United States Department of Agriculture
Research, Education, and Economics

Switchgrass for ethanol and lipid production

- Field based ethanol yields from established SG plots
- Yeast discovery for single cell oil production from SG hydrolysates

Bruce S. Dien*
Patricia. J. Slininger
Josh Quarterman
Patricia J. O'Bryan
Michael Casler
Robert Mitchell
Ken Vogel
Gene Lester (NPS

Contact information; bruce.dien@ars.usda.gov

ARS National Program 213 Biorefineries

Agriculture and Food Research Initiative Competitive Grant No. 2011-68005-30411 from the USDA National Institute of Food and Agriculture (CenUSA; field operations and phenotypic measurements)

AICHE, Boston
December 13, 2016

A BILLION DRY TONS OF BIOMASS

HAS THE POTENTIAL TO SUSTAINABLY PRODUCE

1.5 MILLION JOBS and keep about **$200 BILLION** dollars in the U.S. every year.

80 BILLION kWh of electricity to power **7 MILLION** households, and almost **1400 TRILLION BTUs** of thermal energy.

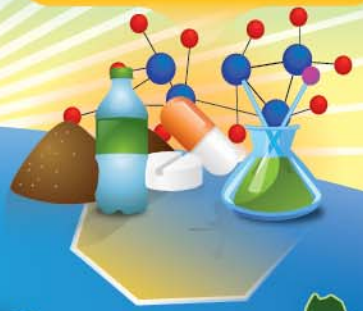
AND

50 BILLION gallons of biofuels displacing almost **30%** of all transportation fuels.

AND

45 BILLION POUNDS of biobased chemicals and bio-products, replacing a significant portion of the chemical market.

550 MILLION TONS of CO_2e reductions every year.



STEPS TO BUILDING THE BIOECONOMY

1. Accelerate research & technology development
2. Develop production, conversion and distribution infrastructure
3. Deploy technology
4. Create markets and delivery methods

Projections based on:

- 2011 Billion Ton Study Report
- EIA 2015 AEO
- Various data sources

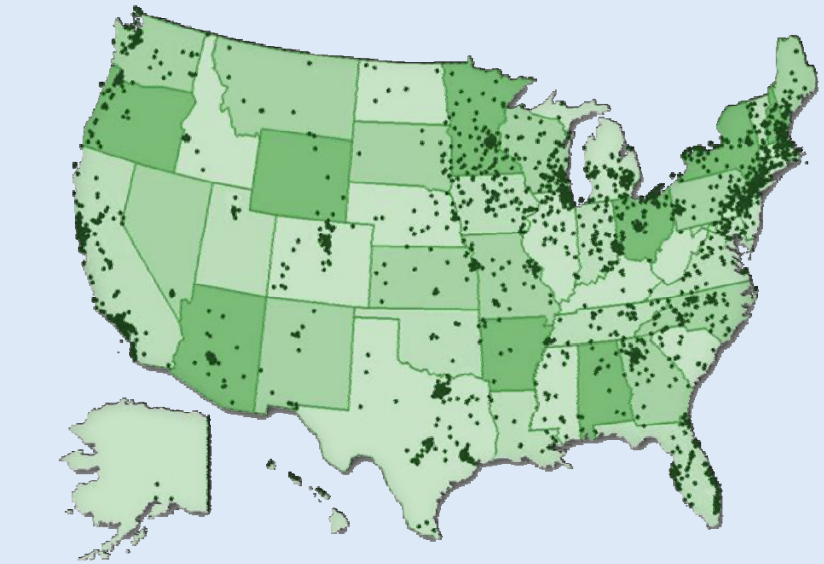
*Estimates are based on a future usage of 1.3 billion dry tons

United States Department of Agriculture

Commodity Credit Corporation
BioPreferred Program

USDA Certified Biobased Label

- Quantification of product's new carbon measurement
- FP on label indicates Federal Procurement Preference
- 2,500 products certified/labeled (2015)



ARS Biological Refining Technology Centers

Eastern Regional Research Center
Wyndmoor, PA

National Center for Agricultural
Utilization Research, Peoria, IL

Southern Regional Research
Center, New Orleans

Western Regional Research
Center, Albany, CA

United States Department of Agriculture
Research, Education, and Economics

Switchgrass for production on marginal farmland.

Mike Casler

Rob Mitchell

Ken Vogel

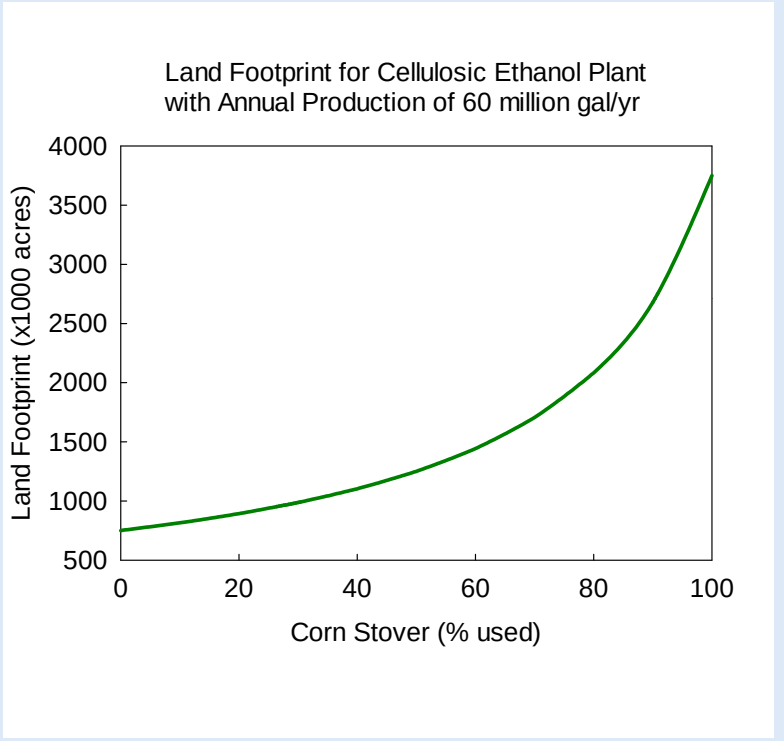
CENUSA: Agriculture and Food Research Initiative Competitive Grant No. 2011-68005-30411

United States Department of Agriculture
Research, Education, and Economics

Why Switchgrass?

