After 100 years of tomato bacterial spot research, what have we learned (in Florida)?

HORT. SCIENCES IN-SERVICE TRAINING #32032 – FEB. 23, 2022

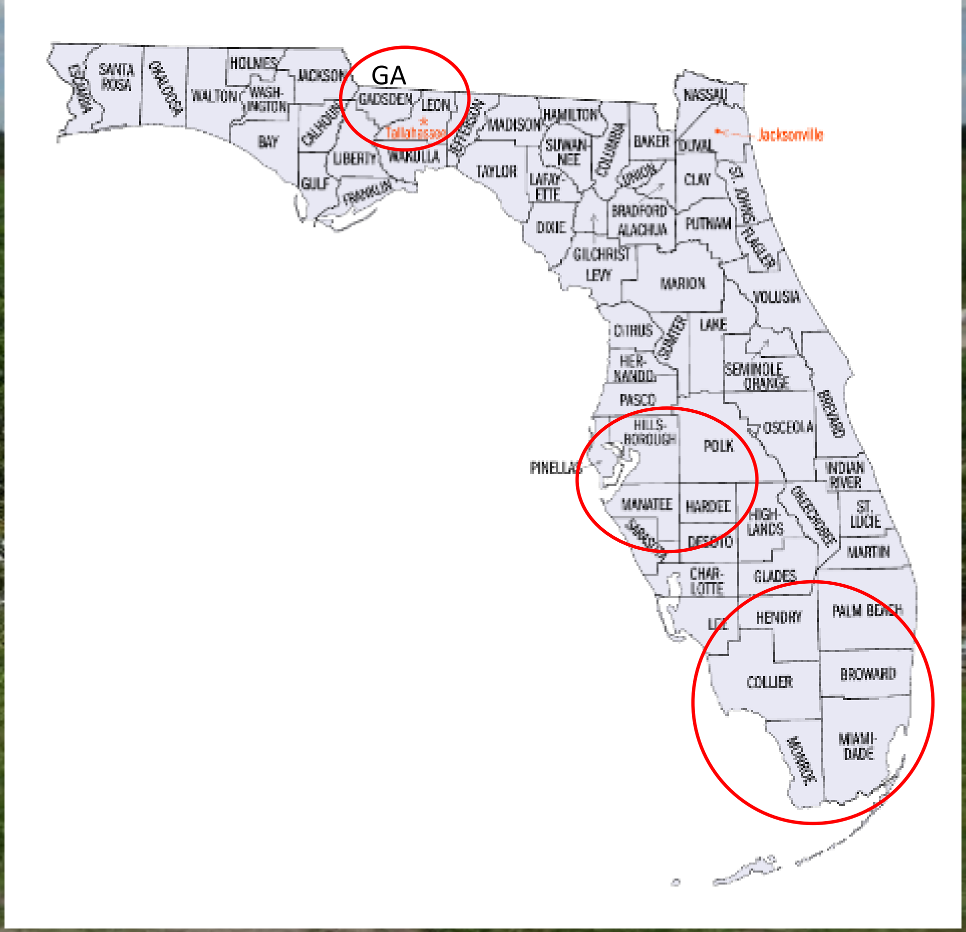
DR. GARY E. VALLAD, PROFESSOR

UF/IFAS GULF COAST REC, BALM, FL

GVALLAD@UFL.EDU

Florida Tomato Production:

- 32,ooo production acreage
- $456 million production value
- Nearly year-long production



Bacterial spot is a major disease of tomato.

Under favorable weather conditions it can cause ‘major’ damage.

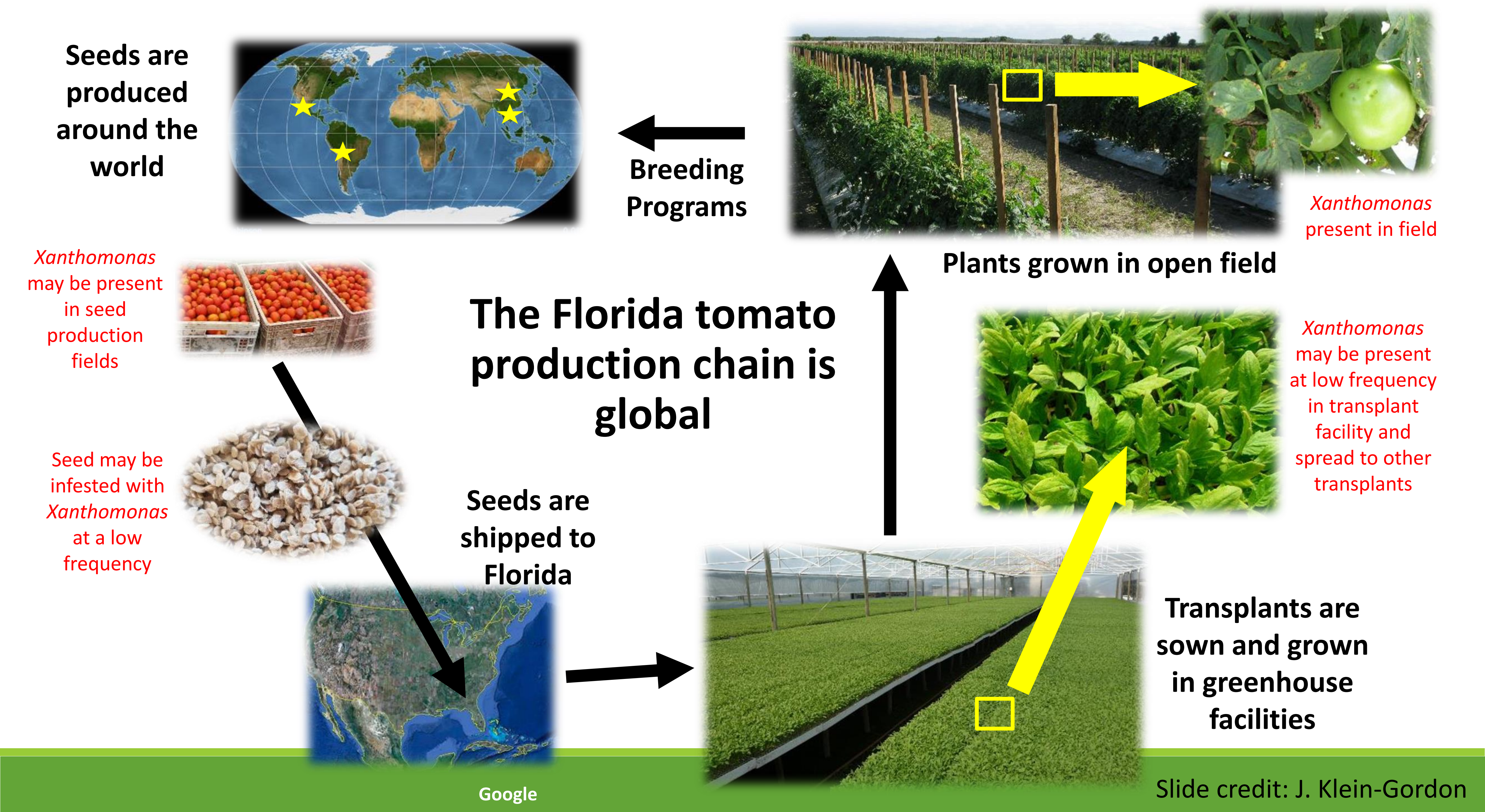
Can cause up to 50% yield loss

Tomato Bacterial Spot - A Florida Perspective

- **Caused by *Xanthomonas perforans***
  - Favored by warm (>80ºF) and humid conditions.
  - Easily spread by wind-driven rains and handling wet plant tissues.
    - **<u>Avoid field operations when foliage is wet!</u>**
  - Seedborne pathogen
  - Copper tolerance is prevalent among Florida strains; limiting the usefulness of copper bactericides.
- **Symptoms**
  - Water-soaked lesions ~ 5 days after infection
  - Lesions become necrotic ~ 7 – 14 days later, depending on temperature & humidity.
  - Fruit infections rare with *X. perforans,* but make fruit unmarketable

Tomato Bacterial Spot - A Florida Perspective

- **Challenges to BLS management:**
  - Weather – favorable weather leads to explosive epidemics
  - Breeding – changing *Xanthomonas* population
  - Movement of infested plant materials
    - Seed – global seed production (long distance)
    - Transplants (regional)
  - Widespread tolerance/resistance to bactericidal compounds



The Florida tomato production chain is global
Seeds are produced around the world
Breeding Programs
Plants grown in open field
Xanthomonas present in field
Xanthomonas may be present in seed production fields
Seed may be infested with *Xanthomonas* at a low frequency
Seeds are shipped to Florida
Google
Transplants are sown and grown in greenhouse facilities
Xanthomonas may be present at low frequency in transplant facility and spread to other transplants
Slide credit: J. Klein-Gordon

Globally, bacterial spot of tomato is caused by 4 species of *Xanthomonas*.

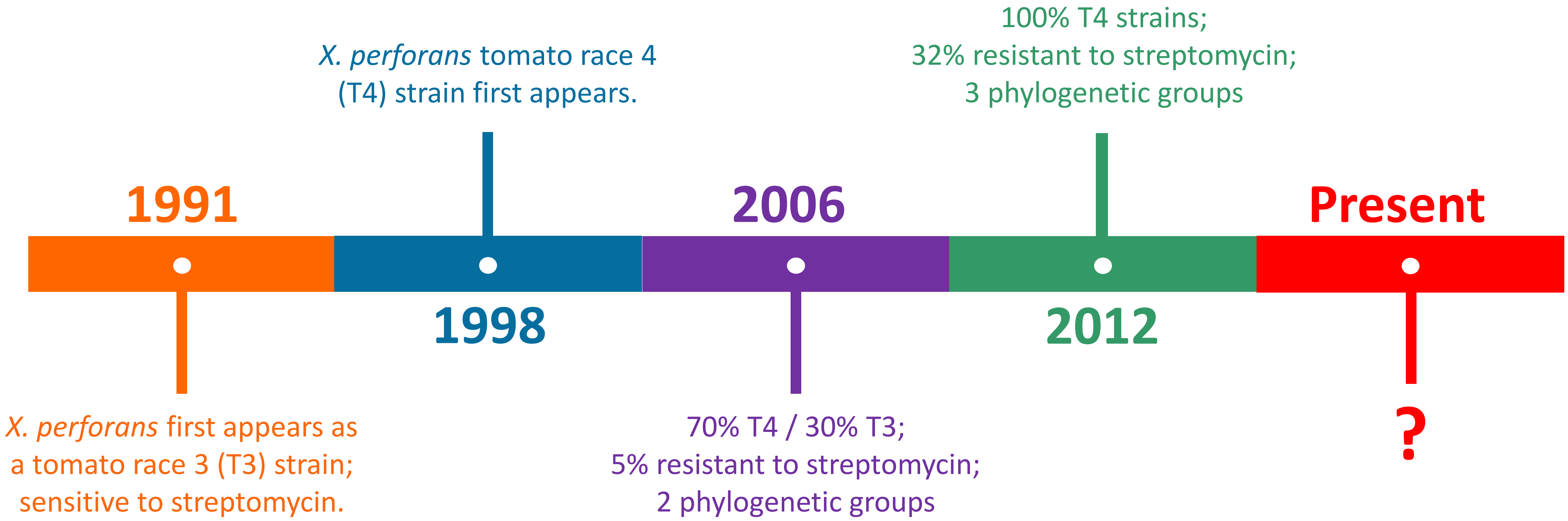
X. euvesicatoria (T1)
X. gardneri (T2)
X. perforans (T3 & T4)
X. vesicatoria (T2)

Pathogen race	Resistance genes		
	rx1, rx2, rx3	Xv3	Xv4
T1	**HR**	Sus	Sus
T2	Sus	Sus	Sus
T3	Sus	**HR**	**HR**
T4	Sus	Sus	**HR**

Population changes have affected efficacy of host resistance and bactericides in the field.

Xanthomonas perforans in Florida over time

<u>Prior to 1991</u>, *X. euvesicatoria* was the cause of bacterial spot on tomato in Florida.
X. perforans slowly displaced *X. euvesicatoria; associated with bacteriocin production*.

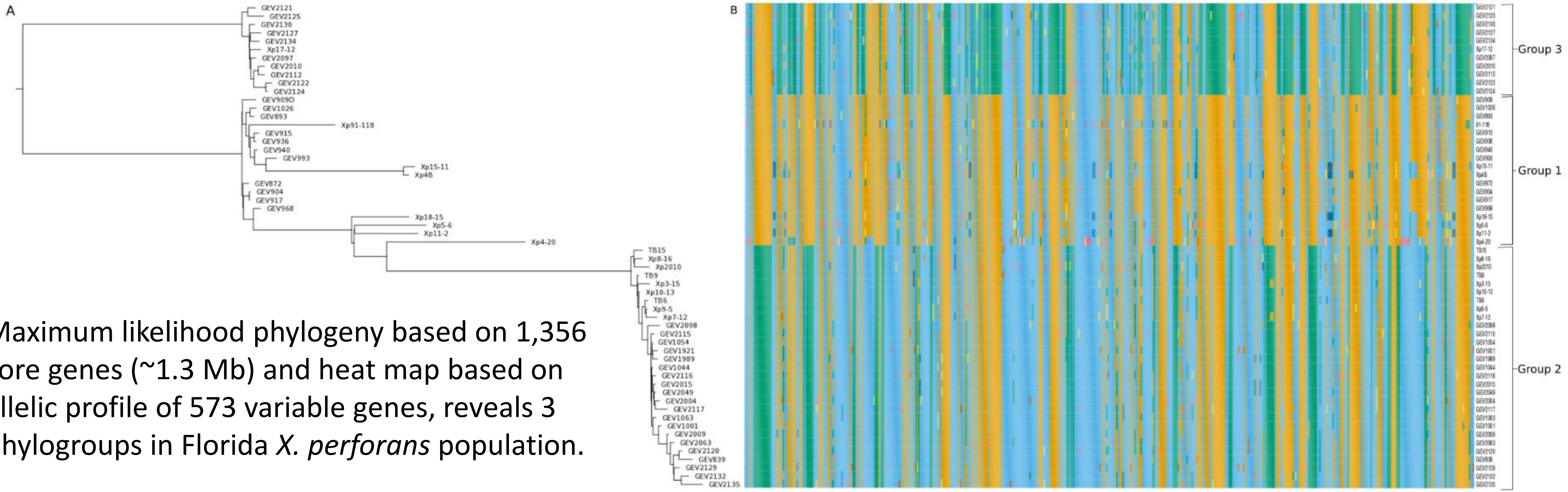


These population changes have occurred in the absence of any commercially deployed resistance to bacterial spot.

Timilsina *et al.* (2016, 2019)

Phylogenetic analysis of *Xanthomonas perforans*

cgMLST phylogeny revealed three groups among *Xp* population



Maximum likelihood phylogeny based on 1,356 core genes (~1.3 Mb) and heat map based on allelic profile of 573 variable genes, reveals 3 phylogroups in Florida *X. perforans* population.

Bacterial recombination plays a larger role in *X. perforans* diversity than mutations.

Timilsina et al. 2019. Front Microbiol10: 448.

