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ACKNOWLEDGMENT

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NOTICE

This IPREFER project quarterly report was prepared by Western Illinois University (WIU) and IPREFER research colleagues from Agricultural Utilization Research Institute (AURI), CoverCress, Inc., Illinois State University (ISU), McLean County Soil and Water Conservation District (MCSWCD), Southern Illinois University (SIU), The Ohio State University (OSU), United States Department of Agriculture-Agricultural Research Service (USDA-ARS), University of Illinois (UI), University of Minnesota (UMN), and the University of Wisconsin-Platteville (UW Platteville) in the course of performing academic research supported by Agriculture and Food Research Initiative Competitive Grant No. 2019-69012-29851 from the United States Department of Agriculture National Institute of Food and Agriculture (“USDA-NIFA”).

The opinions expressed in this report do not necessarily reflect those of WIU and IPREFER research colleagues from AURI, CoverCress, Inc., ISU, MCSWCD, SIU, OSU, USDA-ARS, UI, UMN, and the UW Platteville and reference to any specific product, service, process, or method does not constitute an implied or expressed recommendation or endorsement of it.

Further, WIU and IPREFER research colleagues from AURI, CoverCress, Inc., ISU, MCSWCD, SIU, OSU, USDA-ARS, UI, UMN, and the UW Platteville make no warranties or representations, expressed or implied, as to the fitness for a particular purpose or merchantability of any product, apparatus, or service, or the usefulness, completeness, or accuracy of any processes, methods, or other information contained, described, disclosed, or referred to in this report.

WIU and IPREFER research colleagues from AURI, CoverCress, Inc., ISU, MCSWCD, SIU, OSU, USDA-ARS, UI, UMN, and the UW Platteville and the authors make no representation that the use of any product, apparatus, process, method, or other information will not infringe privately owned rights and will assume no liability for any loss, injury, or damage resulting from or occurring in connection with, the use of the information contained, described, disclosed, or referred to in this report.
PROJECT ADMINISTRATION AND GOVERNANCE

We accomplished the following project governance and administrative tasks during the second quarter of Year 2.

1. Planned Activities

   • Continue to improve the project website and integration with social media platforms.
   
   • Begin uploading project data to UMN GEMS web-based platform with the ultimate goal of having the final project data reside in the MN DRUM data repository.
   
   • Interview and make selections for the summer IPREFER internships with collaborators.
   
   • Continue organization of YR3 annual meeting of IPREFER to occur in August 2022 in St. Louis.

2. Actual Accomplishments

   • Completed social media updates and posting of spring field day events and press releases on recent CoverCress, Inc. supply chain funding.
   
   • Selected two interns (John Kelly and Joseph Brandhorst) for the 2022 summer internship program.
   
   • Hired six undergraduate students (Alex Shaw, Hannah Carlock, Tommy Wood, Alivia Lantz, and Darby Dempsey) to assist with laboratory, greenhouse, experiments, and research plot maintenance at the WIU research farm.
   
   • Completed guidelines and nomenclature updates for CoverCress™ seed and company name.
   
   • Completed time-lapse video of developing pennycress plant. Available on the IPREFER website
   
   • Continued organization of YR3 annual meeting of IPREFER to occur in August 2022 at the Danforth Center in St. Louis.
3. Explanation of Variance

Western Illinois University is beginning to loosen COVID-19 restrictions. All spring field days and travel are allowed to proceed with no restrictions. Undergraduate student research may also proceed with no restrictions.

4. Plans for Next Quarter

- Complete organization of 3rd annual meeting of IPREFER to occur in August 2022.
- Adjust the IPREFER Advisory board by adding representatives for large scale producers for both southern and northern regions
- Initiate YR 4 funding sub-awards to collaborators.
- ELT voted to add additional funding to the CoverCress, Inc. subaward to complete supply chain experiments in years 4 and 5 with U of South Dakota, Lewis Seed, and AURI.

5. Publications, Presentations, and Proposals Submitted

- Professional Conferences /Meetings

Quarterly Progress Report: February 2022 – April 2022


Photo 2. Plant breeding student and former 2021 IPREFER Undergraduate Intern (Tommy Wood (WIU)) presenting his research at the WIU Undergraduate Research Day (Apr. 20, 2022).
• **Education Presentations**
  o Classroom lecture in AGRN-476 Crop Improvement and Biotechnology regarding pennycress breeding and gene editing. Six undergraduate students, Apr. 2022. Conducted experiment demonstrating the use of gene editing.

• **Outreach Presentations**
  o Hosted Illinois State Lieutenant Governor, Juliana Stratton for a greenhouse tour of the IPREFER project (Apr. 18, 2022).
  
  o Hosted Illinois Department of Agriculture Director, Jerry Costello for laboratory and greenhouse tour of IPREFER project (Apr. 27, 2022).

**Objective 3.1 - Agronomic Management**

A cold, wet spring delayed pennycress growth and field operations across the agronomy network. With the exception of USDA Morris, all sites have now completed spring sampling events in CRM, DISK, and PELLET and established the new Oilseed systems trial to run 2022-
24. All datasets are up to date with results from sample analyses completed this quarter. Dr. Heller has begun work on the DISK (cycle 1) manuscript with plans to publish in the upcoming quarter. With few exceptions, all agronomic management objectives are being met and continue to move forward as outlined in the grant.

1. Yield Trade-off Leveraging Corn Relative Maturity and Desiccants (“CRM” / Objective 3.1.1)¹

Recent work suggests that corn grain yield and grain moisture at harvest are increased by 88 kg ha⁻¹ (1.4 bu ac⁻¹) and 0.5%, respectively, per one-day increase in corn relative maturity (CRM). Although early-maturing corn hybrids have lower yield potential than full-season counterparts, they can be harvested earlier (e.g., in late August). The application of desiccants can further shorten the time for corn to reach harvestability. The team will assess corn and pennycress yield trade-offs through evaluating a range of corn CRM by assessing varieties differing in relative maturity in Ohio, Minnesota, and Illinois during Project Years 1 – 3. Minnesota (USDA and RROC), Western Illinois University, Ohio State University, and Illinois State University. This report covers activities from November 2021 to the end of January 2022.

