

# Understanding the origin and dispersal of the taproot decline and Cercospora leaf blight pathogens



# Identifying sources of inoculum to determine effective management strategies for **Cercospora Leaf Blight** and **Purple Seed Stain**



# CLB, PSS, and *C. kikuchii*

日本植物病理學會報

第一卷 第六號

大豆の紫斑粒に関する研究

松 本 巍

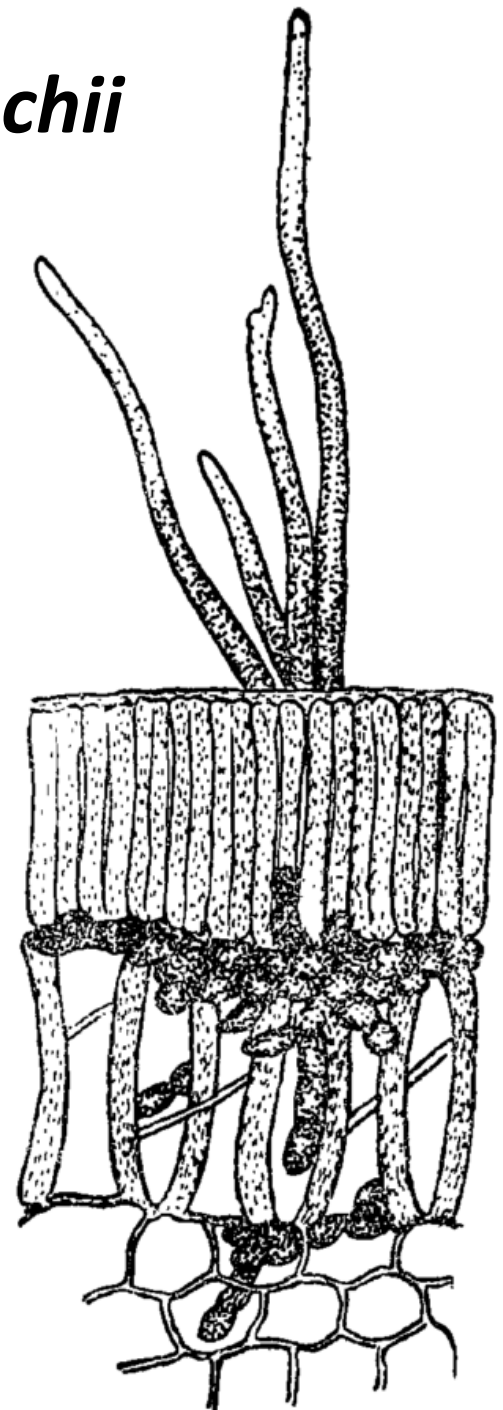
友 安 亮 一

STUDIES ON PURPLE SPECK OF  
SOYBEAN SEED

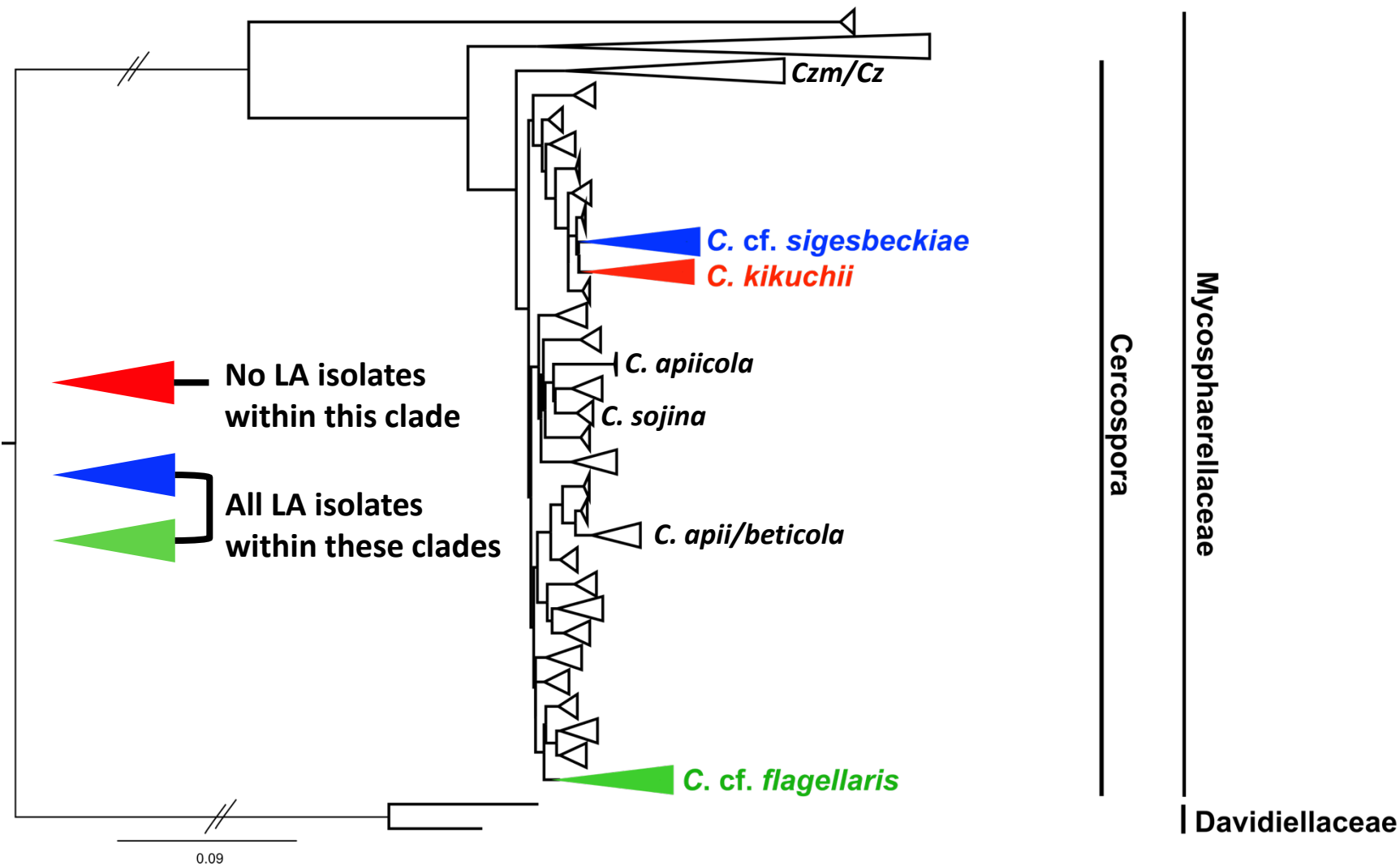
by

Takashi Matsumoto and Ryoichi Tomoyasu

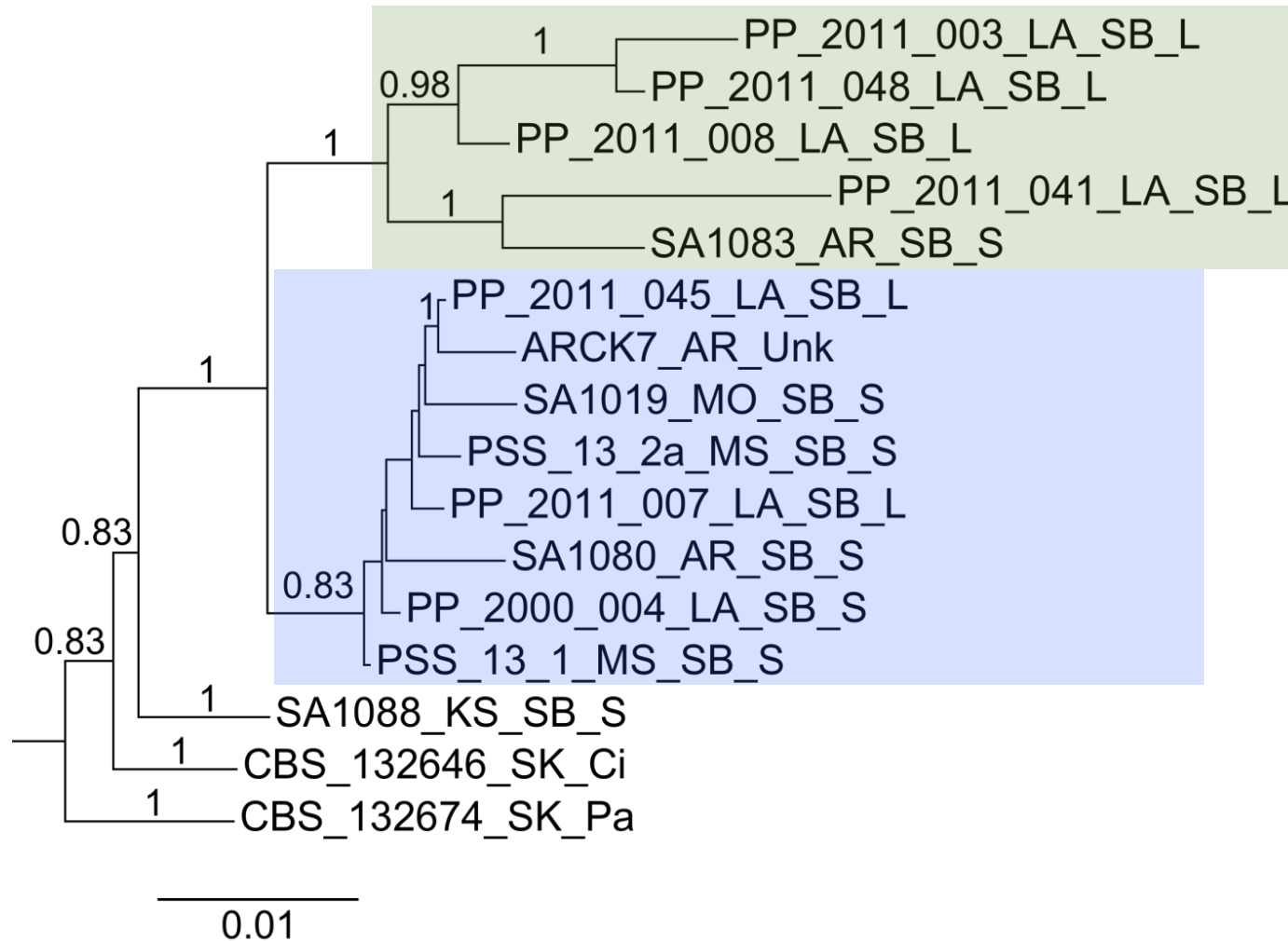
1925



# We found 2 *Cercospora* Lineages associated with CLB and PSS in Louisiana



# *Cercospora flagellaris* has a broad host range and high levels of genetic diversity.



**Generalist**  
**Louisiana\***  
**Arkansas\***  
**Missouri\***  
**Mississippi\***  
**Kansas\***  
**Japan**  
**Argentina**

## **Developing effective management strategies**

**Do alternative hosts serve as a significant source of inoculum**

**or**

**is seed the primary source of inoculum for all manifestations of the disease (CLB, PSS)?