

Developing Regionally-Adapted, Resilient Alfalfa Germplasm Pools

E. Charles Brummer, Heathcliffe Riday, Donald Viands, Julie Hansen, Virginia Moore, Brian Irish, Dan Putnam, Esteban Rios, Surya Acharya, Annie Claessens, and Arvid Boe



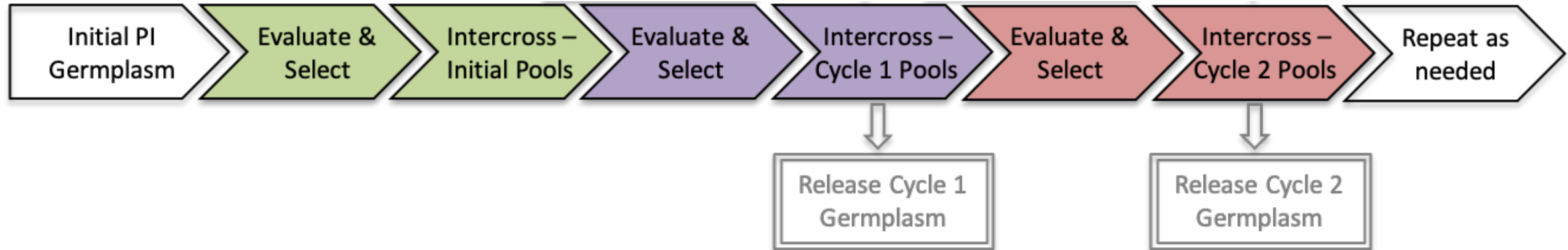
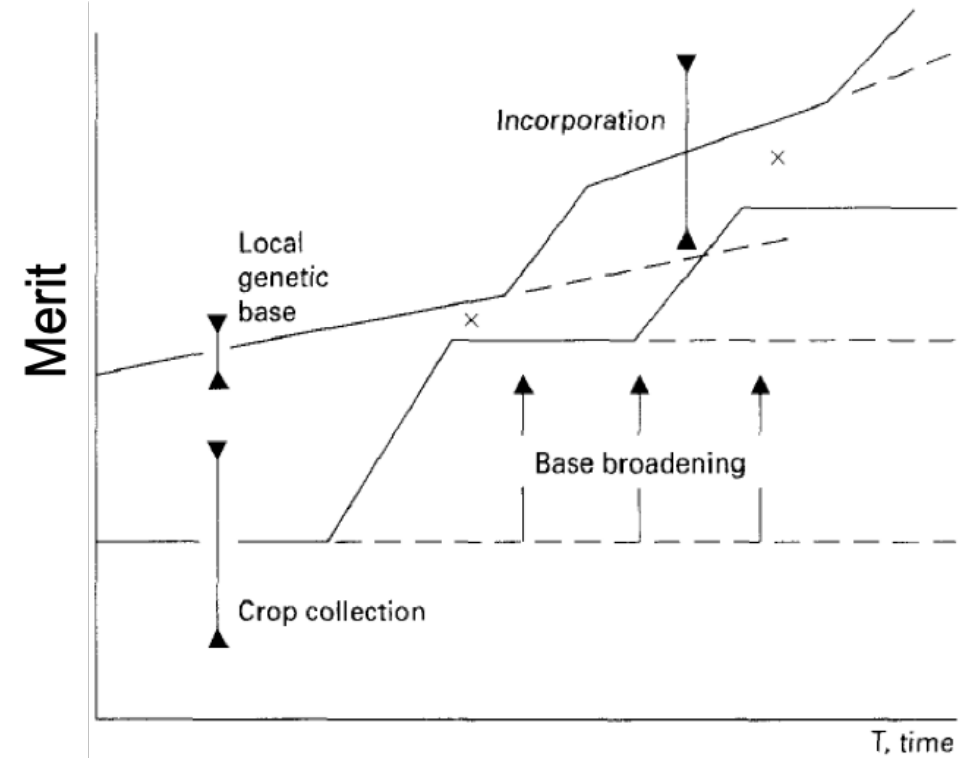
Base-Broadening, Pre-Breeding, or Germplasm Enhancement

INTROGRESSION AND INCORPORATION. STRATEGIES FOR THE USE OF CROP GENETIC RESOURCES

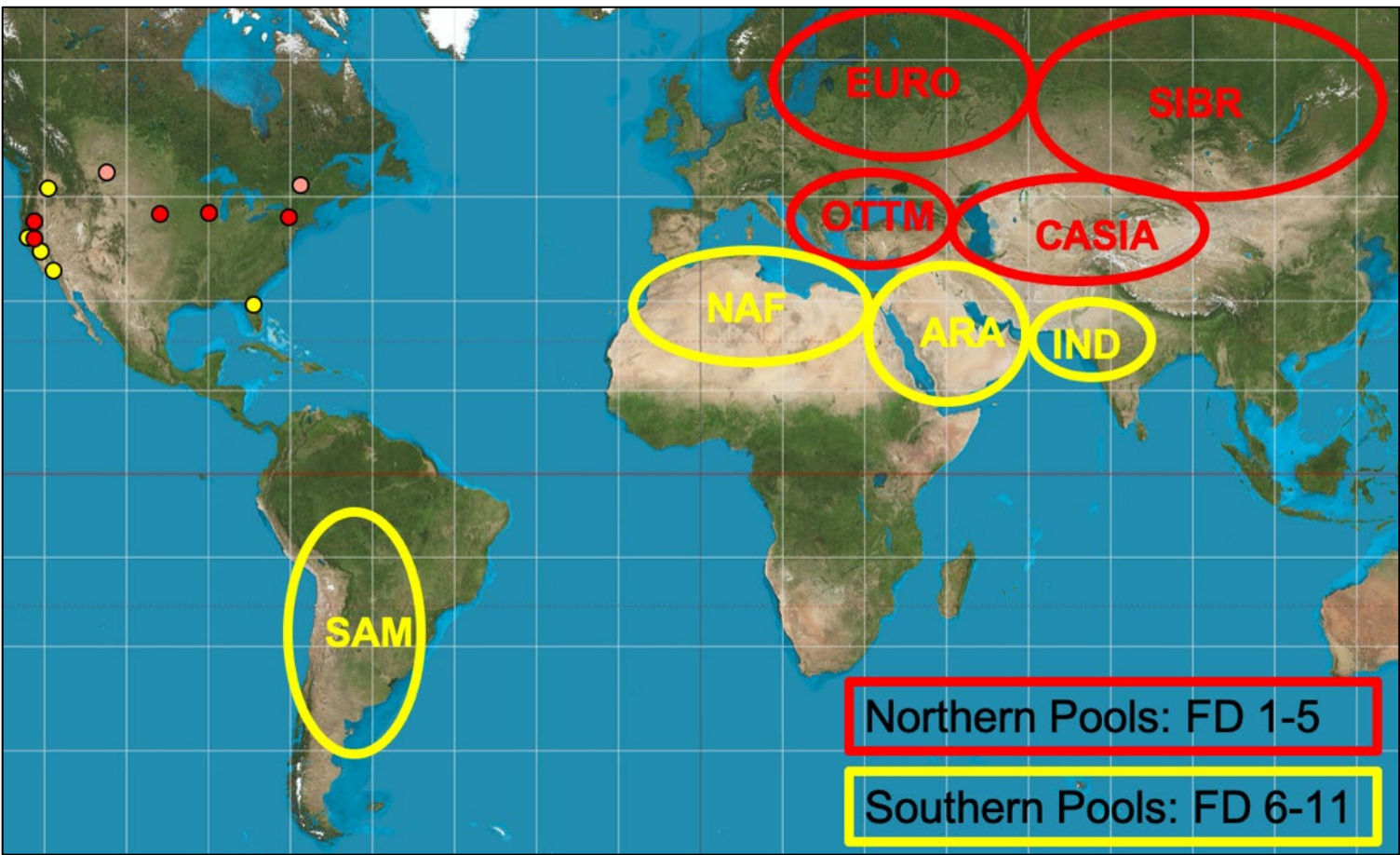
By N. W. SIMMONDS

University of Edinburgh, Institute of Ecology and Resource Management,

Biol. Rev. (1993), 68, pp. 539-562



To summarize, then, the fundamental needs of an effective 'Incorporation' ('Base-broadening') programme are: large scale; wide range of entries; independence of local base; rough, quick, cheap mass-selection methods adapted to the biology of the crop; enhanced recombination; continuity over a long time-scale.



