Alfalfa Breeding at FGI

• Improved forage yield
  – Yield per se
  – Persistence per se
  – Changes in FD/WH relationship

• Improved forage quality
  – Conventional breeding
  – GE traits

• Increased tolerance to biotic/abiotic stresses
  – Multiple pest resistance
  – Selection strategies for tolerance to abiotic stresses
FGI Research – New Traits

• Native Genes
  – Abiotic stress (Salt/Drought/Cold)
  – Pest resistance
  – Traditional and molecular approaches

• Novel alleles – mutations

• GE traits
  – Herbicide tolerance (RRA)
  – Quality traits
Re-Designing Alfalfa for Improved Forage Quality

Mark McCaslin
Forage Genetics
Re-Designing Alfalfa for Improved Forage Quality

• The CAI concept:
  – Interdisciplinary, inter-institutional collaboration
  – Ruminant nutrition
  – Biochemistry
  – Plant molecular biology
  – Plant breeding/agronomy

• CAI target traits – “re-designing alfalfa”
  – Increased cell wall digestibility
  – Improved efficiency of protein utilization
Reduced Lignin Alfalfa

- Lignin increases with advanced maturity in alfalfa.
- Lignin is indigestible, and binds with cellulose/hemicellulose – reducing fiber digestibility.
- Reducing lignin content should increase fiber digestibility and alter change in quality w/ maturity.
- Genetic engineering can be used to reduce lignin content in alfalfa
  - “knockout” genes for key enzymes in the lignin biosynthetic pathway.
Gene knockout to modify lignin pathway

Use genetic engineering to knockout/silence “lignin gene(s)” in alfalfa

Compound A

\[ \text{Enzyme 1} \quad \text{Gene 1} \]

Compound B

\[ \text{Enzyme 2} \quad \text{Gene 2} \]

Lignin

Reduced lignin alfalfa
2008 Summary – RL1 Alfalfa

- 28% lignin reduction
- 24% increase in fiber digestibility

Note: lower stem samples, CAI equation, average of 25 elite events
Proof of Concept FGI Reduced Lignin Alfalfa

April 27, 2007
USDFRC Sheep Feeding Studies
RL1 Alfalfa
Changes in NDF Digestibility over Time

RL Alfalfa = Increased flexibility in harvest timing

NDFD%

Reduced lignin
Null control

Early bud Late bud 10% bloom
Yield/Quality Tradeoff

Putnam and Orloff, 2013
2011-12 Combined Data

Yield - Lbs per plot

- RR/RL
- 54H11
- Liberator

FGI/Monsanto Confidential
2011 RL Cutting Management Test
Touchet, WA

Six Harvests

Five Harvests

Spring Regrowth: 03-05-2013
2011 RL Cutting Management Test
Boone, IA

Photo: May 7, 2013

4 cuts/35d cutting schedule  5 cuts/28d cutting schedule
2011-12 Combined Data

NDFD

RR/RL  54H11  Liberator

7 Cuts
9 Cuts

FGI/Monsanto Confidential
HarvXtra™ Alfalfa

- In an effort to bring the industry’s first quality-enhancing trait to market, FGI would like to announce that the reduced lignin trait will be known as HarvXtra™ alfalfa.
HarvXtra™ Alfalfa Product Concept

- 10-15% increase in whole plant NDFD*  
  - enables a delayed harvest
- Breeding stack w/ RRA  
  - 90% trait purity – both traits
- Competitive agronomic performance  
  - Excellent forage yield  
  - No increase in lodging incidence  
  - MPR, WH and persistence = best in class

*NDFD should not be interpreted as a direct measurement or prediction of animal performance potential, but simply as one of several forage quality metrics commonly used by the forage community.
HarvXtra FD Exptl vs Checks

NDFD

2013/14 - 5 locations/37 total harvests

ADL

% Checks

HarvXtra ND Exptl vs Checks

2014 - 2 locations/15 total harvests

% Checks


NDFD

ADL
HarvXtra™ Alfalfa Potential Benefits

• Delayed harvest advantages
  – Fewer harvests
  – Higher forage yield
  – Improved persistence
  – Unique forage quality parameters
  – Increased harvest timing flexibility

• Forage quality advantage
  – Higher likelihood of harvesting premium quality hay
HarvXtra™ Timeline

- Q4 2014 Deregulation U.S./Canada
- Deregulation pending:
  - Hay export markets
  - MX and Argentina
- 2015 on-farm testing in Midwest
- 2016 limited launch, pending global approvals.
- 2017 broader commercial launch
Tannin Alfalfa

• FGI/Noble/USDFRC collaboration to improve efficiency of alfalfa protein utilization for dairy.

• Tannins bind with protein, slowing rate of rumen degradation and increasing RUP (bypass protein).
  – Increasing RUP 20% → 30% would eliminate need for protein supplements

• Condensed tannins can be found in alfalfa seed coats, but not leaves or stems.
  – Genetic engineering can be used to modify gene expression, producing Tannin Alfalfa.
Condensed Tannin Biosynthesis

Hancock et al, 2012

Proanthocyanidins (Condensed tannins)
Condensed Tannin Biosynthesis

Evaluation of various CT pathway regulatory genes

Hancock et al., 2012

Proanthocyanidins (Condensed tannins)
DMACA Staining for CT in Alfalfa

Control
CT transgenic
Pardee BFT
Tannin Alfalfa Next Steps

• Improve CT characterization
  – Content and composition

• Assay development – RUP effect
  – *in vitro* assay
  – Small scale animal feeding trial late 2015

• Pre-breeding and trait development to fully test CT Product Concept
Tannin Alfalfa

- USDFRC MW dairy model for tannin alfalfa:
  - 60% reduction in protein feed supplementation
  - Up to 12% increase in net return for dairy
  - 25% reduction in N losses
  - Increase value of alfalfa silage by $23/T

- Tannin containing forages are non-bloating
  - Worldwide alfalfa related bloat losses > $200M

Consortium for Alfalfa Improvement
Collaboration Team Leaders

- Noble Fdn – Rick Dixon/Fang Chen
- USDFRC – Martin/Riday/Mertens
- AgCanada – Margie Gruber
- U of Victoria – Peter Constable
- NZ AgResearch – Kerry Hancock
- UCD – Dan Putnam
- UW – Dan Undersander
- LOL/Purina – David Weakley
- Pioneer – Dave Miller
- FGI – Temple/Whalen/McCaslin