- Good Biomass Productivity
- Low Inputs
- Native grass
- Winter Adapted
- Established Management
- Genetic Diversity
- Molecular Knowledge

Co-Feeding Switchgrass & Corn Stover



Estimates harvests of 10 ton/acre for SG & 2 ton/acre for corn stover with ethanol yields of 80 gal/ton.

Switchgrass + corn stover allows for better scaling.

United States Department of Agriculture
Research, Education, and Economics

'Liberty' Switchgrass: Biomass Yield

Cultivar	HZ 5a DEK, IL 42°N	HZ 4b ARL, WI 43°N	HZ 4a MSH, WI 45°N	HZ 3b SPN, WI 46°N	Mean
	Mg/ha	Mg/ha	Mg/ha	Mg/ha	Mg/ha
Summer	8.48	7.24	8.31	9.20	8.31
Kanlow	9.20	4.57	3.16	2.52	4.87
Liberty	**16.38**	**9.05**	**11.11**	**12.45**	**12.25**
P-value	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)
% Increase	78%	25%	34%	36%	38%

Vogel KP, Mitchell RB, Casler MD, Sarath G. Registration of Liberty's switchgrass. Journal of Plant Registrations. 2014;8(3):242-7.

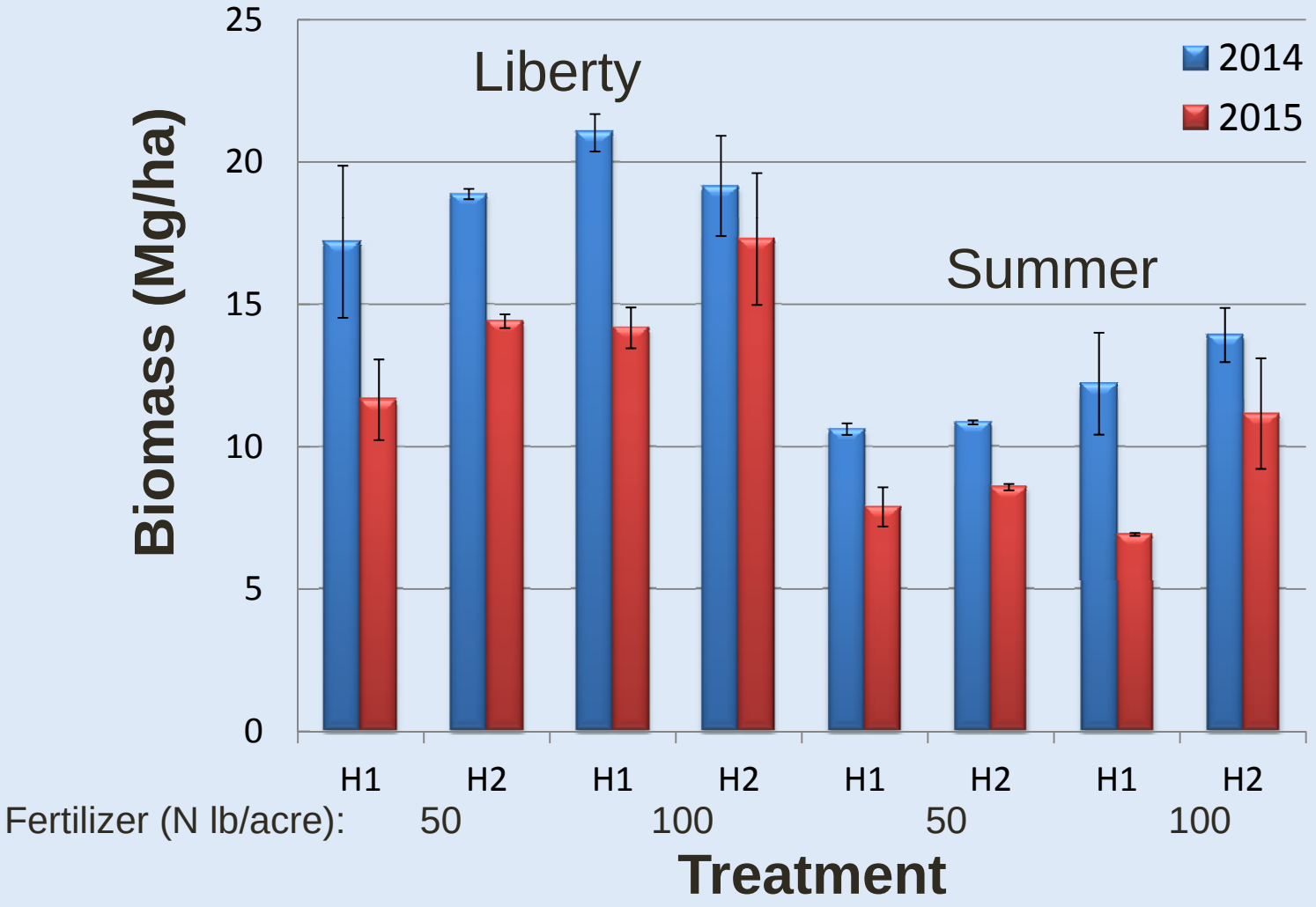
Crop management and land-based ethanol yields

Objectives

- Determine yield response of Summer & Kanlow versus Liberty
- Determine fertilizer application for 50 vs. 100 lb N/acre
- Determine harvest time for anthesis versus post-frost
- Responses: biomass, chemical composition, & enzymatic yield of sugars (e.g. ethanol)

