fermented CaM, along with unfermented CaM and soybean meal as controls, were fed to 8-week-old male mice in a 3-week trial. The CaM inclusion rate increased each week: 10% in week 1, 20% in week 2, and 30% in week 3. Among the fermented CaM groups, AO feeding resulted in better body weight gain and feed intake compared to PO and TV. TV feeding decreased body weight and increased the hepatosomatic ratio with higher inclusion rates, while PO feeding initially increased body weight but caused a decline later. These results suggest that AO-fermented CaM may offer superior nutritional value as an animal feed ingredient.

Pennycress Meal (PeM) fermentation was done using AO, TV, PO, *Rhizopus oryzae* (RO), and *Trichoderma reesei* (TR). Proximate and chemometric analysis revealed comparable crude protein and amino acid levels between the fermented PeM and the unfermented control. AO and RO were most effective in degrading sinigrin, the primary glucosinolate in PeM. The study proved the feasibility of AO-fermentation in improving the feeding value and detoxification of pennycress meal and camelina meal.

(This activity marked as complete as of this status update)

Activity 7

This project involved three cycles of selection for seedling vigor starting with a diverse collection of Silphium germplasm. As of Fall 2025, the group is initiating Cycle 3 by planting individuals by November 2025. Based on the first two cycles, they found the selection differential for leaf area (proxy for seedling vigor) between the selected and the entire population for leaf area was highest in the Best Overall treatment, and lower in the Best Individual and Random groups. This trend was observed in both cycles of selection and suggests effective execution of the selection strategy. Evaluating gain will start once three cycles of selection are completed by 2026.

Second, the group investigated the genetics of seedling traits using the Silphium Domestication Panel using genotyping by sequencing (GBS) technology. GBS generated a marker data set that unexpectedly produced insufficient marker coverage and large amounts of missing data that was insufficient for genetic mapping studies. We are currently

exploring two strategies to overcome this challenge: (1) use imputation to fill in missing data, and (2) is to develop a targeted marker assay. Once this genotyping challenge has been resolved, we will conduct genetic mapping studies utilizing the comprehensive phenotypic datasets previously collected.

(This activity marked as complete as of this status update)

Activity 8

Objective 1. Develop high yielding perennial inbred lines: Over the course of this project, the team developed inbred lines through self-pollination. This approach resulted in the identification of perennial lines with high sexual fertility (i.e., those that develop higher numbers of seeds). Recurrent selfing and selection has resulted in development of eighteen lines that are self-fertile and perennial. Five inbred lines had seed yield over 1000 kg/ha during the 2024 field evaluation in St. Paul, reducing the gap between the perennial sunflower yield and that of the conventional annual sunflower varieties.

Objective 2. Add genetic diversity by restoring fertility to poor fertility lines: The goal of restoring fertility to low-fertility lines was accomplished by inter-mating with the improved fertility lines developed in Objective 1. Progeny of these crosses were self-pollinated for two cycles. Some of the resulting (F1S2) plants were found to have self-fertility, perennialism, and other desired traits. Additional cycles of self-pollination have been performed on selected plants. Seven new high-fertility lines showed excellent single headed types, with larger flowers than current improved fertility inbreds, possibly increasing yield potential. Four new lines developed by inter-mating of restored fertility lines are being evaluated for yield in 2025.

(This activity marked as complete as of this status update)

Dissemination

A major dissemination event at the end of this reporting period was the Forever Green Forum, a two-day event focused on reshaping the future of agriculture through long-term, systemic change. FGI acknowledged LCCMR's support often throughout this event. The Forum opened with a Field Day on May 28, where participants toured research plots and engaged with scientists and growers working on CLC crops. The following day, a diverse group of experts in agronomy, economics, ecology, food systems, and rural development shared insights on the science, policy, and market strategies needed to scale CLC systems.

Individual sub-projects acknowledged LCCMR funding through other dissemination activities, including: (1) the Forever Green Seminar Series, which draws 30-50 people weekly, (2) Government and Legislator information sessions and tours for MN Senate and House Agriculture Committee where researchers presented CLC crops, (3) presentations and field tours for many groups, including the Minnesota Agricultural Water Quality Certification Program, the Clean Water Council, and the Friends of the Mississippi River, (4) presentations at local and national conferences, including the American Phytopathological Society, the Minnesota Organic Conference, and the ASA, CSSA, and SSSA International Annual Meeting and (5) scientific studies in Fermentation and the Journal of ASABE.

