NE 1710 Agenda

Improving Forage and Bioenergy Crops for Better Adaptation, Resilience, and Flexibility

Meeting: 10th August half-day PM starting at 1:15 PM EDST

11th August half day PM starting at 1:15 PM EDST

Link - Microsoft Teams is the meeting link.

Day 1: https://teams.microsoft.com/l/meetup-join/19%3ameeting_NjJkYWUwYzctMDQyZi00ZjQwLWFjMjYtNTBiZGQ4MGMzM2Fm%40thread.v2/0?context=%7b%22Tid%22%3a%221c177758-4d6b-43dc-aaeb-3b9c42562967%22%2c%22Oid%22%3a%220d7dc633-9ee0-43bb-9902-b503f03cef36%22%7d

Day 2: https://teams.microsoft.com/l/meetup-join/19%3ameeting_MWY0M2Q3OTItZWMxMi00MTg1LWI5MGQtYjgxZmU1NTE2ZDlj%40thread.v2/0?context=%7b%22Tid%22%3a%221c177758-4d6b-43dc-aaeb-3b9c42562967%22%2c%22Oid%22%3a%220d7dc633-9ee0-43bb-9902-b503f03cef36%22%7d

Welcome to the meeting

Introduction of members

Eric Bishop von Wettberg, University of Vermont (Co-host); Brian Baldwin, University of Mississippi (co-host); Arvin Boe, South Dakota State University; Ginny Moore, Cornell; Ali Missaoui, University of Georgia; Gary Bergstrom, Cornell; Charlie Brummer, UC Davis; Nancy Jo Ehlke, University of Minnesota; Yousef Papadopoulos, AgriFood Canada (NS); Mike Peel, USDA ARS; Healthcliffe Riday, USDA ARS; Kathleen Glover, AgriFood Canada (NS); Brian Irish, USDA ARS; Esteban Rios, University of Florida; Rebecca Brown, University of Rhode Island; Bill Biligetu, University of Saskatchewan; Joe Robbins, USDA ARS; Stacy Bonos, Rutgers; Annie Claessens, AgriFood Canada; Emmanuel Brefo, University of Vermont (Notes); Emma Parks, University of Vermont (Notes). Noticeably absent were the members formerly employed at Noble Foundation.

Item 1: Status Reports

1.1 Alfalfa testing – Heathcliff (northern group) and Charlie (southern group).

Northern Germplasm

- Locations Cornell, Madison, Davis, Nova Scotia, Quebec.
- Selection of crossing blocks and generations of diverse populations in a northern group and a southern group.

- The main activity has been developing germplasm pools. Charlie Brummer South, Heathcliff in Wisconsin for northern.
- Seeds come from ARS- WSU or WISC. Heathcliff or Brian Irish. More Heathcliff, as he handles the northern germplasm.
- Over the past year, seeds have been sent to a number of partners in network. Quebec, Lethbridge, Florida, Davis, Georgia, Dakotas, Logan Utah.
- We are working with four populations- Central Asian, Russian, Ottoman and Siberian. Populations have been kept separate for seed increase.
- Most western sites are having severe drought. Some delays due to COVID
- Southern alfalfa has been grown at Davis and Fresno in Ca and near Gainsville in Florida. Fresno is behind due to water problems Esteban Rios, UF, has the Florida sites underway. We did not hear about the Georgia site this year
- 384 accessions of southern material suffering Fusarium in Washington in southern material, along with bacterial wilt.
- Breeding insight group using field book and trait ontologies. Breeding insight is doing genotyping – 10-15$ sample to Australia, for 1K or more snps
- Issues with getting seed into Canada reported last year persist, and are made worse by the pandemic

1.2 Fungal investigations in switchgrass

- We discussed the work of David Lowry (MSU) and Tom Jeunger (UT, and their shared postdoc, Acer). They are working with at least 10 sites along latitudinal gradient
- We discussed the need to identifying different species of fungi associated with plants.
- We continued a discussion from last year about characterizing head-smut fungus. With no known sexual cycle wondering if the disease is caused by a single clone of the fungus.
- Gary Bergstrom at Cornell is moving towards retirement – will have a smaller role in the next multi-state.
- The group is inclined to keep switchgrass in the multi-state tent.
- Kathleen glover in Canada is doing some switchgrass work, with breeding
- Gary mentioned Calvin Ernst and their conservation seeds
- Mike Casler, working on breeding switchgrass to go farther north. Adapting southern lowland switchgrass to winter hardiness. This is part of an increasing need for warm season grasses in northern systems.
- Ali M discussed switchgrass quality analysis, nutrient mobilization. We noted this as an item we want to highlight in the next proposal.
- Annie C. in Quebec is breeding for cold adaptation and biomass yield.

1.3 Developing resilient cool-season grasses adapted to variable climatic conditions.

- Locations testing – Utah – 2 locations; western Canada – 2 locations, Quebec -2 locations.
- Focal species are tall fescue, meadowbrome, timothy, and orchardgrass,
- The group is doing selections, and moving on to next cycle.
- Winter hardiness has been an issue, particularly in SK. The Saskatchewan group is looking at sugar accumulation as an indicator of cold tolerance. They are doing RNA-seq at times around cold accumulation.
- Brian Baldwin is looking at high temperature germination in the south. The 5th cycle of selection is done in MS. He is looking for heat tolerance in the segregating material. He is getting some increase in persistence when his group selects for heat tolerance. This is an improvement over the original population, where he has lost plants in the summer.
- Ali M in GA is doing some tall fescue and orchardgrass breeding, some things off to companies and other lines near final stages. He is also screening perennial ryegrass across locations in GA.

