Increasing the Yield of Fuel Ethanol from Barley with β-Glucanases and β-Glucosidases

EASTERN REGIONAL RESEARCH CENTER

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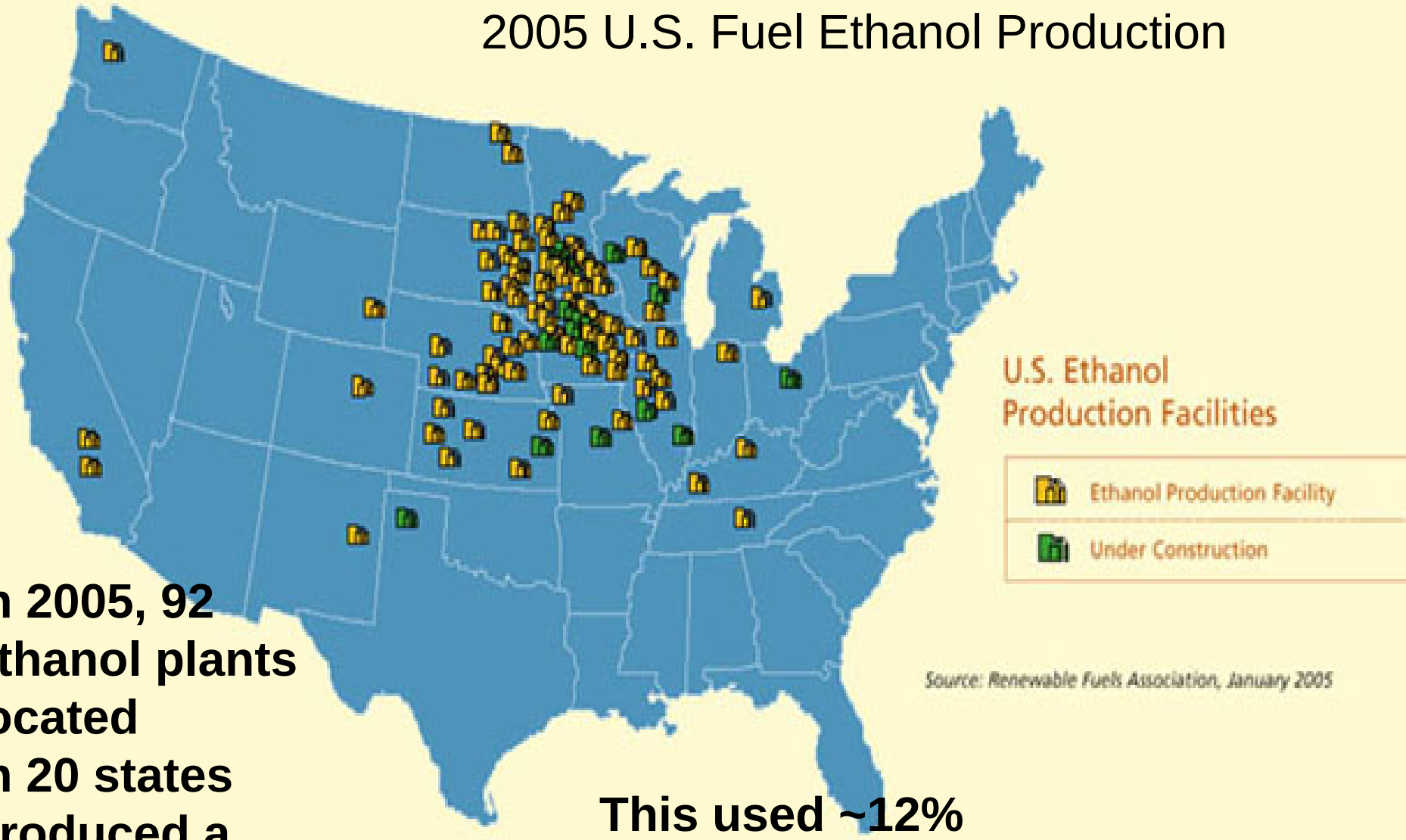
Summary of Presentation

- Why Consider Barley for Fuel Ethanol?
- Technical Problems with Barley as a Fuel Ethanol Feedstock
- How We are Using Enzymes to Solve these Problems

2005 U.S. Fuel Ethanol Production

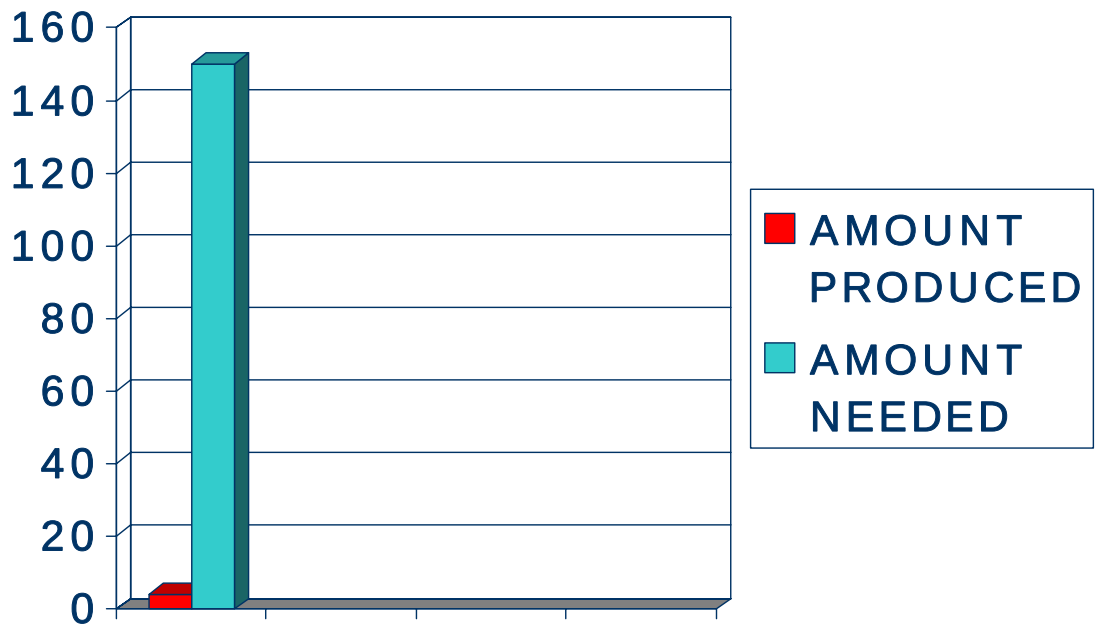


In 2005, 92 ethanol plants located in 20 states produced a record 3.9 billion gallons!

This used ~12% (>12 billion bushels) of the US corn supply!

DO WE HAVE ENOUGH CORN TO MAKE A SIGNIFICANT IMPACT IN OUR FUEL SUPPLY?

