Genotypic Evaluation of Bermudagrass for Conversion to Ethanol

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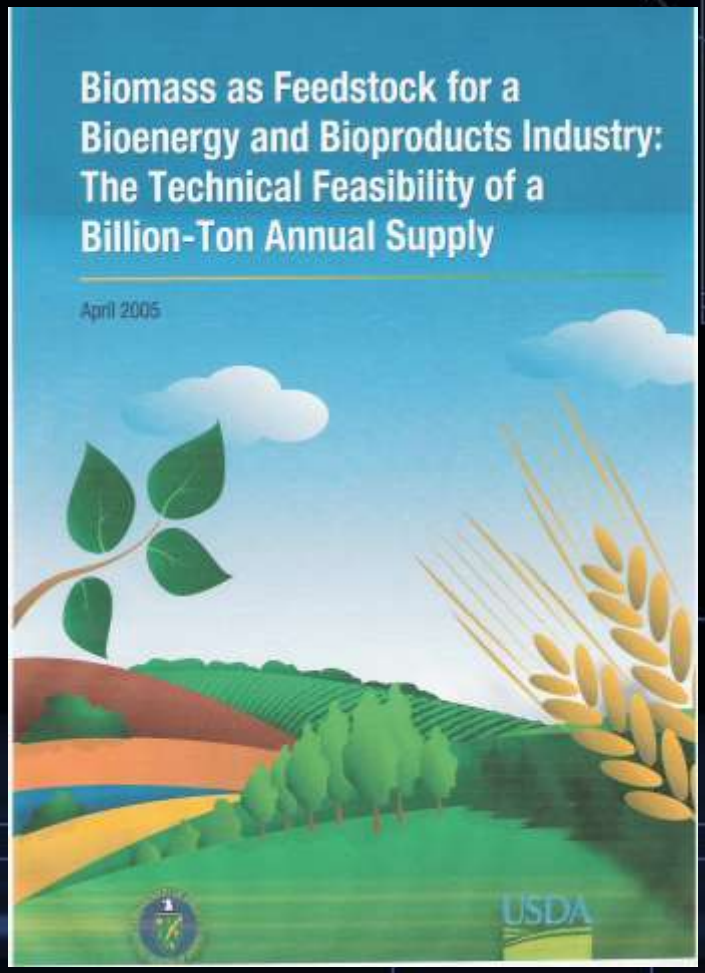
USDA/ARS – Tifton, GA

