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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 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”).

Any opinions, findings, conclusions, or recommendations expressed in this report do not necessarily reflect those of the U.S. Department of Agriculture, 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.

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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

1. Planned Activities

- Conduct an in-person annual meeting on July 31-August 2, 2022.
- Close out summer 2022 summer interns and post student projects to the website.
- Complete evaluations of the annual meeting and provide feedback to organizers, collaborators, and Advisory Board members.
- Evaluate the expansion of the advisory board to include producers.
- Work with UMN to complete Data Management protocols for all working teams.
- Initiate YR 4 funding sub-awards to collaborators.
- Complete evaluations of the annual meeting and provide feedback to organizers, collaborators, and the Advisory Board.
- Complete 3rd annual report to NIFA and ReePORT.
- Continue to conduct weekly meetings with the program manager, monthly meetings with the ELT, and quarterly meetings with the Advisory Board.
- Continue communicating quarterly commercialization updates from CoverCress to the entire research team to identify potential needs and improve the transparency of the commercialization effort.
- Post flyer for 2023 summer interns on the project website.

2. Actual Accomplishments.

- We conducted our third annual meeting on August 2-3, 2022. This was our first in-person all-hands meeting since our kick-off meeting in August 2019. The focus was on highlighting previous years' accomplishments and organizing Year 4 activities.
We initiated the organization of an in-person annual meeting for Year 4, which will be held at the University of Minnesota July 30-31, 2023.

We completed all Year 4 sub-awards with all collaborators. We re-budgeted the UMN sub-award to accommodate a retiring collaborator and onboarding a new collaborator, Julia Zhang, to the IPREFER project.

Selected Julia Zhang of UMN to represent the Northern breeding team on the Executive Leadership Team.

Completed third annual report to NIFA and ReePORT.

3. **Explanation of Variance**

Western Illinois University is back to normal. All students are free to conduct experiments in the field and buildings. Student workers are now fully employed and assisting in field, greenhouse, and laboratory experiments.

4. **Plans for Next Quarter**

- Post the 2022 intern posters on the project website.
- Finalize the protocols for data management deposits to UMN data banks.
- Continue organization of the in-person Year 4 annual meeting hosted by UMN.

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

- **Education Presentations**
  - Phippen, Win. School of Agriculture open house Demonstration and presentation on pennycress and the IPREFER project to 15 potential incoming undergraduate students and their parents. Included a demonstration of the new plot planter and the IPREFER project. Macomb, IL, Sep. 16, 2022. (2 - 45 min presentations)
  - Phippen, Win. Classroom guest lecturer in *Agriculture in Today's Society* (WIU AGRI-120 WIU AGRI-120) regarding pennycress production and the IPREFER project. 50 undergraduate students, 50 min lectures to 2 sections of the class. Included laboratory tour of seed cleaning and chemical analysis equipment. Macomb, IL, Sep. 26, 2022.
- Phippen, Win. Western Illinois University School of Agriculture demonstration and presentation on pennycress and the IPREFER project to 17 potential incoming undergraduate students and their parents from the Columbus Junction (Iowa) Community High School. Included a demonstration of the optical seed sorter and seed testing lab. Macomb, IL, Oct. 24, 2022 (30 min. presentation).

- Phippen, Win. Western Illinois University School of Agriculture demonstration and presentation on pennycress and the IPREFER project to 24 potential incoming undergraduate students and their parents from Wilton (Iowa) Community High School. Included a demonstration of the optical seed sorter and seed testing lab. Macomb, IL, Oct. 24, 2022. (30 min. presentation).

- Stake Holder Engagement

  Along with our regular outreach activities we were very pleased to see IPREFER featured in the NIFA Update in the article “A New Cash Oilseed Cover Crop for Midwest Corn and Soybean Rotations. (Sep. 20, 2022).

  - Sent a 5 lb. seed bag of Golden Pennycress to Donald Danforth Center researchers for 1-acre planting and demonstration plots.
  
  - Sent 1 liter of Golden seeded pennycress oil and 1 liter of wild pennycress oil to Texas A&M for testing oil properties.
  
  - Sent 10g seed packets of pennycress lines to a potential collaborator at the University of Connecticut for cellulosic stem research.
  
  - Sent 15g of black-seeded spring-type pennycress to a new collaborator at UMN for molecular experiments.

**OBJECTIVE 3.1 - AGRONOMIC MANAGEMENT**

This quarter, CRM, Residue Management, and the Select projects conclude following soybean harvest; data analysis and manuscript drafting are underway. The co-authors are currently reviewing the second manuscript from the PELLET research. The manuscript should be submitted to *Crop Science* by the year’s end. A new systems study and golden pennycress seeding date study are underway across the network and will be evaluated for establishment prior to freeze-up this fall. At this point, fall emergence is reported as variable due to wide-ranging moisture conditions across the Midwest.
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., 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 by 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 August 1, 2022, to October 31, 2022.

A. Planned Activities

- Process pennycress samples in the lab for an oil test.
- Compile pennycress data from all sites and enter these data into the database.
- Harvest double-crop soybean.
- Produce one draft manuscript evaluating the effect of preceding corn hybrids on pennycress establishment using data from all four sites and two crop seasons.
- Circulate this draft manuscript to all collaborators and co-authors for review.

