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NOTICE

This IPREFER project quarterly report was prepared by Western Illinois University and IPREFER research colleagues from CoverCress, Inc., Illinois State University, McLean County Soil and Water Conservation District, Southern Illinois University, The Ohio State University, United States Department of Agriculture-Agricultural Research Service, University of Illinois, University of Minnesota, and the University of Wisconsin-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”).

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PROJECT ADMINISTRATION AND GOVERNANCE

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

1. Planned Activities

- Conduct a virtual second annual meeting on August 3-4, 2020. We will highlight Year 1 accomplishments and organize Year 2 activities.

- Improve the project website (www.iprefercap.org), and grow the Twitter account (@IPREFER_CAP) and YouTube Channel (https://www.youtube.com/channel/UClIn60frdoFUyXGF1pVH05Q?view_as=public)

- Organize bi-annual research meetings for each objective team during the winter 2021 months.

- Organize and post a public and team (private) web-based project calendar indicating when all teams are meeting, as well as significant project dates.

- Work more closely with corporate partner CoverCress to better understand the required commercialization needs and how to integrate these needs into the university research setting.

- Oversee implementation of a data management plan. We want to avoid so-called “data dumps” to the academic and commercial communities and the general public.

- Complete first annual report to NIFA and ReePORT.

2. Actual Accomplishments

- We held a virtual annual meeting on August 3-4, 2020. We had excellent participation from the Advisory Board, project collaborators, and 2020 Integrated Plant Systems – Undergraduate Research Experience students.

- We completed the annual meeting evaluations and provided feedback to organizers, collaborators, and Advisory Board members (See Exhibit A). We revised and improved
the project website, attracted new followers to the Twitter account (+33% in Yr. 2, Q1), and added additional videos to the YouTube Channel (10 videos added during Q2).

- We closed out the summer 2020 summer interns and posted student projects to the website (See: https://www.iprefercap.org/about/undergraduate-research-internship/).
- We will deploy the project calendar in January 2021.
- We continued communications with CoverCress regarding commercialization priorities.
- We distributed finalized Material Transfer Agreements and Non-Disclosure Agreements with all collaborating partners.
- We distributed the Year 1 report to Advisory Board and NIFA officials and completed the USDA-NIFA ReePORT.

3. Explanation of Variance

Project participants continue to operate under various COVID-19 restrictions. Support for undergraduate student projects and independent studies has been restricted. However, we have met the majority of project milestones (See reports from each project objective throughout this report).

4. Plans for Next Quarter

- We will post the 2021 call for summer IPREFER 2021 Integrated Plant Systems – Undergraduate Research Experience interns on the project website.
- We will finalize protocols for data management deposits to UMN data banks.
- We will finalize an agreement with Purdue University to conduct land-use change assessments for Carbon Intensity scoring.

5. Publications, Presentations, and Proposals Submitted

- Education Presentations.
  - Classroom guest lecturer in AGRI-120 Agriculture in Today’s Society (WIU) regarding pennycress production and the IPREFER project. 50 undergraduate students, 50 min lectures to 2 sections of the class. Oct. 14, 2020. Included farm tour of research plots.
  - WIU School of Agriculture open house demonstration and presentation on pennycress and the IPREFER project to 20 potential incoming undergraduate students and their
parents. 2 - 45 min presentations on Oct. 9, 2020. Included demonstration of new plot combine and seed handling.

**OBJECTIVE 3.1 - AGRONOMIC MANAGEMENT**

1. **Yield Trade-off Leveraging Corn Relative Maturity and Desiccants (Objective 3.1.1)**

   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 through evaluating a range of corn CRM by assessing varieties differing in relative maturity in Ohio, Minnesota, and Illinois during Project Years 1 – 3.

   **A. Planned Activities**

   - **Minnesota (USDA and RROC)**
     - Collect 1-m biomass samples for all corn hybrids.
     - Harvest corn grain (except silage treatment) for determining grain moisture, yield, and test weight.
     - Plant pennycress.

   - **Illinois State University**
     - Collect 1-m biomass samples for all corn hybrids.
     - Harvest corn grain (except silage treatment) for determining grain moisture, yield, and test weight.
     - Plant pennycress.