Feedstock for Ethanol

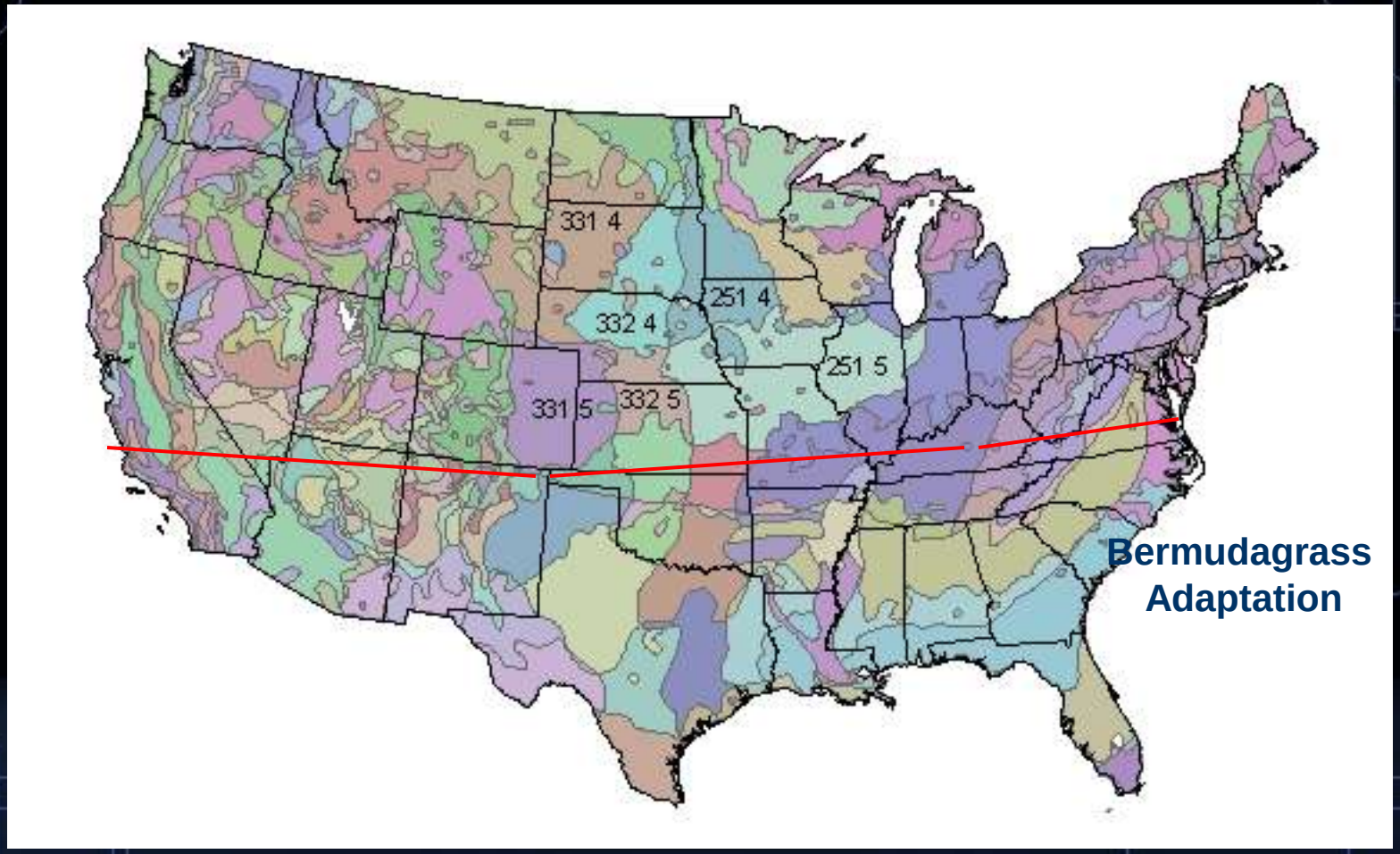
- Sucrose (Brazil) – sugar cane
- Starch (currently in United States) – grains
- Cellulose
  - Biomass
    - Crop residues
    - Trees and timber residue
    - Dedicated energy crops

U.S. Departments of Energy and Agriculture plan - 2005

- Forest biomass – 120 million tons
- Crop residues – 446 million tons
- **Perennial crops** – 377 million tons
- Agricultural grains – 87 million tons
- Animal wastes – 87 million tons