Status Update Reporting

Status Update March 1, 2025

Date Submitted: February 28, 2025

Date Approved: February 28, 2025

Overall Update

Since the last update, all subprojects have been making progress toward their objectives, except Activity 4, which is complete.

This work is improving our understanding of crop genomes, enhancing our breeding programs, improving knowledge of management practices, allowing us to understand pest interactions, and advancing the functional characteristics of the crops.

Activity 1

This activity was previously marked complete.

(This activity marked as complete as of this status update)

Activity 2

A budget amendment was submitted on January 20th to reflect changes in spending that resulted from the normal process of adaptively managing research projects, addressing challenges as they arise and accommodating changes in personnel.

I have worked with all subproject leaders to ensure that they supply timely progress reports, both to MDA and LCCMR. I have consistently reminded them of LCCMR acknowledgement requirements.

Activity 3

Pennycress is a harvestable winter oilseed crop intended for inclusion in Midwest corn and soybean rotations. However, pennycress is a host of the soybean cyst nematode (SCN), a significant pathogen of soybean. Consequently, incorporating pennycress into these rotations may increase the risk to soybean productivity. The goal of this project is to generate the knowledge necessary to manage this risk, with a focus on evaluating SCN resistance in pennycress. Specifically, we are investigating the genotype interaction between pennycress and SCN to determine resistance levels. Fourteen pennycress lines, selected based on previous studies for their low and high levels of SCN reproduction, are being evaluated for resistance against 20 SCN inbred lines. These SCN lines, derived from populations collected across soybean-growing regions in Minnesota, represent various virulence types found in SCN-resistant soybeans. The experiments have been conducted in a greenhouse, and data collection has been completed for most pennycress-SCN combinations. However, a few pennycress lines exhibited poor germination and require retesting. Approximately 500 retest pots have been prepared in the greenhouse, and the experiment is expected to conclude by the end of March 2025.

Activity 4

One of the major goals of this objective was to evaluate a large PC-Rye population for perenniability. We observed true perenniability in 786 plants which were transplanted to a field in St. Paul in fall 2023 and evaluated in summer 2024. Traits evaluated so far are grain yield, plant height, resistance to lodging and diseases, and seed size. Genotyping of this population is also complete. DNA markers were discovered by comparing the sequenced reads against the diploid *Secale cereale* genome sequence. We have discovered more than 20,000 high quality DNA markers through this approach. While this activity is officially complete, the rye breeding project will continue to improve populations and develop

varieties suitable for MN and neighboring regions.

(This activity marked as complete as of this status update)

Activity 5

1) We produced enough layers to complete planting of four replicated trials in Minnesota, with at least three replications of 30 selections at St. Paul and Lamberton, 26 selections at Staples, and 23 selections at Waseca. We also produced enough to complete one trial at Spooner, WI and establish a new trial at Arlington, WI with at least three replications of 23 selections. We also sent stock plants to two propagation researchers to start mass propagation if we eventually decide to commercialize these selections.

2) We added one new selection to the replicated trials, based on 2023 data, and continued to collect data on younger cohorts of controlled cross seedlings, which we will use to make selections for future replicated trials.

3) Yields of our top Midwest selections in the “Joint Performance Trial” are outstanding this year compared to top selections from other breeding programs in eastern North America. Although the selections from Grimo Nut Nursery may be outstanding for Ontario, and the selections from the Hazelnut Consortium may be outstanding for MO and NE, our selections are outperforming those selections here. We are still waiting to see how the selections from NJ perform here, because they are too young.

Activity 6

Camelina Meal (CaM) fermentation was carried out by inoculating three industrial fungi: *Aspergillus oryzae* (AO), *Trametes versicolor* (TV), and *Pleurotus ostreatus* (PO). The fermented CaM, along with unfermented CaM and soybean meal as controls, were fed to 8-week-old male C57Bl/6 mice in a 3-week trial. The CaM inclusion rate increased each week: 10% in week 1, 20% in week 2, and 30% in week 3. Among the fermented CaM groups, AO feeding resulted in better body weight gain and feed intake compared to PO and TV. TV feeding decreased body weight and increased the hepatosomatic ratio with higher inclusion rates, while PO feeding initially increased body weight but caused a decline later. These results suggest that AO-fermented CaM may offer superior nutritional value as a feed ingredient for animal production.