1.4 Genetic variability in BFT

- Locations Madison, Nova Scotia, Cornell, Logan.
- Coordinated by Yousef.
- For Yousef P. this is part of a bigger project in Canada. Birdsfoot trefoil from different regions were combined, in a five-location trial. They are looking at bioactive compounds that will control ruminant intestinal parasites. As part of this, they see a need to measure tannin profiles. Because of slow downs in measuring tannins in closed laboratories (due to COVID), they might need to extend this for two more years.
- Locations at Logan UT, Cornell/Ithaca NY, RI, Madison, WI, Nova Scotia were established last year. First cut freeze dried and saved for assessing tannin values. Samples currently being dried
- Rebecca Brown at URI is looking at some activity of tannins in vivo to determine if BFT tissue extracts impact nematode growth.
- Heathcliff at WI: These plots established somewhat poorly, but otherwise survived. They lost a set of samples due to freezer failure. Fortunately they have both forced-air (hay) and a freeze-dried. They do have a sample backlog due to a lab closure
- Mike Peel- saw rodent damage in Utah. They usually they only see rodents in alfalfa.
- NIR and tannins: Heathcliff has 3 different trefoil projects, and needs to update their tannin estimation technology. He is moving to an oven dried approach. The group asked whether tannins can change over time in storage? Heathcliff is working with a chemist to narrow down the tannin species that occur. Epi-catechin and different soluable and insoluable tannins, condensed tannin, etc. It was discussed whether there is some room for Canada and WI collaboration
- Is there Fusarium in the material? It has been a challenge in Vermont, elsewhere.
- Ginny Moore notes that Carol Hansen was not on call.

- Nancy Elke at UMN has been most successful at getting seed. Historical production in northern MN. They get reasonable yields.
- We discussed pollination of BFT, and whether native pollinators do better. There have been issues with leaf cutter or honeybees being effective pollinators.
  We also discussed the general low market demand for BFT. Some seed production has moved to Argentina and Uruguay. In North American there is also an issue with Canada thistle and competition with red clover
- We discussed the extent to which seed shattering is a problem.
- Some varieties may be harder to grow and seeds may be harder to find.

2.0 G X E for Tall fescue, orchardgrass, red clover

- Georgia: 14 entries of tall fescue, made by germplasm entries from Georgia, Nova Scotia. There are 2-3 years of data, then cross location analysese to be performed. Ali has received data from Kathleen Glover and Julie at Cornell.
- Orchardgrass: Mississippi generated heat-tolerant orchardgrass in hopes of increasing persistence.
- Red clover: This project never fully began. Later discussion showed some interest in it for the renewal.
- Joe Robbins- doing G*E on cool season grasses.
- For Canadian partners- projects fit mandate. They like the multi-site capability. They can't fund it from Canada easily. Our Canadian partners have asked that their names be added to the Participants Directory of the NIMSS - NE1710 web page, it would allow them to refer to it in their grant applications.

Discussions for the next 5-year multistate project period (led by Eric von Wettberg and Brian Baldwin)

We engaged in a broad ranging discussion to see where community interest resides, and then focused on a few areas for future writing.

1. Climate change (diseases not experienced before)
    - Advantage of this project is the extreme diversity of environments.
    - Resilience
    - Stability
    - Carbon sequestration
    - Pre-breeding to get to that point
    - Disease resistance ahead of climate change – new diseases

- We can leverage sites in multi-state as extreme sites given shifting climates- necessary for broad adaptation

2. Go back to more small projects? This was discussed as a need for many members of the multi-state Hatch team, with Jerusalem artichoke and other perennials as one example, and several warm season annuals as another.
   - Teff
   - Sorghum sudangrass
   - Cover crops
   - Cowpea-mungbean

   Perennials
   - Jerusalem artichoke
   - *Silphium* – gets huge in MS = 8.5 to 15 feet tall. It is used as a forage in Europe, and has been a focus at the Land Institute for its high biomass and oil content.

3. We discussed The Noble Foundation reorienting its focus, and abandoning many staff

4. We discussed that we are in the 4th year of this 5-year Hatch.
   - We have aew lead author, which we understand should be from the Northeast region
   - Coauthors are being solicited.
   - New chairs for upcoming years

5. NEXT NE1710 MEETING in conjunction with Michigan (East Lansing) is hosting 2022 NAPIA in June
   - Administrative advisor: Eric Bishop von Wettberg (U. Vermont)

6. Specific form for the renewal proposal
   a. Objectives – current, 1.1 stays; Heathcliff and Zhanyou include genomic selection?
   b. Does 1.2 –switchgrass- stay?

Day 2: Writing day. On this day we continued discussion of topics that interest us, and laid out a plan to write a renewal proposal.

1. As on the day before we discussed climate change and the need for warm season crops.
   a. Warm season annuals: Teff, Sorghum sudangrass, Cowpea-mungbean, cover crops
   b. Perennials: Jerusalem artichoke and *Silphiu,*

2. Discussion of Giant Miscanthus (*Miscanthus x gigantius*) – grows well, but is a poor forage- even goats do not like it. This species is particularly interesting to Yousef.

Rebecca Brown noted that it is considered invasive in RI, MI, depending on the species. Diploid (*M. sinesis*) and tetraploid can be invasive. *Miscanthus gigantius* is a seed sterile triploid. The genus is a ploidy series, with polyploids one can recreate. Stacy and Brian have screened it ,but are not actively breeding. Brian had been working with a breeder at U. Illinois, Erik Sacks. Brian has several thousand miscanthus funding from DOE, which is a good reason to keep it in the NE1710 renewal project. The chemical constituents that cause vitrification and fouling of xeolite catalysts when burned or gasified need more investigation. Leaf senescence, which is related to this, might be an interesting focus.

3. Writing
    a. Discussion of keeping our multi-state Hatch title the same
    b. We went through the proposal to discuss which areas to keep in a renewal.
        i. Alfalfa regionally adapted climate resilient germplasm. We keep this. Add UVM and Brian Irish. Also needs to add Esteban Rios in Florida. Drop Ardmore. Charlie and Heathcliff will lead writing this section
        ii. Switchgrass. Expand to list switchgrass and other natives. Has a definite keep. The status of Kessler was discussed. A point that came up is diseases, and finding resistances
        iii. We moved to change the old aim 1.2 to developing switchgrass and other natives for disease and pest resistance, which makes it more general, and allows it to focus on yield and biomass and other nutritional characteristcs Writers: Brian Baldwin, Stacey Bonos, Arvid Boe, Annie Claessens.
        iv. Cool season grasses: Bill Bigetu in Saskatoon, Annie Claessen in Quebec, Yousef and Kathleen in Nova Scotia. The last proposal had some germplasm exchange, and on this one we would like to bring in more sites.
        v. Birdsfoot trefoil – The last project moved more slowly than hoped, so the group wanted to continue working on it in the renewal. The next step is looking at G*E for tannin. To do this we need to have more sampling at each cut.
        vi. Miscanthus. This is a new section, with Brian Baldwin and Stacey Bonos
        vii. New species. Among those interested were Ginny Moore, Kathleen Glover, UVM, Rebecca Brown, Eric von Wettberg. Pea, hairy vetch, crimson clover (which has establishment challenges). Looking for nitrogen fixation, weed suppression, and erosion control. Around veggies in RI and VT. Also as a replacement of a perennial forage, or companion to forages to boost production in the first year. One way to phrase is as species we can use for a rapidly changing climate. Similarly, Teff and cowpea as summer pasture intersown species, or summer cover crops. In the northeast these species can get potato leaf hopper infestations.
        viii. G*E. This was the second objective of the past proposal. We agreed to keep it, as it is an important way in which this project links across multiple

states and provinces, and keeps the small forage breeding community together. We expect this to update the past proposal, but also break new ground.