A. Planned Activities

- Collect plant count and green cover.
- Fertilize pennycress
- Process corn samples collected from collaborators (OH, IL, MN (Rosemount)) for total N & C testing
- Process corn and soybean samples collected from the Morris, MN site for protein determination

B. Actual Accomplishments

- Corn samples collected from collaborators [Ohio, IL and MN (Rosemont)] ground and submitted to the chemistry lab for N testing.
- Corn and soybean samples from Morris’ site were submitted to the lab for protein determination and waiting for the result.
- Corn samples collected from different locations of the CRM study were processed

¹ Russ Gesch (USDA-ARS) leads the CRM team.
and submitted for total N & C testing.

C. Explanation of Variance

- The weather and the soil at Morris, MN, have been too wet and cold to perform field activities: including:
  - Fertilization of pennycress both for CRM, crop residue management, and pellet studies.
  - Collection of spring pennycress establishment data (count and green cover).

D. Plans for Next Quarter

- Fertilize pennycress as soon as possible when the soil has dried out enough to get into the field.
- Complete spring stand establishment and percent green cover measurements at each site as soon as possible.
- Follow-up and get N testing results from the chem lab and compile the data in standardized spreadsheets for samples collected from:
  - Collaborators (Ohio, Illinois, Minnesota (Rosemount) for corn samples
  - Morris (corn and soybean samples)
- Compiled spring data (count and green cover) from all sites and input it into standardized spreadsheets.
- Summarize spring pennycress establishment data (count and green cover) from all sites.

E. Publications

Update on the project was submitted to local newspapers (Grant County Herald and Chokio Review) (See Exhibit A) We believe this will help to introduce the project about pennycress to the surrounding stakeholders, and in general, to the public.

2. Corn Residue Management (Objective 3.1.2 / "DISC")

2 DISC is the abbreviation we use for the IPREFER corn stover management project. Nicholas Heller (University of Minnesota) leads this IPREFER objective.
Due to ecological and economic benefits, an increasing number of farms are now practicing no-tillage and other conservation tillage farming. Establishing pennycress in no-till fields is a struggle primarily due to the sheer amount of corn residue after harvest. Several corn residue treatments will test the hypothesis that the residue can be sized small enough to not interfere with pennycress establishment. Pennycress establishment and subsequent seed yield in high-residue environments will be compared to reduced-surface stover treatments where its production has proven successful (e.g., silage corn removal and prepared seedbeds).

Minnesota (USDA and RROC), Western Illinois University,

A. Planned Activities

- Process corn samples collected by collaborators from Ohio, IL, and MN (Rosemont) locations for quality tests.
- Finalize protein determination for corn and soybean samples from Morris site.
- Complete spring stand establishment and percent green cover measurements at each site.
- Fertilize pennycress with N fertilizer at each site.
- Summarize spring pennycress establishment data (count and green cover) from all sites.
- Compiled spring data (count and green cover) from all sites and input it into standardized spreadsheets.

B. Actual Accomplishments

- Corn samples collected from collaborators [Ohio, IL, and MN (Rosemont)] ground and submitted to the chemistry lab for N testing.
- Corn and soybean samples from Morris’ site were submitted to the lab for protein determination and waiting for the result.
- Completed spring stand establishment and percent green cover measurements at Southern sites, Northern sites waiting on Spring to arrive.
- Fertilized pennycress with N fertilizer at each site.
Bethany Wohrley\textsuperscript{3}, an IPREFER second-year grad student in the ISU Department of Agriculture, was recently recognized for several achievements. Ms. Wohrley was named a 2022 ISU Gradbird Scholar (see https://www.youtube.com/watch?v=9vNEFUPoAjo). The GradBird Scholar is an initiative recognizing ISU graduate students for their scholarly endeavors. Wohrley also won the Illinois State University CAST (College of Applied Science and Technology) 3-Minute Thesis competition. She was the runner-up in the Image of Research competition at Illinois State University with her photo “Worth Every Penny: Establishing Pennycress in Illinois Agricultural Systems.”

C. Explanation of Variance

- The weather and the soil at Morris, MN, have been too wet and cold to perform field activities, including
  - Fertilization of pennycress.
  - Collection of spring pennycress establishment data (count and green cover).
- Data will be compiled after it is collected.

D. Plans for Next Quarter

- Fertilize pennycress as soon as possible when the soil has dried out enough to get into the field.
- Complete spring stand establishment and percent green cover measurements at each site as soon as possible.
- Follow up and get N testing results from the chem lab and compile the data in standardized spreadsheets for samples collected from:
  - Collaborators (Ohio, Illinois, Minnesota (Rosemount) for corn samples
  - Morris (corn and soybean samples)
- Compiled spring data (count and green cover) from all sites and input it into standardized spreadsheets.

\textsuperscript{3} Ms. Wohrley’s research involves pennycress and developing it as a cover crop for farmers to fill the gap after their main crop has been harvested.
• Summarize spring pennycress establishment data (count and green cover) from all sites.

• Harvest pennycress and plant the following cash crop.

• Analyze Cycle 1 data and send out a rough draft of the manuscript.

Photo 4. Agronomy Studies at ISU in Lexington, IL taken via UAV (Apr. 17, 2022). The study in the foreground is the Corn Residue Management study where all treatments were broadcast seeded. Four replications are shown of golden-seeded pc and black-seeded pc after silage corn (the more solid green strips) and following regionally adapted corn hybrid harvested for grain. All experimental units were planted on the same day. Further back is the CRM study where black-seeded pc was drilled immediately following grain corn harvest of a range of corn maturities. A silage treatment was included as a control and was planted when it was harvested which was the same day as the earliest corn hybrid grain harvest. (The ISU compost site is to the right, and winter wheat is to the left of the pennycress study).
Photo 5. The Multi-State Variety Trial (MSVT) in Normal, IL, and some collected, bred, and improved lines from Dr. Heller and Dr. Sedbrook were taken via a UAV (Apr 19, 2022). From the left, a bulk of golden-seeded pc is planted to border the MSVT trial. The next 40 plots are the MSVT where most varieties produced a uniform stand. Henbit is present in some plots underneath the pennycress plants and appears as a purple-brown where there is a gap in the pennycress. The right 40 plots are the collected, breeding lines, and improved lines.
Photo 6. Flowering pennycress at the Normal, IL site (Apt. 19, 2022). In the foreground, a plot of pennycress has begun flowering. This plot is compared to the plot behind it which does not yet have any open flowers and the one back and right which is already flowering also. In the very foreground is an example of the henbit and red deadnettle present in some plots under the pennycress.
Photo 7. Pennycress in the Agronomy Study, in Lexington, IL (Apr. 27, 2022). The pennycress has grown a lot in the past two weeks and is mid-flower.

