

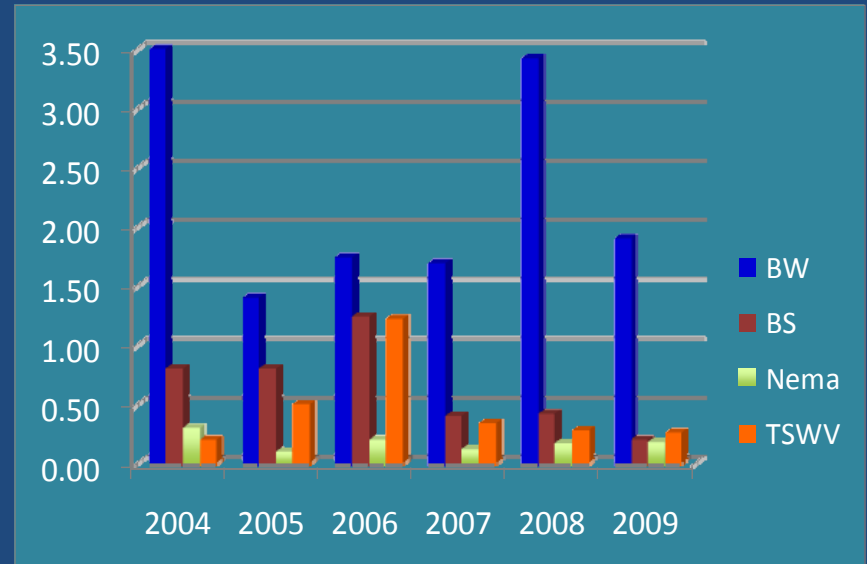


Genetic Diversity in *Ralstonia solanacearum* and Implications for Mechanical Transmission in Tobacco

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Bacterial Wilt in SC

- Bacterial wilt is our major tobacco disease problem in South Carolina.
- Bacterial wilt is also a regional issue – occurs from Virginia to Florida on tobacco & tomato.
- Tobacco losses are focused primarily in North & South Carolina because temperature limits geographical range north & south of these states.



Mechanization & the Spread/Severity of Bacterial Wilt in SC

- A major factor in the increase in bacterial wilt in SC is the shift to mechanization in flower and leaf removal.
- Expanded production acreage has resulted in a rapid shift to mechanization.
- Mechanical topping & harvesting can spread the pathogen rapidly from local centers of infection through entire fields.
 - Contamination of a topper blade can infest the next 50-100 plants.
 - Mechanical harvesting increases disease (2% to 75%) with a single pass of a mechanical harvester.
- **Result: Massive Epidemics & late season collapse of large fields in recent years.**

Root vs Stem Infection



R. solanacearum



Natural infection -
direct penetration of roots
(via wounds or root hairs)

Mechanical Infection –
topping wounds, leaf scars and
stem abrasions caused by topper blades, leaf
defoliators, and harvester guides




1×10^8 cells/ml



1×10^6 cells/ml

Background Studies

- Previous studies by Robertson *et al* (2001) classified *R. solanacearum* isolates into different aggressiveness groups based on root inoculations in controlled environment chambers.
 - Differences among genetically diverse *R. solanacearum* isolates from tobacco and tomato (NC, SC, FL, GA) were recorded.
 - Isolates groupings from Robertson *et al* were:
 1. Highly aggressive (from tobacco)
 2. Aggressive (from tobacco)
 3. Moderately aggressive (from tomato)
 4. Weakly aggressive (from tomato)

Avr gene mutation
 - 5. Non-pathogenic on tobacco (from tomato) = Functional *Avr* gene
- Aggressiveness groups relate to the presence/absence of an *Avr* gene mutation.