**

# Identifying sources of inoculum to determine effective management strategies for **Cercospora Leaf Blight** and **Purple Seed Stain**

## Objectives:

- i) Evaluate strategies for seed disinfestation of soybean and determine if these approaches reduce the contribution of seed-borne inoculum to disease development (incidence and severity).
- ii) Determine the relationship between seed-borne inoculum (PSS – seed lots) and pathogens isolated from blighted leaves (CLB) and harvested seed.
- iii). Determine the potential of alternative host species to contribute to the pathogen population on soybean.

Is seed the primary source of inoculum?

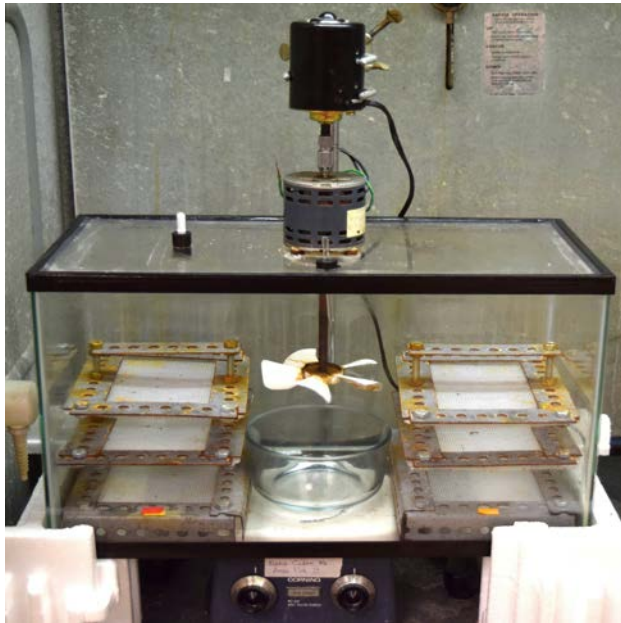
## Objective I

Determine whether seed disinfestation reduces disease incidence and disease severity.





# Chlorine gas eliminates pathogens from seed without phytotoxicity



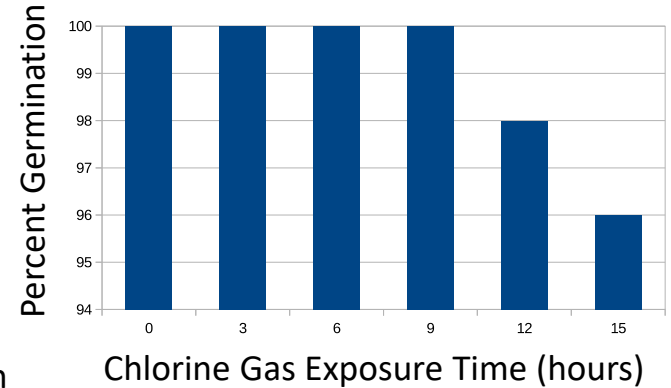
Variety  
AG4632

Lots  
RH1B (TN)  
GB22 (AR)