Pennycress Meal (PeM) fermentation was done using AO, TV, PO, *Rhizopus oryzae* (RO), and *Trichoderma reesei* (TR). Proximate and chemometric analysis revealed comparable crude protein and amino acid levels between the fermented PeM and the unfermented control. AO and RO were most effective in degrading sinigrin, the primary glucosinolate in PeM. Based on these results, AO and RO were selected for further feeding trials.

Activity 7

This project involves three cycles of selection for vigorous seedling growth starting with a diverse collection of Silflower germplasm. We selected 72 individuals for each treatment group in Cycle 2 and are growing the plants to maturity in late Spring 2025. Selected plants from Cycle 0 and Cycle 1 were planted into isolation plots for seed increase to be used in a future field experiment. Data collected on selected individuals include petiole color, stem height, stem diameter and leaf thickness. Tissue was sampled from Cycle 0, Cycle 1 and Cycle 2 to isolate DNA from all selected individuals to use for genotyping and genetic mapping. We are processing GBS data for Cycle 0 and Cycle 1 with a new single nucleotide polymorphism (SNP) calling pipeline. A second part of our project is to investigate genetics of seedling traits using the silphium domestication panel (SDP). We have completed a seed germination and dormancy experiment on the SDP and will repeat the experiment with a selected number of individuals in Summer 2025. We are currently running SDP GBS data through our analysis pipeline to identify SNP markers. Once we have generated and curated the SNP data, we will begin mapping seedling vigor.

Activity 8

Objective 1. Develop high yielding perennial inbred lines derived from *Helianthus tuberosus* x *H. annuus* hybrids: In 2024, eighteen inbred lines were evaluated in a yield trial on the St. Paul campus of the U of MN. Inbred lines developed with 4 to 5 cycles of self-pollination with good seed yield and perennial habit were seeded into 2 row plots with 3 replications. Perennial inbred lines were compared to conventional annual sunflower lines. Seed yield of perennial

inbred lines ranged from 120-2054 kg/ha. Conventional annual checks had seed yields of 2186 and 2996 kg/ha for inbred and hybrid lines, respectively.

Objective 2. Add genetic diversity to the *H. tuberosus* x *H. annuus* breeding pool by restoring fertility to poor fertility lines: By crossing non-fertile lines with pollen from fertile inbreds, additional self-fertile lines have been developed that add desirable traits (e.g., large flower size, disease resistance) to the breeding pool. Eight lines developed using this strategy were included in the 2024 yield trial; four of these lines had yields exceeding 1000 kg/ha.

Activities for the 2025 field and greenhouse will focus on intercrossing best yielding lines and identifying combinations that further improve seed yield and winterhardiness.

Dissemination

Since the subprojects on this grant are still in process, dissemination has been limited. We acknowledged LCCMR support in an article was published in "Minnesota Crop News" by UMN Extension (<https://blog-crop-news.extension.umn.edu/2024/12/perennial-cereal-rye-grain-dual-use.html>). These projects also acknowledge LCCMR funding in the weekly Forever Green Seminar Series, which draws 30-50 people. Additional dissemination activities going forward will acknowledge LCCMR funding.

Status Update Reporting

Status Update September 1, 2024

Date Submitted: February 28, 2025

Date Approved: February 28, 2025

Overall Update

Since the last update, all subprojects have been making progress toward their objectives. Activity 3/Subproject 1 (Anderson) has changed its work plan for both personnel and scientific reasons. It is making progress on the revised work plan, as described below.

This work is improving our understanding of crop genomes, enhancing our breeding programs, improving knowledge of management practices, allowing us to understand pest interactions, and advancing the functional characteristics of the crops.

Activity 1

This activity was previously marked complete.

(This activity marked as complete as of this status update)

Activity 2

The workplan revision to add individual subprojects was completed on time. I have worked with all subproject leaders to ensure that they supply timely progress reports, both to MDA and LCCMR. I have consistently reminded them of LCCMR acknowledgement requirements.