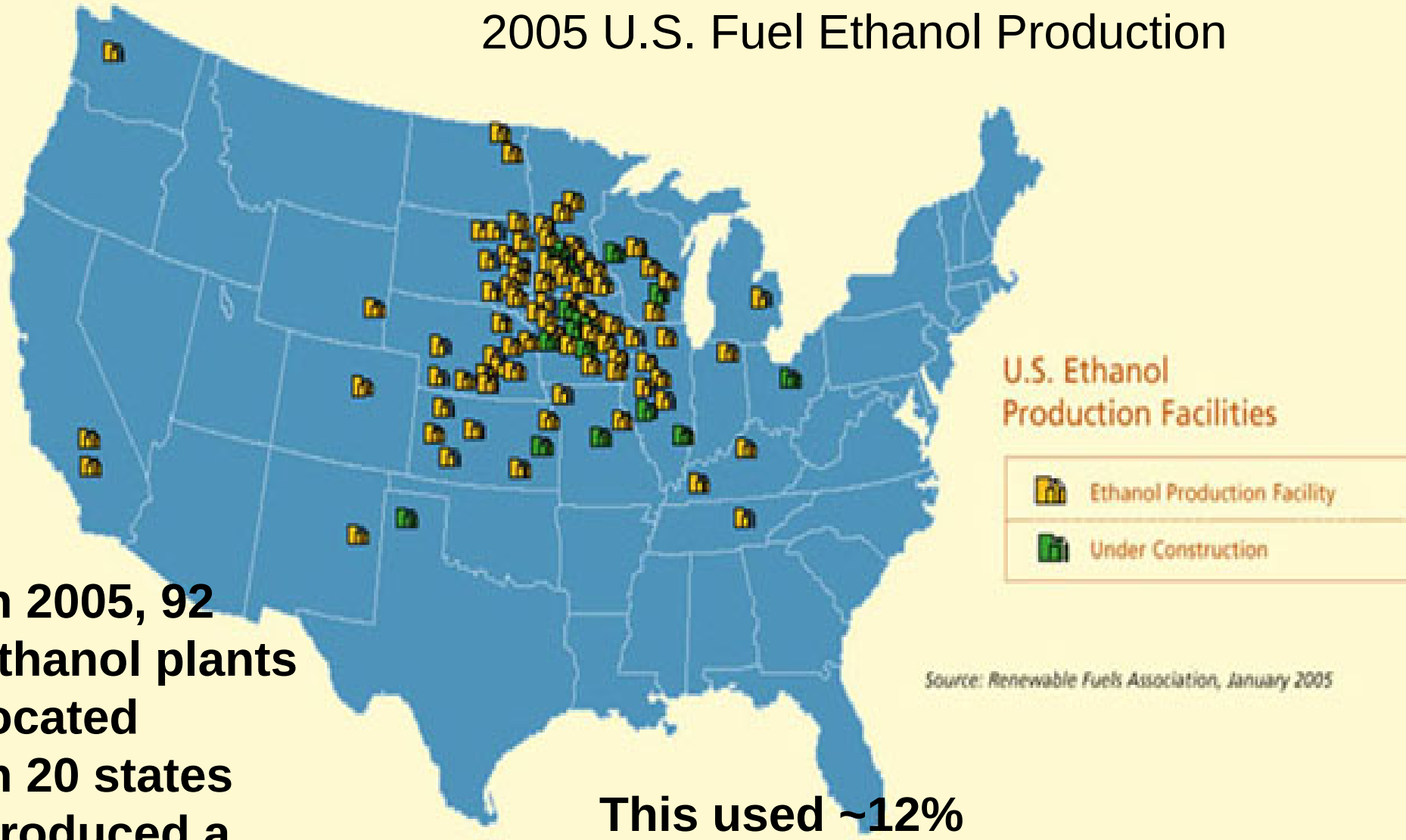
Last year we made ~4 billion gallons of ethanol from 12% of the U.S. corn supply. We need at least 150 billion gallons for automobile fuel!



HOW CAN WE INCREASE PRODUCTION OF ETHANOL WITHOUT DEPLETING CORN SUPPLIES?

- USE BIOMASS?
- USE HYDROGEN?
- USE CANE OR BEET SUGAR? YES BUT NOT IN THE U.S. UNLESS PRICE SUPPORTS ARE CHANGED.
- OTHER GRAINS? BEST SHORT TERM SOLUTION TO THE PROBLEM AND A GREAT OPPORTUNITY FOR U.S. FARMERS.

2005 U.S. Fuel Ethanol Production



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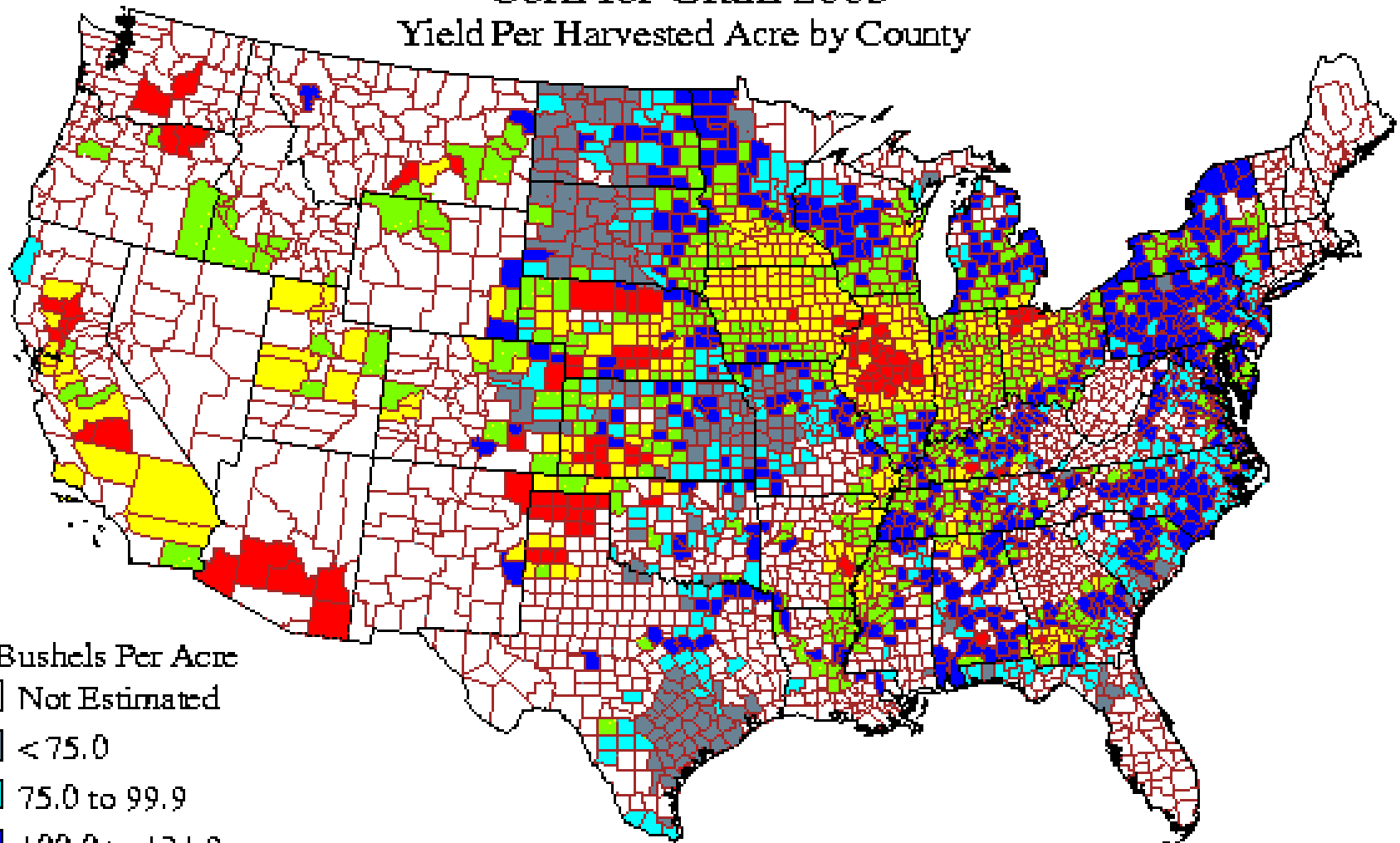
Corn for Grain 2003

