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Reduction of bacterial speck (*Pseudomonas syringae* pv. *tomato*) of tomato by combined treatments of plant growth-promoting bacterium, *Azospirillum brasilense*, streptomycin sulfate, and chemo-thermal seed treatment

Yoav Bashan[1] and Luz E. de-Bashan[1,2]
[1]*Environmental Microbiology, The Center for Biological Research of the Northwest (CIB), POB 128, La Paz, BCS 23000, Mexico (Fax: +52 (612) 1254710; E-mail: bashan@cibnor.mx);*
[2]*Department of Biology, Pontificia Universidad Javeriana, Santafe de Bogota, Colombia*

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Abstract

Inoculation of tomato seeds with the plant growth-promoting bacterium *Azospirillum brasilense*, or spraying tomato foliage with *A. brasilense*, streptomycin sulfate, or commercial copper bactericides, separately, before or after inoculation with *Pseudomonas syringae* pv. *tomato*, the casual agent of bacterial speck of tomato, had no lasting effect on disease severity or on plant height and dry weight. Seed inoculation with *A. brasilense* combined with a single streptomycin foliar treatment and two foliar 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, and significantly slowed disease development under mist conditions. *A. brasilense* did not induce significant systemic resistance against the pathogen although the level of salicylic acid increased in inoculated plants. Treatment of tomato seeds that were artificially inoculated with *P. syringae* pv. *tomato,* with a combination of mild chemo-thermal treatment, *A. brasilense* seed inoculation, and later, a single foliar application of a copper bactericide, nearly eliminated bacterial leaf speck even when the plants were grown under mist for 6 weeks. This study shows that a combination of otherwise ineffective disease management tactics, when applied in concert, can reduce bacterial speck intensity in tomatoes under mist conditions.

Introduction

Although many studies have demonstrated the efficacy of copper compounds and streptomycin sprays against bacterial speck of tomato caused by *Pseudomonas syringae* pv. *tomato* (Conlin and McCarter, 1983; Cooksey, 1988; Jardine and Stephens, 1987), contemporary control methods are inefficient (Bashan, 1997). This is mainly because the pathogen has acquired resistance to copper compounds (Cooksey, 1990; Cooksey and Azad, 1992; Pernezny et al., 1995), which were the most common antibacterial agents used in disease prevention programs (Yunis et al., 1980b). Antibiotics are usually effective, however, large-scale application of antibiotics in tomato cultivation is restricted. Although bacterial speck of tomato is not considered to have a major economic impact (Bashan et al., 1978; Colin and Chafic, 1986), outbreaks can inflict severe damage to tomato plants and can reduce crop yields and fruit quality when the plants are grown under plastic covers or in greenhouses (Yunis et al., 1980a). Alternatives to chemical control include keeping the foliage as dry as possible by increasing ventilation in greenhouses and insertion of pathogen-resistance genes into tomato cultivars (Bashan et al., 1981; Gu and Martin, 1998; Sotirova et al., 1994;

Stockinger and Walling, 1994; Yunis et al., 1980a), either by traditional cross-breeding or by genetic engineering (Fallik et al., 1983; Oldroyd and Staskawicz, 1998).

Biological control of *P. syringae* pv. *tomato* has been largely unexplored. Control of other bacterial and viral leaf pathogens, such as cucumber mosaic virus, tobacco necrosis virus and bacterial leaf pathogens of cucumbers, mulberry, soybean and rice by biocontrol agents has been proposed (Alström, 1991; Liu et al., 1995; Maurhofer et al., 1994; Vidhyasekaram et al., 2001; Völksch and May, 2001; Wei et al., 1996; Zehnder et al., 2000). Biocontrol agents, mainly bacterial inoculants, among other mechanisms of biological control (antibiosis, siderophores, competition, hydrolytic enzymes), are believed to induce systemic resistance in the plants. Although *Azospirillum brasilense,* a well-known plant growth-promoting bacterium (PGPB; Bashan and Holguin, 1998), is not known as a biocontrol-PGPB (Bashan and Holguin, 1997), it has minor biocontrol capabilities against crown gall disease (Bakanchikova et al., 1993) and bacterial leaf blight of mulberry (Sudhakar et al., 2000). In addition, *A. brasilense* can restrict the proliferation of other nonpathogenic rhizosphere bacteria (Holguin and Bashan, 1996; Oliveira and Drozdowicz, 1987), and pathogens such as *P. syringae* pv. *tomato* (Bashan and de-Bashan, 2002), probably by competition.

