Old World Bollworm:
Assessing A Potential New Threat.
(Plus, a bonus fungal endophyte update)

Gregory A. Sword
Department of Entomology, Texas A&M University, College Station, TX

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With help from:
Jason Wulff, Charles Allen, Stephen Biles, Megha Parajulee, Pat Porter & Suhas Vyavhare

TEXAS A&M AGRILIFE

Invasion of the Old World bollworm

(*Helicoverpa armigera*)

Known knowns:

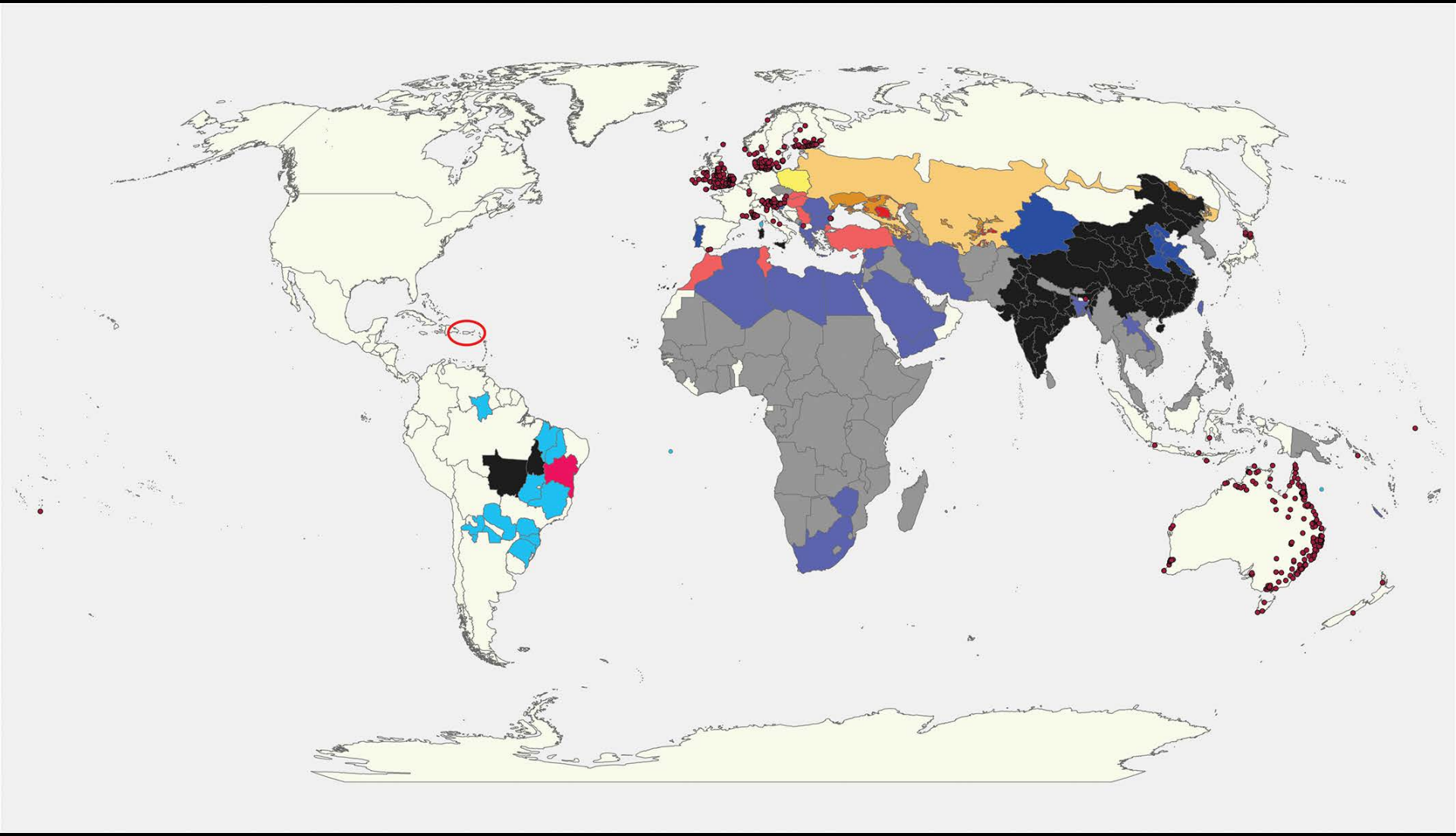
- Brazil 2013; Puerto Rico ’14; Florida ‘15
- Infamous agricultural pest
- Broad host range
- High dispersal capacity
- Insecticide resistance
- Huge potential range

Known *unknowns:*

- If, when, where, and how US establishment will occur
- Extent of invasion
- Severity of impact
- Management response

Unknown unknowns?

Helicoverpa armigera global distribution



Helicoverpa armigera is a severe economic pest

H. armigera **feeds on over 200 different plants in 45 different families.**

Considered a severe economic pest in most places.

Target of over 50% of all insecticides in India and China.

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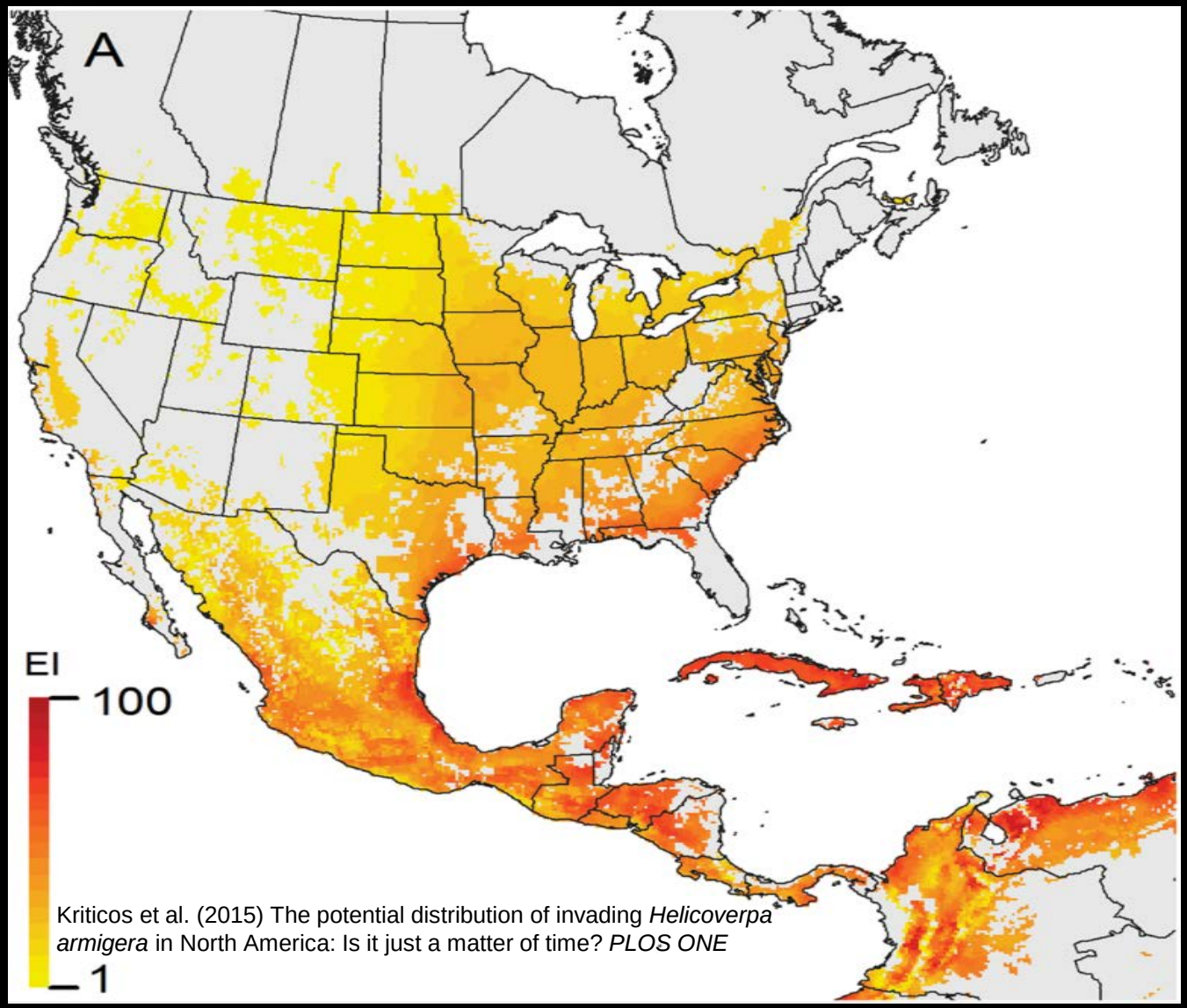
Major hosts:

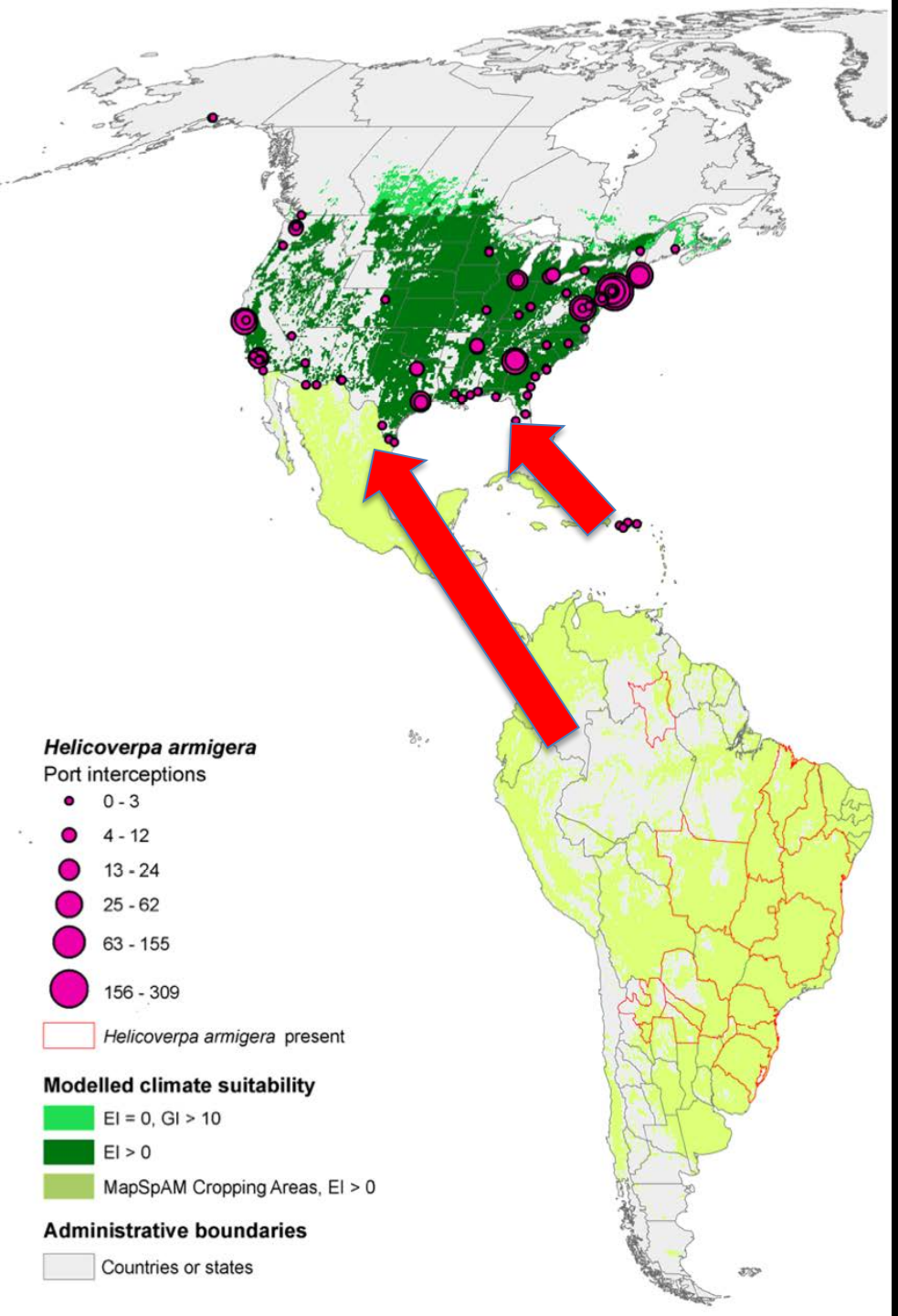
artichokes	okra
beans and forage legumes	peas
bell peppers	potatoes
cacao	rice, sorghum, and sugarcane
chrysanthemums	sunflowers
cotton	tobacco
maize, wheat, and other small grains	tomatoes

Predicted suitable range in N. America



Kriticos et al. (2015) The potential distribution of invading *Helicoverpa armigera* in North America: Is it just a matter of time? *PLOS ONE*

$78 billion annually in crops at risk



Invasion pathways

Accidental

- 1017 mainland border interceptions (1998-2014)

Dispersal

- Expanding S. American range – Overland to SW US?
- Found in Puerto Rico – Sept. 2014 (USDA-APHIS)
  - Island hopping to SE US?
  - Florida 2015

Kriticos et al. (2015) The potential distribution of invading *Helicoverpa armigera* in North America: Is it just a matter of time? *PLOS ONE*

Old world bollworm detected in Florida

Posted by Jennifer Carr on July 22, 2015 Leave a Comment

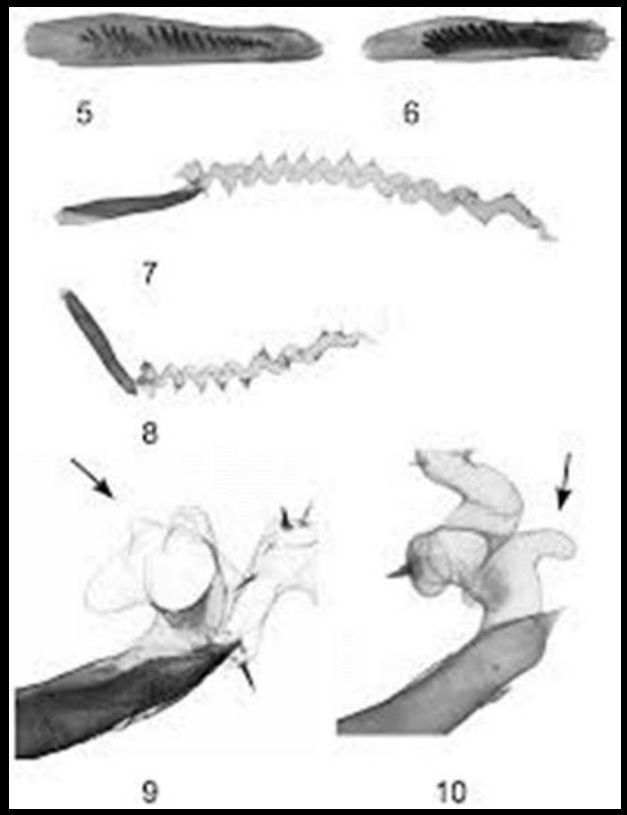
Figure 1. Adult old world bollworm. Credit: Gyorgy Csoka, Hungary Forest Research Institute, Bugwood.org

Old World vs New World bollworm identification

(*Helicoverpa armigera vs H. zea*)

Adults males can be distinguished by morphology.

- Microscopic dissection of male genitalia



Caterpillars are indistinguishable.

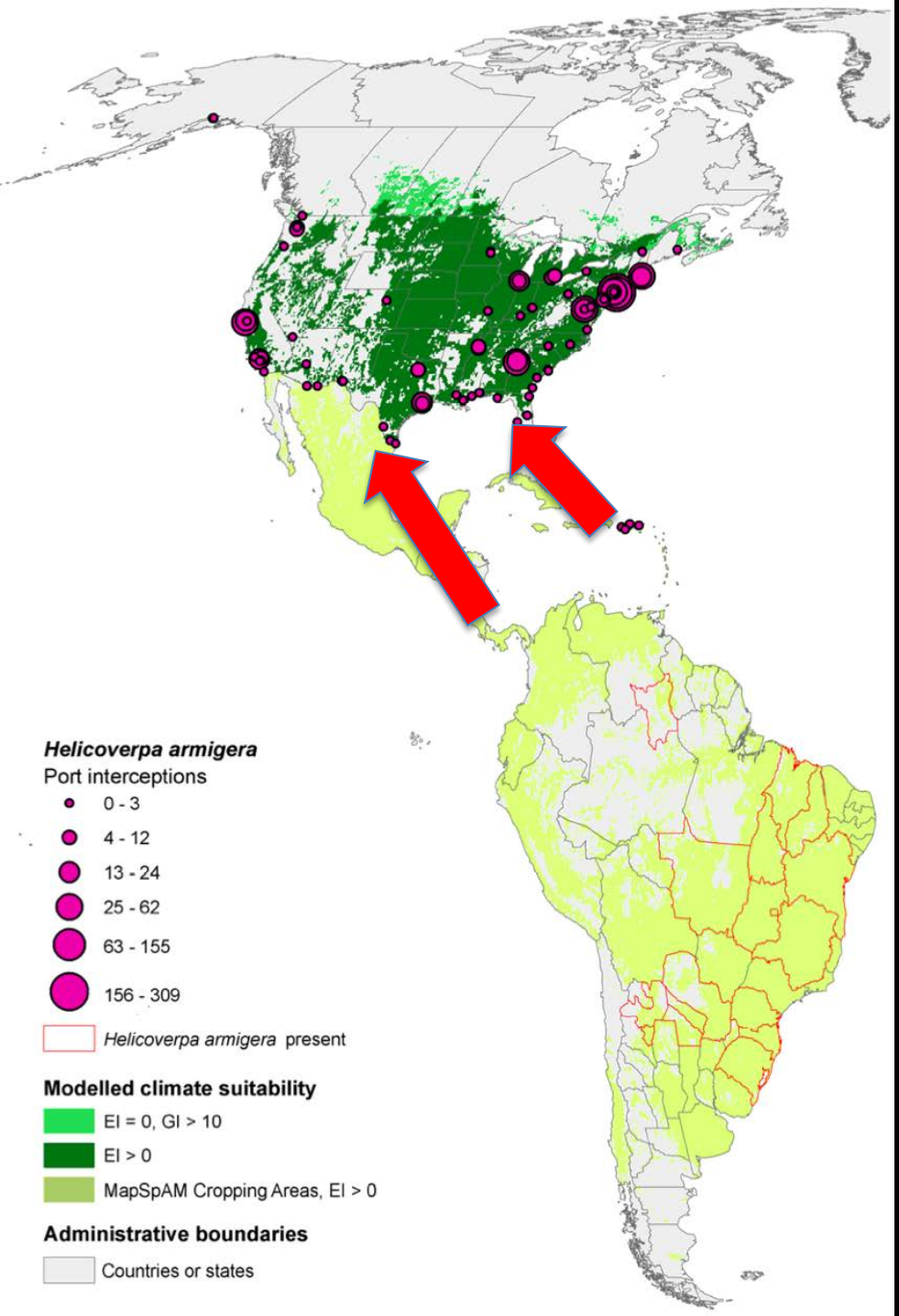
- DNA only

Hybrids?

- Hybridization already detected in Brazil
- DNA only

***Invasive genomes* vs invasive species**

- *H. armigera* genes could already be here in *H. zea*



Helicoverpa IPM in the US

Facts:

- Old World Bollworm is coming
- *H. zea* X *armigera* hybridization is occurring
- Species ID by morphology unreliable
- Need to monitor '*Invasive Genomes*'

Complications:

- Where & when are unknown
- Huge area to monitor
- Genetic testing costs $$$
- Risk and costs are not specific to any particular commodity, agency or industry.