United States Department of Agriculture
Research, Education, and Economics

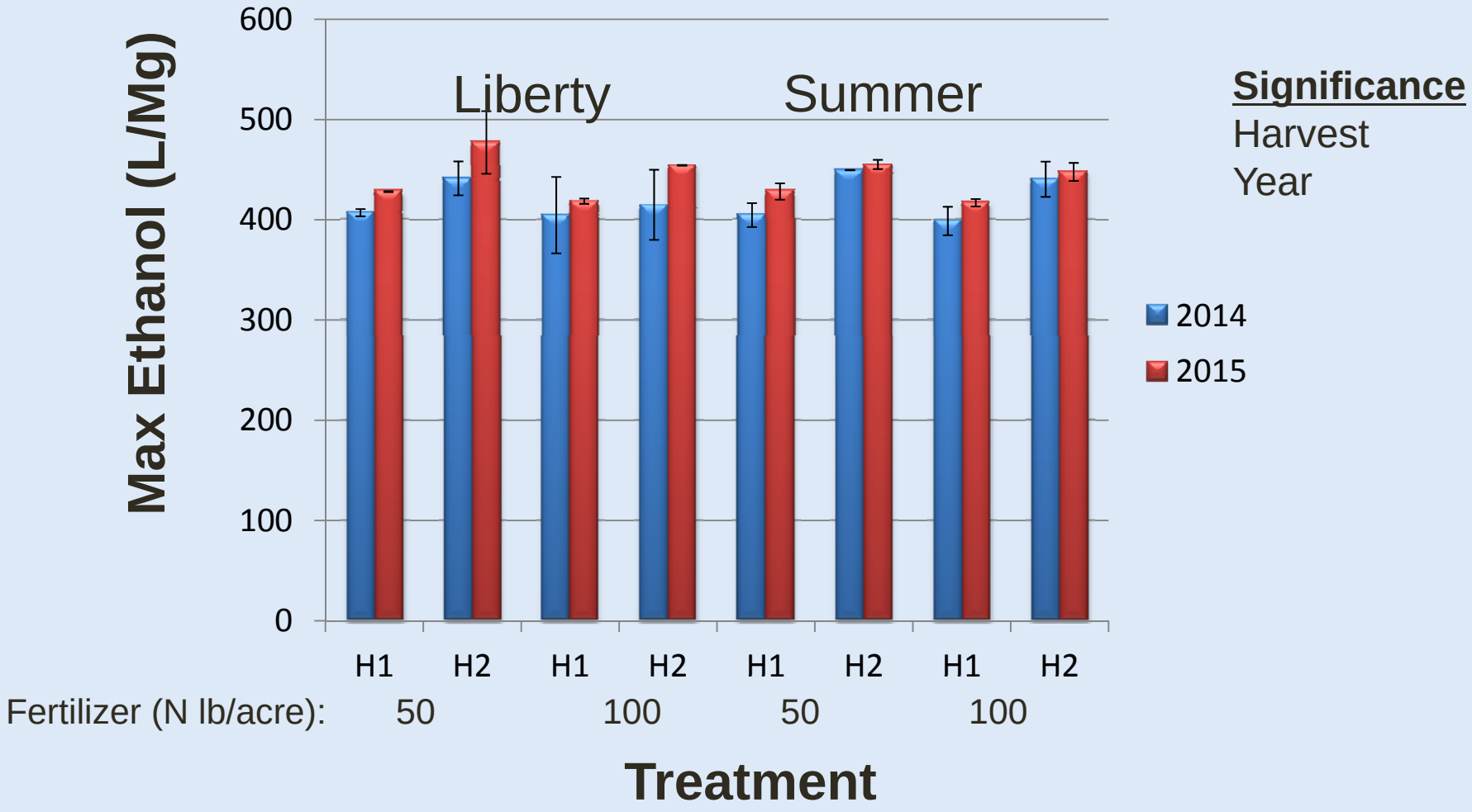
Switchgrass Biomass Production



Significance
Variety
Year
Fertilizer
Harvest

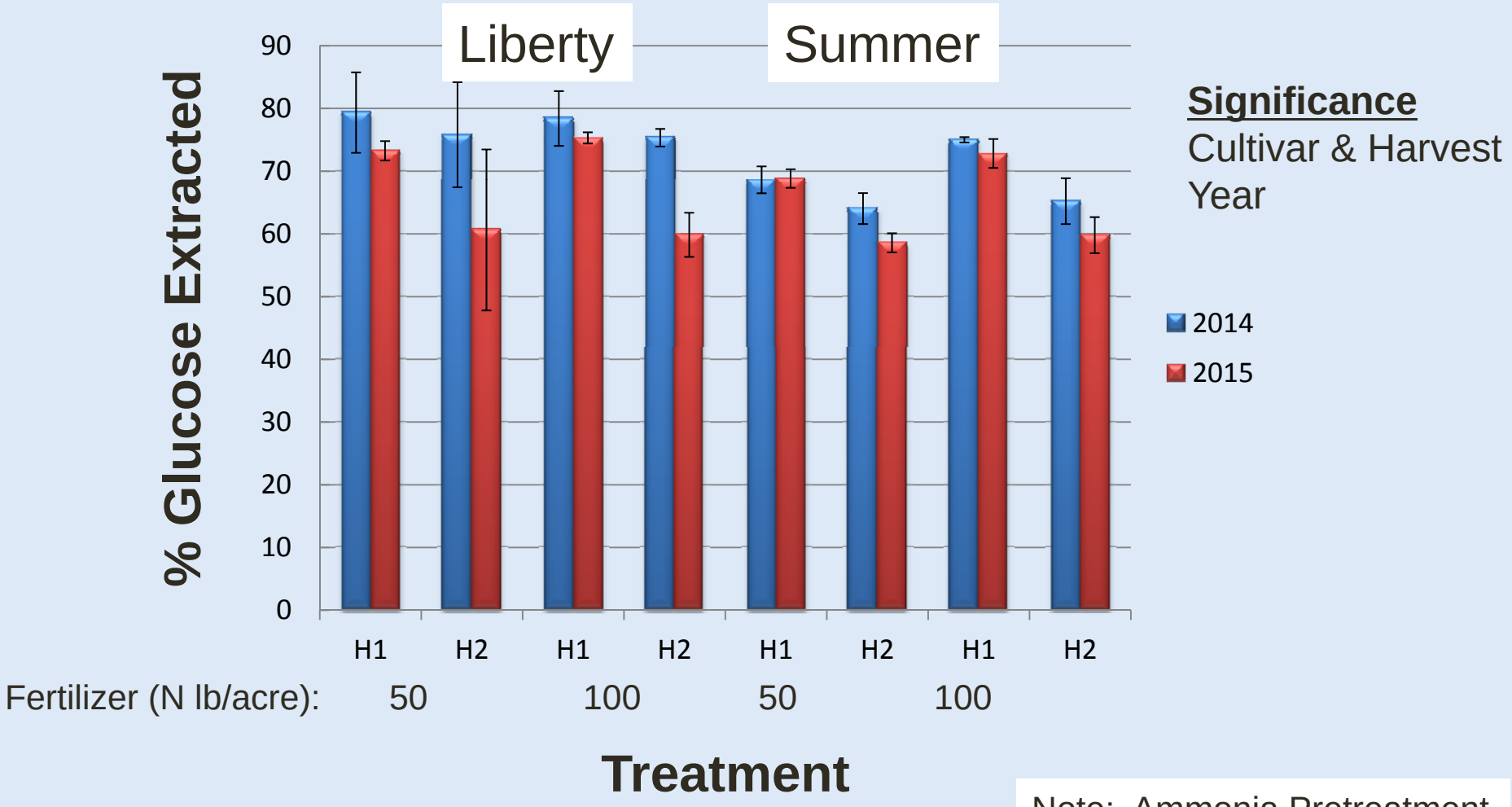
United States Department of Agriculture
Research, Education, and Economics