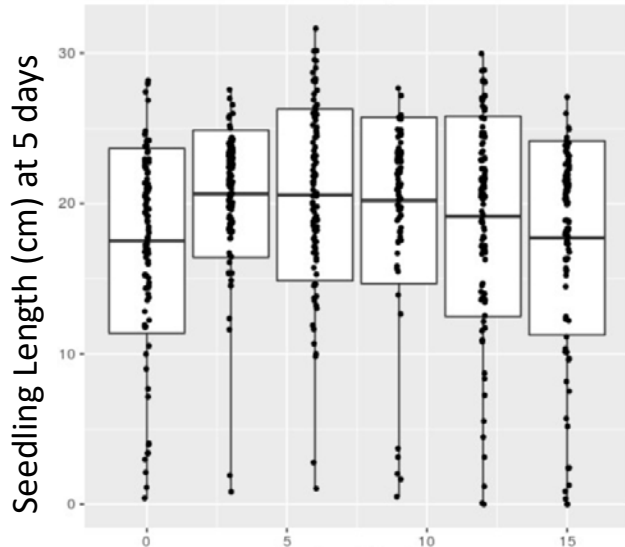
Treatment Times (hrs)  
0,3,6,9,12,15

Chlorine Concentration  
~200 ppm

## Chlorine gas has minimal impact on germination rate

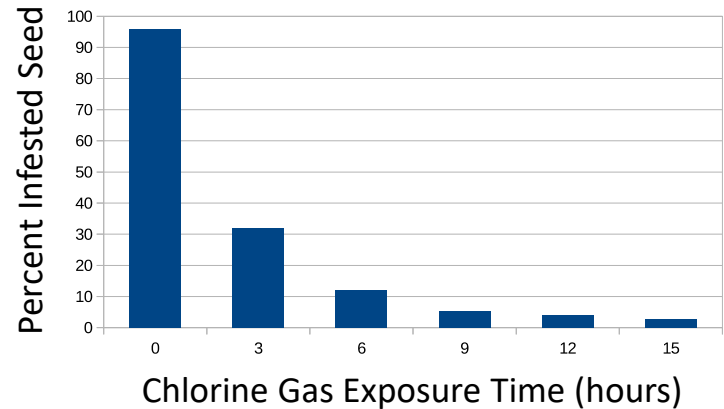


GB22 Seedling Vigor after Cl<sub>2</sub>

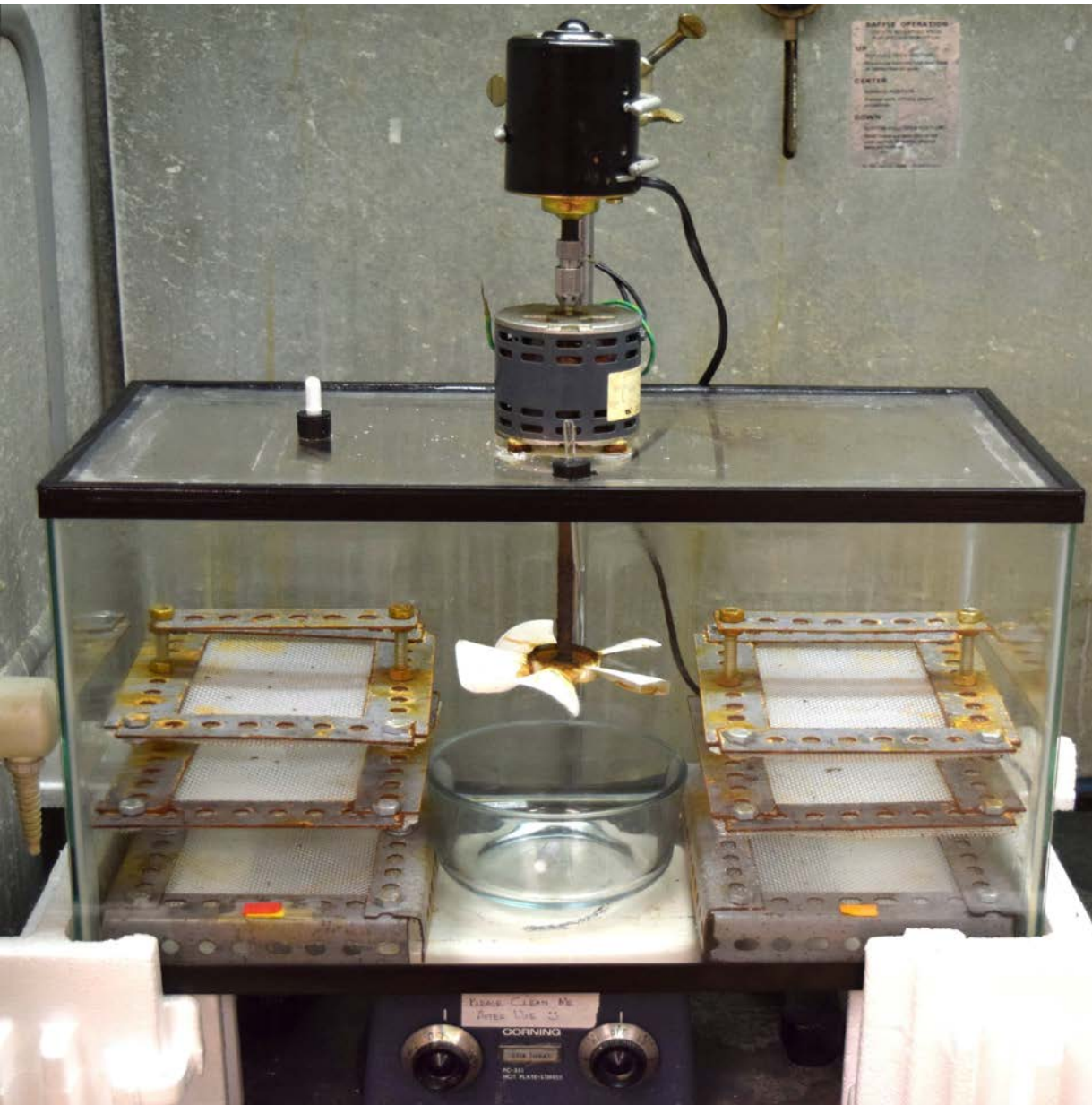


Chlorine Gas Exposure Time (hours)

## Chlorine gas eliminates microbes (including *Cercospora*)



# Field Trials conducted in 2016 and 2017



**Variety**  
AG4632

**Lots**  
Kentucky  
Arkansas  
Tennessee

Treatment Time (hrs)  
**9**

**Locations:**  
Winnsboro, LA  
Macon Ridge Research Station  
**2016 & 2017**

Alexandria, LA  
Dean Lee Research Station  
**2017**

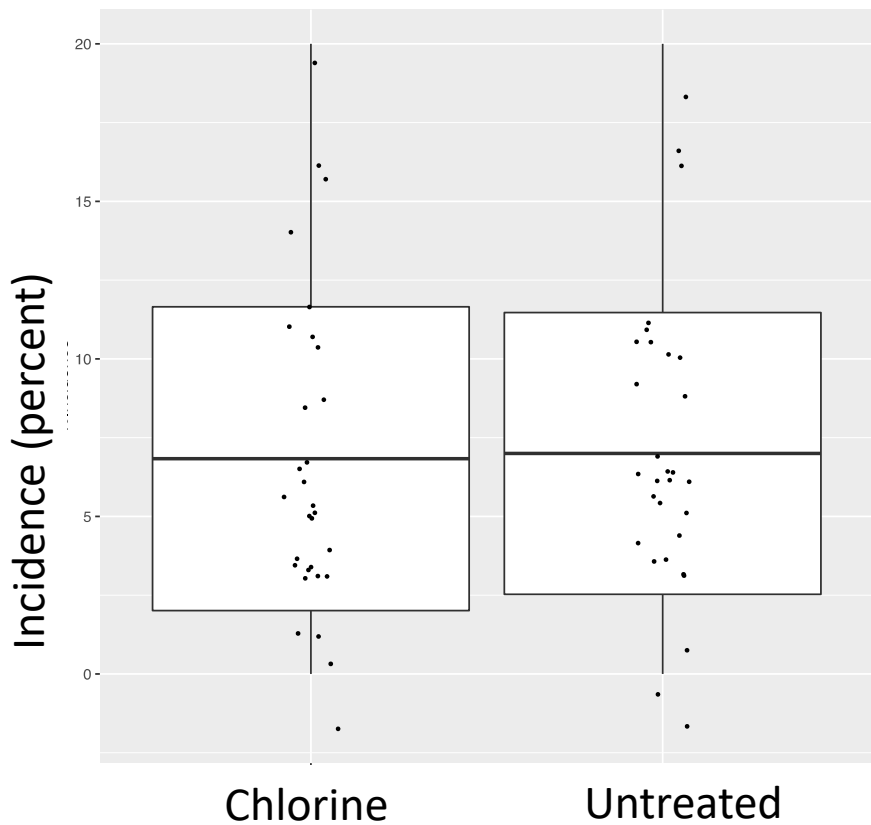
**Planting Date:**  
mid-June 2017 and 2018

