Evaluation of Novel Biological Control Rhizobacteria for Management of Angular Leaf Spot in Dark Tobacco

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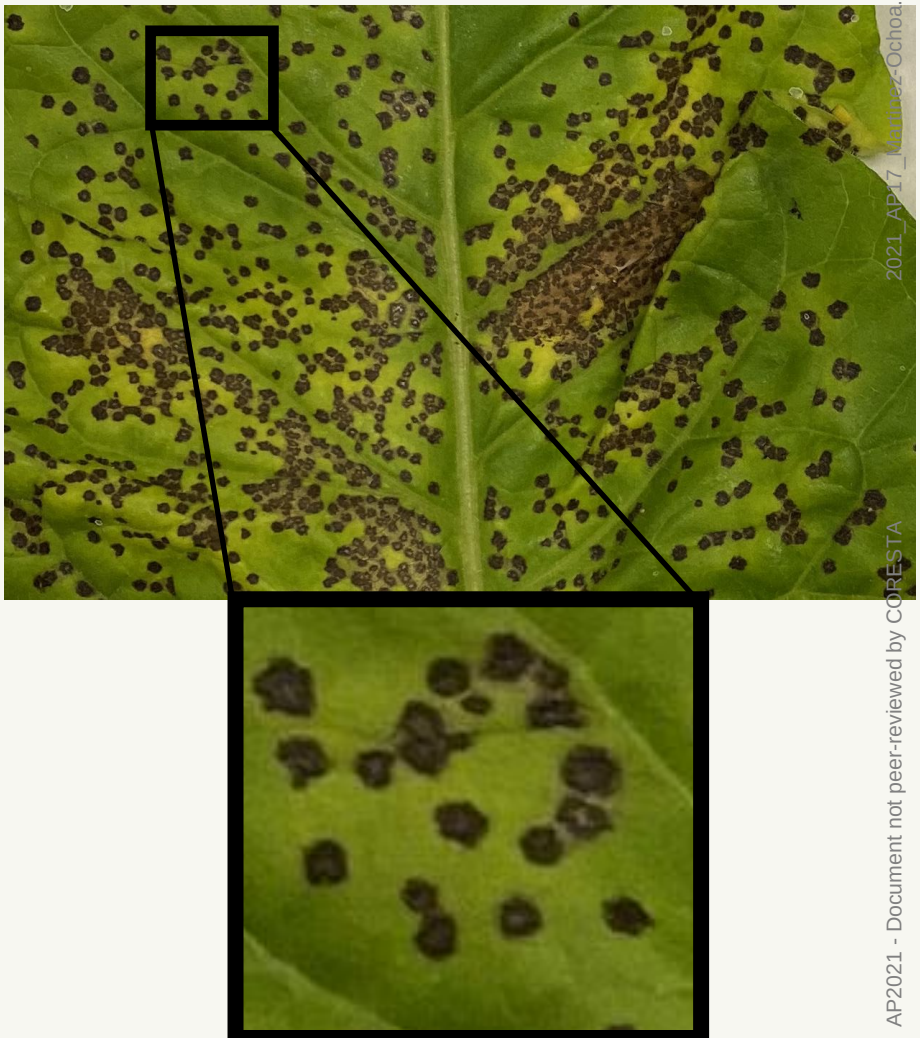
Angular Leaf Spot

- ***Pseudomonas syringae* pv. *tabaci*** (tox-)
- **Foliar** Disease
- Increasing in Kentucky since 2016
- Bacteria are spread in **water droplets**
- Favored by cloudy wet weather
  - We obtained *Pst.* isolate #455 from Dr. Emily Pfeufer
- **Streptomycin** currently used
  - Environmental Antibiotic Resistance