Kriticos et al. (2015) The potential distribution of invading *Helicoverpa armigera* in North America: Is it just a matter of time? *PLOS ONE*

Current *Helicoverpa zea* IPM practices in the south-central United States.

Cotton (cotton bollworm):

1. Largely controlled with pyramided *Bt*
2. Thresholds exist for both *Bt* and non-*Bt*
3. Scouted from first bloom to boll maturation (peak bloom: most susceptible)
4. <1/4 inch worms generally ignored
5. No yield gain from overspray
6. Pyrethroids (some diamides)

Current *Helicoverpa zea* IPM practices in the south-central United States.

Corn (corn earworm):

1. Not considered an economic pest
2. Transgenics with Vip3a are near high dose
3. Low refuge compliance in the south (cotton zone)
4. Seed blends becoming more common
   - Resistance risk?
5. Possible whorl stage risk (FAW and CEW) - diamides

Current *Helicoverpa zea* IPM practices in the south-central United States.

Sorghum (sorghum headworm):

1. Headworm complex with Fall armyworm (FAW)
2. Some treatments every year
3. Pyrethroids – both CEW and FAW susceptible
4. Complicated by sugarcane aphids
   - Pyrethroids flare aphids
   - Diamides recommended - $$$

CEW pyrethroid resistance in treated sorghum could be a 'canary in the coal mine' for OWB or its genes.

What are ‘we’ doing *now* about OWB?

One federal agency's opinion on preventative action

Major proposal submitted in 2015 to the USDA Food Security Program

- $4 million project
- International collaboration
- Population genetic monitoring across the US
- Efficacy assessments of major IPM tools
  - *H. armigera* and *H. zea* under quarantine
  - Insecticides
  - Bt transgenincs
  - Biological control

One federal agency's opinion on preventative action

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  - Bt transgenincs
  - Biological control

Panel Summary comment:

"The project focuses significant resources on a pest that is not yet found in the US. Funds will be better spent in dealing with more relevant and existing pest issues."

What are ‘we’ doing *now* about OWB?

1. **USDA-APHIS Old World Bollworm Strategic Planning Workshop**
   - April 4-6, 2016, Gainesville, FL
   - Strategic Response Plan
     - Diagnostics
     - Monitoring
     - Communication

2. **Texas High Plains monitoring efforts**
   - Led by Meg Parajulee
   - TSSC & AgriLife supported

3. **Sword lab and APHIS collaboration**
   - Molecular diagnostics
   - Farm Bill FY2016

Do we have OWB in the High Plains?

- Megha Parajulee

- **There is NO evidence of OWB invasion in Texas or Texas High Plains.**
  - Monitoring via pheromone trapping and dissection since 2015 (in Texas High Plains)
    - Four locations
    - Two trap designs (Texas trap vs. bucket trap)
    - Two lure types (USDA lure vs. Trécé lure)

- This effort is partially supported by Texas State Support Committee

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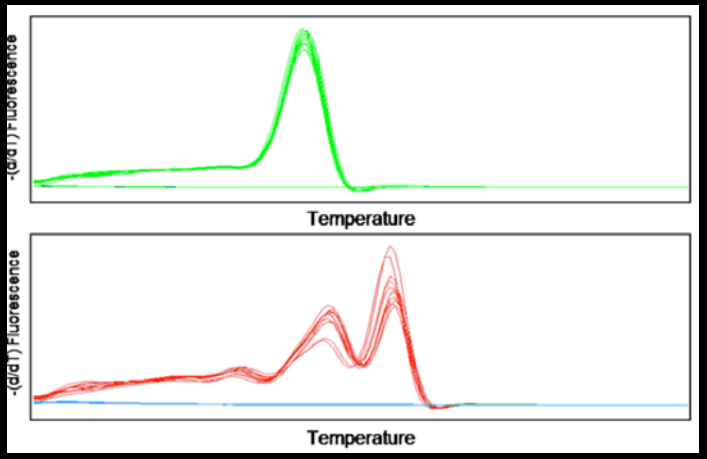
Current Molecular Detection Assays

- **Two currently accepted USDA-APHIS tests**
- **Both rely on variation in *one* DNA marker**
- **Internal transcribed spacer (ITS) region (Nuclear)**
  - qPCR based detection
  - Probe binding ITS2 (Gilligan et al. 2015)
  - Amplicon length ITS1 (Perera et al. 2015)



H. zea

H. armigera

Genotyping of 'suspicious' *Helicoverpa* in the US.

LOCATION	COLLECTOR	DATE	# EXTRACTIONS
Beltsville, MD	G. Dively	9/15/2015	18
Beltsville, MD	G. Dively	8/20/2015	23
Comanche County, TX	C. Valencia	10/4/2015	2
College Station, TX	A. Tessnow	9/19/2015	7
White Bear Lake, MN	C. Deans	9/30/2015	27
Redwood County, MN	B. Potter	10/9/2015	24
Thorndale, TX	C. Valencia	6/1/2016	12
Nueces County, TX	Robert Bowling	6/21/2016	12
Wharton, TX	Robert Bowling	7/12/2016	12

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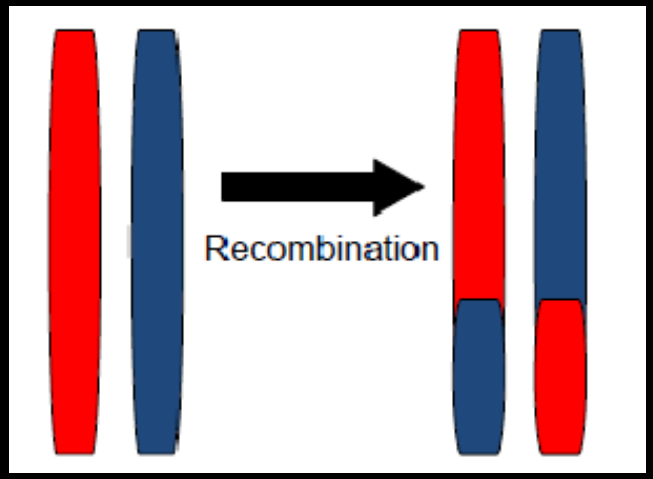
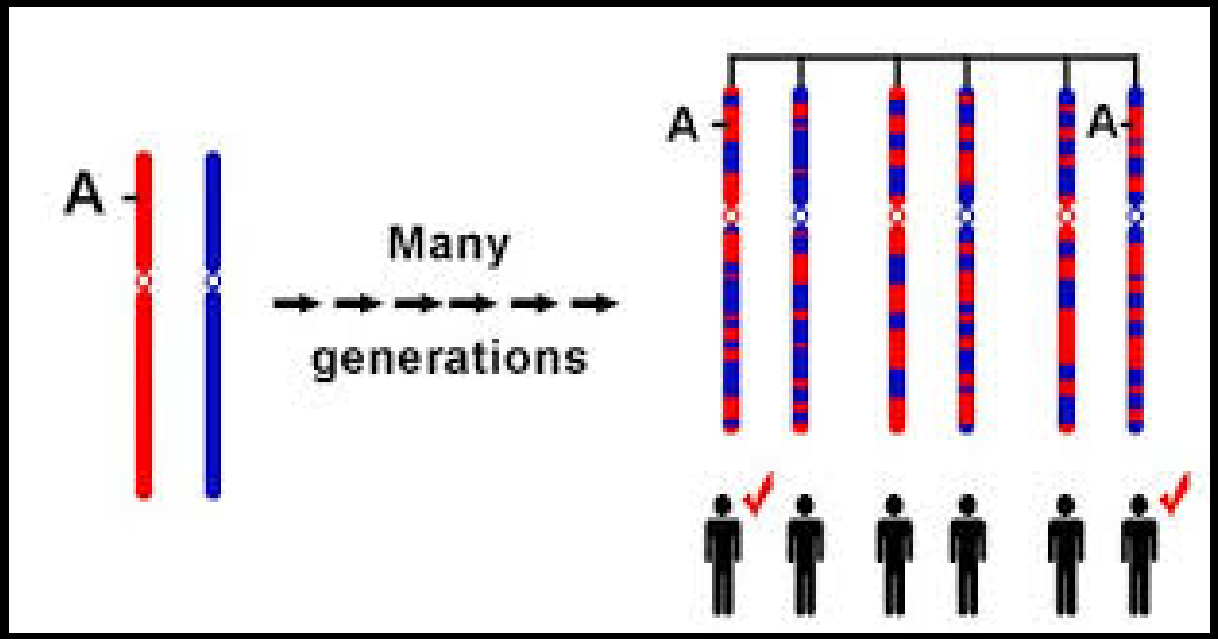
- **All *H. zea*.**
- **Two 'blind' positive *H. armigera* were detected.**
- **Does not rule out possibility of hybrids**
- **Based on 1 individual per sample (lots of time and $$$)**

Detecting *H. armigera* x *zea* hybrids?