Yield Per Harvested Acre by County



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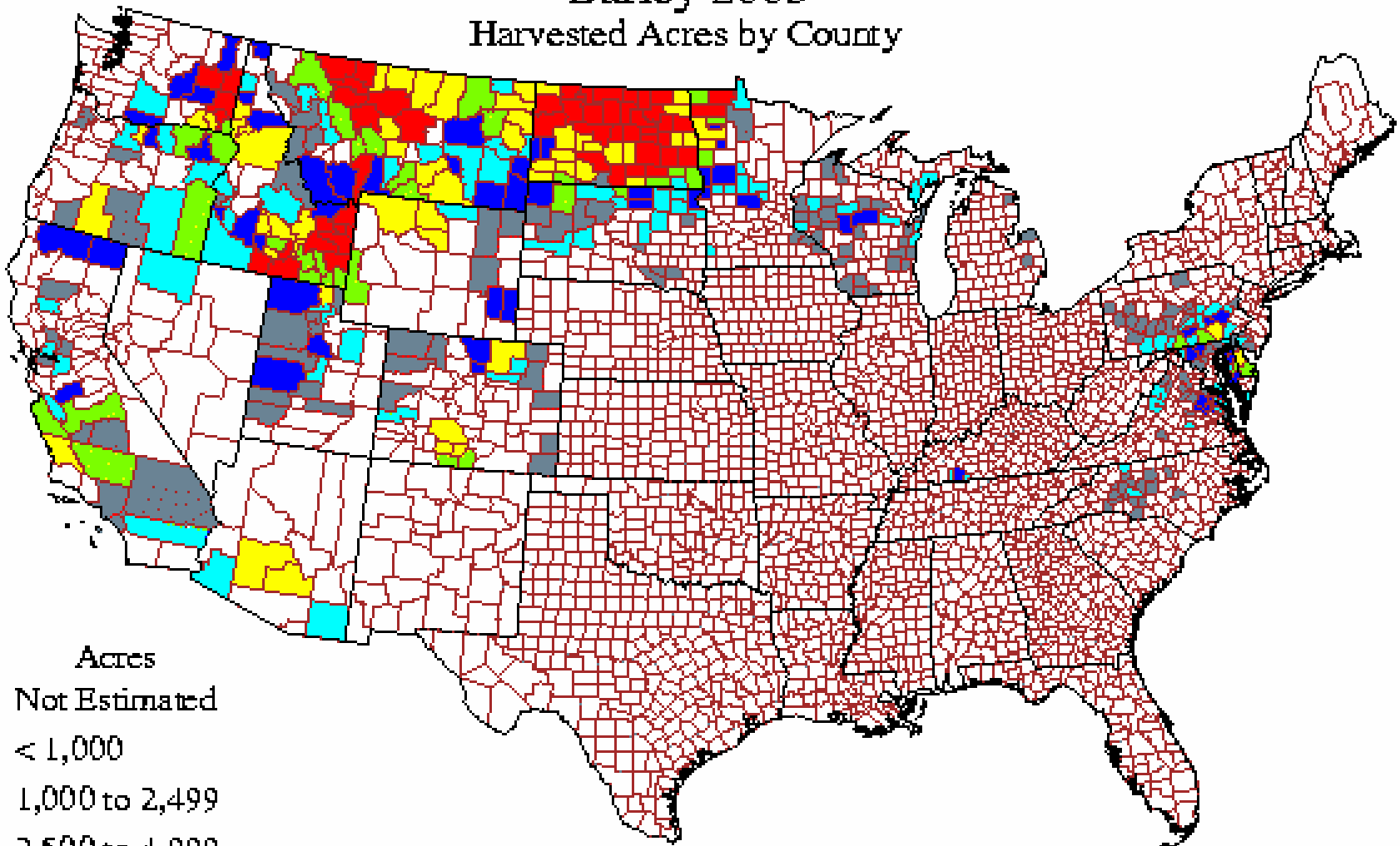
Statistics Service