The aim of this study was to determine whether several relatively ineffective disease management tactics could act synergistically or additively when used in concert to reduce bacterial speck of tomato.

Material and methods

Organisms and growth conditions

A. brasilense Cd (ATCC 29710) and a natural, triple antibiotic resistant mutant (oxytetracycline, rifampicin, kanamycin, 200 µg/ml of each) of *P. syringae* pv. *tomato* (WT-1-ORK from our laboratory culture collection, originally isolated from infected tomato plants growing in a glasshouse during an epidemic in 1975 (Bashan et al., 1978)), were used in this study. Bacteria were grown as described previously (Bashan, 1998). In addition to antibiotic resistance, *P. syringae* pv. *tomato* WT-1-ORK is also moderately resistant to copper compounds (inhibited by a concentration greater than 0.3 mM $CuSO_4 \cdot 5H_2O$ (Cooksey and Azad, 1992) in *in vitro* tests (Bashan Y., unpublished data)). In greenhouse and growth chamber tests, there was no difference in virulence between *P. syringae* pv. *tomato* WT-1-ORK used in this study and wild type *P. syringae* pv. *tomato* WT-1 (unpublished data).

Tomato plants (*Lycopersicon esculentum* Mill) of the susceptible fresh market cultivar Pik Red (Joseph Harris Co. Rochester, NY; Jardine and Stephenens, 1987) were grown in small pots (black, 500 ml) containing the commercial potting substrate, Sunshine Mix 3, special fine (Fisons Horticulture, Mississauga, Ontario), in the greenhouse as previously described (Bashan et al., 1989).

Inoculation and detection techniques

Seeds and leaves were inoculated with *A. brasilense* Cd and *P. syringae* pv. *tomato* (Bashan, 1998; Bashan et al., 1978). Leaves were inoculated at the five to seven-true-leaves stage with a hand-held pneumatic sprayer (Rohm and Hass, Philadelphia) from a height of 25–35 cm. Plants were sprayed until run-off. Tomato seeds or leaves were inoculated with each bacterium separately or with a mixture of both, either at the same time or consecutively. In each case, the total inoculation level was 10^6 cfu/ml (Bashan 1986; Bashan et al., 1978). These concentrations are optimal for plant growth promotion by *Azospirillum* sp. (Bashan et al., 1989), to avoid growth inhibition known to be induced by high cell concentrations (Bashan, 1986), and to prevent atypical symptom formation caused by high concentrations of *P. syringae* pv. *tomato* (Bashan et al., 1978). Plants were incubated under mist conditions in the greenhouse with a temperature regime of 28/22 °C (day/night) and natural illumination (Diab et al., 1982). Mist was applied with mist-diffuser jets for 5 s at 30-min intervals to create permanently wet leaves with minimal leaf surface run-off. Possible cross-contamination of *A. brasilense* and *P. syringae* pv. *tomato* via air currents (Bashan, 1991) was avoided by separating noninoculated pots from inoculated pots by thin, vertical, transparent plastic sheets and placing the diffuser jets high above the experimental area, thus creating a delicate fog. Since all experiments occupied the same greenhouse bench top, which was located in the center of large greenhouse, environmental conditions for all the experiments and for all repetitions within each experiment were similar. Bacteria were specifically detected and enumerated by

an ELISA technique for *A. brasilense* Cd (Levanony et al., 1987), and with a combination of antibiotics (200 μg of each antibiotic (oxytetracycline, rifampicin, kanamycin)/ml medium) supplemented on nutrient agar (Sigma) plates for *P. syringae* pv. *tomato* by the plate count method. Counts were conducted after disinfecting and triturating the leaves (Sharon et al., 1982).

