Year 3 Annual Objective Executive Summaries
Aug 2021 - July 2022
TABLE OF CONTENTS

Acknowledgment........................................................................................................................................... v

Legal Notice ...................................................................................................................................................... v

YEAR 3 – EXECUTIVE SUMMARIES

Project Administration and Governance ........................................................................................................ 1

Agronomy and Crop Management ................................................................................................................. 4

Breeding and Genomics ................................................................................................................................. 13

Pennycress Ecosystem Services .................................................................................................................... 17

Supply Chain Development .......................................................................................................................... 25

Outreach, Extension, and Education ............................................................................................................ 27

Figures

Figure 1. Two-year average pennycress seed yield at different sites ............................................................... 5

Figure 2. Variations in pennycress flowering, maturity, and harvesting ....................................................... 6

Figure 3. Representative plot views and aerial images from the Corn Residue Management Study, Cycle I and Version II in Lexington, IL ................................................................................................................. 8

Figure 4. Spring ground coverage prior to bolting ......................................................................................... 8

Figure 5. Cumulative germination (symbols) and regressed descriptive models (lines) depicting cumulative germination curve for pennycress (Thlaspi arvense L.) for each treatment ............................................. 10

Figure 6. Assessing legacy of soil-applied residual herbicides on pennycress growth and development ............................................................................................................................................................. 11

Figure 7. Average soybean yield of 40 soybean genotypes in a pennycress intercropping system versus monoculture system across three environments ......................................................................................... 12

Figure 8. Flowering dynamics of wild type and golden pennycress ................................................................ 19

Figure 9. Cumulative (three sampling dates) abundances of four groups of insects caught in pan traps placed in adjacent golden-seeded and black-seeded pennycress plots or fields during flowering in spring 2022 .......................................................................................................................... 20
Figure 10. Fall to spring net carbon sequestered by pennycress, cereal rye, and a pea clover, radish, and oat mix compared to no cover crops .................................................................21

Figure 11A. Pennycress biomass from spring 2022 .............................................................22

Figure 11B. Soil porewater nitrate n reductions by pennycress relative to reference plots.........22

Figure 12. Spring soil nitrate reductions by pennycress relative to fallow plots over the course of the experiment.................................................................22

Tables

Table 1. Cycle 1 CRM Study Treatments dynamics of wild type and golden pennycress........7
Table 2. Version II, all planted after grain harvest ..............................................................7

Photos

Photo 1. Intern Jack Kelly optically cleaning seed samples for oil analysis at WIU in Macomb, IL .......................................................................................................................2

Photo 2. Intern Joe Brandhorst running Marvin Seed analyzer to determine 1000 seed weight and seed dimensions at WIU in Macomb, IL .................................................................3

Photo 3. WIU harvest team getting ready to begin 2022 harvest season at Macomb, IL .........17

Photo 4. Native honeybees on pennycress ........................................................................19

Photo 5. Intern Jack Kelly assisting with data collection with Dr. Win Phippen in pennycress research plots at WIU in Macomb, IL .................................................................29

Photo 6. Intern Joe Brandhorst combine harvesting pennycress with Dr. Win Phippen at WIU in Macomb, IL. Photo taken June 5, 2022 ........................................................................29
ACKNOWLEDGMENT

IPREFER is supported by Agriculture and Food Research Initiative Competitive Grant No. 2019-69012-29851 from the National Institute of Food and Agriculture.

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”).

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.

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.
Integrated Pennycress Research Enabling Farm and Energy Resilience
(AFRI-CAP 2019-69012-29851)

Year 3 Executive Summaries
August 1, 2021 – July 31, 2022

Project Administration and Governance

■ Win Phippen, Western Illinois University, wb-hippen@wiu.edu
■ Anne Kinzel, anne.kinzel@iprefercap.org

The IPREFER project was initiated with the specific goal of commercializing pennycress production by 2021. With several University and USDA collaborators and a single corporate partner CoverCress, Inc., we are adapting the project to unforeseen challenges. We are well on our way to meeting our pennycress commercialization goal. In our third year, we redoubled our focus on preparing for a pennycress commercial launch. We accomplished this while having to continue to respond and adjust to COVID-19 impacts on higher education.

Year 3. Accomplishments

• IPREFER personnel have been involved in the development of several pennycress patents:
  
  
  
  
  https://patents.justia.com/search?q=Low+Glucosinolate+Pennycress+Meal+and+Methods+of+Making

- IPREFER continues to be featured in the agricultural press, including a featured story in Prairie Farmer magazine (Spangler, Holly. Pennycress: The First Cash Cover Crop. Prairie Farmer, July 2022, 
  https://editions.mydigitalpublication.com/publication/?m=67558&i=752196&p=6&ver=html5. See also Additional information regarding IPREFER media coverage is available at https://www.iprefercap.org/news-events/newsletter/.

- We hosted eight undergraduate interns in our Integrated Plant Systems – Undergraduate Research Experience. Information on each of the interns’ projects is available at https://www.iprefercap.org/about/2022-undergraduate-research-experience/. The interns will be presenting their work in a poster session at our 2022 Annual Meeting.