Barley 2003

Harvested Acres by County



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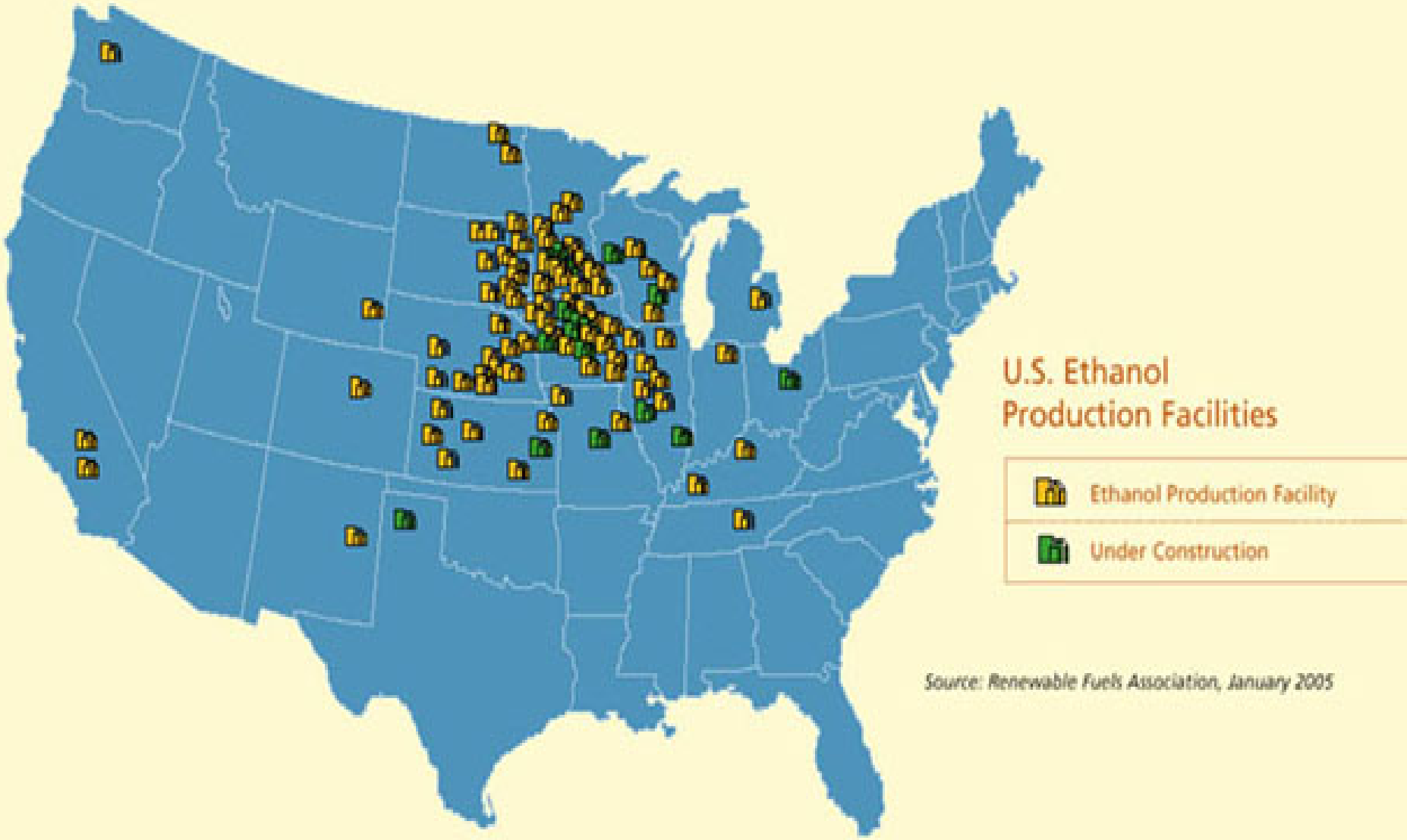
IS THERE ENOUGH BARLEY TO MAKE A SIGNIFICANT AMOUNT OF FUEL ETHANOL?

- U.S. Barley Production (All Types)
  - 5-8 Million Metric Tons = 0.2-0.4 Billion Bushels
- Canadian Barley Production (All Types)
  - ~14 million metric tons = 0.6 Billion Bushels
- Using 30-50% of Available Barley in the U.S. and Canada for Fuel Ethanol Could Yield an Additional 0.5-0.8 Billion Gallons of Ethanol.
- Significantly More Barley can Be Grown in the U.S. if Markets are there.
- Bottom Line: ~1 Billion Gallons of Ethanol from Barley



Number of U.S. Fuel Ethanol Plants that Use Barley as Feedstock = 0

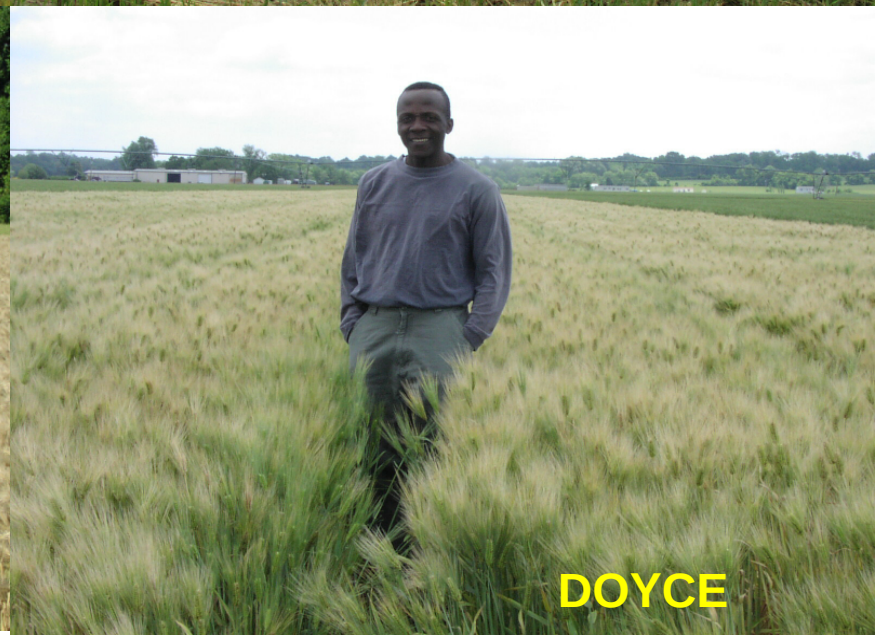
Technical Issues with Barley as a Fuel Ethanol Feedstock

- Abrasive Hull
- Low Starch Content (50-55%) Compared to Corn's (72%)
- High Viscosity of Mash due to β-Glucans
- β-Glucans in DDGS Limit their Applications to Mainly Ruminants

Research Plan to Solve Barley Issue 1

- How to Deal with Abrasive Hull?
  - Use Hulless Barley
  - Use Pearling and/or Milling Processes to Remove Hull and Create High-Starch Fractions and Low-Starch Coproducts

Barley Production at Virginia Tech

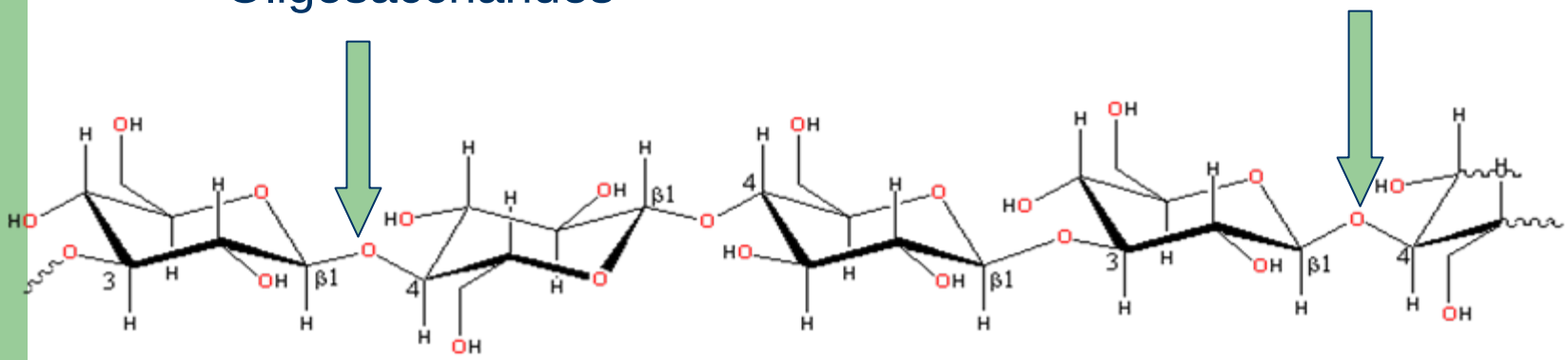


Performance of “Doyce”