Plant Adaptation

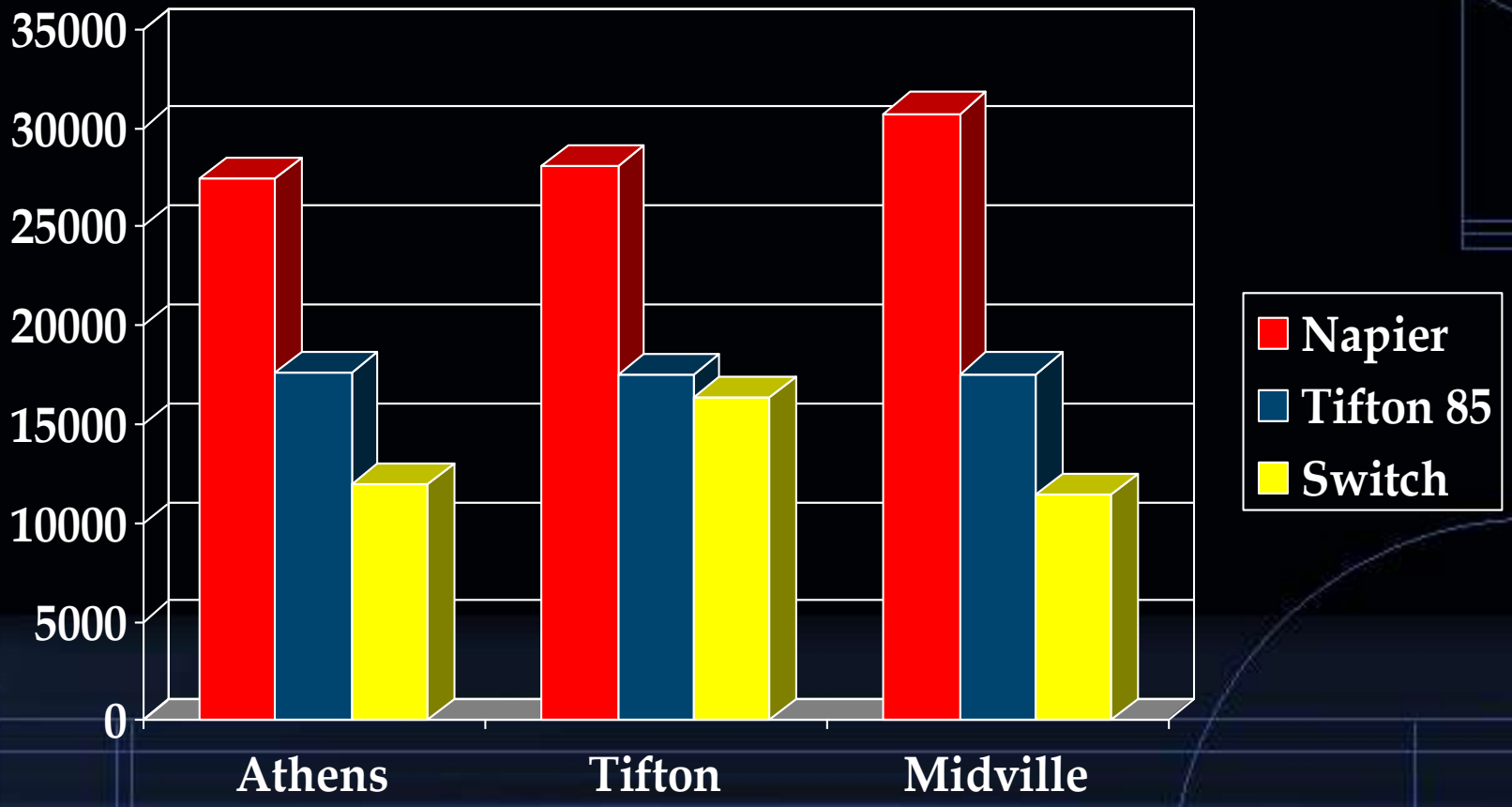


Vogel USDA/ARS

Bermudagrass – Primary Pasture of the South

Grass Yields – 5 year average (kg/ha) 1997-2001 - Georgia

(J. Bouton)



Comparison of Ethanol Efficiency

- Bermudagrass (Tifton 85, Coastal) at 12 weeks, Napiergrass (Merkeron, N 190), Giant reed (Two clones) at full season
- Leaf and stem separated, dried, ground
- Dilute sulfuric acid pretreatment – autoclaved
- Neutralized, cellulase and yeast (SSF)
- Tested for ethanol after 72 hrs

Grass comparison Conversion efficiency

Species	Genotype	Tissue	ADL	Ethanol mg/g
Bermudagrass	**Tifton 85**	**Leaf**	**2.93 a**	**139.6 a**
Bermudagrass	**Tifton 85**	**Stem**	**4.04 b**	**141.1 a**
Bermudagrass	**Coastal**	**Leaf**	**3.85 b**	**121.7 b**
Giant reed	**Cicily**	**Leaf**	**3.82 b**	**109.0 bc**
Napiergrass	**Merkeron**	**Leaf**	**3.04 a**	**106.7 bc**
Napiergrass	**Merkeron**	**Stem**	**6.95 c**	**105.3 c**
Napiergrass	**N 190**	**Leaf**	**3.53 ab**	**96.7 cd**
Giant reed	**Fitzgerald**	**Leaf**	**4.14 b**	**84.8 d**
Napiergrass	**N 190**	**Stem**	**7.90 d**	**84.0 d**
Giant reed	**Fitzgerald**	**Stem**	**8.98 e**	**47.2 e**
Giant reed	**Cicily**	**Stem**	**8.67 e**	**44.2 e**

