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Reduction of Bacterial Leaf Speck Disease (*Pseudomonas syringae* pv *tomato*) in Tomatoes Treated with a Combination of *Azospirillum brasilense*, Bactericides, and Mild Heat

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Introduction

Current methods to control bacterial speck disease of tomato caused by *Pseudomonas syringae* pv *tomato* (*P. s.* pv *tomato*) are inefficient (1) because the pathogen is relatively resistant to the copper compounds (4,9) commonly used in disease prevention programs (13). Although this disease is usually minor, outbreaks can cause severe damage to tomato plants and reduce crop yields when the plants are grown under plastic cover or in greenhouses (12). Alternatives to chemical control include keeping the foliage as dry as possible and insertion of pathogen resistance genes (3,6,10,11,12), either by traditional cross-breeding or by genetic engineering (5,8).

Biological control of *P. syringae* pv *tomato* has been largely unexplored. Although *A. brasilense*, a well-known plant growth-promoting bacteria (PGPB), is not known as a biocontrol-PGPB, it is known to produce antimicrobial agents such as bacteriocins and siderophores. In addition, *A. brasilense* can restrict the proliferation of other rhizosphere bacteria including nonpathogenic bacteria (7), and pathogens such as *P. syringae* pv *tomato* (2), probably by out-competing with them. The aim of this study was to determine if several relatively ineffective pathogen control agents could act synergistically when used in combination to improve control of bacterial leaf speck disease of tomato.

Material and Methods

Organisms, growth conditions, inoculation techniques, and evaluation of disease development and severity are as described elsewhere in this volume (2).

Bacteriocide, antibiotic, and heat treatment of seeds. Commercial copper bacteriocide (a mixture of 0.5 % copper hydroxide and 0.3% copper oxychloride) and commercial streptomycin sulfate (0.02 %) were applied as an aerosol using a commercial garden sprayer. Seeds were inoculated with bacteria after the pesticide spray had dried. For heat treatments, infected seeds with *P. s.* pv *tomato* were incubated in a circulating water bath (42-45 ^{0}C for 2 h). Immediately after the excess water had drained, heat-treated seeds were inoculated with bacteria.

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Results and Discussion

The goal of this study was to reduce the chemical pesticide load on tomato plants infected with *P. s.* pv *tomato* by partially replacing copper bacteriocides with *Azospirillum brasilense* inoculation and with mild heat treatment of seeds. Inoculation of tomato seeds with either *A. brasilense* alone, or spraying tomato foliage with *A. brasilense*, streptomycin sulfate, or a commercial copper bactericide before or after inoculation with *P. syringae* pv *tomato* had no significant lasting effect on disease severity or on plant height and dry weight (Table 1).

Table 1. Effect of various disease control agents on bacterial speck disease development, and height and dry weight of tomato seedlings.

Treatment	Disease development after 6 days (0-3)	Disease development after 15 days (0-3)	Plant height after 15 days (mm)	Plant dry weight after 15 days (mg)
Before inoculation with *P. s.* pv *tomato*				
Seed inoculation with *A. brasilense*	1.85a A	2.88a B	67 b	85b
Foliar inoculation with *A. brasilense*[1]	1.67a A	2.57a B	57 ab	72ab
Streptomycin sulfate	0.12b A	2.61a B	49 a	61a
Copper bactericide	1.77a A	2.81a B	48 a	64a
Untreated plants	1.91a A	2.95a B	53 a	66a
After Inoculation with *P. s.* pv *tomato*				
Seed inoculation with *A. brasilense*[2]	2.25a A	2. 92a B	59 a	69b
Foliar inoculation with *A. brasilense*	2.35a A	2.84a B	50 a	61a
Streptomycin sulfate	0.21b A	2.74a B	58 a	58a
Copper bactericide	2.43a A	2.88a B	49 a	57a
Untreated plants	2.55a A	2.93a B	55 a	64a

P. s. pv *tomato* - *Pseudomonas syringae* pv. *tomato*

[1] *P. s.* pv *tomato* was applied 30 min after application of *A. brasilense*

[2] *A. brasilense* was applied immediately after *P. s.* pv *tomato* application

[3] Numbers in each column, and in each section, denoted by a different lower case letter, differ significantly at $P \leq 0.05$ in ANOVA, and numbers in each row (Disease severity) denoted by a different capital letter differ significantly at $P \leq 0.05$ in Student's *t*-test.

Seed inoculation with *A. brasilense* combined with a single antibiotic treatment and two bactericide applications at 5-day intervals (a third or less of the recommended commercial dose) reduced disease severity in tomato seedlings by over 90% after 4 weeks (Table 2), and significantly slowed disease development under mist chamber conditions (data not shown). Later, after these plants were transferred from the mist chamber to dry conditions, there was no further development of the disease and damage to plant foliage was minimal compared to inoculation with the pathogen alone (Table 2).

Table 2. Bacterial speck disease development and dry weight of tomato seedlings after seed inoculation with *A. brasilense* combined with a single antibiotic treatment and two bactericide applications at 5-day intervals.

Treatment	Disease severity (0-3, after 30 days in mist)	Disease severity (0-3, after additional 30 days in dry)	Plant dry weight (g) after 60 days
*P.s.*pv *tomato* + Combined control agents	0.26a	0.11a	14.9b
P. s. pv *tomato* (no control agents)	2.78b	1. 47b	9.8 a
Uninoculated (no control agents)	None	None	16.5b
Uninoculated + Combined Control Agents	None	None	15.6b

P. s. pv *tomato* - *Pseudomonas syringae* pv *tomato*
Numbers in each column denoted by a different lower case letter differ significantly at $P \leq 0.05$ in ANOVA or Student's *t*-test.

Treatment of tomato seeds infected with P. *syringae* pv *tomato* with a combination of mild heat (42-45 ^{0}C), *A. brasilense* inoculation, and later a single application of a copper bactericide, almost eliminated bacterial leaf speck disease even when the plants were grown in a mist chamber for 6 weeks (Table 3). Plants treated in this manner grew similarly to uninfected plants (Table 3).

This study demonstrates that a combination of several ineffective disease control treatments may reduce the development and severity of bacterial leaf speck disease in tomato.

Table 3. Bacterial speck disease development and dry weight of tomato seedlings after seed treatment with mild heat (42-45 °C), *A. brasilense* and a single bactericide application.

Treatment	Disease severity (0-3, after 42 days in mist)	Plant dry weight (g) after 60 days
Combined control agents	0.35a	17.2a
Inoculated *P. s.* pv *tomato*	2.84b	10.8b
Noninoculated	None	17.7a
Noninoculated and treated	None	18.1a

P. s. pv *tomato* - *Pseudomonas syringae* pv. *tomato*
Numbers in each column denoted by a different lower case letter differ significantly at $P \leq 0.05$ in ANOVA or Student's *t*-test.

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