Doyce Sample	Starch	β-Glucan	Oil	Protein	Test Weight & Yield
2002/2003 Crop, 9 East Coast Locations	**62.28** (2.03)	**3.85** (0.09)	**1.83** (0.04)	**11.39** (0.80)	**55.16** (1.98) ~85 bu/acre*
2003/2004, 15 East Coast Locations	**65.58** (1.56)	**4.67** (0.33)	**2.06** (.09)	**9.49** (0.83)	**52.81** (2.63)

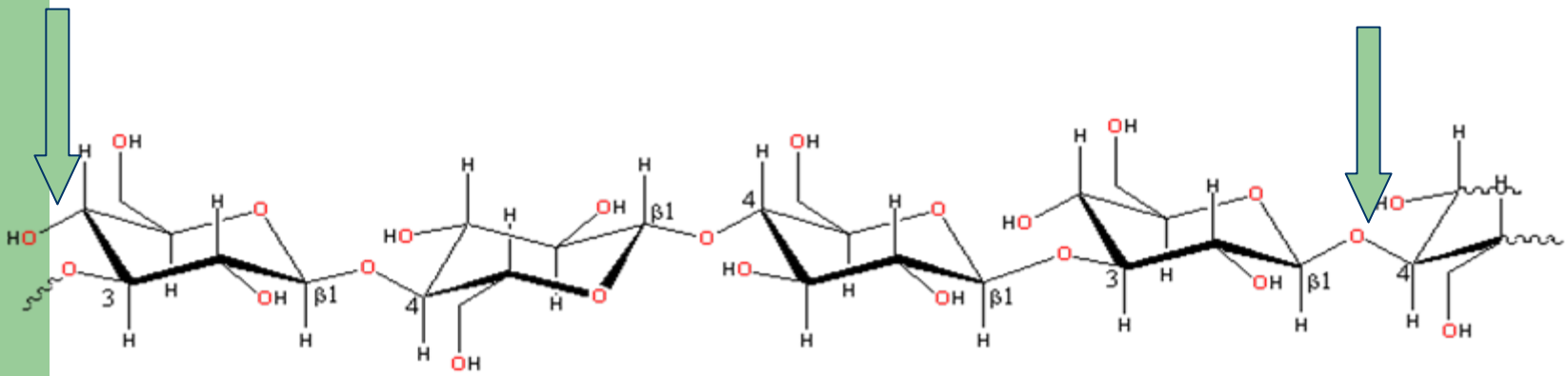
Research Plan to Solve Barley Issue 3

- **How to Deal with High Viscosity of Mash due to β-Glucans**
  - Fractional Milling May Be a Partial Solution by Removing β-Glucan-enriched Fractions Prior to Fermentation
  - Greatest Gains Will Be Made by Use of β-Glucanase Enzymes that Break down High-Viscosity β-Glucans to Low MWT Oligosaccharides



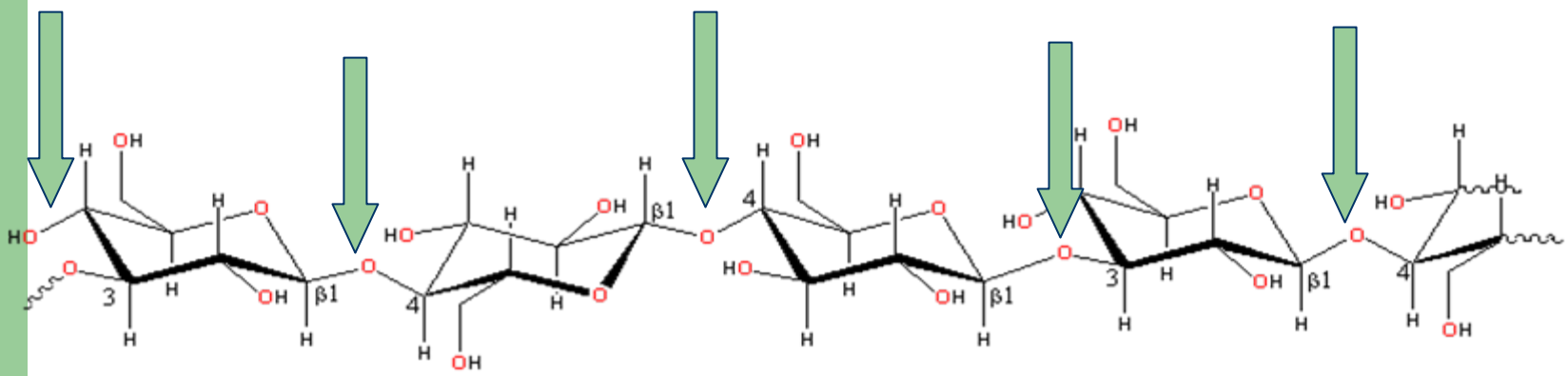
Commercially Available β-Glucanases Can Lower Viscosity of Barley Mashes

- **Commercial Enzyme Preparations Contain Mixtures of Enzymes that Can Partially Degrade Viscous High Molecular Weight β-Glucans, Producing Low Viscosity Oligosaccharides in the Process.**



Are there Commercially Available β-Glucanases that can Completely Hydrolyze β-Glucan to Glucose?

- **What Would be the Benefits?**
  - Major Reduction of Viscosity
  - Additional Glucose Produced Would Be Fermented to Ethanol, Increasing Yields



Benefits of Complete Hydrolysis of β-Glucans

- Even Greater Reduction of Mash Viscosity
- Elimination of β-Glucan in DDGS (Increasing Value)
- Increase in Ethanol Yields:
  - Barley with 65% Starch and 5% β-Glucan would be Equivalent to Grain having 70% Starch!