Means with the same letter are not significantly different (p=0.05)

Genotypic Variability Bermudagrass

Bermudagrass Yields Across the South

Dry matter yield, kg/ha

Cultivar	Gulf Coast, AL 2002	Gulf Coast, AL 2003	Griffin, GA 2003	Griffin, GA 2004	Tifton, GA 2004	Tifton, GA 2006	Brooksville, FL 2004
Coastal	9128	8353	10797	15400	13518	12966	12241
Tifton 44	8701	6557	9555	10236	14860	12148	14150
Russell	8420	7140	12909	18410	15968	18119	13700
Tifton 85	9201	8775	13755	15996	15617	23343	17704

Forage Quality

- Rumen digestibility (*In vitro* Dry Matter Digestibility) IVDMD
- Neutral Detergent Fiber (NDF)
- Acid Detergent Fiber (ADF)
- Acid Detergent Lignin (ADL)

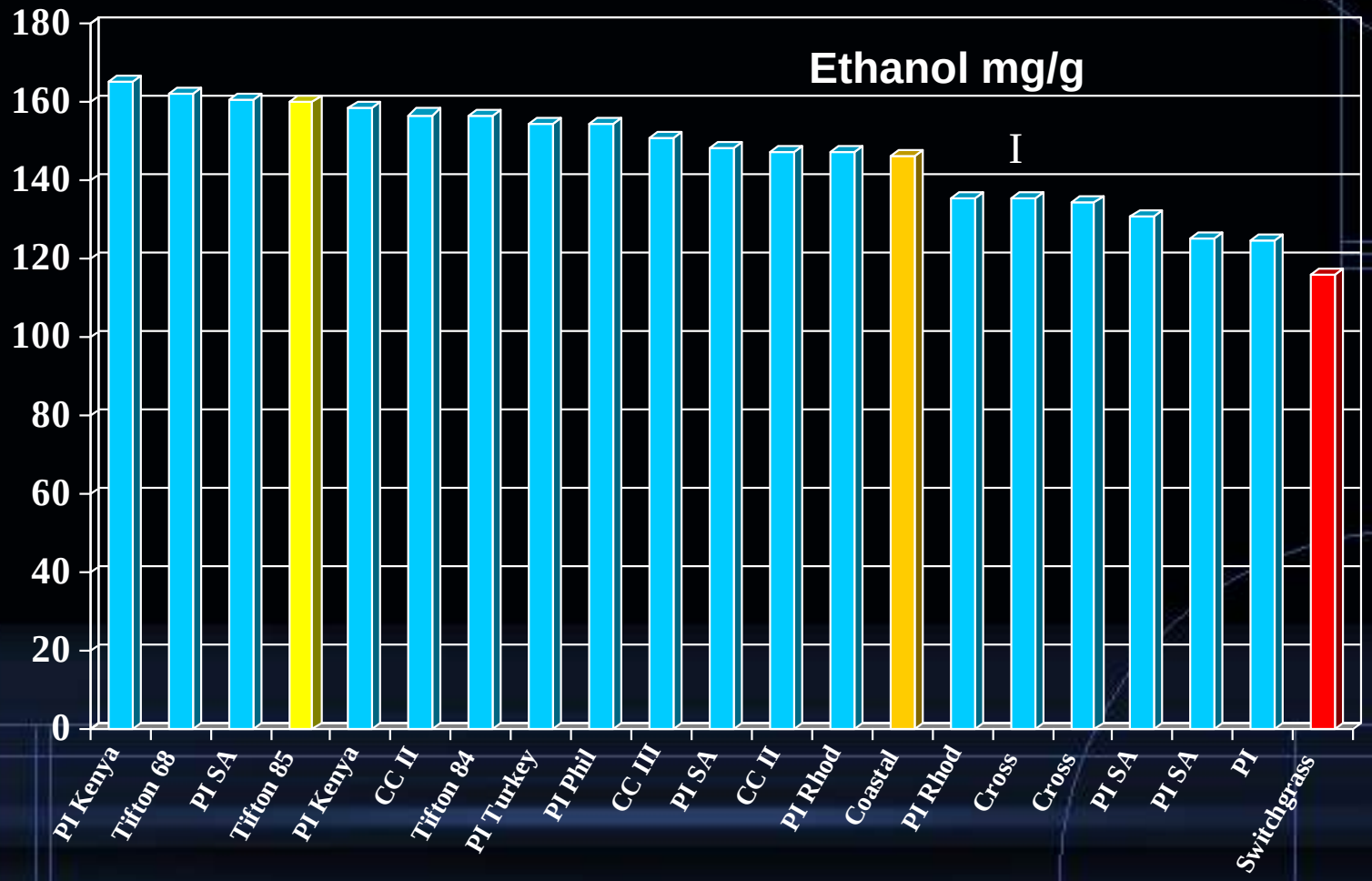
Variation in Rumen Digestibility and Fiber