c. In working on the renewal, we want to make sure in writing this that we have the forage focus, but allow work on other uses under the umbrella of forage and bioenergy crops. Species that are used as cover crops fit this bill, as they may be

4. Timeline: We need a complete proposal at the end of last year. Working backward, we would need completed section mid-September. This requires we start working soon.

Annual Report to NE 1710
Brian S. Baldwin, Jesse I. Morrison
Mississippi State University, Mississippi State MS USA

Heat tolerant annual ryegrass and orchardgrass
Brian Baldwin, Jesse Morrison, and Eric Billman

Abiotic stress tolerance and biotic stress resistance have long been targets for trait improvement in the field of plant breeding. To date, much of the target crop focus has been centered on commodity crops such as corn, soybean, wheat, and rice. However, little work has been conducted on improvement of these traits in forage grasses. This is due to a number of issues, particularly that most species are obligately outcrossing, the traits are governed by many genes at unknown loci, and are greatly affected by environmental variation. This creates major complications in successfully selecting and breeding populations of forage grasses tolerant to extreme high or low temperatures, as well as disease resistance. Recurrent phenotypic selection was used to select elite individuals of annual ryegrass (*Lolium multiflorum* Lam.) and orchardgrass (*Dactylis glomerata* L.) that expressed improved germination at high temperature. Selections were conducted within growth chambers at fixed temperature and light regimes (40/30°C, 12/12 hr, light/darkness) to eliminate environmental variation. Following three cycles of selection, we observed gains ($P < 0.001$) in selection over the base population for both species. Annual ryegrass mean cumulative germination for cycle 3 peaked at 45.8%, and orchardgrass mean cumulative germination for cycle 3 peaked at 82.67%. Further selection of annual ryegrass for freezing tolerance was also conducted. Flats of unselected germplasm were grown to the three-leaf stage, and then frozen for nine hours. Significant differences ($P < 0.05$) in freezing tolerance were observed between selected germplasm in both cycle 1 (0.076%) and cycle 2 (0.125%) over the unselected cycle 0 (0.025%). Finally, initial stages of resistance breeding work were conducted involving gray leaf spot (causal agent *Pyricularia grisea* Cke. [Sacc.]) on annual ryegrass. Isolates of the pathogen were obtained and stored for future use. It was determined that the actual pathogen species responsible was *Pyricularia oryzae* Cavara. Future work for annual ryegrass and orchardgrass germplasm that germinates at high temperatures will involve variety testing and cultivar release. Freezing tolerance and disease resistance work will require larger-scale screening methodology that was able to be conducted in this work to acquire sufficient population sizes for breeding.

PVP of 'Espresso' (PI 687202; rapidly germinating lowland switchgrass)
Brian Baldwin and J. Brett Rushing

Espresso switchgrass was evaluated for germination and seedling establishment in replicated laboratory and field experiments, which included commercially available lowland cultivars.

Laboratory Germination Testing

Populations were evaluated at the end of each growing season by screening seed from the appropriate crossing block. Seed germination was tested under laboratory conditions (Rules for Testing Seeds, AOSA, 2017) by comparing Espresso to commercial varieties grown in the same environment. For the years 2011 and 2012, three lowland [Alamo, Kanlow, Tusca

II (Miss. State germplasm; patent applied for) and EG1101] cultivars and one upland type (Trailblazer) were used as known controls for germination testing. Blocks of these four lowland cultivars were established at the H.H. Leveck Animal Research Unit at Mississippi State University in Starkville, MS in the summer of 2010 from transplants. At the end of the growing season seed was harvested from each cultivar/block for germination testing. Six replications of 100 seed of each of these four cultivar and all antecedent generations were placed into petri dishes (100 x 20 mm). The petri dishes with two layers of germination paper were wetted with 15 mls water and placed into the germination chamber under a 30ºC light (16 hr) / 20ºC dark (8 hr) temperature and light regime (Shaidaee et al. 1969). Seed were checked for radical emergence (germination) every two days for a total of 28 days. Seed was considered germinated when radical emergence was ≥2 mm and coleoptile/1st leaf was 3mm. All seedlings that germinated during this period were counted and recorded (Table B.1 and B.2). Progress of selection, and cultivar comparison was accomplished using germination percentages evaluated by PROC GLM (SAS Institute, 2009). Mean separations were made on the basis of Tukey's protected least significant difference (LSD) and differences were considered significant at the 0.05 probability level.

Field Testing

Replicated field trials were established in the spring of 2013 at five locations across Mississippi. These sites included, from north to south: Holly Springs (34º49'21.25" N, 89º25'55.09" W), Starkville (33º25'13.93" N, 88º47'24.40" W), Brooksville (33º15'38.72: N, 88º33'14.37" W), Newton (32º20'07.42" N, 89º04'56.28" W), and Poplarville 30º47'53.87" N, 89º41'26.49" W). Soils at each site are as follows: Holly Springs (Grenada fine-silty, mixed, active, thermic Oxyaquic Fraglossudalfs), Starkville (Marietta fine-loamy, siliceous, active, thermic Fluvaquentic Eutrodepts), Brooksville (Okolona fine, smectitic, thermic Oxyaquic Hapluderts), Newton (Prentiss coarse-loamy, siliceous, semiactive, thermic Glossic Fragiudults), and Poplarville (Basin coarse-loamy, siliceous, semiactive, thermic Fragiaquic Paleudults). All sites received a pre-plant burn down of glyphosate at 3.08 kg a.i. ha^{-1} prior to planting. Alamo (Ernst Conservation Seeds, Meadville, PA 16335) and Espresso switchgrass seed were sown in a randomized complete block design at 9 kg/ha pure live seed (PLS) using an ALMACO no-till drill. Plots measured 1.8 x 3.6 m. Sowing dates were as follows: Holly Springs (May 31), Starkville (May 30), Brooksville (May 24), Newton (May 24), Poplarville (May 29).