Next Steps for β-Glucanase Studies

β-Glucanase Studies – Model System

- Commercial Enzymes Evaluated
  - Alltech: **Allglucanase**, Heat Stable β-Glucanase from *Trichoderma Longibrachiatum and* **Rhyzozyme**, from *Rhizopus* sp.
  - GNC Bioferm: **Endo Feed**, Feed Enzyme from *Aspergillus sp*.
  - Novozyme: **Viscozyme L**, Multi-Enzyme Mixture from *Aspergillus* sp. (Obtained from Sigma)
  - Genencor: **GC 220, GC 440 and GC 880**, Developmental "Cellulases" from *Trichoderma*

β-Glucanase Studies: Model System

- Method of Evaluation of Commercial Enzymes
  - Reacted Aqueous Solutions (1% w/w) of Soluble Barley β-Glucan with Commercial Enzymes using pH and Temperature as Recommend by the Manufacturer
  - Used Excess of Enzymes
  - Measured the Levels of Oligosaccharides (HPAEC-PAD) and Glucose (YSI Glucose Analyzer) Produced Over Time

β-Glucanase Studies: Model System

Enzyme	Temperature ^{0}C	pH	Dosage g./g.βG
Endo-Feed	45	5	0.193
Multifect-B	45	5	0.73
Viscozyme L	45	5	0.71
GC 220	45	5	0.34
GC 440	55	5	0.35
GC 880	55	5	0.35
Rhizozyme	50	5	0.12
Allglucanase	80	5	0.32

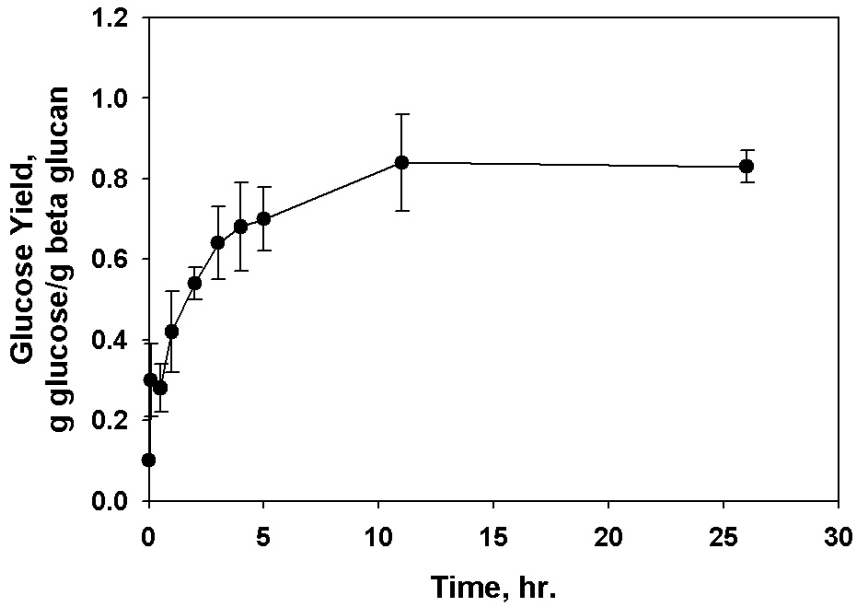
β-Glucanase Studies: Model System - Results (1 hr reaction time)

Enzyme	Glucose Yield g glu/g βG (Mean Value + error)
Endo-Feed	0.03 + .009
Multifect-B	0.05 + .058
Viscozyme L	0.06 + .022
GC 220	0.24 + .091
GC 440	0.28 + .045
GC 880	0.40 + .082
Rhizozyme	0.05 + .053
Allglucanase	0.06 + .030

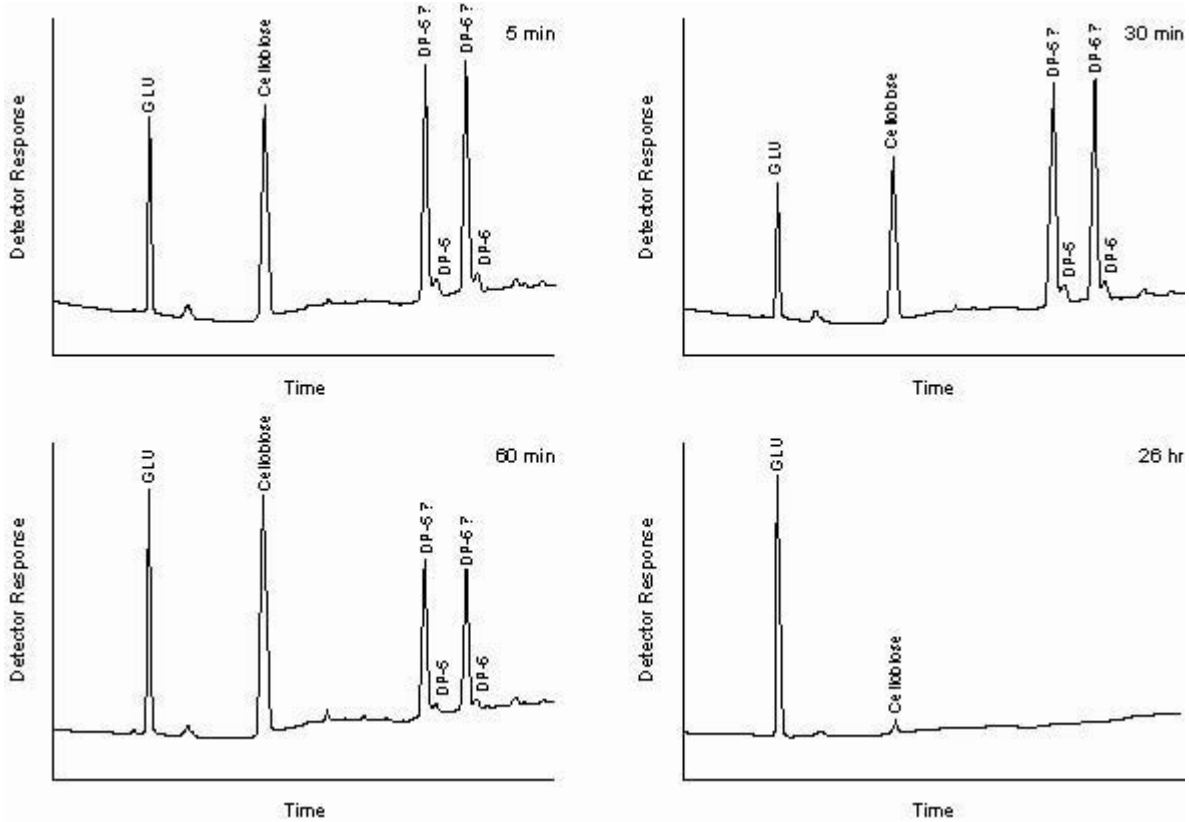
β-Glucanase Studies: Model System - Results

- All Enzymes Dramatically Reduced Viscosity
- Several Enzymes Led to Production of Measurable Levels of Glucose
- Numerous Fermentations were Conducted but Increased Levels of Ethanol Were Not Statistically Significant.

