The Effect of Growth Regulators on the Mechanical Transmission of Bacterial Wilt

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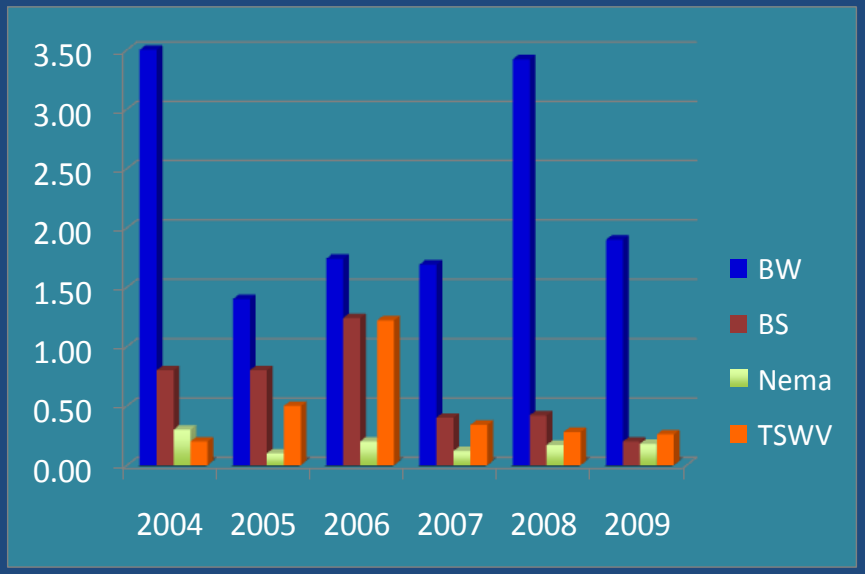
Bacterial Wilt in South Carolina

- Bacterial wilt, caused by *Ralstonia solanacearum,* is the major tobacco disease problem in South Carolina.
- Bacterial wilt is also a regional issue – occurs from Virginia to Florida.
- Losses are focused in North & South Carolina because temperature limits geographical range north & south of these states.



Mechanization & the Spread/Severity of Bacterial Wilt in SC

Background

- Mechanical transmission of *R. solanacearum* during flower and leaf removal also coincides with the time when maleic hydrazide (MH) is applied to arrest axillary shoot growth (suckers).
- Previous observations suggested MH application may suppress mechanical transmission of bacterial wilt during mechanical topping and leaf removal.
- Growth regulators have been shown to effect disease development.

Previous studies with MH

- Evaluated the effect of MH application on *R. solanacearum* establishment and disease development following mechanical transmission of the bacterium using:
  - Growth Chambers
  - Greenhouse

Previous results with MH

- MH suppresses the severity of bacterial wilt.
- Disease suppression is strongly linked to MH application timing.

Previous Results with MH

Inoc. with *R. solanacearum* (inoculum)

Early MH + inoculum

MH + inoculum

Late MH + inoculum

Current Objective

To compare the effect of MH against other growth regulators on *R. solanacearum* establishment and disease development following mechanical transmission of the bacterium.

Materials and Methods

- Experiments were conducted at Clemson's Research and Education Center in Florence, SC.
- Seedlings of K346 were grown under standard agronomic practices for South Carolina (traditional float bays).
- Plants were transferred to 15 cm diameter pots and grown in a greenhouse on a 12-hour photoperiod – artificial light supplement until they reached 30 cm tall.
- Plants were transferred to controlled environment chambers at 30°C, 68% RH on a 12-hour photoperiod.
- Experimental design was a randomized complete block with three replications repeated in 3 different runs.

Inoculation

- An isolate of *R. solanacearum* was grown and suspended in deionized water at Optical Density$_{600}$= 0.2 = 10^8 cells/ml and used as a stock culture.
- The 10^8 suspension was diluted to 2x10^6 cells/ml for inoculation.
- Growth regulator treatments were applied 4 days prior to inoculation with *R. solanacearum*
- *R. solanacearum* inoculation simulated mechanical flower removal.
- Mechanical topping was simulated by removing the apical bud with a scalpel and 100 µl of inoculum pipetted on to the cut stem.

Treatments

Treatment	Application rates
Flumetralin (Prime +) + inoculation	5 ml/500 ml or 2 qt/A
Naphthalene acetic acid (NAA; Sucker Stopper) + inoculation	5 ml/500
Indole-3-butyric acid (IBA) + inoculation	100 ppm
Indole-3-acetic acid (IAA) + inoculation	100 ppm
Maleic Hydrazide (Royal MH 30) + inoculation	15 ml/500 ml or 1.5 gal/A
Inoculated untreated	
Non inoculated, untreated	

****Each treatment was paired with a treated/non-inoculated control.**

Disease Assessment

- *R. solanacearum* was positively confirmed using immunological testing strips (Agdia Pathoscreen Kit).
- Plants were assessed for disease severity every 3 to 5 days starting 7 days post-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.
- All data were subjected to ANOVA using JMP software (SAS).