E. Publications
3. Novel Seed Treatment for Improved Pennycress Performance (“PELLET”) (Objective 3.1.3)\(^4\)

Minnesota (USDA and RROC), Western Illinois University, Ohio State University, Illinois State University, and the University of Wisconsin-Platteville.

**A. Planned Activities - PELLET**

- Complete spring assessment and management of plots.
- All establishment data will be entered in a standardized spreadsheet and returned to OSU for preliminary analysis.
- Submit first PELLET manuscript.

**B. Actual Accomplishments - PELLET**

- Spring establishment has been assessed at most sites but experiencing about 50% successful establishment for the 10 sites.
- All fall data has been received from all collaborators.
- First PELLET manuscript accepted for publication in *Seed Science and Technology*.

**C. Explanation of Variance - PELLET**

- Fall conditions contributed to variable establishment
- Also had issues with natural populations affecting establishment data

**D. Plans for Next Quarter - PELLET**

- Analyze spring establishment data
- collect spring measurements and harvest data

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\(^4\) “PELLET” is the abbreviation we use for the “Novel Seed Treatment for Improve Pennycress Performance” part of the project. This IPREFER objective is led by Alex Lindsey at Ohio State University.
• Analyze Phase 1 data and send the manuscript to co-authors, planned submission to Crop Science

• Nasib Koirala (MS) should be set to complete and defend his thesis

4. Tools for Integrated Weed Management (“WEEDS”) 3.1.4\(^5\)

A. Western Illinois University

• Planned Activities
  o Complete greenhouse herbicide carryover dose-response bioassays
  o Collect stand counts and canopyo measurements in field studies

• Actual Accomplishments
  o Collected spring Canopeo images and stand counts in field studies
  o Completed greenhouse herbicide carryover dose-response bioassays for single active ingredient herbicides in herbicide mode of action Groups 2, 3, 4, 5, 14, and 27.
  o Began greenhouse herbicide carryover dose response bioassay for multiple active ingredient corn herbicides

• Explanation of Variance
  o None noted at this time
  o To this point, our research has focused on carryover from corn herbicides. We have observed much better soybean stands in field areas following soybean and intend to conduct bioassays using soybean herbicides next fall/winter when greenhouse conditions are favorable for Covercress™ growth.

• Plans for Next Quarter
  o Analyze greenhouse bioassay data.
  o Collect yield from 2021 herbicide carryover plots.
  o Establish herbicide carryover plots for 2022.

\(^5\) Mark Bernards (Western Illinois University) leads the WEEDS Team.
Publications, Presentations, Proposals Submitted, and Stakeholder Engagement

We began work on two pennycress-related projects funded by the Illinois Soybean Association. The first is examining the effect of planting soybean or corn into Covercress™ before bolting (mid-late March) or shortly after bolting begins (early to mid-April) compared to waiting to plant corn or soybean until after Covercress™ is harvested (late May-early June).

The second study is looking at soybean maturity group selection of soybean planted in April 2022 (MG 1.7, 2.9, or 4.1). Covercress™ will be sowed three times (Aug 15, Sept 1, Sept 15). If the soybeans have been harvested, Covercress™ will be drilled; otherwise, seed will be broadcast.

Fig. 1. Poster – IPREFER Collaborator Mark L. Bernards.
5. **Contribute to the identification and development of soybean varieties specifically adapted to pennycress inter-cropping systems (“SELECT”) (Objective 3.1.5)**

For farmers to adopt pennycress as a cash cover crop, research needs to be done to characterize how this winter annual can be incorporated into summer annual rotations. In the Midwest, the relay species in double cropping systems is typically soybeans due to their high levels of plasticity. In the upper Midwest, the shorter growing season necessitates this double cropping shift to an intercropping system where soybeans are planted into pennycress stands before flowering. This environment, under a pennycress canopy, is stressful and necessitates the development of soybean varieties adapted to a pennycress intercropping system. Further, some soybean varieties might be highly competitive and decrease the yield potential of the cover crop. Taken together, those findings highlight the possibility of optimizing the cover crop-cash crop associations by identifying summer crop-cover crop cultivar combinations that maximize yields.

To address the yield gap, a soybean selection (i.e., SELECT) was initiated fall of 2019 and planted into skip rows of the pennycress cultivar ‘MN106-non shatter.’ Forty soybean varieties, representing diverse accessions and industry cultivars, are slated for relay cropping into pennycress for three years, starting spring of 2020, with the expressed goal of revealing large variations in soybean yield response to relay planting. A plethora of soybean traits will be recorded to characterize how pennycress intercropping affects soybean development. These results, accompanied by agronomic data, will identify superior cultivars for this system. Minnesota (RROC and USDA)

- **Planned Activities**
  - Finish imaging and extracting data from the 2,880 plants collected during 2021.
  - Lucas Roberts will present a research seminar titled: Battle of the Oilseeds: Characterization and Genetic Variation for Soybean Traits Relevant to a Soybean-Pennycress Intercropping System at the University of Minnesota’s Applied Plant Sciences Seminar Series on Mar. 21, 2022
  - Package and plant the SELECT experiment in late April 2022 in Rosemount and Morris, MN.

- **Actual Accomplishments**
  - Progress was made on imaging plants from 2021.

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6 Aaron Lorenz (University of Minnesota) leads the SELECT Team.
The field design was finalized, and the seed was packaged for the field trial.