Bactericide and antibiotic application, and chemo-thermal treatment of seeds

A commercial copper bactericide (a mixture of 0.5% copper hydroxide (Kocide-101; Kocide Chemicals, Houston) and 0.3% copper oxychloride (Cuprox-50, Machteshim, Israel); Yunis et al., 1980b) and commercial streptomycin sulfate (Agri-Mycin 17, 0.02%) were applied as aerosols using a calibrated commercial garden sprayer (Ace Corp. Model 3133, Oak Brook, Ill). A volume of 10 ml was applied to each plant to simulate commercial field spraying (250 gal/ha). Chemicals were applied, using the regimes indicated in the Results section. Post-inoculation sprays were applied following removal of the plants from the mist and then maintaining a 1-h drying period at 35% RH and $30 \pm 2\,^{\circ}$C. After spraying with the chemicals, the plants were again allowed to dry until no liquid droplets were visible. The plants were then returned to the mist. For chemo-thermal treatments, seeds inoculated with *P. syringae* pv. *tomato* (inoculated as indicated above) were incubated in a circulating water bath at $35 \pm 2\,^{\circ}$C for 1 h. One gram of seeds was incubated in 1 liter distilled water containing: cupric acetate, 2.0 g; glacial acetic acid, 1 ml; pentachloronitrobenzene, 23% (w/v); 6% 5-ethoxy-3 (trichloromethyl)-1,2,4-thiadiazole, 4.5 ml; and Triton x-100, 0.2 ml. This treatment has been reported to eradicate *P. syringae* pv. *tomato* from tomato seeds (Kritzman, 1993). After treatment, the seeds were washed eleven times with sterile, distilled water; this level of washing was sufficient to remove the bactericides. Immediately after draining the excess water, chemothermally-treated seeds were inoculated with *A. brasilense*.

Evaluation of disease intensity

Disease intensity was evaluated visually, and scored using a disease index (DI of 0–3 with: 0 = no lesions, 1 = 2–5 specks together or spread all over the leaf, 2 = 6–10 specks, 3 = more than 11 specks per leaf) (Yunis et al., 1980a).

Induction of systemic resistance

Seeds were inoculated with *A. brasilense* (Bashan, 1986) and plants were grown to the 3–5 leaf stage. The day before challenge with the pathogen, the plants were transferred to mist conditions, inoculated with *P. syringae* pv. *tomato* and maintained under these conditions for an additional 5 days. The disease index was used to evaluate disease progress. The level of salicylic acid in the leaves was measured: leaves were frozen in liquid air and later pulverized with a sterile mortar and pestle. Free and conjugated salicylic acid was extracted and quantified (Meuwly and Métraux, 1993) using 200 ng of the internal standard ortho-anisic acid per gram fresh weight of leaves.

Experimental design and statistical analysis

Plants subjected to the various treatments were placed in the greenhouse and under mist, using a random number distribution table for random distribution of pots. Each treatment had 5 replicates, where 3 pots served as a single replicate; each pot contained 2 plants (30 plants per treatment). Data from the 3 pots were combined and the entire experiment was analyzed by Analysis of Variance (ANOVA) or by Student's t-test at $P \leq 0.05$. All experiments were repeated two or three times. Initially, each experiment was statistically analyzed separately. However, since the repetitions of the experiments yielded very similar results, data from all experiments on the same topic were combined. Results presented are the average of the 2 (or 3) repetitions of each experiment.

Results

Effect of individual disease control agents on bacterial speck intensity, and height and dry weight of tomato seedlings

Inoculation of tomato seeds with *A. brasilense* alone, or by spraying tomato foliage, separately, with either *A. brasilense*, streptomycin sulfate, or a commercial copper bactericides (Kocide 101 + Cuprox 50), before or after inoculation with *P. syringae* pv. *tomato*, had no significant effect on disease severity or on plant height and dry weight. Only streptomycin sulfate reduced bacterial speck intensity 6 days after inoculation. However, the effect faded 15 days after inoculation. The only

significant effect was an increase in the dry weight of plants following seed inoculation with *A. brasilense* (Table 1).

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

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, from disease intensity (DI) of 2.78 to 0.26 (Table 2), and significantly slowed disease development under mist conditions (Figure 1). After plants were transferred from mist to dry bench-top conditions, which simulated the cyclic wet and dry periods common in commercial tomato production in greenhouses, disease failed to develop further and damage to plant foliage was minimal, compared to inoculated controls (Table 2).

Induced systemic resistance (ISR) by A. brasilense *inoculated onto seeds*

The potential of *A. brasilense* to induce systemic resistance was assessed in two ways: by evaluating the degree of protection against challenge inoculation with *P. syringae* pv. *tomato* and by measuring the accumulation of salicylic acid in the leaves of host plants. Inoculation of tomato seeds with *A. brasilense* before challenging the plants with *P. syringae* pv. *tomato* only marginally reduced disease development in the leaves. Concomitantly, the level of salicylic acid in the leaves, although significantly higher than in the noninoculated plants, was still below the threshold level required to induce systemic resistance against *P. syringae* pv. *tomato* (Table 3).

