

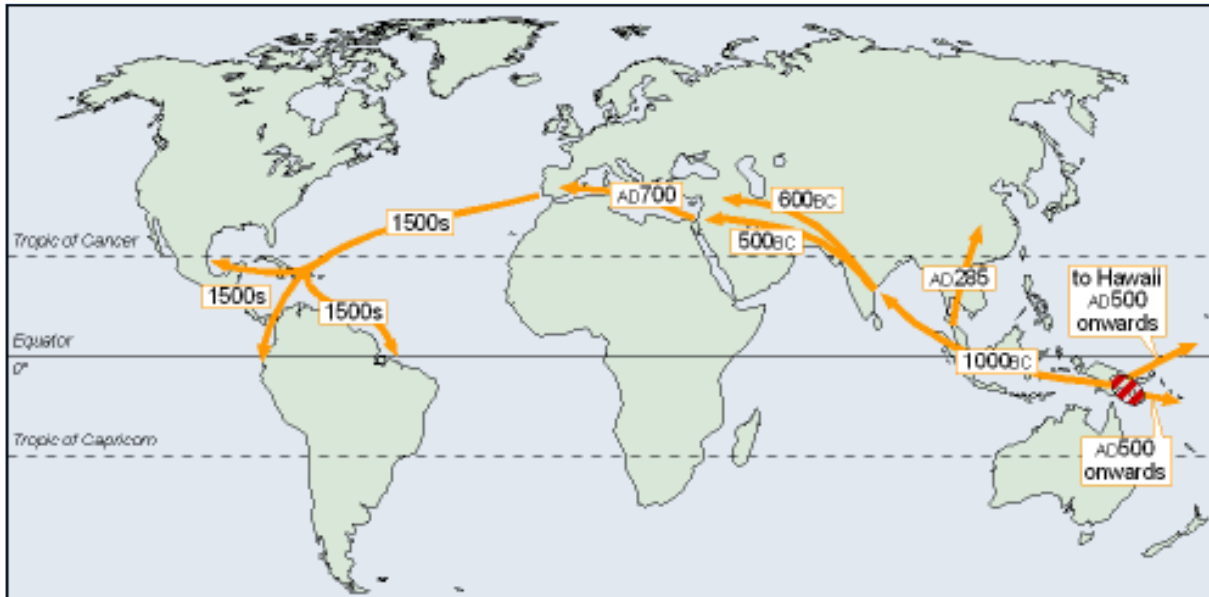
Potential of Diazorhic, Endophytic Bacteria Associated with Sugarcane for Energycane Production

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Origin and Spread of Sugarcane



6000 BC Domestication of sugarcane in New Guinea

1000 BC Traders began spreading sugarcane westward

1493 Brought by Columbus to Hispaniola from Canary Islands

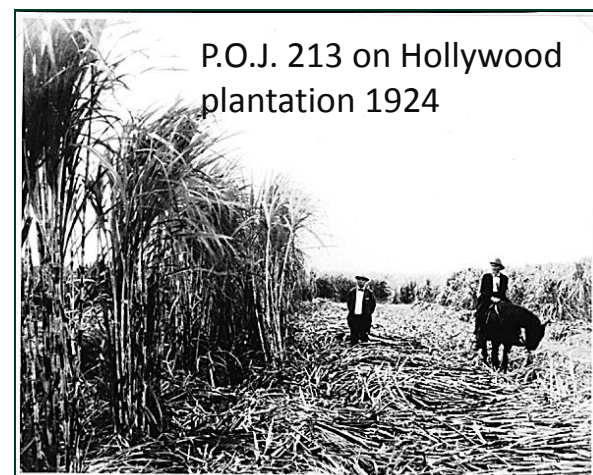
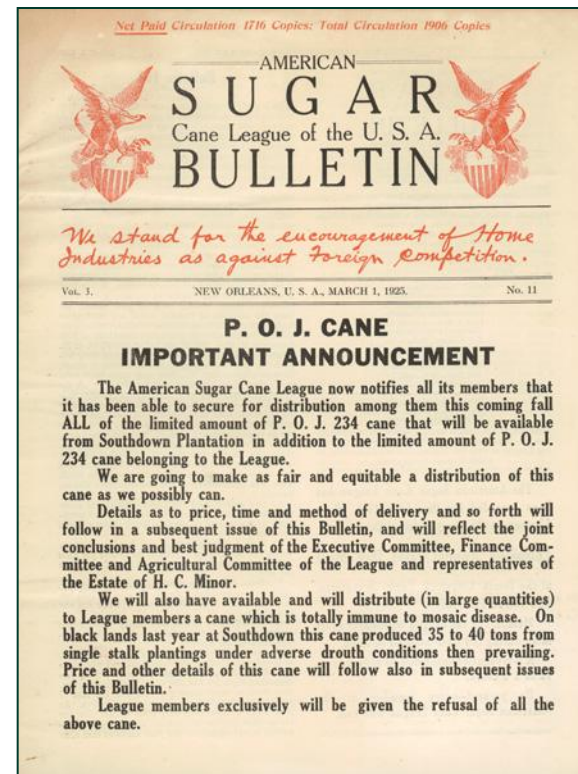
1500s Spanish and Portuguese explorers bring sugarcane to Americas

