

Environmental Factors and Management Practices Associated with Angular Leaf Spot Incidence in Dark Tobacco

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Background

- *Pseudomonas syringae* pv. *Tabaci* is the casual agent of ALS, a bacterial disease
- Most significant foliar disease in dark tobacco since 2015 in Kentucky and Tennessee
- Streptomycin sulfate has been the standard control
- Documented resistance to streptomycin
 - PDDL 2015-2021 data: 28 out of 113 samples with resistance to streptomycin (~25%)



Objective

Evaluate environmental factors and grower management practices to determine if there were correlations with disease onset and spread of angular leaf spot.



Materials and Methods

- Field observational study was conducted in 2020, 2021, and 2022
- 30 fields observed each year
 - Six counties in Western KY: Calloway, Christian, Graves, Logan, Todd, and Trigg
 - One county in Northwestern TN: Henry



Variables of Interest

ENVIRONMENTAL FACTORS

- Soil temperature
- Air temperature
- Rainfall amount
 - Soil type
 - Soil nutrients
- Tissue nutrients

MANAGEMENT FACTORS

- Dark tobacco variety
 - Nitrogen fertilizer application timing
- Field history of ALS
 - Tillage methods
 - Transplanting date
- Previous crop rotation



Field and Plot Establishment

- Dark tobacco producers were not asked to implement or change anything in their management plan
 - Maximum of two fields per grower (>1.6 km)
- Fields were transplanted from early May to early July
- Fields ranged in size from 2 to 32 ha across the three-year study
- Three individual plots were established in each field (representative as possible)
 - Plot A
 - Plot B
 - Plot C

Plot Management - First Field Visit

Approximately two weeks after transplanting



Soil temperature sensor buried 7.6 cm below soil surface, logged temperature every hour



Air temperature sensor 30.5 cm above soil surface, logged temperature every hour, 15 cm soil sample