SG Theoretical Ethanol Yield per Mg



United States Department of Agriculture
Research, Education, and Economics

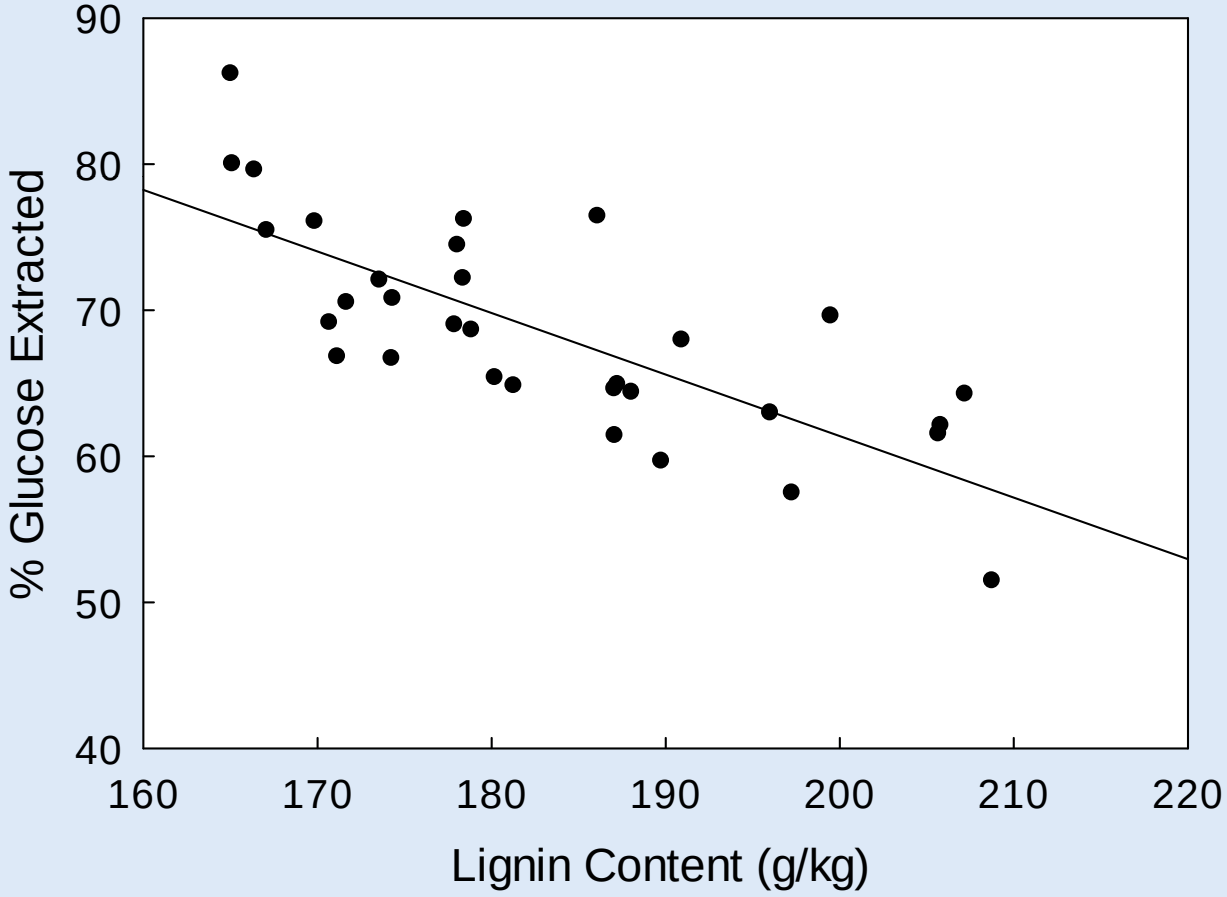
Cellulase Treatment Efficiency



Note: Ammonia Pretreatment

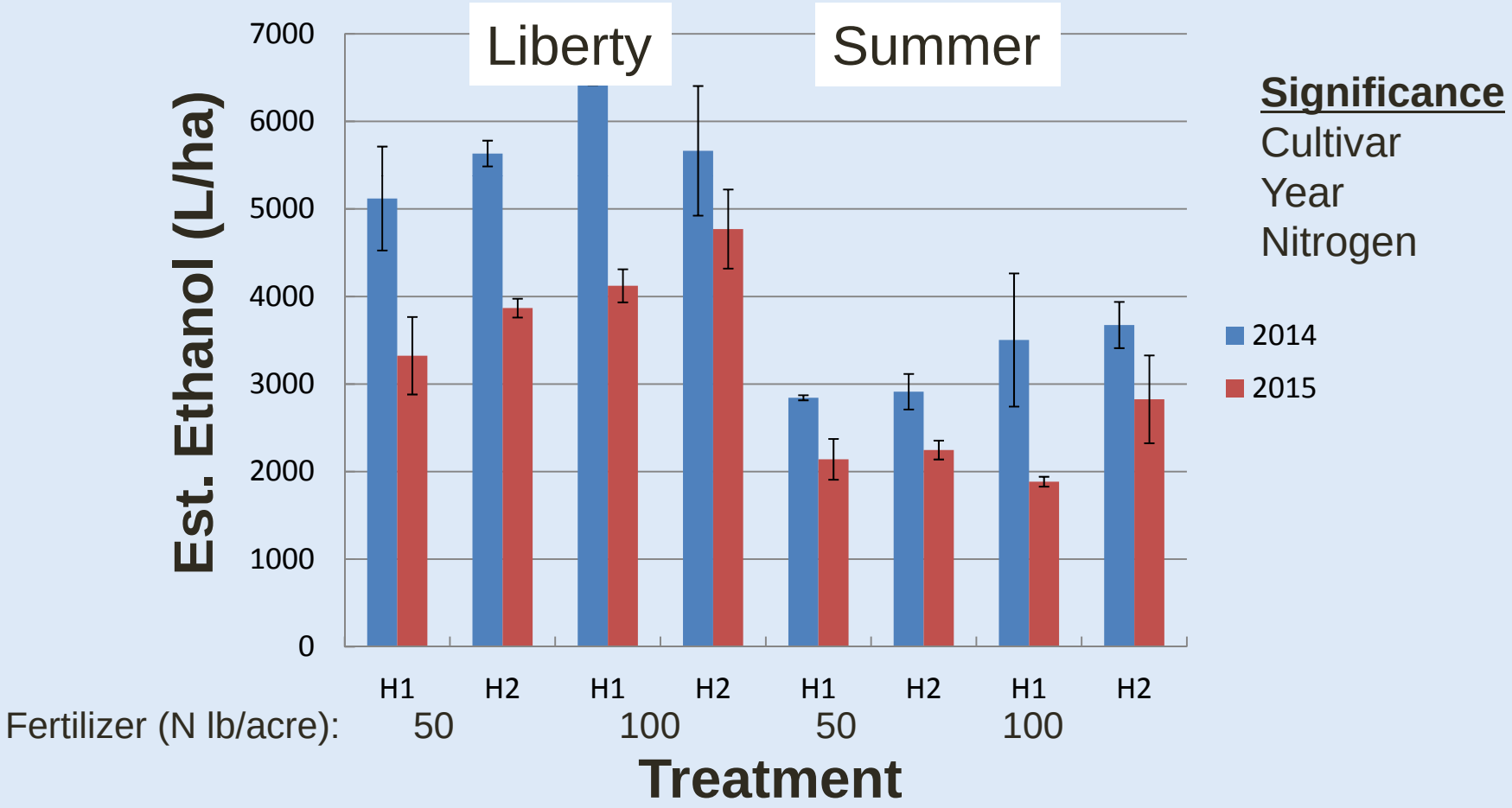
United States Department of Agriculture
Research, Education, and Economics