Data collection included taking seedling counts once a week following initial rainfall for three weeks at each site, which was sufficient for seedling emergence (Robocker et al., 1953). Within each plot, 4 random 0.3 m linear rows were used to determine counts (Table B.3). These rows were used consistently throughout the 3 weeks. Number of seedlings for each entry was compared for each count at each location using PROC GLM (SAS Institute, 2009). Mean separations were made on the basis of Tukey's protected least significant difference (LSD) and differences were considered significant at the 0.05 probability level.

Discussion

Laboratory Germination Testing

Germination differences were observed in 2011 and 2012 testing. For 2011, Espresso had greater germination percentages than all other released cultivars tested with a mean of 68.5% (Table B.1). Espresso's germination was followed by Kanlow, |Alamo, and EG1011

(54.8, 50.8, and 40.8%, respectively). Percentages for all entries decreased for the 2012 experiment (Table B.2). Espresso and Tusca (MSU unreleased germplasm) were statistically similar in 2012, with germination percentages of 57.0 and 53.9, respectively. The upland cultivar were the lowest of each year's testing, generating germination percentages in the single digits.

Field Testing

Differences were observed for seedling counts at all locations for the 2013 field trial (Table B.3). Espresso outperformed Alamo for each count and location, except the first count at Brooksville (Espresso 3.8 vs. Alamo 6.9) and the second (Espresso 6.8 vs. Alamo 10.9) and third (Espresso 8.3 vs. Alamo 11.9) counts at Holly Springs. Of the 15 count x location combinations, Espresso was significantly greater than Alamo in mean number of seedlings per 0.3 m row for 12 of the 15. The greater germination and establishment success of Espresso switchgrass makes it a potential perennial grass choice for the biofuel, forage, wildlife, and soil reclamation arenas.

Long-term Laboratory Germination Testing

Because seed dormancy is so highly controlled by environment during seed ripening, pre- stratification percentages can vary by year. All seed of the different test cultivars was produced at Starkville, MS. Table B.4 shows the change in pre-stratification germination percentages for identical clones composing each block/cycle. In 2010 seed was harvested too early for all cycles, but Cycle 5, and in an effort to loose minimal seed in 2011, Cycle 7 (Espresso) was harvested too early. A comparison of the most recent four year of seed collection show Espresso with 71% mean germination; Alamo with 21% mean germination and Kanlow with 13% mean germination. Espresso has a 3.4 fold greater mean 28-day germination than Alamo and a 5.68 fold greater mean germination than Kanlow.

Velocity of Germination (VOG)

Technically selection for pre-stratification germination should include all seedlings that germinated without stratification. However, by selecting the first 100 seedlings to germinate, we inadvertently selected in favor of faster germination (VOG). Data presented in Figure B.1 shows a comparison of Alamo and Espresso for their first seven days of germination. The slope of the trend line for each is an indication of VOG. The steeper the slope, the faster germination. Espresso slope equation of $y = 10.3 x - 7.7$, indicating that for every day of germination there is roughly an increase of 10% in germination. The same data for Alamo indicates roughly a 4% increase in germination per day 5.7% ($y = 4.1 x - 5.7$).

Table B.1. Statistical mean separation of 28-day pre-stratified germination for all breeding generations, three commercial lowland and one upland (Trailblazer) ecotype of switchgrass. Seed grown and harvested in fall 2011 at Starkville, MS.

Entry	Mean Germination (%)	Significant Difference
Cycle 6	90.3	A[†]
Tusca II	70.5	B
Espresso (Cycle 7)	68.5	B
Cycle 5	68.2	B
Kanlow	54.8	C
Alamo	50.8	C
EG1101	40.8	D
Cycle 3	37.0	DE
Cycle 4	32.7	DE
Cycle 1	30.0	E
Cycle 2	20.2	F
Trailblazer	7.7	G
Cycle 0	0.0	G
$LSD_{(0.05)}$ = 8.6		

[†]Means followed by the same letter are not significantly different at α=0.05

Table B.2. Statistical mean separation of 28-day pre-stratified germination for all breeding generations, three commercial lowland and one upland (Trailblazer) ecotypes of switchgrass. Seed grown and harvested in fall 2012 at Starkville, MS.

Entry	Mean Germination (%)	Significant Difference
Cycle 6	86.8	A[†]
Espresso (Cycle 7)	57.0	B
Tusca II	53.9	BC
Cycle 4	50.5	BC
Cycle 5	48.5	C
Cycle 1	39.5	D
Cycle 2	25.7	E
EG1101	25.4	E
Cycle 3	24.3	E
Kanlow	14.7	F
Alamo	5.9	G
Trailblazer	4.8	G
Cycle 0	0.0	G
$LSD_{(0.05)}$ = 8.39		

Table B.3. Mean field emergence in 2013 at five Mississippi locations, one, two and three weeks after planting. (Only Brooksville week 1; Holly Springs week 2 and 3 are NOT significantly different.)