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1 Russ Gesch (USDA-ARS) leads the CRM team.
B. Actual Accomplishments

- **Minnesota (USDA and RROC).**
  - Collected 1-m biomass samples for all corn hybrids.
  - Harvested corn grain (except silage treatment) for determining grain moisture, yield, and test weight.
  - Planted pennycress.

- **Illinois State University.**
  - Collected 1-m biomass samples for 90-day hybrid used in the study.
  - Harvested corn grain from all treatments (except silage treatment) for determining grain moisture, yield, and test weight.
  - Planted pennycress.

C. Explanation of Variance

- **Minnesota (USDA and RROC).** None noted at both sites.

- **Illinois State University:** None noted.

D. Plans for Next Quarter

- **Minnesota (USDA and RROC)**
  Determine pennycress plant stands (two 1-m of row counting area) and percent canopy cover (using Canopeo) at or before winter freeze.

- **Illinois (Illinois State University)**
  Determine pennycress plant stands (two 1-m of row counting area) and percent canopy cover (using Canopeo) at or before winter freeze.

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

An increasing number of farms are now practicing no-tillage and other conservation tillage farming due to ecological and economic benefits. Establishing pennycress in no-till fields is a struggle, due primarily to the sheer amount of corn residue remaining after harvest. Several

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2 DISC is the abbreviation we use for the IPREFER corn stover management project. Nicholas Heller (University of Minnesota) leads this IPREFER objective.
corn residue treatments will test the hypothesis that the residue can be sized small enough to not interfere with pennycress establishment. Pennycress establishment and subsequent seed yield in high-residue environments will be compared to reduced-surface stover treatments where its production has been proven to be successful (e.g., silage corn removal and prepared seedbeds).

A. Planned Activities

- **Minnesota (USDA and RROC)**
  - Collect 1-m biomass samples for 90 d hybrid used in study.
  - Harvest corn grain from all treatments (except silage treatment) for determining grain moisture, yield, and test weight.
  - Plant pennycress.

- **Illinois State University**
  - Collect 1-m biomass samples for 90 d hybrid used in study.
  - Harvest corn grain from all treatments (except silage treatment) for determining grain moisture, yield, and test weight.
  - Plant pennycress.

B. Actual Accomplishments

- **Minnesota (USDA and RROC).**
  - Collected 1-m biomass samples for 90 d hybrid used in study.
  - Harvested corn grain from all treatments (except silage treatment) for determining grain moisture, yield, and test weight.
  - Planted pennycress.

- **Illinois State University**.
  - Collected 1-m biomass samples for 90 d hybrid used in study.
  - Harvested corn grain from all treatments (except silage treatment) for determining grain moisture, yield, and test weight.
  - Planted pennycress.
C. Explanation of Variance

- **Minnesota (USDA and RROC).** None noted at either site.
- **Illinois.** None noted.

D. Plans for Next Quarter

- **Minnesota (USDA and RROC).** Determine pennycress plant stands (two 1-m of row counting area) and percent canopy cover (using Canopeo) at or before winter freeze.
- **Illinois.** Determine pennycress plant stands (two 1-m of row counting area) and percent canopy cover (using Canopeo) at or before winter freeze.

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

   The use of seed coating and fungicide treatments in concert with certain strategies may improve establishment, especially if planted in fall when rain can be sporadic. In other species like *Brassica juncea*, hydro-priming (controlled pre-planting hydration) improves germination even in water-stressed environments. Another issue that may impact pennycress production is crown disease. Deterioration of the crown may impact maturation, induce lodging, and hamper mechanical harvest. Preventing infection of a pathogen causing crown rot or other seedling pathogens in the fall through the use of seed treatment may improve establishment, crown integrity and minimize losses at harvest due to lodging. A field trial will be established in Ohio, Illinois, and Minnesota to determine the impact of seed treatments on fall establishment, spring crown integrity, and lodging.

   **A. Planned Activities**

   - **Minnesota (USDA and RROC).** None.
   - **Illinois.** None.
   - **Ohio State University.**
     - Obtain the necessary equipment to facilitate research activities.
     - Plant agronomy trials with pennycress from Summer 2020.