- Range among 168 entry bermudagrass core collection:
  - IVDMD - 48.3% to 71.0%
  - NDF - 64.3% to 77.3% (R^2 = -0.86**)
  - ADF - 24.1% to 34.5% (R^2 = -0.58**)
  - ADL - 3.3% to 8.9% (R^2 = NS)

Variability in Ethanol Production among Bermudagrass

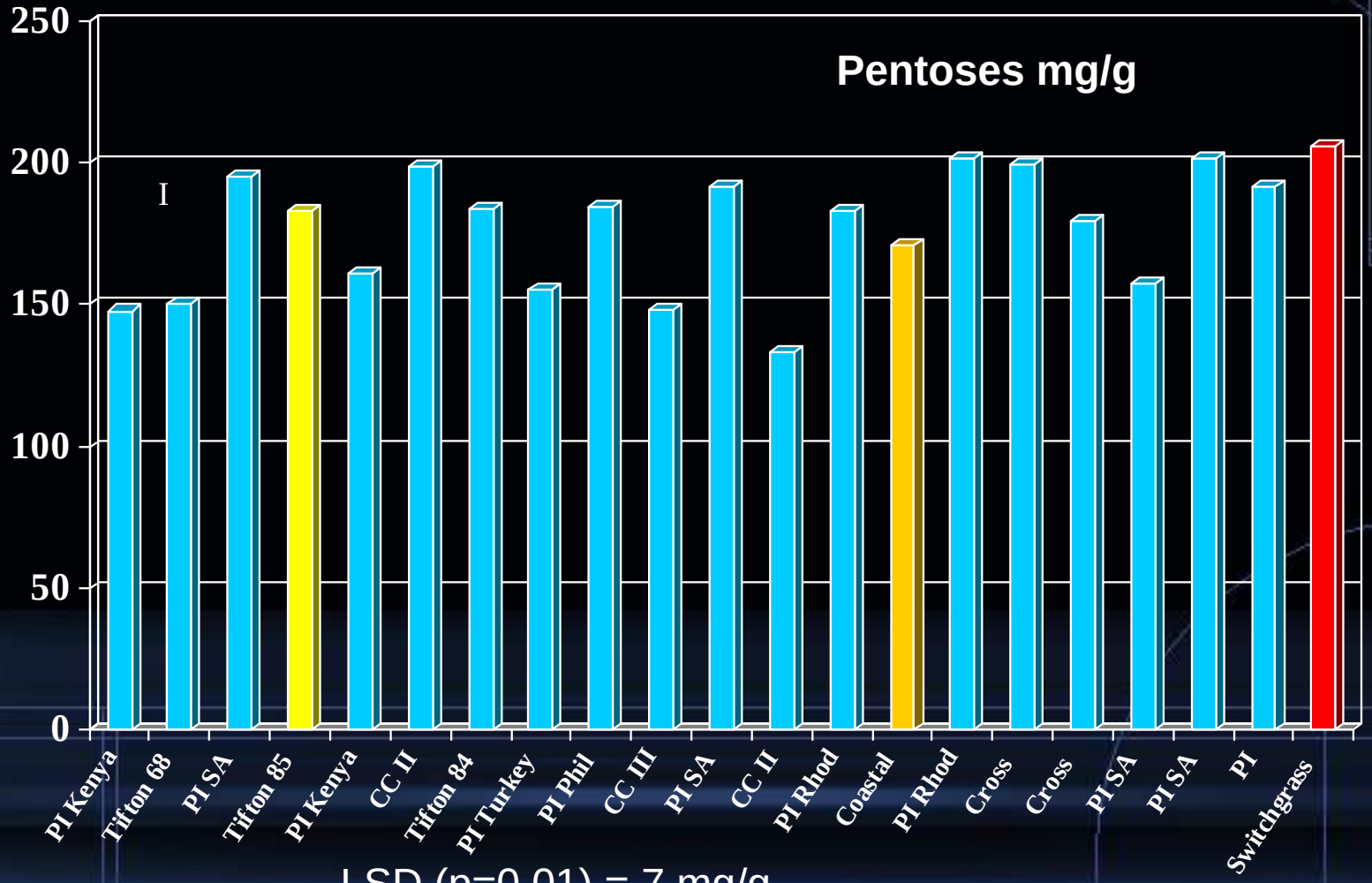
- Bermudagrass entries selected from core collection with diverse IVDMD, NDF, ADF, and ADL – compared with MPV2 Switchgrass
- Subjected to Dilute Acid Hydrolysis Pre-treatment (1.75% sulfuric acid - 121ºC for 1 hour)
- Underwent SSF – cellulase, Novozyme 188 cellobiase and *Saccharomyces cerevisiae* D5A
- HPLC for ethanol and pentoses after 72 hr