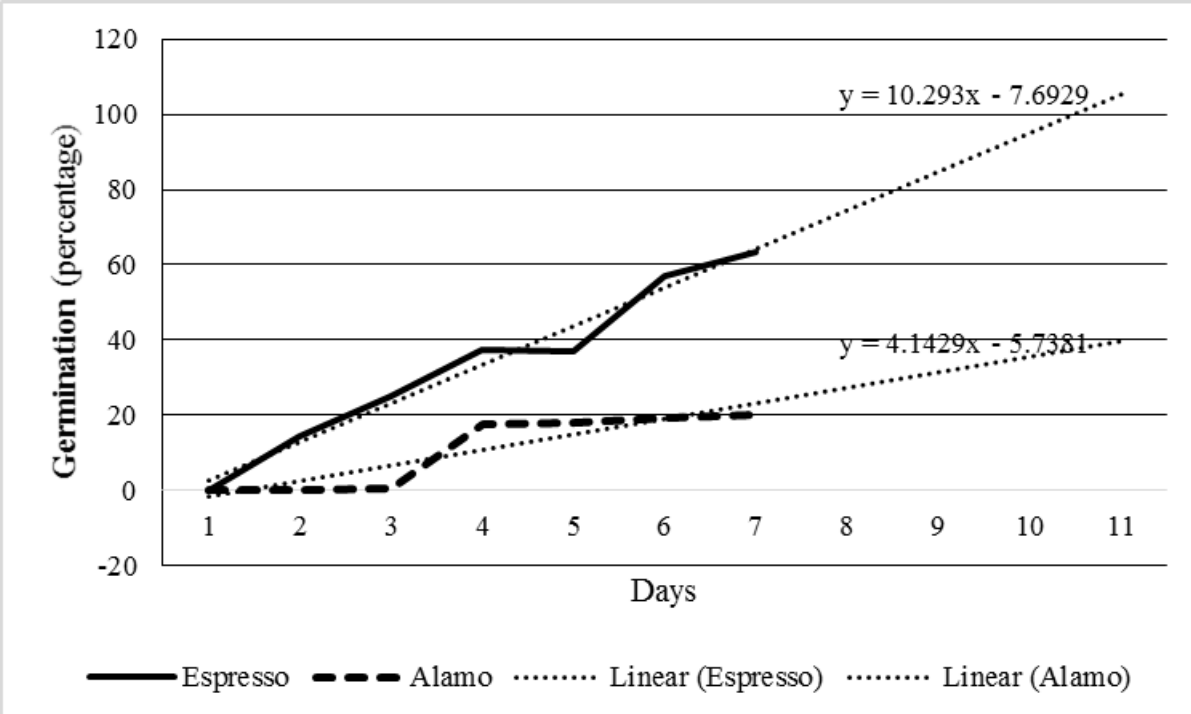
Location	Variety	Week 1	Week 2	Week 3
		Mean number of seedling linear 0.3m $^{-1}$ row		
Holly Springs	Alamo	4.00a†	6.75a	8.25a
	Espresso	8.75b	10.88a	11.88a
Starkville	Alamo	8.19a	10.13a	9.69a
	Espresso	17.06b	26.06b	20.75b
Brooksville	Alamo	3.81a	4.53a	5.34a
	Espresso	6.91 a	11.00b	11.44b
Newton	Alamo	2.00a	2.25a	1.69a
	Espresso	4.25b	5.00b	5.13b
Poplarville	Alamo	12.88a	13.31a	9.75a
	Espresso	23.06b	24.00b	20.75b

†For each data pairing at each location, numbers followed by the same letter are not significantly different at α=0.05

Table B.4 Mean 28-day germination percentages for Espresso (Cycle 7) and its antecedent generations from 2008 – 2017 as well as a direct comparison of Espresso, Alamo, and Kanlow. Seed of all entries produced at Starkville, MS.

Cycle	Year										
	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	Mean
0	0.83	5.34	0.00	0.00	0.00	0.17	3.67	0.17	0.50	2.00	1.00
1	35.50	14.66	2.34	30.00	39.50	37.33	57.67	81.00	39.33	53.83	29.94
2	34.30	45.66	0.34	20.17	25.67	55.33	50.67	83.33	42.17	81.83	41.06
3	21.17	48.34	0.66	37.00	24.33	25.83	59.17	67.50	46.33	76.00	39.72
4	60.00	50.00	1.00	32.67	57.50	14.83	81.00	81.00	69.17	81.83	50.64
5	41.17	74.00	17.34	68.17	50.50	58.17	80.33	81.00	81.33	89.17	62.10
6	66.17	65.66	4.00	90.33	86.83	62.00	67.83	71.00	62.67	62.50	64.48
7 (Espresso)	84.17	94.00	1.34	20.67	57.00	52.17	80.17	59.67	71.17	73.17	59.35
Espresso							80.17	59.67	71.17	73.17	71.04
Alamo							67.50	6.50	8.17	2.67	21.21
Kanlow							45.83	1.50	2.50	0.17	12.50

Figure B.1. Seven-day velocity of germination for Espresso and Alamo expressed a slope of cumulative germination.



Patent of the switchgrass cultivar PANIR ('Tusca'; imazapic resistant lowland switchgrass)
Brian Baldwin and J. Brett Rushing

The present invention provides for a new cultivar of switchgrass that has naturally occurring herbicide resistance. The new switchgrass cultivar has been given the experimental designation 'LL PANVI AL IR' (PanIR). The inventors have developed the improved cultivar of switchgrass having resistance to the imidazolinone herbicide imazapic (5-methyl-2-[4-methyl-5-oxo-4-(propan-2-yl)-4,5-dihydro-1H-imidazol-2-yl]pyridine-3-carboxylic acid) that can be used more efficiently as feedstock for biofuels and that allows this herbicide to be applied over the top of a mixture of the new cultivar PanIR and other imazapic-resistant native grass species. After screening approximately 364,650 individuals of the publicly-released cultivar Alamo, seventy-eight (78) individuals survived the initial screening applying 8 oz/A of imazapic. A second screening was conducted, exposing the 78 individuals to the total allowable annual rate of imazapic in a one-time application (equivalent to 14 oz/A). Fifteen (15) of the first 78 either died or were stunted and dropped from the base population (resulting in 63 individuals). This base population is assembled as a crossing block at the Mississippi State University W.B. 20 Andrews Agricultural Systems Research Facility (AKA MSU Agricultural Experiment Station). This base population and the subsequent generations were limited to derivations of the seven (7) most resistant to imazapic and were vegetatively propagated as the parents of a population of progeny that comprise the present invention. These individuals have naturally occurring resistance to imazapic arising from spontaneous mutations and are not transgenic. The inventors have assembled the novel individuals together to give rise to a new population of individuals resistant to imazapic from a switchgrass cultivar already adapted to the central and southern United States

2021Annual Report for NE1710 Multistate Project: Improving Forage and Bioenergy Crops for Better Adaptation, Resilience, and Flexibility

Michael D. Peel 2021 Progress Report

USDA-ARS, Forage and Range Research Lab, Logan, Utah 84322

Project Title: Evaluating genetic variability of bioactive components in birdsfoot trefoil as they relate to tolerance to parasitic nematodes.