“Creole,” a noble sugarcane, grown for 250 years in the Americas

1750s Jesuits brought sugarcane to Louisiana “Otaheite”

1794 First commercial sugarcane in Louisiana

- **1800s** Noble varieties such as “Louisiana Purple,” “Louisiana Stripe,” and “D74” grown in Louisiana
- **Early 1900s** Devastating crop failures in Louisiana sugarcane industry
- **1919** Dr. Elmer W. Brandes, USDA, identified mosaic as major cause of poor yields
- **1922** Interspecific hybrid P.O.J. varieties imported from Java, key to saving industry
- **1924** Supply of P.O.J. 234 available for distribution through the American Sugarcane League
- **1928** 85% of the state’s acreage was P.O.J. varieties



Flagging plots for harvest operator



Breeding



- **Participating with the LSU Ag Center and the American Sugar Cane League in developing new commercial varieties**
- **Using wild relatives of sugarcane to broaden the genetic base of our parental material**
- **Developing “energy cane” varieties**
- **Developing and utilizing DNA-based molecular markers to fingerprint varieties and to improve selection efficiencies.**

Energycanes



- Hybrids (F_1 and BC_1) between cultivated sugarcane and wild relatives (*Saccharum*, *Miscanthus*, *Erianthus*)
- Vegetatively propagated perennial with better cold tolerance than sugarcane
- Higher fiber and better ratooning ability
- Developed specifically as a bioenergy crop
 - Type I – Dual-purpose sugar and lignocellulosic crop
 - Type II – Primarily lignocellulosic



Breeding with wild relatives:

✓ *Saccharum spontaneum*

✓ *Miscanthus*

✓ *Erianthus*



Energycane



Miscanthus

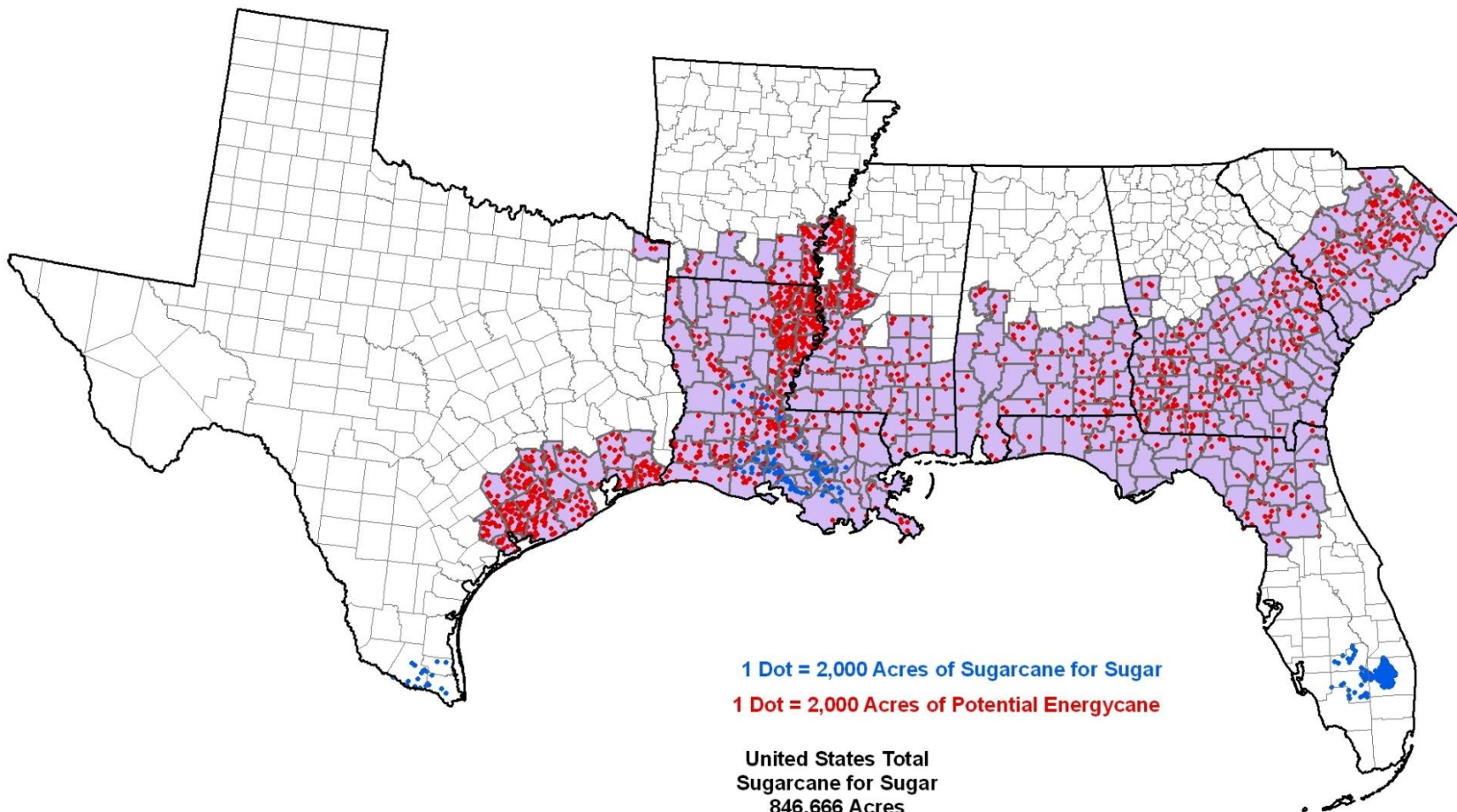


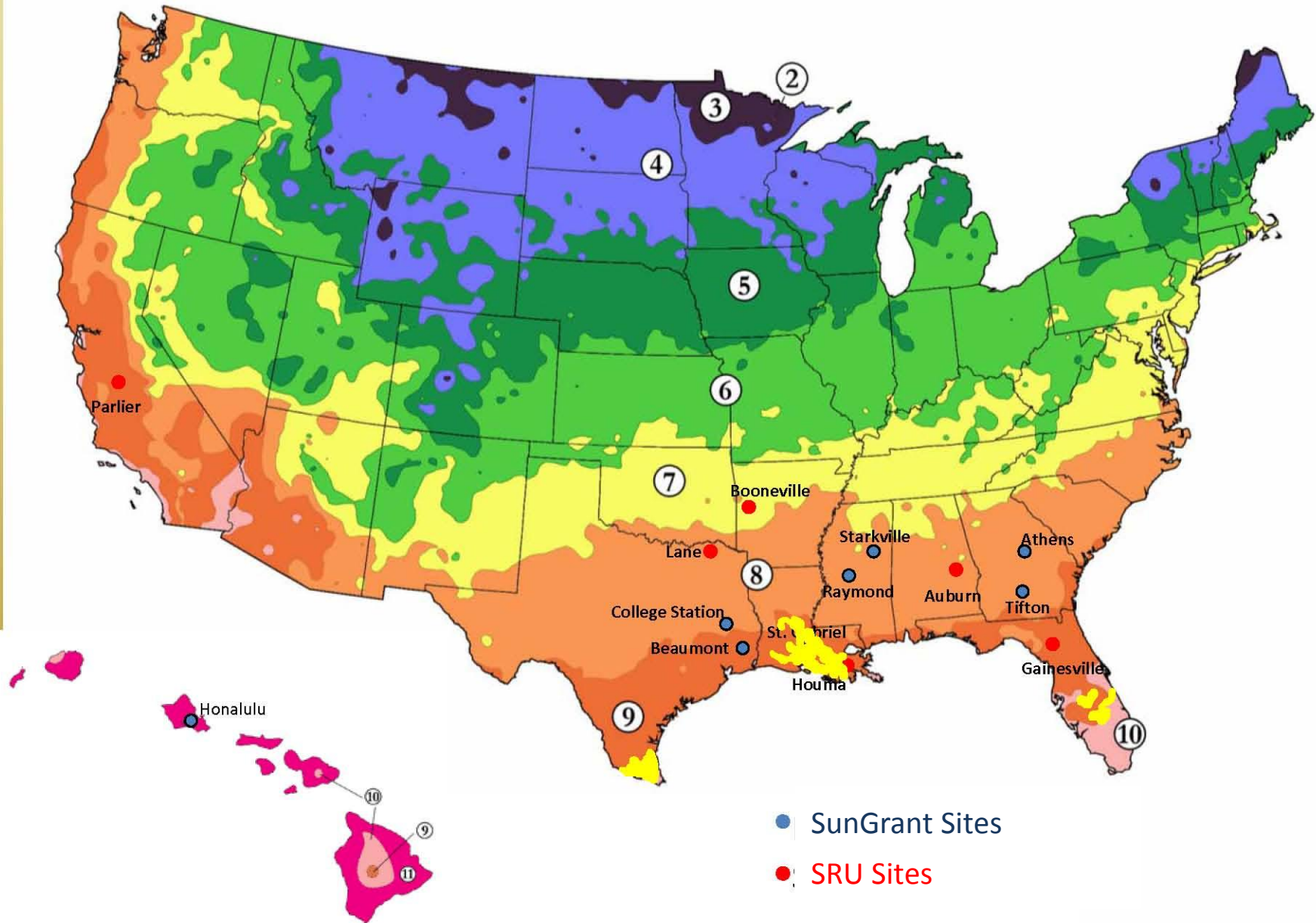






10/01/2001





Common Energycane Germplasm

Germplasm Line*	Pedigree
Ho 02-147	F ₁ (Wild Cane x Sugarcane)
Ho 02-144	F ₁ (Wild Cane x Sugarcane)
US 72-114	BC ₁ with Sugarcane
Ho 06-9001	BC ₁ with Wild Cane
Ho 06-9002	BC ₁ with Wild Cane
Ho 00-961	R-MS, Be-TX & HI
Ho 95-988	HI
Ho 00-07	HI