Activity 3

Pennycress is a harvestable winter oilseed crop intended for inclusion in Midwest corn and soybean rotations. However, pennycress is a host of the soybean cyst nematode (SCN), a significant pathogen of soybean. Consequently, incorporating pennycress into these rotations may increase the risk to soybean productivity. The goal of this project is to generate the knowledge necessary to manage this risk, with a focus on evaluating SCN resistance in pennycress. Three experiments have been conducted to refine the procedures for assessing SCN resistance. Specifically, we are studying the genotype interaction between pennycress and SCN to determine the resistance levels. Fourteen pennycress lines, selected for their low and high levels of SCN reproduction based on previous studies, are being evaluated for resistance against 20 SCN inbred lines. These SCN lines, derived from populations collected across soybean-growing areas in Minnesota, represent different virulence types to SCN-resistant soybean. Seeds from individual plants and multiple plants per line were developed during the winter of 2023 and spring of 2024. The experiments were established in August 2024, and data on SCN reproduction in pennycress lines will be collected to assess the genotype interaction.

Activity 4

The perennial cereal rye (PC-Rye) population established in fall 2023 was evaluated for several traits in spring 2023. Example traits include winter hardiness, plant height, and resistance to lodging and diseases. Harvesting was completed in July 2023 and we are in the process of measuring grain yield and seed size on this population. The population will be maintained in the field in St. Paul, MN for an additional year and re-evaluated in spring-summer 2025. The population has also been genotyped using genotyping by sequencing (GBS) approach. Specifically, DNA was extracted from tissues collected in early May 2024 and two restriction enzymes (PstI and Mspl) were used to create sequencing libraries. These libraries were sequenced using high-throughput sequencing approach at the University of Minnesota Genomics Center in July 2024. Genome-wide markers will be discovered by aligning the sequenced reads to the rye reference genome in early fall 2024. Following this, genetic mapping and training of genomic selection models will be carried out in late fall of

2024.

(This activity marked as complete as of this status update)

Activity 5

- 1) We established four replicated trials in Minnesota (at St. Paul, Staples, Lamberton and Waseca) and one at Spooner Wisconsin with layers of 28 of the top 31 selections from our 2nd generation Midwest by European controlled-cross seedlings and thus far survival has been excellent. We re-layered those selections for which we did not obtain enough layers for a full four to five replications at all five sites, with the objective of filling out the trials next year.
- 2) Based on 2023 yield data, we selected just one to add to the replicated trials. Instead, we used the top 11 selections as maternal parents for pollinations, backcrossing them to our top 1st generation selections. The progeny developed should be of an intermediate stature favorable for machine harvest.
- 3) Nut loads in our “Joint Performance Trial”, in which we are comparing our Midwest top selections with top selections from other breeding programs in eastern North America look outstanding this year, thanks to this year’s abundant rain. We look forward to the first harvest in which we will finally be able to compare our own 1st generation selections against top selections from Grimo Nut Nursery in Ontario.

Activity 6

Pennycress and camelina meal (PM, CM) were fermented with five fungal strains separately under solid state. *Aspergillus oryzae* (AO) and *Rhizopus oryzae* (RO) degraded sinigrin by 68-83% and 30% during 12 days of incubation of PM. AO and *Trametes versicolor* (TV) degraded glucocamelinin in CM by 96% after 9 days and 97% after 12 days of incubation, respectively. Chemometric analysis confirmed that both sinigrin and glucocamelinin can be degraded by AO, while TV transformed glucocamelinin into some unknown compounds. CM fermentation with AO and TV was then scaled up in 1 kg spawn bags to produce feed for mice feeding trial. Three weeks of feeding trial was performed using standard soybean meal diet (control), standard diet with 10% (week 1), 20% (week 2), and 30% (week 3) inclusion of un-fermented CM, AO fermented CM and TV fermented CM. The mice body weight was the highest with standard soybean meal diet, followed by AO fermented CM and un-fermented CM, while TV fermented CM reduced body weight. Average daily gain and liver/body weight showed similar trends. This indicates that TV fermented CM could be toxic and the future research will be focusing on fermentation of CM and PM using AO.

Activity 7

This project involves three cycles of selection for vigorous seedling growth starting with a diverse collection of Silflower germplasm. We successfully selected 72 individuals for each treatment group in Cycle 1 and grew the plants to maturity. Cross-pollination of Cycle 1 plants was successful and we obtained a good seed set on the selected plants for each group. We measured petiole color, stem height, stem diameter and leaf thickness on the Cycle 1 selected individuals. Over the summer, flowering time data was taken on Cycle 0 plants that were planted into respective isolation plots within the field the previous Fall. We sampled tissue to isolate DNA from Cycle 1 selected individuals to use for genotyping and eventually characterizing genetic change resulting from recurrent selection. We have received this GBS data and it awaits processing. A second part of our project is to investigate genetics of seedling traits using the silphium domestication panel (SDP). We have completed a seed germination and dormancy experiment on the SDP genotypes. We are currently running SDP GBS data through our analysis pipeline to identify single nucleotide polymorphism (SNP) markers. Once we have generated and curated the SNP data, we will begin genetic mapping seedling vigor.