Field Book app for data collection

Seed increase in Prosser, WA

Composite populations across locations



Germplasm screened:

250 Northern PI accessions

400 Southern PI accessions

Populations created:

24 Northern Pools, Round 1

4 Composite Round 1 increases

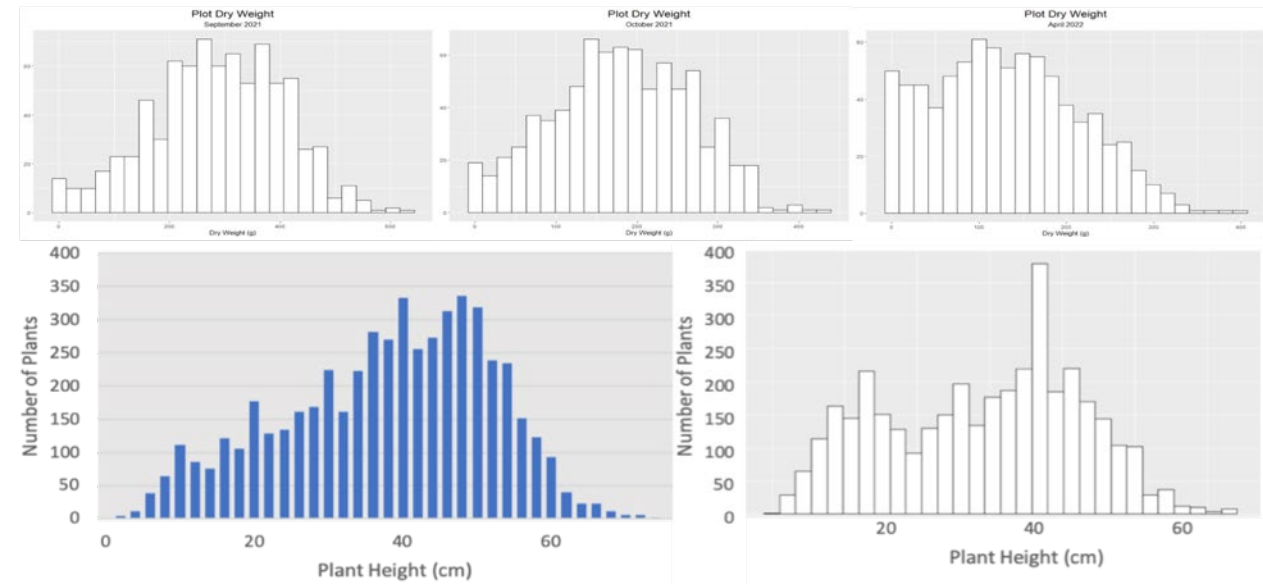
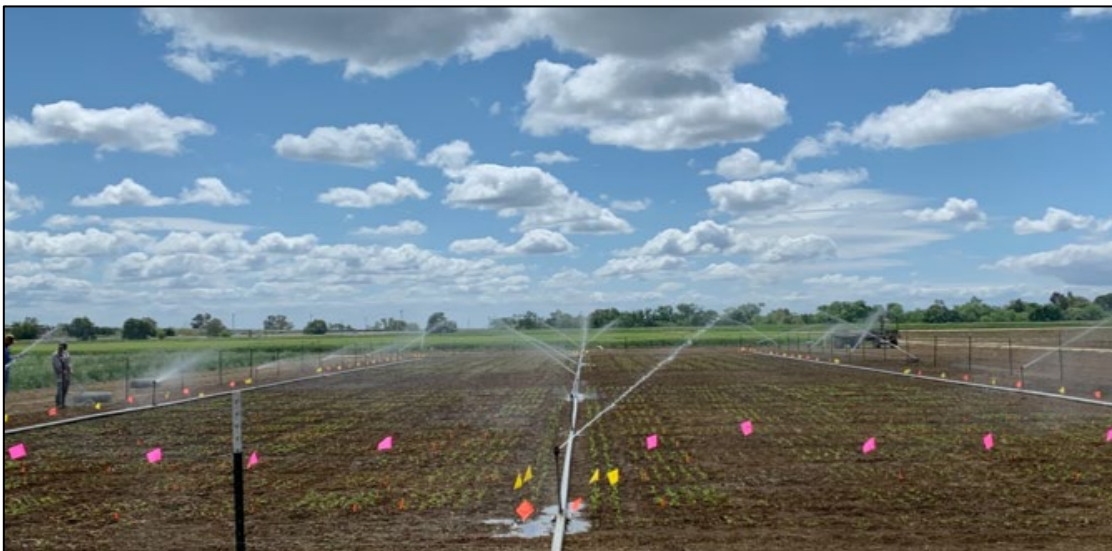
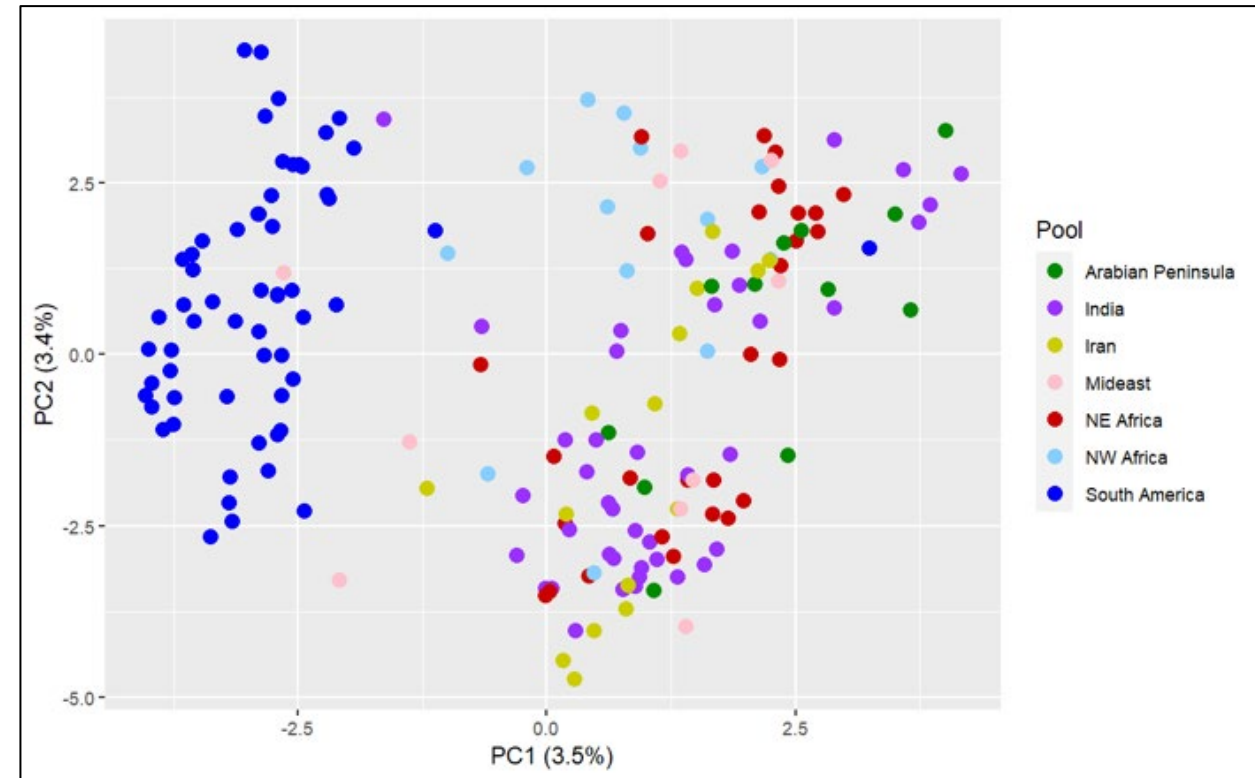
16 Northern Pools, Round 2

