NE1010 MULTISTATE PROJECT

Breeding and Genetics of Forage Crops to Improve Productivity, Quality, and Industrial Uses

October 1, 2002 to September 30, 2017

Period Covered: October 2016 to September 2017

Annual Meeting Date: July 25, 2017

LOCATION: Atlantic Food and Horticulture Research Centre, Agriculture and Agri-Food Canada
Kentville, Nova Scotia, Canada

Y. Papadopoulo (AAFC), Chair and Host
S. Kenaley (Cornell Univ.), Secretary

Attendees (20) – Institutions
Don Viands – Cornell Univ. (New York, U.S.A)
Bill Biligetu – Univ. Saskatchewan (CAN)
Heathcliffe Riday – USDA-Dairy Forage Research Center (Wisconsin, U.S.A)
Donn Vellkson – Univ. Minnesota (U.S.A)
Nancy Ehlke – Univ. Minnesota (U.S.A)
Michael Peel – USDA-Agricultural Research Service (Utah, U.S.A)
Tim Phillips – Univ. Kentucky (U.S.A.)
Charlie Brummer – Univ. California – Davis (U.S.A.)
Shawn Kenaley – Cornell Univ. (New York, U.S.A)
Arvid Boe – South Dakota State Univ. (U.S.A.)
Rebecca Brown – Univ. Rhode Island (U.S.A.)
Jesse Morrison – Mississippi State Univ. (U.S.A.)
Ali Missaoui – Univ. Georgia (U.S.A.)
Solen Rocher – Agriculture and Agri-Food Canada (Quebec, CAN)
Annie Claessens – Agriculture and Agri-Food Canada (Quebec, CAN)
Surya Acharya – Agriculture and Agri-Food Canada (Alberta, CAN)
Abdelali Hannoufa – Agriculture and Agri-Food Canada (Ontario, CAN)
Kathleen Glover – Agriculture and Agri-Food Canada (Nova Scotia, CAN)
Yousef Papadopoulo – Agriculture and Agri-Food Canada (Nova Scotia, CAN)
Sherry Stillmore – Agriculture and Agri-Food Canada (Nova Scotia, CAN)

MINUTES

Opening – Introductions
1. Call to order, 9:48 AM
2. Dale Hebb (Acting Assoc. Director): welcome and introduction to the Atlantic Food and Horticulture Research Centre.
   a. Mission
   b. Facilities
   c. Agriculture education
   d. Research activities
   e. Research support
3. Y. Papadopoulo (Chair and host): welcome, introductions, and meeting agenda
Open discussion on NE1010 projects by objectives and forage species

Objective 1: Evaluate new plant characters and develop germplasm and cultivars with these characters to improve perennial forage species as livestock feed and biofuel uses to enhance rural vitality and promote more secure energy sources

1.1 Alfalfa

1.1.3. Breeding for resistance to alfalfa snout beetle in alfalfa

- **D. Viands** (Cornell): discussed breeding resistance to alfalfa snout beetle
  - Indicated that, after several cycles of selection, resistance appears to be quantitative
  - At present, effective snout beetle control can be achieved via a combination of entomopathogenic nematodes (e.g., *Steinernema* spp. and *Heterorhabditis bacteriophora*) and resistant cultivar.
  - The new resistant cultivar, Seedway 9558 SBR, is being used in Northern NY.
  - The 14th cycle of selection for resistance has been completed in six alfalfa populations.

1.1.4. Developing alafa germplasm with potato leafhopper resistance from three diverse genetic sources

- **D. Viands** (Cornell)
  - Discussed that effective breeding is capable of producing resistance gains to potato leafhopper.

1.1.6. Aluminum tolerance in tetraploid alfalfa

- **A. Missaoui** (Univ. Georgia) and **Y. Papadopoulos** (AAFC): discussed the promise of breeding and selecting from aluminum tolerance in alfalfa.
  - In so doing, **Y. Papadopoulos** noted that he will provide electronic copies of outputs for his related work.
  - Importantly they noted that a number of selections of tetraploid alfalfa with enhanced tolerance to aluminum.