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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.
o Begin seed dormancy and pelleting work.

o Begin a plant breeding trial: Fall 2020.

B. Actual Accomplishments

• Ohio State University.
  o Added a scientist specializing in biopolymers to Nasib’s Koirala MS research committee.
  o Obtained additional research and travel exemption to conduct planned agronomic trials for 2020-2021.
  o Collected final biomass and corn yield data for CRM and DISK. Grain samples were shipped to Russ Gesch at USDA for corn C and N content (grain only).
  o Expanded the pelleting trial work (establishing an SOP, working with different binders, etc.).
  o Planted CRM and DISK pennycress (stand assessment will be done Nov. 19, 2020).
  o Planted a breeding trial in September 2020.

C. Explanation of Variance

• Minnesota and Illinois. For the Minnesota and Illinois sites, all field experiments are postponed until next year.

• Illinois. None noted.

• Ohio State University. None noted.

D. Plans for Next Quarter

• Minnesota. If necessary, obtain experimental seeds from Ohio and run control-environment studies to ID best treatments for 2021 field study.

• Illinois. Contribute to planning 2021 experiment.

• Ohio State University.
  o We will finalize standard operating procedures for seed pelletingizing.
- We will assess pennycress stand in the breeding trial, CRM, and DISK.
- We will begin controlled environment pelleting assessments.

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

Where herbicides are used as the primary weed management tool, there will be herbicide residues in the soil that may impact the successful establishment of pennycress similar to the impact that herbicide residues may have on the establishment of more traditional winter cover crops. We will evaluate commercial pennycress varieties for their tolerance to likely concentrations of common corn herbicides that will be found in the soils of pennycress fields. Identifying herbicides to which pennycress is less sensitive will allow us to develop herbicide recommendations for the preceding crop that will decrease the likelihood of pennycress injury or death during its establishment. We will test the effects of common corn herbicides used to control summer annual weeds and quantify pennycress population density and biomass in the fall, survival of plants the following spring, and pennycress seed yield.

A. Western Illinois University

- Planned Activities

  None to report this quarter

5. Monitor Soybean Cyst Nematode (*Heterodera Glycines*, SCN) in the cropping rotation (“SCN”) (Objective 3.1.5)

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

A. Minnesota

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4 Mark Bernards (Western Illinois University) leads the WEEDS Team.
5 Cody Hoerning and Senyu Chen (University of Minnesota) lead the SCN Team.

Quarterly Progress Report: August 2020 – October 2020
• Planned Activities

Proceed with planting and experiment development at four sites—two in Minnesota (Lamberton and Rosemount) and two in Illinois (Belleville and Carbondale). During this period, the plan is to soil sample sites, plant pennycress treatments, and assess the agronomic characteristics of pennycress stands.

• Actual Accomplishments

The Minnesota sites were planted with the three planting dates prescribed as treatments in the study. All soil SCN samples and agronomic measurements were completed for these experiments.

Logistical concerns regarding labor availability and COVID-19 concerns have delayed set up at the Carbondale and Belleville, IL sites. The experiment will begin at those sites in 2021. Experimentation with planting methods was completed to ready equipment for the 2021 start at these locations. In addition, microplots were set up to examine fall reproduction and review sampling methodology.

• Explanation of Variance

Labor concerns from COVID-19 and low initial SCN counts in Illinois' selected fields brought about a team decision to delay implementing the full research experiments at these sites until 2021.

Plans for Next Quarter

The data collected this fall will be analyzed. Protocols and experimental data collection needs will be assessed for the upcoming 2021 field season.

• Publications, Presentations, and Proposals Submitted

  o Presentations


  o Publications. None at this time.

  o Proposals
Minnesota Department of Agriculture AGRI Crop Research Grant. **Proposal Title:** Investigating Pest Risks in the Emerging Cash Cover Crop Pennycress (Thlaspi arvense). Cody Hoerning, Co-PI’s Kathryn Bushley, and Robert Koch.