**Photo 1.** Intern Jack Kelly optically cleaning seed samples for oil analysis at WIU in Macomb, IL
We created a special page on our website dedicated to graduate students, post docs and fellows participating in the IPREFER project (See https://www.iprefercap.org/about/iprefer-graduate-students-postdocs-fellows/). Four Ph.D. students and four Master’s level students completed their educational programs while participating in the IPREFER project (See https://www.iprefercap.org/about/iprefer-graduate-students-postdocs-fellows/).

Year 4. Planned Activities, Outcomes, and Impacts

- During the 4th Quarter we completed all the planning for our Year 3 Annual Meeting (all hands) which will be held July 31-Aug 2, 2022, in St. Louis at the Danforth Center. Project partner CoverCress, Inc. is serving as our local host (See Exhibit A, 2022 PREFER Annual Meeting Agenda).
- We will provide Year 4 funding to all the project subcontractors.
- We will host a Year 4 2023 Integrated Plant Systems – Undergraduate Research Experience program. We will start taking application on November 1, 2022, via the website. We have already secured mentor commitments from throughout the project.
- We will continue to hold monthly Executive Leadership Team (ELT) meetings and quarterly Advisory Board meetings. CoverCress will continue to share updates with the ELT throughout the year.
Now that the project is accumulating significantly more data, we are working with CoPD Samantha Wells on data protocols for our project database.

We will continue to publish our internal *PennyPulse* newsletter.

Our Year 4 Annual Meeting will be held at the University of Minnesota Twin Cities campus. CoPd Samantha Wells will be our local host.

**OBJECTIVE 3.1 – AGRONOMY AND CROP MANAGEMENT**

Co-Project Directors

- Alexander (Alex) Lindsey, The Ohio State University, lindsey.227@osu.edu
- Samantha Wells, University, University of Minnesota, mswells@umn.edu

**CORN RELATIVE MATURITY (CRM) STUDY: FACILITATING TIMELY ESTABLISHMENT OF PENNYCRESS**

- **PI:** Russ Gesch, USDA-ARS-NCSCRL, Morris, MN (russ.gesch@ars.usda.gov)
- **Collaborators:** Yesuf Assen Mohammed (USDA-ARS-NCSCRL, Morris, MN), Samantha Wells (University of Minnesota), Alexander Lindsey (The Ohio State University, Nicholas Heller (Illinois State University), and Alexander Hard (University of Minnesota)

Pennycress (*Thlaspi arvense L.*) is a new oilseed crop being developed as a cash cover crop to help diversify corn-soybean systems in the Midwest Corn Belt while providing new economic opportunities and ecosystem services. Because of corn’s long growing season, establishing pennycress after grain-corn harvest is challenging, especially in the northern Corn Belt. We hypothesized that early maturing corn hybrids may provide more time for pennycress establishment before soil freeze with minimal effects on corn grain yield. The study’s objectives were to evaluate different corn relative maturity hybrids (CRM) on pennycress establishment and yield and assess yield tradeoffs between corn and pennycress and productivity of double-crop soybean. We conducted the study in 2020/21 and 2021/2022 at Custar (Ohio), Lexington (IL), Morris (MN), and Rosemount (MN). The corn relative maturity hybrids ranged from 95 to 113 days for the Ohio and Illinois sites (113-day corn representing full season) and from 76 to 95 days for the two Minnesota sites (95-day corn representing full season). The check was the full-season corn hybrid for each site harvested for silage.

Results showed that earlier corn harvest facilitated by earlier CRMs led to timely pennycress planting that improved fall stand establishment. Except for the Illinois site, there was a
tendency for pennycress seed yield to increase with earlier planting (i.e., earlier corn harvest). Also, earlier pennycress planting facilitated earlier harvest the following summer, leading to earlier soybean planting, which can help improve double-crop productivity. Using the 86-day corn for Minnesota sites and 113-day corn for the Illinois and Ohio sites minimized corn gain yield penalty while facilitating timely pennycress planting, which improved establishment and yield. Enhancing pennycress adoption into corn-soybean systems in the Midwest may require using mid-range RM corn hybrids to improve pennycress establishment, yields, and overall system productivity.

Figure 1. Two-year average pennycress seed yield at different sites. "Si is silage treatment.
The corn residue management study aimed to assess best management practices in the fall transition from corn to pennycress. For the first cycle of the experiment, only black-seeded pennycress was available for use in the study. We found that none of the methods of managing corn residue were effective at improving pennycress establishment except for removing the residue altogether. The silage control was the best at all sites in the study. After completing the first cycle of the experiment, modifications were deemed prudent to learn from the data already collected and to utilize recently made-available golden-seeded pennycress seed for the study. Version II included a comparison of black-seeded pennycress to golden pennycress following grain corn and silage corn. See Table 1 for study treatments.