- **Explanation of Variance**
  
  2021 data was analyzed and visualized for the above seminar.

- **Plans for Next Quarter**
  
  o Plant the field trial in Rosemount and Morris.
  
  o Start UAV flights weekly to record canopy coverage.
  
  o Design greenhouse and growth chamber experiment studying shading tolerance in soybeans as part of an IPREFER summer internship supervised research project.
  
  o Record soybean stand counts and take notes on flower color and pubescence color.
  
  o Collect fresh pennycress leaves for a sinigrin extraction protocol to make an assay to test soybean tolerance to allelopathy.

6. **Publications, Presentations, and Proposals Submitted**


**OBJECTIVE 3.2 - BREEDING AND GENOMICS – PENNYCRESS IMPROVEMENT**

1. **Illinois State University**

   A. **Planned Activities**

   Our research teams have generated high-yielding pennycress breeding lines through multi-state testing and identified/validated trait-improving mutations and natural variants. These were our planned activities for the second quarter:

   - Utilize marker-assisted selection and CRISPR gene editing to complete the introgression of these traits into elite breeding lines.
   
   - Extend our replicated yield testing program to a cooperative regional program and rapidly identify the best lines for each Midwest location (IL, MN, OH, WI) in conjunction with a commercial launch.
   
   - Perform field evaluations and seed increases of lines with commercial potential.
• Develop additional genetic/genomic resources for long-term breeding programs success.

B. Actual Accomplishments

• Research
  o We have been collecting growth data on plants in field plots having various genotypes, including those conferring reduced glucosinolate, reduced dormancy, larger seed size, as well as natural populations collected at different latitudes; we are obtaining useful data. Of interest, plants that had poor seed set in growth chambers are performing well in the field. Conversely, a few plant lines that had appeared healthy in growth chambers are maturing later than normal.
  
o We continue making progress in stacking new genetic combinations aimed at attaining target levels of glucosinolates in combination with other core domestication traits. We are also exploring plant fitness, including heat tolerance of pennycress lines having increased oleic acid in seed oil.
  
o Multi-state variety trial plots at the ISU Horticulture Center site look very good and are providing useful data, including stand establishment metrics and times to first flower.

C. Explanation of Variance

Research is progressing well.

D. Plans for Next Quarter

Continue work on the aims stated above.

E. Publications, Presentations, Proposals Submitted, and Stakeholder Engagement


• Sedbrook, John. “Developing pennycress into an oilseed cash cover crop named Covercress.” Donald Danforth Center Seminar Series, St. Louis, MO (Mar. 2, 2022).
• Sedbrook, John. "Using agricultural biotechnology to rapidly domesticate pennycress and the opportunity that this cover crop and oilseed crop has to mitigate climate change" Asia-Pacific Economic Cooperation High Level Policy Dialogue on Agricultural Biotechnology. Agriculture & Food Systems Institute, Malaysia. (via Zoom, Apr. 19, 2022).

• Sedbrook, John. "Developing pennycress \textit{(Thlaspi arvense)} into an oilseed cash cover crop named Covercress," Daybreak Rotary Club, Bloomington/Normal, IL. Zoom presentation (Feb. 2, 2022).


• Illinois State University Field Day. ISU Lexington, IL farm (Apr. 22, 2022).

2. University of Minnesota

A. Planned Activities

Our research teams have generated high-yielding pennycress breeding lines through multi-state testing and identified/validated trait-improving mutations and natural variants. These were our planned activities:

• Utilize marker-assisted selection and CRISPR gene editing to complete the introgression of these traits into elite breeding lines.

• Extend our replicated yield testing program to a cooperative regional program and rapidly identify the best lines for each Midwest location (IL, MN, OH, WI) in conjunction with a commercial launch.

• Perform field evaluations and seed increases of lines with commercial potential.

• Develop additional genetic/genomic resources for long-term breeding program success.

B. Actual Accomplishments

• Dr. Jim Anderson has hired Dr. Julia Zhang as a replacement for Dr. Katherine Frels. Her initial focus will be on introgressing the domestications traits into five elite pennycress lines that were generated via traditional breeding, starting with wild pennycress isolates. Her email address is Zhang0521@umn.edu. The yield trial was planted in St. Paul. It has been an extremely cold and wet spring in Minnesota.
Pennycress flowered about one month later than in an average year. The flowering dates of the breeding lines were compressed into a 10-day window (May 9-20, 2022).

- Dr. Jim Anderson has hired Dr. Antony Brusa as a replacement for Dr. Ratan Chopra. His initial project focuses on identifying the causative mutation responsible for reducing glucosinolate levels in line E5-444 to below 40 without causing any obvious detrimental effects. While the nature of the causative gene mutation has not been identified, Dr. Brusa has been able to map the chromosomal location of the mutation and develop a genetic marker that has allowed us to begin to introgress this mutation into lines harboring our domestication traits. His email address is abrusa@umn.edu.

- We are in the process of stacking useful traits into MN106 using allele-specific markers (KASP). We have focused on the traits that improve the seed quality by reducing erucic acid, glucosinolates, poly-unsaturated fatty acids, improving germination, hastening flowering and maturation, reducing seed fiber, and reducing seedpod shatter. We now have several plants with all of these traits stacked into a single line (MN106 background). These will be put through evaluation studies starting fall of 2022. All these lines were created using EMS mutagenesis and carry residual mutations from the EMS treatments. For this reason, the lines are also being backcrossed to MN106. These backcrossed lines with fewer mutations will be used for restacking the traits to recreate improved stacked lines in the future.

- Over the past two years, new mutant EMS populations have been created. DNA from over 500 lines has been submitted for whole genome sequencing. Dr. Chopra is continuing this work. All the sequencing has now been completed. This new data is in the process of being combined with data from existing sequenced lines. In total, the population of sequenced lines will carry multiple mutations in every gene in pennycress. This population will serve as a new source of useful traits and will be shared with the scientific community to serve as a resource to address basic questions in plant science. This spring, seeds from 20+ lines harboring potential beneficial mutations were planted in the field for evaluation.