6. **Contribute to the identification and development of soybean varieties specifically adapted to pennycress inter-cropping systems ("SELECT") (Objective 3.1.6)**

Minimizing yield trade-offs between pennycress and soybean in a relay cropping system is paramount. Recent research detected sizeable differences in light transmittance-related properties between pennycress crop canopies. This indicates that both canopies represent different stress environments for the relay-planted soybean, potentially requiring different soybean cultivars to be relay-planted. Further, the good performance of the relay-planted soybean cultivars suggests a stronger competitive ability that might 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 with the bulk planting of pennycress ‘MN106’. A large number of soybean cultivars are slated for relay cropping into the pennycress spring of 2020 with the expressed goal of revealing large variations in soybean yield response to relay-planting. A large number of cultivars will, therefore, increase chances to identify uniquely superior cultivars. In addition, such group size will maximize the chances of identifying superior cultivars.

**A. Minnesota (RROC)**

- **Planned Activities**
  - Seedbed preparation for SELECT’s second season.
  - Plant pennycress w/Interseeder using 30” skip-rows.
  - Collect data on soybean plots for maturity date, plant height, lodging, yield, and plant samples for yield components.
  - Use unmanned aerial vehicle flown to capture images for final canopy coverage estimates on plots.

- **Actual Accomplishments**
  - Seedbed prepared for SELECT’s second season.

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6 Aaron Lorenz (University of Minnesota) leads the SELECT Team.

**Quarterly Progress Report:** August 2020 – October 2020
- Planted pennycress w/Interseeder using 30” skip-rows.
- Collected data on soybean plots for maturity date, plant height, lodging, yield, and plant samples for yield components.
- Used unmanned aerial vehicle was flown to capture images for final canopy coverage estimates on plots.
- Overall, soybean lines were significantly shorter when grown under pennycress. We will be focusing on lines that had minimal shortening.

![Figure 1. 2020 Intercropping Soybean Height.](image)

- Maturity date data were collected on all 240 plots. The average maturity date of the pennycress treatment was significantly later than the control treatment, although the difference was very small.
A worry was that plants would be more etiolated prior to the harvest of pennycress and thus might be more prone to lodging. This was not the case, with no significant differences in lodging seen when comparing treatments.

Overall, there were significant reductions in yield observed with the pennycress treatment, but there were interesting exceptions with a few lines having larger average yields when intercropped. We will focus on explaining these differences for the next quarterly report.
**Explanation of Variance.** None noted.

**Plans for Next Quarter**

- Assess pennycress stand establishment prior to freeze up.
- Process plant samples to study changes in soybean yield components under pennycress treatment and the variation among different soybean genotypes. Anecdotally, the first few internode lengths were twice as long when a given line was grown with pennycress. We will quantify this difference with image processing.
Process UAS imagery to quantify canopy coverage.

Continue to analyze field trial data (yield, ht, etc.)

7. Publications, Presentations and Proposals Submitted (Agronomic Management Objective)

- Professional Conferences /Meetings


- Proposals

  The USDA-ARS Innovation Fund funded a grant proposal to study the effects of pennycress and winter camelina on nodulation and nitrogen fixation in soybean was funded ($25,000), September 3, 2020.

OBJECTIVE 3.2 - BREEDING AND GENOMICS – PENNYCRESS IMPROVEMENT

1. Illinois State University

   A. Planned Activities

   Our research teams have generated high-yielding pennycress breeding lines through multi-state testing and identified/validated trait-improving mutations and natural variants. These were our planned activities for the first quarter (Yr. 2):
• 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 a commercial launch.

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

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

B. Actual Accomplishments

• We continue to stack and phenotypically evaluate various mutant combinations conferring the core domestication traits (low erucic, low fiber, low glucosinolate) and value-added traits that will improve the economics of pennycress as a crop. We have found that mutations conferring the reduced glucosinolate trait are causing growth phenotypes when the plants are grown in laboratory conditions, which are not being observed in the field, which highlights the importance of field evaluations. Given some uncertainties with many of our glucosinolate mutants' robustness, we continue to explore new gene targets, having found a new target that meaningfully reduces glucosinolate content with the plants showing no obvious growth phenotypes.

• We planted numerous mutants, mutant combinations, and wild-type controls in replicated field plots and are gathering phenotypic data to complement and compare to growth chamber data. Mutations-based traits and trait combinations that were planted and are being evaluated include core domestication traits, herbicide carryover tolerance, higher total seed oil content, larger seed size, high-oleic seed oil, and better germination/stand establishment, etc.