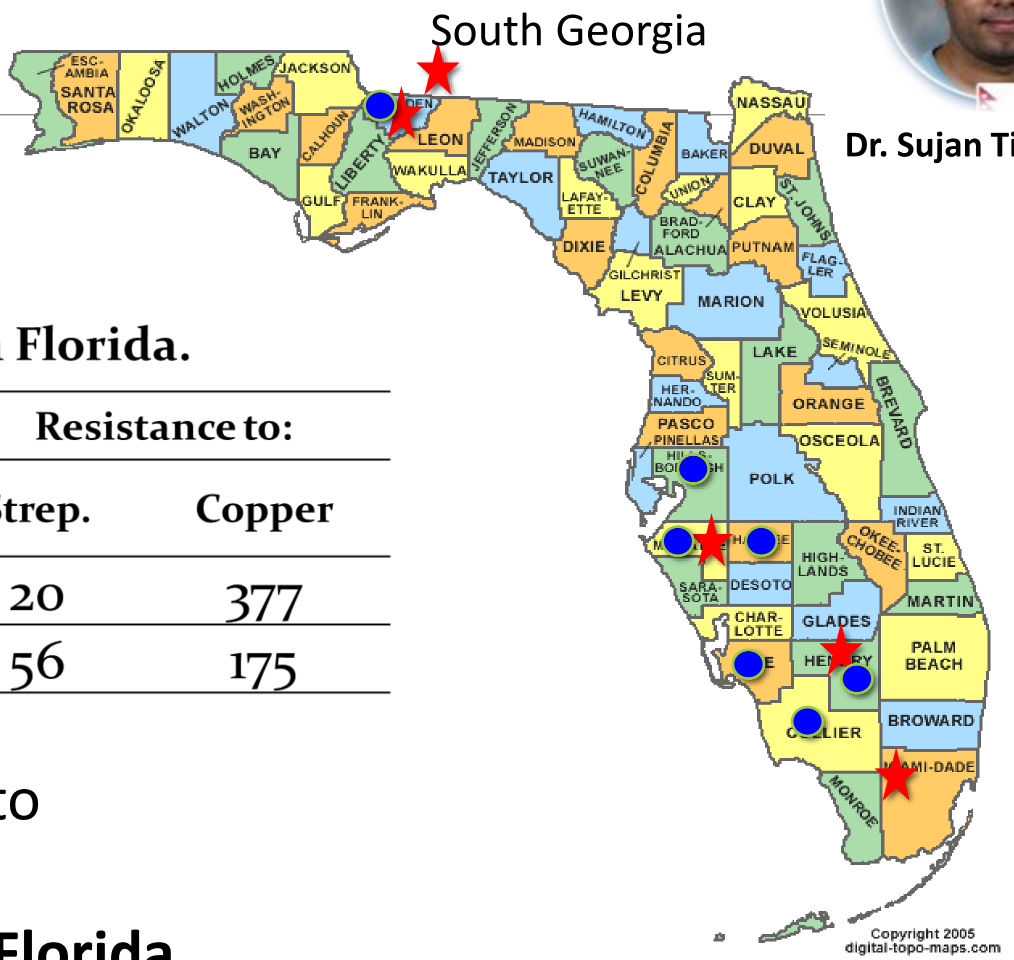
Xanthomonas perforans in Florida

Dr. Sujan Timilsina

State-wide Surveys

Field surveys in 2006 ★

Field surveys in 2012 and 2013 ●



Characterization of tomato BLS strains in Florida.

		Tomato race:			Resistance to:	
	No. of Strains	T1	T3	T4	Strep.	Copper
2006-07*	377	0	116	261	20	377
2011-12	175	0	0	175	56	175

* Horvath et al. 2012

- Only *X. perforans* found on tomato
- Conversion from race T3 to T4
- **Copper tolerance is the norm in Florida…**
- Increasing resistance to streptomycin…86% of transplant strains

Implications of copper tolerance…

On-Farm Trial, Parrish, FL Tomato 'Marianna' plums – 6 reps/trt

	Disease Severity (% foliage):				
Treatment, rate /100 gal	**7-Oct**	**17-Oct**	**3-Nov**	**18-Nov**	**AUDPC**
Actigard, 0.75 oz	7.3 d	27.5 c	50.0 e	65.9 d	1955 d
Actigard, 0.75 oz; Firewall, 16 oz **	2.8 e	11.8 d	10.4 f	15.0 f	542 e
Actigard, 0.75 oz; ManKocide, 4 lbs	18.5 c	54.2 b	75.6 cd	75.6 cd	3064 c
Agriphage 2 pt (once a week)	37.5 a	69.3 a	89.6 ab	86.6 a	4026 a
Agriphage, 2 pt (twice a week)	24.4 bc	68.7 ab	83.3 bc	78.6 abc	3553 b
ManKocide, 4 lbs	41.6 a	72.5 a	83.3 bc	85.0 ab	4042 a
Water-treated Control	38.1 a	72.5 a	91.8 a	85.0 ab	4118 a
Non-treated Control	27.5 b	71.9 a	85.0 b	77.6 bc	3680 ab
P =	*< 0.0001*	*< 0.0001*	*< 0.0001*	*< 0.0001*	*< 0.0001*

****Firewall is not labelled for field use!**

Implications of copper tolerance…

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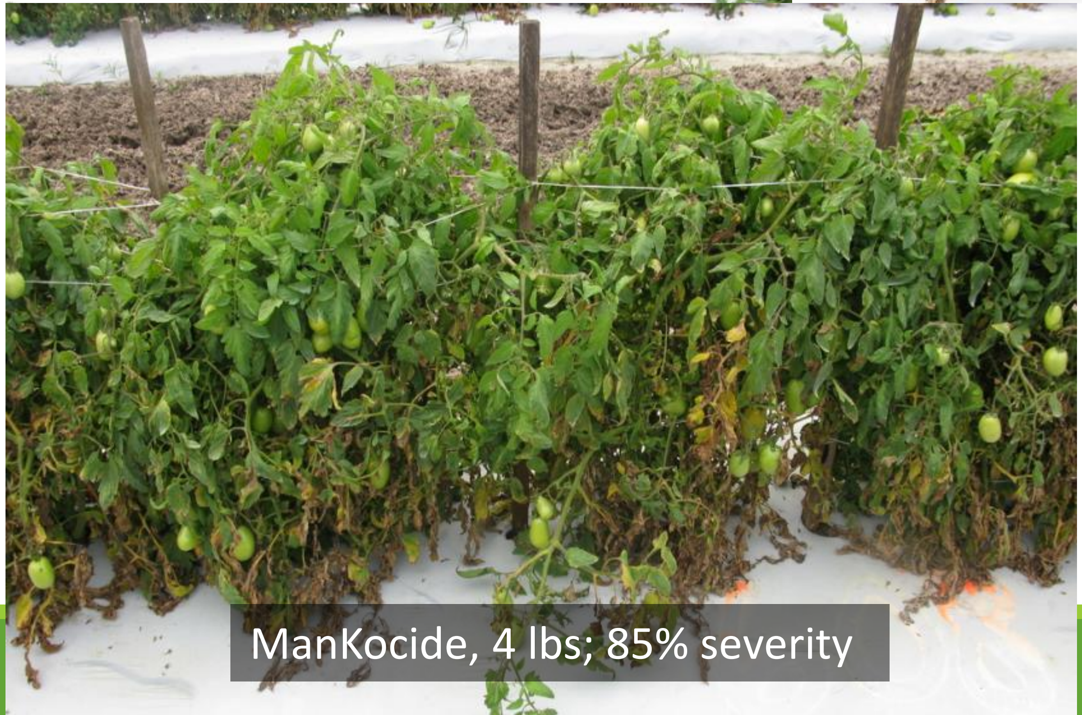
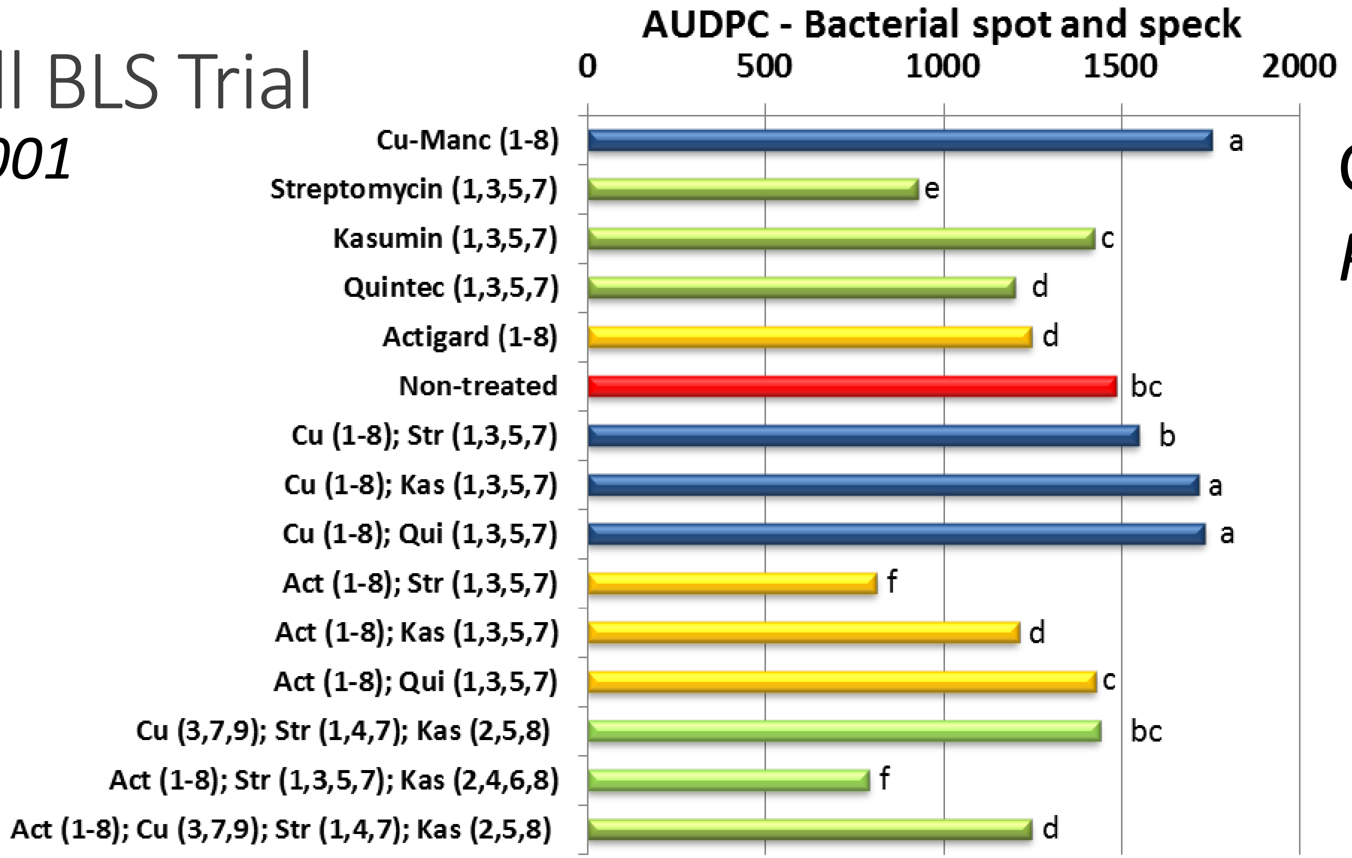


↑CONTROL ≠ ↑ YIELD

Tomato Bacterial Spot - A Florida Perspective

2012 Fall BLS Trial

P < 0.0001



Cu-manc vs. Actigard

P = < 0.0001

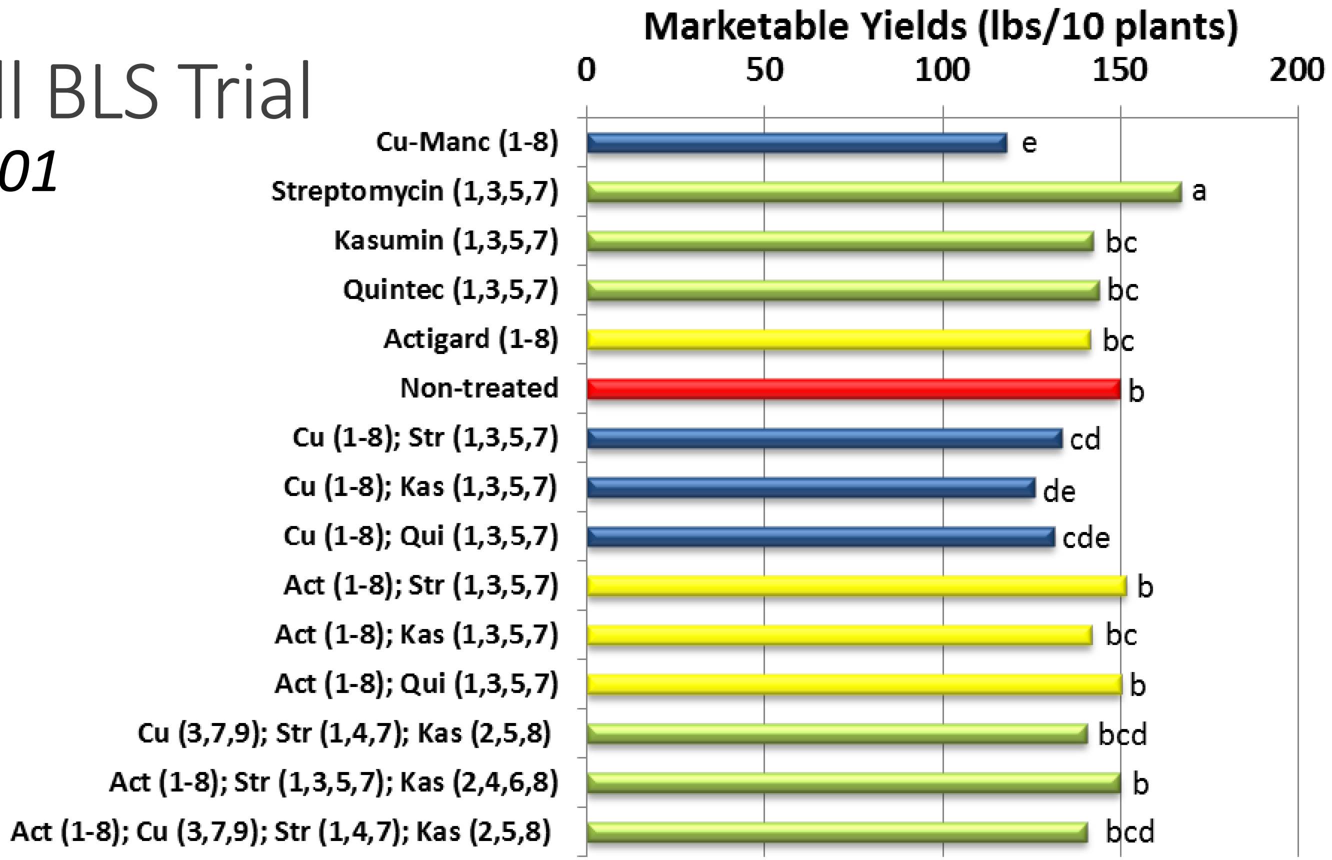
Efficacy: Streptomycin , Kasumin, Quintec, and Actigard were more effective than copper sulfate–mancozeb standard.

Regardless of product, combining with copper-mancozeb increased disease.

Tomato Bacterial Spot - A Florida Perspective

2012 Fall BLS Trial

P < 0.0001



Cu-manc vs. Actigard

P = < 0.0001

↑CONTROL ≠ ↑ YIELD

Streptomycin , Kasumin, Quintec, and Actigard statistically improved total marketable yields over copper sulfate–mancozeb standard. Other than streptomycin little improvement in marketable yields.

Regardless of product, combining with copper-mancozeb decreased marketable yield.

Tomato Bacterial Spot - A Florida Perspective

Field management of BLS shown limited success!

- **Field management**
  - Copper-tolerance is widespread
  - Plant defense activators (like Actigard) are **relatively** effective
  - Regardless of product, little to no yield improvement relative to non-treated control

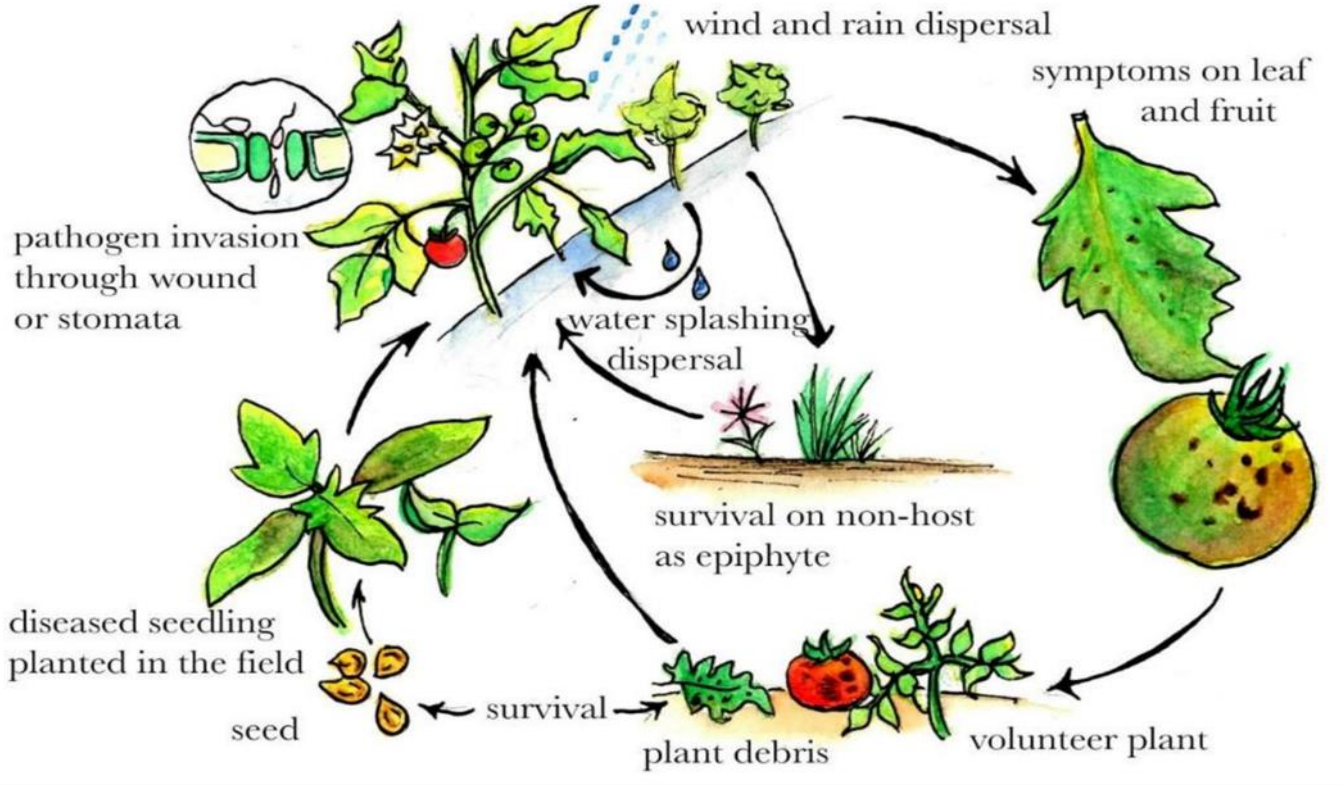


Image credits: Ying-Yu Liao

Tomato Bacterial Spot - A Florida Perspective

- **Transplant Production**
  - *X. perforans* is seedborne
  - Transplant production environment is ideal for BLS
    - High plant density
    - High humidity
    - Frequent overhead watering with high-pressure boom
  - Few effective controls available
  - Rely on physical roguing
  - Source of field inoculum??