**Figure 2. Variations in pennycress flowering, maturity and harvesting.**
Table 1. Cycle 1 CRM Study Treatments dynamics of wild type and golden pennycress

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Previous Crop</th>
<th>Crop Residue</th>
<th>Tillage Type</th>
<th>Pennycress Planting Method</th>
<th>Pennycress Planting Date*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Grain Corn</td>
<td>Chopped</td>
<td>No-till</td>
<td>No-till Drill</td>
<td>11 Oct 2020</td>
</tr>
<tr>
<td>2</td>
<td>Silage Corn</td>
<td>Removed</td>
<td>No-till</td>
<td>No-till Drill</td>
<td>17 Sept 2020</td>
</tr>
<tr>
<td>3</td>
<td>Grain Corn</td>
<td>Chopped</td>
<td>No-till</td>
<td>Broadcast after corn harvest</td>
<td>11 Oct 2020</td>
</tr>
<tr>
<td>4</td>
<td>Grain Corn</td>
<td>Chopped</td>
<td>Vertical Tillage</td>
<td>Broadcast</td>
<td>11 Oct 2020</td>
</tr>
<tr>
<td>5</td>
<td>Grain Corn</td>
<td>Chopped</td>
<td>No-till</td>
<td>Broadcast ahead of corn harvest</td>
<td>11 Oct 2020</td>
</tr>
</tbody>
</table>

* Dates for Lexington, IL site

Table 2. Version II, all planted after grain harvest

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Previous Crop</th>
<th>Crop Residue</th>
<th>Tillage Type</th>
<th>Pennycress Planting Method</th>
<th>Pennycress Seed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Grain Corn</td>
<td>Chopped</td>
<td>Vertical Tillage</td>
<td>Broadcast</td>
<td>Black-seeded Pennycress</td>
</tr>
<tr>
<td>2</td>
<td>Grain Corn</td>
<td>Chopped</td>
<td>Vertical Tillage</td>
<td>Broadcast</td>
<td>Golden Pennycress</td>
</tr>
<tr>
<td>3</td>
<td>Silage Corn</td>
<td>Chopped</td>
<td>Vertical Tillage</td>
<td>Broadcast</td>
<td>Black-seeded Pennycress</td>
</tr>
<tr>
<td>4</td>
<td>Silage Corn</td>
<td>Chopped</td>
<td>Vertical Tillage</td>
<td>Broadcast</td>
<td>Golden Pennycress</td>
</tr>
</tbody>
</table>

All sites in this study (Lexington, IL; Macomb, IL; Hoytville, OH; Rosemount, MN; Morris, MN) are north of 40 degrees N latitude. Removal of corn residue was the treatment that resulted in the best pennycress establishment in both Cycle 1 (Table 1) and Version II (Table 1) of this experiment. At press time, harvest data are still coming in, but prior studies have documented a correlation between establishment and seed yield in pennycress.
Figure 3. Representative plot views and aerial images from the Corn Residue Management Study, Cycle 1 and Version II in Lexington, IL. Corn residue coupled with later planting in Cycle 1 prevented establishment of pennycress compared to the residue-free control; however, in Version II all pennycress was seeded on the same date highlighting the role of corn residue on pennycress establishment for both golden and black-seeded pennycress. Photo credit: Nicholas Heller and Austin Nakaerts.

Figure 4. Spring ground coverage prior to bolting. Data from Cycle 1 of the Corn Residue Management study quantify the advantage in pennycress establishment. Here the percentage green cover, for treatment 2 which was the silage control where corn stover was removed from the plots. Treatments 1, 3, 4, and 5 were all following grain corn where corn residue lessened the pennycress establishment.
NOVEL SEED TREATMENT FOR IMPROVED PENNYCRESS PERFORMANCE (PELLET STUDY)

- **PI:** Alexander J. Lindsey, The Ohio State University, (lindsey.227@osu.edu)
- **Collaborator:** Nasib Koirala (The Ohio State University, Graduate RA)

**Update: Seed Treatment Effects on Germination and Establishment of Oilseed Pennycress**

Fall establishment has been identified as a major challenge for pennycress that should be addressed prior to widespread crop release. This study focused on improving germination and establishment using seed treatments, including seed pelleting. The objectives were to assess the effect of seed treatments and storage on seed germination and vigor of pennycress varieties in the laboratory and field environments. A lab experiment with four pennycress varieties and four seed treatments with an added component of seed storage for up to 12 months in different temperatures was conducted during 2021/22. Seed pelleting was found to improve germination characteristics. Still, those benefits diminished when stored for more than three months, eventually leading to loss of seed vigor and germination compared to non-pelleted treatments. The loss of vigor over time was more prominent in cold storage. Results indicated seed treatment with GA can increase germination for black-seeded pennycress, though it may be less effective for golden pennycress. A field experiment at nine sites over four states in the Midwestern US in the year 2021 was conducted to determine how GA treatment, seed pelleting, and addition of a fungicide would affect fall establishment of a black-seeded and golden pennycress line. The establishment of the black-seeded pennycress variety was unchanged or improved with treatment under broadcast sowing, while no such benefit was observed for the gold-seeded variety. Almost all seed pelleting treatments negatively impacted the establishment of the golden pennycress variety. The study indicates that using GA as a soak treatment in black-seeded pennycress for commercial uses could be effective. This study also highlights the need for further study with seed pelleting in pennycress and recommends dormancy breaking breeding programs in the case of black-seeded pennycress line as it had higher vigor and establishment compared to golden seeded pennycress line.
The crop rotation currently proposed for pennycress is to follow corn and precede soybean. Residual herbicides provide more consistent weed control in corn but may impact the growth and productivity of the following pennycress crop. Greenhouse experiments were conducted to assess the impact of single active ingredient herbicides on pennycress establishment and growth. Field experiments were conducted to assess the potential for commercial, multi-active ingredient herbicides applied in a corn crop to impact pennycress stand and yield. Pendimethalin and trifluralin (Group 3 herbicides) were the only herbicides that did not reduce pennycress stand or biomass when applied at full-use rates in the greenhouse. The interval between spring or summer herbicide applications in corn and pennycress planting in the fall will typically be greater than 75 days. Single active ingredient herbicides applied at rates equivalent to what would be in the soil after 75 days (based on published soil degradation half-life values) did not impact pennycress stand or biomass. This was true for the following herbicides: Group 2 (rimsulfuron, thiencarbazone-methyl), Group 4 (2,4-D,
clopyralid, dicamba), Group 5 (atrazine), Group 14 (flumioxazin, saflufenacil), Group 15 (acetochlor, dimethenamid-P, flufenacet, S-metolachlor, pyroxasulfone), and Group 27 (isoxaflutole, mesotrione, tembotrione, tolpyralate, topramezone). In field studies conducted over three different years in Macomb, no herbicides applied at 1X or 2X rates in corn reduced the yield of the following pennycress crop compared to the control (no residual herbicide) treatment. Products tested included Aatrex, Zidua, Callisto, Balance Flexx, Laudis, Impact, Acuron, Corvus, Capreno, Halext GT, and Verdict. However, based on current herbicide labels, pennycress may not be planted following corn treated with any of these herbicides. It will be critical for the widespread planting of pennycress to work with herbicide registrants to develop the data necessary to allow the use of residual herbicides in the crop preceding pennycress.