Randomized Complete Block Design  
5 blocks  
2 rows per treatment per lot

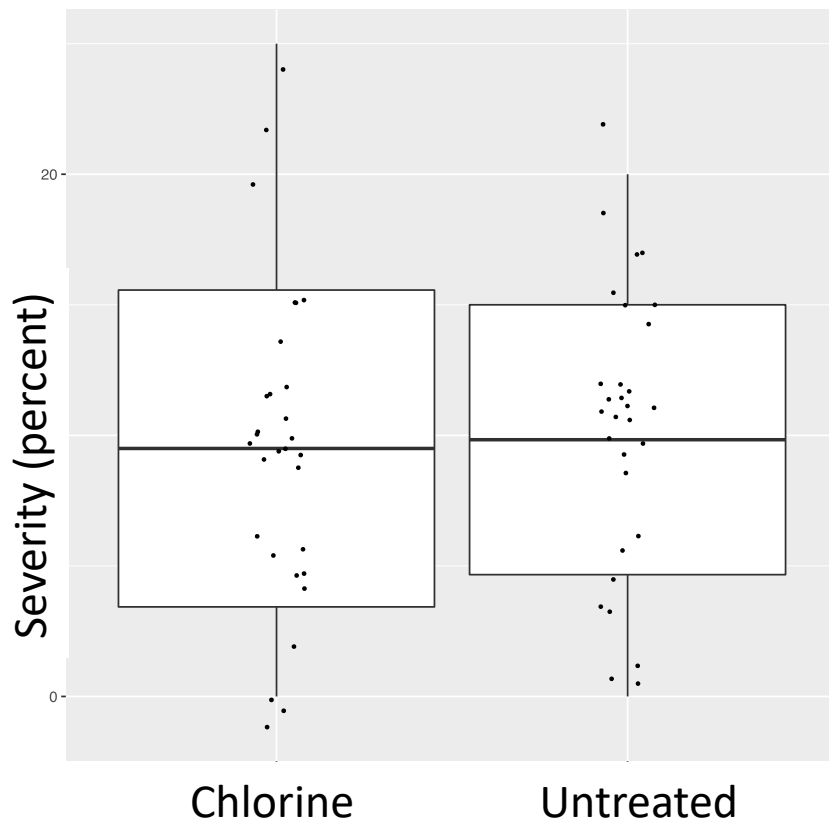
4320 seed treated per location

# Chlorine treatment did not reduce incidence or severity in 2016

**Winnsboro**

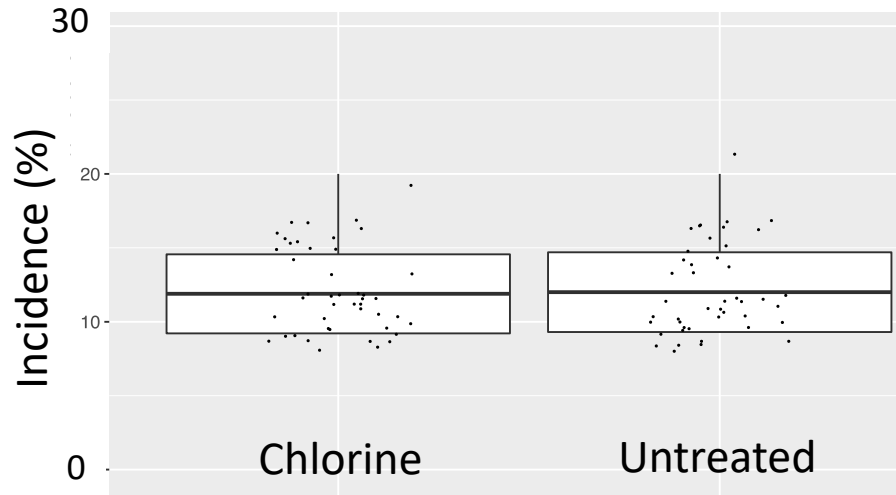


**Winnsboro**

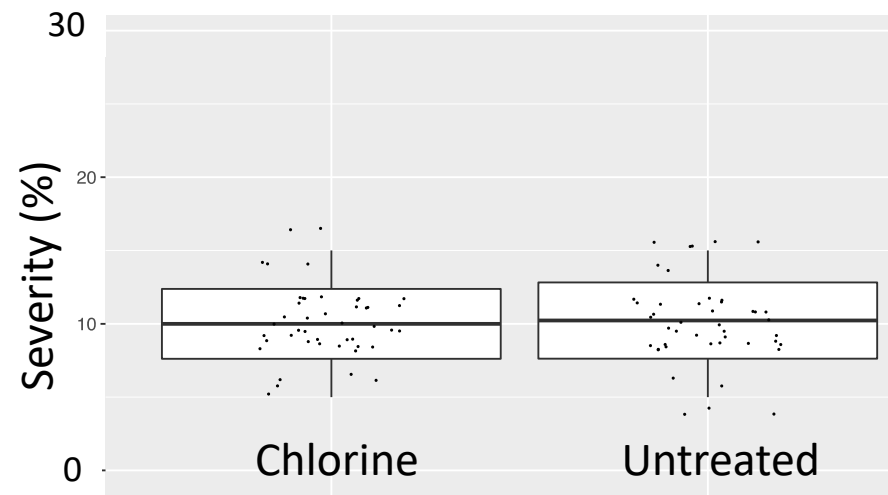