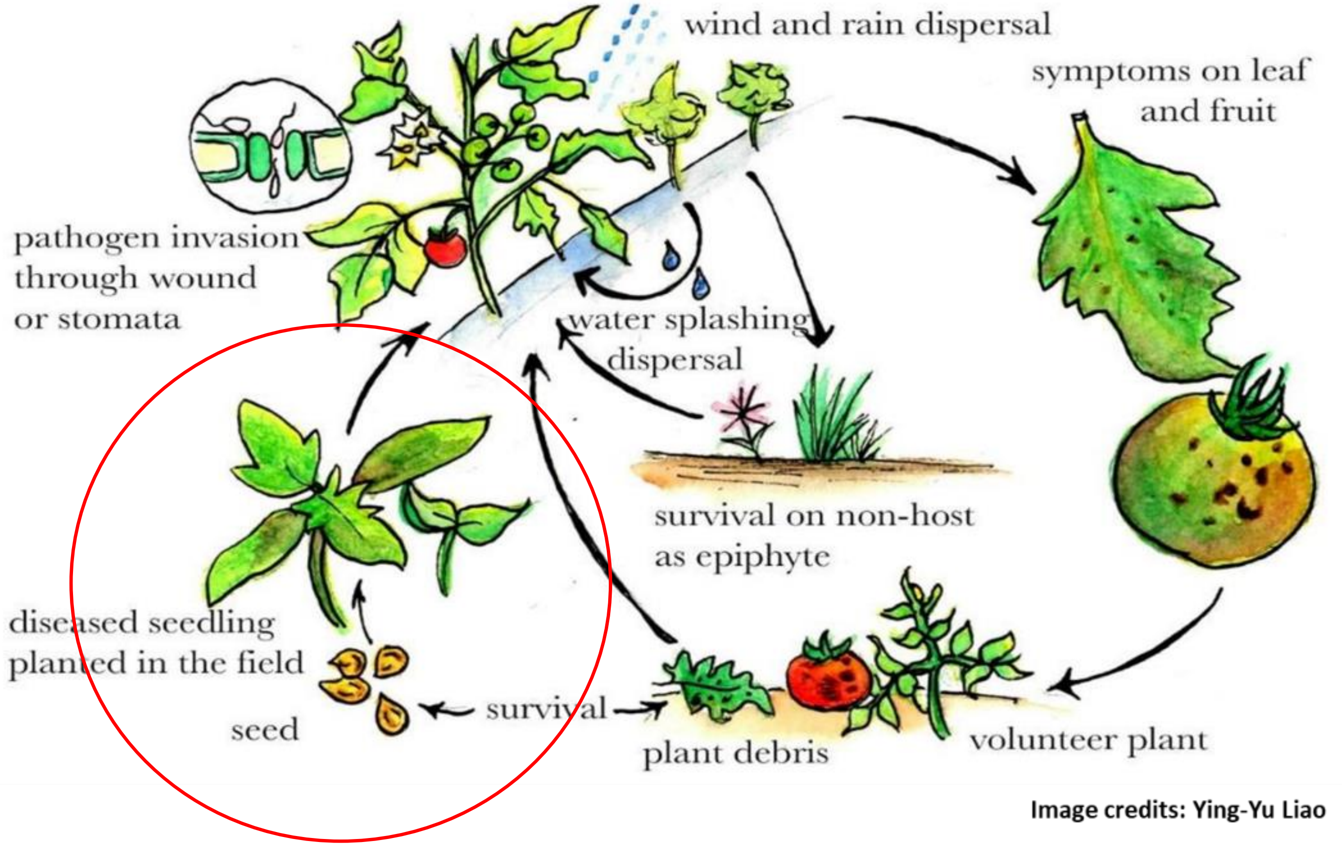
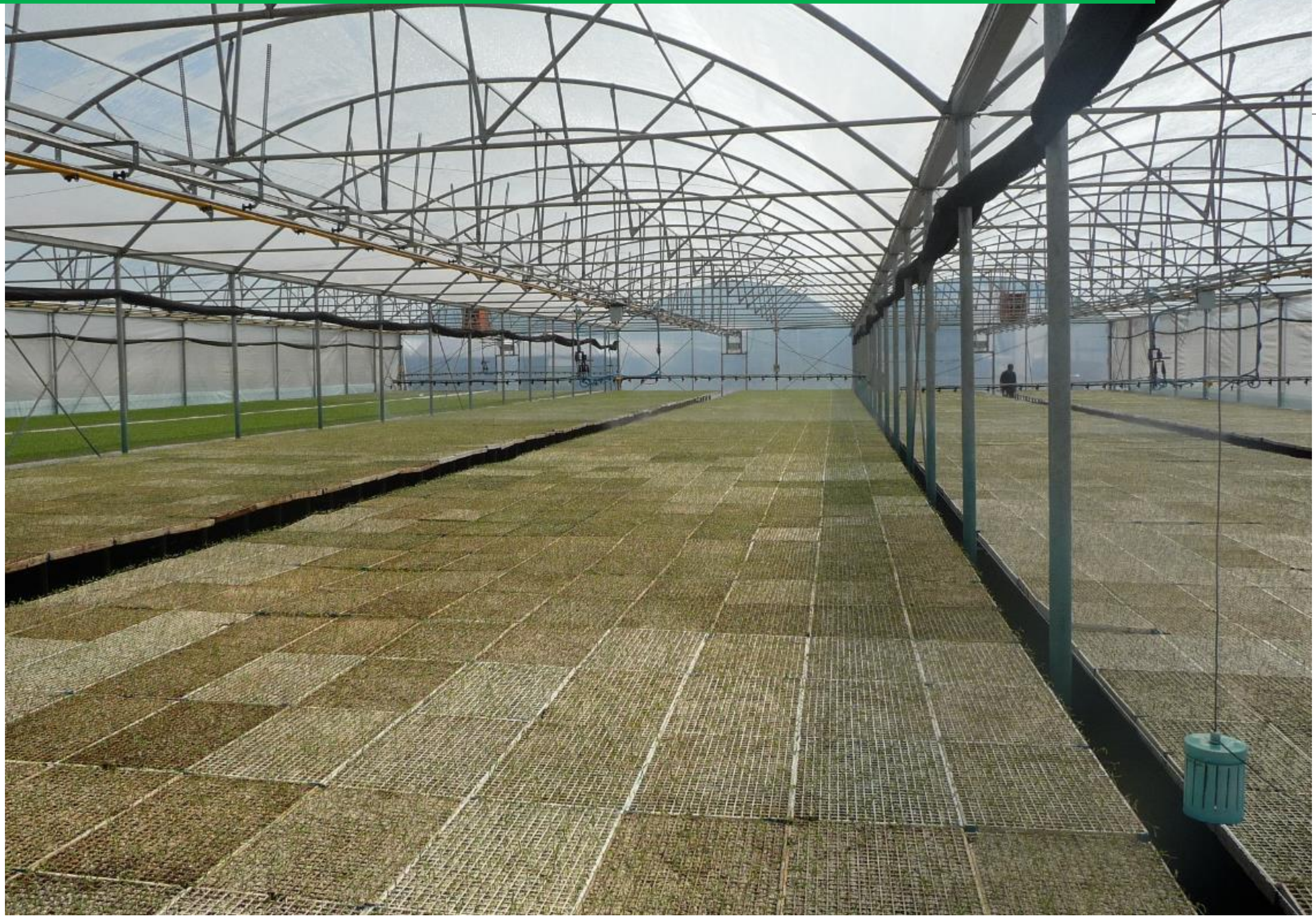


Image credits: Ying-Yu Liao

Transplant Management

Bacterial spot on tomato transplants



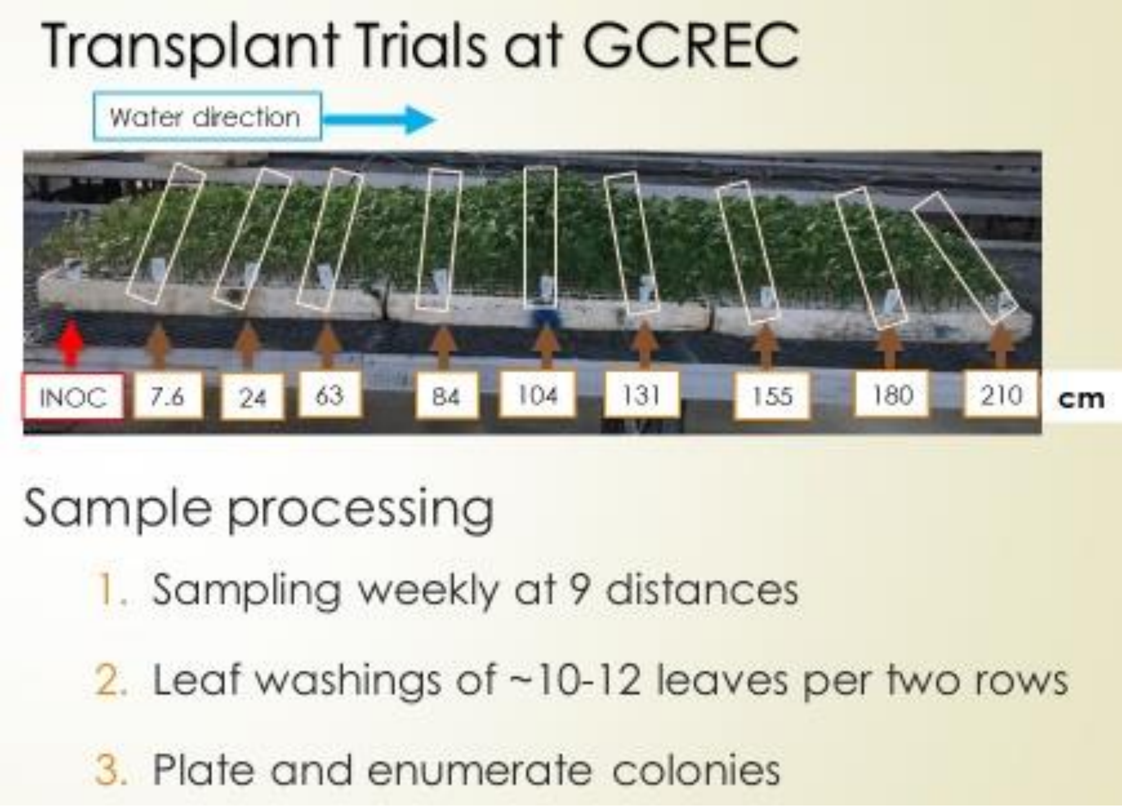
Dr. Peter Abrahamian

Dr. Anuj Sharma

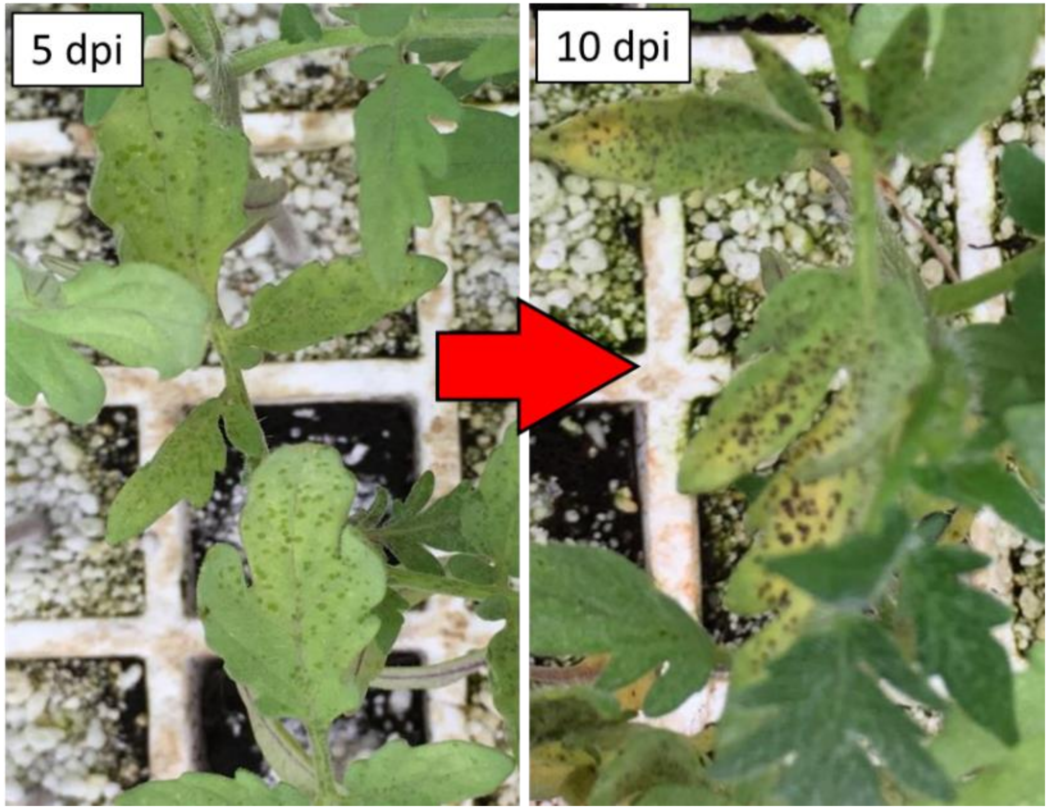
Bacterial spot on tomato transplants

Determine the movement and epiphytic survival of pathogenic Xanthomonas spp. on tomato seedlings during transplant production and field establishment.

Evaluated movement of *X. perforans* on tomato seedlings during transplant production:



Use a rifampicin resistant *X. perforans* strain.