Figure 6. Assessing legacy of soil applied residual herbicides on pennycress growth and development.

**Breeding Soybeans for Intercropping with Pennycress (SOYSELECT)**

- **PI:** Aaron Lorenz, University of Minnesota, lore0149@umn.edu
- **Collaborators:** Seth Naeve & Samantha Wells (University of Minnesota)
- **Graduate Student:** Lucas Roberts (University of Minnesota)

In the current soybean-pennycress intercropping system envisioned for the Upper Midwest, soybeans are planted into a stand of pennycress at the rosette or early bolting phase in early- to mid-May. This places the soybean crop into direct competition with the pennycress crop for four to eight weeks until the pennycress is harvested in mid- to late June. A yield penalty on soybeans is expected given the competition for light and water (if precipitation is limited) and other potential biotic interactions such as allelopathy imposed by the pennycress. Yield
reductions in soybean would negatively affect the profitability of the entire system, perhaps even making the system financially unsustainable. Variation for many traits in soybean exists, making it possible that soybean varieties could be identified, or new soybean varieties bred, that tolerate stresses imposed by pennycress and hence minimize yield penalties. Prior to achieving the long-term goal of soybean variety development for pennycress intercropping, knowledge on the extent of cropping system-by-variety interactions and traits important for selection need to be developed. Given this background, the SOYSELECT project objectives are 1) Determine the extent of cropping system-by-variety interactions in soybean germplasm adapted to the Upper Midwest; and 2) Identify traits that may be important for adapting soybeans to a pennycress intercropping system that can be selected upon in a monoculture system. To address these objectives, we have been growing 40 soybean genotypes in a split design with cropping system (pennycress intercropping versus monoculture) as the whole-plot factor. Traits such as yield, maturity date, lodging, protein, oil, stand, yield components, shoot architecture, and canopy coverage are being measured. Most of the data analysis is still underway, but key findings to date include a strong cropping system-by-variety interaction and the finding that traits such as maturity and plant height drive yield more in the intercropping treatment compared to the monoculture treatment. More detailed morphological and physiological measurements were taken on ten varieties spanning the variation in response to the intercropping yield penalty. Preliminary results suggest differences among varieties exist for traits such as etiolation under shading stress, chlorophyll content under intercropping stress, and resistance to allelopathy.

Figure 7. Average soybean yield of 40 soybean genotypes in a pennycress intercropping system versus monoculture system across three environments (Rosemount in 2020; Rosemount and Morris in 2021).
OBJECTIVE 3.2 – BREEDING AND GENOMICS – PENNYCRESS IMPROVEMENT

Co-Project Directors

- M. David Marks, (LEAD Northern Genetics), University of Minnesota, marks004@umn.edu
- John Sedbrook (LEAD Southern Genetics), Illinois State University, jcsedbr@ilstu.edu

Northern Breeding – PennyCress Improvement (University of Minnesota)

- PI: M. David Marks, University of Minnesota (marks004@umn.edu)
- Collaborators: Ratan Chopra (CoverCress, Inc.), James Anderson, and Julia Zhang (University of Minnesota)
- Graduate Students: Matthew A. Ott, Zenith Tandukar, & Brett Heim (University of Minnesota)

Year 3 Accomplishments

- State Trials

  While final results are not in for this year, the state trials included four pennyCress varieties developed at MN. The most advanced line contains three mutations, including two that reduce erucic acid and that greatly increase oleic acid content and reduce shatter. In the past, we found that this triple variant line consistently performed better than wild-type (MN106) and produced, on average, 20% or more seed yield across eight experimental trials. We believe this is primarily due to the reduced shatter trait and partly due to the phenotypic selection made on an individual plant after backcrossing, which is under investigation.