*from USDA-ARS-SRU, Houma, LA

Second Year Field

Ho 06-9001

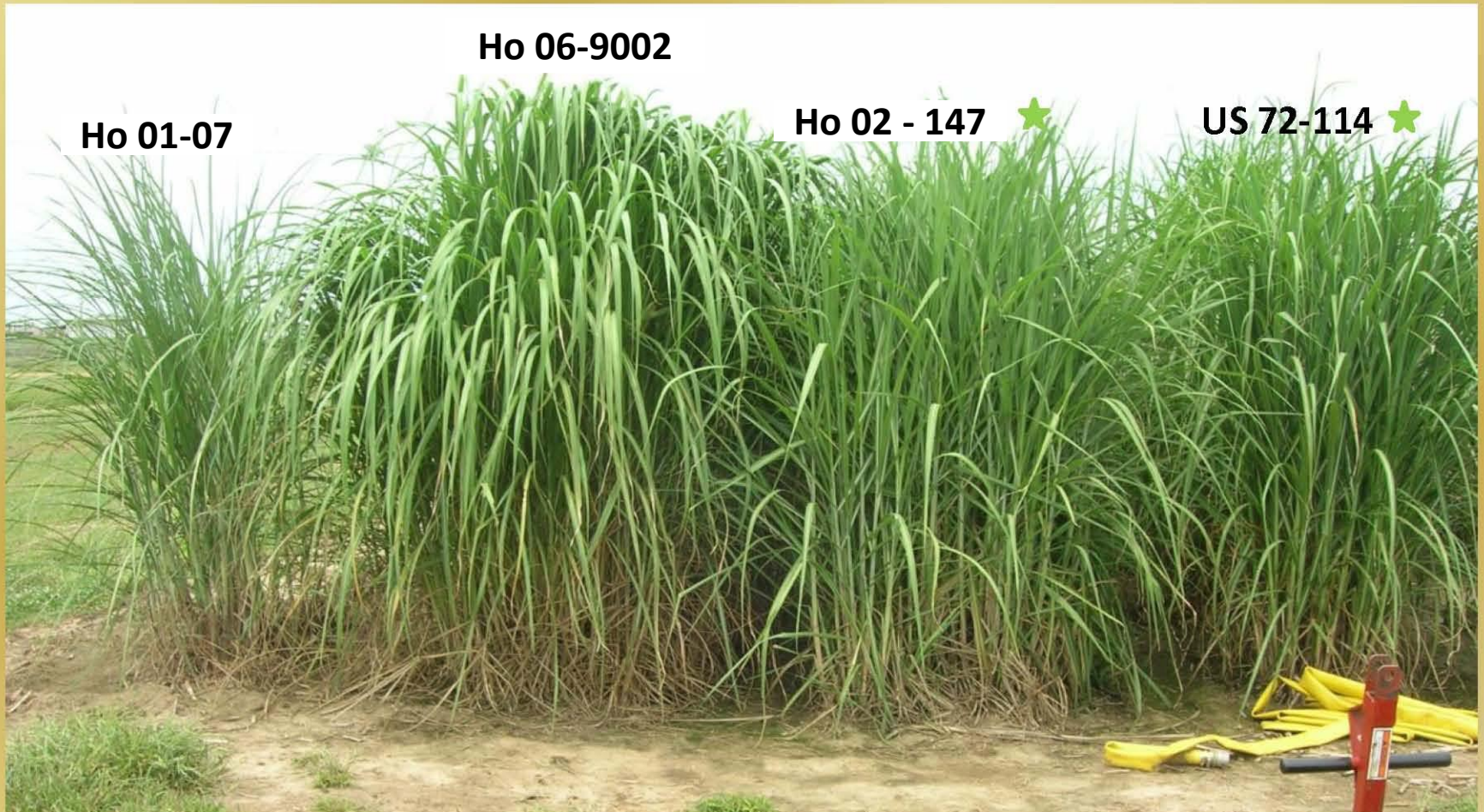
Ho 01-07

Ho 00-961



Starkville, MS; Aug 2008

Second Year Field

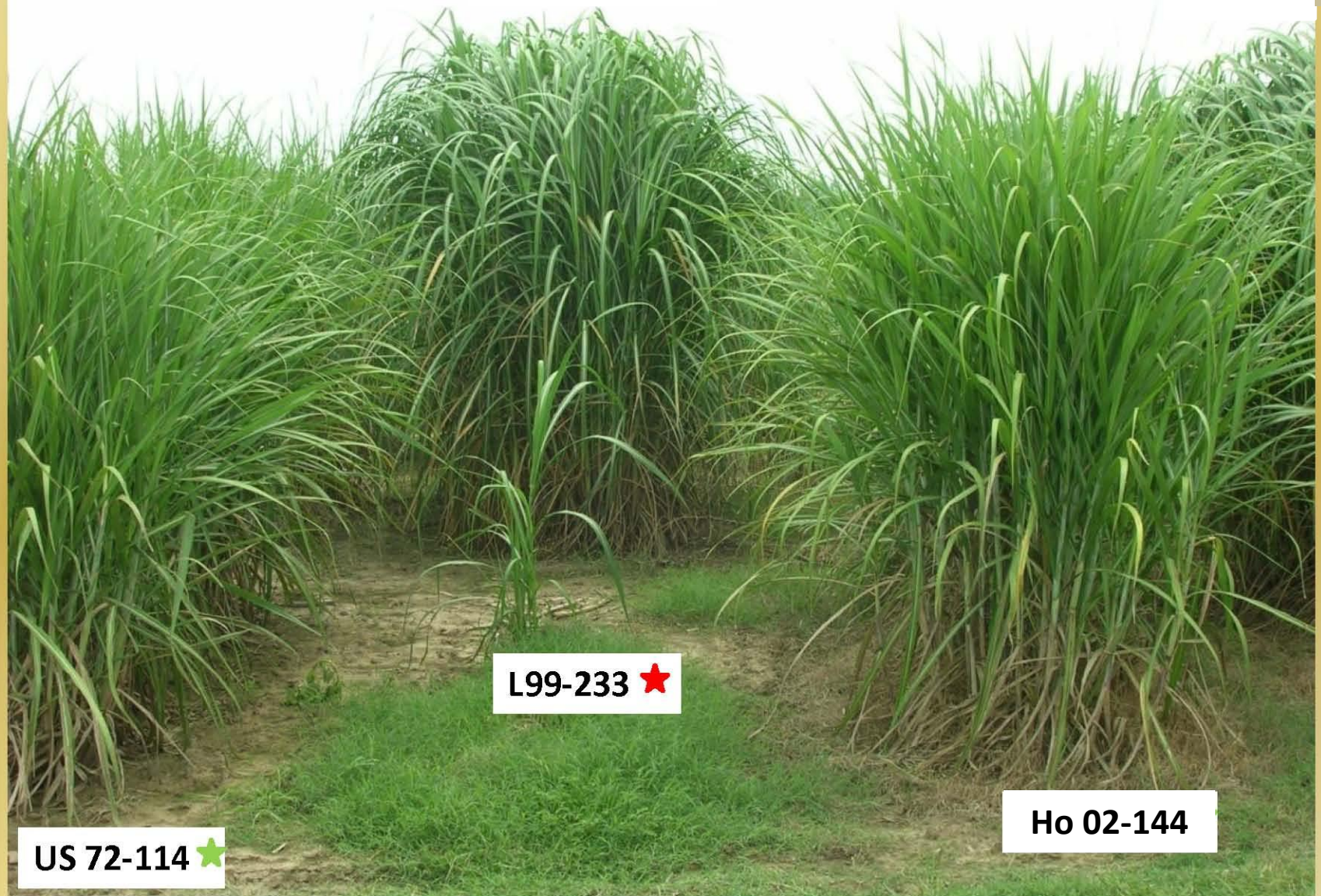


Courtesy of Brian Baldwin, MSU

Starkville, MS; Aug 2008

Second Year Field

Ho 06-9002



L99-233 ★

Ho 02-144

US 72-114 ★



Courtesy of Brian Baldwin, MSU

Starkville - February 2008



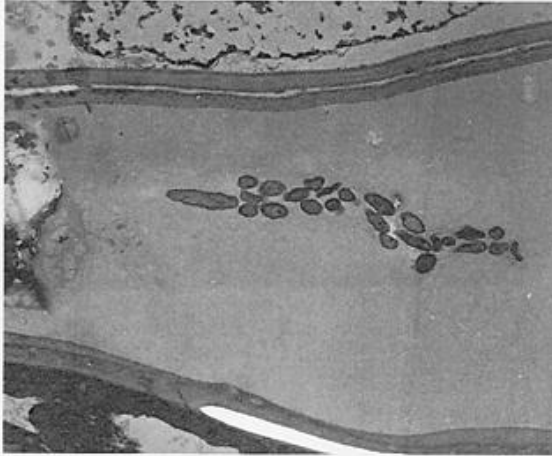
Introduction