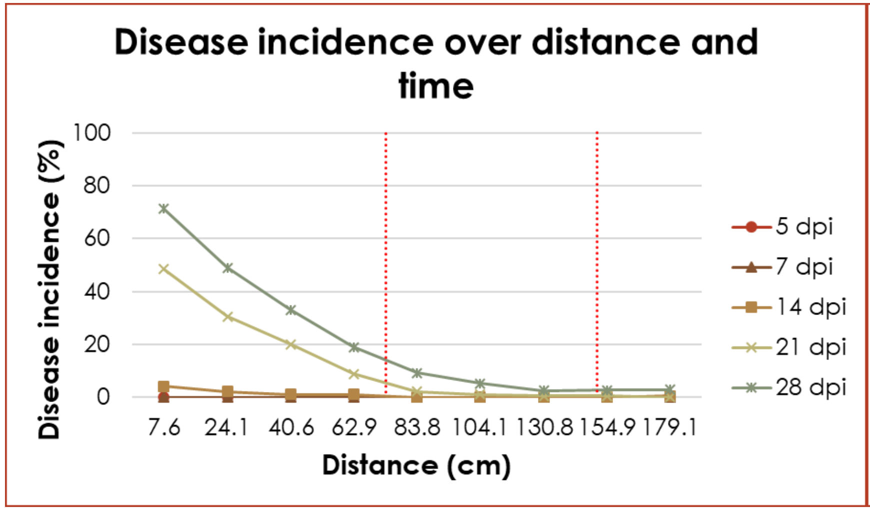


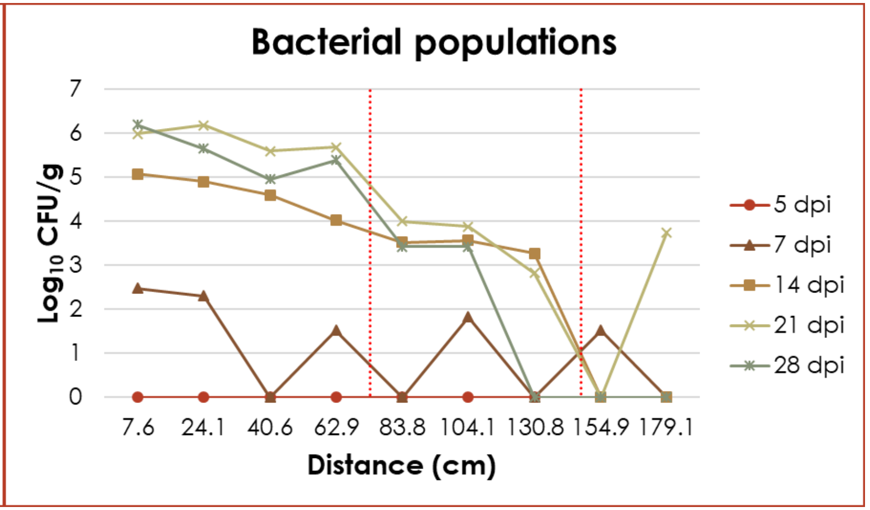
Bacterial spot on tomato transplants

Determine the movement and epiphytic survival of pathogenic Xanthomonas spp. on tomato seedlings during transplant production and field establishment.

Evaluated movement of *X. perforans* on tomato seedlings during transplant production:

GCREC Greenhouse

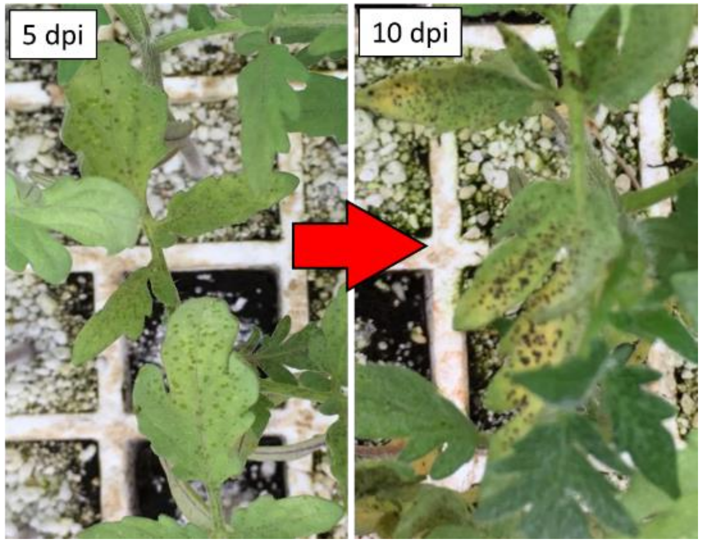




1.3 in / day

Standard tray = 26.6 in x 13.6 in (67.6 cm x 34.6 cm)

Bacterial spot on tomato transplants
(8 to 12 day latent period)



Bacterial spot on tomato transplants

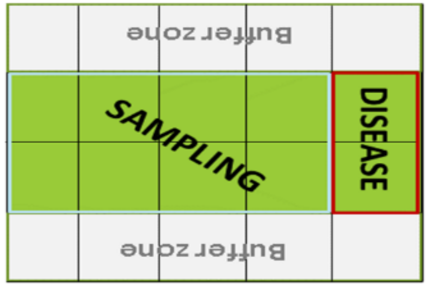
Determine the movement and epiphytic survival of pathogenic Xanthomonas spp. on tomato seedlings during transplant production and field establishment.

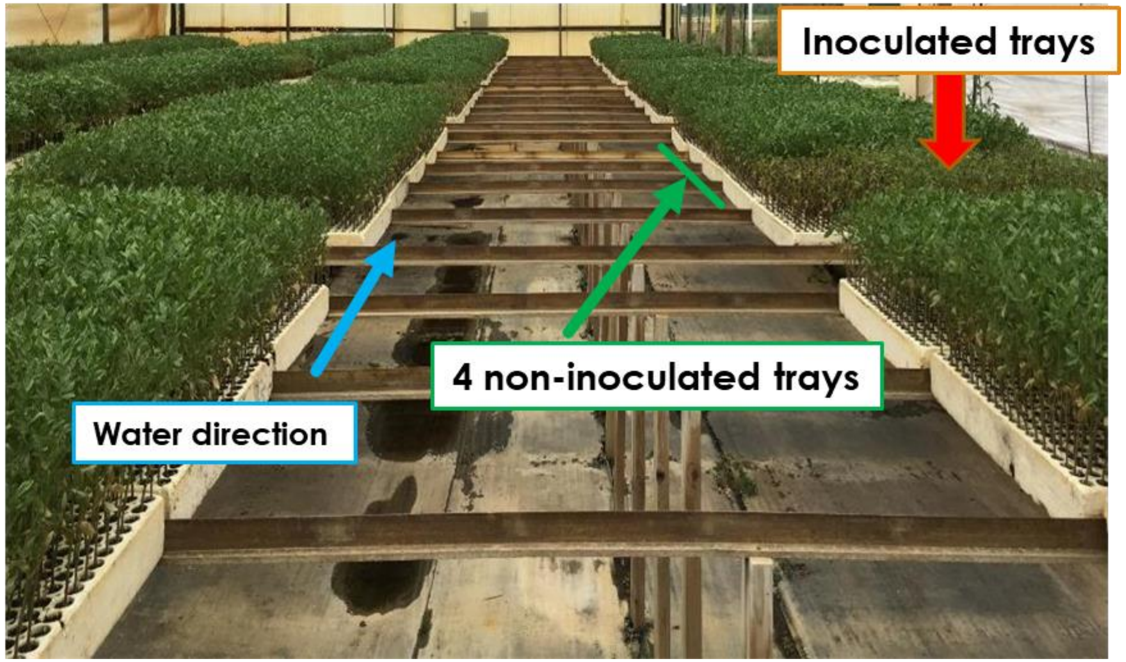
Evaluated movement of *X. perforans* on tomato seedlings during transplant production:

Trials at Commercial Facility

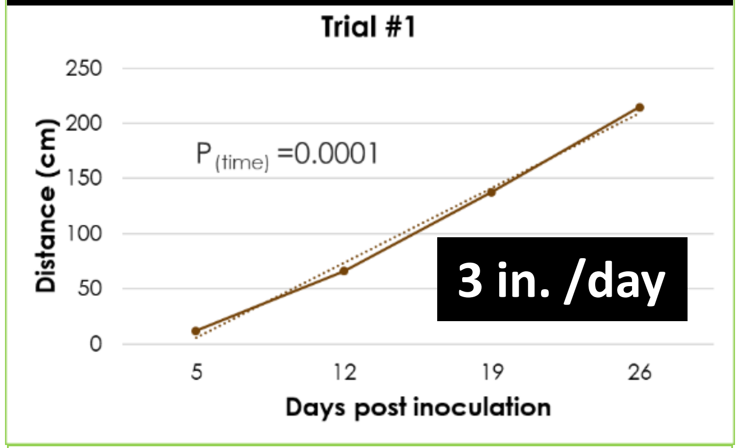
Sample processing

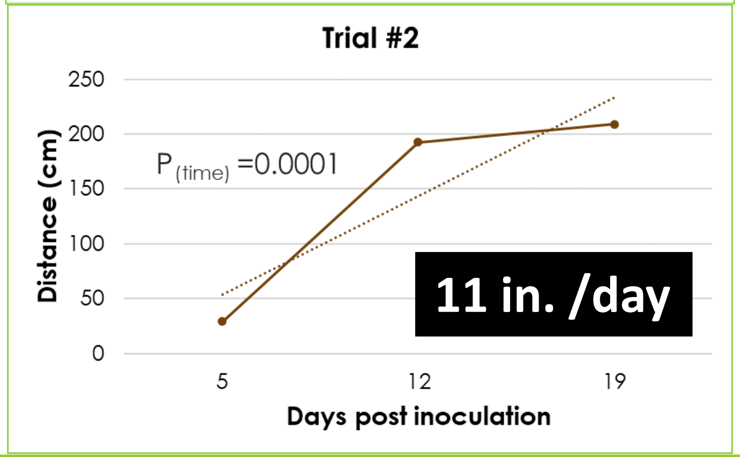
1. Sampling weekly at 9 distances
2. Leaf washings of ~10-12 leaves per two rows
3. Plate and enumerate colonies





Commercial Transplant Facility



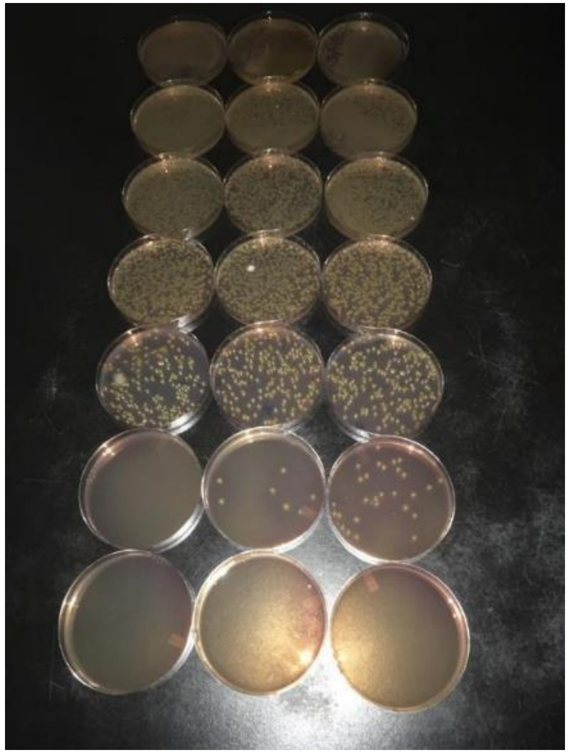


Bacterial spot on tomato transplants

Determine the movement and epiphytic survival of pathogenic Xanthomonas spp. on tomato seedlings during transplant production and field establishment.

Evaluated movement of *X. perforans* from tomato seedlings caused by overhead irrigation:

- Dip inoculated 5-week-old seedlings
- Used a rifampicin resistant *X. perforans* strain
- Watered daily - beginning 1 DPI
- Ran overhead watering boom for 3 seconds – kept boom stationary
- Captured aerosol 'downwind' from boom.



***X. perforans* spreads via aerosols!**

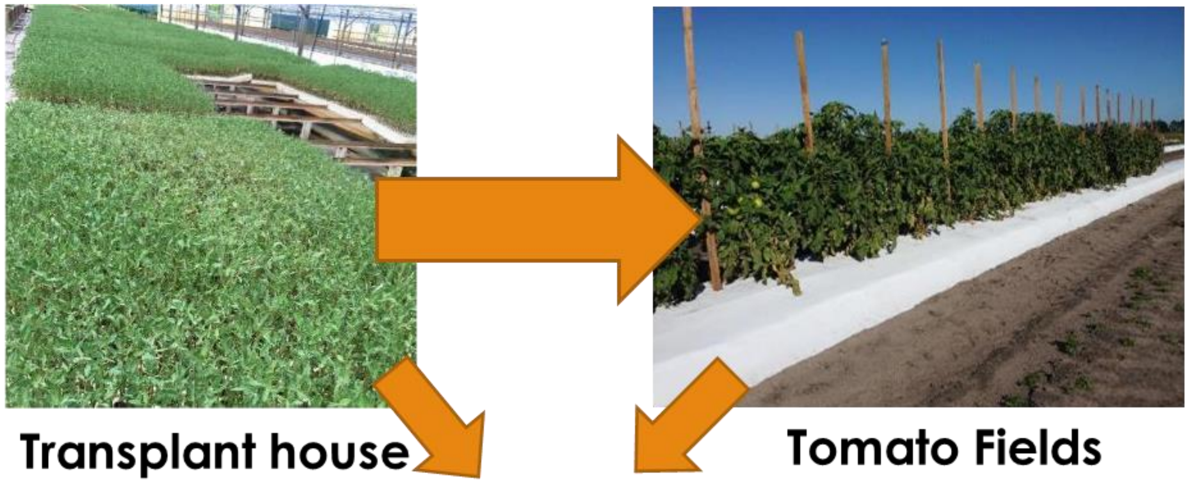
Bacterial spot on tomato transplants

Characterize movement of bacterial strains from transplants to the field :

Dr. Peter Abrahamian

Dr. Sujan Timilsina

Collected *X. perforans* strains from 2 grower operations



Transplant house

Tomato Fields
(prior to 1st harvest)

67 isolates

20 isolates from grower 'A'

47 isolates from grower 'B'

Strains collected:

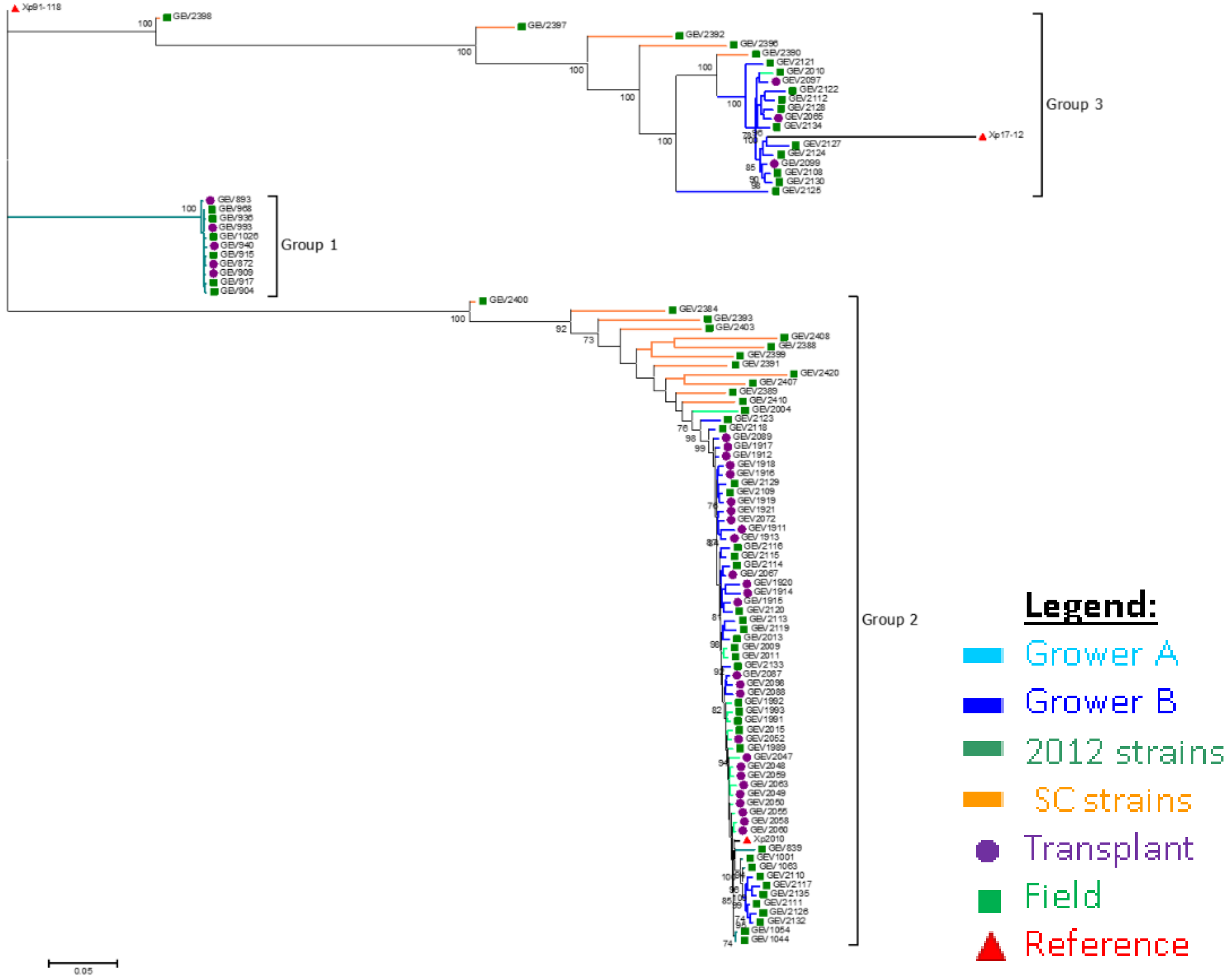
- Prior to rogueing diseased seedlings (~1 week to shipping). Only sampled seedlings once.
- Prior to first harvest in the field (~8 weeks later)
- Both grower operations produce transplants in-house for field operations.