- A seed increase is currently underway for a pennycress line that carried mutations that confer high oleic acid content in the seeds along with reduced seedpod shatter and seed fiber. We are working with a company that will use the high oleic oil to make precursors for the synthesis of biodegradable plastics. In addition, several new collaborations are being developed to further characterize this oil's utility.

- An on-campus field site in St. Paul is being used to conduct the multistate IPREFER pennycress trials in 2022. In all four replicates of, ten lines from various sources have
been planted. All of the plots exhibited excellent winter survival, and they are showing reproducible variation in date to flower and date to begin seed set.

C. Explanation of Variance

The departures of Dr. Frels (moved on to a tenure track assistant professor position at the University of Nebraska as a wheat breeder), and Dr. Chopra (moved on to a lead research position at CoverCress, Inc. to continue working with pennycress), have slowed down progress. In addition, this past spring has been much cooler than normal, delaying the pennycress harvest in Minnesota till later in June 2022.

D. Plans for Next Quarter

We will continue working on the aims stated above.

A. Publications, Presentations, Proposals Submitted, and Stakeholder Engagement

- **Publications**
  

- **Stakeholder Engagement**
  
  AURI will host an oil seed field day in Waseca, MN, on May 19, 2022, which was well attended by other members of the pennycress team from IL, MN government officials, representatives from Cargill Inc., and others.

3. Western Illinois University

A. Planned Activities

- Transplant winter and spring type advanced breeding lines to the WIU greenhouse.

- Initiate fertilization experiment investigating nitrogen and phosphorus effects on pennycress plant structure, flowering time, overall seed yield, and seed oil traits.

- Initiate screening of 30 EMS mutant lines for heat stress tolerance in controlled growth chambers.

- Conduct controlled crosses of advantageous traits into golden pennycress lines.
• Initiate selections of increased rosette diameters from 146 wild pennycress populations.

• Complete installation of automation system on TD-NMR

• Organize in-person field days at WIU farms for May 26, 2022.

• Begin spring assessment of WIU research plots for winter survival and flowering dates.

• Maintain research plots with hand weeding and plot labeling.

B. Actual Accomplishments

• Completed initial winter survival and flowering notes for all field experiments in Macomb. Stand establishment of all plots was excellent, with only a few lost plots due to field flooding.

• Experiments at the Macomb site include state variety trials of top 10 breeding lines, GA treatments on black and golden seed, nitrogen application timing on black and golden seed, winter, and spring line variety trials, spring line evaluations of new GE traits, planting methods, seed pelting experiment, planting depth experiment, pre-plant Treflan experiment, large 1-acre seed grow out of triple stacked GE pennycress line for supply chain experiments.

• Completed grow outs in the WIU greenhouses of selected breeding lines containing traits of interest, including early flowering, low erucic acid, low glucosinolates, low fiber, high oil, large seed size, and thick stems (Photo 10).

• Completed shade cloth experiment with three pennycress varieties looking at stem elongation.

• Completed crosses of large-seeded and large rosettes with golden seeded breeding lines.

• Completed large undergraduate research experiment investigating fertilizer treatments with combinations of nitrogen and phosphorus on three different breeding lines of pennycress in the WIU greenhouses. Waiting to complete seed and seed oil analyses.

• Completed EMS mutant pennycress screen in the growth chambers for heat tolerance in collaboration with the IPReP pennycress project.

• Completed grow out of 148 lines of wild populations of pennycress to generate sufficient seed for field experiments in 2023.
- Attended field day at ISU research plots on Apr. 22, 2022, in Lexington, IL.
- Conducted greenhouse and research plot tours for prospective students. Mar. 25, 2022. (40 participants).

Photo 8. WIU research plots in Macomb, Illinois. Plots are in bloom and looking great (Apr. 28, 2022).

Photo 9. IPREFER Research Technician Tad Wesley completing flowering notes on pennycress trials in Macomb, IL.
C. Explanation of Variance

Western Illinois University is beginning to loosen COVID-19 restrictions. All spring field days and travel are allowed to proceed with no restrictions. Undergraduate student research may also proceed with no restrictions.

D. Plans for Next Quarter

- Complete harvesting of winter and spring type advanced breeding lines at the WIU greenhouse.
- Complete harvesting fertilization experiment investigating nitrogen and phosphorus effects on pennycress plant structure, flowering time, overall seed yield, and seed oil traits at the WIU greenhouse.
- Harvest large EMS mutant screen for heat stress tolerant pennycress lines in growth chambers.
- Organize and conduct an in-person field day at the WIU research farm on May 26, 2022.
- Dedicate the new IPREFER storage building on May 26, 2022.
- Maintain research plots with hand weeding and plot labeling.
• Prepare plot combine for harvesting research plots at WIU, OSU, ISU, CoverCress, Inc., and UW in May and June 2022.

• Host summer interns John Kelly and Joseph Brandhorst to complete pennycress studies.

**E. Publications, Presentations, and Proposals Submitted**

• **Presentations**
  

  o Classroom lecture in AGRN-476 Crop Improvement and Biotechnology regarding pennycress breeding and gene editing. Six undergraduate students, April 2022. Conducted experiment demonstrating the use of gene editing.

**OBJECTIVE 3.3 – CHARACTERIZATION OF PENNYCRESS ECOSYSTEM SERVICES**

The goal of the water quality component of the Ecosystem Services group is to assess pennycress’s potential to function as a cover crop.7

1. **Nutrient Sequestration**

   A. **Planned Activities**

   • **Nitrogen Immobilization by Cover Crops**

     o Assess biomass accrual of golden pennycress and wild-type pennycress both growing in

     o Assess effects of pennycress biomass on weed biomass

     o Use drone imagery to determine plant biomass on plots and compare NDVI estimates of biomass to manual estimates of biomass collections.

   • **Soil Porewater and Soil Fertility**

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7 Bill Perry (Illinois State University) leads the Characterization of Pennycress Ecosystem Services Objective. Frank Forcella (UMN) leads the Pollinator Group, and Jason Bond (SIU) leads the SCN group.
Examine effects of pennycress on soil porewater after rainstorms (>0.5” accumulation).