B. Actual Accomplishments

- Pennycress samples were processed in the lab for an oil test.
- We compiled pennycress data from all four sites, and this data was entered into the database.
- The double-crop soybean was harvested.
- One draft manuscript, “Preceding corn hybrid evaluation to enhance early pennycress establishment for increased crop diversification and intensification in the upper Midwest US,” has been prepared. We are working on finalizing the draft prior to circulation with the team.

¹ Russ Gesch (USDA-ARS) leads the CRM team.
C. Explanation of Variance

There is a little delay in preparing the draft manuscript for publication due to the overlap of field activities. Still, it will be finished and submitted in the upcoming quarter as field activities will slow down.

D. Plans for Next Quarter

- Process double-crop soybean samples in the lab.
- Double-crop soybean data will be received from all collaborators and compiled, and these data will be entered into the database.
- The manuscript will be circulated among co-authors, their inputs will be incorporated, and the manuscript will be updated and submitted to peer review journal for publication.
- Results from this project will be presented at ASA-CSSA-SSSA annual meeting in Baltimore, MD (Nov. 6-9, 2022).
- Pennycress establishment data (plant density and green cover) from pennycress seeding date and system studies will be collected before soil freeze.

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

- **Award – USDA 2022 Mission Area Award**

  IPREFER CoPds Russ Gesch and Frank Forcella were part of a team recognized with a Technology Transfer Award at the 2022 USDA-NIFA Mission Area Awards ceremony. The ARS Pennycress Research Team (Steven C. Cermak, Team Leader, Roque L. Evangelista, Milla Hojilla Evangelista, **Russ Gesch, Frank Forcella**, Candice Gardner, and Terry A. Isbell /Peoria, Illinois Category) received their award “For the advancement of pennycress as a commercial cover crop in the U.S” (See Fig. 1).

- CRM results (oral and poster) were presented at the IPREFER 3rd annual meeting (St. Louis, MO, Aug. 1-3, 2022).
- Two presentations about pennycress research results and updates at the Association for the Advancement of Industrial Crops (AAIC) 33rd Annual Meeting, Bozeman, MT, Oct. 8-12, 2022).
2. **Corn Residue Management (Objective 3.1.2 / "DISC")**

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 remaining after harvest. Several corn residue treatments will test the hypothesis that the residue can be sized small enough not to 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, Ohio State University, and Illinois State University.

**A. Planned Activities**

Harvest soybean at all sites.

**B. Actual Accomplishments**

- Spring data compiled and shared
- Soybean harvested at all locations

**C. Explanation of Variance**

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DISC is the abbreviation we use for the IPREFER corn stover management project. Nicholas Heller (University of Minnesota) leads this IPREFER objective.
None noted.

D. Plans for Next Quarter

- Analyze Cycle 1 data and send out a rough draft of a manuscript.
- Compile Harvested Soybean data to finish Res Mngt Version II

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

None this quarter.

3. Novel Seed Treatment for Improved Pennycress Performance (“PELLET”) (Objective 3.1.3)³

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

A. Planned Activities

- Analyze spring establishment and yield data from Phase 3
  - Obtain data from WIU, UW Platteville.
- Re-package Phase 1 data and send the manuscript to co-authors with a planned submission to the journal *Crop Science*.
- Reformat data from Phases 1, 2 and 3 to GEMS format
  - Draft two intern positions for completing the final PELLET experimental steps.

B. Actual Accomplishments

- All data has been collated from all three studies.
- Datasets formatted and submitted to GEMS.
- Two IPREFER intern positions were posted.

C. Explanation of Variance

³ “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.

Yr. 4 – Quarterly Progress Report: August – October 2022
Substantial figure creation is taking a while to generate, given other research and teaching duties.

D. Plans for Next Quarter

- Re-package Phase 1 and 3 data and send manuscripts to co-authors, planned submission to Crop Science
- Begin intern screening for the IPREFER internship program

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

A. Western Illinois University

- Planned Activities
  - Begin greenhouse herbicide carryover dose-response bioassays for single active ingredient soybean herbicides
  - Plant pennycress into corn and soybean herbicide carryover studies
  - Plant pennycress and apply preemergence herbicide to measure pennycress response and winter annual weed control
  - Analyze greenhouse bioassay data

- Actual Accomplishments

Planted and sprayed corn and soybean herbicide carryover studies (Year 3 of corn carryover, year 1 of soybean carryover)

- Explanation of Variance
  - The oilseed cropping system study was abandoned because of extreme damage from wildlife (especially white-tailed deer)
  - We still need to finish the greenhouse bioassay data analysis. Will be completed in the next quarter for a poster to be presented at the ASA-CSSA-SSSA annual meeting

- Plans for Next Quarter

4 Mark Bernards (Western Illinois University) leads the WEEDS Team.
- Begin greenhouse herbicide carryover dose-response bioassays for single active ingredient soybean herbicides

- Analyze greenhouse bioassay data

- **Publications, Presentations, Proposals Submitted, and Stakeholder Engagement**

  None this quarter.