Status. This was successfully established the fall of 2019 using the design provided by Yousef. It was harvested three times during the **2020** growing season, June July and August. Prior to harvesting the first plot a subsample of each plot was obtained and flash frozen in a -80 freezer. These 2020 samples have been freeze dried and are ready for grinding. In 2021 the trial has been harvested twice. Prior to the first harvest a subsample was obtained from each plot and frozen in a -80 freezer. These are currently being freeze dried by another project that is allowed in our building. Prior to each harvest I have also recorded flowering data.

I currently plan a third harvest but we are in a severe drought and our irrigation water has been shut off. A third harvest I expect will have a reduced production and may impact the longevity of the trial if no regrowth occurs following the harvest. Any thoughts?

I still have the soil samples that were collected prior to establishing the nursery. I have probably been told what were we specifically supposed to have these tested for? My copy of the project description has that information omitted.

Project title: 2021 Alfalfa Pre-Breeding Nurseries

I received the seed from Heathcliffe to plant this study. My current intent is to start the plants in the greenhouse this winter and plant next spring. Disclaimer, It appears our unit is going back into greater lockdown. If we have the moratorium on new project like the last two years who knows when it will get planted. :-/|\

Tall fescue GxE trial

Ali Missaoui (University of Georgia)

A set of 14 entries consisting of cultivars and experimental populations selected in southern and northern breeding stations were planted in 2019 at the University of Georgia, Cornell University, and Nova Scotia for evaluation of yield and persistence. The preliminary data for Cornell and Georgia are in the table below. Nova Scotia data summery is in the report provided by Kathleen Glover. Data collection will continue for at least another year before we undertake summary across years and locations to dissect the GxE aspect and stability of the entries.

Summary of Cornell 2020 data

variety	Total Yield (t/acre)
GALA1502T	2.669
Jesup Minus	2.6362
Kokanee	2.5688
KYFA 9821 / AR584TF	2.5556
Texoma Max Q II	2.5318
KYFA 1405	2.4661
JesupMaxQ	2.4542
GA29	2.3872
Lacefield MaxQ II	2.384
KYFA 9611	2.3746
GA186	2.3507
GA5	2.2557
GALA1503T	2.1996
KY 31+	1.4126
LSD	0.3
CV%	8.91

Summary of UGA 2021 data

Accession	Total Yield (kg/ha)
GALA1502T	8689.3
GALA16029	8553.6
KY31+	8409.6
BarOptima	8184.6
GALA1402	8181.4
JesupMaxQ	8086.2
GA186	7785.4
GA5	7638
KY32	7622.2
KYFA 9821 / AR584TF	7533.9
Lacefield MaxQ II	7372.9
TexomaMaxQII	7276
GALA 1503T	7191.6
CajunII	7166.9
GA29	7102.5
KYFA 1405	7061.7
KYFA 9611	6995.4

Barianne	6699.3
JesupMinus	6459.5
Kokanne	3924.7
LSD	1471.9
CV%	15.79%

NE1710: Improving Forage and Bioenergy Crops for
Better Adaptation, Resilience, and Flexibility
(April 1- 2019 –March 31 2020)

Annie Claessens, Joe Robins, Solen Rocher, Bill Biligetu

Objective 1. Developing broadly adapted, climate resilient forages for sustainable cropping systems

1.3. **Developing resilient cool-season grasses adapted to variable climatic conditions.**
Cooperating locations: AES: Cornell Univ.; South Dakota State Univ.; Univ. California, Davis; Univ. Kentucky, and Univ. Minnesota; USDA-ARS: Logan, UT [co-lead] and Madison, WI; AAFC: Québec, QC and Saskatoon, SK [co-lead].

1.3.1: Cold acclimation, Identification of candidate genes associated with non-structural carbohydrate accumulation in orchardgrass. (AAFC: Québec, QC and Saskatoon, SK USDA-ARS: Logan)

To determine water soluble carbohydrate (WSC) content, 11 genotypes of orchardgrass cultivar Killarney was cloned and a new nursery was transplanted in early spring 2019 at Saint-Augustin, Quebec and Saskatoon, SK. At each site, plots deign was a RCBD with four replications. The clones were grouped into three sampling dates of 1-wk-before, during (day temp. -2oC for more than 5 hours), and 1-wk- after a killing frost. At each sampling, clones were dug out and rinsed off the soil before the stem bases (2-3g) were collected for WSC and RNAseq analysis. The sampling dates were on September19, September 28 and October 4 2019 at Saskatoon site, and September 27, November 8 and November 13 2019 at Quebec site. The crown samples for RNAseq were collected in liquid nitrogen from the field then they were stored under -80^{o}C in the laboratory. The additional crown samples for WSC were stored in container with ice during the field sampling prior to being freeze-dried in the laboratory.

1.3.2. **Developing resilient cool-season grasses**

Plant Materials:

Meadow brome (10 entries): Armada, Border, Cache, Paddock, QBP1201, QBP1301, S9498, S9549, S9522 and Salt
Tall fescue (7 entries): Arido, Courtney, Cowgirl, Fawns, Kokanee, S9582 and Tower
Orchardgrass (9 entries): Early Artic, Killarney, Latar, Okay, S9485, SFD-9601, SFD200102, UTDG-101 and UTWH-1020.
Timothy (10 entries): AC Alliance, Aurora, Climax, QF0901, QF1701, QF1702, S9530, ST1, TM1601, TM1602.

Experimental design

Plots of meadow brome, tall fescue and orchardgrass were established at Saskatoon, Swift Current SK, Saint-Augustin and Normandin, QB, and Logan, Utah (USA), UT by transplanting 8 weeks old seedlings in 2018. The experimental design at each location was randomized complete block design with 4 replications. Plot size was 1.4 x 2m, with 4 rows containing 12 plants/row. At Swift Current, plants were transplanted in a spaced nursery at 1 m row spacing. For orchardgrass, an additional field test was carried out at Beaverlodge, Alberta. In 2019, data for all four species were collected for winter survival, plant height, forage yield, and forage nutritive value (ADF, NDF and CP).

Results

Meadow bromegrass

Winter survival: As expected, there was almost no winter kill in meadow brome in 2019. winter survival rate of meadow brome populations varied from 96% to 100% across all sites. There was no difference in winter survival rate at any of the four locations in the study.