8 Southern Pools, Round 1

Evaluation Trials of New Populations:

Northern Pools: NY, WI, SD, CA (x2), QC

Southern Pools: CA (x2)



2014 - 2016

2017

2018

2019

2020

2021

2022

2023

2024

2025

2026+

Northern Pools Round 1 (NP R1) – 150 PI accessions evaluated in WI; selections formed four R1 **Cycle 0 (C0)** base pool pops

NP R1C0 – 4 pops – Spaced plant selection nurseries in WI, NY, CA, QC, AB

NP Round 1
Selection and intercrossing
5 locs x 4 pops = 20 C1 pops

NP R1C1 – WA
seed increase of
4 C1 pool
composite pops

NP R1C1 – 20 C1 pops + base pops – Spaced plant evaluation trials WI, NY, CA(x2), QC, NS

Release of 4 NP
R1C1 composite
pool pops

NP R1 – Selection and
intercrossing, 6 locs x 4
pops = 24 C2 pops

Northern Pools Round 2 (NP R2)
100 PI accessions evaluated in WI, CA, NY

NP R2 – 12 C1 populations
C1 spaced evaluation trials WI, NY, CA(x2)

NP R2 – Selection and
intercrossing, 3 locs x 4
pops = 12 R2C1 pops

NP R2C1 – WA
seed increase of 4
composite pops

NP R1C1 & R2C1 – 8 composite pops
Sward evaluation trials (WI, CA, NY)

Release of 4 NP
R2C1 composite
pool pops

NP R2 – Selection
and intercrossing, 4
locs x 4 pops = 16
R2C2 pops

Boxes with dashed lines are completed
Boxes with solid lines are included in
this proposal

Northern Pools (SIBR, OTTM, CASIA, EURO)

Southern Pools Round 1 (SP R1)
191 PI accessions evaluated in CA(x2)

SP R1 – One
composite C1
population from
EI Centro, CA

SP R1 – Three
pool C1
populations from
Westside, CA

SP R1C1: Evaluation trials of four populations in CA(x2)

SP R1C1 – Seed
increase in WA
of 1 pop

SP R1C1 – Seed
increase in WA
of 3 pool pops

Release of 4 SP
R1C1 pool pops

SP R1 – Selection
and intercrossing 4
C2 pops

Southern Pools Round 2 (SP R2)
383 PI accessions evaluated in CA(x2), WA, FL

SP R2C1 spaced plant evaluation
CA(x2), FL

SP R2
Selection and intercrossing
4 locs x 4 pops = 16 C1 pops

SP R2C1 Seed
increase in WA of 4
pool composite pops

Southern Pools (IND, ARA, NAF, SAM)

Precision Agriculture Tools for Optimizing Alfalfa Production and Marketing



← Kim Cassida, A. Pouyan Nejadhashemi, Kyla Dahlin, & Babak Saravi
Yoana Newman →



Alfalfa & Forage Research Program



Goals

- 1) Development of landscape-scale remote sensing technologies for pre-harvest estimation of alfalfa yield and quality
- 2) Evaluate use of hand-held and small farm-scale benchtop units to estimate post-harvest forage nutritive value

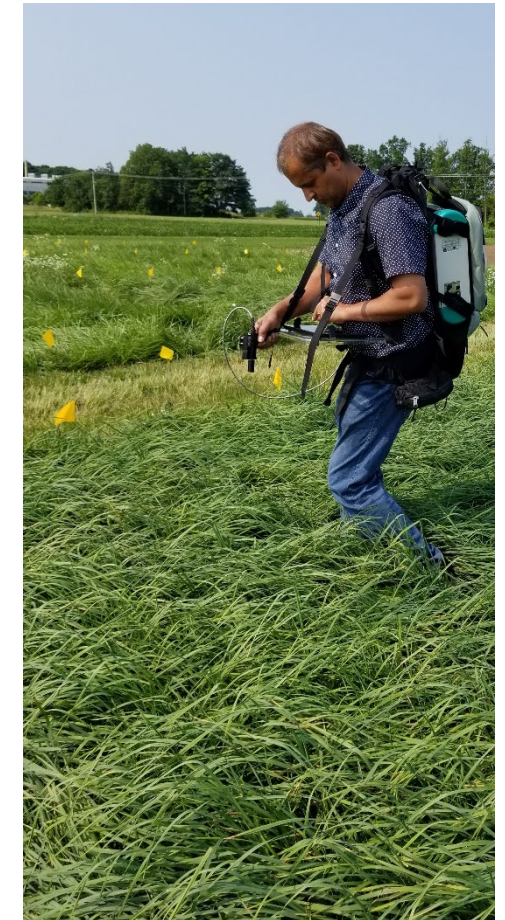
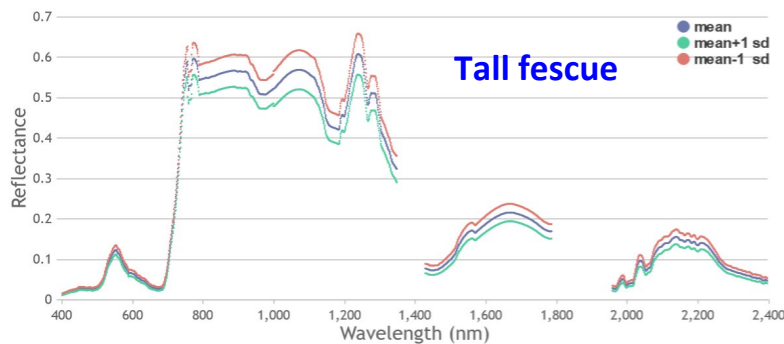
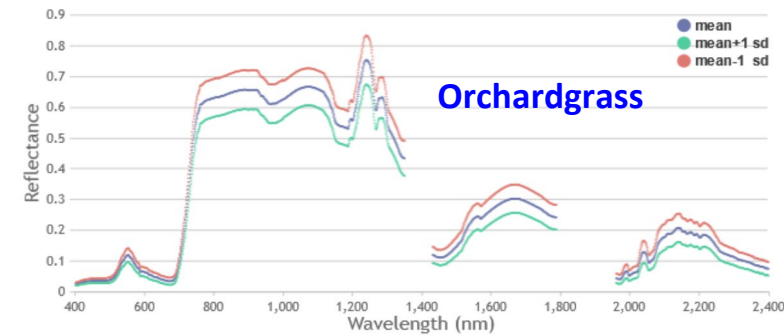
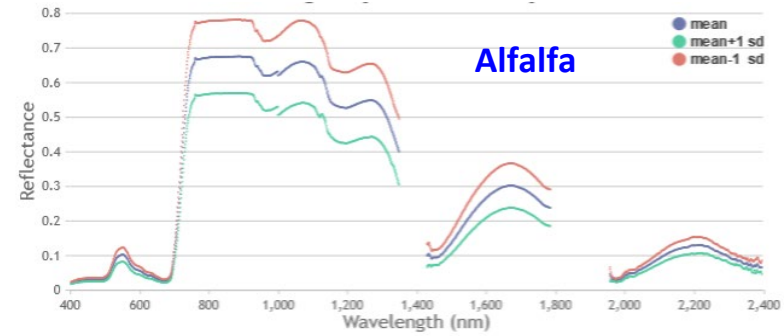
Objective 1. Identify spectral signatures for pre-harvest alfalfa, companion grasses, and mixtures

Spectra collected in 2019 from replicated multiple genotypes of alfalfa, orchardgrass, and tall fescue monocultures