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. The shorter growing season in the upper Midwest 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-nonshatter’. 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)

**A. Planned Activities**

- Lucas Roberts and Haley Reuter will present research posters at the annual meeting in St. Louis on August 1-2, 2022.

- Lucas Roberts will provide a 10-minute research talk about the SELECT project at the annual meeting in St. Louis.

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Aaron Lorenz (University of Minnesota) leads the SELECT Team.
• Research plots will be harvested, and post-harvest quality analysis will be done on soybean seeds on a plot basis.

• Plants will be pulled for future image analysis to quantify the number of nodes, internode spacing, branch numbers, and pod numbers to better characterize how pennycress affects soybean growth and morphology.

• Lucas Roberts will be a guest lecturer for Walking Research Tours (AGRO 1661W). He will present the cropping system and current SoySELECT research to 70 undergrad plant science majors on September 14, 2022.

• SoySELECT 2.0 will be planted in Rosemount and St. Paul in late September. This experiment will utilize three pennycress genotypes and eight soybean genotypes. The closer locations and fewer number of plots will enable more frequent growth and developmental measurements to be taken on soybeans.

B. Actual Accomplishments

• Field trials were harvested in Rosemount and Morris. Soybean seed quality analysis for protein, oil, and predicted amino acid content is ongoing.

• Image analysis began on the 2022 plant pulls. This analysis will characterize how pennycress affects soybean plant architecture by quantifying branch number, internode spacing, and pod placement.

• SoySELECT 2.0 was planted in Rosemount and St. Paul.

C. Explanation of Variance

• 2022 data is being compiled for analysis next quarter.

• Lucas Roberts has begun writing the manuscript for the SoySELECT experiment, as this is the third year of data collection.

D. Plans for Next Quarter

• The imaging of 2022 soybean plants will be completed.

• Quantifying soybean seed quality will finish.

• A greenhouse experiment characterizing the genetic architecture of soybean allelopathy tolerance will commence.
• Lucas will present a research poster about the SELECT experiment at the Plant and Animal Genome Conference in January.

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

• Lucas Roberts (Graduate Student) and Hayley Reuter (2022 Undergraduate Intern) presented posters at the annual meeting in St. Louis. Roberts also presented his poster at the World Food Prize graduate student poster contest in Des Moines, IA, in October 2022 (See Fig. 2 and 3).

• Lucas Roberts was a guest lecturer for AGRO 1661W – Walking Research Tours. He presented the cropping system and current research of SELECT to 70 University of Minnesota undergrad plant science majors, Sep. 14, 2022.

Figure 3. Research poster Lucas Roberts presented at the annual meeting in St. Louis and the World Food Prize graduate student poster contest in Des Moines, IA, in October 2022.
6. Evaluating agronomic and economic performance of pennycress in regionally adapted cropping systems (“OILSYSTEMS”) (Objective 3.1.6)

Minnesota (RROC, USDA), Western Illinois University, Ohio State University, and Illinois State University

A. Planned Activities

- Harvest grain and biomass from the Corn/Soy/Wheat plots.
- Apply burndown herbicides to ensure plots start weed free.
- Plant Golden Pennycress (tt8-ARV1) at 10lbs/ac using no-till drill.

B. Actual Accomplishments

Crops were harvested and the pennycress was planted at 4/5 agronomy network sites.

C. Explanation of Variance

WIU site abandoned due to pest damage
D. Plans for Next Quarter

- Perform fall establishment assessment prior to freeze-up
- Collate fall establishment data from all sites

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

None at this time.

7. Pennycress Seeding Date Trial using the golden pennycress (“SDT”)

The golden pennycress has several better qualities than MN106, but information on seeding date for the golden pennycress is missing. With more fall establishment than spring, Pennycress tends to show an increased pennycress seed yield than spring germination. This increased germination in fall may depend on the type of pennycress cultivar used. So far, most of the pennycress seeding date trials were evaluated using the pennycress line ‘MN106”. The objective of this study is to evaluate several seeding dates ranging from the end of August to the first week of November. Collaborators are from Minnesota (RROC, USDA-Morris), Illinois State University, Ohio State University, and the University of Wisconsin-Platteville.

A. Planned Activities

- Developed research protocol
- Share the research protocol with collaborators, get inputs, and update the research protocol.
- Share research protocol and data collection template with collaborators.
- Plant goldenccress following treatment structure.

B. Actual Accomplishments

- Draft research protocol developed.
- This research protocol was shared among collaborators, and their inputs were incorporated.
- The updated research protocol and data collection template were shared with collaborators.
- Goldenccress seeded following treatment structure (the first seeding date being Aug. 30 at the Morris, MN site) has shown good establishment so far.
C. Explanation of Variance

None noted.

D. Plans for Next Quarter

- Plant density (count) and green cover data will be collected.
- Fall-collected data will be received from different sites and compiled.
- Compiled data will be entered into the database

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

None this quarter.