Add microplots of cereal rye to compare the effects of rye cover crops to pennycress on soil porewater nutrients.

**Subsurface Drainage**

Determine loads of nitrogen and phosphorus leaving the fields in subsurface drainage for each rainstorm where subsurface drainage flows – this is a combination of the volume of water lost multiplied by nutrient concentration leading to load or mass per unit time.

**Mineralization of Pennycress Residue**

We estimated the decomposition of pennycress and gene-edited pennycress relative to cereal and annual rye on two common soil types.

**B. Actual Accomplishments**

**Nitrogen Immobilization by Cover Crops**

In the spring of 2022, pennycress biomass established well in the fall and has bolted and is already setting seed which is the best establishment we have had (Fig. 1 and Photo 11).

We have collected biomass from pennycress and nitrogen-amended pennycress on two dates, and the plants are being dried and weighed. We will have one more collection date this year. The biomass present in the field at the start of the season was significantly greater than in past seasons.

All carbon and nitrogen analyses of pennycress biomass from the 2021 season have been completed at the Morris Minnesota Agricultural Research Station by Russ Gesch and his team. We are now analyzing the data and combining it with biomass to determine total N uptake by pennycress compared to the effect observed in soil nitrogen concentrations.

Aerial imagery of NDVI and nitrogen content has been processed, and we are now estimating the biomass and N immobilization near where manual estimates of biomass were taken.

Analyses are being conducted, and after this cover crop season, we will be preparing a manuscript on this.
Fig. 2. Aerial view of cover crops in the replicated ecosystem experiments via drone (Apr. 22, 2022). The north plots have robust establishment (A) while the higher clay soils in the south plots (B) have poorer establishment.
Photo 11. The same photos of the replicated plots show more detail of the establishment on April 21, 2022. Establishment in the north plots (top) was better than in the south (bottom). In the north plots, the effects of fertilization with 50 lb N/acre can be seen in the plot closest to the upper right, where the plots are greener.

- Soil Porewater and Soil Fertility
In the spring of 2022, we have had fewer large rain events, which limited the collection of soil porewater, but we have had ample small rain events, which has benefitted the pennycress biomass growth.

We have collected soil porewater three times this year, and the results have been simply astounding (Fig 3). We have seen an order of magnitude reduction in soil porewater nitrate nitrogen. There have been no significant effects on ammonia or dissolved reactive phosphate levels. This is in stark contrast to the effects of pennycress in the spring of 2021 when the only significant effects on porewater nitrate nitrogen levels were in late May and early June.

We will be collecting soil fertility by Jun. 1, 2022.

Fig. 3. Results from this quarter showing porewater nitrate nitrogen averaged over the first two sampling dates this quarter Feb. 1 – Apr. 30, 2022). This preliminary data shows the differences relative to a rye microplot within our soils. The reference plots were significantly different from all other plots, which were not statistically significantly different.
• **Subsurface Drainage**
  
  o This is the first season where most if not all of the equipment has functioned, and we have successfully collected subsurface flow for two rain events, one of which ended on May 9, 2022.

  o We are working on analyzing the results, but one thing has been apparent – fallow plots lose water quickly, and the volume is high, while pennycress plots have delayed water loss, and the volume is negligible. This will lead to dramatically different nitrogen and phosphorous loads leaving the plots.

• **Mineralization of pennycress residue**

  o The carbon and nitrogen concentrations in the cover crops have been analyzed at the Morris Minnesota Agricultural Research Station. We have begun to analyze this data in relation to the loss of biomass over time.

  o A manuscript for this study is in progress, and we are waiting on the total nitrogen analysis and hope to have it submitted in the summer of 2022.

2. **Soil Carbon Sequestration**

   A. **Planned Activities**

   • Establish plots for examining soil organic matter changes with cover crops

   • Established replicated plots of select cover crops to estimate changes in soil organic matter at cover crop termination and planting of cash crops. The five selected cover crops were: 1) wild type pennycress, 2) golden pennycress, 3) cereal rye, 4) annual rye, and a 5) mix of pea clover radish and oat, all compared to fallow plots.

   B. **Actual Accomplishments**

   • The cover crops of 1) wild type pennycress, 2) golden pennycress, 3) cereal rye, 4) annual rye, and a 5) mix of pea clover radish and oat have been planted in 4 replicates and two blocks.

   • Soil samples will be taken at termination to determine soil organic matter accrual across the profile from 0 to 30 cm deep in sub-sections along the core.

3. **Pollinators and Honeybees**

   A. **Planned Activities**

   Complete analysis of honeybee colony health in 2021.
B. Actual Accomplishments

- A manuscript on field pennycress bee pollinator use has been submitted to the *Journal of Pollination Ecology*.

- A draft manuscript on the fly and other arthropod use of field pennycress has been circulated to coauthors.

- Honeybee hive health has been assessed and is being analyzed

4. Monitor Soybean Cyst Nematode (Heterodera Glycines, SCN) in the Cropping Rotation

Pennycress has been reported to be a poor to moderate host for SCN in greenhouse studies. Research is underway to determine its impact on SCN populations under field conditions in the corn-soybean production system. Preliminary results indicate no increase in SCN population density in pennycress compared with the no cover crop treatment. It is possible that pennycress as a winter cover crop does not support SCN reproduction, or pennycress may even function as a trap crop during the cool season. Currently, funded research is investigating the ability of SCN to infect pennycress in the field and is evaluating the role of temperature in SCN reproduction. Field-scale and microplot experiments are being conducted to evaluate the reproduction and life cycle development of SCN in the fall and spring months. Pennycress’s role as a trap crop or alternate host will be discovered through these experiments.

A. Planned Activities

- **Microplots and Field Trial in Southern Illinois**
  - Complete SCN extraction and elucidation from the samples collected in the field and microplot experiments.
  - Spring assessment of the winter survival and density of the pennycress in the field and microplot experiments.
  - Preparation to sample the soil in microplot experiments that will take place in May 2022.
  - Planning of soybean planting that will take place in late June 2022 in the field and microplot experiments.

B. Actual Accomplishments

- **Microplots and Field Trial in Southern Illinois**
SCN extraction and elucidation from the samples collected in the field and microplot experiment is still in process due to sampling delays with the weather.