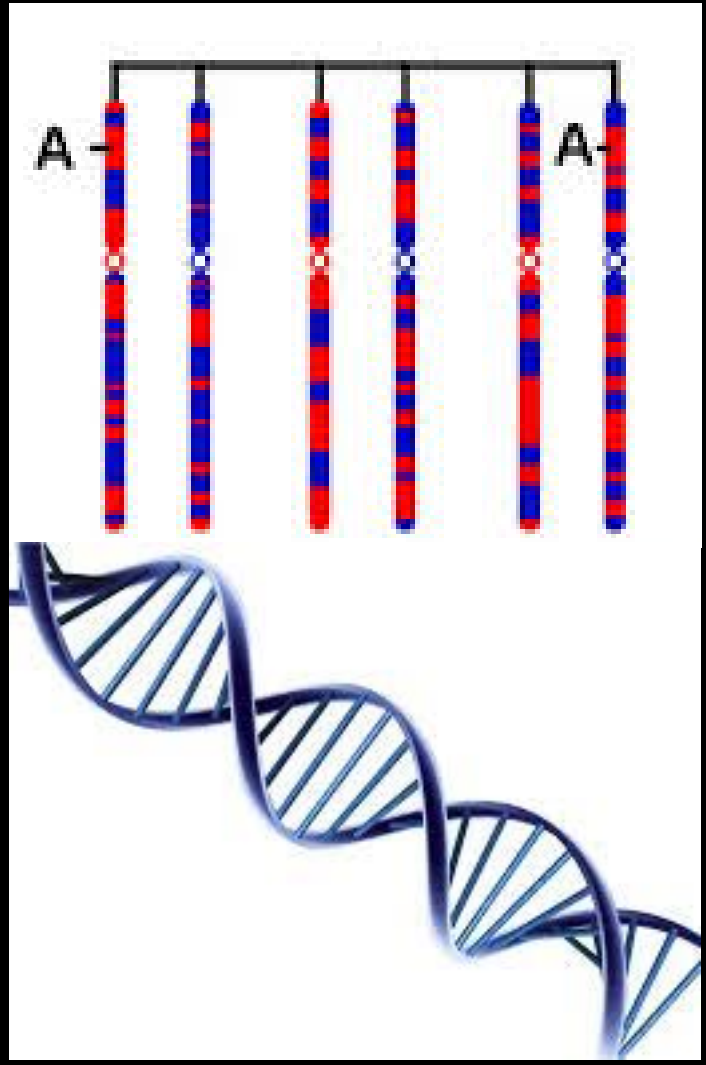


Using only 1 DNA marker is *insufficient*.



Multiple genome-wide markers

- **Cytochrome oxidase 1** (Mitochondrial)
- **Internal transcribed spacer** (ITS) region (Nuclear)
- **Chimeric P450 gene**
  - resistance to fenvalerate, a pyrethroid insecticide
- **SNPs (single nucleotide polymorphisms)**
  - Detect low levels of hybridization and gene flow between *zea* and *armigera*
  - Determine source population of introduction



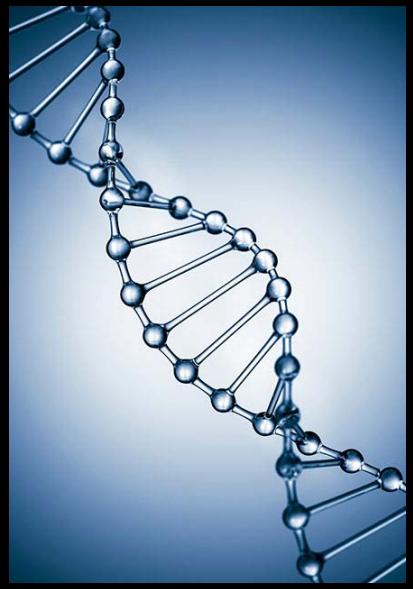
Next Generation Sequencing (NGS)

- **Traditional approaches can only identify one sequence per sample**

- **NGS can sequence multiple genes at the same time**

- **NGS produces millions of DNA reads**
  - Detect even dilute amounts of *H. armigera* DNA obtain from *H. zea* dominant pheromone traps

- **NGS Bulk Detection Assay?**



Molecular-based initiatives to support the Old World Bollworm Strategic Management Plan

(funded by FY16 Farm Bill)

Todd Gilligan - APHIS, Colo. State
Gregory Sword & Jason Wulff - TAMU
O. P. Perera - USDA-ARS
Norman Barr - APHIS-PPQ Mission Lab

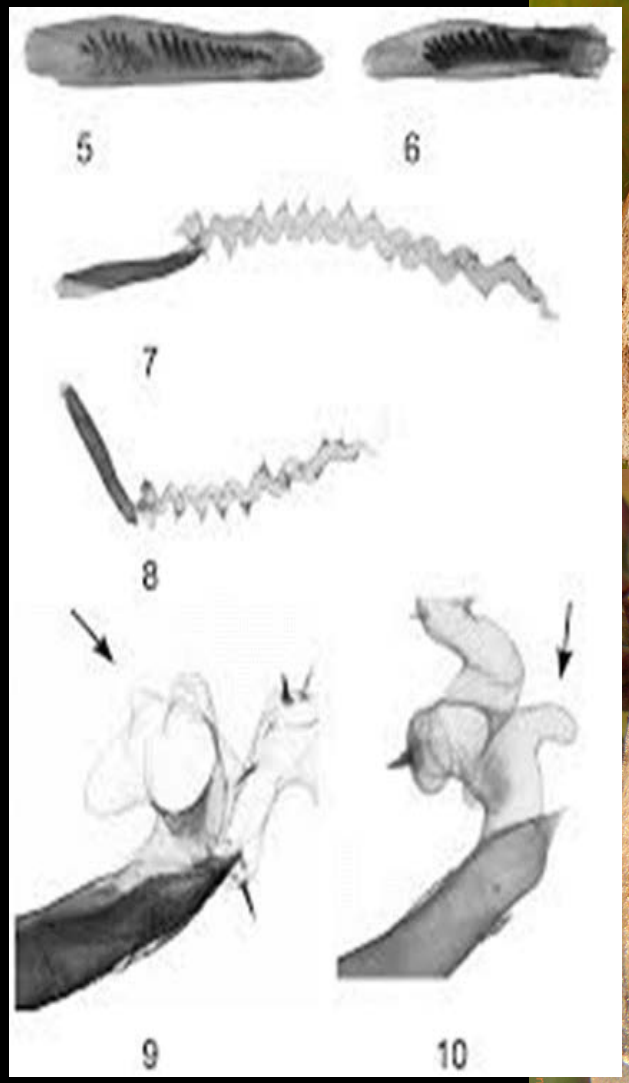
Specific Objective:

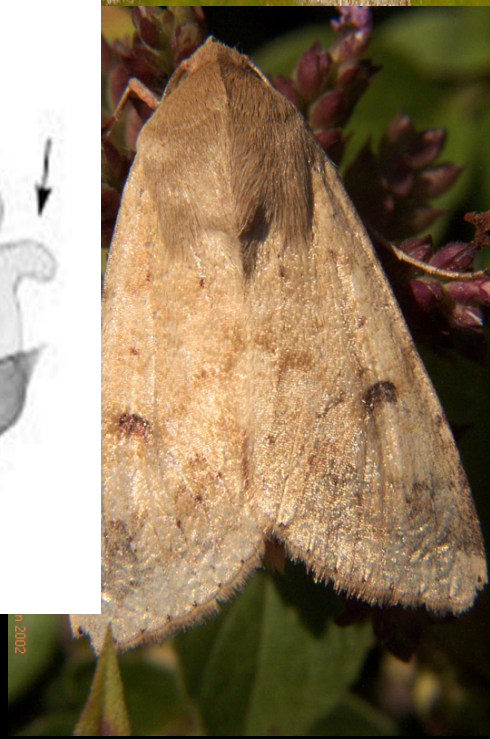
- Develop a next-generation DNA sequencing-based assay for the detection of *H. armigera* in bulk trap samples

Develop a next-generation DNA sequencing-based assay for the detection of *H. armigera* in bulk trap samples

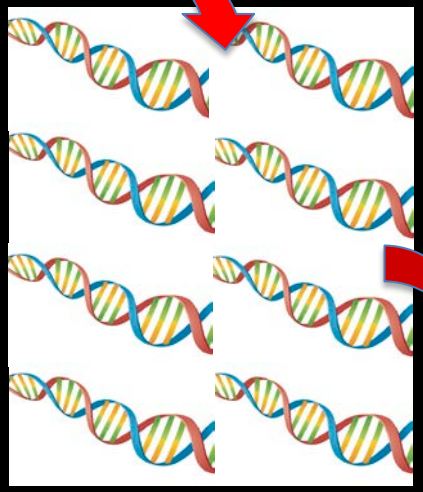
H. zea or *H. armigera*?

- **Visually cannot distinguish between caterpillars or moths**
- **Male moths from both species are attracted to pheromone trap**
- **Dissections**
  - Male genitalia morphology
- **Molecular detection assay?**





NGS Bulk Detection Assay



OWB genes detected?

Yes / No

Focused Monitoring

- Species ID
- Abundance

No worries

Next Gen Sequencing

H. armigera and *H. zea* Field Diagnostic Assay

- **MinION**
  - (Oxford Nanopore Tech.)
- **Portable NGS**
- **Real-time field application**
- **Rapid results**
- **Inexpensive (?)**

Introducing Indigo's First Commercial Product - Improving Cotton's Water Use Efficiency for a Water Constrained World