# Chlorine treatment did not reduce incidence or severity in 2017

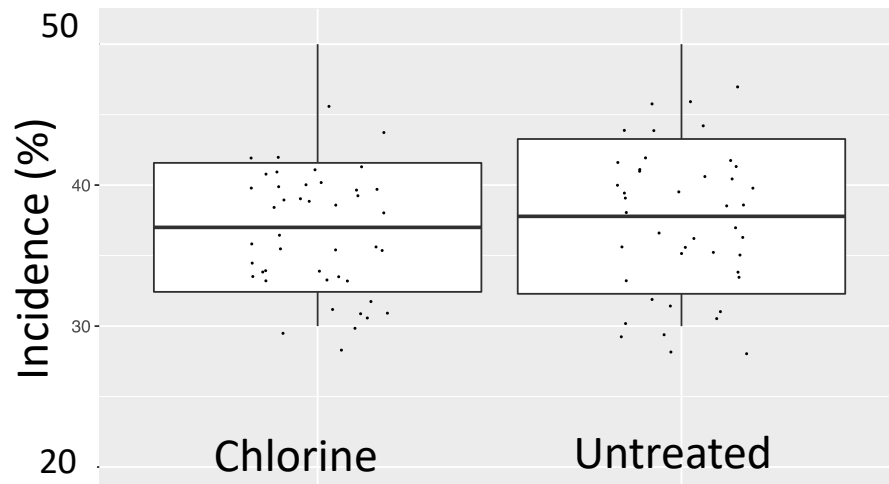
Alexandria



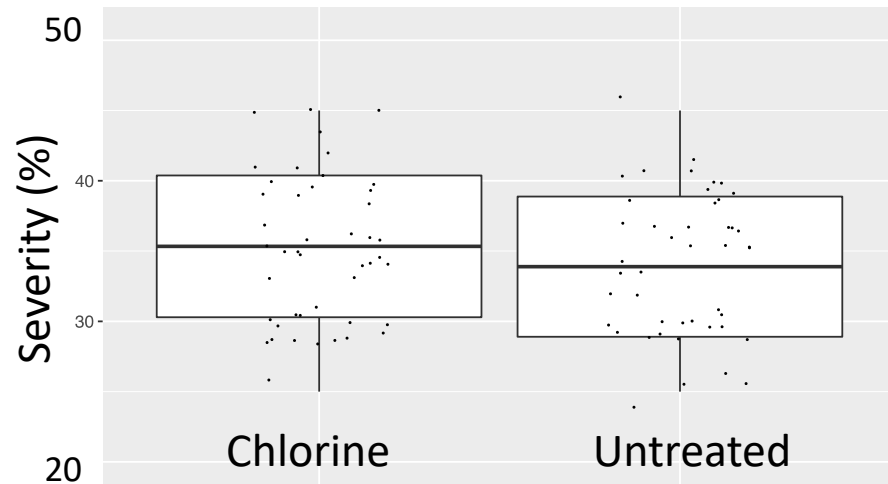
Alexandria



Winnsboro



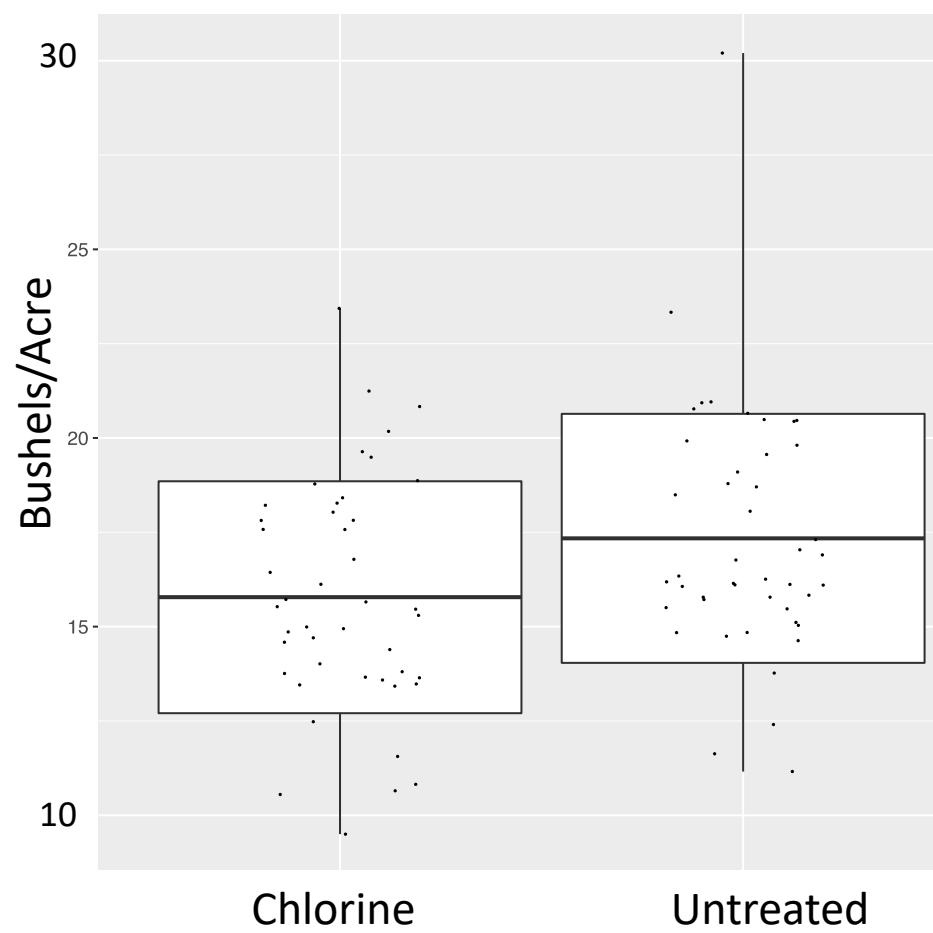
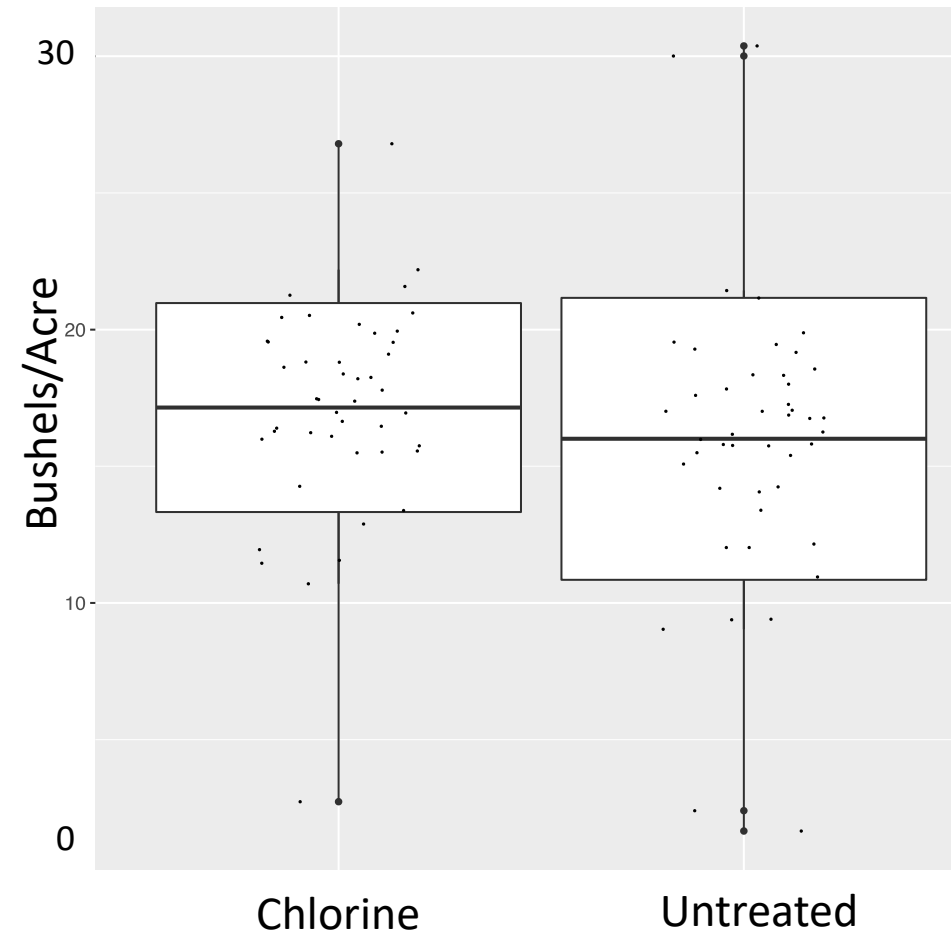
Winnsboro