Objectives

- To evaluate the aggressiveness of genetically diverse *R. solanacearum* isolates when applied to foliar plant parts during flower removal (topping)
- To determine if an *Avr*-induced resistance response similar to what occurs in root tissue also occurs in stem tissue

Materials and Methods

- Experiment was conducted at Clemson's Research and Education Center in Florence, SC, summer 2009.
- Plants of K346 were grown under standard agronomic practices for South Carolina.
- 23 isolates of *R. solanacearum* were selected for differences in genetic diversity and aggressiveness including groupings from Robertson *et al* (2001).
- Plots consisted of a single row of 10 plants, 6m long with a 1.2m row spacing.
- Each row was fumigated with 1,3 D + chloropicrin (10.5 gal/A) 21 days prior to transplanting (Telone C-17).
- Experimental design was a randomized complete block with four replications.

Inoculation

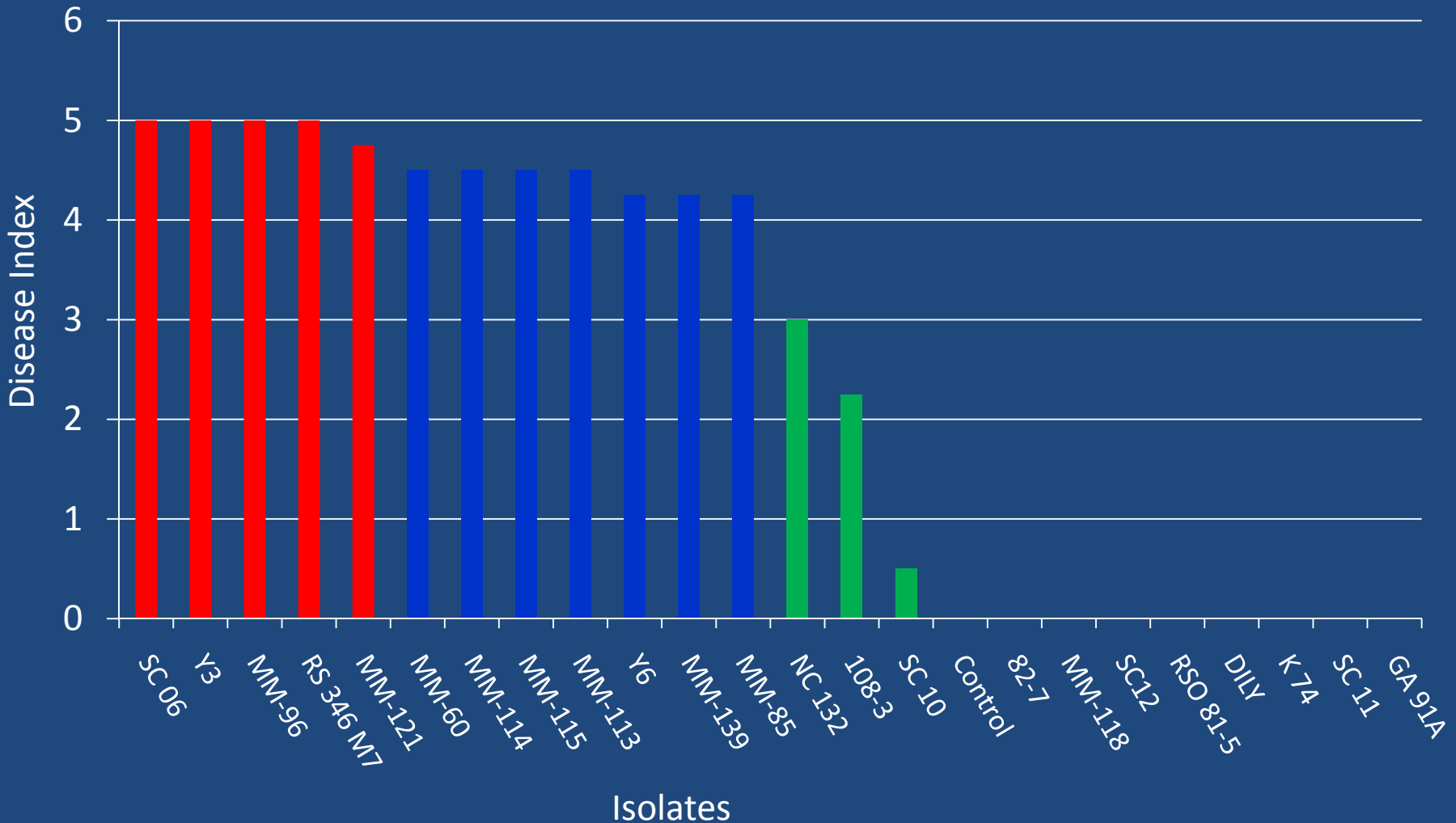
- Isolates of *R. solanacearum* were grown on tetrazolium amended nutrient agar (72 hours), re-suspended in deionized water at Optical Density₆₀₀ = 0.2 = 10⁸ cells/ml and used as a stock culture.
- The 10⁸ suspension was used to make inoculum for a 10⁶ cells/ml dilution.
- Inoculation was performed to simulate mechanical flower removal.
 - A steel cutter blade was misted individually with each isolate suspension and used to top 10 plants (1 plot) 15cm below the inflorescence.
- The control was a water inoculated treatment.