- Louisiana sugarcane receives 90-180 kg N/ha
- Biological N fixation (BNF) may reduce requirement
- In one nitrogen balance study in sugarcane, 70% of biomass from BNF

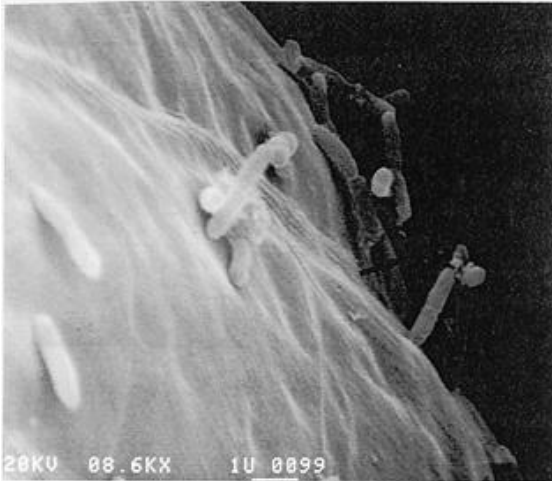
Research Objectives

- **Attempt to isolate diazotrophic, endophytic bacteria from Louisiana-grown sugarcane**
- **Measure how much N can the isolates “fix”**
- **Determine if we can inoculate commercial varieties with N-fixing bacteria**