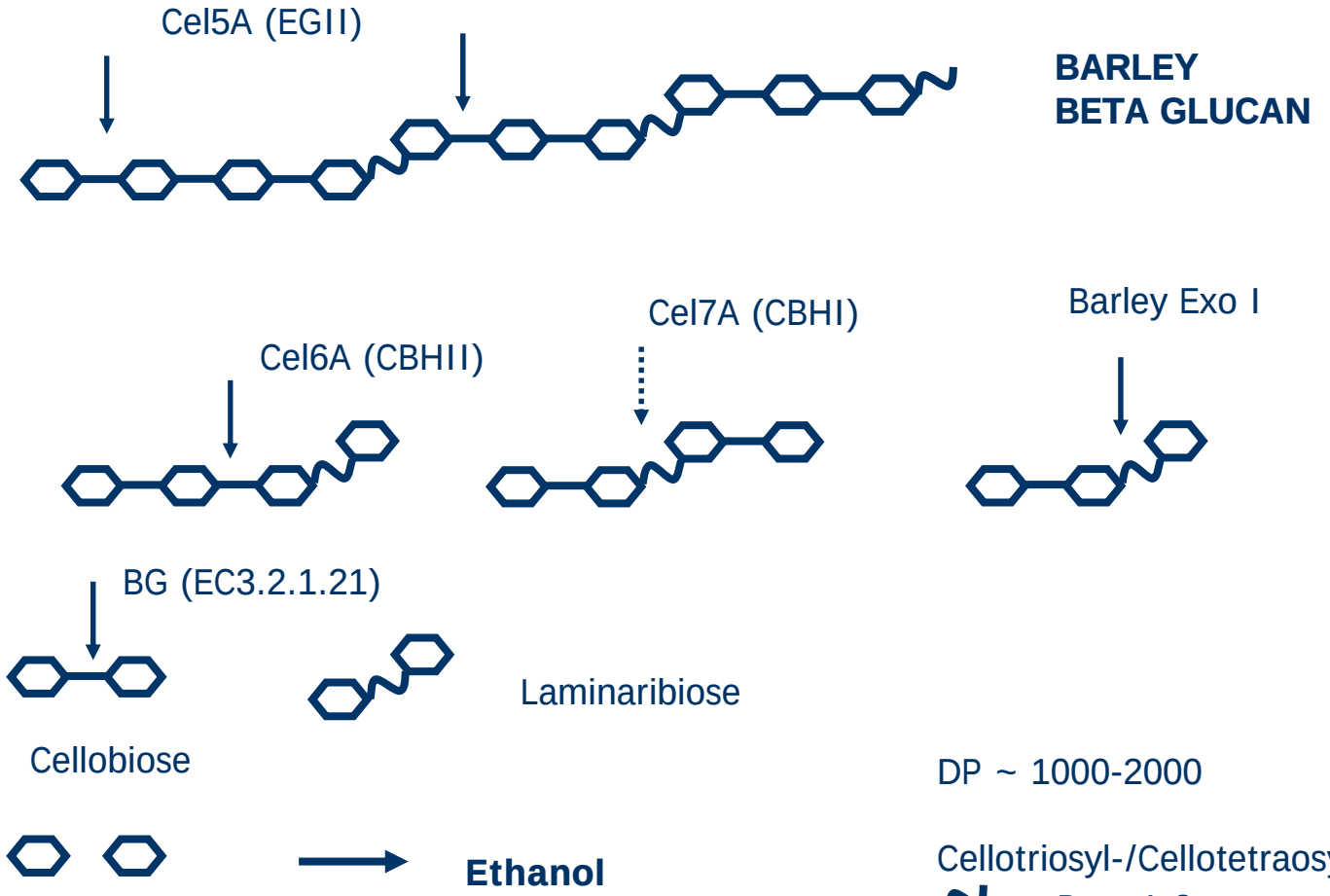
Production of Glucose from β-Glucan by GC 880