We have conducted the assessment of the winter survival and density of the pennycress in the field and microplot experiments.

Preparation to sample the soil in microplot experiments that will take place in May 2022.

Our plans have been completed for soybean planting to take place in early June 2022 in the field and microplot experiments.

5. Plans for Next Quarter

- Microplots and Field Trial in Southern Illinois
  - We will collect soil samples at the time of soybean planting in the field and in microplots.
  - We will plant soybean behind the pennycress at Belleville, IL.
  - We complete all extraction of SCN from samples collected in the Spring.
  - We will complete data analysis for the nematode counts.

- Nitrogen immobilization by Cover Crops
  - We will finish the last sampling of biomass at the end of May or the start of June 2022. The samples will be assessed for biomass and carbon, and nitrogen content. These data will be combined with drone imagery from the same points to determine a relationship to be used in the future.
  - Prepare a manuscript on the relationship between drone imagery and manual estimates of biomass and carbon and nitrogen content and compare the two estimates of total nitrogen immobilized in the replicated plots.

- Soil porewater and Soil Fertility
  - We will have completed porewater sampling by early June 2022 and added to the already developed analyses from 2021 and 2020, which will become part of a manuscript over the summer of 2022.
  - Soil fertility will be sampled during the first week of June 2022, before the planting of soybeans.
We will assess how well these variables relate to one another.

- **Subsurface Drainage**
  - We will continue to monitor subsurface drainage through the fall and also determine if the effects of cover crop continue into the cash crop phase.
  - Ryan Meyer will analyze the data for his thesis, which he will defend in the spring of 2023.

- **Soybean Cyst Nematodes in Replicated Plots in Lexington, Illinois**
  We will sample soybean cyst nematode populations at the same time soil fertility samples are taken in the replicated plots in Lexington, IL.

6. **Publications, Presentations, Proposals Submitted, and Stakeholder Engagement**

- **Professional Conferences / Meetings (Note that the USDA Grant was acknowledged in each presentation)**


  - **Upcoming**

- **Proposals**
USDA Climate Smart Program - Partnership for Adopting Climate-Smart CoverCress™ and Cereal Hybrid Rye. Rob Rhykerd, Nicholas Heller, and William Perry were part of this larger proposal led by win Phippen.

- **Stakeholder Engagement.**
  - Cover crop field day at ISU on Apr. 22, 2022.
  - Forcella, Frank. Winter oilseeds “field day” presentation to producers about pollinators on pennycress and camelina. AURI / Southern Res & Ext Center in Waseca, MN.

**OBJECTIVE 3.4 - LIFE CYCLE SUPPLY CHAIN DEVELOPMENT**

1. **Planned Activities**
   - **CoverCress, Inc.**
   - **AURI**
     - Host Winter Oilseed Field Day with the University of Minnesota at AURI’s Waseca facility on May 18, 2022.
     - Continue long-term grain storage studies and current data from AURI on storage of WT Pennycress and Golden Pennycress grain, oil, and meal.
   - **Illinois State University**
     - We will be harvesting our pennycress plots at the ISU Farm around the first of June 2022.
     - The ISU Farm will host a Field Day for McLean County, IL, on Jul. 12, 2022, which will include an update on the IPREFER project.

2. **Actual Accomplishments**
   - **CoverCress, Inc.**
o Participated in St. Louis Agribusiness Club speaker’s panel (Feb 2022).

o Recognized as a top ten St. Louis “Startups to Watch” and received St. Louis Business Journal Innovation Award (Feb 2022).


o Participated in Biofuels Digest’s ABLC 2022 Conference as a guest speaker for their agriculture and feedstocks program (Mar 2022).

o Attended annual NIFA-CAP grant conference hosted by K-State in KC, MO (Apr. 2022).

o Successfully reached an agreement with the IPREFER ELT to approve additional Supply Chain funding specifically intended to assist CoverCress, Inc. to research/execute go-to-market tactics (Apr. 2022).

o Initiated April Field Days (Apr. 11, 14, 21, 27).


• **AURI**

  o Long-term oil stability is ongoing at refrigerated and room temperature conditions. It appears from current results that room temperature oil is beginning to oxidize after six months.

  o AURI’s long-term storage study for oil and meal (yellow and black pennycress) is underway and is stable, with a quick dissipation of yeast and mold in both groups. The results indicate, for the seed, stability for up to 12 months and a rapid loss of mold and yeast.

• **Illinois State University**

  o ISU hosted a Cover Crop Field Day on Apr. 22, 2022.

  o We collaborated with Win on the USDA-Climate Smart Proposal submitted earlier this month.

  o We submitted a proposal this week (along with Win and Tad at WIU) to the Illinois Nutrient Research and Education Council to evaluate carbon sequestration
and soil and soil porewater N and P under selected cover crops including wild pennycress and golden pennycress.

3. **Explanation of Variance**

No variances were noted.

4. **Plans for Next Quarter**

   - **CoverCress, Inc.**
     - Wrap up CoverCress, Inc. Field Days (May 2022).
     - Harvest is expected to start around May 26, 2022.
     - Initiate SC/CC Priority: *SGS Seed Storage Study* (June 2022).

   - **AURI**
     - AURI’s long-term storage study for oil and seed (yellow and black pennycress) is underway.
     - AURI’s long-term oil study under multiple conditions is ongoing, with additional data to be gathered.
     - We are coordinating with the University of Minnesota to receive seed harvested in the summer of 2022.
     - We are collaborating with the University of Minnesota on field day in May 2022 at AURI’s Waseca facility.

5. **Publications, Presentations, Proposals Submitted, and Stakeholder Engagement**

   - **CoverCress, Inc.**
     - Received award from the City of Creve Coeur, MO for “Most Innovative Company” (May 2022)
o Participated in CAAFI’s Biennial General Meeting – Washington, DC as a guest speaker (June 2022).