# Chlorine treatment did not increase yield in 2017

Alexandria

Winnsboro



**Is seed the primary source of inoculum for all manifestations of the disease?**

## **Objective II**

Determine the relationship between seed-borne inoculum (PSS – seed lots) and pathogens isolated from blighted leaves (CLB) and harvested seed

**Sample from seed lots  
prior to planting**



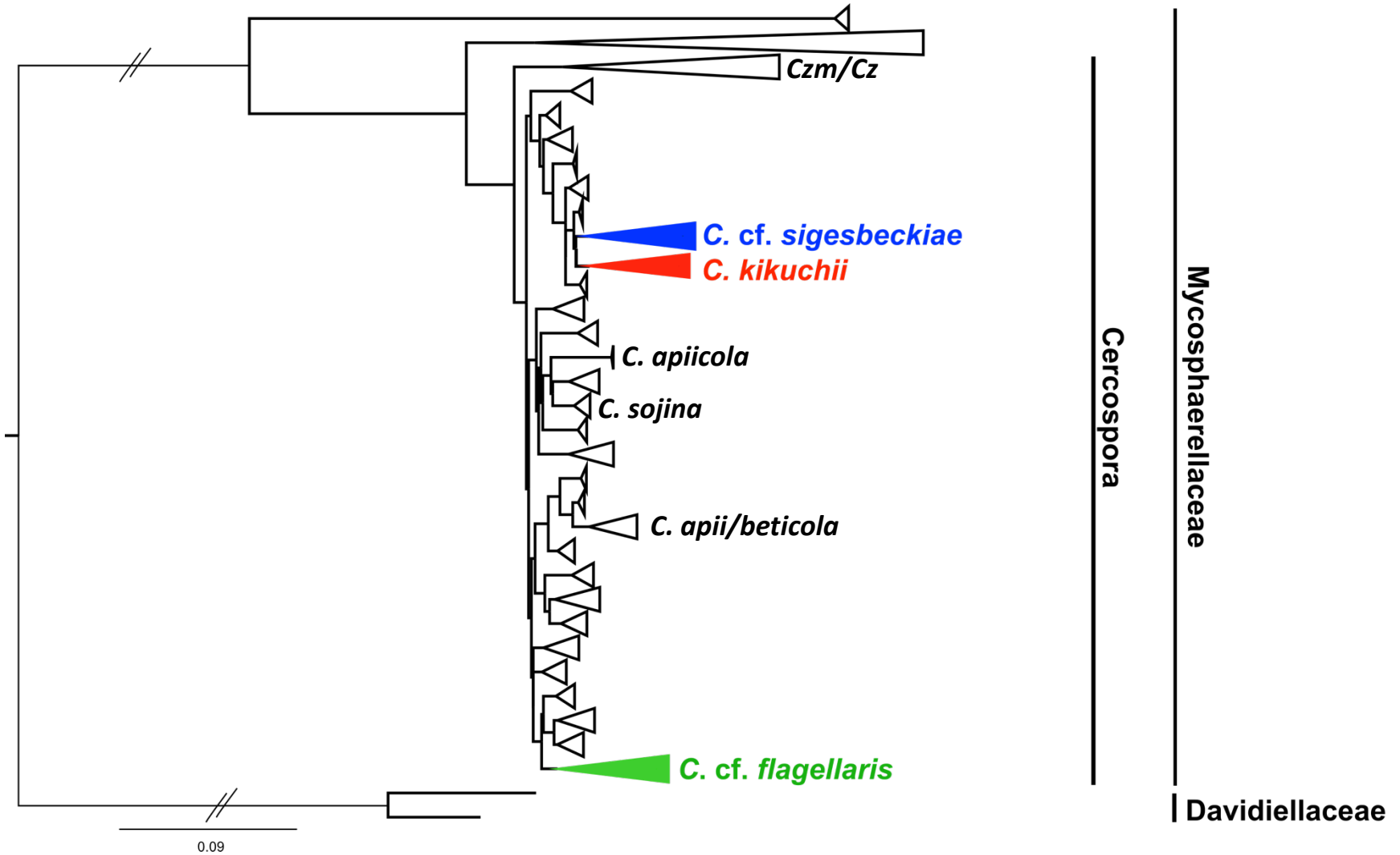
**Sample from  
harvested seed**



**Sample from symptomatic  
leaves**



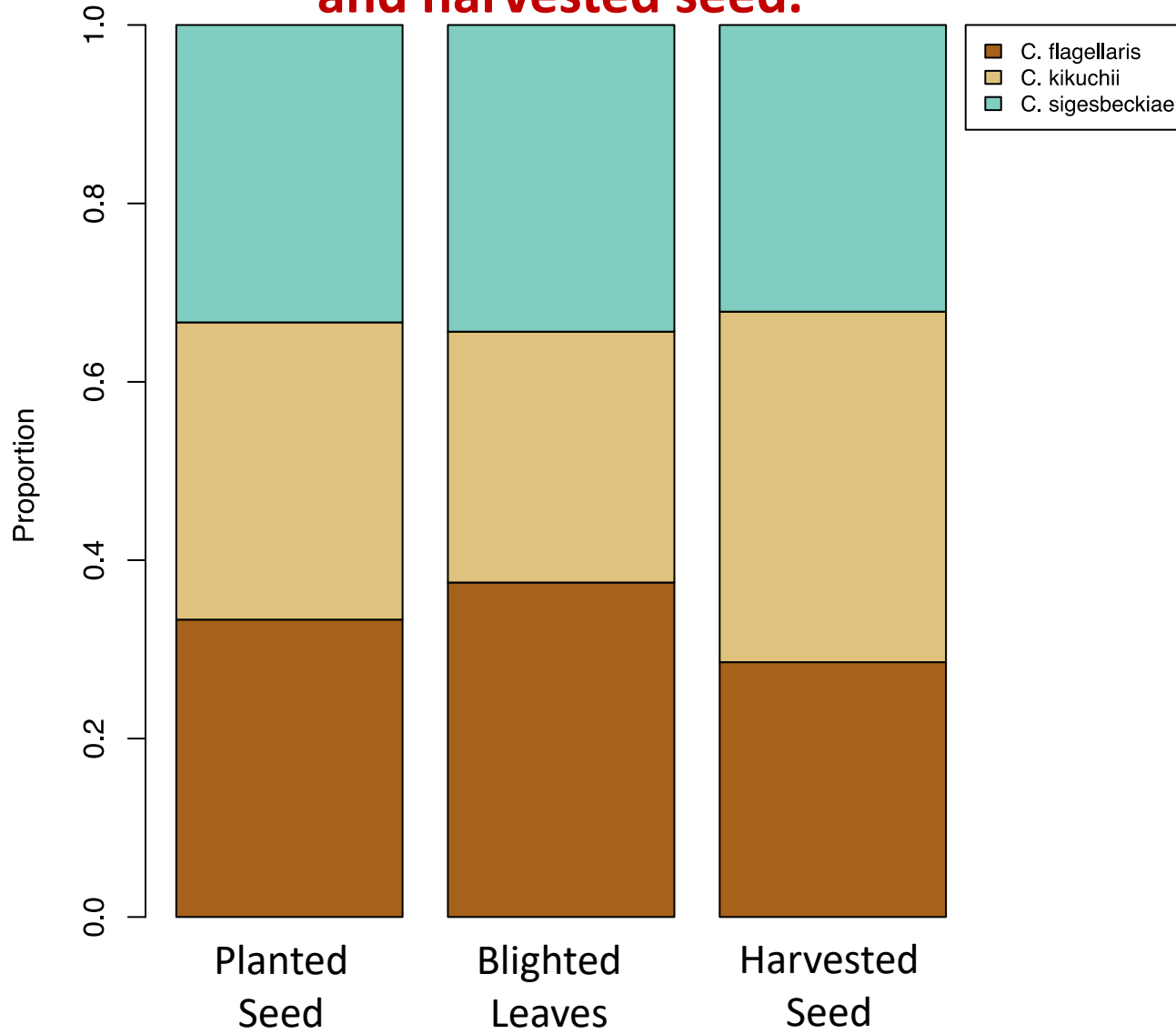
# Seed from producers in Arkansas and Tennessee harbor multiple *Cercospora* species



# What do we expect if seed is the primary source of inoculum?

## Expectations:

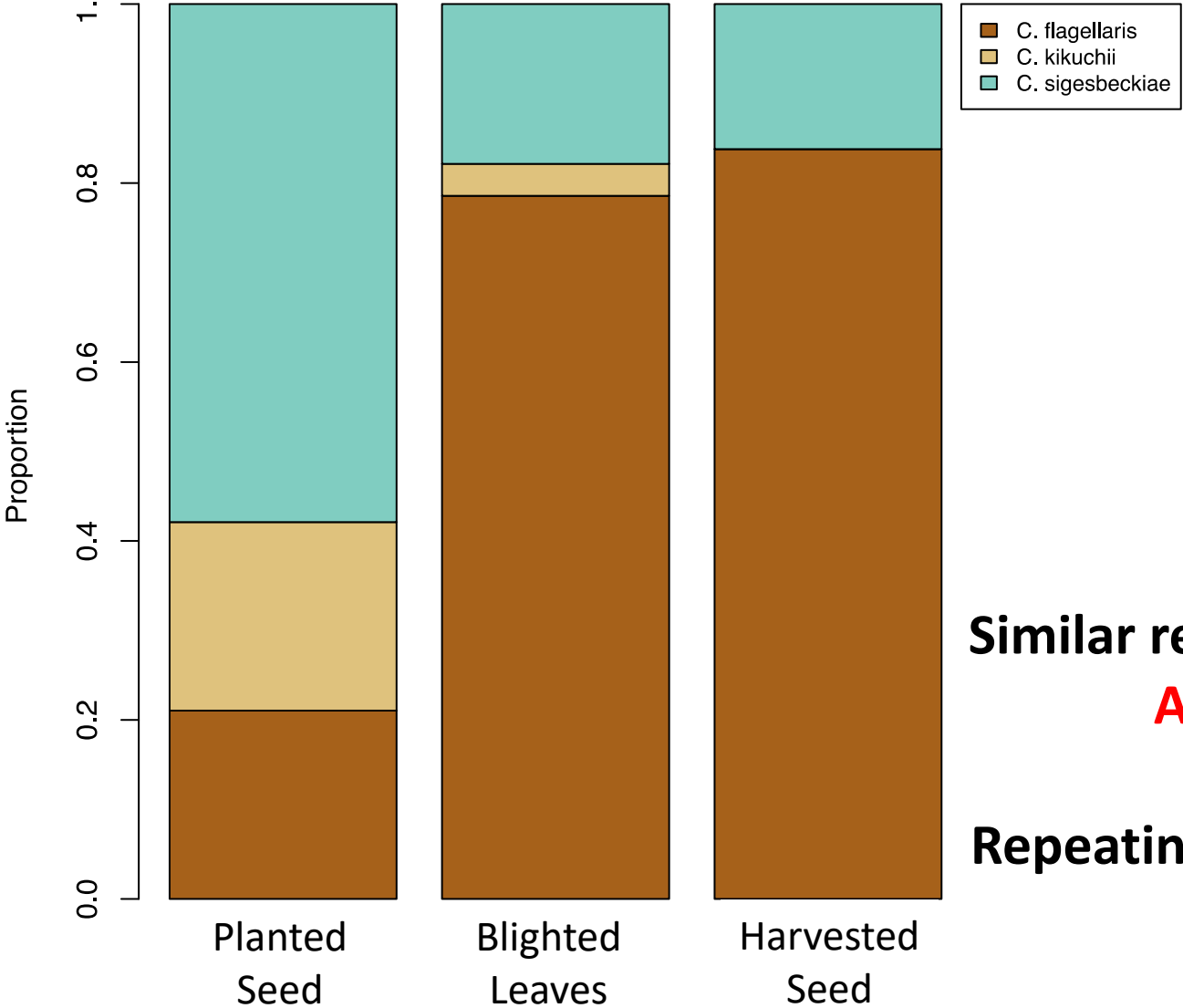
Similar proportions of each species in planted seed, blighted leaves, and harvested seed.





# The proportion of each species in blighted leaves and harvested seed is significantly different from planted seed

**Seed source: Tennessee**



**Similar results for  
AR**

**Repeating in 2017**

## Objective III

Identify potential alternative host species for the CLB and PSS pathogen



Pokeweed  
*Phytolacca*

COMMON POKEBERRY  
*Phytolacca americana* L.  
POKEBERRY FAMILY



TNAU Agritech Portal

Cotton  
*Gossypium*

# Several plant species are potential sources of inoculum

Cotton – *Gossypium*

Jungle Rice – *Echinochloa colona*

Pokeweed – *Phytolacca americana*

Mulberry – *Morus sp.*

Giant Ragweed – *Ambrosia trifida*

Clasping Venus' Looking Glass - *Triodanis perfoliata*

Palmer amaranth – *Amaranthus palmeri*

**Giant Ragweed**



**Pokeweed**



**Triodanis**



**Jungle Rice**



**Giant Ragweed is abundant along roadsides, field margins, and disturbed areas in Louisiana**



# Conclusions

- Disinfesting seed prior to planting does not reduce disease incidence or severity.
- There is a significant change in the community of *Cercospora* species between planting and the appearance of blighted leaves.
- The dominant species associated with CLB and PSS, *C. flagellaris*, can be found on multiple host species that may serve as a source of inoculum.
  - Seed does not appear to be the primary source of inoculum.