Growth Regulator Trial Results (Run 1)

Treatment	Final Stem Necrosis	
	Level	Least Sq Mean
Prime + + inoculation	A	4.33
NAA + inoculation	A	4.00
Inoculated, untreated	A	4.00
IBA + inoculation	AB	3.33
IAA + inoculated	AB	2.67
MH + inoculation	BC	1.33
Non-inoculated, untreated	C	0.00

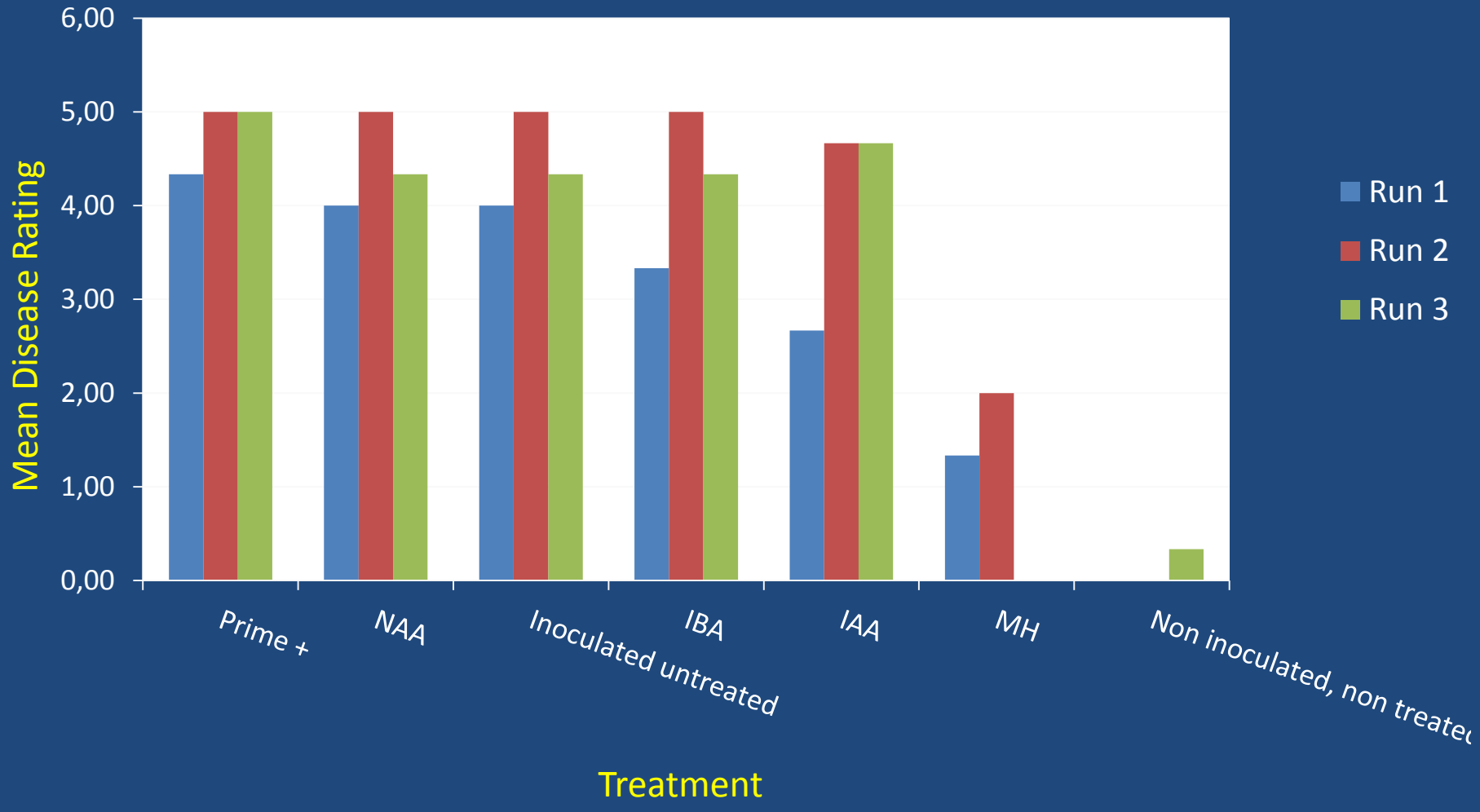
Growth Regulator Trial Results (Run 2)

Treatment	Final Stem Necrosis	
	Level	Least Sq Mean
Prime + + inoculation	A	5.00
Inoculated, untreated	A	5.00
IBA + inoculation	A	5.00
IAA + inoculated	A	5.00
NAA + inoculation	A	4.67
MH + inoculation	B	2.00
Non-inoculated, untreated	C	0.00

Growth Regulator Trial Results (Run 3)

Treatment	Final Stem Necrosis	
	Level	Least Sq Mean
Prime + + inoculation	A	5.00
Inoculated, untreated	A	4.33
IBA + inoculation	A	4.33
NAA + inoculation	A	4.33
IAA + inoculated	A	4.67
MH + inoculation	B	0.33
Non-inoculated, untreated	B	0.00

Growth Regulator Trial Results Combined – Final Stem Necrosis



Summary

- Maleic Hydrazide (MH) significantly suppressed the severity of bacterial wilt.
- Disease severity in the Flumetralin (Prime +) treatment was not significantly different from the inoculated untreated control.
- Application of IAA and IBA showed some reduction in disease severity but levels were not significantly different from untreated control.