Protection against P. syringae *pv.* tomato *infection in leaves after chemo-thermal treatment of seeds combined with* A. brasilense *inoculation*

When tomato seeds were infected with *P. syringae* pv. *tomato* and later subjected to a chemo-thermal treatment followed by inoculation with *A. brasilense*, bacterial speck did not develop in the leaves. However, this treatment combination did not completely protect the plants when challenged a second time with the pathogen. Although the pathogen population was small compared to untreated plants and disease intensity was significantly lower, disease symptoms were still apparent and plant development was negatively affected (Table 4).

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

Treatment	Disease development after 6 days[4]	Disease development after 15 days[4]	Plant height after 15 days (mm)	Plant dry weight after 15 days (mg)
Before inoculation with *P. syringae* pv. *tomato*				
Seed inoculation with *A. brasilense*	1.85 aA	2.88 aB	67 b	85 b
Foliar inoculation with *A. brasilense*[1]	1.67 aA	2.57 aB	57 ab	72 ab
Streptomycin sulfate	0.12 bA	2.61 aB	49 a	61 a
Copper bactericide	1.77 aA	2.81 aB	48 a	64 a
Untreated plants	1.91 aA	2.95 aB	53 a	66 a
After inoculation with *P. syringae* pv. *tomato*				
Seed inoculation with *A. brasilense*[2]	2.25 aA	2.92 aA	59 a	69 b
Foliar inoculation with *A. brasilense*[3]	2.35 aA	2.84 aA	50 a	61 a
Streptomycin sulfate	0.21 bA	2.74 aB	58 a	58 a
Copper bactericide	2.43 aA	2.88 aA	49 a	57 a
Untreated plants	2.55 aA	2.93 aA	55 a	64 a

[1]*P. syringae* pv. *tomato* was applied 30 min after application of *A. brasilense.*
[2]*A. brasilense* was applied immediately after *P. syringae* pv. *tomato* application.
[3]*A. brasilense* was applied one day after *P. syringae* pv. *tomato* application.
[4]Disease index on the scale of 0–3 (0 = free of symptoms).
Numbers in each column, and in each section (before inoculation and after inoculation with *P. syringae* pv. *tomato*), denoted by a different lower case letter, differ significantly at $P \leq 0.05$ in ANOVA analysis. Numbers in each row, denoted by a different upper case letter differ significantly at $P \leq 0.05$ in Student's *t*-test analysis.

Table 2. Bacterial speck intensity and dry weight of tomato seedlings subjected to inoculation with *P. syringae* pv. *tomato* and various management strategies, including streptomycin sulfate, *A. brasilense* inoculation, and bactericidal foliar application, singly or in combination

Treatment	Disease intensity* (after 6 days in mist)	Disease intensity* (after 30 days in mist)	Disease intensity* (after additional 30 days in dry)	Plant dry weight (g) after 60 days
P. syringae pv. *tomato* + seed inoculation with *A. brasilense* + one streptomycin treatment + two bactericide applications at 5-day intervals	0.22 a	0.26 a	0.11 a	14.9 b
P. syringae pv. *tomato* (no control agents)	2.06 c	2.78 b	1.47 b	9.8 a
Uninoculated (no control agents)	0 a	0 a	0 a	16.5 b
Noninoculated + seed inoculation with *A. brasilense* + one streptomycin treatment + two bactericide applications at 5-day intervals	0 a	0 a	0 a	15.6 b
P. syringae pv. *tomato* + streptomycin	0.17 a	2.73 b	0.14 a	10.2 a
P. syringae pv. *tomato* + bactericide	1.67 b	2.86 b	1.26 b	9.6 a
P. syringae pv. *tomato*+ streptomycin + bactericide	0.19 a	2.58 b	0.15 a	10.3 a
P. syringae pv. *tomato* + *A. brasilense*	1.89 bc	2.71 b	1.44 b	11.4 a

Numbers in each column denoted by a different lower case letter differ significantly at $P \leq 0.05$ in ANOVA.
*Disease index on the scale of 0–3 (0 = free of symptoms).