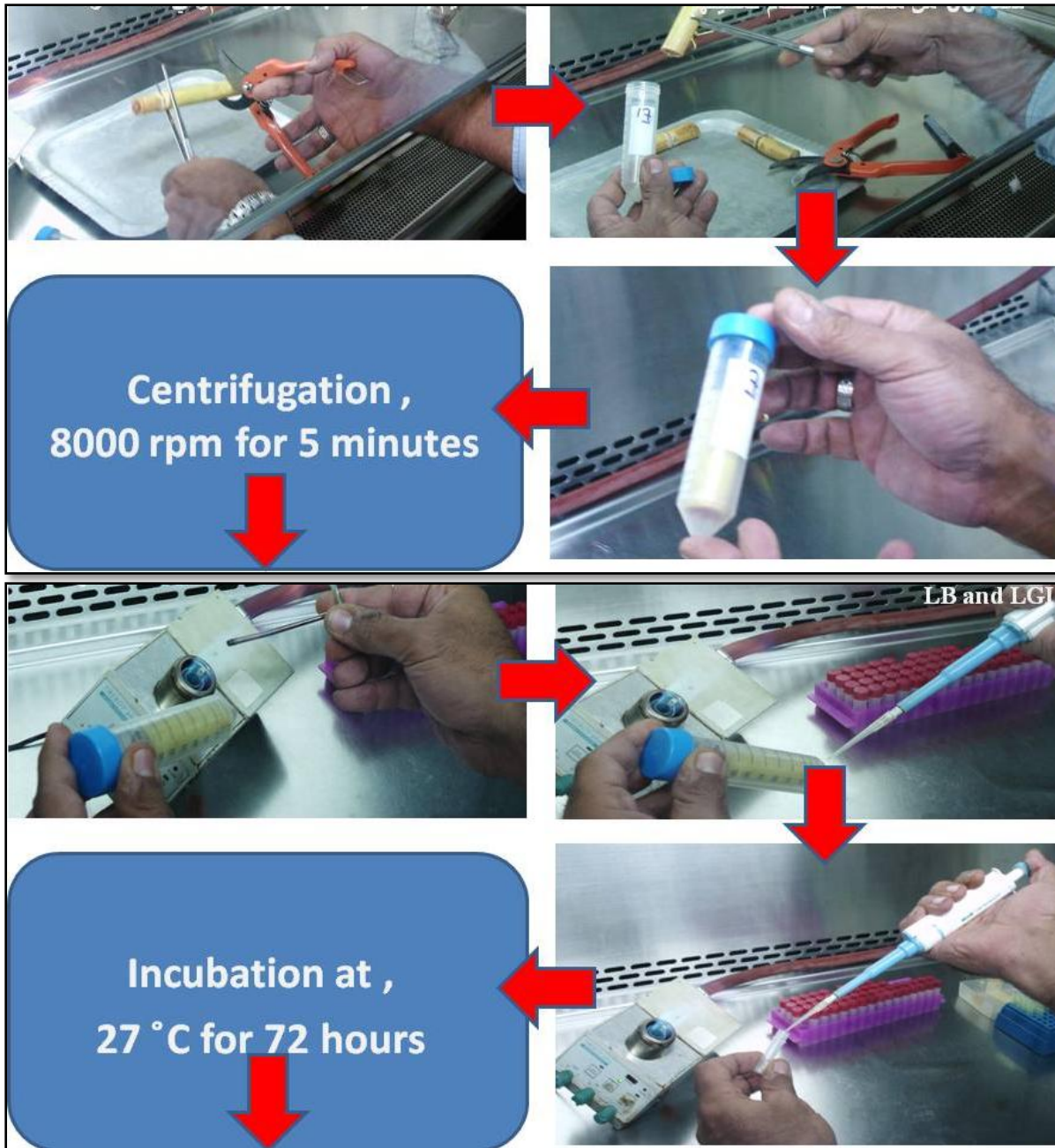
BNF Process



- Endophytic bacteria (live among cells of plant tissue)
- Convert atmospheric N to plant-available N
- Some may be antagonistic to pathogens