Ethanol Production Among Diverse Bermudagrass – SSF after 72 hr



LSD (p=0.01) = 7 mg/g

Xylose and Arabinose Yields Among Diverse Bermudagrass – SSF after 72 hr



LSD (p=0.01) = 7 mg/g

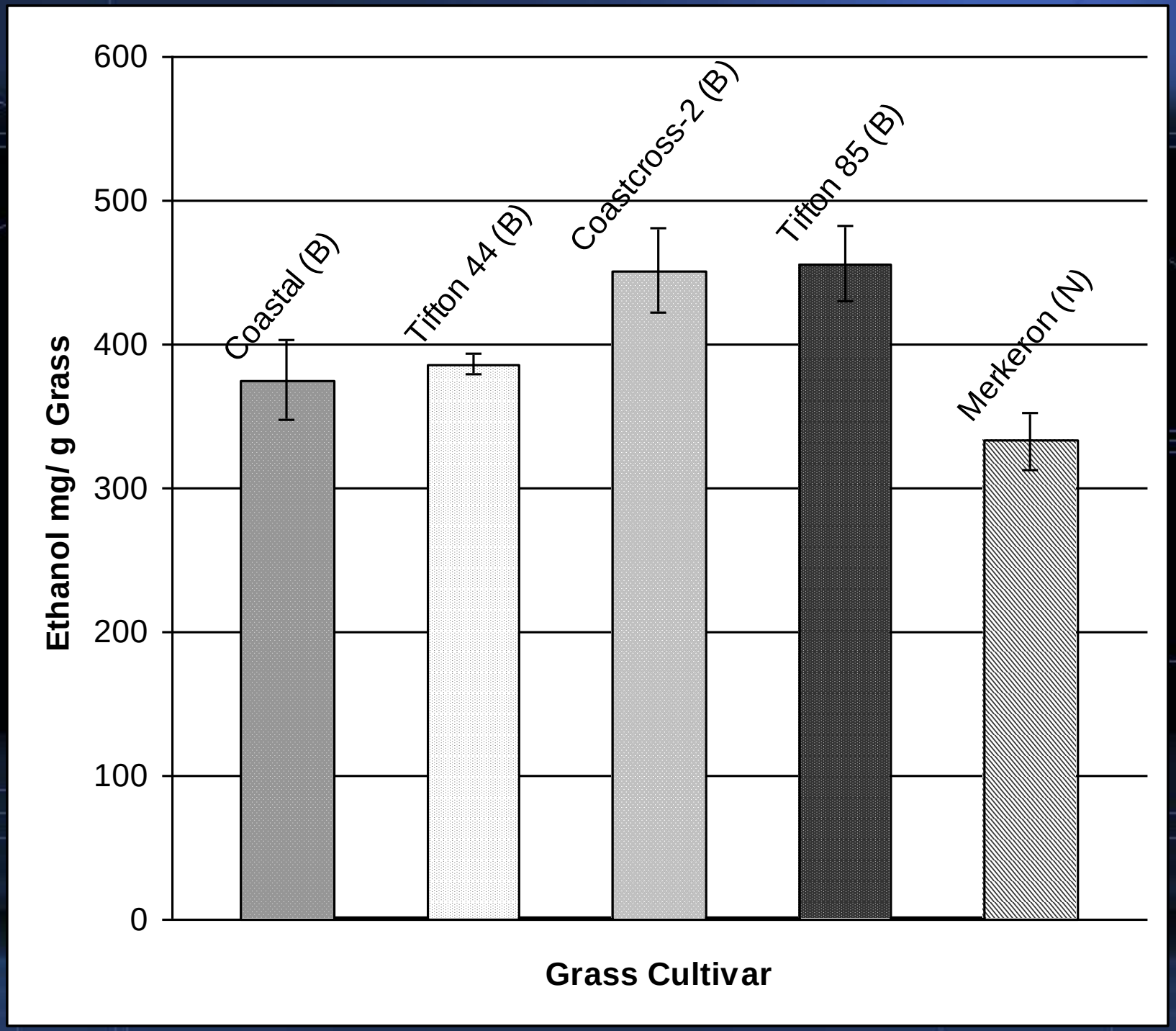
Correlation Coefficients among Forage Traits and Ethanol Production

	Pentose Sugars	IVDMD	NDF	ADF	ADL	NDF-ADF	ADF-ADL
Ethanol Produced	- 0.51*	0.57*	-0.41	-0.18	-0.45	-0.48	-0.04
Pentose Sugars		-0.63**	0.71**	0.33	0.54*	0.79**	0.16

* R^2 significant at p=0.05 ** R^2 significant at p=0.01

Esterase + Cellulase Pretreatment Study

- Samples from 4 week old bermudagrass samples – cut, dried and ground (1mm)
- Esterase then cellulase pretreatments (Anderson et al., 2005)
- Combined esterase and cellulase-treated samples
- Fermentation at 35^{o} for 24 hours
- Ethanol measured by GC


600
500
400
300
200
100
0
Ethanol mg/ g Grass
Coastal (B)
Tifton 44 (B)
Coastcross-2 (B)
Tifton 85 (B)
Merkeron (N)
Grass Cultivar

Conclusions

- Bermudagrass has superior rates of conversion to ethanol compared to other semi-tropical grasses
- Conversion rates and rumen digestibility are moderately correlated but not with NDF, ADF, or ADL
- Sufficient variability exists within the germplasm pool to allow for genetic improvement through breeding

Is Bermudagrass a Viable Feedstock?

- Price of hay in 2006 was $90 - $100/short ton
- Bermudagrass requires water and N, P, K for high yields
- However, older - unusable forage hay may be sufficient for biofuel industry as evidenced by conversion of 12 week old growth

Future work

- Evaluate other germplasm
- Develop NIR calibration sets for fast evaluation of breeding material
- Develop genetic markers for improved breeding efficiency