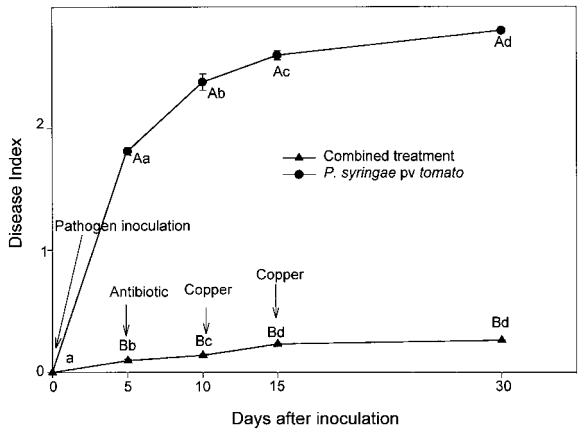

Figure 1. Development of bacterial speck of tomato under mist conditions for 30 days after a combined treatment that included seed inoculation with *A. brasilense*, foliar infestation by *P. syringae* pv. *tomato,* combined with a single foliar streptomycin treatment and two foliar bactericide applications at 5-day intervals. Points in each line (disease development over time) denoted by a different lower case letter differ significantly at $P \leq 0.05$ in ANOVA. Points in each day after inoculation (indicating the difference between the two treatments) denoted by a different capital letter differ significantly at $P \leq 0.05$ in Student's t-test. Bars represent standard error (SE). Missing bars indicate that the SE is smaller than the point. Arrows indicate the time of application of each treatment. Disease index on a scale of 0–3 (0 = free of symptoms).

Table 3. Endogenous levels of free and conjugated salicylic acid in tomato leaves growing from seeds inoculated with *A. brasilense* and the severity of bacterial speck after challenging with *P. syringae* pv. *tomato*

Treatment	Free SA (ng/g fw)	Conjugated SA (ng/g fw)	Disease index after 5 days*
Uninoculated control	21 ± 3 a	276 ± 23 a	0 a
P. syringae pv. *tomato* inoculated control	62 ± 5 c	608 ± 12 c	2.63 b
Inoculated with *A. brasilense* + challenge by *P. syringae* pv. *tomato*	32 ± 4 b	355 ± 8 b	2.38 b

SA = Salicylic acid; fw = Fresh weight.
Numbers in each column denoted by a different letter differ significantly at $P \leq 0.05$ in ANOVA analysis.
*Disease index on the scale of 0–3 (0 = free of symptoms).

Bacterial speck development and dry weight of tomato seedlings after seed treatment with mild chemo-thermal treatment, A. brasilense *and a single bactericide application*

Treatment of tomato seeds inoculated with *P. syringae* pv. *tomato* with a combination of mild chemo-thermal treatment, *A. brasilense* inoculation, and later a single application of a copper bactericide, nearly eliminated

Table 4. Effect of chemo-thermal treatment of seeds with and without *A. brasilense* inoculation on bacterial speck intensity, *P. syringae* pv. *tomato* population and plant dry weight

Treatment	Disease intensity (5 days after inoculation in mist)*	*P. syringae* pv. *tomato* (cfu/g dw), 5 days after inoculation in mist	Plant dry weight (g) after 60 days
Control: Chemo-thermal only	0 d	0	15.7 b
Control: *P. syringae* pv. *tomato* alone in leaves	2.54 a	$6.9 \pm 0.7 \times 10^8$	9.1 a
Control: Chemo-thermal plus *A. brasilense*	0 d	0	17.3 c
P. syringae pv.*tomato* in seeds plus chemo-thermal	0.5 c	$4.3 \pm 0.9 \times 10^7$	15.2 b
P. syringae pv. *tomato* in seeds plus chemo-thermal plus *A. brasilense* on seeds	0 d	0	16.5 bc
P. syringae pv. *tomato* in seeds plus chemo-thermal plus *A. brasilense* on seeds plus *P. syringae* pv. *tomato* on leaves	2.02 b	$9.6 \pm 1.1 \times 10^7$	9.9 a

dw – dry weight. Numbers in each column denoted by a different letter differ significantly at $P \leq 0.05$ in ANOVA. *Disease index on a scale of 0–3 (0 = free of symptoms).

bacterial speck even when the plants were grown under mist for 6 weeks, a condition that was highly conducive for disease development (Table 5). Plants treated in this manner grew similar to noninoculated plants (Table 5). This combined treatment against *P. syringae* pv. *tomato* reduced foliar populations of this bacteria (Figure 2) and reduced disease development (Figure 3).