Single nucleotide polymorphism

- Single nucleotide polymorphism (SNP) is a variation in single nucleotide observed at a specific position in the genome.

Mapping	SNP calling	Merge	Phylogenetic analysis
All *X. perforans* strains mapped using Bowtie2 with complete genome of Xp91-118 as reference.	Genome Analysis Toolkit (GATK) and Geneious software used for SNP calling and realignments	Concatenating SNPs from individual strains into concatenated SNP calling file.	Neighbor joining and Maximum likelihood trees constructed using Geneious.

Phylogenetic tree based on 11,007 SNPs



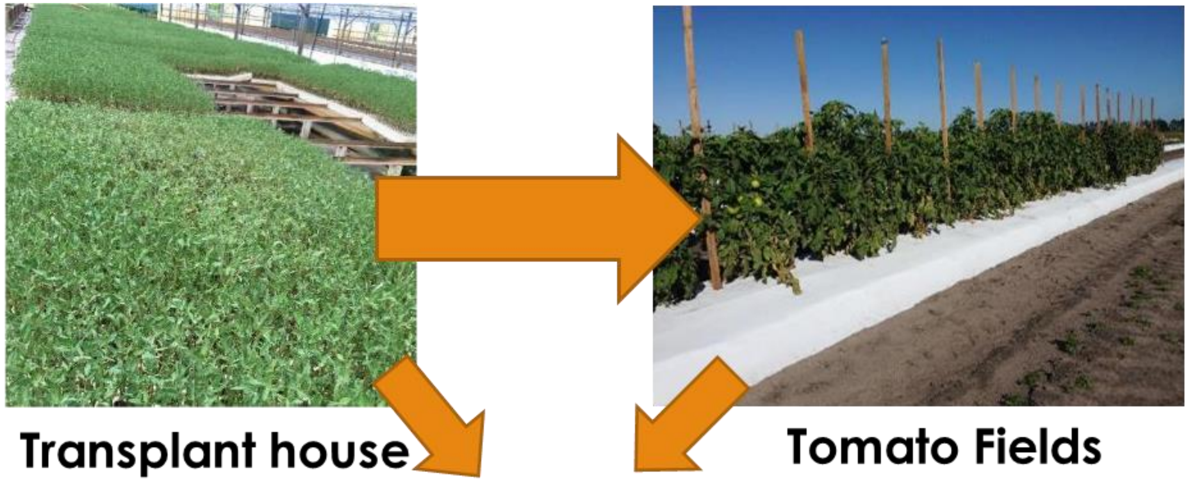
- RAxML (Rapid bootstrapping; 100 bootstraps)
- Model: GTR +G
- SNPs at 8x coverage
- 11,007 SNP concatenated
- Group 1: ~1600 SNPs
- Others: ~5000-6000 SNPs
- 103 strains
- Grower A: Central FL
- Grower B: South FL
- SC strains: South Carolina

Abrahamian et al. 2019 App. Eniv. Micro.

Bacterial spot on tomato transplants

Characterize movement of bacterial strains from transplants to the field :

Collected *X. perforans* strains from 2 grower operations



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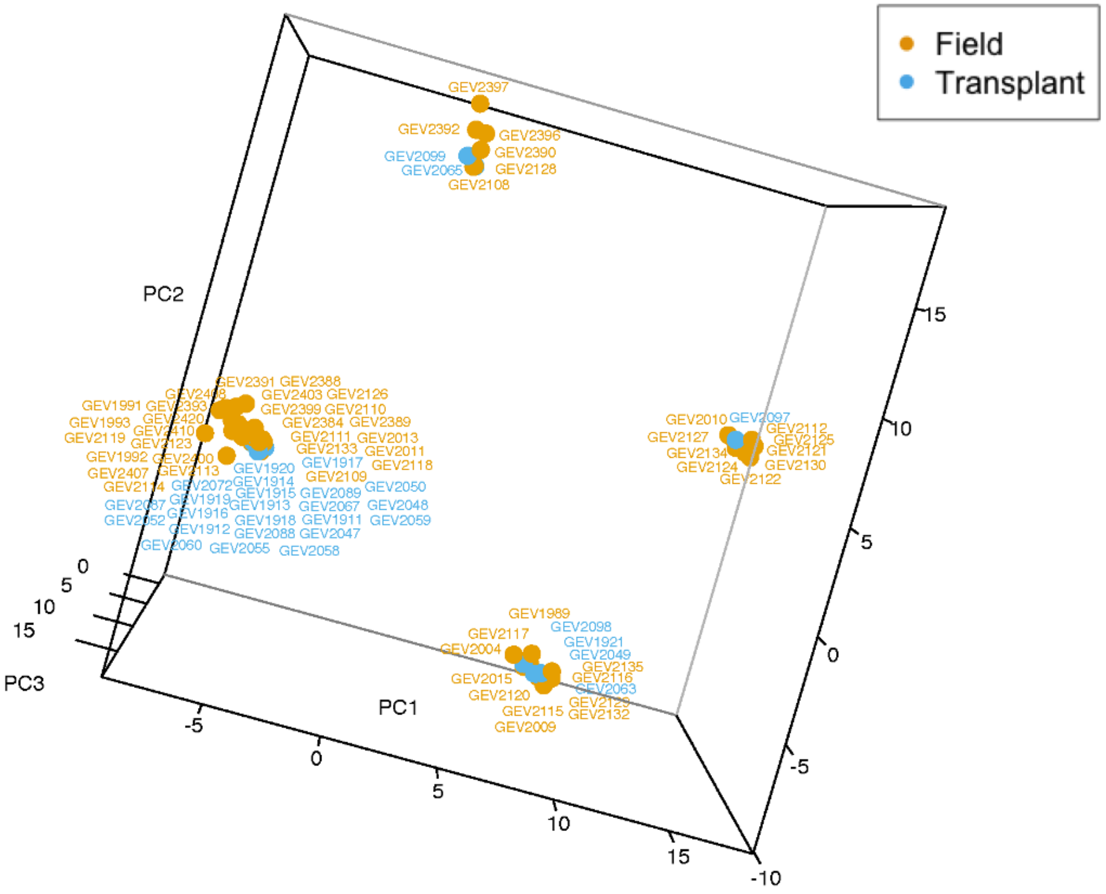


Principal-component analysis of strains based on cgMLST of 1,356 genes (Field vs. Transplant strains)

Bacterial spot on tomato transplants

Characterize movement of bacterial strains from transplants to the field :

- 60 to 100% of strains isolated from the field **likely** originated from seedlings grown in transplant house.
- Could differentiate growers based on strains isolated from transplant source and field operation.
- A few strains were common to both **field** sites and some field strains that didn’t correspond to any transplant strain.
- **Results stress the importance of transplants as a primary source of inoculum.**

Abrahamian et al. 2019 App. Eniv. Micro.

Bacterial spot on tomato transplants

Challenge:

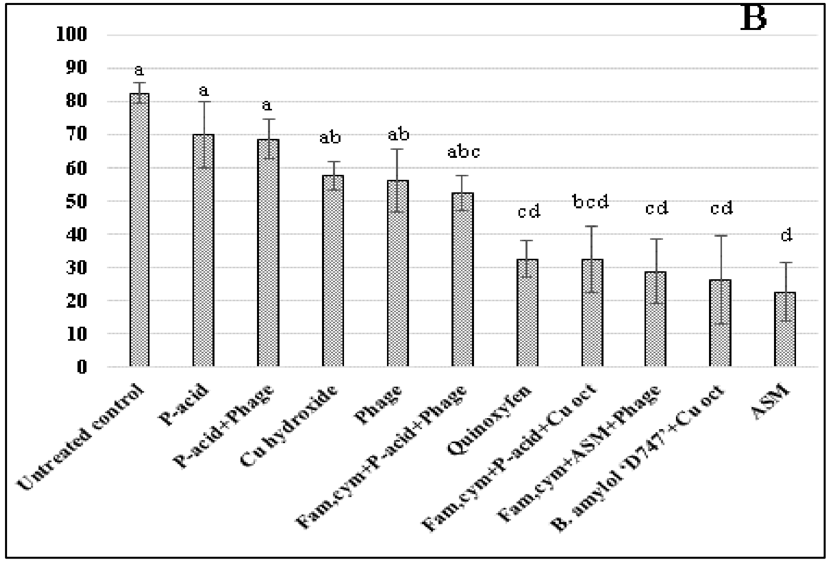
- BST widely resistant to copper
- Mixed resistance among strains against antibiotics
- Lack of good chemical alternatives for greenhouse application
- Potential reduction of BST disease (inoculum) introduced into tomato fields

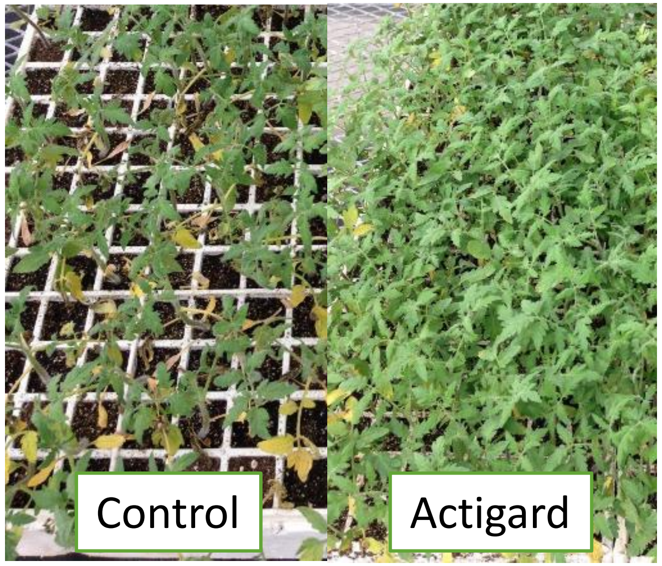
Dr. Peter Abrahamian

Bacterial spot on tomato transplants

Evaluate the integrated use of bactericides, Actigard, and other non-copper alternatives for the improved management of bacterial spot in transplant operations.

Evaluated 13 products across 8 trials with tomato transplants:

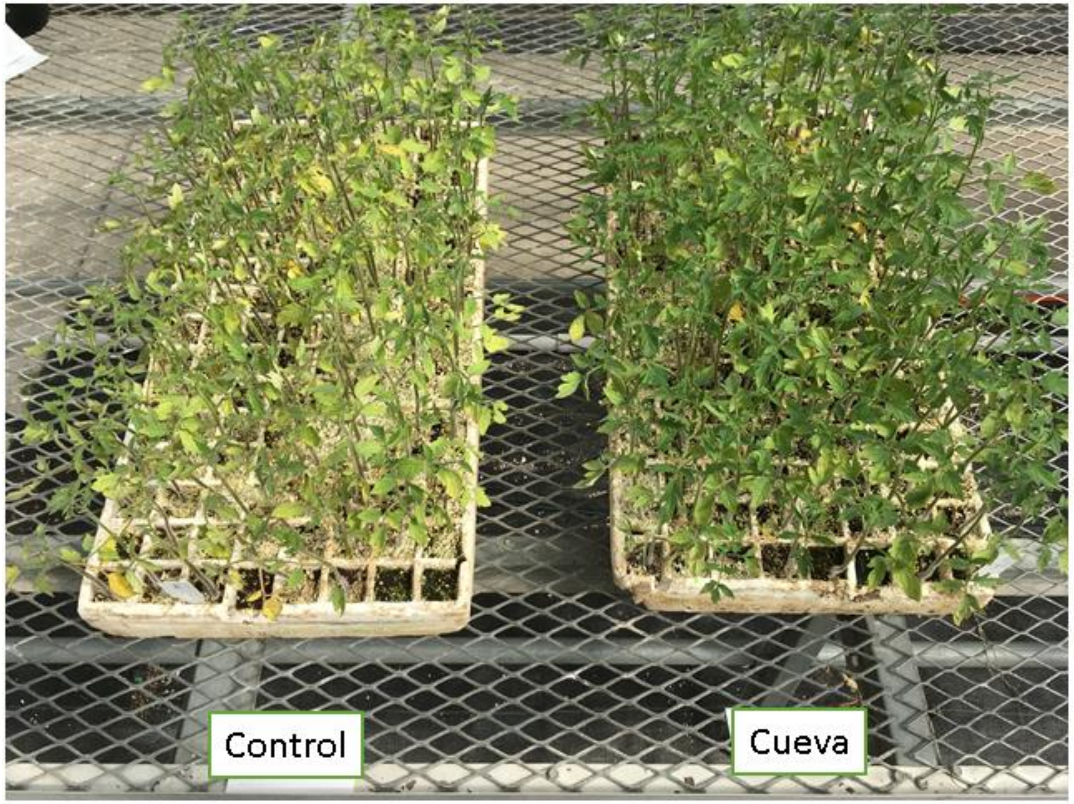




Product name	Active ingredient
Actigard®	acibenzolar-s-methyl (ASM)
Agress®	oxysilver nitrate (OSN)
AgreGuard™-1	pentasilver hexaoxoiodate (Ag_5IO_6)
Agri-mycin® 17	streptomycin
AgriPhage™	bacteriophage
Cueva™	copper octanoate
Cuprofix® Ultra 40D	copper sulfate
Double Nickel 55™	*Bacillus amyloliquefaciens* 'D747'
KleenGrow™	ammonium chloride
Kocide® 3000	copper hydroxide
K-Phite®	mono- and di- sodium phosphoric acid
Mycoshield®	oxytetracycline
Milstop®	potassium bicarbonate ($KHCO_3$)
Penncozeb® 75DF	mancozeb
Quintec®	quinoxyfen
Sil-matrix™	potassium silicate
Serenade® Opti	*Bacillus subtilis* 'QST 713'
Tanos®	famoxodone, cymoxanil
USF2018A[a]	-

- For transplants, copper octanoate, oxysilver nitrate, acibenzolar-S-methyl alone or in combinations were superior
- Chemical performance of same products varied in the field:
  - Field applications not very effective
  - No effect on yield – fruit infection extremely low

Bacterial spot on tomato transplants

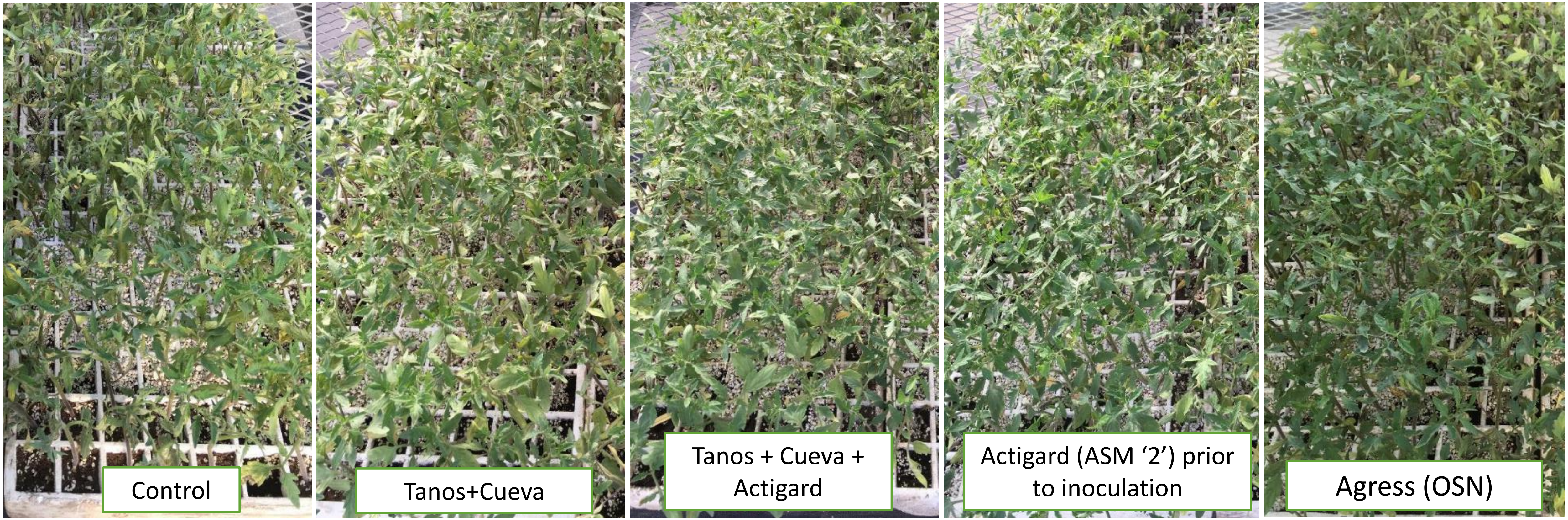


Interesting…

Cueva outperformed Kocide 3000, but unlikely related to copper content alone. Cueva consists of copper octanoate, which contains only 1.8% metallic copper equivalent compared to 30% in Kocide 3000 (copper hydroxide).