Cellulase efficiency negatively correlated with lignin content



United States Department of Agriculture
Research, Education, and Economics

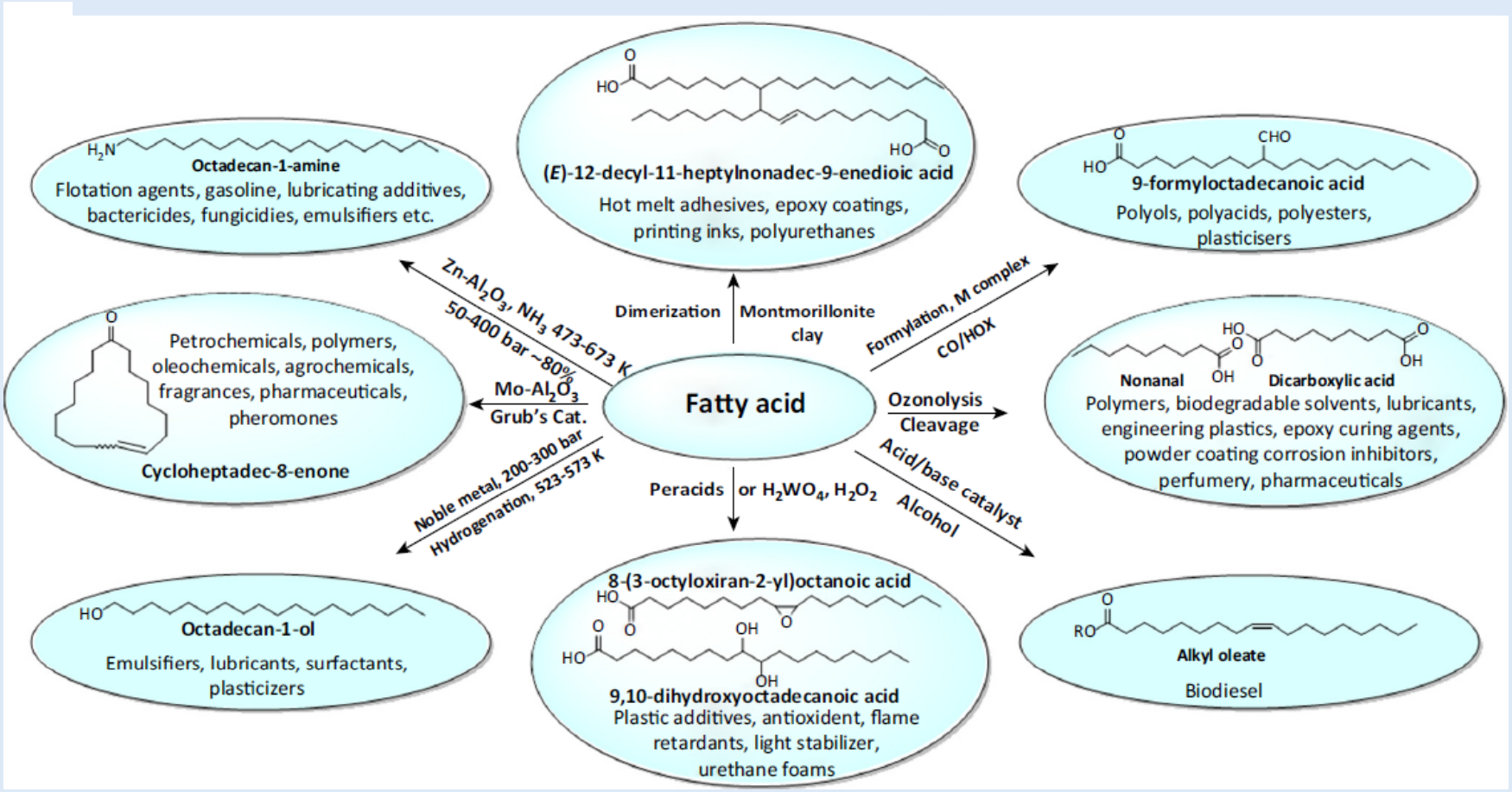
Estimated Ethanol Land Productivity



Yield of corn ethanol = 200 bu/acre x 2.8 gal/bu = 560 gal/acre **5300 L/ha**

United States Department of Agriculture

Beyond Ethanol with Lipid Production



Jin et al., 2015

United States Department of Agriculture
Research, Education, and Economics

Lipid Producing Yeast

Plan: To screen Lipomyces and Yarrowia **yeasts** on dilute-acid pretreated switchgrass sugar hydrolysates

Lipomyces: better lipid producer & ARS culture collection has diverse collection.

Yarrowia: widely accepted commercial yeast, GRAS, & developed transformation system.

Advantage of oleaginous yeast

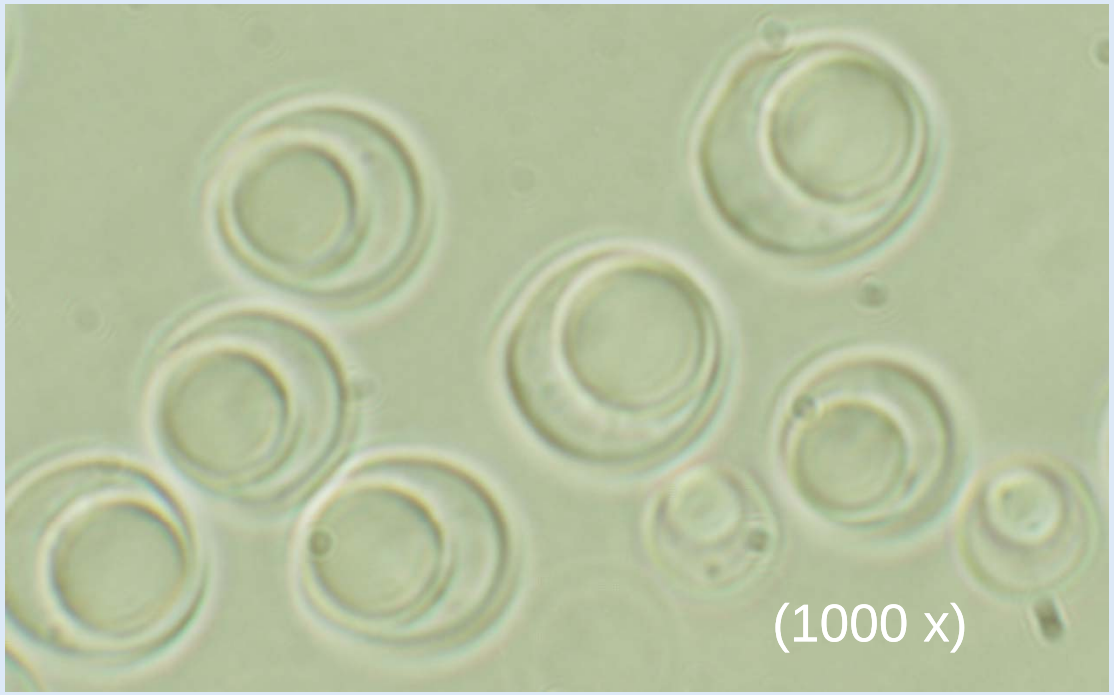
- Accumulate 20-70+% of their biomass as lipids
- In general, oleaginous yeast have faster growth, higher density growth, and allow lower pH conditions than microalgae
- Similar in fatty acid composition to seed oils