- Developing Germplasm

  We have successfully developed germplasm using mutagenesis and natural germplasm. We have 2,160 M3 individual plants and 270 M2 pools available for forward screens. In addition, two recombinant inbred populations have been developed for QTL mapping by Zenith Tandukar, namely x17014 and x16017. X17014 is a cross between Ames32867 (Armenia) and MN106 (the reference genotype), whereas x16017 is a cross between wild accessions Ames31501 (Canada) and MN77 (USA). These populations have been phenotyped for various traits, including seed size, oil content, protein content, glucosinolate content, plant height, and days to flowering.

- Sequencing Support
Dr. Chopra successfully led the sequencing support that was granted by JGI. This support will allow us to whole-genome sequence ~1,000 pennycress samples and provide much-needed information for making breeding decisions in the future. This included 500 natural accessions and 500 additional mutagenized lines. Overall, more than 700 EMS mutagenized lines have been sequenced. In total, mutations in approximately 90% of the genes have been identified.

In most cases, multiple alleles have been identified for these genes. Currently, an allelic series of the AOP2 mutations required for glucosinolate biosynthesis, and FAD2 mutations required for the biosynthesis of linoleic and linolenic fatty acids, are under investigation. In addition, this sequence information has been used to identify potential early maturing mutants, mutants with altered root architecture, mutants that flower early, and more.

- **Overcoming Lodging Issues**

To overcome lodging issues in pennycress, we are developing semi-dwarf lines. To understand the effectiveness of the semi-dwarves in terms of standability, we conducted experiments with the wild-type and a semi-dwarf line along with different nitrogen regimes. We found that pennycress yields are positively correlated with nitrogen rates, but lodging was severe in the wild type (MN106). However, the semi-dwarf pennycress line did not lodge even with high nitrogen application rates. However, the original semi-dwarf population was segregated for plants exhibiting reduced vigor, and the population matured later than MN106. Thus, original semi-dwarf used in these experiments has been backcrossed to MN106 to remove detrimental mutations, and an early maturing trait has been added to produce an early maturing version of the semi-dwarf line. These new lines will be retested this coming fall.

- **Improving Establishment**

Establishment in pennycress can be improved using the yellow-seed trait. Matthew A. Ott found that tt2-1 allele germinates better than MN106, which was reflected in the total seed yields. We have now created lines containing tt2, fae1, rod1, aop2, and ind-1. These are all the key domestication traits, and these lines will be evaluated over the next few years to assess their potential for commercialization.

- **Southern State Performance**

We also found that one of the breeding lines – MN17077-2 from the UMN breeding program, performed well in the southern states on the IPREFER trials. We believe this line could be more adapted to those climates. We will focus on MN17077-2 to better understand the traits that impart better performance and to develop markers that will aid in the introgression of domestication traits into this line.
• Year 4 Planned Activities, Outcomes, and Impacts

We will continue to introgression of domestication traits in elite breeding lines. These lines will form the foundation of the UMN breeding program and will contribute to improved yields and increased stability under variable environmental conditions.

We will perform replicated yield testing trials for the best lines identified from various breeding programs. We will also continue to:

- Test and stack in value-added traits, including higher total seed oil, larger seed size, and high oleic oil.
- Exploit the utility of the mutant gene index and other plant genetic material.
- Test for heritability of the phenotypic traits identified in Year 4 within the mutant populations and natural crosses.
- We will continue to provide quarterly progress reports and prepare and submit journal articles.

Southern Breeding – Pennycress Improvement: Illinois State University and Western Illinois University

Co-Project Directors

- Win Phippen, Western Illinois University, wb-phippen@wiu.edu
- John Sedbrook, Illinois State University, jcsedbr@ilstu.edu

We had a successful 2021-2022 pennycress field season, collecting growth data on plants in field plots having various genotypes (single CRISPR-induced mutations and combinations), including those conferring reduced glucosinolate, reduced dormancy, better stand establishment, larger seed size, higher total seed oil, as well as natural populations collected at different latitudes and environments. Of interest, some plant lines with poor seed set in growth chambers performed well in the field. Conversely, a few plant lines that appeared indistinguishable from wild type in growth chambers matured later than wild type in the field.

We made good progress in stacking new genetic combinations aimed at attaining target levels of glucosinolates in combination with other core domestication traits. We also explored plant fitness, including heat tolerance of pennycress lines having increased oleic acid in seed oil, obtaining encouraging results.
A single mutation and double mutation have been identified and confirmed to reduce pennycress seed glucosinolate levels by 30 to 40 percent each without impacting plant growth and seed yields. These mutations were combined by genetic crossing and CRISPR genome editing in both laboratory lines and commercial varieties. Determinations are underway to determine whether the combined mutations have additive effects on reducing glucosinolate and if the plants remain healthy. Other mutation combinations were also generated with the same end goal of reducing seed glucosinolate levels to near or below 30 umol/gm. These mutations are also being stacked with the core pennycress domestication traits necessary for commercialization.

Whole genome sequence data has been generated by JGI for nearly 500 natural pennycress populations worldwide and will be made publicly available. The corresponding germplasm is being deposited in and curated by the Arabidopsis Biological Resource Center (ABRC) and the Nottingham Arabidopsis Stock Centre (NASC) for public dissemination.