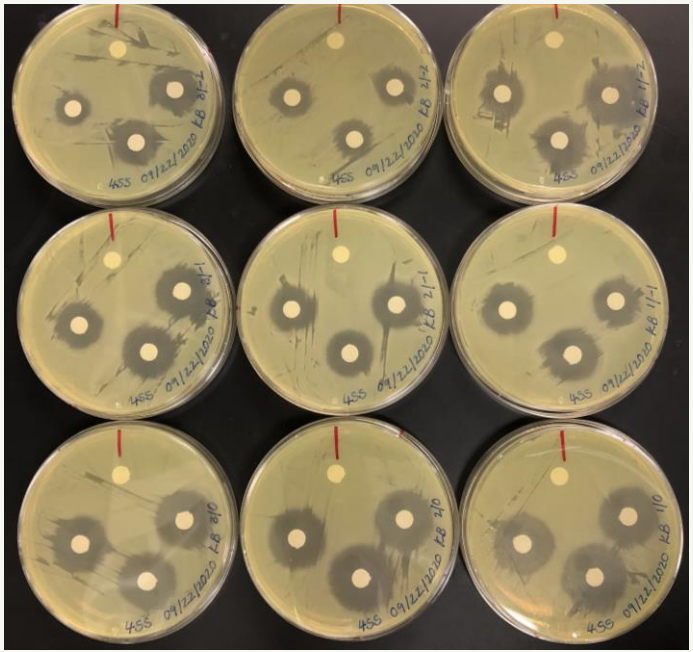


Bacterial Selection

- Chose list of **32 bacterial strains** to be tested against *Pseudomonas syringae* pv. *tabaci*
  - Collection of rhizobacteria from Dr. Joseph W. Kloepper at Auburn University (by Material Transfer Agreement). These have shown to induced systemic resistance in several crops.
  - Most of genus ***Bacillus***-Commonly used for Biological Control
    - Other genera include:
      - ***Serratia***
      - ***Burkholderia***
      - ***Strenotrophomas***
      - *Microbacterium*

Overview of Methods

In-vitro antibiosis



Greenhouse Trials

In-vitro Antibiosis Experimental Design

Method: Disk diffusion on solid media

Media: Trypticase Soy Agar (TSA)

Pathogen: *Pseudomonas syringae* pv. *tabaci* (Pst.)

Treatment on disks: Rhizobacterial strains

Experimental Unit: One TSA plate with 3 disks of rhizobacteria and a water control disk

Replications: 3

Arrangement: Completely randomized

Environment: 28 ℃

In-vitro Antibiosis Results

Table 1

In-vitro antibiosis of AU rhizobacteria vs. *Pseudomonas syringae* pv. *tabaci* on TSA

	Average Inhibition Zone (mm)		
Strains/dilutions	**0**	**-1**	**-2**
Bacillus cereus/proteolyticus (AP-94)	0	0	1.72
Bacillus safensis (AP-110)	1.78	3.08	4.78

Other Strains chosen for greenhouse testing based on history of biocontrol with other bacterial pathogens in tomato:

- *Serratia marcescens* (AP-4)
- *Bacillus altitudinis* (AP-281)

Hypothesis

One or more of the selected rhizobacteria will reduce the incidence of Angular Leaf Spot (ALS) in dark tobacco

Greenhouse Experimental Design

- **Tobacco Variety:** Dark Tobacco KT D8 (most susceptible to ALS)
- **Environment:** Greenhouse at 27 °C and 50 - 70 % relative humidity
- **Treatments:**
  - Healthy Check (Untreated Control)
  - Disease Check (Pathogen Only)
  - Rhizobacterial Strains
- **Experimental Unit:** One Plant
- **Replications:** 5
- **Arrangement:** Randomized Complete Block (RCB)

Rhizobacteria Inoculation Methods

Spray Method

- 2 TSA plates of rhizobacteria diluted in 1 L DI water
- 20µl **Silwet**
- Spray 35 - 50 mL per plant
- Arrange according to RCB labels

Soil Drench Method

- 2 TSA plates of Rhizobacteria diluted in 500 mL DI water
- 100 mL poured per plant