β-Glucanase Studies: Model System - HPAEC Results



Many Enzymes Are Used by *Trichoderma* sp. to Hydrolyze β-Glucan



DP ~ 1000-2000

Cellotriosyl-/Cellotetraosyl ratio:3/1

Beta 1-3

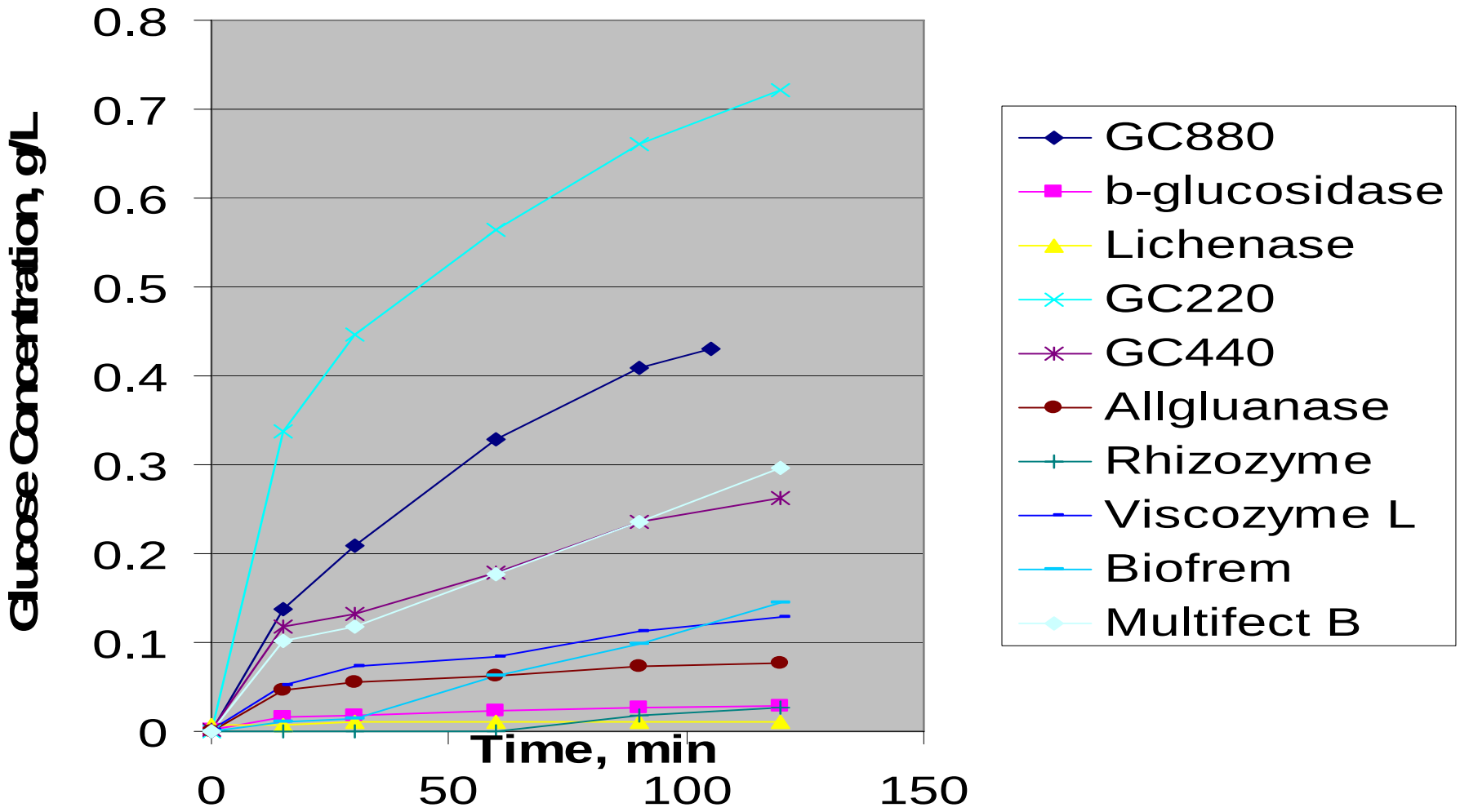
Beta 1-4

Graphics Courtesy of Genencor

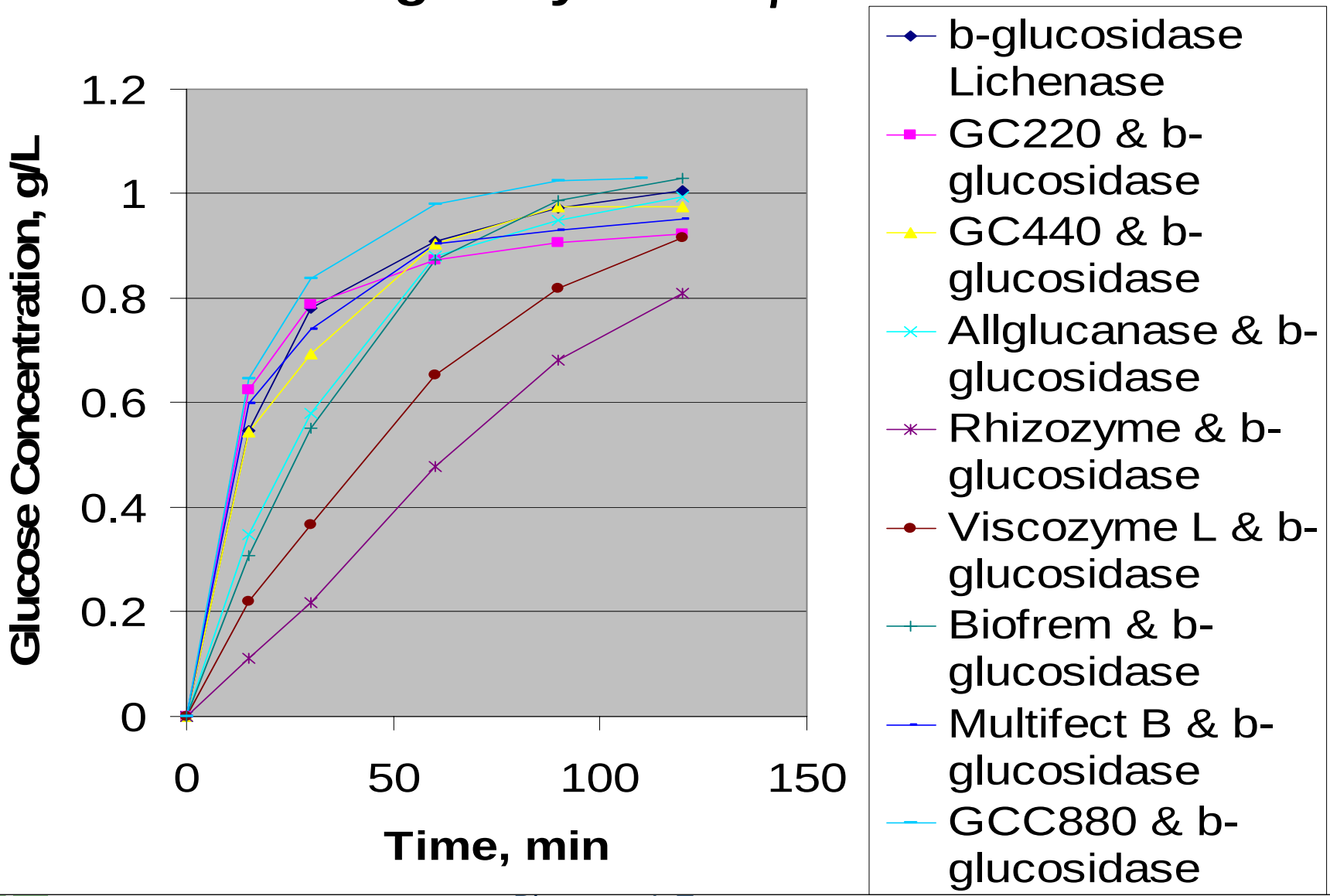
Can We Find the Missing Enzymes?

YES!

Conversion of Beta-Glucan using Single Enzyme



Conversion of Beta-Glucan to Glucose using Enzymes + β-G



Conclusions for β-Glucanase Studies

- We Have Determined the Missing Components of Commercial β-Glucanases
- Addition of β-Glucosidase to Even the Cheapest β-Glucanases Should be able to Convert all β-Glucan to Glucose and During Fermentations,
  - Increase Ethanol Yield
  - Remove All Beta-Glucans from DDGS

Summary of Fermentation Studies Without Beta-Glucanases

- Doyce Hulless Barley and a Typical Hulled Barley Appeared to Ferment at Approximately Similar Rates
- Doyce Barley Produced Higher Ethanol Yields
  - (2.27 versus 1.64 gal/bushel)
- Doyce Produced Less DDGS, but the Composition was Higher in Protein (More Valuable) than Hulled Barley DDGS
  - ~30% versus ~23% Protein
- Without use of β-Glucanases, both Doyce and Hulless Barley DDGS Contained Significant Levels of β-Glucans
  - ~7-8% Beta Glucans in DDGS

Next Steps for β-Glucanase Studies

- Conduct Fermentation Experiments to Validate Viscosity Decrease, Ethanol Yield Increase and Lack of β-Glucans in DDGS, and Other Process Requirements
- Determine Economic Feasibility by Building a 40 MGY Barley Ethanol Plant SuperPro™ Model
- Transfer the Technology to Users

Acknowledgements

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  - Jerry Senske

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The Future Grows Here 50

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