  - Four selections performed by the program of **Y. Papadopoulos** have demonstrated enhanced tolerance; thus, **Y. Papadopoulos** suggested regional trials should be conducted to validate yield potential and aluminum tolerance of these selections across latitudes and longitudes.
    - Produced four novel acid tolerant populations (TA08-1003, TA08-1004, TA08-1005 and TA08-1004).
    - A newly developed synthetic TA08-1003 was evaluated in the Atlantic Forage registration trial and was supported for registration in 2014.
    - Complete a second cycle of selection and produced an addition novel synthetic (TA13-1012) which included plants with enhanced vigor and heritage with NY9627 and NA60-1002.
    - TA13-1012 will be placed into registration trials in 2018.
  - **Y. Papadopoulos** also noted that the latter (multisite regional testing) as well as the exchange of germplasm has been paramount to the success and coordination of NE1010 projects, and, hence, breeding products have value-added to the plant breeding community at large.
1.2 Birdsfoot trefoil
1.2.1. Rhizomatous birdsfoot trefoil for yield improvement
   • **D. Viands** (Cornell)
     o Slow trait to select from wild background
       ▪ Noted that after four cycles of selection rhizomatous plants demonstrated poor vigor.
     o Recently backcrossed to enhance trait but remains a slow trait to select.
     o Plants will be selected for vigor and rhizome production in fall 2017.

1.2.2. Plot evaluation of birdsfoot trefoil for vigor
   • **M. Peel** (USDA-ARS, Utah)
     o Discussed plot evaluations on dryland sites with little to no summer precipitation.
     o Several promising selections with drought tolerance and high/sufficient vigor were identified, and, hence, warrant additional study.
     o These studies have concluded; successfully fulfilling deliverables.

1.5 Multiple Species
1.5.1. Selection of fiber digestibility and cell wall pectin
   • **D. Viands** (Cornell)
     o Discuss his previous selection studies to identify traits for forage quality
       ▪ Selecting for pectin concentration after two years significantly reduced forage yield
     o Presently, working to combine alfalfa forage quality with resistance to potato leafhopper
   • **A. Claessens**
     o Sugar content and cell wall digestibility. Still working on lower digestibility of alfalfa.
   • **C. Brummer** (UC-Davis)
     o Posed the question: “Is there a relationship between cell wall digestibility and fall dormancy?”
     o Noted that increasing dormancy (producing more dormant plants) likely will increase forage quality.

1.6 Marker-assisted selection
1.6.1. Red Clover marker-assisted selection. To be developed.
   • **H. Riday** (USDA-DFRC, Wisconsin)
     o Utilized 60 SSRs per PCR per genotype sequencing (Illumina)
     o Working with new RNAseq data
     o Improved assembly platforms and SSR marker discovery
       ▪ For the discovery and assessment of cheaper markers, **H. Riday** and colleagues would like to move away from Illumina to Nano pore technology

1.6.2. Kura clover: Paternity testing
   • **H. Riday** (USDA-DFRC, Wisconsin) – finished work and have since published findings

1.6.3. Alfalfa: Marker-assisted selection
   • **H. Riday** (USDA-DFRC, Wisconsin)
     o Tracked selfing and paternity
     o SSRs not associated with a particular trait(s)
No confirmed loci; however, QTLs have been mapped.
Present SSR markers provide an improved basic set of tools; yet, do not lend themselves to pure marker-assisted selection.

1.7 Kura Clover: Vigor and spreadability
- Unrealized project, no significant deliverables
- Y. Papadopoulos (AAFC)
  - Stated that plant vigor in Kura clover is a significant challenge
  - Suggested that discussion among the NE1010 group should be held to determine who should lead this project.
  - Questioned whether the goal should be to focus on promising germplasm and their downstream release
- H. Riday (USDA-DFRC, Wisconsin)
  - In response to Y. Papadopoulos’ call for promising germplasm, H. Riday replied that he and his collaborators have 15 lines of Kura clover for possible vigor and spreadability testing
- Rebecca Brown (Univ. Rhode Island)
  - Responded to H. Riday comment about his 15 lines inquiring about the seed production and seed sources of Kura clover with demonstrated vigor and spreadability. She has several projects looking to utilize Kura Clover.

Objective 2: Build on previous research to evaluate additional breeding methods for improving yield and persistence of alfalfa, red clover, orchardgrass, and other forage species to make production agriculture more economical and sustainable.