Activity 8

Objective 1. Develop high yielding perennial inbred lines derived from *Helianthus tuberosus* x *H. annuus* hybrids: The best inbred lines are being evaluated in a yield trial on the St. Paul campus of the U of MN. Ten inbred lines from 4 to 5 cycles of self-pollination with good seed yield potential and perennial habit were seeded into 2 row plots with 3 replications. Lines were selected based on 2023 field and greenhouse observations. In fall of 2024 the seed yield of

selected tetraploid lines will be compared to each other and conventional annual sunflower inbreds.

Objective 2. Add genetic diversity to the *H. tuberosus* x *H. annuus* breeding pool by restoring fertility to poor fertility lines: By crossing non-fertile lines with pollen from fertile inbreds, additional self-fertile lines have been developed that add desirable traits (e.g., large flower size, disease resistance) to the breeding pool. Self-fertile individuals selected from this procedure have undergone two to three cycles of self-pollination and selection. Seven self-fertile lines developed using this strategy are also being tested in the replicated yield trial. The best performing lines will be intercrossed in winter 2024-25 to identify lines with good combining ability and generate new improved

Dissemination

Since the subprojects on this grant are still in process, dissemination has been limited. One article was published in UMN Extension "Minnesota Crop News" (<https://blog-crop-news.extension.umn.edu/2024/12/perennial-cereal-rye-grain-dual-use.html>). LCCMR support was not acknowledged but I have reached out to the subproject PI to ensure that it is added.

Status Update Reporting

Status Update March 1, 2024

Date Submitted: March 1, 2024

Date Approved: May 8, 2024

Overall Update

Since the last update, all subprojects have been making progress toward their objectives. Activity 3/Subproject 1 (Anderson) has changed its work plan for both personnel and scientific reasons. It is making progress on the revised work plan, as described below.

This work is improving our understanding of crop genomes, enhancing our breeding programs, improving knowledge of management practices, allowing us to understand pest interactions, and advancing the functional characteristics of the crops.

Activity 1

This activity was previously marked complete.

(This activity marked as complete as of this status update)

Activity 2

The workplan revision to add individual subprojects was completed on time. I have worked with all subproject leaders to ensure that they supplied timeline progress reports, both to MDA and LCCMR. I have consistently reminded them of LCCMR acknowledgement requirements.

Activity 3

Our original proposal had two objectives: (1) refine our understanding of SCN overwinter survival in pennycress, and 2) identify pennycress accessions with the greatest and least resistance to SCN, to enable future work identifying resistance genes. We changed work scope of the two objectives because we realized that it was critical to focus on SCN resistance evaluation, which must be done right so that subsequent objectives can be achieved. Milestones 1 and 3 were part of Objective 1 which is no longer a part of this project – that activity is being performed using other funding. Milestone #2, Evaluate at least 30 pennycress lines for SCN resistance in inoculated cone-tainers has been expanded to assess resistance against different SCN races. Three experiments have been conducted (one has not been finished) to refine the procedures for evaluation of SCN resistance/susceptibility in pennycress. We plan to test 17 pennycress lines for their resistance/susceptibility to 20 SCN populations or lines that represent all SCN HG Types or races found in Minnesota. We are currently preparing the seeds of pennycress lines and inoculums of the SCN lines and populations for the experiment.

Activity 4

1) One of our major goals was to evaluate a large PC-Rye population for perenniability. Of the 2160 accessions planted in fall 2022 and taken through two vernalization rounds in a cold chamber, 786 plants survived. While this was slightly lower than an anticipated number of approximately 1,200 plants, we still have a sufficiently large population. A lower-than-expected number also suggests that the selection process worked as planned. All plants were transplanted into a field in St. Paul in 09/2023. No plant deaths were observed due to transplant shock. This population will be evaluated in 2024 for several traits such as winter hardiness, grain yield, seed size, plant height, and resistance to lodging and diseases. 2) The population (n = 786) that was transplanted to the field in 09/2023 will be genotyped using a sequencing-based approach during May-June 2024. Tissues will be collected in late April or early May 2024 and sequencing will be done in June. Genome-wide markers will be discovered by aligning the sequenced reads to the rye reference genome

during June-July 2024. We will carry out genetic mapping and genomic selection after trait data is collected in summer-early fall.