Challenging with *Pseudomonas syringae* pv. *tabaci*

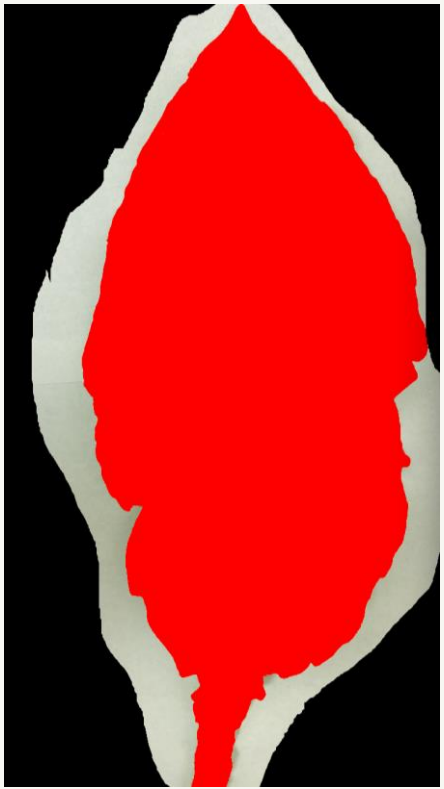
- **Spray inoculation**: challenged with *Pst* after **24 hours**
- **Soil drench inoculation**: challenged with ALS after **7 days**
- Light water mist applied first
- **Solution**: 2 KB plates of pathogen per Liter of DI water + 20µl of Silwet (adjuvant)
- 35 - 50 mL **sprayed** per plant
- Arranged in trays using RCB

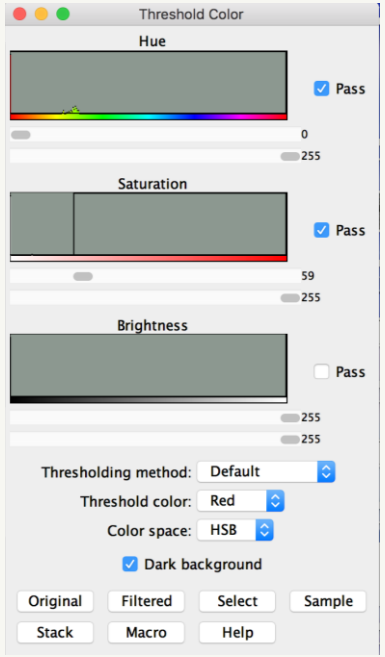
Greenhouse evaluations

- Counting number of lesions after **7 days**
- Determine total disease area in each leaf after **14 days**