2.1. Alfalfa
2.1.2. Replicated clonal selection for improving forage yield of alfalfa
- D. Viands (Cornell)
  - Indicated that this study concluded and all outcomes were achieved.
  - Called for three-year data to be submitted to him for analysis
  - Discussed that genotype x environment interaction was a big issue and was reflected in multi-site data.
  - Three-year data to be analyzed shortly
- C. Brummer (UC-Davis)
  - Discussed clonal selection trial in CA including ten reps of promised germplasm across two sites
  - Lines were scored based on phenotype, resulting in yield gains

2.2. Orchardgrass
2.2.1. Clonal selection in orchardgrass for broad adaptation
- T. Phillips (Univ. Kentucky)
  - Discussed four germplasm sources that were inter-mated and selected
  - Noted a thrips-transmitted virus ravaged these selections and, hence, no products (i.e., broadly adapted clones) were realized.
2.2.2. Non-heading orchardgrass research
- Michael Casler (USDA-ARS, Wisconsin) – NOT PRESENT
  - General discussion among attendees, particularly the observation that flowering in orchardgrass is remarkably different by region.

2.3. Red clover
2.3.1. Selection for general adaptation in red clover
- **Y. Papadopoulos** (AAFC)
  - Evaluated the theory of general adaptation (genotype x environment interactions) in red clover
  - Data were collected in 2013, 2014 and 2015 growing seasons and data analysis is currently underway.
- **Y. Papadopoulos** (AAFC) and **H. Riday** (USDA-DFRC, Wisconsin)
  - Noted:
    - Varieties in Quebec, NY State, and WI clustered together
    - No evidence of local adaptation
    - Varieties with high performance were the high performers across sites
    - Selecting populations with high performance across latitudes is possible
    - Publication of results in prep

2.3.2. Selection for persistence in red clover using half-sib families
- **H. Riday** (USDA-DFRC, Wisconsin)
  - Data in place for the selection of persistence in red clover using half-sib families
  - Tried demonstrating selection and yield gain among half-sib and phenotypic selections
  - Selection for yield and vigor is most advantageous rather than for persistence

2.3.3. Red clover biofuels
- **Y. Papadopoulos** (AAFC)
  - Co-seeded switchgrass and red clover
  - Switchgrass failed to establish

2.4. Reed canarygrass: Methods to improve reed canarygrass
- **Michael Casler** (USDA-ARS, Wisconsin) – NOT PRESENT, no update

*Objective 3: Evaluate new experimental populations and cultivars of perennial forage species for characteristics necessary for breeders, seed companies, see and forage producers, and crop consultants to make decisions on commercial use over large regions.*

3.1 Alfalfa
3.1.1. Evaluation of new *M. sativa* subsp. *falcata* populations
- **M. Peel** (USDA-ARS, Utah)
  - Populations have been evaluated.

3.1.2. Hybrid alfalfa evaluation
- General discussion as to who will continue this work as the former lead (Lamb, USDA-MN) is no longer actively executing this study. Group indicated that Deborah Samac (USDA-ARS, Minnesota) is planning to continue the work of Lamb.

3.1.3. Evaluation of salt tolerant alfalfa
- **S. Acharya** (AAFC)
  - Provided brief update, no significant products in 2016
  - Genomic selections will be challenged under controlled conditions.
  - Selections have been completed and will evaluate them. Mike has a huge effort in salt tolerance. Surya and Mike will exchange GP to evaluate. Annie volunteered to evaluate too. Mike will be ready for another evaluation trial in two to three years.
- **H. Riday** (USDA-DFRC, Wisconsin)
  - Noted that there is a pressing need for an improved salt germination test for high-throughput screening of genomic selections
At present, “our community also doesn’t have a very good salt tolerant check.”

- **Y. Papadopoulos** (AAFC)
  - Salt tolerance is a pressing issue – one that should be explored and, thus, emphasized in new NE1710

### 3.3 Multiple species

#### 3.3.1. Biomass alfalfa/grass mixture evaluation

- **General discussion as Lead Lamb – NOT PRESENT**
  - Monoculture and mixture trials were conducted
  - Data for project possessed by Deborah Samac (USDA-ARS, Minnesota) should be procured and circulated among collaborators

#### 3.3.2. Meadow and hybrid bromegrass

- **B. Coulman**
  - Developed one population
  - Will send abstract indicating results.
  - Bruce will write a manuscript within the next two years.
  - One population yields 13% more than Fleet in 5-year trials.