INDIGO AGRICULTURE / JULY 21, 2016

COMMODITIES | Fri Jul 22, 2016 6:39pm EDT

Ag-tech startup Indigo raises $100 million new funding round

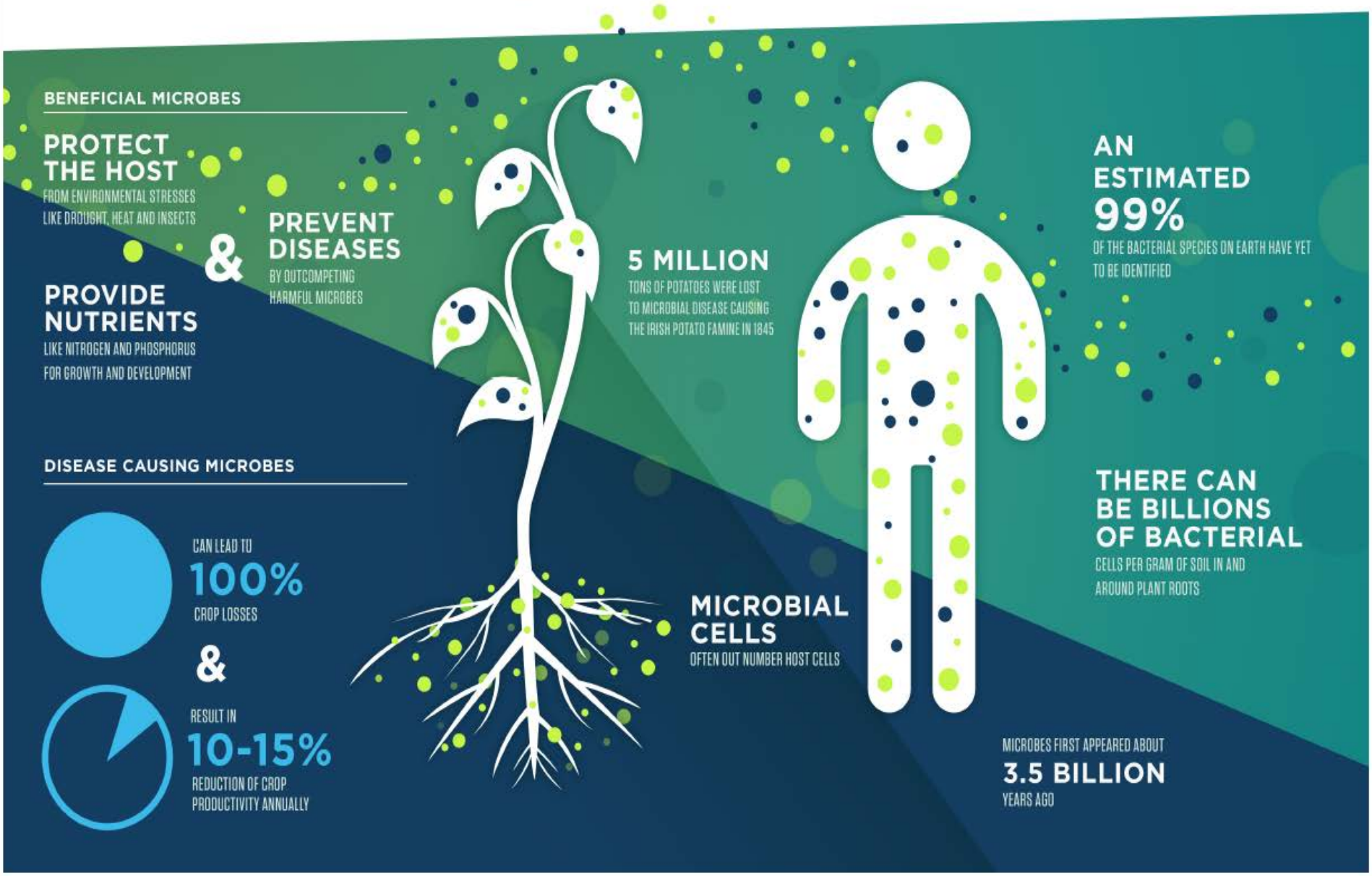
REUTERS

By **Heather Somerville** | SAN FRANCISCO

Agriculture technology startup Indigo has raised $100 million in a new round of funding, bringing its total financing to more than $150 million, a sign of growing investor interest in new ways to ease

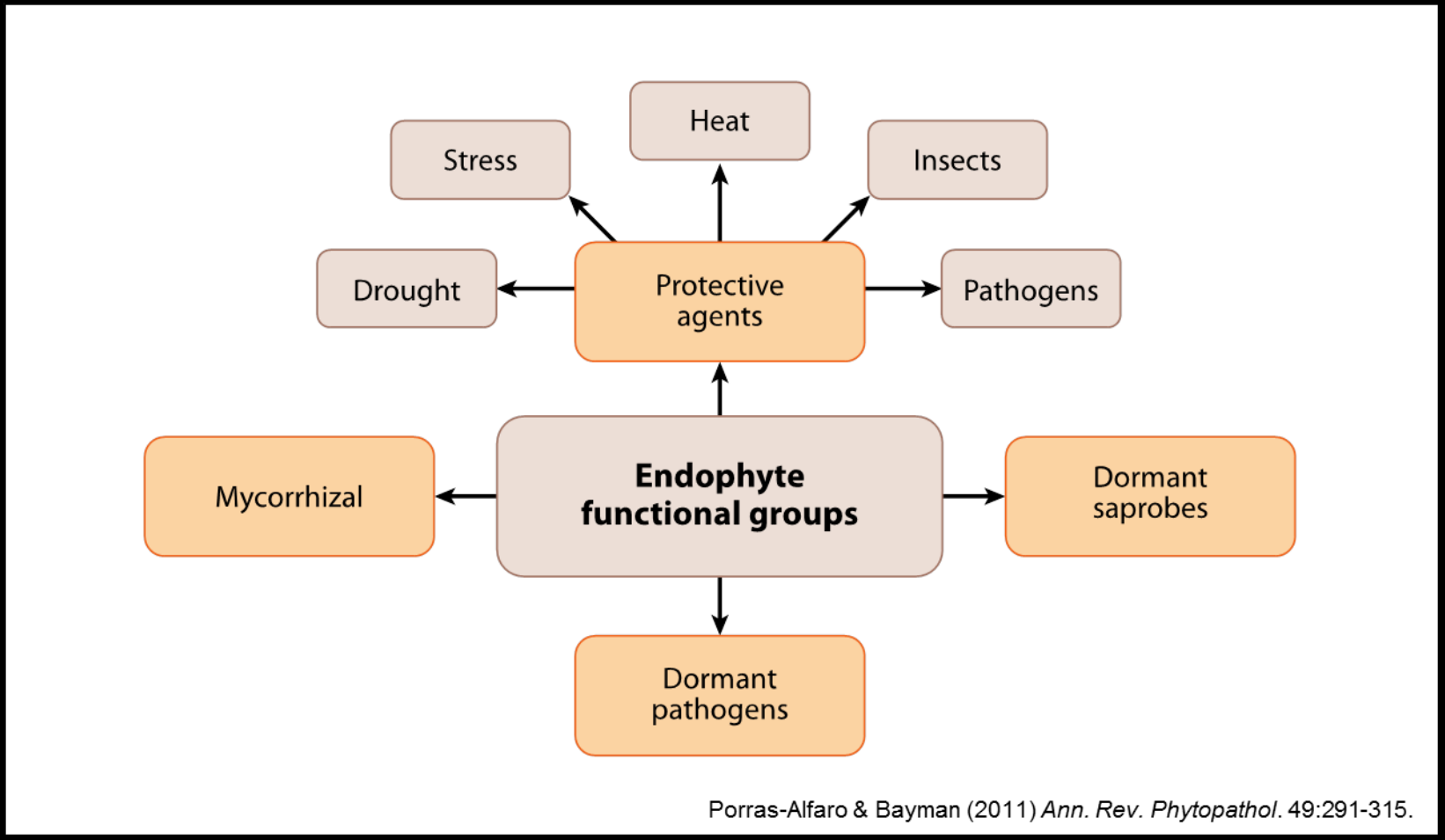
The Influence of Microbes on Agriculture Productivity

DONALD DANFORTH PLANT SCIENCE CENTER
DISCOVERY | COMMUNITY | IMPACT



Fungal endophytes

Fungi that live asymptomatically in plant tissues for at least part of their life cycle.



Can we manipulate the cotton microbiome?

OPEN ACCESS Freely available online

PLOS ONE

Spatial and Temporal Variation in Fungal Endophyte Communities Isolated from Cultivated Cotton (*Gossypium hirsutum*)

María J. Ek-Ramos[1]*, Wenqing Zhou[1], César U. Valencia[1], Josephine B. Antwi[1], Lauren L. Kalns[1], Gaylon D. Morgan[2], David L. Kerns[1,3¤], Gregory A. Sword[1]

1 Department of Entomology, Texas A & M University, College Station, Texas, United States of America, **2** Department of Soil and Crop Sciences and Texas AgriLife Extension, Texas A & M University, College Station, Texas, United States of America, **3** AgriLife Extension Service, Texas A & M University, Lubbock, Texas, United States of America

Endophyte survey summary:

- 3000 isolates
- 69 fungal taxa (OTUs)
- 44 genera
- 706 isolates in library

Greenhouse and field trials of candidate beneficial isolates are ongoing.

Endophytes can negatively affect both above- and belowground herbivores.

OPEN ACCESS Freely available online

PLOS ONE

The Entomopathogenic Fungal Endophytes *Purpureocillium lilacinum* (Formerly *Paecilomyces lilacinus*) and *Beauveria bassiana* Negatively Affect Cotton Aphid Reproduction under Both Greenhouse and Field Conditions

Diana Castillo Lopez[1]*, Keyan Zhu-Salzman[1], Maria Julissa Ek-Ramos[2], Gregory A. Sword[1]

1 Department of Entomology, Texas A&M University, College Station, Texas, United States of America, 2 Department of Immunology and Microbiology, Autonomous University of Nuevo Leon, San Nicolás de los Garza, Nuevo Leon, Mexico

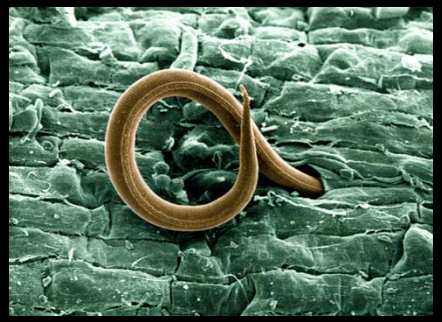
RESEARCH ARTICLE

FEMS Microbiology Ecology, 92, 2016,

The fungal endophyte *Chaetomium globosum* negatively affects both above- and belowground herbivores in cotton

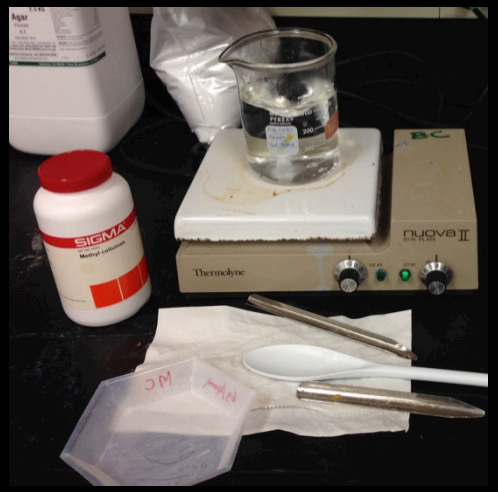
Wenqing Zhou[1,†], James L. Starr[2], Janice L. Krumm[3] and Gregory A. Sword[1,*]