Activity 5

1) We successfully propagated via stool-bed layering 28 of the top 31 hazelnut plants selected from our 2nd generation Midwest by European controlled-cross seedlings. Rooting percentages were significantly higher than for our 1st generation Midwest germplasm, suggesting that propagation of this 2nd generation germplasm may not be as big a challenge as it was for the 1st generation. The 530 field-ready layers generated will be planted into replicated trials at six locations this spring. We should be able to completely fill these trials with plants generated from two seasons of layering. 2) We harvested another 29 plants from the 2025 cohort of controlled cross seedlings, plus another 183 seedlings from younger cohorts to identify additional selections to add to the replicated trials, but we have not yet finished nut evaluation. 3) We harvested the first significant crop of nuts from our “Joint Performance Trial”, in which we are comparing our Midwest top selections with top selections from other breeding programs in eastern North America. One selection that was planted in 2017 from Grimo Nut Nursery in Ontario is proving itself to be a winner for Minnesota, though several of our own 1st generation selections are also proving to be competitive.

Activity 6

Camelina and pennycress as two cover crops producing oil seeds which can be separated into oil and protein-rich meal. However, the anti-nutritional factors in camelina (glucocamelinin) and pennycress (sinigrin) meals prevent their use as nutrition supply for animal and human. Wild type *Aspergillus oryzae* and *Trametes versicolor* each was tested to degrade glucocamelinin and sinigrin by over 95% in their corresponding meal, potentially transforming both meals into low-risk materials for possible feeding or food ingredients. *A. oryzae* has long been used for food production in Asia such as miso, soy source, rice vinegar, and demonstrated safe for consumption. *T. versicolor* is a type of white rot fungi capable of degrading fiber. *T. versicolor* fermentation of camelina meal mixed with fiber-rich soybean hull and wheat bran greatly reduced fiber components and increased in vitro digestibility. Chemometric analysis of fermented camelina meal by *A. oryzae* and *T. versicolor* demonstrated near complete removal of glucocamelinin while generation of new compounds that could be the degraded products from glucocamelinin. Further chemometric analysis and mouse feeding test will define those formed compounds and if fermented meal from both pennycress and camelina have positive or negative effects on the mouse performance when included in the diets.

Activity 7

This project involves three cycles of selection for vigorous seedling growth starting with a diverse collection of Silflower germplasm. We successfully completed the cross-pollination for cycle 1 and obtained good seed set on the selected plants, treated the cycle 1 seed to break dormancy and initiated growing ~720 plants from each of the three selection strategies. We measured leaf number and size as an indication of seedling vigor and have selecting 72 plants from each of the three strategies to be intermated to create cycle 2. We sampled tissue to isolate DNA to use for genotyping and eventually characterizing genetic change resulting from recurrent selection. A second part of our project is to investigate genetics of seedling traits using the silphium domestication panel (SDP). We harvested seed from the SDP at the end of this summer and have used it to initiate a dormancy experiment. We have received from the UM Genomics Center DNA sequence data of the individuals in the SDP and are currently running that data through our analysis pipeline to identify single nucleotide polymorphism (SNP) markers. Once we have generated and curated the SNP data, we will begin genetic mapping seedling vigor and other important breeding traits.

Activity 8

Objectives: (1) Develop high seed production perennial inbred lines derived from *Helianthus tuberosus* x *H. annuus* hybrids; (2) Expand genetic diversity of the *H. tuberosus* x *H. annuus* breeding pool by restoring fertility to poor fertility lines. Objective 1 progress: Ten S5 and S6 lines appear to be uniform in desired seed yield potential and perennial habit; these have been tentatively selected for yield evaluation. Lines currently in the greenhouse that exhibit satisfactory seed yield and demonstrate development of rhizomes will be planted for field yield evaluations in spring of 2024. In 2024 replicated yield trials, selected tetraploid lines will be compared to one another and representative oilseed sunflower

varieties. Yield trials will be conducted at Waseca, MN (SROC) and Rosemount, MN (UMORE). Objective 2 progress: Several non-fertile lines have desirable traits that would be beneficial in varietal development, such as large flower size, large seeds, vigorous tuber development, and resistance to fungal pathogens. We have restored fertility to some of these underutilized lines by introducing fertility traits through inter-mating with improved fertility lines developed in Objective 1. Seven lines appear to have acquired self-fertility after three rounds of self-pollination; if stable, these will undergo yield trials in 2025.