Bacterial spot on tomato transplants

Transplant Trial 3



- None of the mixtures improved BST control compared to the most effective compound in the mixture (Actigard & Cueva).
- Newer formulation of Agress (OSN) did not cause any phytotoxicity (spotting) of leaves.

Bacterial spot on tomato transplants

Field Trial
Fall 2016

Program, rate/100 gal (week of application)	AUDPC
Cueva + Tanos (1,4,7,10); Serenade Opti + Milstop (2,3,5,6,8,9,11,12)	1954a
Cueva + Tanos (1,4,7,10); Double Nickel 55 (2,3,5,6,8,9,11,12)	1850ab
Cueva (1-12); Tanos (1,3,5,7,9,11)	1804abc
Cuprofix 40D + Penncozeb 75DF (1-12)	1782abc
Cueva (1-12); Tanos (1,4,7,10)	1697bcd
Cueva + Tanos (1,4,7,10); K-Phite (2,5,8,11); Agriphage (3,6,9,12)	1677bcd
Cueva + Tanos (1,4,7,10); Agriphage (2,3,5,6,8,9,11,12)	1657cd
Cueva + Tanos (1,4,7,10); Agriphage (3,6,9,12); Actigard (1-8)	1651cde
Nontreated control	1602de
Cueva (1-12); Tanos (1,4,7,10); K-Phite (2,5,8,11); Actigard (1-8)	1550de
Cueva (1,4,7,10); Tanos (1,4,7,10); K-Phite (2,5,8,11); Actigard (1-8)	1487e
Actigard, (1-8)	1321f
P	< 0.0001

1st treatment was also applied to transplants prior to planting…

Results:

- Cu-manc ineffective
- Tanos & Cueva ineffective
- Only Actigard alone was effective, and improved ineffective programs
- **Regardless, no improved yields!**

Bacterial spot on tomato transplants

SUMMARY

Copper octanoate, oxysilver nitrate, acibenzolar-S-methyl either alone or in combinations were effective against BST on transplants (equivalent or superior to standard copper hydroxide)

Field applications not very effective

- Chemical performance varied across experiments
- Actigard & copper-mancozeb standard reduced disease (Actigard was typically equivalent or superior to copper-mancozeb standard)
- Experimentals showed some promise (Ag-based & USF2018A)
- Copper octanoate (Cueva) & Tanos were ineffective in the field

In field trials, no effect on marketable yield – fruit infection extremely low

Tracking *Xanthomonas perforans* strains linked to a seasonal outbreak of bacterial spot using whole genome sequencing

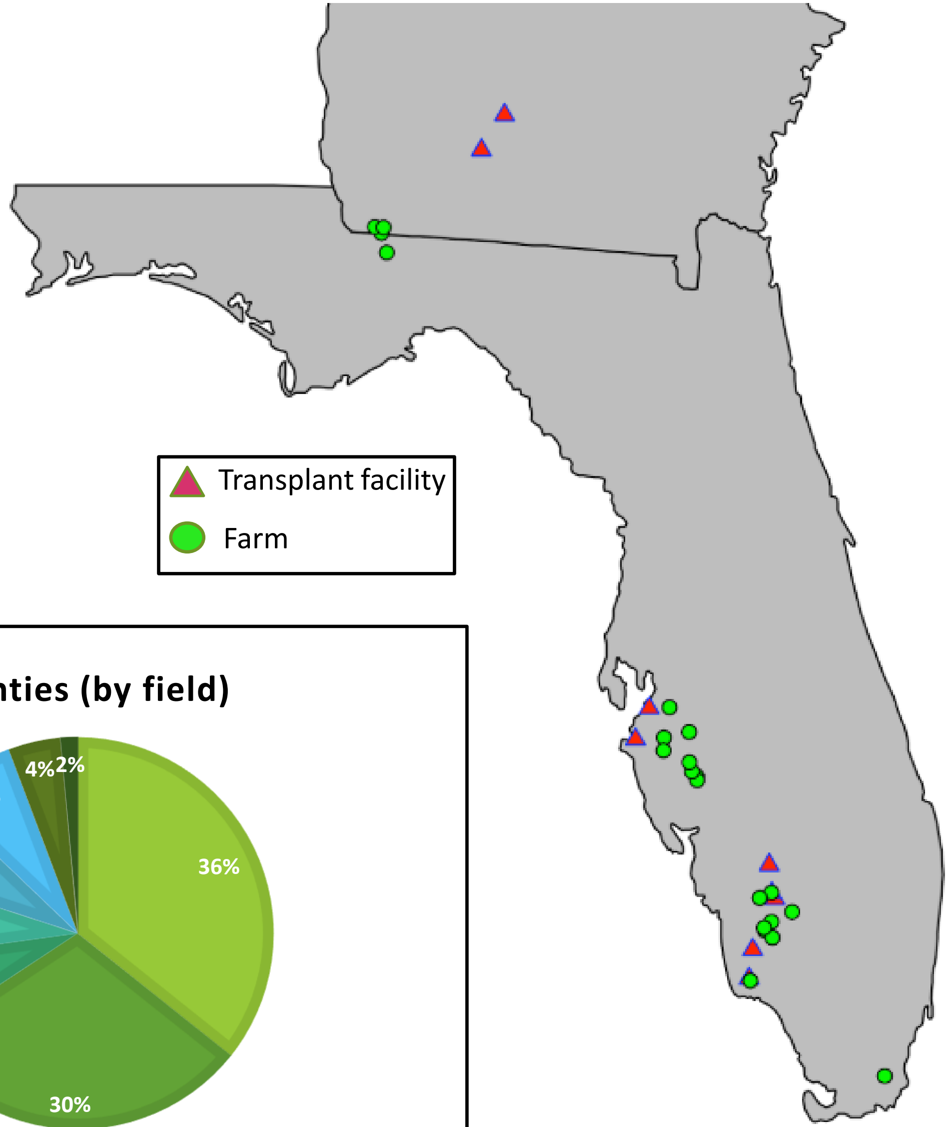
Objectives:

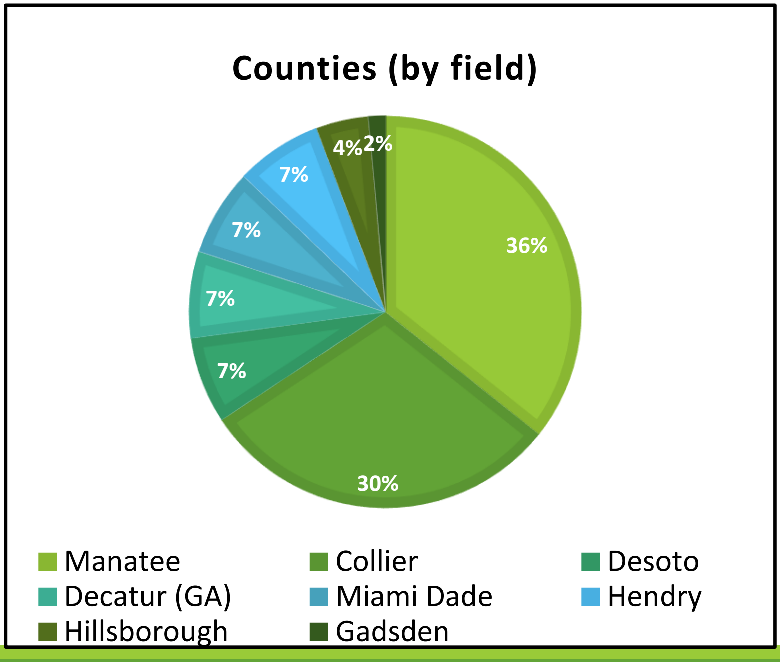
1. Assess strain diversity through copper and streptomycin resistance, pathogen race, and presence or absence of effectors of potential interest to breeding programs.
2. Infer origin of field isolates via genetic relatedness of bacterial strains by seed producer, cultivar, transplant facility, geographic location, and grower or farm.

2017-18 Florida collection

- 585 *Xanthomonas* strains, from field-grown tomatoes planted Fall 2017, representing:
  - 70 fields
  - 22 farms
  - 15 grower operations
  - 8 transplant facilities
  - 8 counties
  - 23 cultivars
  - 8 seed producers
- Overall, this collection represents relative proportions of tomato production by county.
- Largest single season collection:
  - Copper tolerance
  - Streptomycin resistance
  - Tomato race
  - Phylogenetic group
  - Bacteriocin production

Dr. Jeannie Klein-Gordon

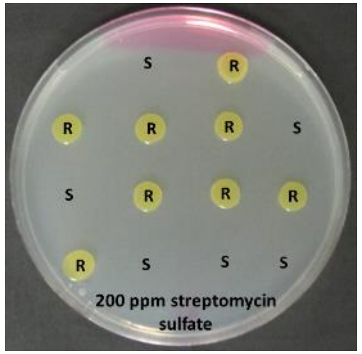




Xanthomonas perforans in Florida

Collection year	# of strains	# of collection sites	Species	Race T3	Race T4	Copper sulfate resistance (200 ppm)	Streptomycin resistance (200 ppm)
2006	377	20	*X. perforans*	33%	67%	100%	5.3%
2011-12	176	46	*X. perforans*	0%	100%	99.4%	32% (14% from field)
2017-18	585	70	*X. perforans*	8%	92%	99.8%	25% *

*40% of fields had at least one streptomycin resistant isolate

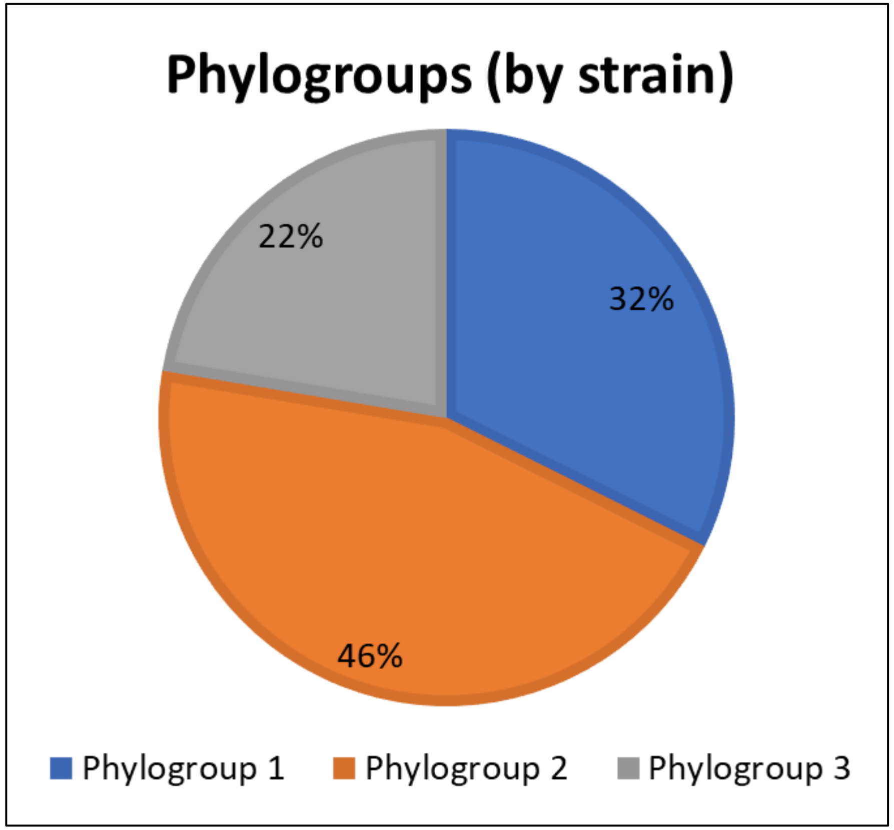


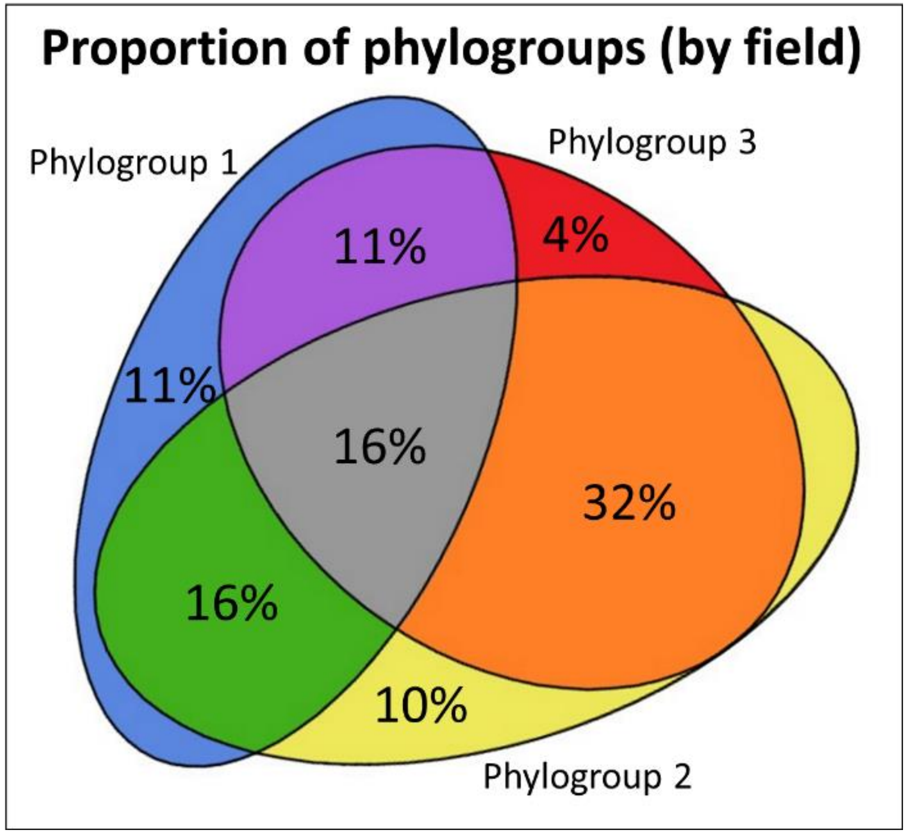
Xanthomonas perforans in Florida

Phylogenetic group proportions for 2017-18 collection

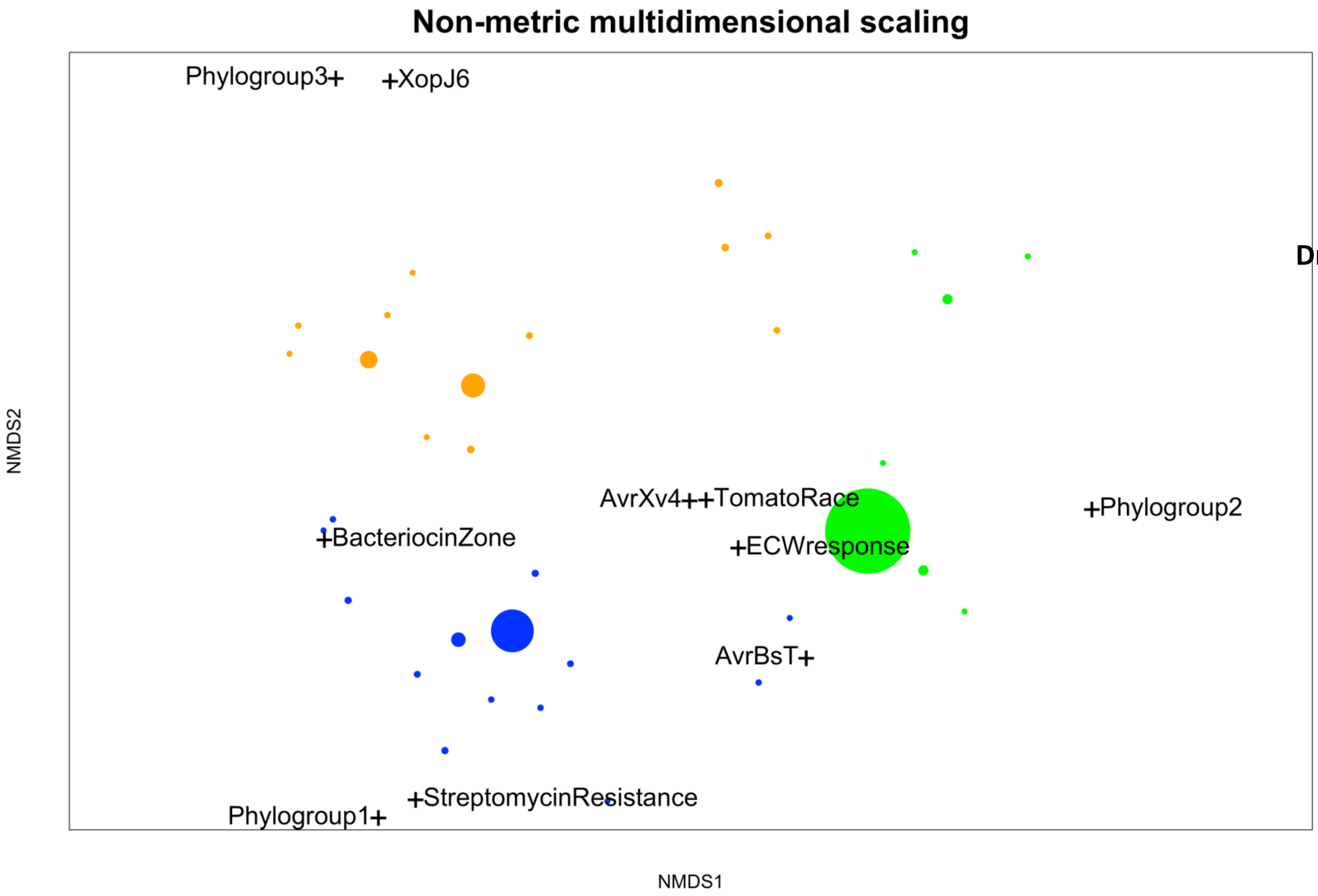
Dr. Jeannie Klein-Gordon

Assigned strains into three phylogroups identified in prior studies, based on several SNPs from portions of two genes.