[1]Department of Entomology, Texas A&M University, College Station, TX 77843-2475, USA, [2]Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843-2132, USA and [3]Biology Department, Widener University, Chester, PA 19013, USA



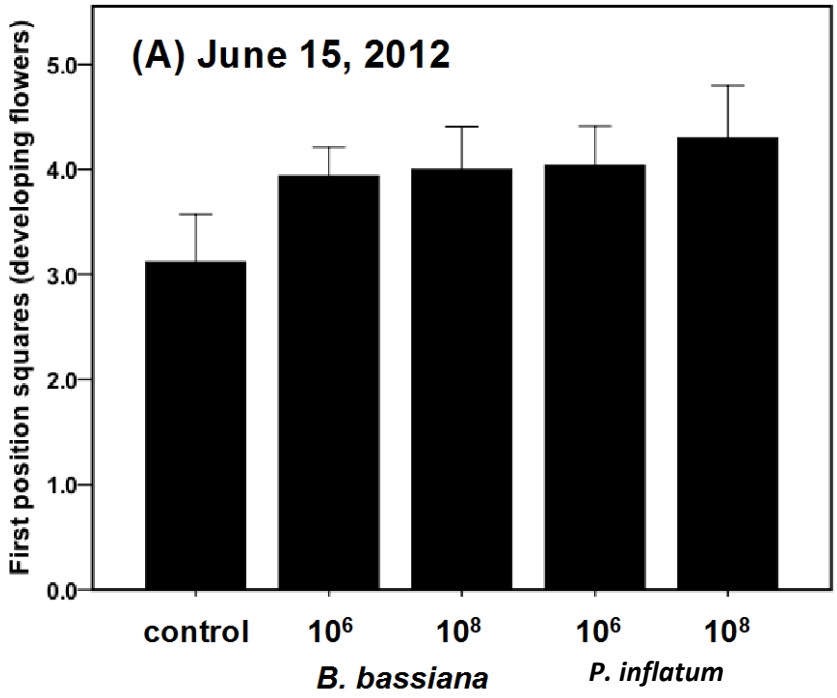
Seed treatment evaluations

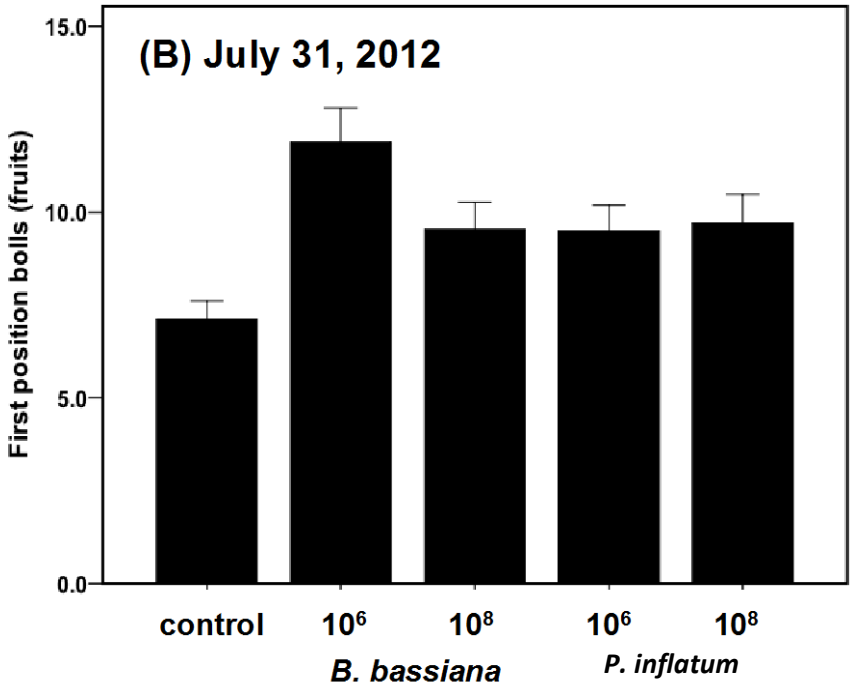
1. **Inoculum**
   - Fungal biomass
   - Spores
2. **Soaking**
   - Seeds soaked overnight in 10^7spore/ml solutions
3. **Stickers**
   - 1% methylcellulose + 10^7spore/ml
   - 10% polyvinyl alcohol + 10^7spore/ml



Cotton Endophyte Field Trials

More squares & bolls retained in *all* endophyte treatments. (2012 field trial)

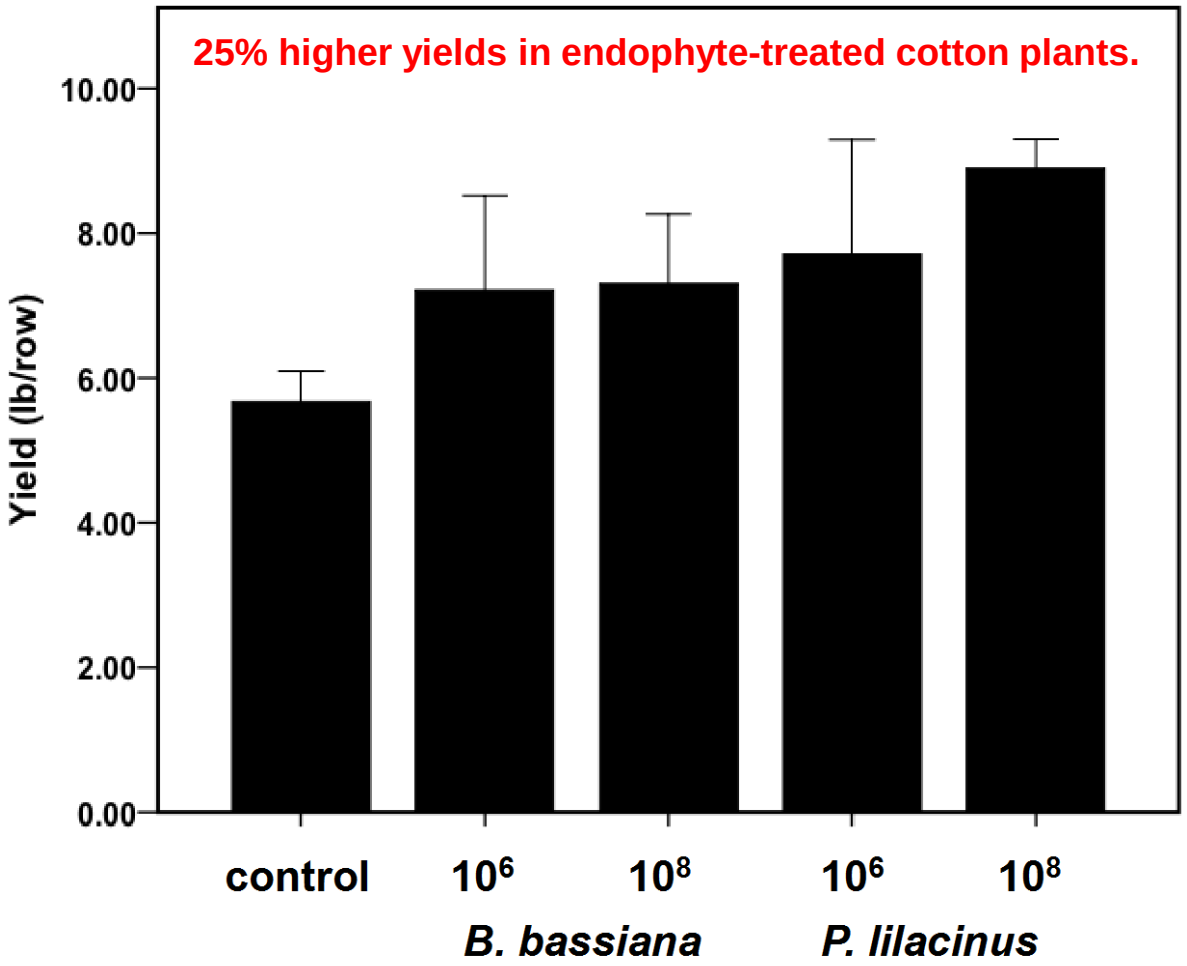




Repeated measures ANOVA (Time, $P < 0.001$; Time*Endophyte, $P = 0.045$, Endophyte, $P = 0.003$)

Positive effects of endophytes on plant reproductive traits.

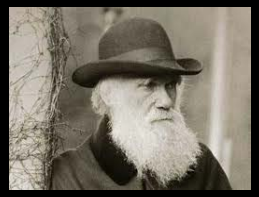
Endophytes can positively affect yields.