• We continue working to find a CRISPR-based solution to reducing pod shatter by carrying out “promoter bashing” and base substitution experiments. We have found that total knockout mutations reduced pod shatter too much (the pods will not break open with a combine), so partial loss of function mutations are necessary. Transformants are in hand and under analysis.

• Project Director Win Phippen planted this season’s strip plots at the ISU Horticulture Center on Sep. 5, 2020. Those plots were evaluated for germination and stand establishment. This fall's warm temperatures have resulted in good establishment and growth, actually too good for the spring-type lines as they flowered before freezing temperatures induced dormancy, resulting in those plants ill-prepared for overwintering.
It is worth noting that spring-type is a laboratory tool for more rapid scientific progress (being that spring-type lines do not require a 21-day cold treatment to flower) and is not a trait that will be in commercial lines, so this type of damage will not be a risk commercially.

C. Explanation of Variance

Laboratory work remains reduced to about 80% of pre-COVID levels due to the need for social distancing. Students in the lab work in two shifts.

D. Plans for Next Quarter

Continue work on the aims stated above. One manuscript has been submitted this quarter, another will be submitted before the end of the year, and three more are in the works for submission in the first half of 2021.

E. Publications, Presentations and Proposals Submitted

- **Grant Proposal Awarded.**
  
  Joint Genome Institute Community Science Program (JGI CSP). Proposal Title: Pennycress - A solution for global food security, renewable energy and ecosystem benefits. **Proposers’ Names:** Ratan Chopra, M. David Marks, John Sedbrook, Katherine Frels. **Award:** Whole-genome sequencing of a total of 1,000 unique populations and lines (500 pennycress natural populations and 500 EMS mutant lines). Submitted Jun. 23, 2020; Awarded Sep. 10, 2020.

- **Patent Awarded**
  
  
  [https://patentimages.storage.googleapis.com/60/aa/e0/77abc56873dd05/US10709151.pdf](https://patentimages.storage.googleapis.com/60/aa/e0/77abc56873dd05/US10709151.pdf)

- **Presentation**
  

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7 Tim Ulmasov, John C. Sedbrook, Michael David Marks, Ratan Chopra, and Maliheh Esfahanian are all part of the IPREFER project.
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 for Year 2-Q1:

- 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 a commercial launch.
- Perform field evaluations and seed increases of lines with commercial potential.
- Develop additional genetic/genomic resources for long-term breeding program success.

B. Actual Accomplishments
• We remain on track with stacking core domestication traits into MN106 and other top breeding lines. We planted two independent triple and quadruple mutants in FY20-21 and the plots look well established. These triple and quadruple mutants contain low erucic, low glucosinolate, and reduced shatter traits.

![Field plots established during Fall 2020.](image)

Figure 7. Field plots established during Fall 2020. Report Formatting – Pics No significant differences were observed in the growth among the wild-type and stacked mutants.

• We continue to evaluate new EMS-induced mutations conferring high-priority traits, including the core domestication traits, and value-added traits, including higher total seed oil content, larger seed size, and high-oleic seed oil. We planted over 1,000 plots of newly developed EMS lines to select for variants in the Spring of 2021.

• We have started testing some of the top breeding lines and MN106 with improved traits in four Minnesota locations. All locations were planted in mid-September 2020.
We scored emergence on all pennycress plots using a 0-10 scale (0% emergence to 100%). Average emergence in IPREFER variety trials was moderate to good. The UMN lines emerged the best in these trials as they are well adapted to this environment. We also measured rosette width on IPREFER variety trials after the first frost. The plants were larger this year compared to fall 2019 as we had more growing degree days prior to the first frost.

C. Explanation of Variance

Laboratory work remains reduced to about 50% of pre-COVID levels due to the need for social distancing. Students in the lab work in shifts.

D. Plans for Next Quarter

We will continue working on the aims stated above. One manuscript will be submitted before the end of the year, and three more are in the works for submission in the first half of 2021.