United States Department of Agriculture
Research, Education, and Economics

Lipomyces

In C:N 60:1 dilute acid pretreated switch grass hydrolyzate

24 h



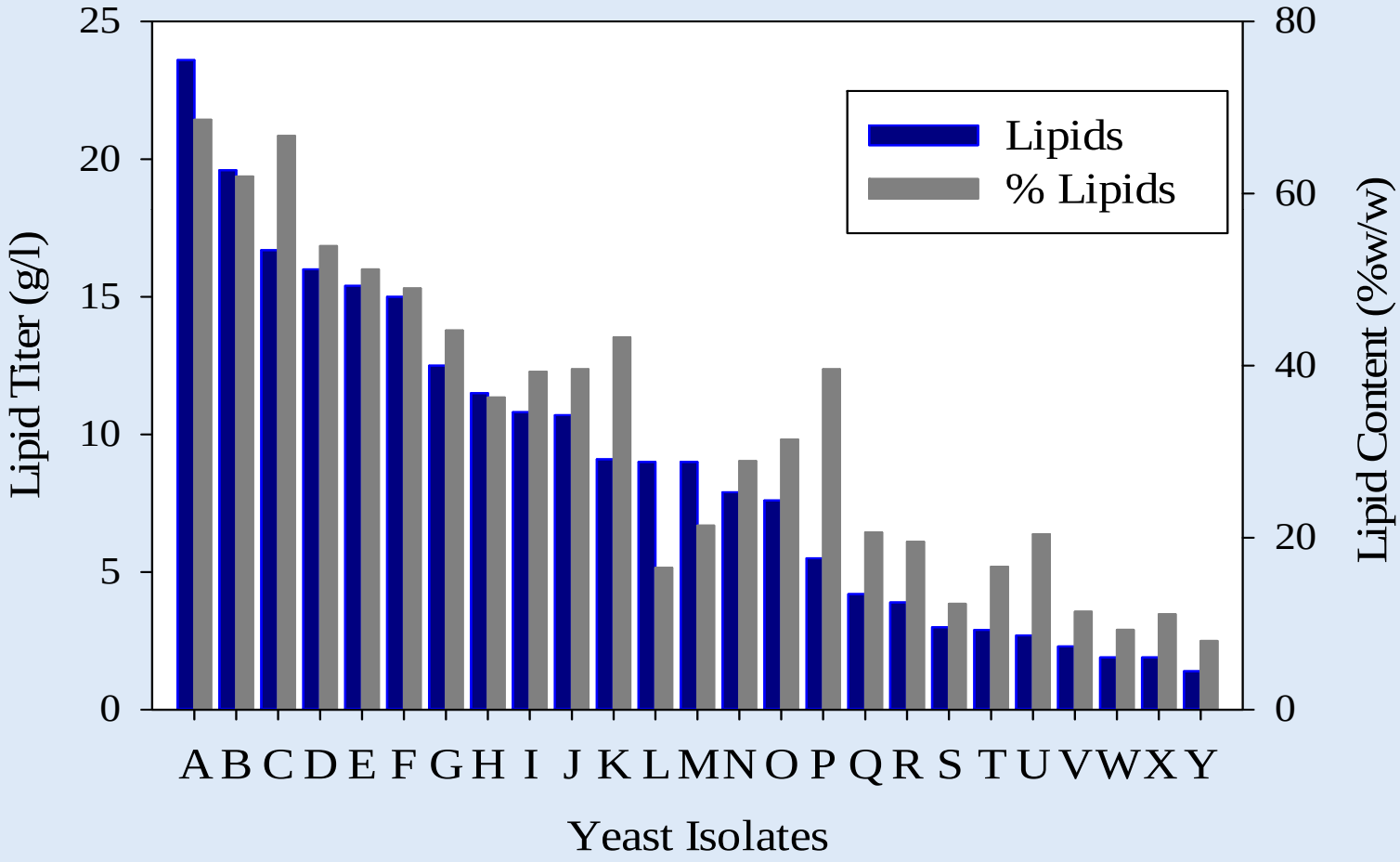
120 h

144h

United States Department of Agriculture
Research, Education, and Economics

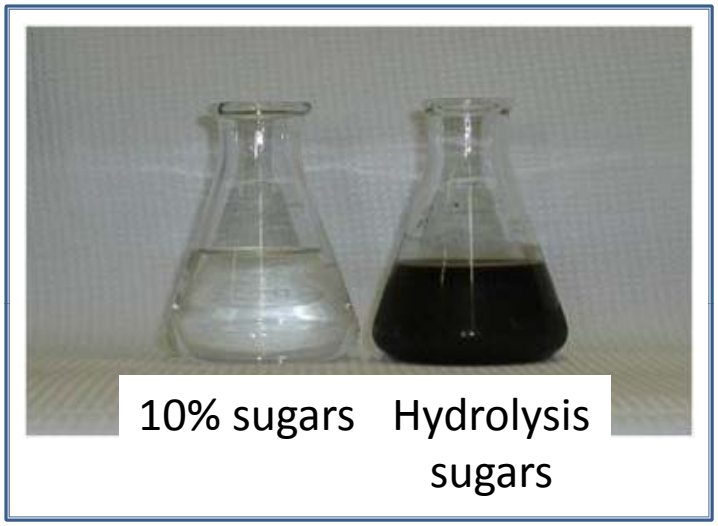
Lipomyces clade screening

Screening Lipid Production on 100 g/l Lipid Glucose Production Medium

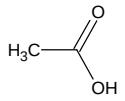


Winners: ***L. tetrasporous, L. spencer-martin , L. lipofer, & Rhodosporidium toruloides***

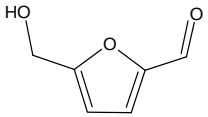
Biomass hydrolysates are challenging to ferment because they contain inhibitors.