# Building the framework to develop integrated management strategies for taproot decline



# Foliar symptoms of taproot decline are similar to many other soilborne diseases and nutritional deficiencies.

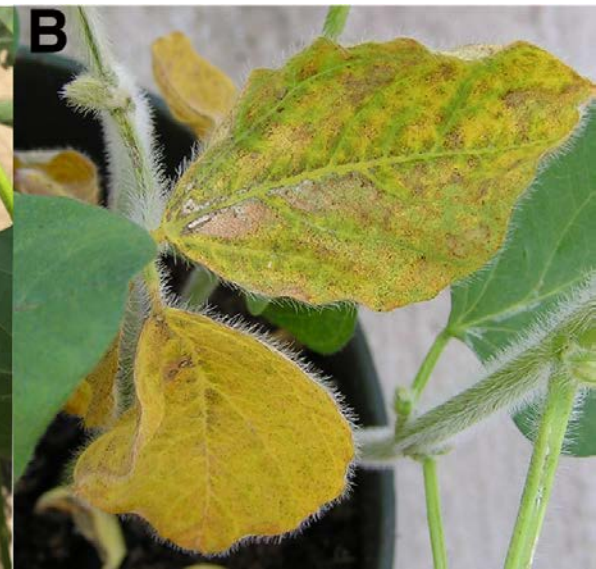
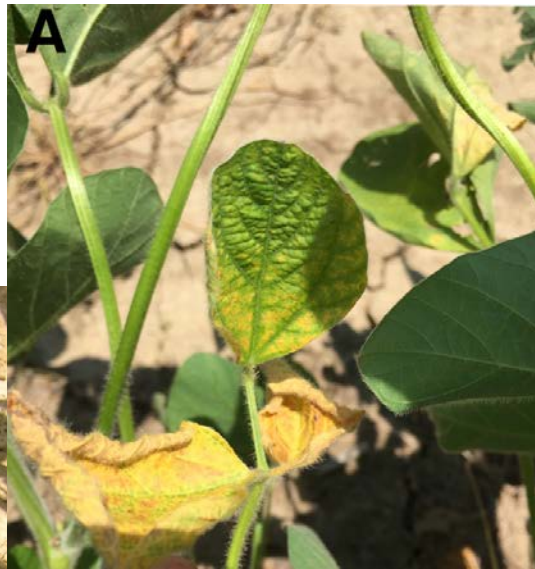


Sudden Death Syndrome  
*Fusarium virguliforme*



Taproot Decline  
*Xylaria* sp.

# Impacts can be seen from seedling to full seed (R6)





# Signs of the pathogen occur on and in the tap and lateral roots



Allen, ..., Doyle, Price, Singh, et al. 2017. Plant Health Progress

T. Garcia

# Likely overwinters on crop debris



T. Garcia

Corn debris



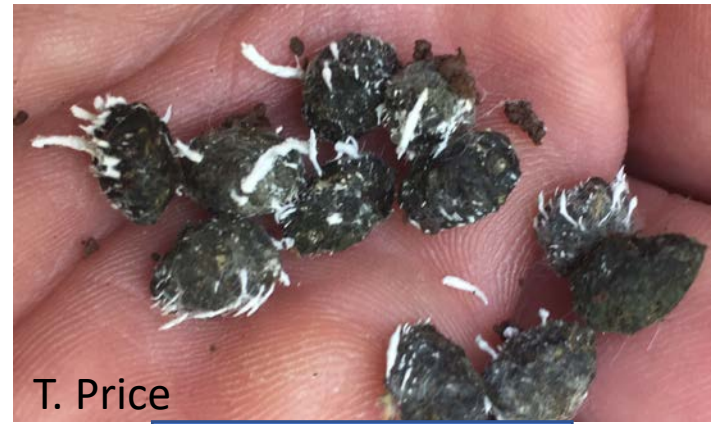
T. Garcia

Soybean debris



T. Price

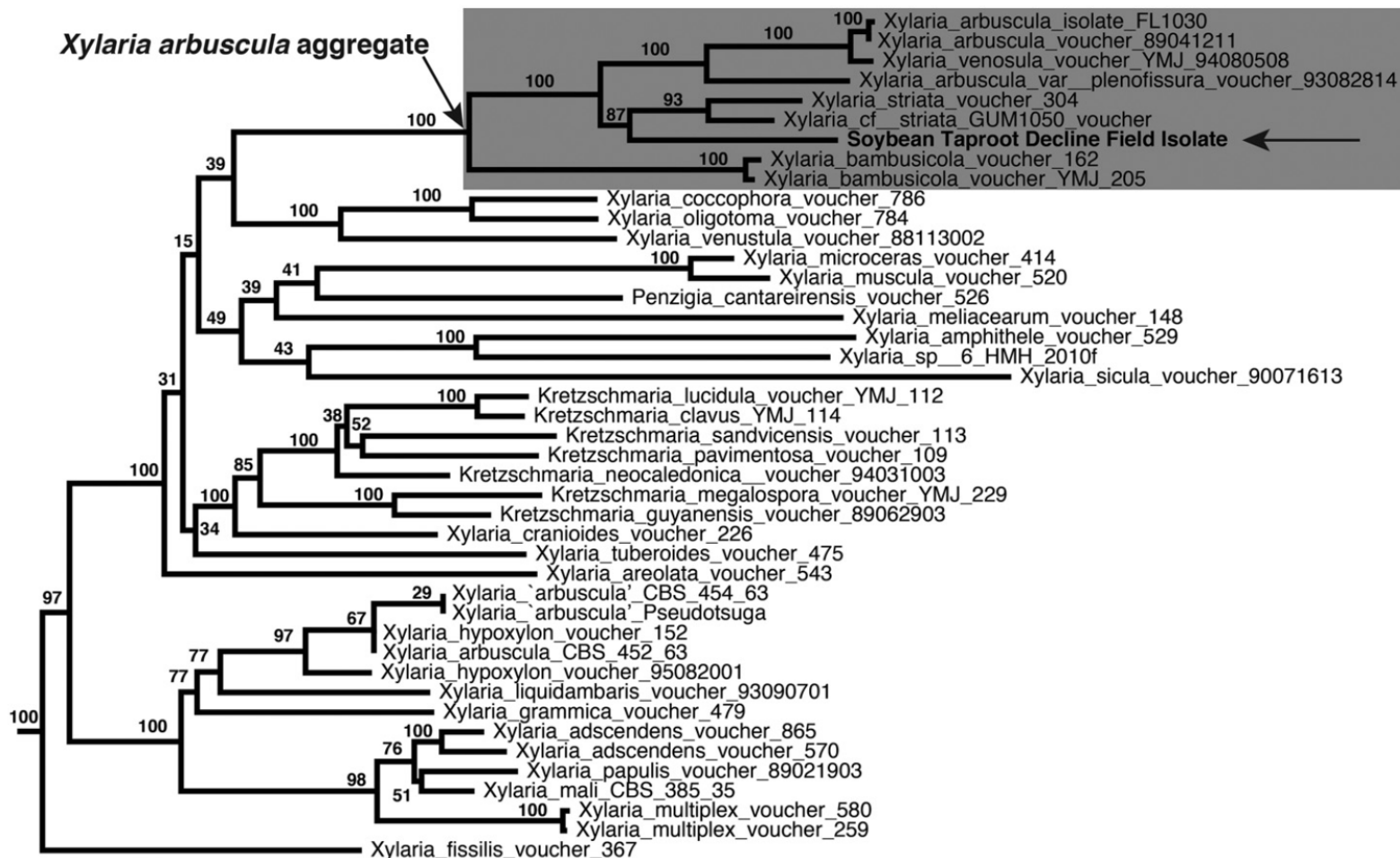
Corn after soybean



T. Price

Soybean seed

# The taproot decline pathogen is likely an undescribed species of *Xylaria*.



# More information is needed to develop management approaches

What isolates should we select to represent the pathogen population for cultivar screening and breeding?

- Genetic diversity of TRD
- Maintenance of culture collection

What is the potential for this pathogen to adapt to changing management regimes and cultivars?

- Genetic diversity of TRD
- Sexual reproduction

Was this pathogen introduced or did it emerge due to a change in the genetic base of the cultivars being used today?