Traits such as streptomycin resistance are associated with a phylogroup, while other traits are not associated with a single phylogroup



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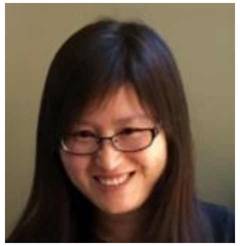
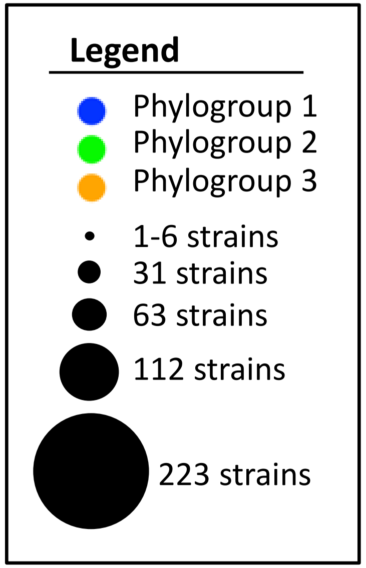
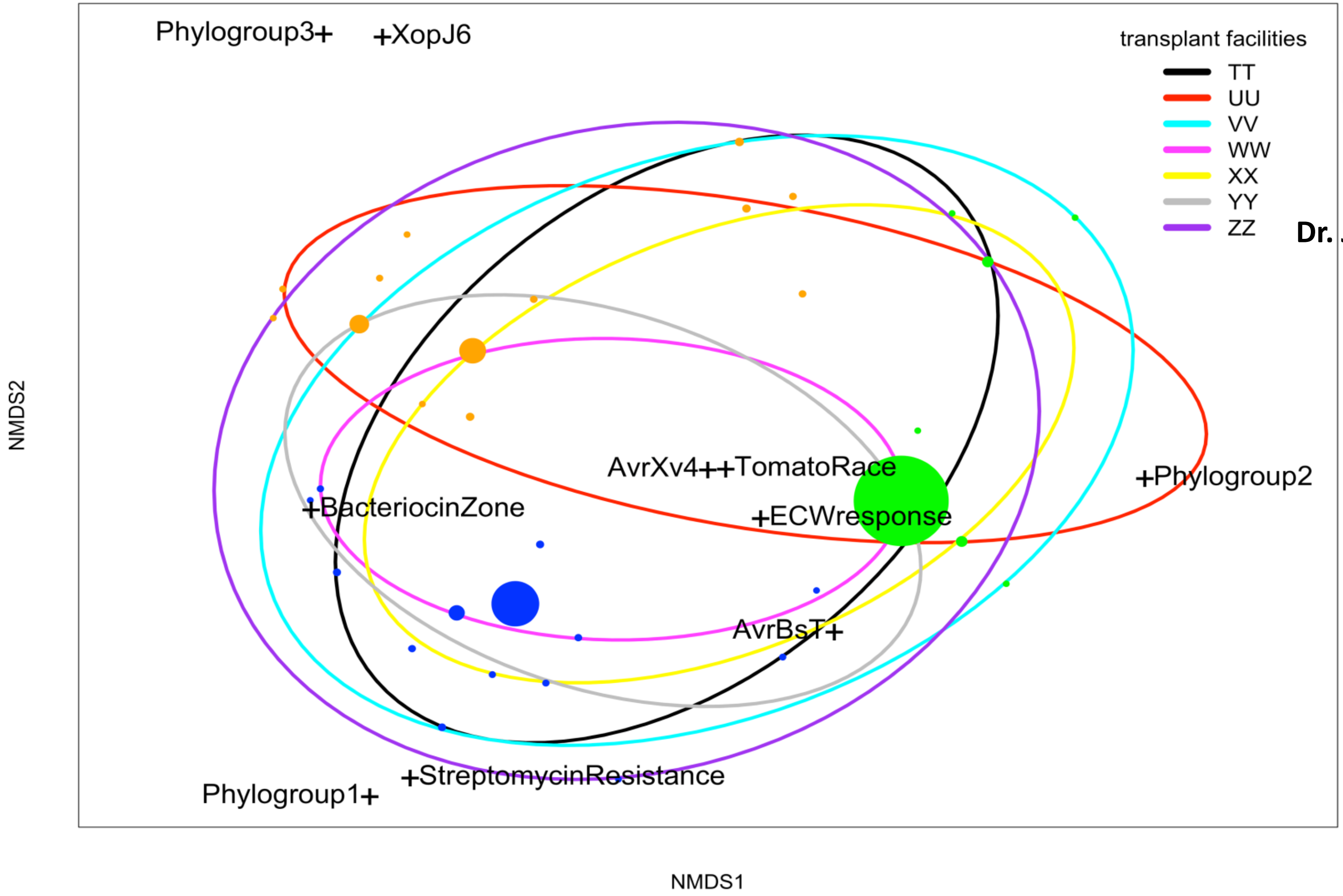


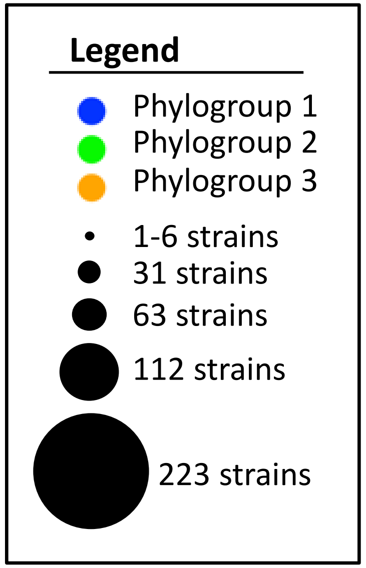
Yanru Xing
Dr. Karen Garett's Lab



There is some evidence that plants originating from certain transplant facilities tend to be infected by *Xanthomonas perforans* with certain characteristics

Non-metric multidimensional scaling by transplant facilities





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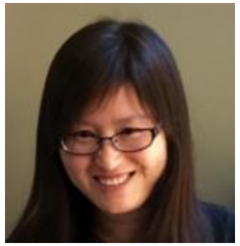

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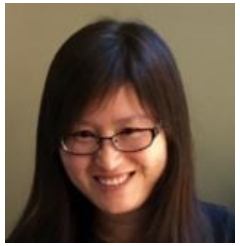
Xanthomonas perforans in Florida

2017-18 Florida collection

- 585 *Xanthomonas* strains, from field-grown tomatoes planted Fall 2017, representing:
    - 70 fields
    - 22 farms
    - 15 grower operations
    - 8 transplant facilities
    - 8 counties
    - 23 cultivars
    - 8 seed producers

- **Sequenced 366 strains.**
- **281 strains selected from five most common cultivars for analyses.**

Jeannie Klein



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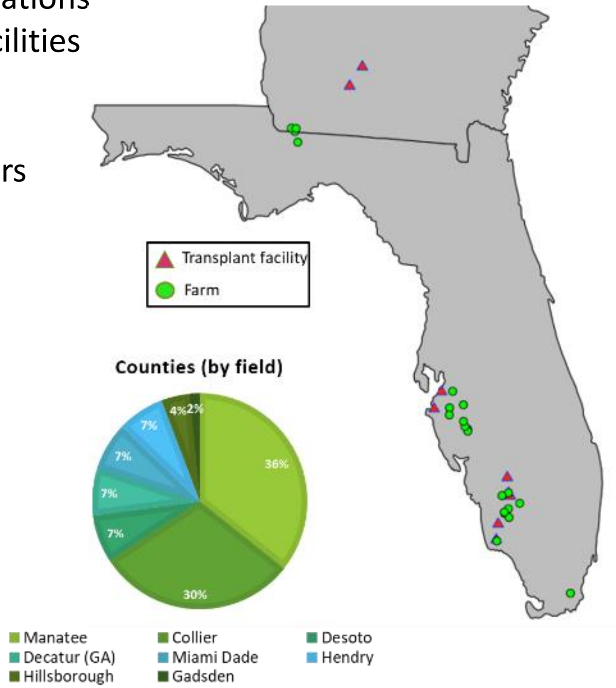

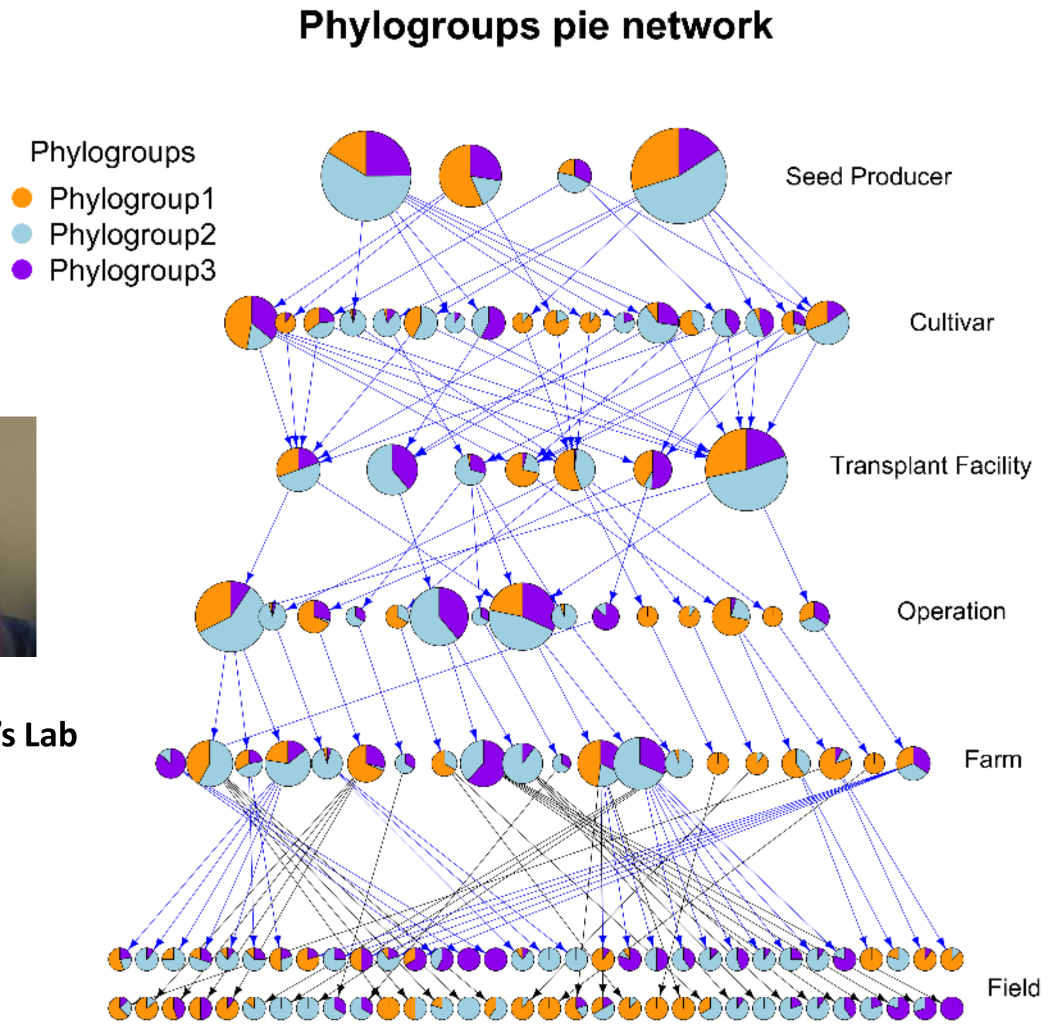

Tracking *Xanthomonas perforans* strains linked to a seasonal outbreak of bacterial spot using whole genome sequencing

Possible complication to the study…

Infer origin of field isolates via genetic relatedness of bacterial strains by seed producer, cultivar, transplant facility, geographic location, and grower or farm.

Xanthomonas perforans in Florida

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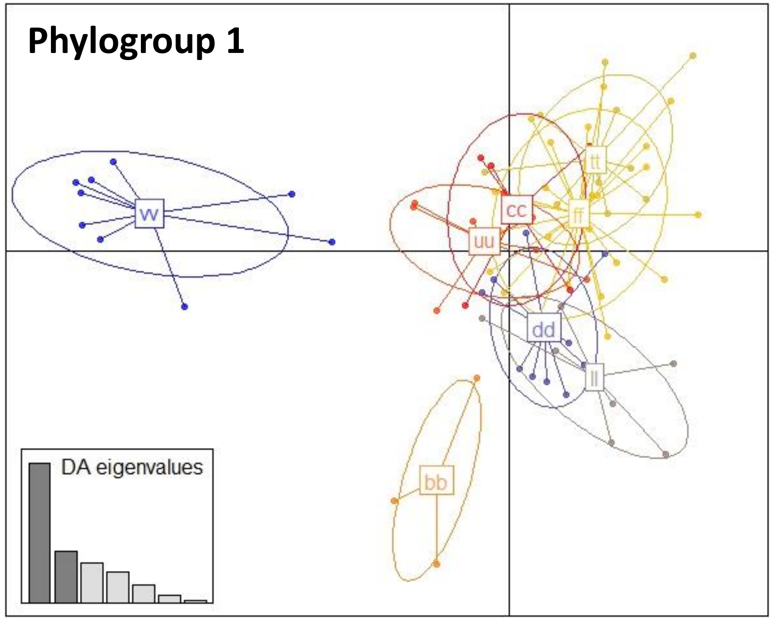
Jeannie Klein

Discriminant Analysis of Principal Components (DAPC)
Jombart et al. 2010



Strains grouped by Farm

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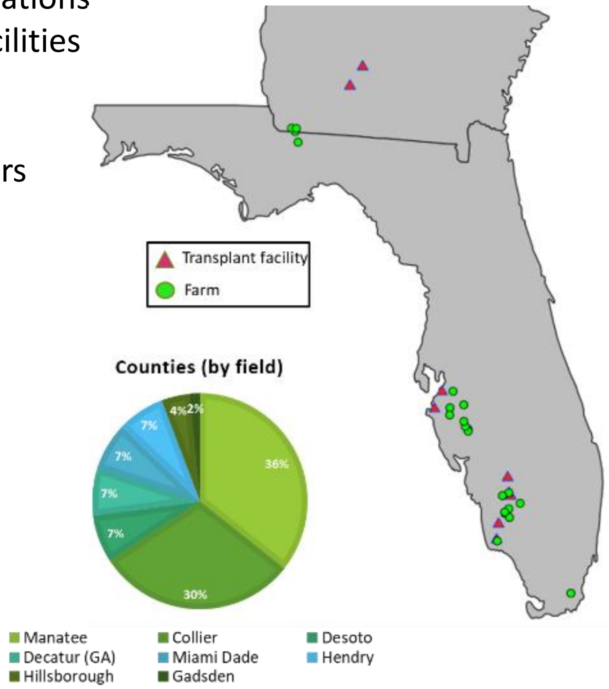