Dissemination

Since the subprojects on this grant are still in process, we have not done any dissemination yet.

Status Update Reporting

Status Update September 1, 2023

Date Submitted: August 31, 2023

Date Approved: September 27, 2023

Overall Update

Since the last update, we have amended the workplan and budget has been allocated to the subprojects. All subprojects have begun their activities and are making progress toward their objectives. This work is improving our understanding of crop genomes, enhancing our breeding programs, improving knowledge of management practices, allowing us to understand pest interactions, and advancing the functional characteristics of the crops.

Activity 1

This activity was previously marked complete.

(This activity marked as complete as of this status update)

Activity 2

The workplan revision to add individual subprojects was completed on time. I have worked with all subproject leaders to ensure that they supplied timeline progress reports, both to MDA and LCCMR. I have consistently reminded them of LCCMR acknowledgement requirements.

Activity 3

During this period, we have focused on establishing a reliable screening method of soybean cyst nematode (SCN). We are comparing St. Paul greenhouse and Waseca growth room environments for their effects on pennycress growth, and SCN variability in a few winter and spring pennycress lines. We experimented to germinate the seed without GA3 treatment. The yellow-seeded breeding lines had 60%-90% germination rate when grown in moist filter paper or sand and soil mixture, but the germination rate of the wild collections (black seed) varied from 0%-40%. Based on the initial findings, we compared the germination rate of the wild collections by pre-germination treatments (GA3 soaking and water soaking) and growing the seed on moist filter paper in petri dishes and in sand and soil mixture in cone-trainers. The data suggests soaking the seed in GA3 for 24 hrs, then germinating them in water-moist filter papers in petri dishes was the most reliable method for seedling establishment in SCN screening. Based on the experiments, we will soak the seed in GA3 for 24hrs, then grow on moist filter paper in petri dishes for 7d. The germinated seedlings will be transplanted to soil/sand mixture for SCN inoculation and incubation.

Activity 4

Our work plan on initiating a Perennial Cereal Rye breeding program for MN environments is progressing as expected and we are meeting our objectives in timely manner. One of the major goals of this objective was to evaluate a large PC-Rye population for perenniability. We planted 2160 accessions in fall 2022 and introduced them to two rounds of vernalization treatment in a cold chamber (Figure 1). This has led to reduction in the population size by approximately 30%; remaining plants are currently being maintained in an outdoor nursery. From the remaining plants, a population of 1200 genotypes will be transplanted to a field in St. Paul in September 2023. This population will be evaluated for several traits such as winter hardiness, grain yield, seed size, plant height, and resistance to lodging and diseases in summer 2024. The population (n = 1200) that will be transplanted to the field in September 2023 will be genotyped using a sequencing based approach during November-December 2023. Genome-wide markers will be discovered by aligning the sequenced reads to the rye reference genome during January-March 2024. We will carry out genetic mapping and genomic selection after trait data is collected in summer 2024.

Activity 5

Evaluating 2nd Generation Hybrid Hazelnut Germplasm for Minnesota, Phase Two:

We selected the top 31 hazelnut plants from our 2014 and 2015 controlled cross seedlings at Rosemount and propagated them for layering in June. We expect to dig the layers in early November and are preparing land to plant replicated trials using these layers at St. Paul, Staples, Lamberton and Waseca. We marked another 37 seedlings from the 2015 cohort and approximately 100 from the 2016 cohort to identify those to harvest for nut quality traits in August; the winners of this process will be added to the replicated trials in future years. We also installed a drip irrigation system in the “Joint Performance Trial” in which we are comparing our Midwest top selections with top selections from other breeding programs in eastern North America. Already the irrigation system is making a huge difference: these plants growing on a thin rocky soil are looking the best they have ever looked, in spite of this year’s drought, with many of them coming into nut bearing this year, which will bring us closer to being able to advise growers on which are best for Minnesota.