Western Illinois University Year 3 Accomplishments

- Win Phippen (wb-philpen@wiu.edu) & Tad Wesley, Western Illinois University (tl-wesley@wiu.edu)
  
  - **Multi-State Variety Trials**
    
    Completed eight location grow out in Illinois, Ohio, Wisconsin, and Minnesota of top 10 breeding lines, including three commercial varieties from CoverCress. All three commercial Lines, B3WG, B28WG, and B48WG outperformed all lines at all locations.

  - **Breeding Line Trials**
    
    Thirty winter and 27 spring varieties were replicated in field plots. Evaluations were done throughout the growing season to determine germination rate, rosette size, date of first flower, pod height, plant height, maturity date, yield, oil content, and seed characteristics. The top performers were tt8-t/ARV1, ARV2032, and Eliza-338-1 yielding over 1,900 lbs. per acre.

  - **Bulk Seed Increase**
    
    We planted seed increase plots for tt8-t/ARV1 and MN106+fae1/rod1/ind1-1. WIU increased 1,500 lbs. of tt8-t/ARV1 to supply the agronomic and supply chain studies and 4H/FFA experiments for next year. Bulk harvest of MN106+fae1/rod1/ind1-1 yielded 1,800 lbs.

  - **EMS Trial**
We evaluated 10 EMS varieties from the previous year with improved seed yield, thick stems, and early maturity, including a tan seed variety and condensed floral stem.

- **Controlled Crosses**
  Conducted controlled crosses in the greenhouse for the introgression of the tt8 gene into selected wild lines to increase seed size and rosette diameter.

- **Explanation of Variance**
  Laboratory activities and progress are on track. COVID, while causing delays and canceled in-person meetings, was primarily a minor to moderate nuisance.

- **Plans for Year 4**
  In Year 4, we will continue work on the aims stated above, along with exploring genetic solutions for improving seed germination, stand establishment, early maturity, and harvestable yields.

![Photo 3. WIU harvest team getting ready to begin 2022 harvest season at Macomb, IL](image)

**OBJECTIVE 3.3 – PENNYCRESS ECOSYSTEM SERVICES**

- **PI: William Perry, Illinois State University,** ([wlperry@ilstu.edu](mailto:wlperry@ilstu.edu))
- **Collaborators:** Jason Bond (Southern Illinois University), Rob Rhykerd (Illinois State University), Frank Forcella
- **Graduate Student:** Ryan Meyers (Illinois State University)
Soybean Cyst Nematode (SCN)

- Completed first microplot in 2021 (25.4 cm diam pots containing 15 kg of infested field soil set into depressions in the ground), which revealed that while pennycress allowed SCN invasion and reproduction, the number of eggs per cyst were much lower than observed on hosts like soybean.

- The microplot experiment was repeated in 2022, and SCN population densities are being determined. Two treatments are still ongoing, soybean planting into fallow soil and soybean planting into soil with pennycress in fall and spring.

- A field experiment at Belleville, IL, was planted to soybean following pennycress termination, and SCN densities are being determined amongst three planting dates.

Pollinators

- We submitted a manuscript describing bee abundance and diversity associated with flowering crops of black-seeded pennycress (‘MN106’). The research was performed at four sites: two in Illinois and two in Minnesota.

- We wrote the first draft of the manuscript describing fly abundance and diversity associated with flowering crops of black-seeded pennycress (‘MN106’). The research was performed at four sites: two in Illinois and two in Minnesota.

- We initiated comparisons of insect (pollinator) abundance and diversity between black-seeded pennycress (‘MN106’) and newly bred golden-seeded pennycress (‘tt8’). The research was performed at three sites: one in Illinois and two in Minnesota.
Photo 4. Native honeybees on pennycress

Figure 8. Flowering dynamics of wild type and golden pennycress.
Carbon Sequestration

- Carbon accumulation in the top 3 cm is increasing significantly after three years under pennycress and is comparable to Pea Clover Radish Oat and half of Rye (See Figure 10).

- We collected soil cores in the fall and spring – fall has been processed, and spring is being completed now

- We applied for additional funding from the Illinois Nutrient Research and Education Council.

- We added a golden pennycress treatment.
Cover Crop Potential

- Crops of golden pennycress and wild-type black seed from prior crops formed the first fall germinated and robust spring crops.

- Documented the full potential of pennycress as a cover crop in:
  - Reducing weed biomass which was significant and over 50%
  - Immobilizing nitrate in soils by nearly >45% and soil porewater by >75% while not affecting ammonia or phosphorus.
  - Reducing export of subsurface drainage water by >75% and dissolved nitrates by 50%
  - Completing the pennycress mineralization study. Pennycress decomposes slower than both annual and cereal rye.
Critical Issues to be Resolved Prior to a Pennycress Commercial Launch

We have addressed several critical issues in years 2 and 3 and anticipate continuing to address them in Year 4.

- **SCN**
  - How do planting dates of pennycress and the planting dates of the following soybean crop influence SCN densities?
In each cyst, a large percentage of the eggs are dormant and will not hatch until future years. If not yet addressed, the amount of dormancy in cysts produced on pennycress vs. soybean should be determined.