Rain gauge placed at topping height, measured rainfall during growing season

Plot Management - Second Field Visit

Approximately one to two weeks before topping

Tissue samples of first fully expanded leaf from five plants per plot

ALS symptomology was observed and noted



Plot Management - Third Field Visit

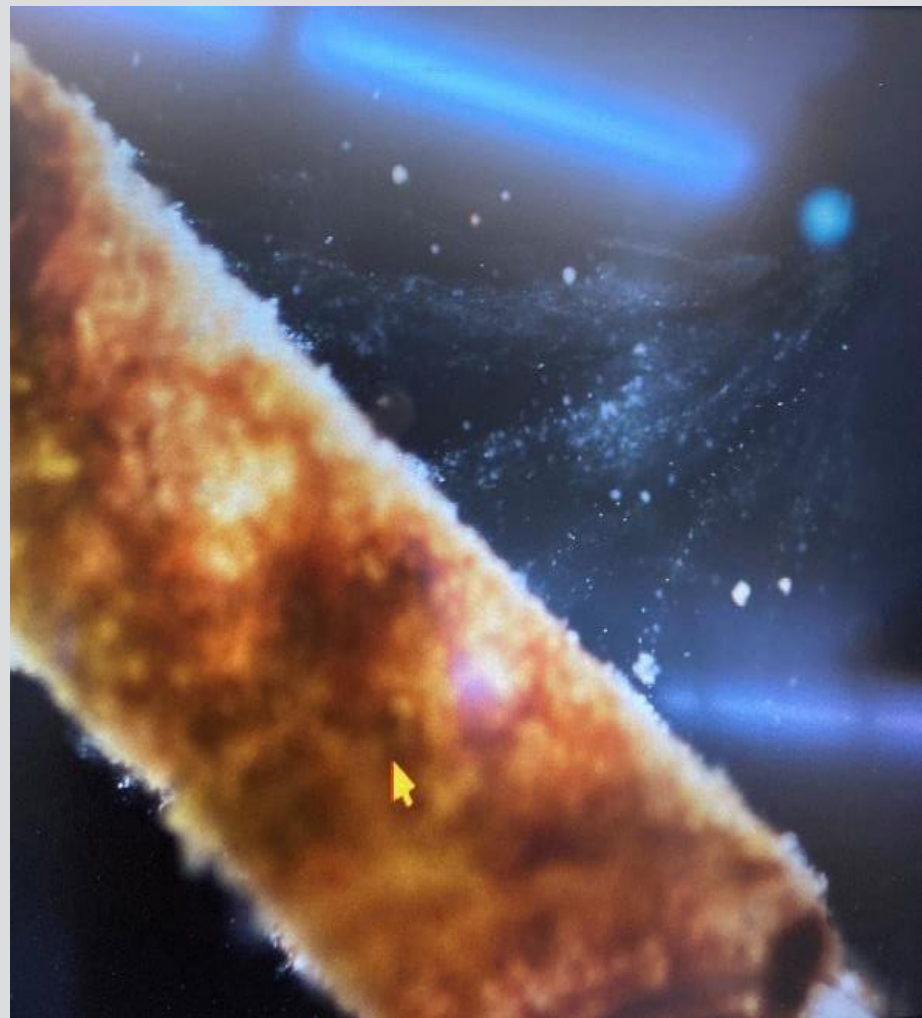
Approximately two weeks before harvest

- Green weights were taken (5 plants/plot)
 - Sensors removed
- Final disease presence noted
 - If ALS was present, tissue sample was taken to confirm bacterial presence



Laboratory Assay to Confirm *Pseudomonas syringae* pv. *tabaci* and to test Streptomycin Sensitivity

- Tissue samples collected were taken to UKREC PDDL in 2021 and 2021, and Lexington PDDL in 2022
- Samples were confirmed to be ALS by confirming bacterial streaming



Laboratory Assay to Confirm *Pseudomonas syringae* pv. *tabaci* and to test Streptomycin Sensitivity

- Isolates were screened for resistance to streptomycin sulfate
- Inhibition and clearing around the disk denotes sensitivity to streptomycin sulfate



Data Analysis

- Statistical analysis software (SAS) version 9.4
- Angular leaf spot presence was used as a binary outcome, as either a 'yes' for fields that had ALS presence and 'no' for fields that did not have ALS presence
- Tissue and soil nutrients were analyzed using a logistic regression with a stepwise procedure to determine which variables to include within the model. Data for all three years were analyzed together using PROC LOGISTICS
- Significance is denoted by an alpha <0.1 , means were separated using least square means



Results

- Overall, 16 of 90 fields observed became infected with ALS
 - 2020: four fields
 - 2021: eight fields
 - 2022: four fields
- Low number of ALS observations limited data analysis
 - Air temperature
 - Tissue and soil nutrients
 - Soil types
 - No differences in other variables



Air Temperature

- With a 1°C increase in average air temperature, there is a 46.9% higher chance of ALS being detected

Point Estimate	<i>p-value</i>
1.469	0.0265

Tissue nutrients that contribute to angular leaf spot probability in 2020, 2021, and 2022

- Average tissue nutrients in fields with ALS and without ALS were compared
- Fields with ALS had higher average levels of P, B, and Cu in tissue than fields that did not have ALS

Nutrient	Unit	Estimate	ALS Possibility	p-value
			%	
Phosphorus	0.1 %	1.691	+69	0.0094
Boron	5 ppm	1.211	+21	0.0389
Copper	50 ppm	1.119	+12	0.0805

Soil nutrients that contribute to angular leaf spot probability in 2020, 2021, and 2022

- Average soil nutrients in fields with ALS and without ALS were compared
 - Fields that had ALS had higher average Mn levels in soil compared to fields without ALS
 - Fields that had ALS had lower average S and Cu levels in soil compared to fields without ALS

Nutrient	Year	Unit	Estimate	ALS Possibility	p-value
		kg ha ⁻¹		%	
Sulfur	2020-2022	11.2	0.783	-22	0.0063
Manganese		112	1.665	+66	0.0006
Copper	2020 and 2022	1.12	0.530	-47	0.0646

Soil types included in analysis to determine correlation to ALS presence in fields

- 18 soil types included in analysis
- Six showed correlation to ALS presence

Soil Type	<i>p</i> -value
Purchase Region of Kentucky	
Calloway-Kurk complex	0.0315
Providence silt loam	0.0586
Pennyrile Region of Kentucky	
Nolin silt loam	0.0772
Pembroke silt loam	0.0069
Sadler silt loam	0.0691
Zanesville silt loam	0.0975



Summary

- Low number of ALS fields (16 of 90 fields)
- Increase in air temperature by one degree unit, can lead to possible increase of ALS by 47%
- With an increase in P, B, and Cu in plant tissue there is increased probability of ALS presence
- With an increase in Mn in soil, there is the possibility to increase ALS presence
- With an increase in S and Cu there is the possibility to reduce ALS presence



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Questions

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