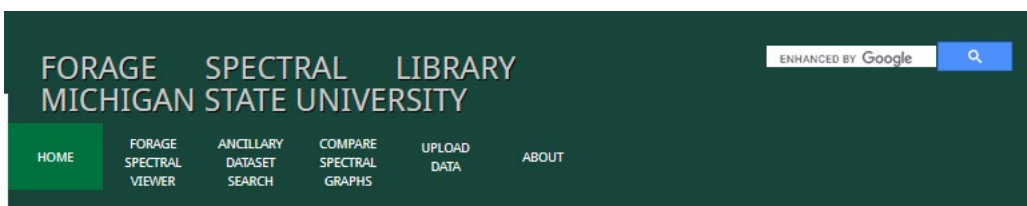
ASD Inc. FieldSpec 4 Hi-Res (spectral range of 350-2500 nm, bandwidth resolution 3 nm at 700 nm and 8 nm at 1400/2100 nm), measured plus/minus 2 hr from solar noon

Ancillary data collected:

- PAR above and below canopy
- Green canopy cover
- Stage of maturity
- Forage dry matter yield
- Volumetric soil moisture
- Nutritive composition



Forage Spectral Library Created



FORAGE SPECTRAL LIBRARY

Collecting spectra information from natural resources is becoming a common practice in remote sensing, resulting in many spectral signatures. However, methods for sharing spectra information are scarce. Here we designed the first forage spectral library. This project is supported by the US Department of Agriculture- National Institute of Food and Agriculture under the grant number 2018-70005-28738.



The Forage Spectral Library comprises four major functions, including:

- **The forage spectral viewer** allows users to graph a spectral library associated with certain plant species, varieties, and locations
- **The ancillary dataset search** permits full dataset query using different combinations of ancillary variables (plant height, PAR, crude protein)
- **The compare spectral graph tool** allows users to simultaneously compare spectral graphs while comparing their associated ancillary data
- **An upload data function** allows users to add a new set of spectral information along with associated ancillary data to the Forage Spectral



Objective 2. Use spectral unmixing algorithms to determine pre-harvest yield, nutritive composition, and abundance of alfalfa and grass in a mixture.



Collect spectra and ancillary data from alfalfa/grass plots for two cuttings at two locations in 2022.

Suitable unmixing algorithms could eventually be applied to drone-collected spectra to guide harvest decisions.



Objective 3. Develop extension materials to assist in adoption of precision technologies in alfalfa production

- Use NIR optical sensing technologies to increase knowledge and skills in forage crop quality testing for current and future producers (UW-RF, ongoing)
- Comparison of portable and research-grade NIRS instruments
- Development of a global NIRS calibration for “as-fed” whole moist alfalfa hay, haylage, and green chop (UW-RF, ongoing)
- Conduct online webinars on the use of remote sensing in alfalfa and forage production (winter 2022/23)



Developing Alfalfa for Intercropping with Intermediate Wheatgrass Towards *Perennial Grain- Forage Systems*

Virginia Moore, Julie Hansen, Jacob Jungers, Jared Goplen,
Valentin Picasso, Heathcliffe Riday, and Brandon Schlautman

2022 North American Alfalfa Improvement Conference

Lansing, MI

Identifying optimal **nitrogen rate** for alfalfa-IWG intercropping systems



N rate trial in Arlington, WI, Oct. 2021 (Picasso/Bures)

Four **locations** in KS, MN, NY, WI, planted in Fall 2021

Partial factorial, with **treatments** including:

- Crop treatments:
 - IWG monoculture
 - Three alfalfa varieties (FD 3-5) in monoculture & intercropped with IWG
- N rates: 0, 40, 80, 120, 160 kg N per ha

Data collection: fall stand count, winter survival, grain & forage yield/quality

Project Funding: *USDA-NIFA Alfalfa Forage Research Program (AFRP) 2021-70005-35692*

Breeding alfalfa for intercropping with IWG



Alfalfa seed increase (Crawford)

Alfalfa **populations selected** from previous alfalfa-IWG intercropping trial

- Selected from three locations (KS, MN, WI)

Timeline:

- *Summer 2021:* selected & dug plants
- *Ongoing:* seed increase in WI/WA
- *Fall 2023:* evaluation of Base & Cycle 1 populations in KS, NY, WI

Project Funding: *USDA-NIFA Alfalfa Forage Research Program (AFRP) 2021-70005-35692*

Extension needs for alfalfa-IWG intercropping systems



Stakeholder needs assessment:

- *Ongoing*: farmer & stakeholder interviews
- *Summer 2022*: field day presentations & facilitated discussions

Planned extension products:

- Fact sheets
- Webinar
- Video

Project Funding: USDA-NIFA Alfalfa Forage Research Program (AFRP) 2021-70005-35692

Project timeline

| | 2021 | 2022 | 2023 | 2024 |
|--------------------------|--|--|--|---|
| N-rate study | Protocol development, planting in KS, MN, NY, WI | Year 1 data collection & analysis | Year 2 data collection & analysis | Year 3 data collection, analysis, publication |
| Alfalfa selection | Alfalfa selection & seed increase | Seed increase | Protocol development, planting in KS, NY, WI | Data collection, analysis, publication, variety release |
| Extension | Hiring | Farmer & industry interviews, field days | Field days; fact sheet, webinar, and video development | Field days; publication and dissemination of materials |

Project Funding: USDA-NIFA Alfalfa Forage Research Program (AFRP) 2021-70005-35692

Advancing the use of alfalfa leaf protein concentrate in aquafeeds to enhance finfish production and reduce environmental impacts in aquaculture production

Deborah A. Samac; USDA-ARS
Dong Fang Deng; University of Wisconsin-Milwaukee
Brian Shepherd; USDA-ARS
Matt Digman; University of Wisconsin-Madison



National Institute of Food and Agriculture

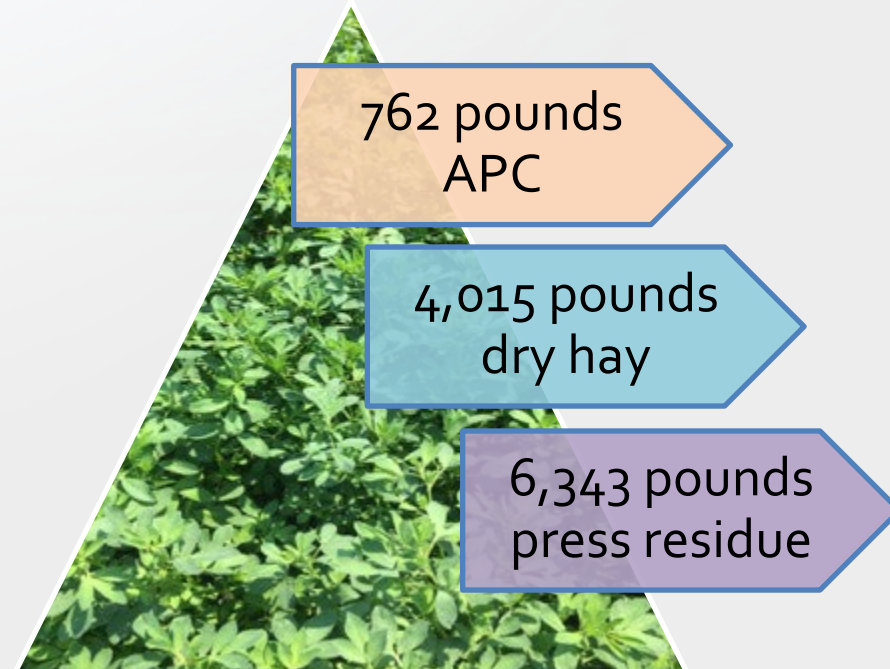
U.S. DEPARTMENT OF AGRICULTURE

Past research on alfalfa protein concentrate (APC)



High lysine, high xanthophyll protein concentrate from juicing fresh alfalfa.