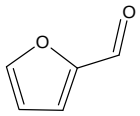
Inhibitors



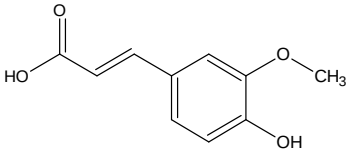
Acetic



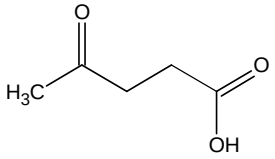
HMF



Furfural



Ferulic



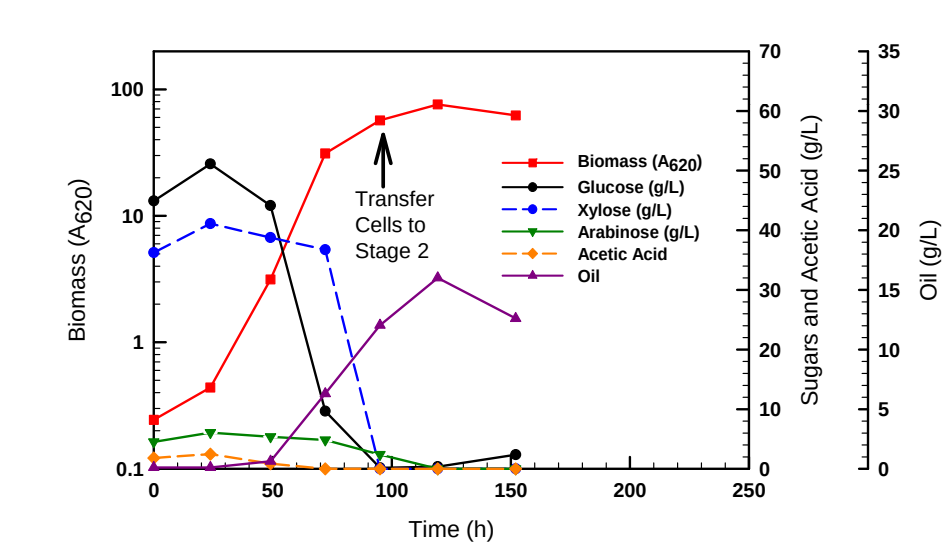
Levulinic

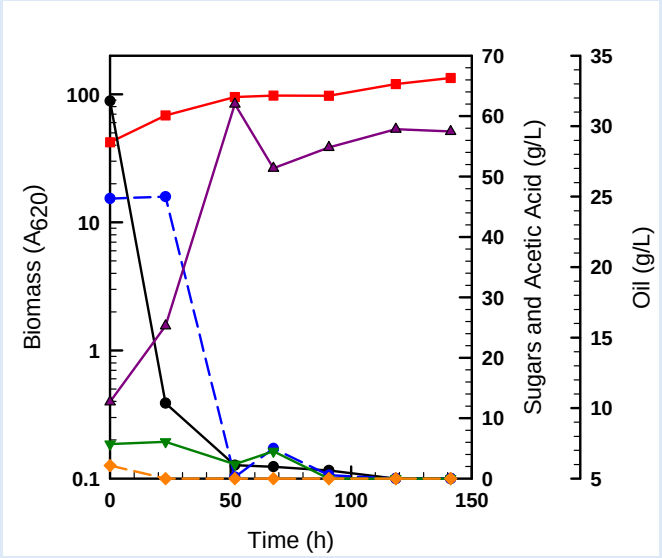
Phenolics

Formic

Dilute-acid pretreated switchgrass
- Superior Strains Utilize Undetoxified Hydrolyzate
- Two Stage Process Optimizes High Lipid Titers

Lipomyces tetrasporus Y-11562



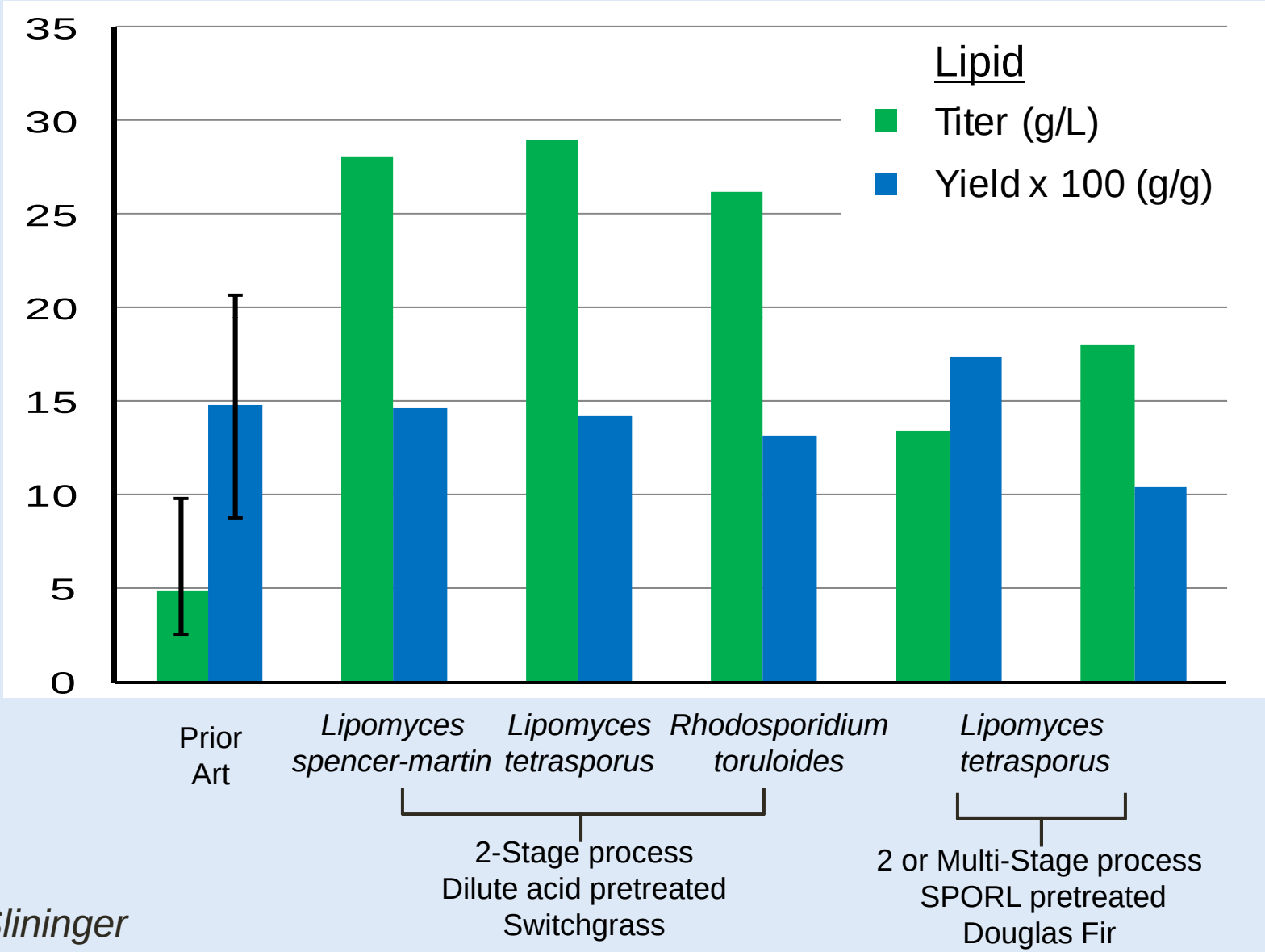


Stage 1 - Growth
75% Switchgrass Hydrolyzate
62:1 C:N

Stage 2 - Lipid Amplification
100% Switchgrass Hydrolyzate
600:1 C:N

Pat Slininger

A 2- to 5-Fold Lipid Titer Improvement by Strain and Process Optimization



Pat Slininger

United States Department of Agriculture
Research, Education, and Economics

Summary

- Switchgrass cultivated on marginal land can compete with corn grain for ethanol production. Biomass productivity controlled overall land-based ethanol yield.
- Demonstrated lipid titers from switchgrass hydrolysate that are 2-5x higher than maximum previously reported for lignocellulose.
- Identified a lesser-known *Yarrowia* species that was superior to the control in terms of lipid content (49.7% of DW) and lipid titer (2.9-fold improvement). Demonstrated successful transformation of top performing strains (not shown).