Activity 6

The work plan is to develop a biorefinery process using naturally available fungi to improve the nutritional values and edibility of pennycress (PM) and camelina (CAM) oilseed meals, and therefore increase the applications and profitability of these cover crops. We focused on the optimization of cultivation of *Aspergillus oryzae* on CAM and now the fermented meal is ready for the chemometric analysis. We will work on two more strains which showed promising results in our preliminary screening process. We are also developing co-culture of CAM together with wheat bran in order increase the fermentability of CAM. Our current study showed that the fermentation can effectively remove almost all glucosinolate, the main anti-nutritional factor in both PM and CAM, and our next step is to figure out what degradation products this project generate. The current master student Kristine is finishing up her thesis, and we are recruiting a new graduate student to work on the project.

Activity 7

This project involves conducting three cycles of selection for vigorous seedling growth starting with a diverse collection of Silflower germplasm. We accomplish cross-pollination of plants selected for vigorous seedling growth using bumble bees in pollination tents. Unfortunately, we had several technical difficulties with deploying and maintaining the bees during pollination of cycle 1 resulting in insufficient and uneven pollination among the plants. Fortunately, as a perennial, Silflower can grow back after cutting back the stems so we were able to “restart” the experiment for cycle 1. After consulting with an entomologist and making some changes to our procedure we successfully completed the cross-pollination for cycle 1 at the beginning of July. We are now finishing harvest of seed from cycle 1 and will initiate cycle 2 in September.

Activity 8

The two objectives of this project are to: (1) Develop high seed production perennial inbred lines derived from *Helianthus tuberosus* x *H. annuus* hybrids, and (2) Expand genetic diversity of the *H. tuberosus* x *H. annuus* breeding pool by restoring fertility to poor fertility lines. Recent progress on Objective 1: At the conclusion of the 2023 greenhouse season, 2064 seeds produced by the most recent round of self-pollinations were germinated and then transplanted to the 2023 field nursery for evaluation. Advanced inbred lines appear to have great phenotypic uniformity, allowing for the initiation of yield trials in replicated small plots to be planted in the spring of 2024. Trials will be done at two or three locations including Rosemount and/or St. Paul and Waseca. Recent progress for Objective 2: We have started the process of restoring fertility by introducing fertility traits through inter-mating with the improved fertility lines developed in Objective 1. Following two rounds of self-pollination, over 1000 plants were transplanted to the 2023 field nursery for an additional cycle of self-pollination. Lines have been identified that show promising early responses to the crossing procedures, with some indication of fertility restoration while retaining desirable characteristics.

Dissemination

Since the activities on this grant are still just getting going, we have not done any dissemination yet.

Status Update Reporting

Status Update March 1, 2023

Date Submitted: March 19, 2023

Date Approved: March 23, 2023

Overall Update

We have run the RFP process and selected sub-projects to receive funding. This is the necessary first step toward delivering on the outcomes. Sub-project budgets have been submitted and we are awaiting feedback.

Activity 1

This activity is complete. We have run the RFP process and selected sub-projects to receive funding. The RFP process was very successful. We received 22 very strong proposals requesting a total of more than \$2,700,000, with \$763,000 available. The Review Committee and Associate Dean Milena Saqui-Salces ultimately selected six proposals for funding, following input from the Forever Green Initiative directors. We learned that we will receive many applications even if the available funding is low and we include a relatively narrow set of priorities in the RFP (in this case, to fill specific funding gaps, e.g. that could lead to the loss of a project collaborator/staff). The Review Committee shared helpful feedback on how the RFP could be made even more detailed and clear in the future to enable more straightforward comparisons among proposals. We will apply these lessons to future RFPs run by the Forever Green Initiative.

(This activity marked as complete as of this status update)

Activity 2

We are in the process of awarding funds to the sub-projects. Each sub-project has been added as an activity in an amended work plan for LCCMR review and approval and the budget template has been uploaded. Funds have been moved from the budget line "to be awarded to sub-projects" to the budget line "awarded to sub-projects" in Professional and Technical Contracts. I have liaised with Margaret Wagner at the Minnesota Department of Agriculture (MDA) to ensure compatibility with the MDA contracting process. A contract with MDA has been developed and will be executed when LCCMR approves the updated workplan and releases funds to MDA.

Activity 3

Awaiting funding to begin activities.

Activity 4

Awaiting funding to begin activities.

Activity 5

Awaiting funding to begin activities.

Activity 6

Awaiting funding to begin activities.

Activity 7

Awaiting funding to begin activities.

Activity 8

Awaiting funding to begin activities.

Dissemination

Dissemination has not begun since funds have not been awarded to sub-projects.