Figure 5. Midwest Drought Map – Drought conditions are variable across the IPREFER network ranging from “Extreme Drought” in Rosemount, MN, to “None” or normal conditions in Platteville, WI. Weather data will be collected from each site and used to interpret fall emergence and establishment at each location. Rosemount, MN, the driest location, saw minimal emergence of September planting dates in the driest September on record.
**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.

   - Utilize marker-assisted selection and CRISPR gene editing to complete 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 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**

   - **Research**

     o Genetic combinations aimed at reducing seed glucosinolate content to near the 30 micromol/gram target have been independently identified by the Sedbrook Lab and another lab. Growth chamber data suggest the plants grow similarly to the wild type. The combination was stacked via genetic crosses by the Sedbrook lab, which allows for field testing, which is currently taking place.

     o Using CRISPR genome editing, we have generated a number of mutant combinations leading to substantially increased pennycress seed size in both wild-type and tt8 mutant backgrounds. Natural variants with relatively larger seed size have also been identified and genetically crossed to decipher possible combinatorial effects.

     o We tilled field plot locations from the past five years with the goal of assessing next spring how many dark-seeded and yellow-seeded pennycress plants emerge. This study is aimed at determining potential weediness issues. Preliminary data show pennycress yellow seeded lines do not persist in the field, likely due to the thin seed coat and lack of protective condensed tannins in the seed coat, which greatly diminishes seed viability in field conditions.
C. Explanation of Variance

Multi-state variety trial plots were not planted this field season due to not getting regulatory approval in time to satisfy the USDA APHIS SECURE rules. The same occurred for CRISPR-induced lines where more than one mutation was introduced at the same time.

D. Plans for Next Quarter

Continue work on the aims stated above.

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

- **Publication**
  
  [https://doi.org/10.3390/fermentation8110617](https://doi.org/10.3390/fermentation8110617)

- **Presentation**
  

- **Stakeholder Engagement**
  

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 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 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

• Dr. David Marks is stacking useful traits in MN106 background using allele specific KASP markers. New mutants showing reduced dormancy, improved oil quality, and stature that may have green revolution characteristics have been discovered. Those new traits are being introgressed into stacked MN106 based lines. Those lines are also being grown in the 2022-23 field season for evaluation and seed increase. Dr. Anthony Brusa, directed by Dr. David Marks, is investigating non-AOP reduced glucosinolate mutants.

• We are developing germplasm strategies to expand the breeding pipeline and accelerate domestication by fast cycle elite germplasm and forward breeding. New crosses using MN elite lines and natural collections are planned and will be made in the winter 2022 greenhouse. We are focusing on introducing EMS mutants of ind1 (shattering resistance), fae1 (zero erucic acid), rod1 (reduced polyunsaturated fatty acids), and tt2 (low fiber and transparent testa) to the breeding pipeline. F2 plants from six crosses with the alleles of those domestication genes are being grown. We will use allele specific KASP markers to select for plants with at least one allele of the target traits. The F2:3 seed from selected plants will be harvested and planted in the 2023-2024 field. Single plant selection will be made within the F3 rows based on phenotypic results of the domestication and agronomic traits.

• Introgression of ind1, fae1, rod1, and tt2 to five elite MN lines was initiated in fall 2021. By fall 2022, F1 seed stacked with alleles of the four domestication genes were obtained. The F1 plants are being grown and will be genotyped with allele specific KASP markers in winter 2022. The selected plants stacked with the four desirable alleles (heterozygote of ind1,fae1,rod1,tt2 alleles) will be crossed to the recurrent parents. One more backcross will be made in spring 2023 after selecting the BC1F1 with KASP markers. We anticipate completion of the backcrossing and marker assisted selection by spring 2024.

• The 2022-2023 pennycress field trials were planted in mid Sep. at five locations across MN. The main field location is in St. Paul. The main nursery contains evaluations of germplasm and domestication traits, F3 rows, yield trials, and seed increase. The multiple location experiments include advanced yield trials and investigation of trait X environment interaction of stacked genes of new EMS mutants.
in MN106 background developed by Dr. David Marks. The fall germination score of each plot was recorded three weeks after planting and before snow cover. Due to historical drought in MN, the fall germination was poor in three of the five locations.

C. Explanation of Variance

None noted.

D. Plans for Next Quarter

We will continue working on the aims stated above.

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

None this quarter.

3. Western Illinois University

A. Planned Activities

• Analyze data from 2022 summer research projects (See Fig. 5).

• Design experiments for the fall 2022 planting season.

• Hire additional undergraduate students to support seed analysis and planting activities.

• Plant all WIU research starting on October 1, 2022, in Macomb, IL.

• Experiments planned include spring-applied nitrogen trials, spring-applied sulfur experiments on low glucosinolate pennycress lines, replicated breeding variety trials of winter lines, breeding line seed increases, and evaluating new traits to improve commercialization efforts.

• Plant 435 wild populations of pennycress for potential trait identification in collaboration with DOE project – IPREP.

• Analyze data from 2021 summer research projects.

• Continue evaluating the seed burial study to determine seed persistence in the soil bank.