2012

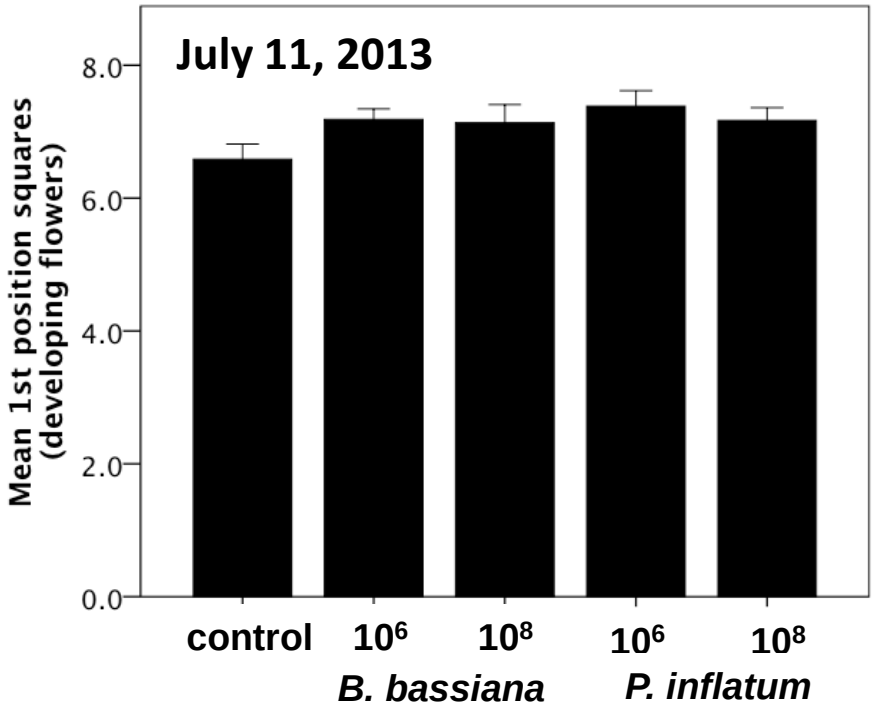
Nested ANOVA
- ENDO: $P = 0.013$
- CONC: NS

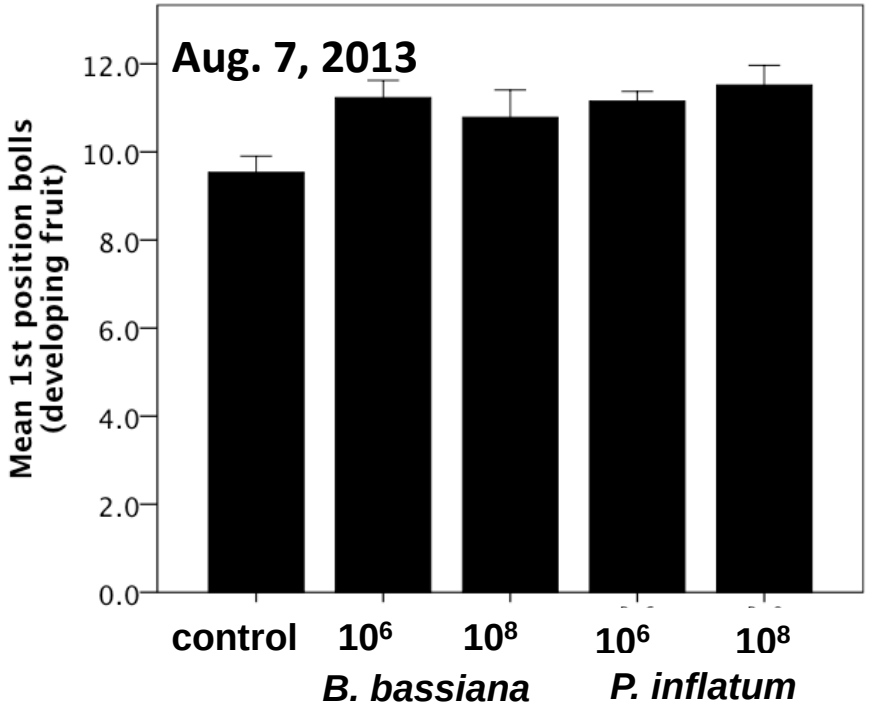
- **Fitness**



- **Yields**

More squares and bolls retained across all endophyte treatments (2013 field trial)



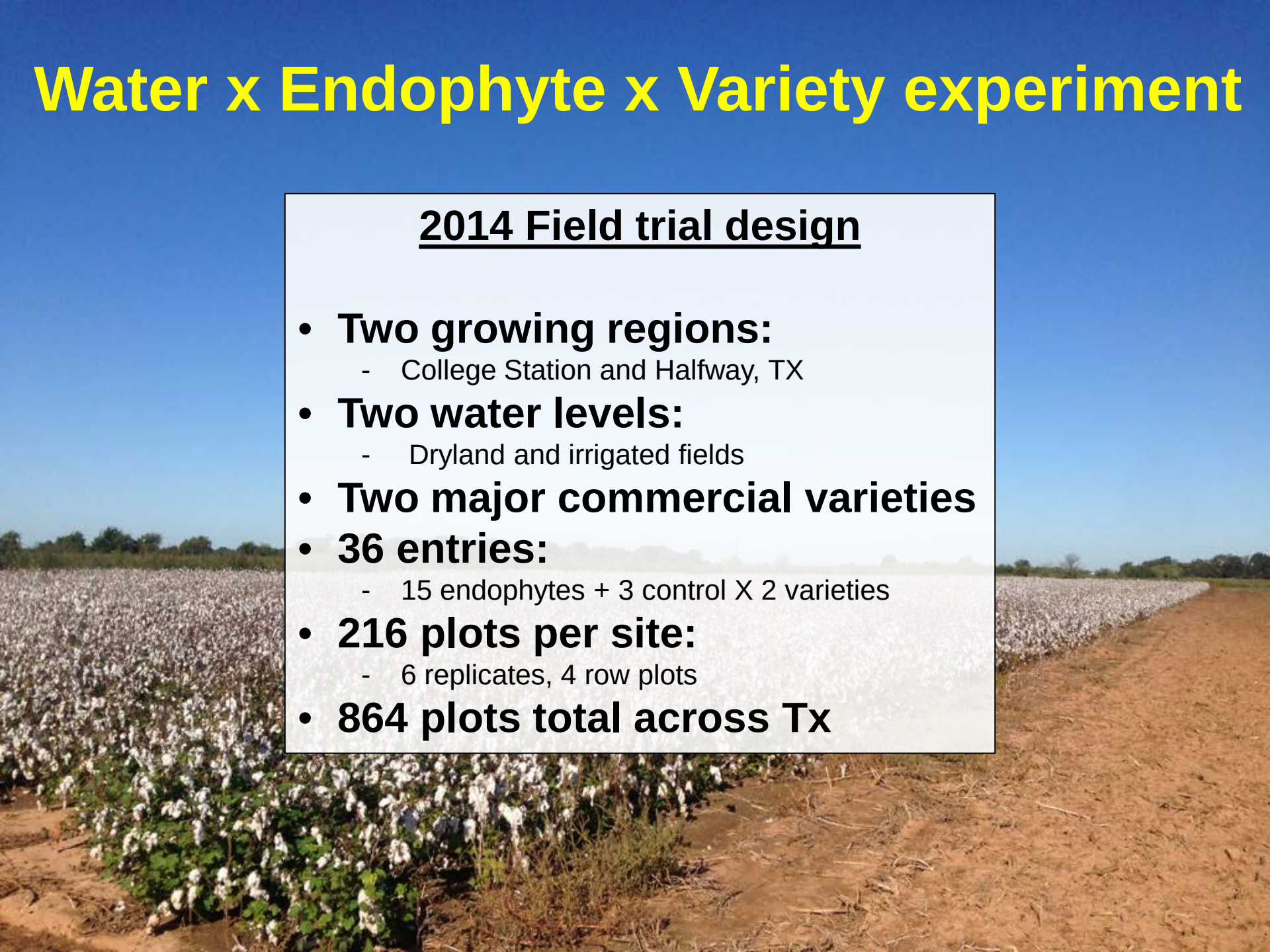


Repeated measures ANOVA (Time, $P < 0.001$; Time*Endophyte, NS, Endophyte, $P = 0.002$)

- **Similar pattern as in 2012.**
- **Positive effects of all endophyte treatments.**
- **Slightly smaller effect size as of Aug. 7.**
- **Lower initial colonization frequencies.**
- **No effect on final yields – late summer rains.**

Endophytes can also confer water stress resistance.