Discussion

The goal of this study was to reduce the amount of chemical pesticide required to protect tomato plants from *P. syringae* pv. *tomato* infection by partially substituting bactericide applications with mild chemo-thermal seed treatment, and with *A. brasilense* seed inoculation. These treatments when applied separately, are ineffective.

Seed transmission is perhaps the main mechanism by which *P. syringae* pv. *tomato* infects tomato (Bashan and Okon, 1981; Pyke et al., 1984) and the main mode of transfer of the disease from field to field (Bashan et al., 1982), therefore eradication protocols should focus on the seeds (Kritzman, 1993). The working hypothesis of this study was that disinfecting contaminated seeds followed by inoculation with plant growth-promoting bacteria, will induce systemic resistance in the plants and allow them to grow more vigorously. This, together with fewer applications of bactericides, will provide better protection against the pathogen than the common chemical control methods.

Salicylic acid accumulation is required for expression of some types of disease resistance and therefore plays a central role in protecting plants against pathogens (Delaney et al., 1994; De Meyer et al., 1999). This is true for systemic acquired resistance (SAR), induced in plants in response to the pathogen, as shown for *P. syringae* pv. *tomato* infection of *Arabidopsis*

Table 5. Effect of different combinations of chemo-thermal seed treatment, *A. brasilense* inoculation of seeds, and a single foliar application of bactericide on bacterial speck intensity and tomato seedling dry weight, when applied to seeds artificially infested with *P. syringae* pv. *tomato*

Treatment	Disease intensity (after 42 days in mist)[2]	Plant dry weight (g) after 60 days
Inoculated *P. syringae* pv. *tomato* in seeds plus combined control[1]	0.35 a	17.2 a
Inoculated *P. syringae* pv. *tomato* in seeds	2.84 c	10.8 c
Inoculated *P. syringae* pv. *tomato* in leaves	2.92 c	9.4 c
Inoculated *P. syringae* pv. *tomato* + chemo-thermal	1.8 b	16.1 b
Inoculated *P. syringae* pv. *tomato* + *Azospirillum* alone	2.68 c	11.3 c
Inoculated *P. syringae* pv. *tomato* + bactericide alone	2.73 c	8.9 c
Inoculated *P. syringae* pv. *tomato* + chemo-thermal+ bactericide	1.85 b	15.1 b
Noninoculated	0 a	17.7 a
Noninoculated and treated	0 a	18.1 a

[1]Combined controls are chemo-thermal seed treatment + *A. brasilense* inoculation of seeds, and a single foliar application of bactericide.
[2]Disease index on a scale of 0–3 (0 = free of symptoms).
Numbers in each column denoted by a different lower case letter differ significantly at $P \leq 0.05$ in ANOVA analysis.