• Distribute planting seed to collaborating institutions and possible new collaborators.
Figure 6. Results of multi-state variety trials for 2022. Gold bars indicate golden-seeded varieties, while gray bars indicate black-seed varieties. Boxplots display Minimum, Q1, Median, Q3, Maximum, and outliers. The number above bars represents the Mean.

B. Actual Accomplishments

- Continued analysis of data from 2022 summer research projects.
- Designed experiments for the fall 2022 planting season.
- Hired seven undergraduate students for the fall and spring semesters to assist with IPREFER projects.
- Planted all WIU research starting on October 1, 2022, in Macomb, IL. (Photos 1-3)
- Experiments included: drilled spring applied nitrogen trials, replicated variety trials of wild pennycress populations, breeding line seed increases, and evaluation plots of
new traits to identify promising traits. Collected data on emergence from all 1,260 research plots.

- Provided 10 lb. seed bags of Golden pennycress to collaborating institutions for all agronomic field studies.
- We delivered 500 lbs. of dry bulk golden seeded pennycress to AURI in Waseca, MN, for seed storage and processing experiments.

C. Explanation of Variance

Western Illinois University is back to normal. All students are free to conduct experiments in the field and buildings. Student workers are now fully employed and assisting in field, greenhouse, and laboratory experiments.

Photo 1. Planting pennycress plots at WIU, Oct 1, 2022 (Credit: Win Phippen).
D. Plans for Next Quarter

- Complete emergence and fall data collection on all field experiments in Macomb
• Meet with Southern breeding team members to identify breeding lines for winter
grow out in the greenhouse during the winter months

• Select and germinate advanced breeding lines with traits including compact flower
stem, early flowering, improved germination, stem thickness, heat tolerance, and
reduced seed coat fibers.

• Complete computer algorithms for the optical seed sorter to recognize seed size and
shape.

• Complete computer algorithms for selecting larger seed size on the Marvin seed
analyzer.

• Continue to develop protocols for analyzing glucosinolates in pennycress seeds and
plant materials.

• Initiate greenhouse growing experiments.

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

• Professional Conferences and Meetings

  o Phippen, Win J., John Sedbrook & W. David Marks. ”Advancements in breeding
pennycress (Thlaspi arvense L.) as a sustainable feed and fuel source.” Bajwa,
D.S. and M.T. Berti Eds. 2022. Integrating technology with industrial crops and
their products for a sustainable bioeconomy. 33rd Annual Meeting of the
Association for the Advancement of Industrial Crops (AAIC). Program and

  o Phippen, Mary & Win Phippen. “Development of a rapid assay for total
glucosinolate content in Pennycress (Thlaspi arvense L.) seed by measurement of
enzymatically released glucose using a blood glucose meter and test strips.”
(Poster) Integrating technology with industrial crops and their products for a
sustainable bioeconomy. 33rd Annual Meeting of the Association for the
Advancement of Industrial Crops (AAIC). Program and Abstracts. Bozeman, MT,
Oct. 9-12.

  o Phippen, Win, & Tad L. Wesley. “Advances in pennycress breeding in a southern
breeding program at WIU.” (Poster). USDA-CAP- IPREFER 3rd annual meeting,


- **Awards**

  Best poster presentation oilseed division, AAIC 2022, Bozeman, MT, Oct. 2022. (See Fig. 6.)
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. We will present findings from our Soybean Cyst Nematode, Nutrient sequestration Soil Carbon Sequestration research activities at local and national meetings throughout Year 4.

1. Nutrient Sequestration

A. Planned Activities
• **Nitrogen Immobilization by Cover Crops**
  
  o Plant golden pennycress in the replicated immobilization plots.
  
  o Observe the production of golden versus black seed pennycress over the growing season.

• **Soil Porewater and Soil Fertility**

Sample soil fertility after soybean harvest and install lysimeters.

• **Subsurface Drainage**

Continue to monitor subsurface drainage through the late fall and remove equipment prior to the winter to prevent damage.

• **Soybean cyst nematodes in replicated plots in Lexington, Illinois**

Sample soybean cyst nematode populations at the same time soil fertility samples are taken in the replicated immobilization plots in Lexington, IL.

### B. Actual Accomplishments

• **Replicated cover crops experiments**

  o Soybeans were harvested from replicated plots in the fall of 2023.
  
  o Golden Pennycress was drilled into each plot with the help of Nicholas Heller on the agronomy team.
  
  o Soil samples at 3 points (homogenates of 20 cores at each point 30-cm deep) were taken in each plot and analyzed at United Soils Inc.
  
  o SCN samples were also taken at a point collocated with soil samples, and the samples are being analyzed now at the University of Illinois Plant Clinic. To date, one block has been counted for SCN eggs per 100 g of soil. There is no difference between SCN in reference, pennycress, or nitrogen-amended pennycress plots. The soybean varieties planted at the ISU farm are not advanced strains, and SCN levels averaged 7600 in the reference plot, 5400 in one pennycress plot, and 3750 in another pennycress plot.
  
  o Due to dry conditions, no water has been sampled, and lysimeters will be placed in the field for porewater analysis in the spring. Tile water monitoring equipment will be removed from the field in the winter.
1. Manuscript Preparation
   - A manuscript has been submitted on the decomposition rates of pennycress relative to cereal and annual rye.
   - Data analysis of pore water responses to pennycress cover crops is nearing submission.