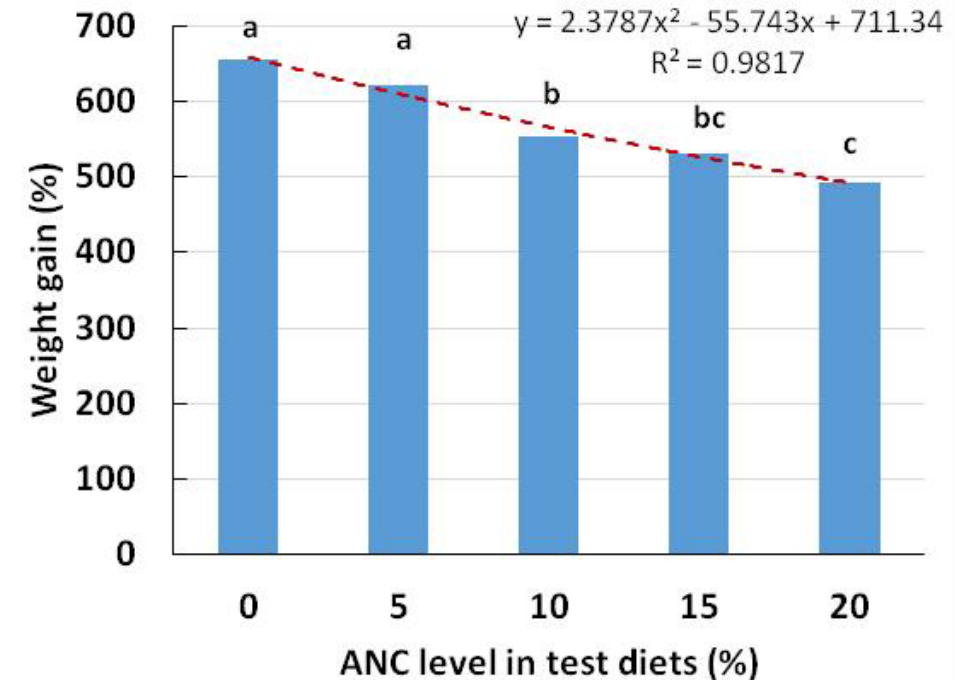
1 Acre of Biomass Type Alfalfa



Preliminary studies with yellow perch successful.

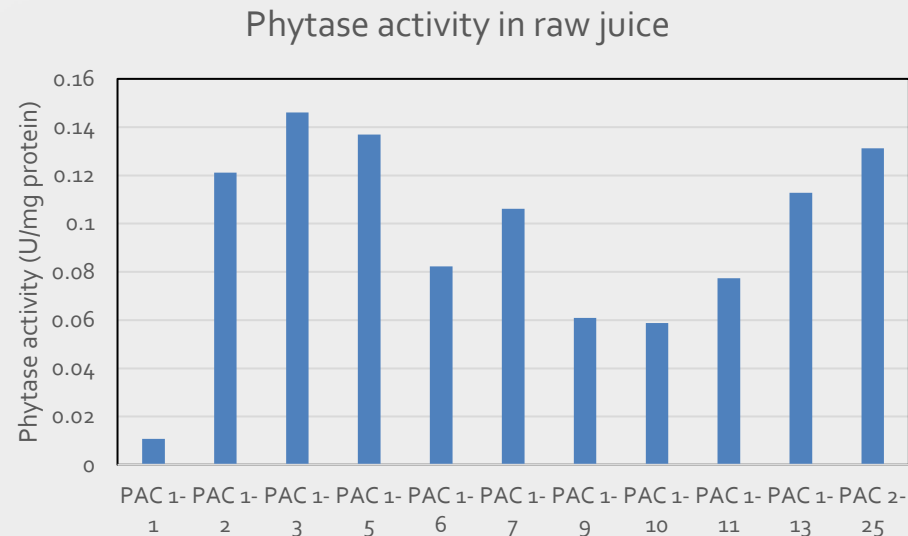
Objective 1: Evaluate the Potential of APC as an Ingredient in Feed Production for Rainbow Trout.

- Effect of APC on physical quality of feed pellets
 - Increasing APC increases density, durability, durability and sinking of pelleted feed
- Effect on feeding response
 - No effect on palatability or feed intake
 - Phosphorus apparent digestibility coefficient was significantly decreased
- Effect on digestibility
 - 20% APC decreased DM digestibility
 - 10-20% APC decreased P digestibility
 - Slowed growth
 - Altered amino acid metabolism
- 12% optimum replacement level



OBJECTIVE 2: EVALUATE THE EFFECT OF APC WITH PHYTASE

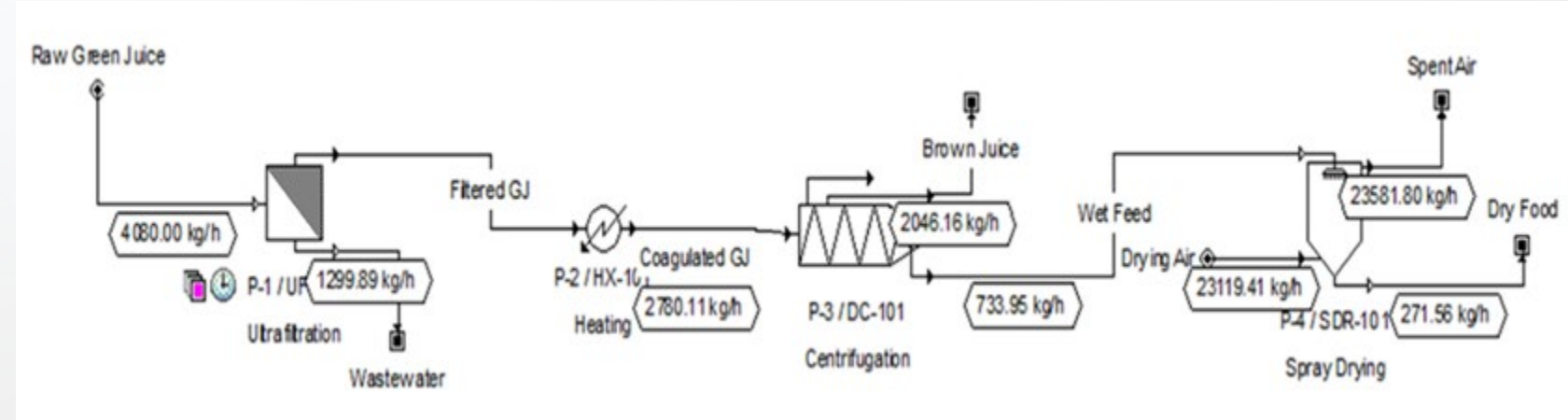
- Phytase releases phosphate from plant feeds
- Use commercial phytase as an additive to trout feed
 - Measure growth, health, and water quality
- Field growth of transgenic alfalfa plants expressing phytase
 - Quantify in plants, juice and protein concentrate



Measure phytase in:

- juice
- reconstituted dried juice
- protein concentrate; dry, freeze dried
- dried herbage

OBJECTIVE 3. CARRY OUT A TECHNOECONOMIC AND MARKET ANALYSIS (TEA) OF APC PRODUCTION



- Equipment used:
 - Forage Harvester w/ direct-cut forage header
 - Tractors and forage wagons
 - Tractor and macerator /screw press machine
 - Tractor and forage bagger for press cake by-product
- Estimated field costs: \$10.74/kg protein concentrate