- **A. Bow** (South Dakota State Univ.)
  - Hybrids were less vigorous and produced less biomass than when compared to non-hybrids.

- **Y. Papadopoulos** (AAFC)
  - Several bromegrass populations are flexible for early grazing

### BREAK - LUNCH

Discussion regarding NE-1710

1. Regionally adapted resilient alfalfa germplasm pool development

- **H. Riday** (USDA-DFRC, Wisconsin)
  - Established four pools – central Asian, Baltic, northeastern European, and Siberia
    - Created seed increases for each pool and have since established nurseries
    - Submitted grant proposal to assist in funding project
  - Pre-breeding will be part of effort
    - Diversity of selections compared to commercially available
  - Movement of germplasm among collaborators will be done via seed
  - Retain tissue samples from the original pools’ members for DNA analysis

- **C. Brummer** (UC-Davis)
  - Established new germplasm for southern germ pools – South Africa
    - Est. in CA
    - Need discussion on workflow and pre-breeding
    - Will do dormancy screening
    - Crop Characteristics will do disease screening
  - Asked for multiple collaborators to submit plants/seeds to include in the initial pools

- **A. Missaoui** (Univ. Georgia)
  - Gardens include germplasm from Iran and North Africa

2. Resilient cool-season grasses adapted to variable climatic conditions

- Multiple collaborators to assess winter survival, yield, and influence of latitude on the former and latter.

3. Meadow bromegrass – low genetic diversity
• **A. Bow** (South Dakota State Univ.) and **D. Viands** (Cornell) would be interested in developing trials for meadow bromegrass
  - Important grass for southern Canada

4. Assessing the varied profile and contents of isoflavoids in birdsfoot trefoil across diverse climatic conditions in the northern latitudes.

• **Y. Papadopoulos** (AAFC)
  - Already demonstrated genotype x environment differences of tannin content between outdoor and greenhouse cultivated plants
  - Looking at tannic acid because of its demonstrated benefits to plants as well as possible suppression in intestinal, sheep nematodes

• **Missaoui, H. Riday, D. Viands, R. Brown** will cooperate.
  - Agreed to establish plot trials in 2018
  - 20-25 entries. Assess plots in 2019 and 2020 for development stage in first two harvests, and send frozen (freeze dried) forage to Yousef, who assay samples. Also take forage yield.

5. Understanding genotype by environmental interactions across multiple forage species

• Group discussion: will look for past and present data to determine factors contributing to combined GxE
  - Candidate plant species: red clover
  - Multi-location evaluations every few years. Multiple species. Lead for each species.
  - **Sherry Stillmore** discussed doing a meta-analysis of previous data to determine the level of G x E in various species. She would be willing to be involved in the analysis if we could get her the data. Maybe begin with one species. Yousef named Mike (lead), Yousef, Sherry, Heathcliffe and Don will identify a species and send data.
  - Red clover: what data are available? Maybe trefoil, too? Yousef to coordinate data. Mike and Yousef co-lead the meta-analysis.

**Planning: 2018 Annual Meeting**
Proposed host site: Logan, UT. Meet the day before the NAAIC meeting.
In 2019, meet in Athens, GA (Ali Mussaoui host)
Appointed chair: S. Kenaley (Cornell)
Secretary: Ali Mussaoui

**Summary of Accomplishments:**

Objective 1:

(1) Four novel acid tolerance alfalfa populations have been developed. One population is targeted to become a cultivar.

(2) The new alfalfa snout beetle cultivar, Seedway 9558 SBR, is being used in northern NY. Field experiments continue to indicate that this cultivar has less root damage and higher forage yield than susceptible cultivars.

(3) Forage quality has been improved in alfalfa and other species.
(4) Research on paternity testing in kura clover has been published.

(5) USDA-UTWH-102 orchardgrass germplasm was released with improved winter hardiness.