Forage Yield: meadow bromegrass populations displayed similar dry matter yield of first cut in all locations in 2019. Meadow brome populations found to be differed for total dry matter yield only at Logan, Utah where plants were cuts for 5 times. Paddock produced total dry matter yield of 6.6 t/ha, which was the lowest among the 8 entries at Logan.

Orchardgrass

Winter survival: winter survival rate significantly ($P<0.001$) varied among the 9 orchardgrass entries in space nursery at Swift Current, SK. The population of S9485 showed the highest (86%) and the population of Latar showed the lowest (26%) winter survival rate.

Forage yield: first cut yield was significant different ($P<0.05$) at Swift Current, Normandin, Quebec and Logan, Utah. The population of S9485 and Latar showed the highest and lowest yield of first cut at Swift Current, respectively. The populations of both S9485 and Killarney showed the highest dry matter yield of first cut, while Latar showed the lowest dry matter yield at Normandin, Quebec. However, under irrigation at Logan, Utah, the populations of Latar and UTWH-102 showed the highest and lowest dry matter yield of first cut, respectively.

Total dry matter yield of orchardgrass did not differ among the 9 entries at any locations except at Logan, Utah. The population of Latar (12.6 t/ha) showed the highest total dry matter yield among the entries examined at Logan. The seven entries of orchardgrass (Early Arctic, Okay, S9485, SFD200102, SFD9601, UTDG101 and UTWH-102) had statistically similar productivity in terms of total dry matter at Logan, Utah under irrigated condition.

Tall fescue

Winter survival: tall fescue populations showed significant variation in winter survival rate at Swift Current ($P<0.001$), which was a spaced nursery. The populations of Courtney, Kokanee and S9582 showed significantly higher winter survival rate (70-83%) than the populations of Cowgirl and Fawn (23-26%) at Swift Current. Winter kill rate is generally high in nursery than a plot trial. At plot level, winter kill rate was also different ($P<0.05$) at Saskatoon, but not at Normandin and Saint-Augustin of Quebec. In Saskatoon site, the population of Courtney and Kokanee showed the highest winter survival rate (100%) and the population of Cowgirl showed the lowest (92%).

Forage yield: the populations of tall fescue exhibited significant variation (P = 0.001) in dry matter yield of first cut at Swift Current, Saskatoon, Normandin, and Logan. The populations of both Courtney and S9582 produced higher dry matter yield than Cowgirl and Fawns at Swift Current. At Saskatoon, Courtney and S9582 produced the highest first cut yield among the populations examined. At Normandin, Quebec the populations of Fawn, Kokanee and Tower had lower first cut dry matter yield than the population of Courtney. The population of Tower showed the lowest dry matter yield of first cut, under irrigation at Logan, Utah.

The total dry matter yields of seven entries of tall fescue were differed significantly (P =0.001) only in three locations (Swift Current, Saskatoon and Normandin). The populations of Courtney, Kakonee and S9582 exhibited higher total dry matter yield than that of Cowgirl and Fawns at Swift Current. At Saskatoon site, the populations of Courtney, Kokanee and S9582 produced the highest total dry matter yield among all entries. However, Courtney displayed higher total dry matter than Arido, Cowgirl, Fawns and Tower at Normandin, Quebec.

Timothy

Winter survival: no winter kill in timothy was observed except cultivar 'Aurora' had a low winter survival rate of 91% at Saint-Augustin, QB in 2019. Winter survival of timothy populations greater than 97% at most of the sites. Plant height was different among the timothy populations at Saskatoon in 2019, but it was similar at Normadin and Saint-Augustin, QB.

Forage Yield: timothy populations had similar first cut and total dry matter yield in 2019.

Title:	**Improving Forage and Bioenergy Crops for Better Adaptation, Resilience, and Flexibility**

Sponsoring Agency	NIFA	**Project Status**	ACTIVE
Funding Source	Hatch/Multi State	**Reporting Frequency**	Annual
Accession No.	1019343	**Project No.**	RI0019-NE1710
		Multistate No.	NE1710
Project Start Date	04/30/2019	**Project End Date**	10/01/2022
Reporting Period Start Date	10/01/2019	**Reporting Period End Date**	09/30/2020
Submitted By	Jane Vieira	**Date Submitted to NIFA**	02/26/2021

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Performing Department
Plant Sciences & Entomology

Non-Technical Summary

Pasture-raised sheep and goats are an increasingly important part of the local agricultural system in the Northeast. However, gastro-intestinal parasites are a major limiting factor for producers. Many farmers in the Northeast do not have sufficient pasture to utilize the 60-day rotations needed to avoid animal exposure to parasites. Parasite infections reduce production of milk, meat and wool, and can cause death of animals. Parasite resistance to synthetic dewormers is widespread, and synthetic dewormers can disrupt the soil microbiological ecosystem in pastures. Birdsfoot trefoil is a regionally adapted forage legume which has been shown to have antheminthic properties. In vitro studies have shown that anthelminthic activity differs between BFT genotypes grown in a common environment, and activity is believed to be related to tannin chemistry. However, the precise active compounds have not been identified. Tannins, like all plant secondary compounds, are affected by environment, but little is known about genotype by environment interactions of secondary compounds in birdsfoot trefoil.
This project will trial eight BFT accessions at six locations for three years. URI will be one of the locations, with others located in Nova Scotia, Ontario, western New York, Utah, and Wisconsin. Each year herbage of each variety at each location will be collected, freeze-dried, and shipped to Nova Scotia for biochemical analysis. At each location data will be collected on forage yield and plant survival. At URI this project will complement additional research on BFT as an anthelminthic for sheep and goats in Organic systems. It will also complement the larger cover crop research program.

Accomplishments

Major goals of the project

(1)
Understanding genotype by environment interactions across multiple forage species

What was accomplished under these goals?

A birdsfoot trefoil variety trial with 12 entries and 3 replications was established in September 2019. Plant count data were collected in November 2019 and percentage ground cover data were collected in June 2020. Yield data were collected at the end of June and again at the end of August (2 cuttings). Plant count data were collected in October 2020 to measure survival. Sub-samples from each harvest were collected for biochemical analysis and antheminthic efficacy testing. Field data and one set of freeze-dried samples will be sent to the trial coordinater (Yousef Papadoupolas, Agriculture Canada, Nova Scotia) for analysis and comparison to the other trial locations. A second set of freeze dried samples and a set of air-dried samples will be used in a complementary project at URI to evaluate anthelminthic efficacy of extracts. The complementary project is funded by the USDA Organic Transitions program and has the objective of evaluating birdsfoot trefoil as a medicinal forage to protect sheep and goats from intestinal parasites.