2. Soil Carbon Sequestration
   A. Planned Activities
      - Harvest corn from the Carbon Sequestration plots
      - Plant cover crops on each treatment with added hybrid rye and golden pennycress treatments.
      - Current treatments are:
         - Fallow
         - Pea, Clover, Radish, Oat
         - Black seed pennycress
         - Golden seed pennycress
         - Cereal Rye
         - Annual Rye
      - Collect soil samples from the carbon sequestration plots for analysis.
   B. Actual Accomplishments
      - Corn was harvested from the Carbon Sequestration plots on October 16.
      - Cover crops were planted on October 16 with Nicholas Heller and Bill Perry.
      - Collected soil samples from the carbon sequestration plots from October 22 – 30, and samples are being air dried and sieved to prepare for analyses.
      - We also presented a poster at the Agronomy meeting titled: “Impact of Cover Crops on Soil Profile Distribution of Organic Matter in Midwest Cropping Systems.”

3. Pollinators and Honeybees
A. Planned Activities

- Organize a multisite comparison of pollinators in golden-seeded and black-seeded pennycress for 2023.
- Locate beehives that can be placed in early spring 2023, immediately adjacent to pennycress fields, and companion hives that are placed far from pennycress fields during pennycress bloom.
- Continue working on manuscripts of bee and fly visitation dynamics on pennycress.

B. Actual Accomplishments

- Black and golden pennycress have been planted at Western Illinois University, Illinois State University, and at one site in Minnesota to compare pollinator visitation between the two varieties in the Spring of 2023.
- This October and November, a small commercial beekeeper who maintains about 150 hives and over-winters them in central Minnesota has agreed to rent eight hives to us. Four hives will be placed adjacent to a pennycress field in early to mid-April before pennycress commences flowering. Another group of four hives will be placed far from flowering pennycress. After flowering, all eight hives will be commingled. Pollen collection by bees will be monitored once weekly in all hives during pennycress flowering and once monthly after that. Hive strength will be examined in autumn. The hypothesis being tested is that access to voluminous amounts of pennycress pollen and nectar in April and early May is beneficial to honeybees and beekeepers in the Upper Midwest.

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

A. Microplots and Field Trial in Southern Illinois

- Planned Activities
  - Complete processing of SCN samples collected in 2022.
  - Analyze data collected from the field study and microplots.
- Actual Accomplishments
  - Completed sampling of the microplot study that was initiated in October 2021. The final samples came from select treatments that had soybean following pennycress.
5. Plans for Next Quarter

- Microplots and Field Trial in Southern Illinois
  - We plan to complete processing our SCN samples collected in 2022.
  - Analyze the data collected from the field study and microplots.

- Nitrogen immobilization by Cover Crops
  
  Replicated Plots
  - Prepare for spring sampling under pennycress relative to fallow plots
  - Plant buffer strips between plots of cereal rye and assess nutrient uptake by rye compared to pennycress
  - Design sampling approach to assess soil temperature and moisture under pennycress compared to cereal rye
  - Analyze data collected in the fall and complete the manuscript on pennycress nutrient immobilization in soils and soil porewater.

6. Explanation of Variance

The studies are all on track, and plans for the fall planting of pennycress are in progress.

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

- Publications


- Proposals
OBJECTIVE 3.4 - LIFE CYCLE SUPPLY CHAIN DEVELOPMENT

1. Planned Activities

A. CoverCress, Inc.

- Complete financing and sale with Bayer, Bunge, and Chevron.
- Host IPREFER Annual Meeting.
- Formalize go-to-market strategy and customer agreements.
- Distribute and plant seed.
- Advance CoverCress™ regulatory status.

B. AURI

- Received ~500 lbs. of pennycress seed and will investigate procedures for hexane extraction of oil and analyze oil and meal characteristics for commercial applications.
- Develop documents on protocols for seed cleaning and handling of pennycress.
- Investigating pennycress oil as a possible feedstock for resins in biobased plastics.
- Long-term grain storage studies and current data from AURI on storage of Golden Pennycress grain, oil, and meal.

2. Actual Accomplishments

A. CoverCress, Inc.

- Closed final investor round and sale of the company resulting in three owners: Bayer, Bunge, and Chevron.
- Hosted the first new Board of Directors meeting.
- Hosted and participated in IPREFER Annual Meeting.
- Finalized CoverCress “Founding Agent” and “Farmer” signed agreements.
- Approved initial Go-to-Market plan based on current Regulatory status.
- Completed bagging and distribution of CoverCress seed to ~90 farmers.
- Nearly ~8,000 acres of CoverCress were planted for the 2022-2023 Demonstration Plot Program that will be terminated in Spring 2023.
- Held a seed production planning meeting with production companies.
- Submitted request for GRAS approval of CoverCress WG product
- Submitted CoverCress grain for pilot-scale processing

B. AURI

- 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.
- 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 up to 12 months and a rapid loss of mold and yeast.
- Presented current research at the 2022 IPREFER annual meeting.