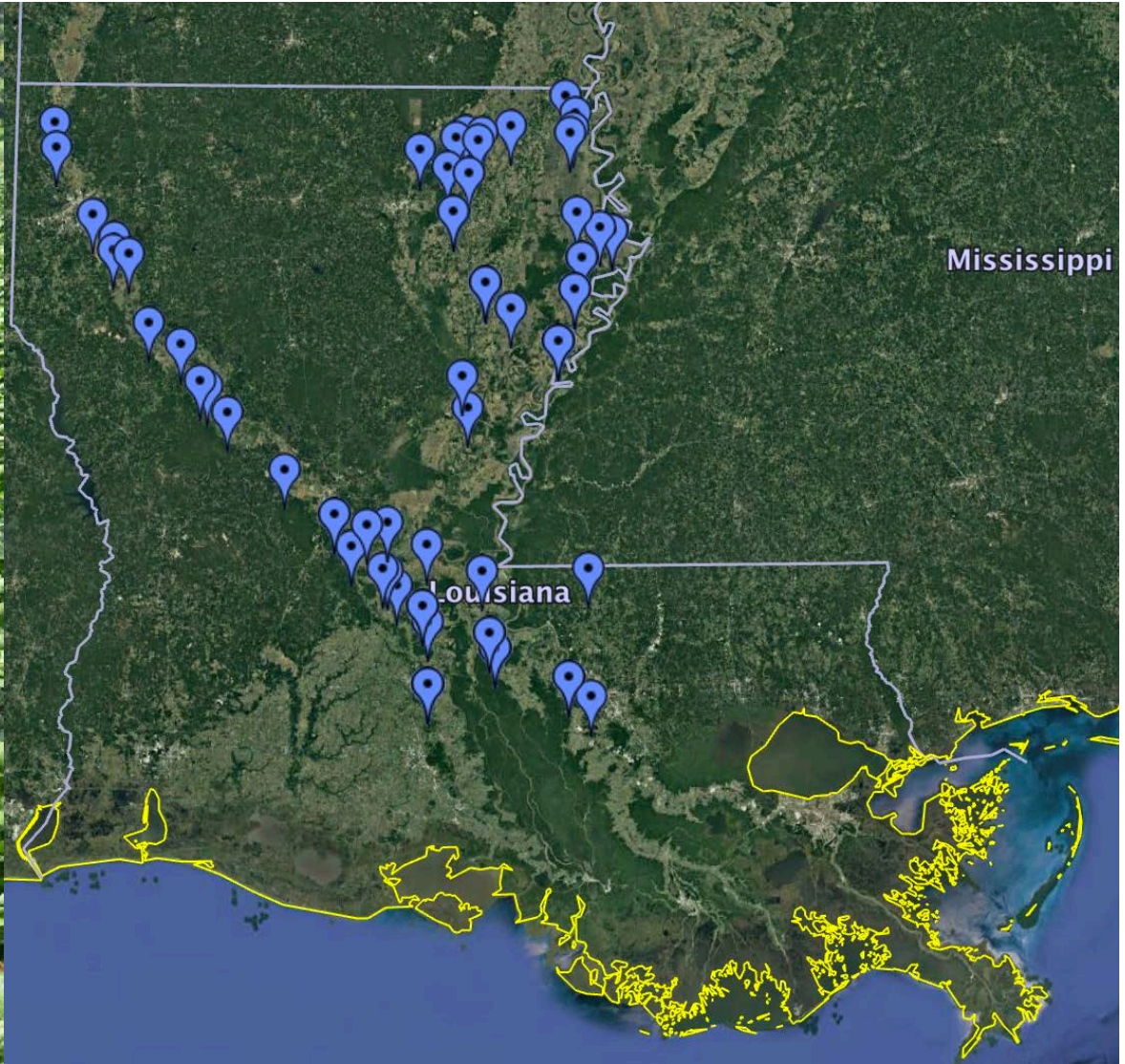
Historical specimens of *Xylaria*  
Broader sampling

Does this pathogen produce mycotoxins that cause foliar symptoms like those of SDS (*Fusarium*)?

# Preliminary Work

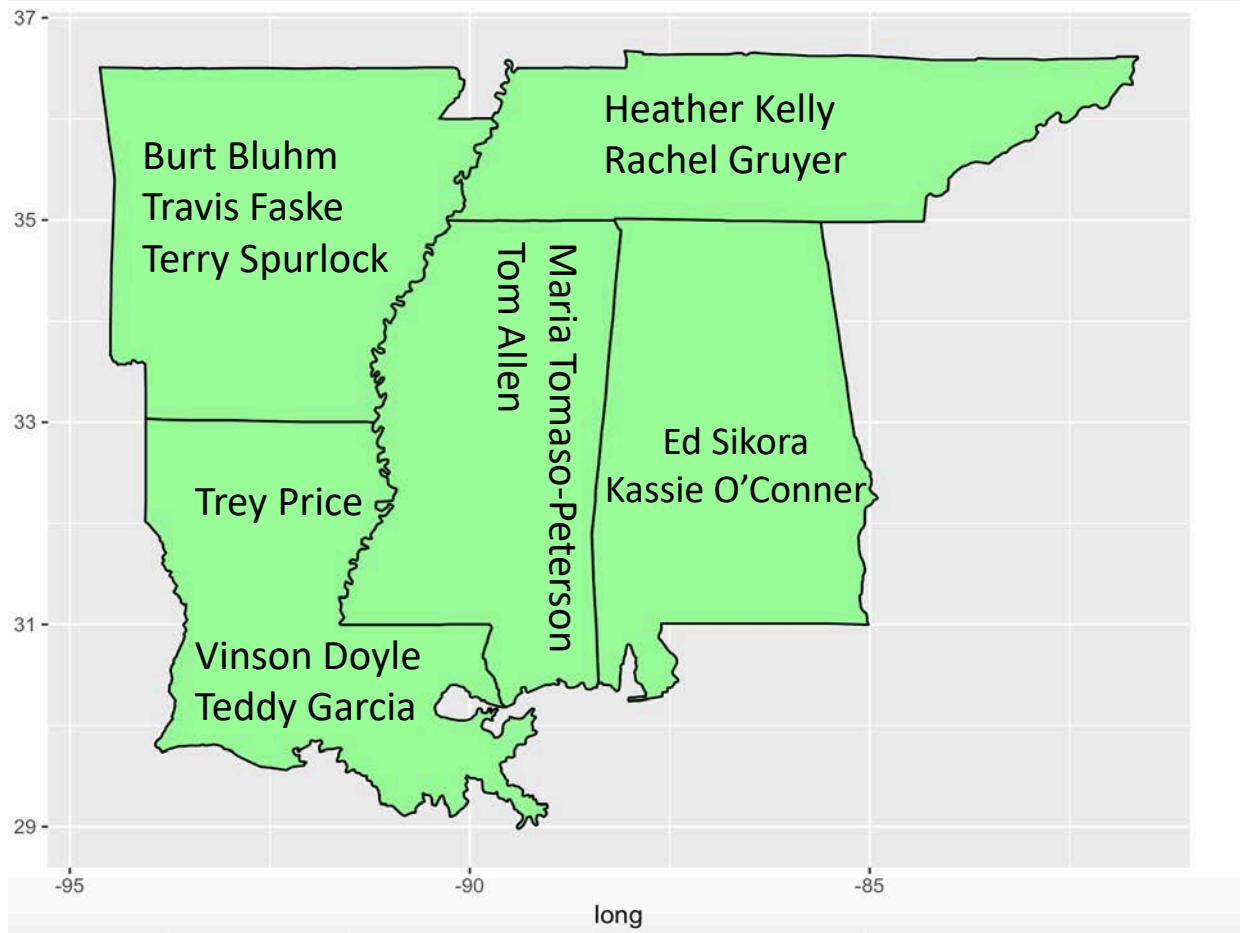


Teddy Garcia



Sampling of taproot decline in 2017

# Taproot Decline Regional Collaborators



# Acknowledgements

## Doyle Lab

Teddy Garcia

Zachary Carver

Elaisa Tubana

Hannah Tonry

Jacob Searight

Veronica Bras

Jose Solarzano

Sara Berrezueta

Emily Rolfes

Dan Cooke

## Price Lab

Trey Price

Myra Purvis

Hunter Pruitt

## Schneider Lab

Ray Schneider

Clark Robertson

Eduardo Chagas

Brian Ward

## Other Collaborators

Marc Cohn



# More information is needed to develop management approaches

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Historical specimens of *Xylaria*  
Broader sampling

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## Research Plans for 2018

- i) Determine the contribution of alternative host species to the pathogen population on soybean
  - Continue survey for alternative hosts of *Cercospora* near soybean fields
  - Genotype *Cercospora flagellaris* from alternative hosts to determine contribution to soybean disease
  
- ii) Develop protocols to re-infest disinfested seed with *Cercospora* and generate inoculum in the lab.
  - Utilize seed disinfestation protocol to rid seed of pathogens and re-infect with selected isolates of *Cercospora* as a means to determine the role of each of these species in disease development (host-fungal interactions, plant breeding)
  - Develop protocols to induce sporulation of *Cercospora* on artificial media for inoculation studies.
  
- iii). Determine the potential for crop debris to serve as a source of inoculum for CLB and PSS.

Survey soybean fields monthly from February – June for soybean and other crop debris and assess as a source of inoculum (morphological and molecular).

# Objectives

- I. Identify the principal lineages of the TRD pathogen in Louisiana and maintain isolates for future cultivar screening and breeding work.
- II. Determine whether the TRD pathogen is sexually reproducing in Louisiana to make predictions about the long-term stability of cultivar resistance.
- III. Determine the TRD pathogens dispersal modes and center of origin to design the most impactful and relevant mitigation strategies.
- IV. Determine whether the symptoms produced by the TRD pathogen is the result of the production and translocation of phytotoxic compounds.

# Projected Outcomes

- I. Characterization of the genetic diversity of TRD in Louisiana and a culture collection to be used for cultivar screening.
- II. An assessment of the long-term potential for TRD to adapt to management regimes.
- III. A determination of whether management practices can be implemented to limit pathogen dispersal.
- IV. A potential rapid cultivar screening method using fungal extracts.