E. Publications, Presentations and Proposals Submitted

- Publications
  - Chopra, Ratan, Folstad, Nicole & M. David Marks. (2020). Combined genotype and fatty-acid analysis of single small field pennycress (*Thlaspi arvense*) seeds


- **Professional Conferences /Meetings**

  None this quarter.

3. **Western Illinois University**

   **A. Planned Activities**

   - Organize experiments for Fall 2020 planting experiments.
   - Collect and distribute seed from top 10 breeding lines to collaborators.
   - Coordinate and plant ISU and WIU research plots.
   - Complete seed oil analysis of the 2020 growing season.
   - Establish 10 seed and single seed non-destructive analysis for total seed oil using pulsed NMR.

   **B. Actual Accomplishments**

   - Analyzed data from 2020 summer research projects and distributed it to colleagues.
   - Designed experiments for the Fall 2020 planting season.
   - Collected and distributed seed packets for multi-state trials to be planted in nine sites: 2 - MN, 2 - WI, 4 - IL, and 1 - OH.
   - Planted state trials and GA experiments at ISU (Normal, IL) on Sep. 6, 2020.
   - Planted all WIU research plots on Sep. 12 and Sep. 27, 2020, in Macomb, IL.
   - Experiments planted include:
     - Timing of spring-applied nitrogen trials on two breeding lines.
     - Gibberellic acid seed treat duration study on two breeding lines.
Replicated variety trials with 54 lines, breeding line seed increases for 12 lines.

Advanced selection of 30 EMS mutants for desirable traits, no-till drill seeding methods, Treflan seed emergence.

- Initiated bulk seed increases for drying and grain handling experiments at AURI.

**C. Explanation of Variance**

WIU is still under COVID-19 restrictions. Undergraduate student support for projects and conducting independent studies have been restricted.

**D. Plans for Next Quarter**

- Complete emergence and fall data collection on all field experiments in Macomb, IL.
- Meet with Southern Breeding team members to identify breeding lines for winter grow out in the greenhouse during the winter 2021 months.
- Select and germinate advanced breeding lines with traits including compact flower stem, early flowering, improved germination, and reduced seed coat fibers.
- Complete computer algorithms for running optical seed sorter to recognize seed size and shape.
- Complete computer algorithms for running optical seed sorter to recognize seed contamination.

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


**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.

**1. Planned Activities**

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8 Bill Perry (Illinois State University) leads the Characterization of Pennycress Ecosystem Services Objective.
The fall objectives center around harvesting the cash crop, ensuring a robust stand of fall germinated pennycress, and sampling soil parameters as they respond to the experimental treatments —pennycress, pennycress with nitrogen amendments, relative to the reference plots. We specifically planned to:

- Establish a robust fall germinated pennycress crop interseeded in soybeans
- Harvest soybeans and determine yields
- Sample soils for ammonia, nitrate, phosphate, organic matter percent, micronutrients
- Sample soybean cyst nematodes to determine changes in populations over time
- Sample soil porewater nutrients using lysimeters after rains
- Establish subsurface drainage samplers and begin nutrient load losses.

2. Actual Accomplishments

We have robust stands of fall germinated black seed coat pennycress established in all treatments (Figure 9) and have harvested soybeans (Figure 10). We have just finished the soybean harvest and are awaiting the analysis of the yields and observations. The harvester's yield monitor that uploaded data to FieldView suggested no significant differences in the pennycress plots relative to the reference plots (Figure 11). Pennycress interseeded in the soybean plots has established well in all plots but was delayed in the south plots, possibly due to low soil organic carbon or denser soybean stands. We will till the reference plots to a depth of 2 cm to remove the extensive weed cover in these plots and use herbicides if the weather is conducive to its use (Figure 12).
Figure 9. Pennycress established in late October in standing soybeans.

Figure 10. Harvesting soybeans with pennycress established by broadcast seeding. Note the patch of pennycress growing where the seeder did not plant soybeans due to issues with mounds of soil.
remaining after tile installation. Bottom – Using a yield monitor sending data to FieldView we will be able to estimate yields in the center acre of all plots.