3. Explanation of Variance

   No variances were noted.

4. Plans for Next Quarter

   A. CoverCress, Inc.

   - Interpret results for pilot scale processing.
   - Initiate RFS workstream
   - Continue ongoing meetings with USDA and EPA
   - Launch customer portals
• Complete the first USDA Field Audit.

• **Engagements**
  - Kick-off Meeting with Bayer.
  - Renewable Fuels Conference (November 2022, St. Louis).
  - No-Till Conference (January 2023, St. Louis).
  - Expand CoverCress headquarters.

B. **AURI**

• Continued outreach and education activities in support of supply chain development.

• AURI’s long-term storage study for oil and seed (yellow and black pennycress) is underway.

• AURI’s long-term oil study at multiple conditions is ongoing, with additional data to be gathered.

• Hexane extraction of oil from pennycress seed at pilot scale to produce low-fat meal comparable to soybean meal. We then plan to identify feeding trials with seed or hexane-extracted meal as part of the feed ration.

• Identify and test golden pennycress oil characteristics for use in bioplastics.

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

A. **CoverCress, Inc.**

• Attended various commercial/industry events: FPS, Husker Harvest, CTIC, USF&R, Danforth Center’s BioBash and AgTech NEXT, BioSTL Tri-Lateral Summit with Israel & UAE.

• Met with USDA and EPA regarding aligning regulatory processes for a new cash crop.

• Held Kick-Off meetings with Commercial Partners: Bunge, Climate Corp.

B. **AURI**

• Dr. Michael Stutelberg attended the Biodiesel Technical Workshop in St. Louis, MO, in early November 2022.
- Yellow pennycress meal was sent to St. John’s University in Minnesota for testing as a fertilizer.

- AURI attended a Central Lakes College meeting and discussed pennycress as a cover crop with stakeholders.

**OBJECTIVE 3.5 – EDUCATION, EXTENSION, AND OUTREACH**

1. **Planned Activities**

   - Post-developed curricular resources to the OSU webpage.
   - Develop six curricular activities for undergraduate classrooms.
   - Showcase pennycress at the Farm Science Review (Sept. 20-22) Extension event at OSU.
   - Solicit internship opportunities from IPREFER collaborators and update the IPREFER website.
   - CCI planned a large 8,000-10,000-acre launch of the CoverCress product.
   - Host an IPREFER field day for local high school students to introduce them to career opportunities stemming from pennycress development at UM.
   - Compile and analyze data collected using a mixed-methods approach to evaluate the impact of the IPREFER internship program on the 2022 intern cohort, including survey responses, artifacts, and observations.
   - Develop an interview tool to obtain post-program reflections from the 2022 IPREFER internship program cohort.
   - Develop promotional materials and begin recruiting participants for the 2023 IPREFER internship program.
   - Continue evaluating and revising Cover Crop Science Project Book based on summer camps and other outreach engagements.
   - Conduct educational outreach at the Illinois State Fair (first during 4-H judging, then during the general admission STEAM Expo) and county fairs (Fulton, Mason, McLean, Peoria, Tazewell).

2. **Actual Accomplishments**
• Posted developed curricular resources to OSU webpage on Aug 10.

• Showcased pennycress at the Farm Science Review (Sept. 20-22) Extension event at OSU.

• Obtained commitments to mentor IPREFER internship participants from eight IPREFER collaborators, including two collaborators who have not previously worked with IPREFER interns.

• Posted six IPREFER internship opportunities on the website by Oct 31.

• Seven positions are currently posted on the IPREFER website.

• Distributed seed to 89 farmers in Missouri, Illinois, Iowa, and Indiana with a planted goal of close to 8,000 acres of seed CoverCress planted for the demonstration program, allowing more farmers to be able to learn firsthand how to seed CoverCress.

• Hosted an IPREFER field day at UM for 140 high school students and school staff. The objectives of this activity were to introduce young people to pennycress/agricultural research and career opportunities stemming from pennycress development and deployment.

• Analyzed survey responses from the 2022 IPREFER intern cohort and developed an interview tool to gain post-internship reflections from the 2022 intern cohort.

• Scheduled post-internship interviews with six students.

• Continue evaluating and revising Cover Crop Science Project Book based on summer camp and other outreach engagements.

• Conduct educational outreach at the Illinois State Fair (first during 4-H judging, then during the general admission STEAM Expo) and county fairs (Fulton, Mason, McLean, Peoria, Tazewell).

3. **Explanation of Variance**

• Undergraduate curricular activities will be built spring-summer 2023 with the help of a new post-doc who is starting April 1, 2023.

• We have not completed the analysis of artifacts (responses to online activities, presentations) and observations of participants obtained during the 2022 IPREFER internship program. Materials have been compiled and analyzed using NVIVO to identify themes related to undergraduate students’ knowledge of the research process, confidence
in their ability to participate in research, and perceptions of the abilities that contribute to collaboration and interdisciplinary competency.

- Submission of the poster abstract is on hold until the data analysis is completed.