- **Pollinators**
  o Do new genetic lines of pennycress (e.g., CoverCress™) affect the timing of pennycress flowering and attractiveness to insect pollinators? To answer this question, in 2022, side-by-side comparisons in small fields or plots were made of (a) flower densities and (b) abundance and diversity of pollinating insects at three sites using the traditional (wild-type) pennycress variety (‘MN106’), which is black-seeded, and the recently developed golden-seeded line, ‘tt8’. These comparisons were made across the breadth of the bloom period at each site. We hypothesized that the chemicals that determine seed color could be present earlier in flowers (including nectar and pollen) and influence pollinator preferences and visitation. This research will continue in 2023.
  o Although bees are probably the most important pollinators of pennycress, flies (Diptera) are the most commonly observed group of insects on pennycress flowers. Unfortunately, Diptera are notoriously difficult to identify to the level of species. Fortunately, our team was able to recruit an entomologist who specializes in Dipteran taxonomy. Dr. Matt Peterson is currently identifying all flies captured in 2020 and 2021 and should have preliminary species lists available for each of the four pennycress research sites by late July 2022.
  o Does the presence of pennycress flowers (i.e., pollen and nectar) in early spring benefit honeybees? We investigated this possibility in 2021 in collaboration with two commercial beekeepers. Our goal was to repeat and slightly revise the experimental protocol in 2022, but extremely abnormal weather conditions prevented our collaborating beekeepers from returning their hives to Minnesota from overwintering sites in southern states until after pennycress flowered. We will attempt to perform the relevant experiments again in 2023.

- **Carbon Sequestration**
  o We established uniform and robust stands of pennycress.
  o We are dealing with herbicide resistance.
  o We are finishing soil sampling and analyses.

- **Cover Crop Potential**
o Establishing uniform and robust stands of fall germinated golden pennycress and harvesting in time to plant cash crops.

o Sensitivity to herbicide use in nearby plots causes issues with weed control.

o Having enough precipitation in spring to sample lysimeters and subsurface drainage and fine-tune sampling.

o Maintaining functional equipment for subsurface drainage.

- Year 4. Planned Activities, Outcomes, and Impacts

  o SCN

    ♦ Complete our second season of microplot research and publish the results from the first two years.

    ♦ Establish field sites at Belleville and Carbondale with golden seed pennycress. For the first two years, the black seeded cultivar struggled and did not create the dense stand we need when monitoring the population densities of such a variable soilborne pathogen.

  o Pollinators

    ♦ In 2023, collect the second year of data comparing black- and golden-seeded pennycress attractiveness to pollinators.

    ♦ In 2023, collect the second year of data comparing honeybee hive strength for hives close to and distant from flowering pennycress fields during spring.

    ♦ Publish a manuscript pertaining to bee diversity and abundance associated with pennycress flowers. Complete the final draft of the manuscript pertaining to fly diversity and abundance associated with pennycress flowers.

  o Carbon Sequestration

    ♦ Conduct an additional year of soil analyses.

    ♦ Begin a manuscript documenting changes after three years.

    ♦ Work to obtain more funding.

  o Cover Crop Potential
Complete manuscripts on nutrient immobilization and plan new experiments based on meeting feedback

Monitor pennycress establishment and effects on nutrients and weeds but emphasize collecting data on subsurface drainage.

Change the N amendment to a rye cover crop, possibly allowing for a direct comparison of pennycress to rye cover crops.

**OBJECTIVE 3.4 – SUPPLY CHAIN DEVELOPMENT**

- **Objective Leader** Matt Luetje, CoverCress, Inc., [mluetje@covercress.com](mailto:mluetje@covercress.com)

- **Collaborators:** Constance (Connie) Carlson (University of Minnesota), Colin Cureton (University of Minnesota), Alexandra Diemer (AURI), Alan Doerning (AURI), Riley Gordon (AURI), Gregg Johnson (University of Minnesota), Matthew Leiphon (AURI), Michaela McGinn (Genetic & Regulatory Consultant), Bill Perry (Illinois State University), Rob Rhykerd (Illinois State University), John C. Sedbrook (Illinois State University), Tim Smith (University of Minnesota), Michael Sparby (AURI), Harold Stanislawski (AURI), Michael Stutelberg (AURI), Jennifer Wagner-Lehr (AURI), Samantha S. Wells (University of Minnesota), Luca Zullo (AURI)

- **Graduate Student:** Kathryn Phillips (University of Minnesota).

**Year 3 Planned Activities**

- The Supply Chain Objective had planned the following accomplishments:
  - Provide Golden Grain Pennycress to Agronomy and Breeding as projects shift to growing pennycress varieties with **improved genetics**.
  - Conduct additional economic modeling.

- Provide grain and oil samples for commercial testing with industry and academic collaborators.

- AURI and CoverCress (CCI) will continue to perform long-term storage and treatment studies.

- CCI will participate in ongoing poultry feed trials by feeding CoverCress™ to broiler chickens to determine if and what grain treatments are necessary for inclusion in feed.

- CCI will secure additional IPREFER Supply Chain funding designated for Year 3 support.
• CCI will conduct the first large-scale grain harvest, including logistics and quality processing.

• CCI will secure strategic business agreements with critical supply chain partners to demonstrate viable commercial pathways.

• CCI will collaborate with a strategic partner to execute a pilot-scale processing trial to determine optimal recommendations for full-scale commercial production.

Year 3 Significant Accomplishments

• CCI successfully raised $26 million through its Series C-1 investment round.

• CCI announced a farm-to-fuel agreement with major value-chain partners Bunge and Chevron.

• CCI executed the largest CoverCress™/pennycress grain harvest in history during Spring 2022.