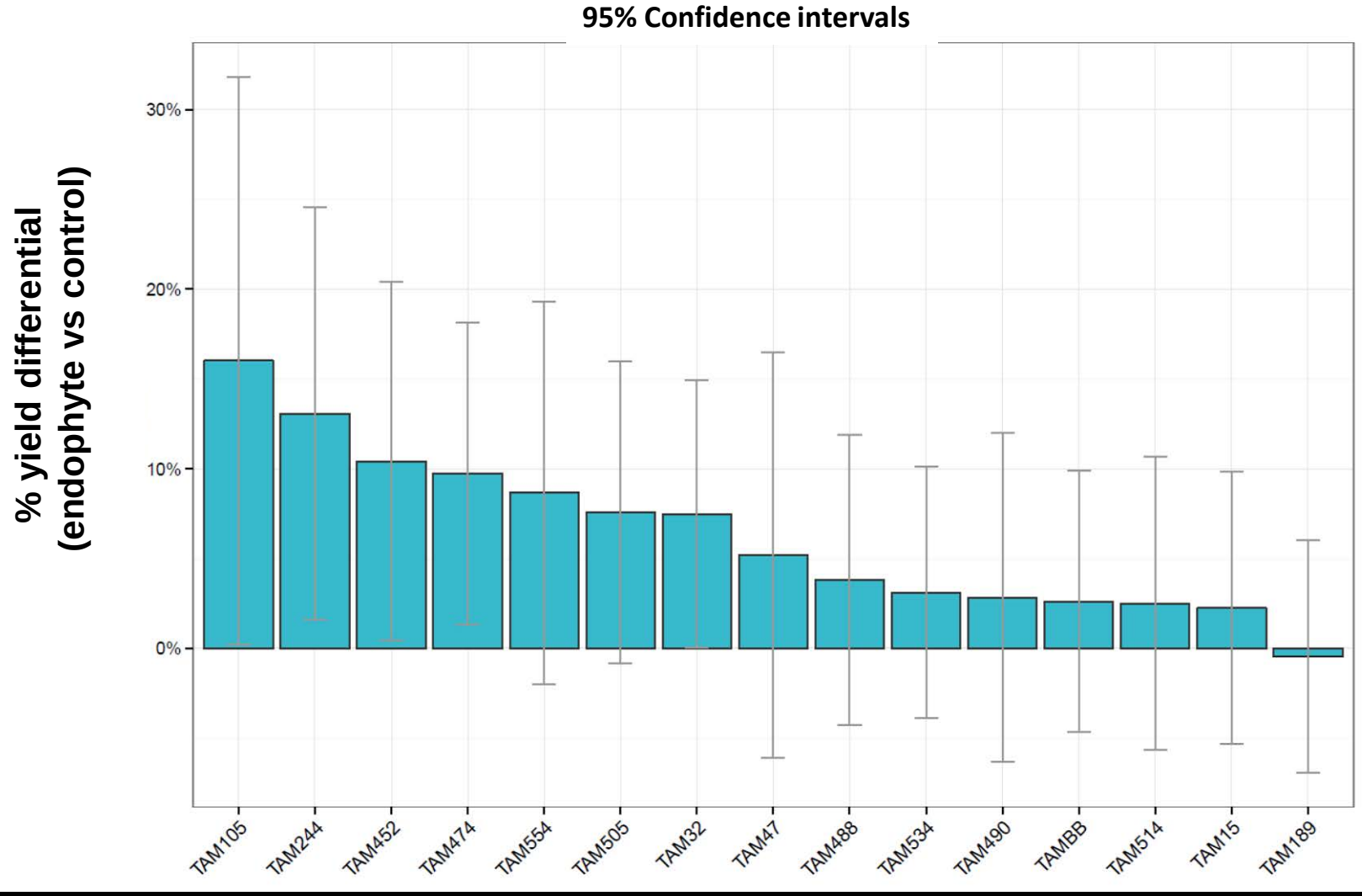
Water x Endophyte x Variety experiment



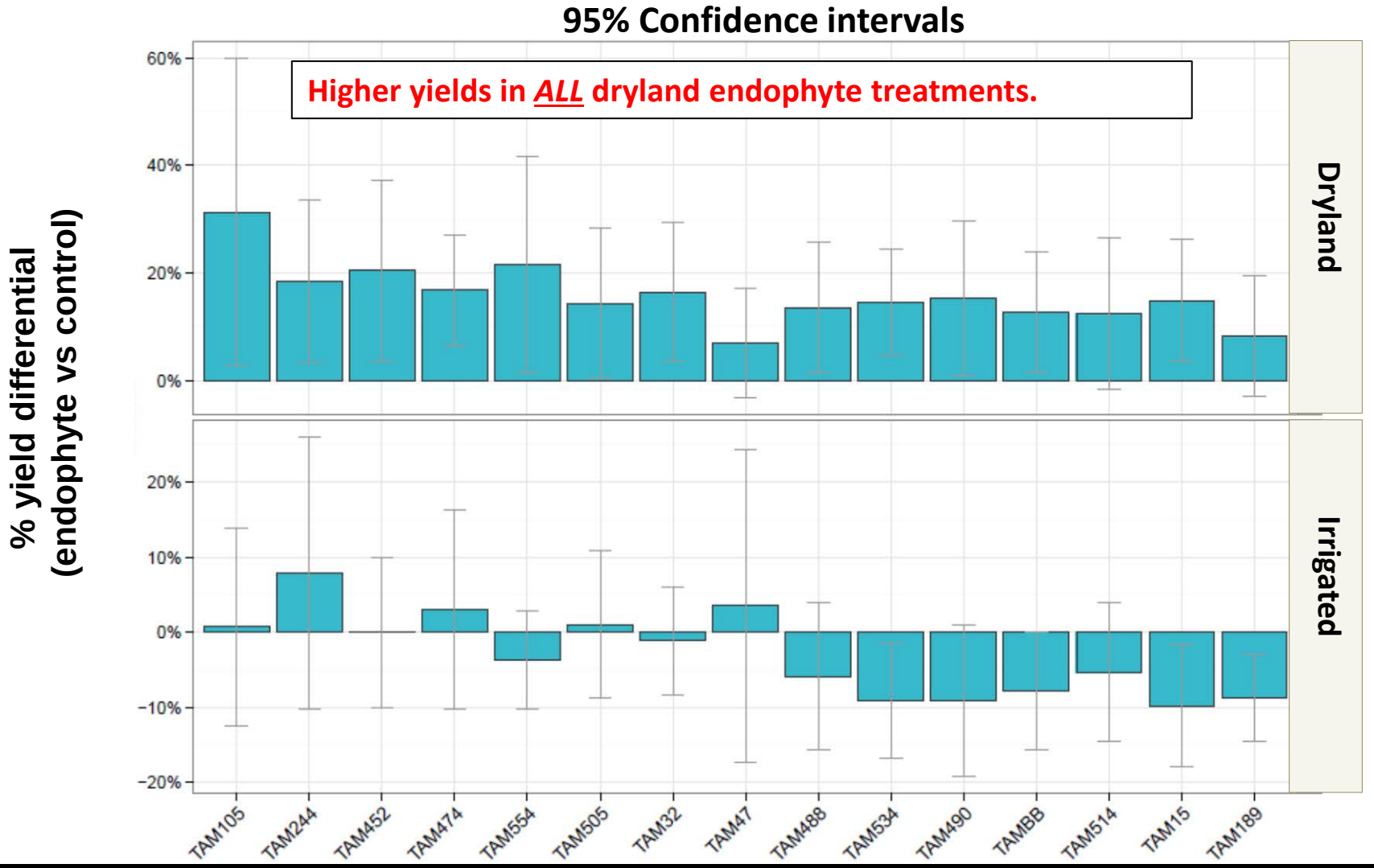
2014 Field trial design

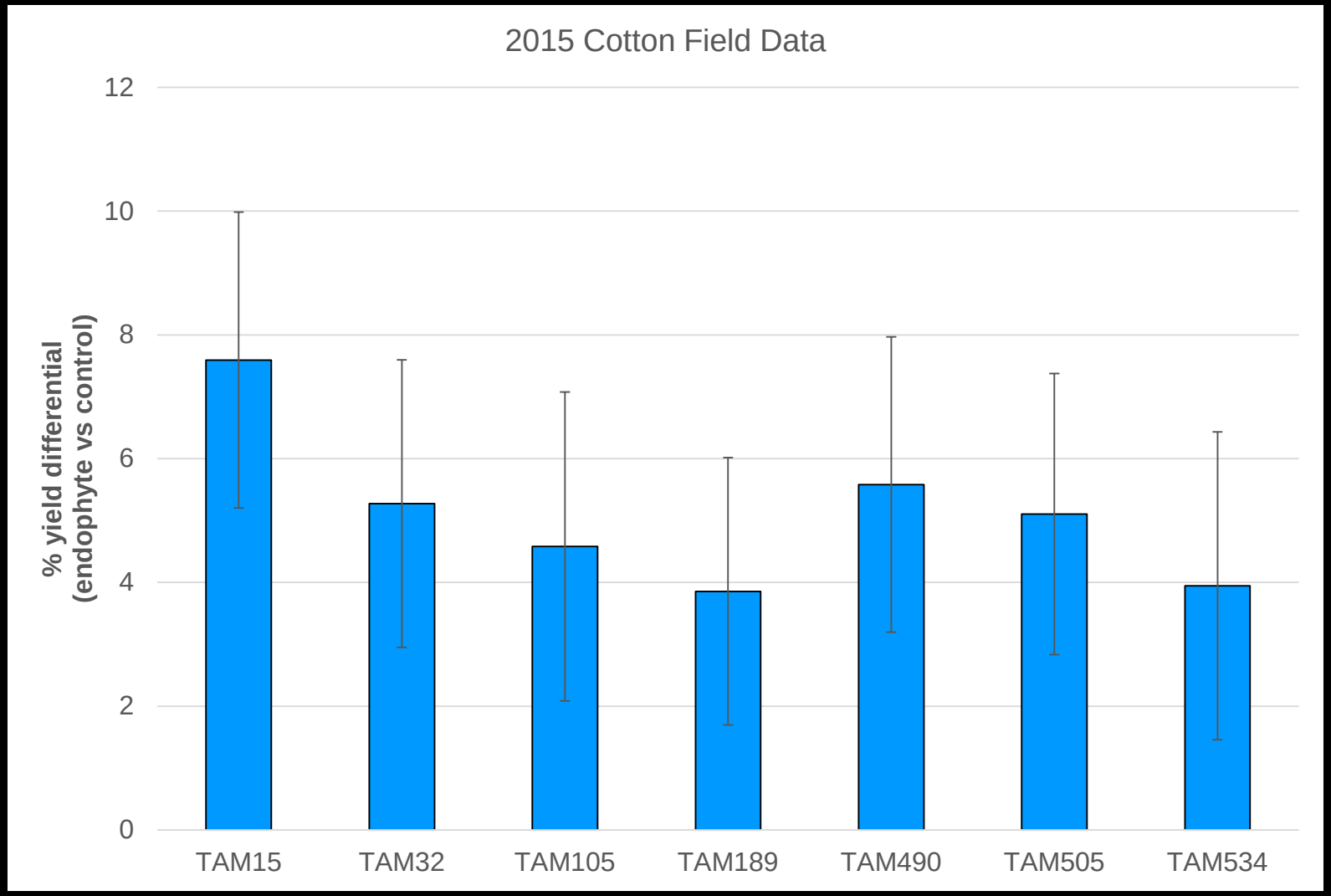
- **Two growing regions:**
  - College Station and Halfway, TX
- **Two water levels:**
  - Dryland and irrigated fields
- **Two major commercial varieties**
- **36 entries:**
  - 15 endophytes + 3 control X 2 varieties
- **216 plots per site:**
  - 6 replicates, 4 row plots
- **864 plots total across Tx**

Yield – Aggregated over both conditions and locations



Total yield by condition at both sites





Two dryland locations, two varieties at each location, 10 reps per treatment (n = 40 for each strain). Bars are standard error.

AP / *January 7, 2012, 9:34 AM*

2011 was Texas' driest year on record

The remains of a cow lay near a watering point in a pasture July 28, 2011 near Tulia, Texas. A severe drought in the region has caused shortages of grass, hay and water, forcing ranchers to thin their herds or risk losing their cattle to the drought.

Scott Olson/Getty Images

HOUSTON — The National Weather Service says 2011 was Texas' driest year on record as well as its second hottest.

The agency said Friday the average rainfall for the drought-stricken state last year was 14.88 inches. The previous driest average total was in 1917 with 14.99 inches.

The weather service says 2011's average temperature was 67.2 degrees. Texas'