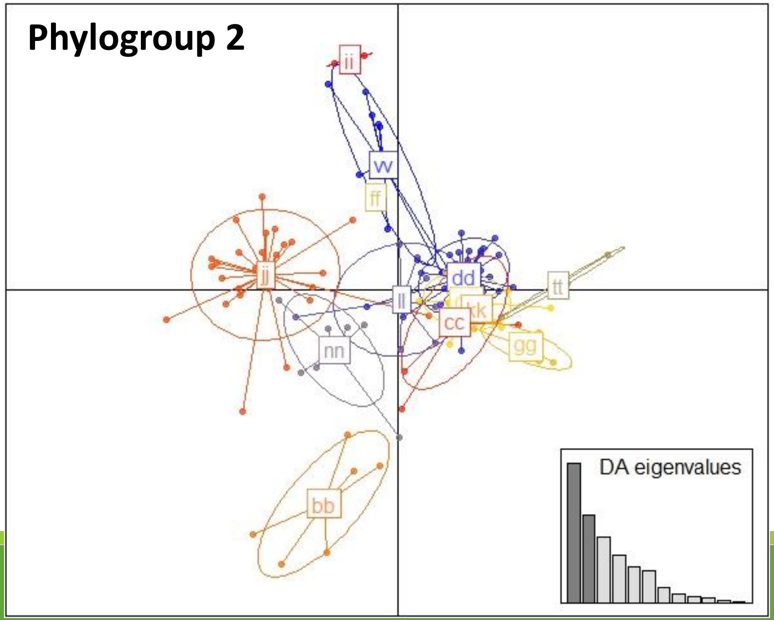

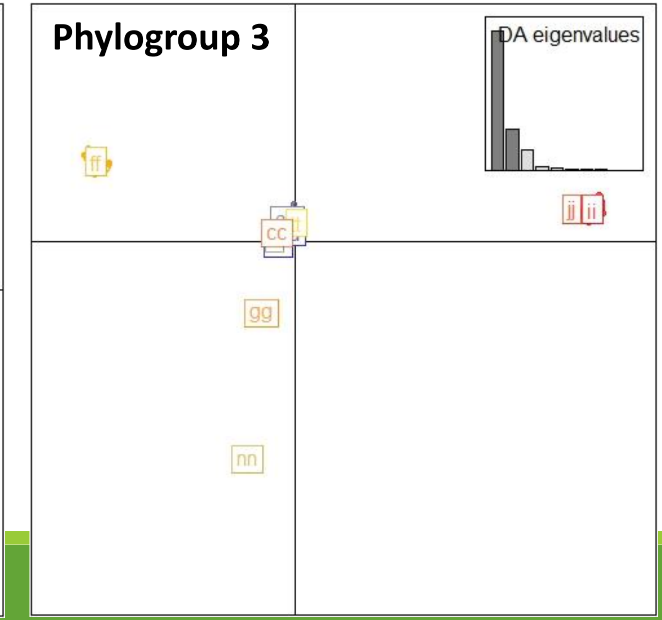

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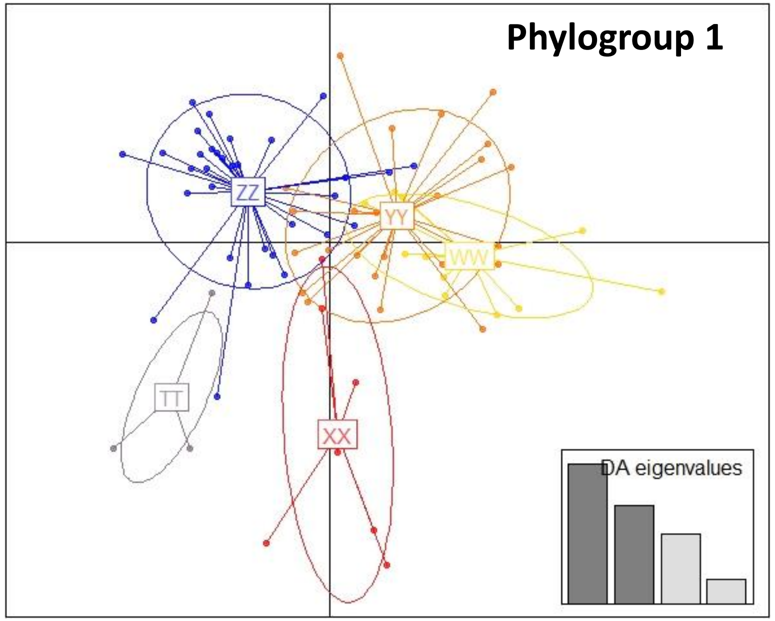
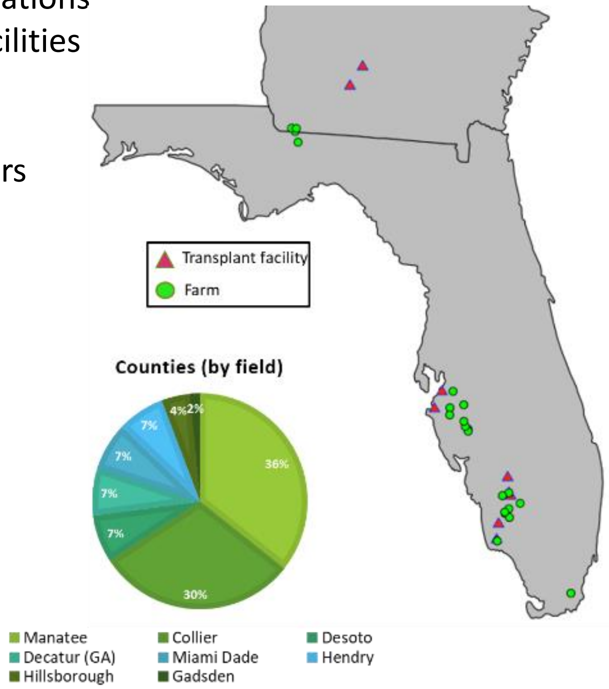


Strains grouped by Transplant Facility



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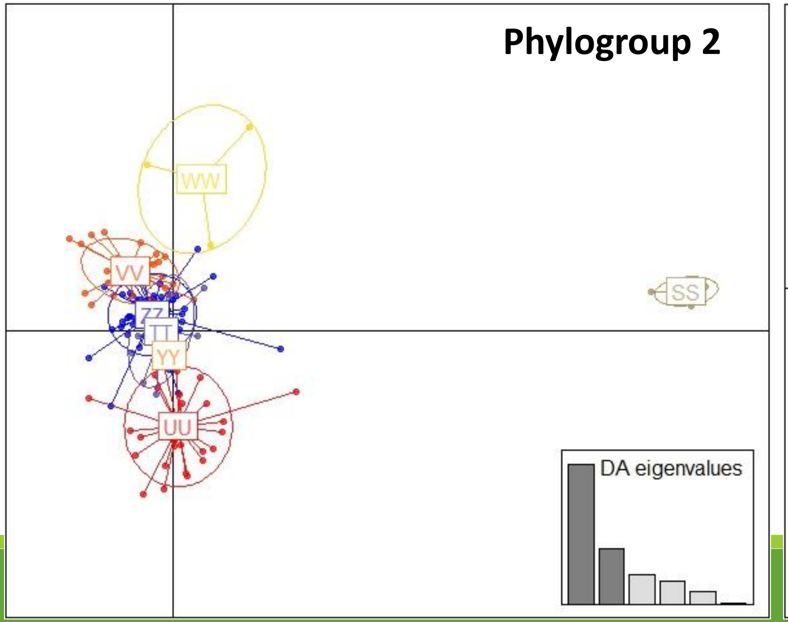
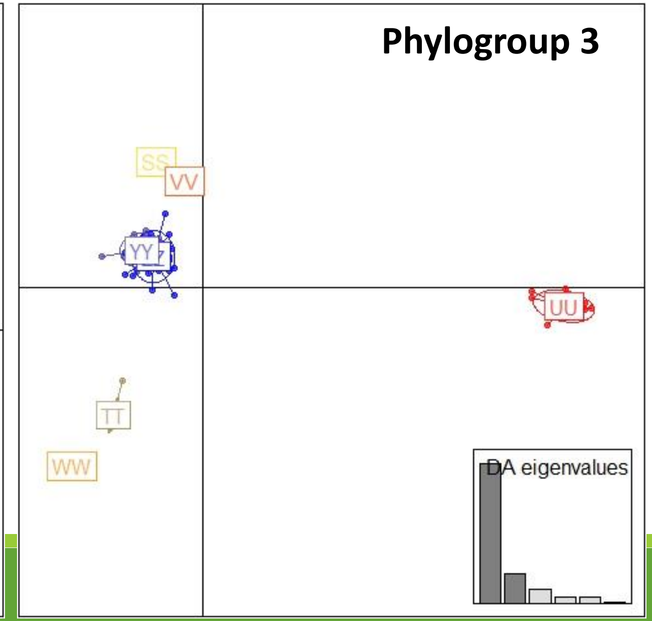
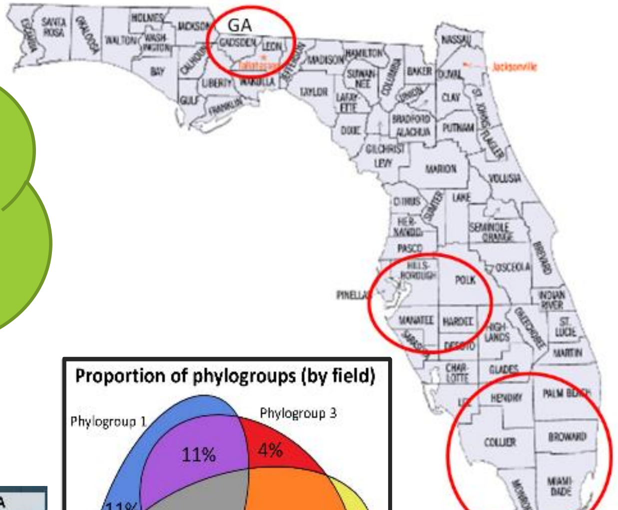
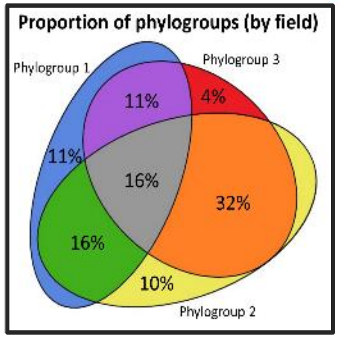
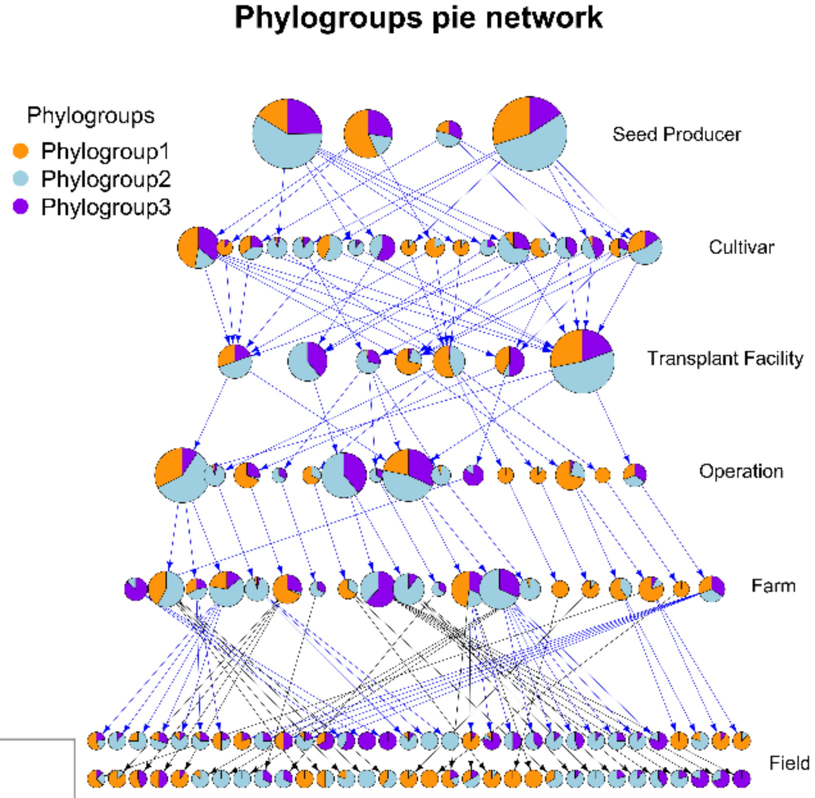


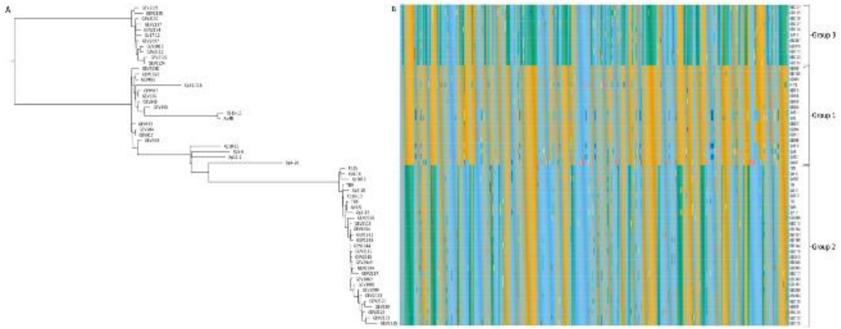


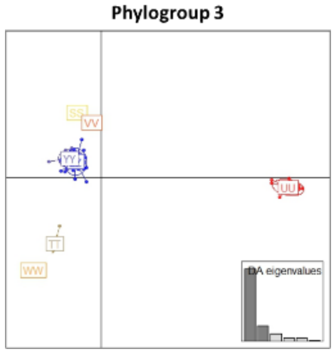
What have we learned about X. perforans in Florida??











It's complicated…

What have we learned about X. perforans in Florida??

SUMMARY

1. BST management in the field is difficult
    - Copper tolerance is the norm
    - Reduced disease ≠ improved yield
    - Mother Nature dictates outcome
2. Transplants play a major role in field epidemics
    1. Production environment favorable for BST
    2. Antibiotic resistance
    3. Better control = bigger impact
3. Role of seed vs. weeds vs. other anthropogenic factors?
    1. X. perforans is genetically diverse - recombination
    2. X. perforans is expanding – fitness vs. human activity
    3. Prior studies based on other BST xanthomonads
4. NexGen sequencing offers a powerful tool to address strain movement & population genetics (breeding)

TRANSGENIC RESISTANCE TO BACTERIAL LEAF SPOT

It truly takes a TEAM!

UF | IFAS
UNIVERSITY of FLORIDA

Co-Investigators

- Dr. Jeff Jones, UF
- Dr. Erica Goss, UF
- Dr. Karen Garrett, UF
- Dr. Sam Hutton, UF
- Dr. Mathews Paret, UF
- Dr. Pam Roberts, UF
- Gerald Minsavage, UF


Dr. Jeff Jones

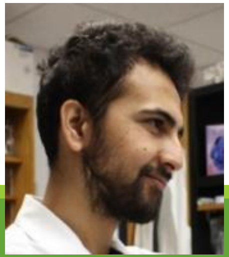

Dr. Erica Goss


Dr. Karen Garrett


Dr. Peter Abrahamian

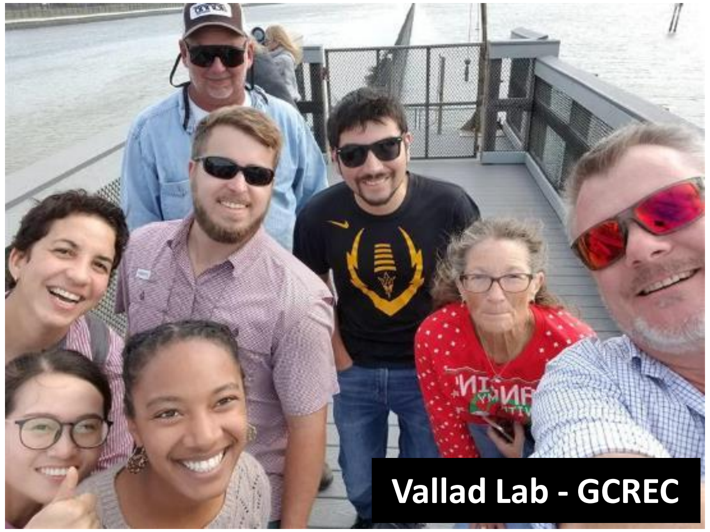

Dr. Sujan Timilsina


Dr. Jeannie Klein-Gordon


Dr. Anuj Sharma


Yanru Xing


Garrett Giles


Vallad Lab - GCREC

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FLORIDA TOMATOES

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