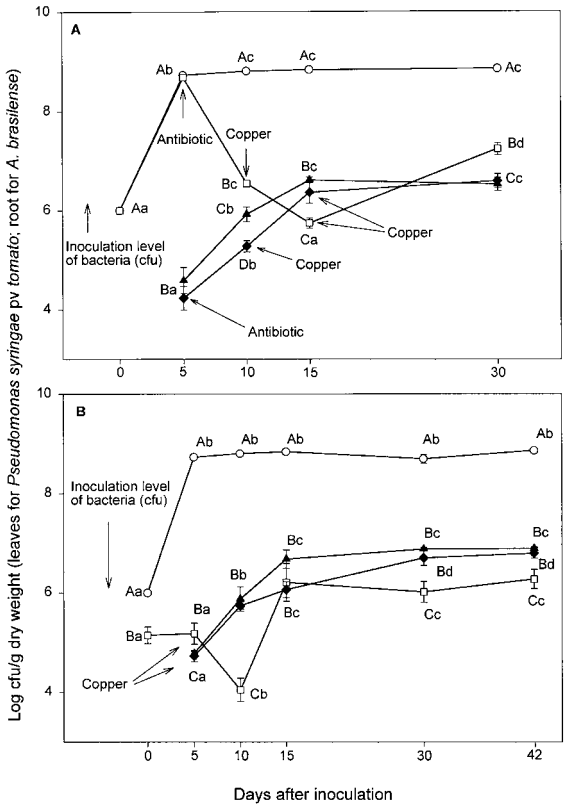

Figure 2. Development of the *P. syringae* pv. *tomato* population on tomato leaves and the *A. brasilense* population in the rhizosphere of the same tomato after two combined control treatments; A. after seed inoculation with *A. brasilense* combined with a single foliar streptomycin treatment and two foliar bactericide applications at 5-day intervals and leaf inoculation by *P. syringae* pv. *tomato.* B. After seed inoculation by *P. syringae* pv. *tomato,* chemo-thermal treatment of seed and inoculation with *A. brasilense* combined with a single foliar bactericide treatment. Inoculation levels were 10^6 cfu/ml (*P. syringae* pv. *tomato* on leaves) and 10^6 cfu/g seed (for *A. brasilense*). Points in each line (growth of bacterial population over time) denoted by a different lower case letter differ significantly at $P \leq 0.05$ in ANOVA. Points in each day after inoculation (comparing the bacterial populations in different treatments) denoted by a different capital letter differ significantly at $P \leq 0.05$ in ANOVA. Bars represent standard error (SE). Missing bars indicate that the SE is smaller than the point. Arrows indicate the time of application of each control treatment. ○ – Untreated control plus *P. syringae* pv. *tomato* inoculation; □ – *P. syringae* pv. *tomato* with combined treatment; ▲ – Untreated control plus *A. brasilense* inoculation; ♦ – *A. brasilense* inoculation plus combined treatment.

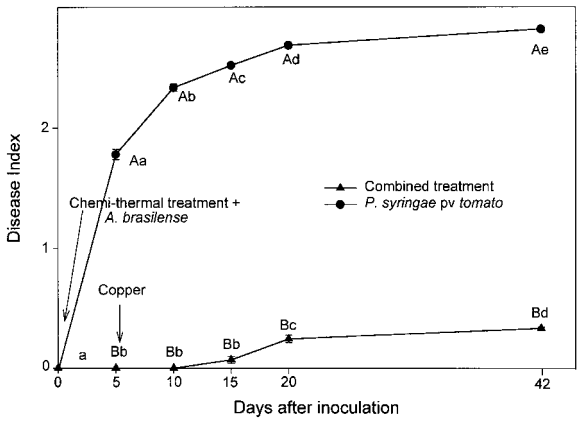

Figure 3. Development of bacterial speck of tomato after a combined treatment of artificially-inoculated tomato seeds with *P. syringae* pv. *tomato,* including sterilization of seeds by chemo-thermal treatment, inoculation with *A. brasilense,* combined with a single bactericide treatment of leaves. Points in each line (disease development over time) denoted by a different lower case letter differ significantly at $P \leq 0.05$ in ANOVA. Points in each day after inoculation (indicating differences between the two treatments) denoted by a different capital letter differ significantly at $P \leq 0.05$ in Student's t-test. Bars represent standard error (SE). Missing bars means that the SE is smaller than the point. Arrows indicate the timing of application of each control treatment. Disease index on a scale of 0–3 (0 = free of symptoms).

plants (van Wees et al., 2000). However, salicylic acid does not always accumulate to a large extent in induced systemic resistance (ISR) that can be induced with biotic and abiotic factors other than plant pathogens (van Loon et al., 1998). Accumulation of salicylic acid may therefore be used as an indicator that the plant is more resistant to infection. Although *A. brasilense* increases the growth of tomato plants (Bashan et al., 1989) and enhances production of salicylic acid (this study), resistance induced by *A. brasilense* alone was insufficient to protect the plants from infection by *P. syringae* pv. *tomato*.

In this study, we were not able to completely protect tomato plants from pathogen infection. When the seeds were initially pathogen-free, the combined treatment reduced disease development and reduced the negative effects on plant growth caused by subsequent leaf inoculation. Similarly, when the combined treatment was applied to infested seeds, the protection achieved was significant. However, the combined treatment did not provide sufficient protection against high-pressure pathogen infections, for example, when seeds and subsequently, leaves were infected in the same cultivation.

In summary, this study demonstrates that a combination of several ineffective management tactics can reduce the development and severity of bacterial speck of tomato.

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