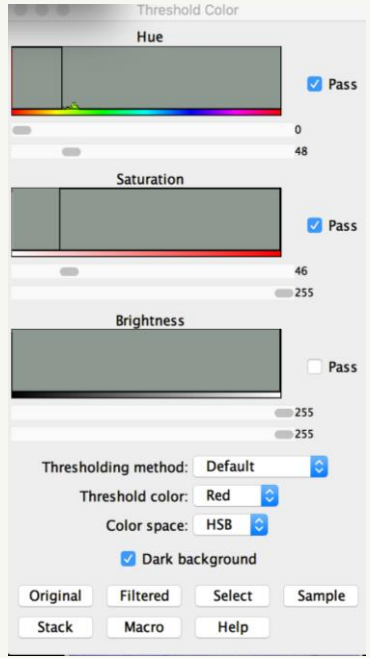
ImageJ

Total Leaf Area





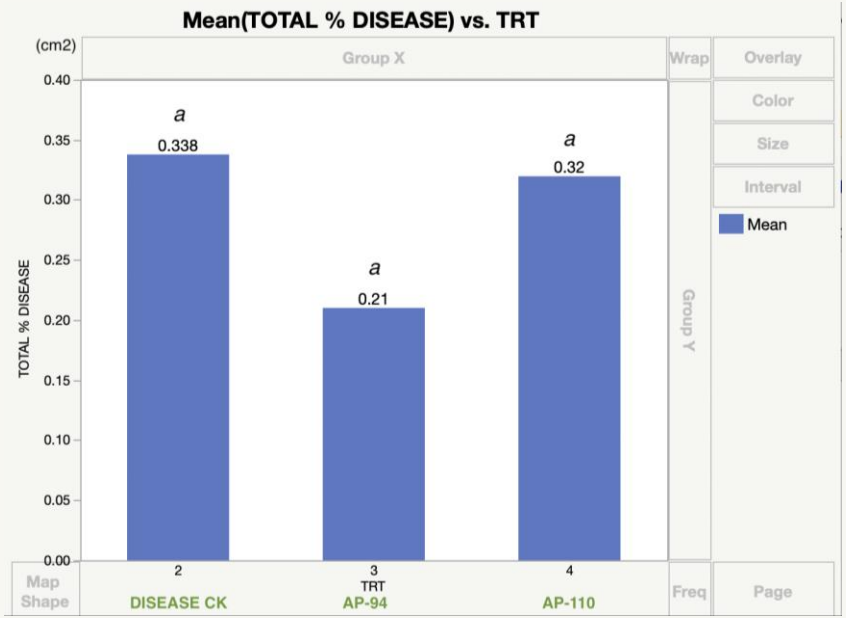
Diseased Leaf Area



Statistical Analysis

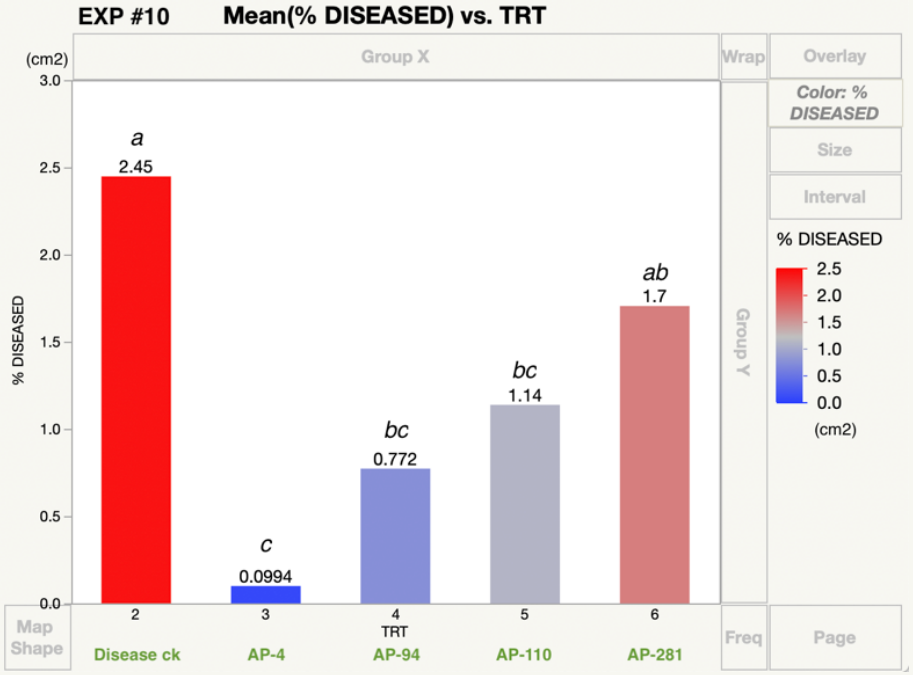
- ANOVA
- Student’s t-test
- Consulted with a statistician

Experiments I-II (Spray)