What opportunities for training and professional development has the project provided?

Four URI undergraduate students and a post-doc were involved in trial establishment, maintenance, harvest and data collection. The trial provided an opportunity for students to learn about experimental design and about growing and managing birdsfoot trefoil using organic methods. A URI graduate student is using the trial as a source of birdsfoot trefoil for her dissertation research on medicinal forages.

How have the results been disseminated to communities of interest?

{Nothing to report}

What do you plan to do during the next reporting period to accomplish the goals?

The birdsfoot trefoil trial is intended to run for three years. In 2021 we expect to collect another round of data and tissue samples.

Participants

Actual FTE's for this Reporting Period

Role	Non-Students or faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Scientist	0.1	0	0	0	0.1
Professional	0	0	0.1	0	0.1
Technical	0	0.1	0	0.1	0.2
Administrative	0	0	0	0	0
Other	0	0	0	0	0
Computed Total	0.1	0.1	0.1	0.1	0.4

Student Count by Classification of Instructional Programs (CIP) Code

Undergraduate	Graduate	Post-Doctorate	CIP Code
3		1	01.11 Plant Sciences.
1			01.09 Animal Sciences.
	1		30.01 Biological and Physical Sciences.

Target Audience

Plant breeders, small ruminant producers

Products

{Nothing to report}

Other Products

Product Type

Data and Research Material

Description

Data were collected on the performance of entries in the Birdsfoot Trefoil Variety trial. Data will benefit the plant breeders who submitted entries to the trial, and will guide cultivar selection advice for farmers.

Accession No. 1019343 **Project No. RI0019-NE1710** **Multistate No. NE1710**

Changes/Problems

{Nothing to report}

REGIONAL RESEARCH TECHNICAL PROJECTS:
NE-1710 2021 ANNUAL REPORT

K. E. Glover, Agriculture and Agri-Food Canada, Kentville Research and Development Center, Truro, N.S., Canada

Objective 2. Understanding genotype by environment interactions across multiple forage species.

This project being lead by Dr. Ali Missaoui, aims to evaluate novel tall fescue germplasm for its productivity and adaptability over multiple sites providing a means to assess genotype by environment interactions with respect to forage yield. Fourteen tall fescue entries were seeded at the Nappan Research Farm, Nappan, N.S., Canada in the spring of 2019. The tall fescue established well and was clipped several times to manage weeds in the seeding year.

In the first production year (2020), access to the field was delayed until mid-June due to Covid-19 restrictions. At this time all tall fescue germplasm had entered the reproductive stage of growth and plots were harvested June 15th, 2020. After first cut 60 kg/ha of CAN 27-0-0 was applied. A second application of CAN 27-0-0 was applied July 10th at a rate of 40 kg/ha, however due to very droughty conditions in the summer of 2020, plots were not harvested again until August 10th. Seasonal, first cut and second cut dry matter yields are provided in the table on the following page. Unfortunately no pictures are available for the trial in 2020. The photo below shows the plots this year taken after plenty of rain in contrast to the drought of 2020 and we anticipate forage yield to be higher in 2021.

Second production year for tall fescue germplasm evaluation: Photo taken July 26, 2021 just before 2nd cut was harvested.

Table 1: Dry Matter Yield of Fescue in Pure Stands for First Production Year (Nappan, Nova Scotia)

Cultivar	seasonal, t/ha	cut 1, t/ha	cut 2, t/ha
GA5	11.7	8.8	2.8
GA29	12.1	9.7	2.4
GA186	13.2	9.6	3.6
GALA1502T	10.7	7.8	3.0
GALA1503T	10.2	7.6	2.6
JesupMaxQ	12.2	8.8	3.5
Jesup Minus	9.7	7.3	2.4
Texoma Max Q II	12.6	9.6	3.0
KY 31+	9.9	6.9	3.0
KYFA 1405	10.9	8.2	2.7
KYFA 9611	11.2	8.3	3.4
KYFA 9821/AR584TF	13.6	10.5	3.1
Lacefield MaxQ II	11.3	8.3	3.0
Kokanee	12.2	9.1	3.1
Pradel	12.2	10.6	1.6
Grand Mean	11.6	8.7	2.9
SEM	1.17	1.16	0.17
F pr			
pCv	ns	ns	<0.001

Notes: N = 3, SEM = Standard error of the Mean, ns = Not significant at P=0.10

REGIONAL RESEARCH TECHNICAL PROJECTS: NE-1710
2021 ANNUAL REPORT

Yousef A. Papadopoulos, Agriculture and Agri-Food Canada, Dalhousie University-Faculty of agriculture Truro, NS, Canada

1.1. Regionally adapted, resilient alfalfa germplasm pool development.

Cooperating locations: Univ. California, Davis [co-lead]; and Madison, WI [co-lead]; Cornell Univ., South Dakota State Univ. Logan, UT; Québec, QC; Saskatoon, SK; Truro, NS; and Noble Foundation, Ardmore OK.

- In Nova Scotia in the fall of 2020, Dr. Papadopoulos completed the selections of plants from each of the 4 alfalfa germplasm pools of this study. Due to a second COVID lockdown access to greenhouse indoor facilities was delayed. Intercrossing plants for each of the newly selected populations to generate syn 1 seeds was initiated in June, 2021.

1.4. Birdsfoot trefoil germplasm with bioactive components to control parasitic nematodes.

Cooperating locations: AAFC: Truro, NS [lead]; Univ. Rhode Island; AES: Cornell Univ.; USDA-ARS: Logan; UT and Madison, WI.

- In 2020, first production year samples were collected and freeze dried from cut 1 at four of the five research sites: Logan, Cornell University, Rhode Island and Madison. Sampling from the second production year at the above locations is currently underway. Due to the very challenging weather conditions during the 2019 growing season and COVID pandemic restrictions in Nova Scotia in 2020, the establishment of this trial in Nova Scotia was delayed. The plots were seeded in the spring of 2021. Dr. Justin Renaud, AAFC in London, Ontario, joined our research team and is currently in the process of conducting the analysis of tannins and isoflavones from the above four sites.