4. Plans for Next Quarter

- Recruit for IPREFER internship from ISU classroom visits, sharing with Minneapolis-St. Paul metro community colleges, etc.
- Post more internship opportunities as IPREFER team members submit them.
- CCI will be providing support for a farmer customer who will be presenting at the National No-Till conference that will be held in St. Louis, MO, in January.
- CCI will also provide training opportunities to the farmers who seed CoverCress in the fall of 2022 through email correspondence.
- Dr. Ratan Chopra, Vice President of Research, CoverCress Inc., will speak on November 9 for ISU’s Energy for a Sustainable Future seminar series. His talk is entitled “Translational Research Provides a Path for Sustainable Feedstocks.”
- Plan and prepare for spring field days, which at UM will focus on introducing community college students to pennycress research, careers in agriculture, and IPREFER undergraduate research opportunities to assist in building a new agricultural workforce.
- Complete analysis of data collected during the 2022 IPREFER internship program, including post-internship reflections. Present results of the analysis at a professional conference.
- Organize a mentor training workshop that will be held in spring 2023.
- Continue to promote and recruit students to the 2023 IPREER internship program.
- Submit 4-H Cover Crop Science Project Book for national 4-H review.
- Complete an informal educator guide to accompany the 4-H Cover Crop Science Project Book.

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

A. Stakeholder Engagement

- Highlighted pennycress with field plots and signs at the Farm Science Review event at OSU had over 100,000 attendees across the three days in September.
• Introduced 15 science teachers to the IPREFER webpage through a workshop called “Ag is STEM,” hosted by OSU.

• IPREFER Field Day event at University of Minnesota Rosemount Research and Outreach Center (Rosemount, MN) with 140 total attendees (130 students grades 9-12, 10 teachers) on Sept 22.
  o Preliminary survey results of 85 student attendees indicate that students had a positive learning experience, with 24% of surveyed students saying that they would definitely consider a career in agriculture, food, and natural resources and 50% saying they would maybe consider a career in agriculture, food, and natural resources after attending the field day. Furthermore, 88% of students said they learned at least a bit about pennycress’s uses, agronomic practices, and environmental benefits, with 35% saying they learned a lot about these topics; none of the attending students were familiar with pennycress prior to the field day. All teachers involved indicated that they were satisfied with the event and would bring students to attend again next year, which we also consider as evidence of a successful field day.

• Had direct interactions with 740 youth at the Illinois State 4-H fair, 180 youth at the Illinois State Fair STEM Expo, 250 youth at various county fairs (Fulton, Mason, McLean, Peoria, Tazewell), and five youths at a library program on natural and artificial selection.

B. Proposal Submitted

“Growing Future Agricultural Professionals: Advancing the Sustainability of the Forever Green Initiative via Youth Engagement and Career Development.” Grant proposal submitted to the University of Minnesota Forever Grant Initiative Grant Program. $140,500. FY23-FY25.

This funding would provide 50% salary funding for our current Education and Outreach Associate who manages our lab’s IPREFER education activities. If funded, this project would overlap well with the IPREFER funding as it would provide funding for additional field days for high school students to learn about pennycress and opportunities in the new agricultural workforce. This would allow IPREFER and FGI resources could be pooled to reach an even larger audience of future undergraduate researchers and pennycress-advancing professionals. In addition, the Forever Green Funding would allow us to develop online educational modules that use videos to tell the pennycress story and the subsequent career opportunities the crop creates. These videos would be distributed to area schools to prepare them for field day attendance and be a resource for general agricultural education activities.
New Cash Cover Crop for Sustainable Aviation Fuel

By the US Federal government, annually towards the 25-year goal of 50 billion gallons of biofuels set

At the Energy for a Sustainable Future Seminar Series

Wednesday, September 21, 2022, at 5:00 p.m. via Zoom

https://illinoisstate.zoom.us/j/95165466322

Meeting ID: 951 6546 6322

Dr. Winthrop Phippen

Professor of Plant Breeding & Genetics, School of Agriculture, Western Illinois University
Dr. Ratan Chopra  
Vice President of Research, CoverCress Inc.

CoverCress Inc. (CCI) is converting field pennycress into a new domesticated variety under the CoverCress™ brand as the third crop in standard corn/soybean rotations, which could be used on up to 10 million acres at maturity in the US. To enable the domestication of field pennycress into a new crop, we are using an approach of translational research and modern genetic improvement tools. The dual purposes of providing a winter/spring cover-crop, but more importantly providing growers with an extra source of income via contracting, growing, and harvesting the oilseed grain produced from CoverCress™ seed, CCI will sell that grain to processors in a closed loop commercial system for use as a renewable fuel feedstock and high-quality animal feed. 

Translational Research Provides a Path for Sustainable Feedstocks

Sponsored by the Energy for a Sustainable Future Seminar Series, the Center for Mathematics, Science & Technology, and the Department of Technology. If you need a special accommodation to participate, please contact Rebekka Darner at rldarne@ilstu.edu.

Thursday, November 9, 2022, at 5:00 P.M. via Zoom

Meeting ID: 982 1055 1444

https://illinoisstate.zoom.us/j/98210551444
Our mission is to optimize off-season pennycress oilseed production by overcoming production and supply chain bottlenecks.