Figure 11. Henbit is currently a cool-season weed that is growing in all plots and competing with pennycress. In the reference plot above, we will use a small plot to disturb the top 2 cm of soil to remove the weeds and also utilize herbicides if weather permits
Figure 12. Sampling soil fertility and soybean cyst nematodes. SCN was sampled by homogenizing 20 cores, each 6-8 inches long at 3 points in each plot. The soil fertility was estimated by homogenizing 20 cores, each 12 inches deep at 3 points in each treatment for soil fertility. In the image are Noah Price, an undergraduate volunteer, and Jack Wang, a graduate student studying soil pore water.

We have also taken 1080 soil probe samples in the plots to estimate soil fertility and soil organic carbon, and soybean cyst nematodes (Figure 13). We sampled 3 points in each plot and homogenized 20 soil cores for soil fertility and a separate set of samples for soybean cyst nematodes. The SCN samples are being analyzed at the University of Illinois Plant Lab, and the soil samples are being analyzed at United Soils, Fairbury, IL. We installed lysimeters to sample soil pore water in early November (Figure 14). We received the first rain on 2020-11-24 of over 2.5 cm, and this is the first rain event to allow soil pore water samples, which were taken on 2020-11-25 and 26. Subsequent samples will be taken after each rain until the soil freezes.
Figure 13. Installing soil pore water samplers – lysimeters in min November with Jack Wang, graduate student (right), and Noah Price, an undergraduate volunteer (left).

The subsurface drainage sampling systems of water discharge and nutrient loads are nearly complete for the reference and pennycress treatments. We have installed the ISCO automated samplers, batteries, and solar panels on the platforms next to Turkey Creek (Figure 8). We are in the process of installing the flow velocity probes this fall, which will be in place for the next several years. We have not received funding to instrument the added pennycress with nitrogen amendment plots and are working on borrowing instruments from collaborators at The Nature Conservancy. We are excited about the progress now that the samplers are in place. The delay will not be an issue as subsurface drainage takes greater than two years to respond to cover crop treatments.
Figure 14. Top - Elevated platforms with solar panels to charge batteries for the ISCO automated samplers with Matt Nugent, an undergraduate working on the carbon sequestration. Bottom – Automated sampler platform into Turkey Creek showing soybeans in the background prior to harvest.

3. Explanation of Variance

The majority of our experiments are on track for examining soil and crop responses to pennycress treatments. The addition of automated sampling of subsurface drainage and the pennycress nitrogen amendment treatments increased the time required to install that equipment and move funds to the appropriate lines.

We have added a side project examining the response of soil organic carbon – carbon sequestration – to the project to compare pennycress to cereal rye, cereal rye, tillage radish and pea, and reference treatments. A stellar undergraduate student, Matthew Nugent, funded by the Department of Agricultural Sciences at ISU, is working with Rob Rhykerd and myself. Matt has completed his first sampling to determine carbon sequestration in the soil.
profile at every 2 cm to 15 cm and from 15 to 20 cm and 20 – 25 cm. We are nearly finished with the analysis of the soil samples. This project's hypothesis is that in no-till systems with cover crops, the top layer of soil will respond faster than the deeps soil layers. Homogenized soil samples of the top 15 cm will possibly miss this response, and his project will examine this.

4. Plans for Next Quarter

The winter quarter plans are to process all samples in the laboratory, write reports, and prepare grant proposals to fund the project's remaining parts. We will prepare for the spring sampling of soils and pennycress and weed biomass.

5. Publications, Presentations and Proposals Submitted

- **Publications**
  
  None this quarter.

- **Professional Conferences /Meetings**

  None this quarter.

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

The IPREFER supply chain team held a conference call to discuss the work being conducted in the second quarter. The University of Minnesota has started specific seed drying tests and will have results by next quarter. CoverCress has offered to share their grain drying results and grain samples from larger bin drying efforts conducted about three years ago.

CoverCress is now establishing efforts to map the whole supply chain needs from the farm to the grain processor and will have updates on modeling and costs by the third quarter.

We are forming a broader supply chain team with other interested individuals in the IPREFER group. The full list of individuals and meeting schedules will be shared next quarter, along with the progress made.