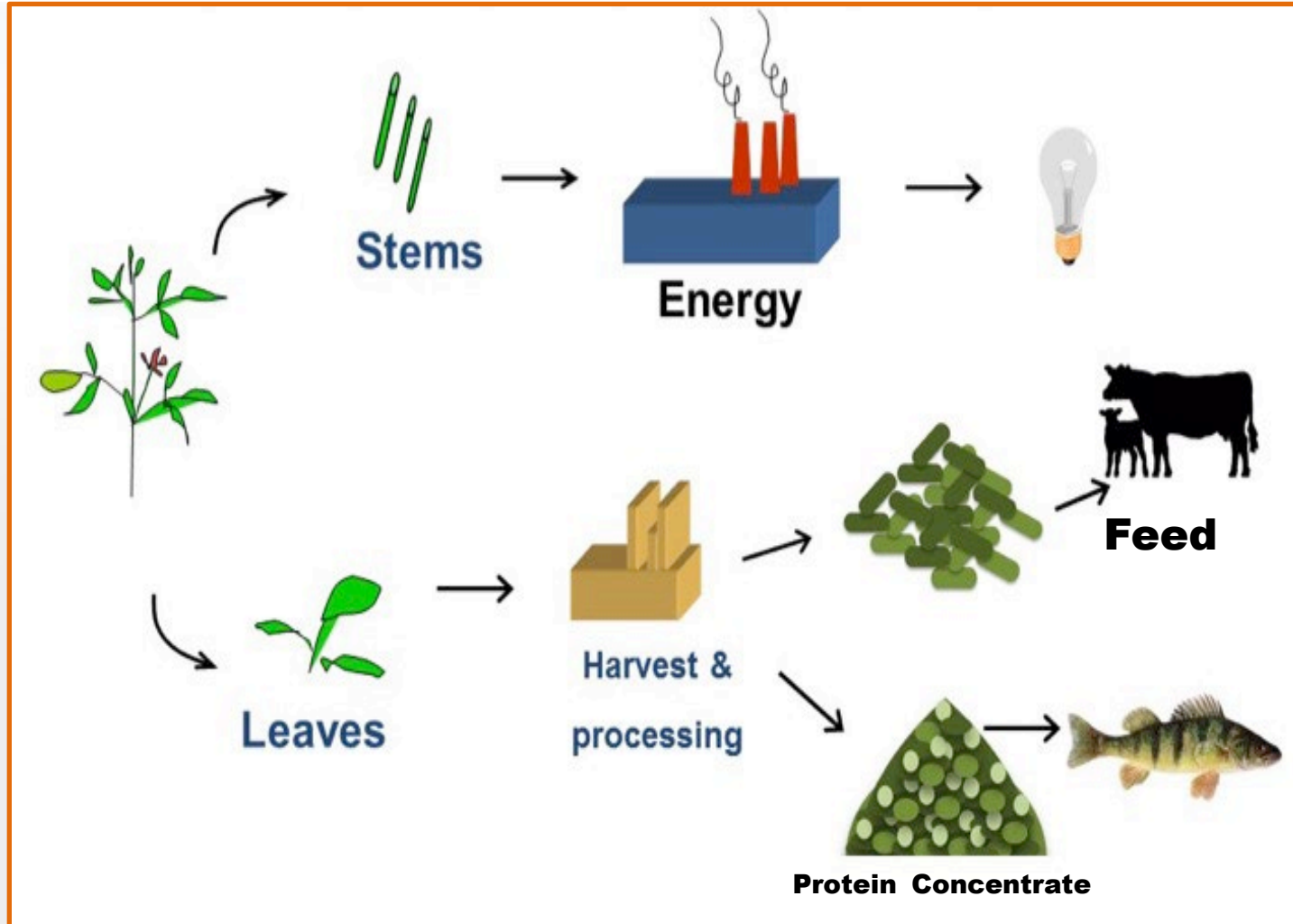
Processing facility

244 kg protein concentrate/hr

Utilizing a continuous flow facility

Costs still preliminary, more refinement is needed

Biorefining of alfalfa



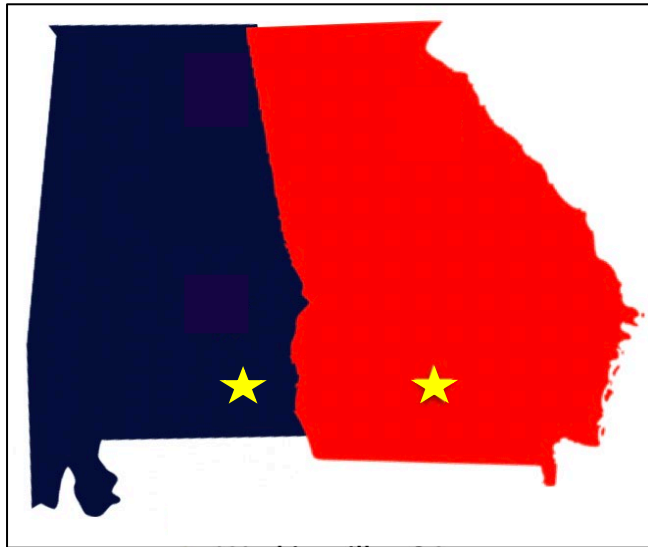
White protein concentrate
Food and food products
Cosmetics

Press cake
Animal feed
Bioenergy feedstock
Paper feedstock
Adhesives

Separation
Enzymes, Dyes
Carbohydrates
Vitamins
Food supplements

Fermentation
Lactic acid
Amino acids
Enzymes
Organic acids
Ethanol
Biogas

Alfalfa Nutrient Preservation, Utilization and Cycling in Sustainable Southeastern Livestock Systems



NIFA ASAFS
2021-06151



United States Department of Agriculture
National Institute of Food and Agriculture

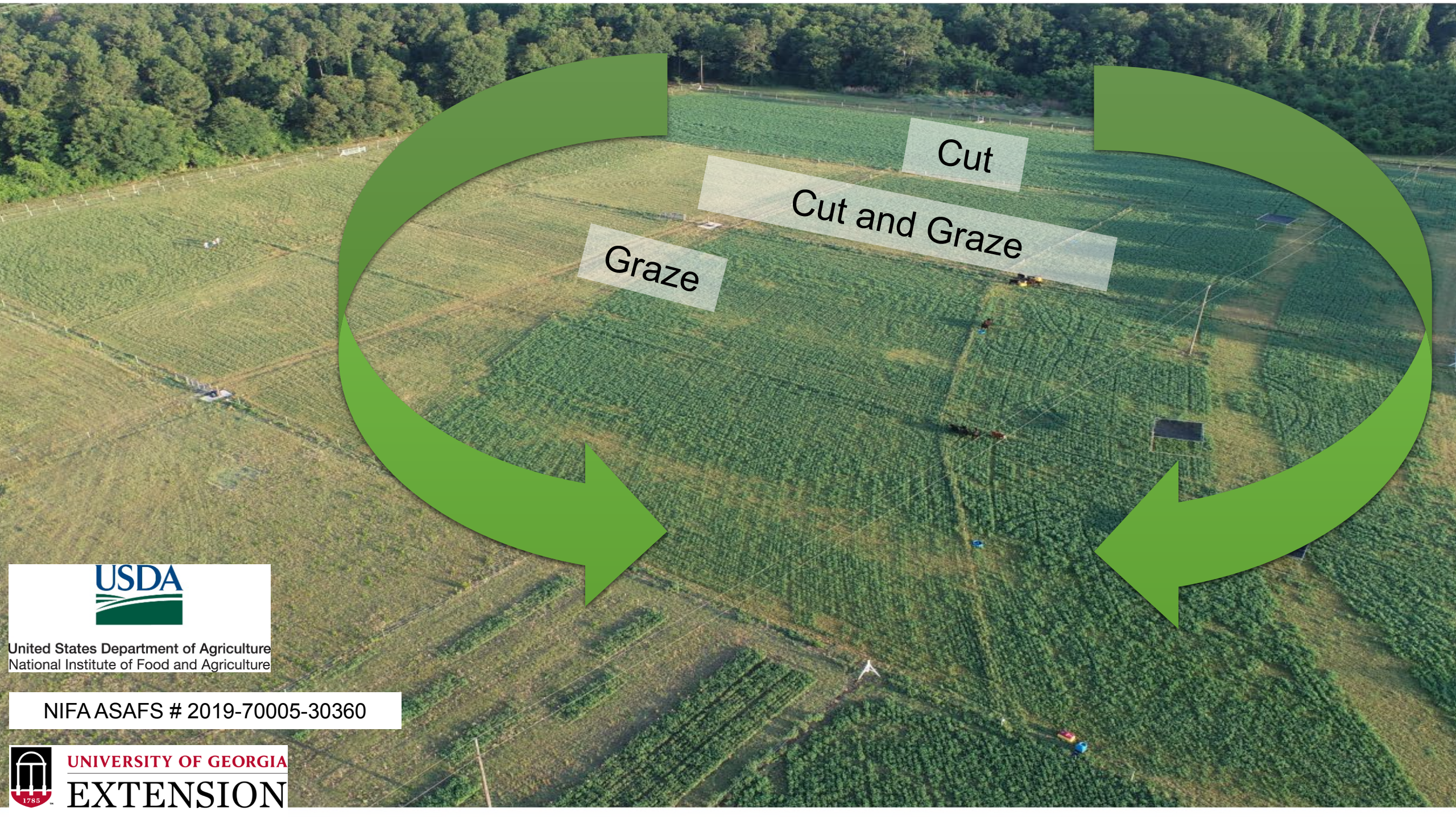
Jennifer J. Tucker, M. Kimberly Mullenix, Chris Prevatt, Liliane S. da Silva, Sandra L. Dillard, Todd Callaway, and Lisa L. Baxter