Assessment

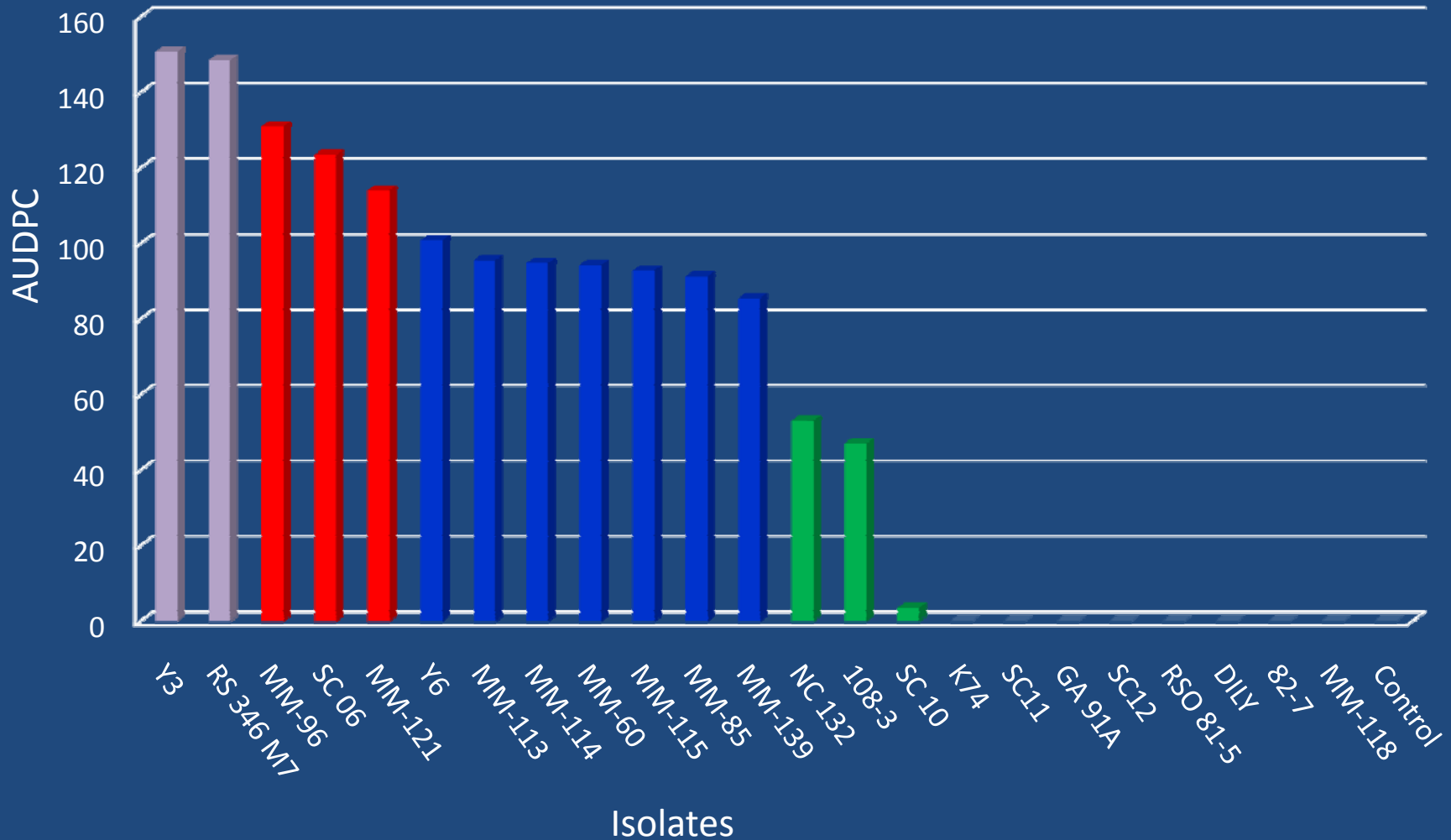
- *R. solanacearum* was positively confirmed using immunological testing strips (Agdia Pathoscreen Kit).
- Plants were assessed weekly for disease severity starting 21 days following inoculation and rated on a 0 to 5 scale (0 = no visible symptoms, 5 = complete collapse of tissue).
- Stem necrosis was recorded on a 0 to 5 scale at final disease assessment date.
- Disease severity data were subjected to ANOVA using JMP software (SAS); AUDPC values were calculated for each treatment.