- AURI

  o Alexandra Diemer, AURI’s Business Development Director of Novel Supply Chains, met with Millborn Seeds out of South Dakota and was invited to attend the Field Day in May.

  o Alexandra Diemer met with the Regenerative Ag Alliance, which works with the poultry industry, including feed, and was also invited to attend the May field day.

  o Michael Stutelberg met with Dr. Eric Cochran at Iowa State University in April 2022 and provided him with a liter of pennycress oil for testing on biobased applications.

**Objective 3.5 and 6 – Education, Extension, and Outreach**

1. Planned Activities

- Continue evaluating and revising the Cover Crop Science project book based on SPIN Clubs and other activities.

- Hold the planned 4-H SPIN (Special Interest) Clubs in East Peoria, IL (serving Peoria and Tazewell counties, IL) and Havana, IL (serving Fulton and Mason counties, IL).

- Hold additional outreach events at the Marquette Heights Public Library and other locations as requested.

- Continue conversations with UMN Extension to pilot Cover Crop Science Project Book activities in Minnesota.

- Plan for summer camps for community organizations to further pilot Project Book activities.

- Begin developing workshop and summer camp implementation guides to accompany the individual-focused project book.

- Adapt staging guide into a PowerPoint slide set

- Submit staging guide for publication in a peer-reviewed journal.
- Determine placement on field day agenda for early summer 2022 at the Northwest Agricultural Research Station as an extension presentation.

- Planned field days. Dates to TBD based on weather and field availability.

- Host small group farmer meetings.

- Continue direct engagement with farmers and other farm-related channels.

- More development of our go-to-market strategy.

- Development of our CoverCress, Inc. website to provide more information to farmers and investors on the value of CoverCress Inc.

- Events scheduled:
  - St. Louis Agribusiness Club Panel Discussion – Mike DeCamp will be on a panel discussion (Feb. 8, 2022).
  - St. Louis Business Journal - St. Louis INNO: Startups to Watch – the event is on Feb 23rd (Feb. 23, 2022).
  - CoverCress, Inc. CEO Mike DeCamp will be part of a panel discussion on renewable fuels at the Bank of America Global Ag and Materials Conference in Ft Lauderdale, FL. (Mar. 3, 2022).
  - Mike DeCamp (or Jerry Steiner, CoverCress, Inc. CEO Emeritus) will speak as part of the agriculture and feedstocks program at the Biofuels Digest ABLC 2022 Conference, Washington, D.C. (Mar. 16, 2022).

- Identify intern participants for 2022.

- Conduct a mentoring workshop for IPREFER project members.

- Develop summer program curriculum and activities.

- Develop tools to evaluate the development of interdisciplinary competency of program participants.

2. Actual Accomplishments

- The staging guide was accepted for publication in April 2022.

- A staging presentation slideset was built, and we are waiting for the article DOI before widespread release.
• Planned field day in Northwest Ohio (Jun. 23, 2022).

• Sept. 20-22, 2022, is the Farm Science Review, and pennycress plots should be established.

• CoverCress, Inc. will have participated in/conducted six field days by the end of April 2022. These events will have contacted at least 70 farmers in Illinois and Missouri.

• CoverCress, Inc. will have conducted as many as 12 farmer-facing luncheons and dinner meetings. These events have contacted as many as 40 farmers.

• CoverCress, Inc. conducted four virtual sessions in late February and early March 2022 that reached over 100 farmers. These 1-hour long evening sessions provided farmers an overview of growing CoverCress™. These events we all recorded and can be made available upon request.

• Marketing materials for the CoverCress, Inc. 2022-2023 Founding Farmers program were also developed and shared with these groups (See Exhibit B. Discover the Benefits of a New Winter Oilseed Cover Crop).

• CoverCress, Inc. has secured verbal commitments to nearly 8000 acres of crop to be seeded in central IL and east central MO for the fall of 2022. This is on target for the projected 10,000 acres of seed that CoverCress, Inc. will have available for the fall of 2022.

• CoverCress, Inc. has also been capturing video and still photography for future marketing and training information for CoverCress, Inc.

• CoverCress, Inc. conducted a harvest training program for seven farmers who will be harvesting the first seed crop of CoverCress™ in the spring of 2022 (See Exhibit C. Determining CoverCress™ Harvest Maturity).

• Fifteen undergraduate students from nine different states applied to the Integrated Plant Systems Undergraduate Research Experience. Eight undergraduate interns from six different institutions (six states) accepted internship positions for the summer 2022 program.

• Dr. Gina Hunter and Kara Baldwin, Illinois State University, led a two-part virtual workshop on Effective Mentoring in Research on Apr. 28 and 29, 2022. Twelve IPREFER collaborators participated on day one, and ten collaborators participated on day two. Four of the five IPREFER researchers who will host interns in 2022 attended. An evaluation of the workshop was not conducted.
• We developed a mixed methods approach to evaluate the interdisciplinary competency of IPREFER research interns during the summer 2022 program. Methods include a 14-item pre- and a post-survey developed by Lattuca et al. (2013), reflective writing, student presentations, and participant observation.  

• An orientation program for the eight undergraduate research interns was developed and will take place May 17 – 20, 2022, in St. Paul, MN.

• We developed summer program activities to develop dimensions of interdisciplinary competency, including interdisciplinary skills, recognizing disciplinary perspectives, and reflective behavior (Lattuca et al., 2013).

3. Explanation of Variance

None noted.

4. Plans for Next Quarter

• Present at Northwest field day

• Answer questions from OSU extension about the Bunge-CoverCress, Inc. announcement

• Discuss the cover crop book and link Ohio teachers with it.

• CoverCress, Inc. will be developing the go-to-market strategies for the fall of 2023

• Complete harvest CoverCress, Inc. field days.

• CoverCress, Inc. will be developing training programs for seeding CoverCress™ that will be presented in August 2022.

• Implement curriculum for the 2022 Integrated Plant Systems – Undergraduate Research Experience.

• Draft a literature review of educational approaches to undergraduate interdisciplinary competency.

• Prepare and submit one conference poster abstract describing the Integrated Plant Systems – Undergraduate Research Experience.

5. Publications, Presentations, and Proposals Submitted

Our mission is to optimize off-season pennycress oilseed production by overcoming production and supply chain bottlenecks.