UNIVERSITY OF GEORGIA
EXTENSION



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EXTENSION



Cut

Cut and Graze

Graze



United States Department of Agriculture
National Institute of Food and Agriculture

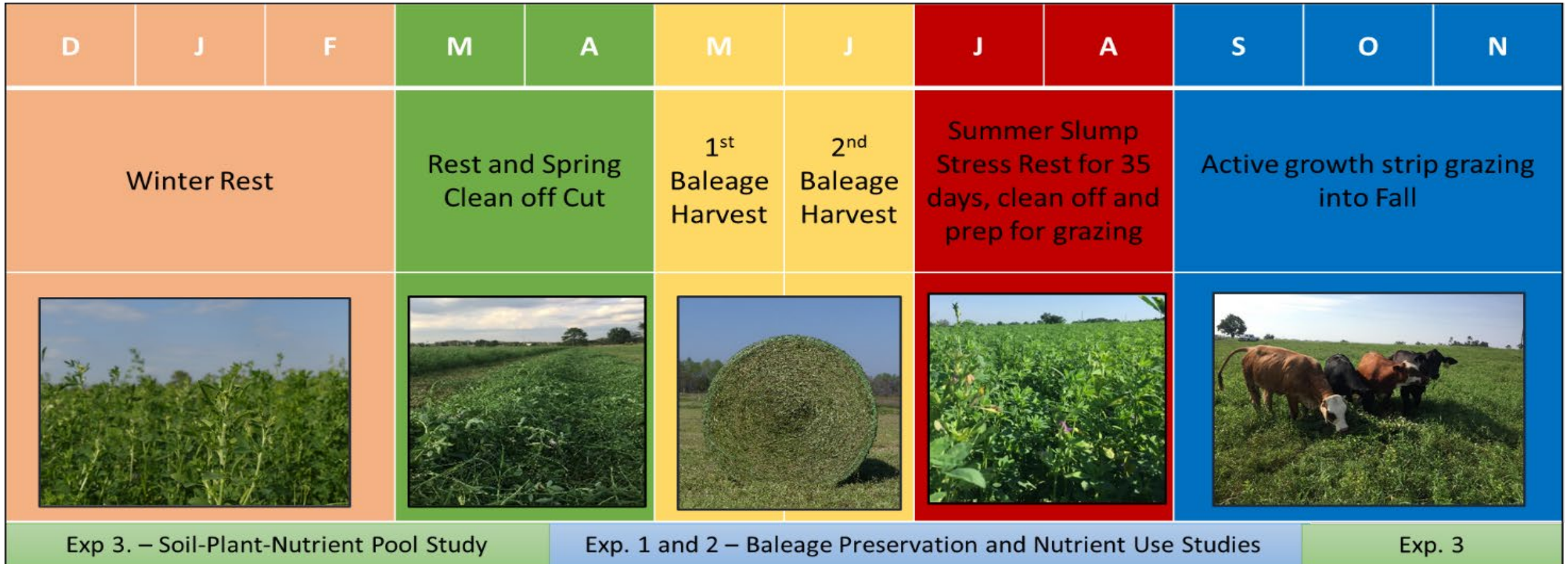
NIFA ASAFS # 2019-70005-30360



UNIVERSITY OF GEORGIA
EXTENSION

Goal:

Translate ancillary system benefits of Alfalfa based systems to producers to enhance adoption, use and sustainability in southern forage-livestock operations



Management/research timeline for dual-purpose cut-and-graze Alfalfa-Bermudagrass mixtures



Objectives:

- Research
 1. Evaluate the use of forage preservatives and inoculants to assess field dry down time and bale package preservation.
 2. Quantify nutrient use efficiency of alfalfa-bermudagrass baleage in livestock systems
 3. Assess forage and soil nutrient pools under alfalfa-bermudagrass pastures.
- Economics & Extension



Benefits:

The data obtained will:

1. Improve product preservation as a high-quality feed for livestock
2. Quantify, define, and illustrate nutrient use and cycling benefits to the animal and pasture
3. Develop economic tools for producers considering alfalfa use under these applications
4. Significantly impact alfalfa production not only in the South but nationwide.





IDENTIFYING MOLECULAR MARKERS ASSOCIATED WITH QUALITY & QUANTIFYING THEIR POTENTIAL TO INCREASE ALFALFA VALUE

**Cesar A. Medina, WSU, Sen Lin, WSU; Long-Xi Yu, ARS;
Geoffrey Zanton, ARS, Glenn Shewmaker, UI; Guojie
Wang, OSU; Don Llewellyn, WSU, Steve Fransen,
WSU; Steve Norberg, WSU;**

Funded
By:



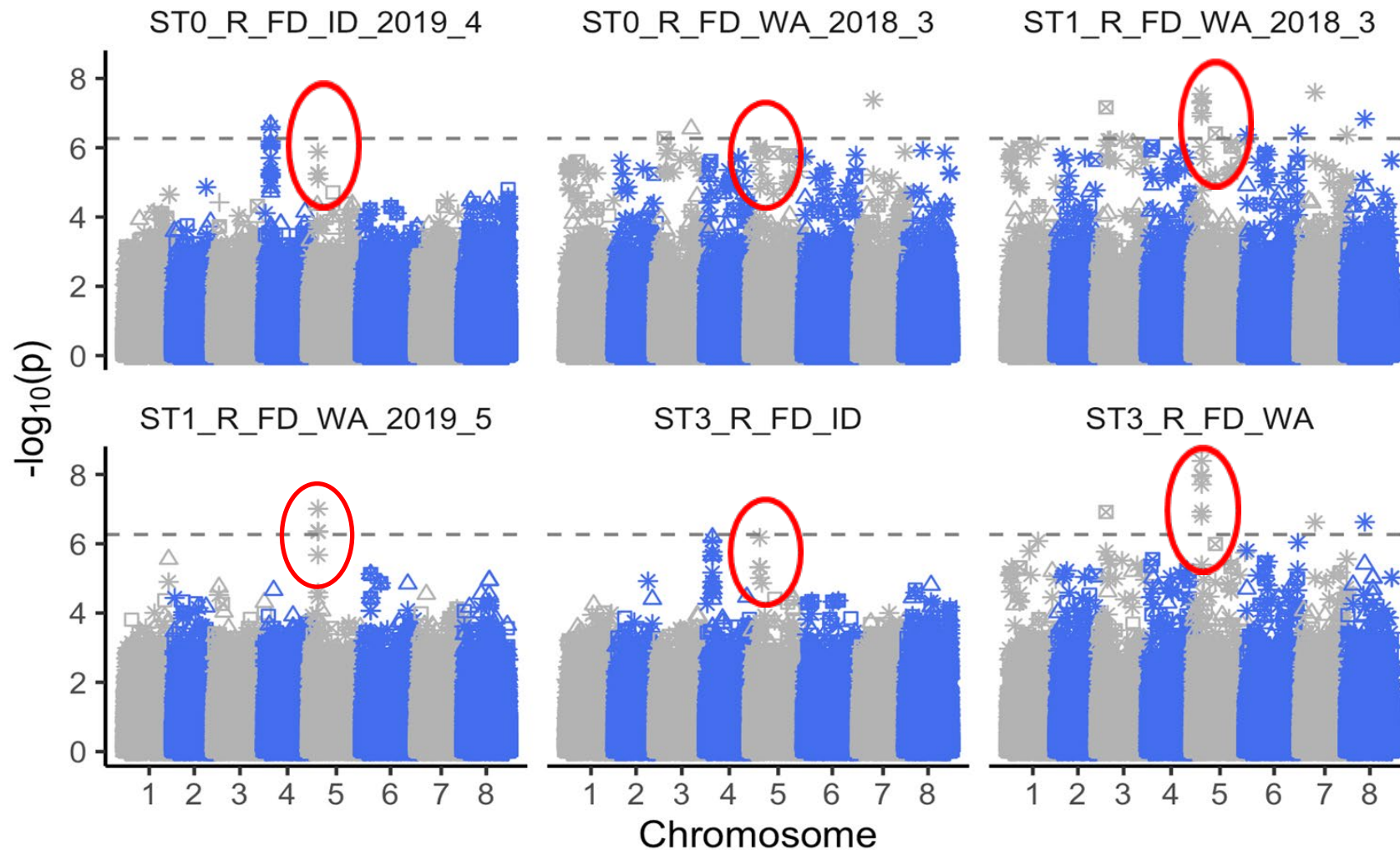
United States
Department of
Agriculture

National Institute
of Food and
Agriculture

Breakdown of the 200 entries in this irrigated study planted in Idaho, Oregon, and Washington

| Region | Country | N |
|----------------|---|-----|
| North America | Canada (21), United States (121) | 138 |
| Turkey | Turkey | 21 |
| Central Asia | Afghanistan, Armenia, Georgia, Kazakhstan, Turkmenistan | 14 |
| Eastern Europe | Belarus (1), Russian Federation (8) | 9 |
| China | China | 8 |
| Central Europe | Czech Republic, Denmark, France, Germany | 4 |
| Mediterranean | Greece, Morocco, Romania, Spain | 4 |
| Other | Australia, Japan | 2 |

Markers strongly associated with fall Dormancy on Chromosome 5



Significant Molecular Markers Found

- Twenty-seven significant markers were associated with ADICP (3), NDICP (4), yield (7), and fall dormancy (13).
- Interestingly, using *Medicago truncatula* genome as reference genome one transcription factor (TUBBY family) and one transcription regulator, (BTB-POZ-MATH family [BPM proteins]) were associated with ADICP.

Significant findings

- Stagewise statistical approach produced more significant markers using GWAS consistently across multiple datasets than when the single-trail approach.
- Using *Medicago truncatula* genome as reference genome for annotation the protein NUCLEAR FUSION DEFECTIVE 4-like (NFD4) in chromosome 5 is responsible for 10 of the 13 molecular markers found for fall dormancy.

Establishing the Value of Alfalfa with Highly Digestible Fiber

Doohong Min*, Rudra Baral, Muhammad Ibraheem, Barry Bradford, Kassidy Buse, Paul Kononoff, and Krishna Jagadish

Kansas State University
Michigan State University
University of Nebraska

Objectives

Physiological and agronomic characterization of field-grown alfalfa cultivars exposed to a range of water-deficit stress conditions during different stages of maturity

Determine impacts of alfalfa hay varying in neutral detergent fiber digestibility (NDFD) on productivity and energetics of lactating dairy COWS

Methodology

Research Institutions

- ✓ Kansas State University, Manhattan KS
- ✓ Michigan State University, E. Lansing, MI
- ✓ University of Nebraska-Lincoln NE

Agronomic trial at Kansas State Univ.

- ✓ 3 water treatments (drought imposed, irrigated and rainfed)
- ✓ 5 commercial varieties (i.e., HybriForce 3400, 54 HVX42, HiGest 460, 455TQRR, and Megatron)
- ✓ 3 different stages of maturity (late bud, early flowering, and 7 d after flowering)



Methodology

Animal performance trial

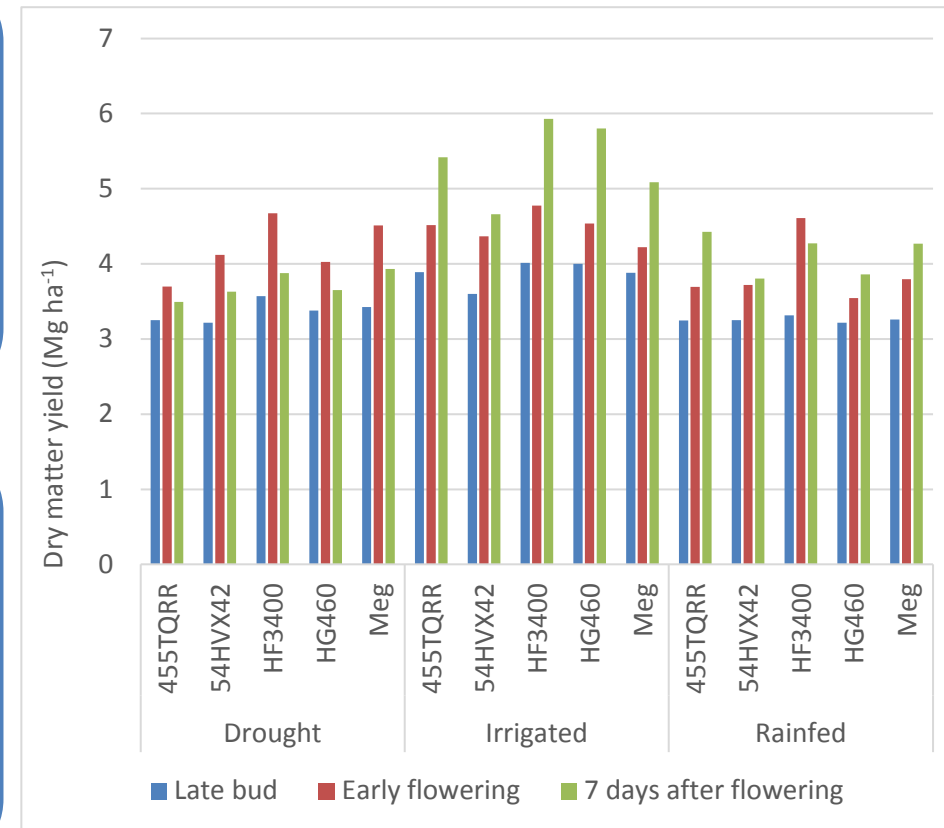
- ✓ 3 alfalfa varieties (HybriForce 3400, 54HVX42, and HiGest 460) were used.
- ✓ 12 multiparous Jersey cows were used in NE.
- ✓ 60 multiparous mid-lactation Holstein cows were used in MI.
- ✓ 2-week baseline period and 6-week treatment period.
- ✓ 3 feeding treatments: Conventional (CON), 50:50 blend of CON and lower-lignin (LL), and LL.



Results

Year 1 result showed the highest DMY in the conventional variety (HF3400) followed by lower-lignin (HG460) harvested 7 days after early flowering grown under irrigation at Kansas State University

On average, conventional variety (HF3400) performed better yield under all 3 water treatments (drought, rainfed, and irrigation) over lower-lignin alfalfa (HG460)



Results

Feeding trial treatments had no impact on milk yield or protein content in both MI and NE.

However, lower-lignin (LL) alfalfa hay linearly decreased milk fat concentration when it replaced a conventional variety in MI.

Total-tract NDF digestibility was also linearly decreased by LL, with no impact on total-tract crude protein (CP) or starch digestibility in both locations.