1. **Planned Activities**

- Team meeting to finalize Carbon Intensity project needs.
2. Actual Accomplishments

- We scoped, signed, and started carbon evaluation projects with Purdue University on Oct. 20, 2020. We are continuing to discuss the appropriate scope of a project with Argonne National Labs (See Exhibit C).

- AURI has scoped grain cleaning, drying, and storing for the 2021 harvest, as well as small crush batches on the same harvest. CoverCress agronomists, as well as four AURI personnel, are in line with sample sizes and needs.

- We presented Supply Chain plans at the October 2020 Executive Team Leadership meeting (See Figure 15).

- We scheduled monthly Supply Chain team meetings for all of 2021.

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**Supply chain team update – 10/16/2020**

**Carbon related efforts:**
- Final discussions with Purdue (Dr. Farzad) to start a multi path analysis on GTAP model.
- This is critical to get a well known researcher to show numbers on CI in pennycress, giving us better perceptions when interacting with other organizations that validate (and possibly price) feedstocks.
- Single pathway with overhead: $20k
- Three pathways with overhead: $45k
- Ongoing interactions with Argonne to use GREET model (no deadline at this time)
- We will start a more detailed work on carbon sequestration with our own data and efforts.

**Grain handling efforts:**
- WIU and CoverCress have successfully planted large grain increases for yellow grain production.
- Conversations started with AURI to have project proposals on how to work with the grain in terms of cleaning, drying, storage and crushing.
- Suggestion: “library of harvested lots”?

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Figure 15. October 2020 Supply Chain Team Update.
3. **Explanation of Variance**

   No variances were noted.

4. **Plans for Next Quarter**

   Support and verify Purdue’s carbon intensity analysis. Start the Carbon Intensity project with Argonne National Lab.

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

   None at this time.

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

1. **Planned Activities**

   - Complete drafts of 12 lessons for the 4-H Cover Crops project book.
   - Share the project book draft with 4-H staff for early review. (Submission for official state-level review will come after activities have been piloted.)
   - Pilot activities from the project book with youth through virtual STEAM workshops in 4-H programs around the state.\(^9\)
   - Pilot activities from the project book with youth through a Cover Crops SPIN Club, dependent on the resumption of in-person 4-H meetings.

   **A. Ohio State University**

   - Participate in Objective conference calls to discuss efforts.
   - Begin planning modules for implementation.

2. **Actual Accomplishments**

   - 12 of 12 lessons for the 4-H Cover Crops project book have been drafted.
   - A 4-H staff member has reviewed lessons.

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\(^9\) STEAM (Science, Technology, Engineering, Art, and Math) is the terminology that Illinois Extension was using for its online workshops.

**Quarterly Progress Report:** August 2020 – October 2020
• Promotion has begun for a 4-H SPecial INterest (SPIN) Club. Starting in January, the club will consist of two in-person meetings and five virtual sessions, allowing us to pilot lessons in a variety of formats.

A. Ohio State University

• Submitted an IPREFER project idea to Mary Brakke for the summer 2021 IPREFER Integrated Plant Systems – Undergraduate Research Experience Presentation.
• Collected images for crop staging material development (early stages).
• Discussed a collaborative plan with UMN to develop a staging guide possibly containing thermal development models.

3. Explanation of Variance

Scheduling of locations and promotional timing made it difficult to begin the SPIN Club this quarter, but we have youth excited to begin after the holidays.

4. Plans for Next Quarter

• Pilot activities from the project book through various hands-on, virtual/synchronous, and asynchronous sessions.
• Complete peer review (with subject matter experts from within IPREFER) and begin a 4-H review of the Cover Crop curriculum.
• Format the lessons into a ready-to-publish project book.
• Work with the Chicago High School for Agricultural Sciences to embed cover crop activities/research in classroom activities and school-based internships. This activity could serve as an opportunity to begin networking with Illinois FFA.

A. Ohio State University

• Begin staging guide discussion (Dec. 2020 target date).
• Support graduate research on seed pelleting.

5. Publications, Presentations, and Proposals Submitted

• Brakke, Mary and Rebekka Darner. 2020. Using undergraduate research experience to enhance interdisciplinary competency in agricultural sciences. Innovative/Learner-Centered Teaching Poster Presentation. American Association of Agricultural Educators

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

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