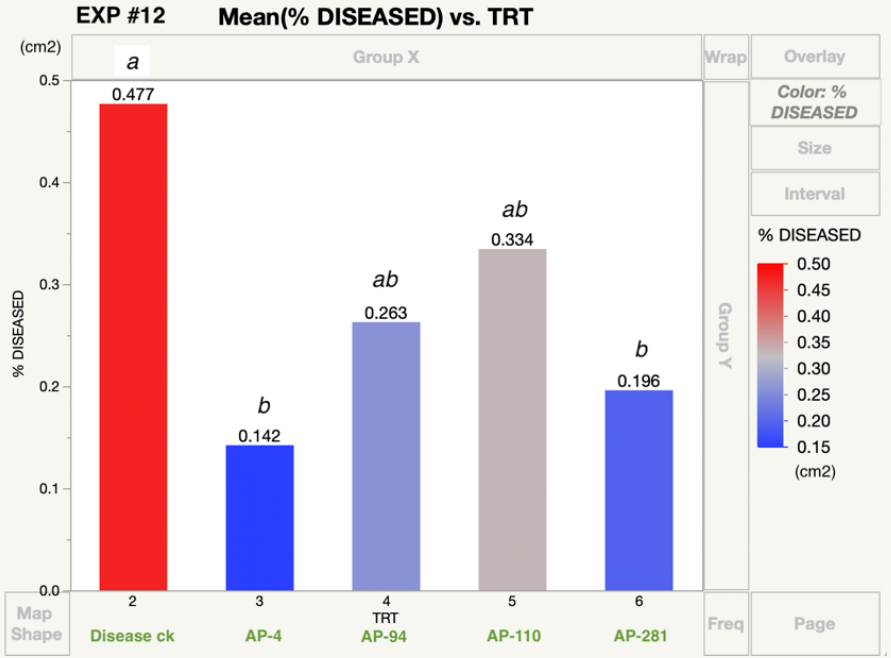
Treatment	Average Total # of lesions	Average total % disease
1: Healthy Check	-	-
2: Disease Check	99	0.34
3: *B. cereus/proteolyticus* (AP-94)	63	0.32
4: *B. safensis* (AP-110)	104	0.21

Experiment I (Soil drench)



Treatment	Average Total # of lesions	Average total % disease
1: Healthy Check	-	-
2: Disease Check	495 a	2.45 a
3: ***S. marcescens*** (AP-4)	**22 b***	**0.10 c***
4: ***B. cereus/proteolyticus*** **(AP-94)**	309 ab	**0.77 bc***
5: ***B. safensis*** **(AP-110)**	347 a	**1.14 bc***
6: *B. altitudinis* (AP-281)	524 a	1.70 ab

Experiment II (Soil drench)



Treatment	Average Total # of lesions	Average total % disease
1: Healthy Check	-	-
2: Disease Check	135 a	0.48 a
3: *S. marcescens* (AP-4)	**49 b***	**0.14 b***
4: *B. cereus/proteolyticus* (AP-94)	99 ab	0.26 ab
5: *B. safensis* (AP-110)	106 ab	0.33 ab
6: *B. altitudinis* (AP-281)	71 ab	**0.20 b***

Conclusions - part I

***Serratia marcescens* (AP-4)**

- The **most promising** of the four rhizobacterial strains tested
- Showed reduction in disease in two experiments with in lesion count **and** % diseased leaf area

***Bacillus cereus/proteolyticus* (AP-94)**

- Inhibited growth of *Pst in-vitro*
- Showed reduction in % diseased leaf area in the first experiment only

***Bacillus safensis* (AP-110)**

- Inhibited growth of *Pst in-vitro*
- Showed reduction in % diseased leaf area in the first experiment only

***Bacillus altitudinis* (AP-281)**

- Showed reduction in % diseased leaf area only in the second experiment only

Conclusions – part II

- Inoculation via **soil drench** worked better than the spray method, confirming the findings in Kloepper's lab, that **induced systemic resistance** could be involved in reducing ALS with rhizobacteria inoculations.

- Further research should be conducted to confirm the results found and improve **consistency of disease incidence** under greenhouse conditions.

- **Future experiments** will include testing mixes of 2 or 3 rhizobacteria and different time intervals biocontrol inoculation-pathogen challenge. The remainder 28 strains will be tested in the greenhouse as well.

- *Serratia marcescens* (AP-4) showed **potential** to be used as a biological control for treating angular leaf spot in dark tobacco, and could be included in **preliminary field trials** in 2022.

Acknowledgements

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Questions?

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