• CCI secured IPREFER Yr.3 Supply Chain funding to support critical operational steps associated with large-scale grain processing.

• CCI partnered with a large poultry integrator to confirm safety and palatability in broiler chickens with a 4% inclusion rate.

• CCI continued to raise significant crop awareness via multiple industry conferences, media appearances, and stakeholder engagements.

• ISU provided seed to a company that assessed the anaerobic digestion of crushed and uncrushed seeds. Their studies found that a crushed yellow seeded variety performed significantly better than crushed wild-type seeds and uncrushed WT and yellow seeds. Additional experiments are planned with oil-extracted meal samples.

• ISU provided wild-type erucic and low-erucic seed oil to a university collaborator who performed friction and wear experiments at a range of temperatures compared to that of oil from canola, rapeseed, and other oilseeds. The pennycress-derived oils performed comparably well, so a company will now assess the performance of green grease they will produce with the pennycress oil.

• UMN/Pennycress LCA: Kathryn Phillips and Tim Smith are developing a pennycress baseline model for the agriculture stage in GREET. Work is ongoing to refine the baseline model and spatialize the rotation data.
• AURI’s **business development** meetings include a Minneapolis-based oilseed processor, a global animal/pet care company, Millborn Seeds (S. Dakota), and the Regenerative Ag Alliance to discuss new opportunities for pennycress oilseed projects.

• AURI’s long-term oil **stability study** 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.

**Year 4 Planned Activities, Impacts, and Outcomes**

• CCI will engage with industry stakeholders and regulatory agencies to secure **final approvals** for the commercialization of CoverCress™

• CCI will complete the build out of its digital **agronomic and business systems** to provide a pathway for commercial

• CCI will increase **growth targets** for participating farmers, commercial acres and yield expectations

• AURI and CoverCress will continue to perform **long-term studies** for storage, stability and treatment of seed, grain, oil and meal

• Supply chain team will continue to share **best practices** for developing pathways and enablers for the commercialization of pennycress and CoverCress.

**OBJECTIVES 3.5 AND 6 – EDUCATION, EXTENSION, AND OUTREACH**

**PIs:**

■ Rebekka Darner, Illinois State University, [rldarne@ilstu.edu](mailto:rldarne@ilstu.edu)

■ Willy Hunter, Illinois State University, [wjhunte@ilstu.edu](mailto:wjhunte@ilstu.edu)

■ **Collaborators:** Chris Aulbach (CoverCress), Katie Black (University of Minnesota), Mary Brakke (University of Minnesota), Matthew Hagaman (Illinois State University), Nicholas Heller (Illinois State University), Frances Lawrenz (University of Minnesota), Alex Lindsey (Ohio State University), Emily Schoenfelder (University of Illinois), Samantha S. Wells

■ **Graduate Students:** Aaron Hauger (University of Minnesota)
Undergraduate Interns: Taylor Irvin (Illinois State University), Layla Jones (Illinois State University), Karyn Rader (Illinois State University)

Year 3 Goals

- Complete national 4-H adoption of the *Cover Crop Science* project book
- Engage 10-20 more 4-H clubs in hands-on activities and invite them to submit *Cover Crop Science* projects to 2022 4-H fairs
- Host three or more Cover Crop Science SPecial INterest (SPIN) Clubs
- Host Cohort #3 of the IPREFER IPS-URE program
- Conduct hands-on activities at the 2021 Illinois State Fair’s STEM Playground
- Collaborate with IPREFER leaders to make field day events family-friendly
- Host hands-on workshops at two or more conferences for educators or professional symposia

Year 3 Accomplishments

- Illinois adoption is complete, and national adoption has begun and will be completed in the fall of 2022.
- 77 youth across Illinois showed cover crop projects in county fairs in 2022.
- A table was hosted at the 2021 Illinois State Fair’s STEM Playground, which was an opportunity to introduce pennycress to a statewide audience, including state legislators.
- In the fall of 2021, Extension educators from across Illinois were trained on the *Cover Crop Science* project book.
- A former IPREFER intern, mentored by A. Lindsey, published a staging guide in *Crop, Forage, and Turfgrass Management*.
- Winter virtual meetings were held from February through March, reaching 240 farmers.
- Field days held throughout central Illinois and Missouri reached over 80 farmers.
- The staging guide was converted to a ppt file for classroom use.
• The third cohort of IPREFER IPS-URE program was hosted across the project, with eight interns, 3 of which conducted educational research with the EEO team.
Research conducted within the context of the IPREFER internship fosters an understanding of how interdisciplinary competence develops during mentored research experiences.

Year 4 Planned Activities, Outcomes, and Impacts

- National 4-H adoption of the Cover Crop Science project book will be obtained in the fall of 2022.
- A Youth Field Day, entitled “Engaging Youth in Sustainable Cropping System Development,” will be offered in September 2022.
- We will host additional field days in 2023.
- We will revise the field day evaluation form to gather data about more specific learning outcomes from field days.
- We will host the fourth cohort of the IPREFER IPS-URE program.
- We will host another table at the 2022 Illinois State Fair.
- We will publish implementation guides for the Cover Crop Science project book for both formal and informal learning spaces.
Our mission is to optimize off-season pennycress oilseed production by overcoming production and supply chain bottlenecks.