• Isolate bacteria on nitrogen-free medium (LGI-P) from stalks



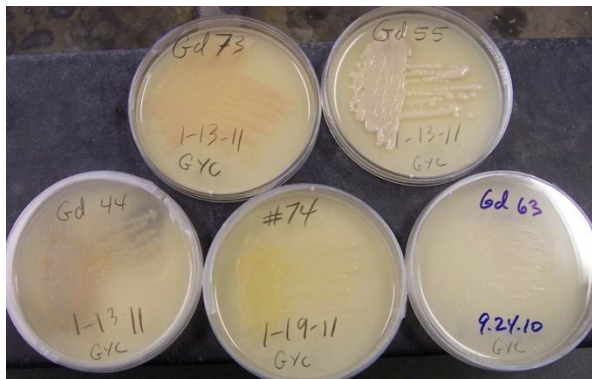
Procedures



- Isolate bacteria on nitrogen-free medium (LGI-P) from stalks
- Identify isolates through rDNA sequences analysis and blasted in NCBI
- Nitrogen-fixation capability testing
- Inoculation studies
- Test effect of N-fixing bacteria on pathogens

Current Progress

- Isolated approx. 100 bacterial isolates from commercial Louisiana varieties
- Isolates grew on N-deleted media

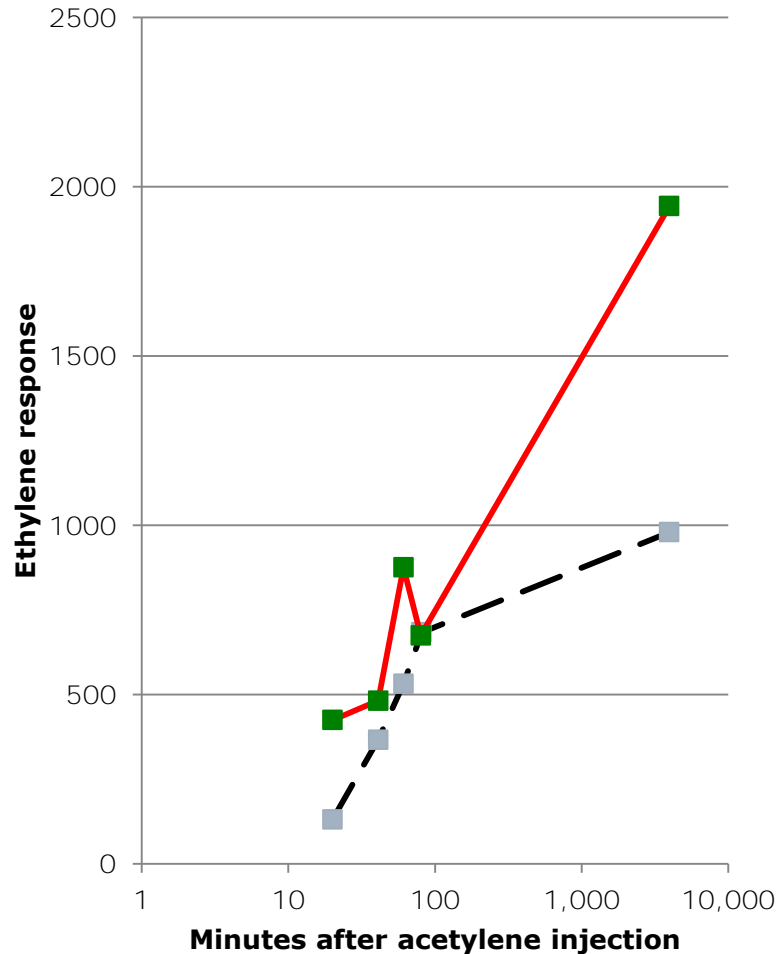


- The identity of some is the same as those from Brazilian sugarcane

Bacterial Isolation

Egyptian Isolates (20)	Louisiana Isolates (85)
Gluconacetobacter*	Gluconacetobacter*
Burkholderia	Burkholderia
	Herbaspirillum
Panoea	Panoea
Enterobacter	Enterobacter
Pseudomonas	Pseudomonas
Frateauria or Dyella	
Aneurinibacillus	
Pectobacterium	
	Xanthomonas oryzae oryzae
* Including G. diazotrophicus	

- **Two *Gluconacetobacter diazotrophicus* isolates demonstrated nitrogen-fixation capability**



Isolates of *G. diazotrophicus* exhibited moderate levels of nitrogenase activity (2 nmol C₂H₄ per hour).

—■— Std culture
—■— Isolated from 00-950



Industry Benefits

- **Lower nitrogen fertility rates**
- **Inoculating tissue-culture propagated plants with endophytic, N-fixing bacteria could:**
 - **improve seed cane germination**
 - **reduce effects of systemic diseases**



Thank You