Results: Final Disease Ratings

(60 Days Post-Inoculation)



Results: AUDPC Values



AUDPC Values – Comparison of Treatment Means

Host	Isolates	Means separation level	Mean AUDPC
Tobacco	Y3	A	150.75
Tobacco	RS 346 M7	A	148.50
Tobacco	MM-96	AB	131.00
Tobacco	SC 06	ABC	123.50
Tobacco	MM-121	ABC	114.00
Tobacco	Y6	BC	100.75
Tobacco	MM-113	BC	95.50
Tobacco	MM-114	BCD	94.75
Tobacco	MM-60	BCD	94.25
Tobacco	MM-115	BCD	92.75
Tobacco	MM-85	BCD	91.25
Tobacco	MM-139	CDE	85.50
Tobacco	NC 132	DE	53.00
Tobacco	108-3	E	47.00
Tobacco	SC 10	F	3.50
Tomato	K74	F	0.00
Tomato	SC11	F	0.00
Tobacco	GA 91A	F	0.00
Tomato	SC12	F	0.00
Tomato	RSO 81-5	F	0.00
Tomato	DILY	F	0.00
Tomato	82-7	F	0.00
Tobacco	MM-118	F	0.00
	Control	F	0.00

Highly Aggressive

Aggressive

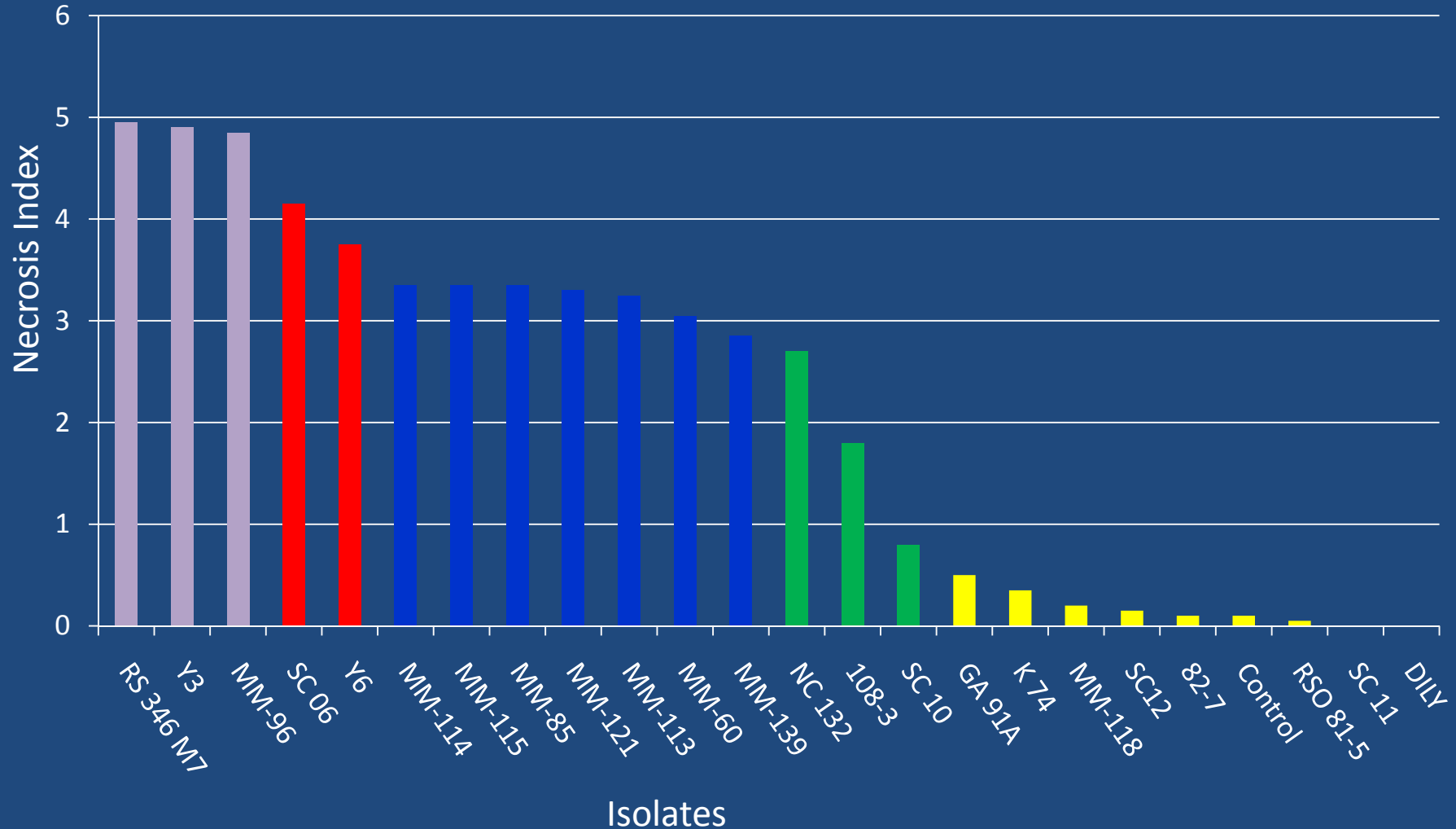
Moderately Aggressive

Weakly Aggressive

Non-pathogenic

Results: Stem Necrosis

(60 Days Post-Inoculation)



Results

- There were significant differences in the amount of disease caused by the selected isolates of *R. solanacearum* when inoculated to foliar plant parts in the field.
- The resistance mechanism that functions against tomato strains in root infections also appears to function in tobacco stem tissue.
- Many of the isolates showed no measurable leaf/plant symptoms (tomato strains).
- Rankings of disease intensity measurements (leaf tissue) and stem necrosis were highly correlated (Correlation=0.987909).
- Rankings of isolates based on the level of disease in inoculated foliar plant parts were highly correlated with rankings shown by Robertson *et al* (2001) in studies using inoculum applied to the soil.