Objective 2:

(1) Comparison of selection methods for biomass yield is continuing at multiple location trials. Data so far indicate that progress from replicated clonal selection for higher yield has resulted in some progress in yield, but only at the location where selection was conducted.

(2) Research has shown that selection of red clover across latitudes is possible. A publication is being prepared.

Objective 3:

(1) Evaluations of multiple species of cool and warm season perennial forages continue to be evaluated for forage yield, persistence, and other agronomic traits at multiple locations in North America.

Impact Statements:

1. The development of new grass and legume cultivars will provide a more reliable source of economical feed for dairy and other livestock industries. For example, alfalfa cultivars with resistance to alfalfa snout beetle and potato leafhopper provide protection of the crop to realize maximum forage yield and quality. A new orchardgrass cultivar that does not produce seed heads in the areas of forage production would provide higher quality grass. Orchardgrass with improved winter hardiness will result in more persistence and productivity.

2. This project improves environmental quality through the development of new cultivars of grasses and legumes with improved persistence, increased resistance to abiotic and biotic stresses, and enhanced soil-binding improvement capabilities (e.g., reduced soil erosion; improved nutrient cycling; less soil surface runoff; increased soil carbon sequestration; reduced atmospheric CO2; reduced use of agricultural chemicals/fertilizers; reduced pollution/contamination of surface and ground waters).

3. Acid soils are very extensive in North America and worldwide. Alfalfa cultivars with tolerance to these soils will provide more economical forage by eliminating or reducing the need for lime applications. New cultivars will convert such problem soils, previously unsuitable for agriculture, into areas of stable high quality forage production. Development of salt-tolerant forage species should expand the use of crops for forage production.
4. With improved forages and biomass crops, increased diversification and sustainability in agricultural ecosystems can be achieved. Identification of breeding methods that will improve forage and biomass yield and quality is essential for improving the economics of these crops in production agriculture and in conservation and wildlife habitat systems.

5. The evaluation of experimental populations is essential to ensure that all stakeholders have the agronomic information to make decisions on use of forage species and cultivars within species. These evaluations across locations and years helps scientists to better understand genotype x environmental interactions.

6. Development of warm season grasses for biofuel use will contribute toward sustainability of energy production. Protecting switchgrass from diseases and insects will help to provide biomass more economically.

**Publications:**


Kenaley, S.C., G.W. Hudler, G.C. Bergstrom. 2016. Detection and phylogenetic relationships of *Puccinia emaculata* and *Uromyces graminicola* (Pucciniales) on switchgrass in New York State using rDNA sequence information. Fungal Biology 120: 791–806


Lee, DoKyoung; Aberle, Ezra; Anderson, Eric; Anderson, William; Baldwin, Brian; Baltensperger, David; Barrett, Michael; Bonos, Stacy; Bouton, Joe; Brummer, Charlie; Burks, Payne; Chen, Chengci; Daly, christopher; Egenolf, Josh; Farris, Rodney; Fike, John; Gaussoin, Roch; Gill, John; Gravois, Kenneth; Halbleib, Michael; Hale, Anna; Hanna, Wayne; Harmoney, Keith; Heaton, Emily; Heiniger, Ron; Hoffman, Lindsey; Hong, Chang; Kakani, Vijaya Gopal; Kallenbach, Robert; Macoon, Bisoodat; Medly, James; Missaoui, Ali; Mitchell, Robert; Moore, Ken; Morrison, Jesse; Odvody, Gary; Ogoshi, Richard; Parrish, Jimmy; Quinn, Lauren; Richard, Ed; Rooney, Bill; Rushing, Bret; Schnell, Ronnie; Sousek, Matt; Staggenborg, Scott; Tew, Thomas; Uehara, Goro; Viands, Donald; Voigt, Thomas; Williams, David; Williams, Linda; Wilson, Lloyd; Wycislo, Andrew; Yang, Yubin; Owens, Vance. 2017. Biomass Production of Herbaceous Energy Crops in the United States: Field Trial Results and Yield Potential Maps from the Multiyear Regional Feedstock Partnership. GCB Bioenergy. Submitted for publication.


Waldron, B.L., M.D. Peel, S.R. Larson, I.W. Mott, and J.E. Creech. 2017. Tall fescue forage