Influence of Timing of Application and Chemical on Control of Bacterial Speck of Tomato

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ABSTRACT

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Greenhouse experiments were conducted to determine the effect of timing of application of selected chemicals on control of bacterial speck on artificially inoculated plants. Streptomycin sulfate, oxytetracycline, and a copper-mancozeb complex were applied at various times before or after inoculation with *Pseudomonas syringae* pv. *tomato*. Only streptomycin provided significant control, and then only if applied within 24–48 hr of inoculation. In field studies, various antibiotic and copper compounds were applied using either a 4- or 7-day spray schedule. The efficacy of all chemicals appeared to be related to environmental conditions; significant ($P = 0.05$) protection was provided only when conditions for disease were limiting. Streptomycin generally provided the highest amount of control. The activity of oxytetracycline was increased by adding an adjuvant.

Bacterial speck of tomato (*Lycopersicon esculentum* Mill.) caused by the bacterium *Pseudomonas syringae* pv. *tomato* (Okabe) Young, Dye, & Wilkie (*P. s.* pv. *tomato*) continues to be a serious problem in Michigan, Ohio, and other tomato production areas of the United States and Canada (5). Diseased plants are characterized by a reduction in quality caused by lesions on the fruit surface and by a reduction in yield (12,15). Some researchers (2,15) have suggested that copper compounds can be used effectively in a preventative control program, whereas others (8,11) have shown chemical control to be ineffective, at least under cool, temperate growing conditions. Copper compounds have traditionally been applied on a 7-day spray schedule. It is well known that the selection of material and timing of application are critical for effective disease control (3). The ineffectiveness of copper sprays in some tests may be due in part to the lack of good preventative spray materials or too lengthy intervals between sprays.

Streptomycin sulfate has shown promise in some tests (2) but is currently only registered for greenhouse use. Information on the effectiveness of other antibiotic control agents is limited (10). The purpose of this study was to evaluate the timing of application of selected chemical control agents and to evaluate selected antibiotics and fixed copper compounds for control of bacterial speck.

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MATERIALS AND METHODS

Inoculum. A naturally occurring rifampicin-resistant isolate of *P. s.* pv. *tomato* (PtFr) was used as the pathogen throughout this study. The rifampicin resistance marker permitted selective isolation from field- and greenhouse-grown, inoculated tomato leaves with rifampicin-amended growth media. In greenhouse tests (*unpublished*), there was no difference in virulence between the PtFr isolate and the wild type. Cultures were grown as a lawn on a complete agar medium (7) for 24 hr at room temperature. Inoculum was prepared by washing cells from the agar surface with 5 ml of sterile distilled water (SDW). Final inoculum concentration was adjusted by dilution with SDW to about 5×10^7 colony-forming units (cfu) per milliliter as determined by standard turbidimetric and dilution plate techniques.

Greenhouse tests. Inoculum was applied to plants at the five- to seven-true-leaves stage with a hand-held pneumatic sprayer from a height of 25–35 cm. Plants were sprayed until runoff. The preinoculation and postinoculation experiments were each repeated five times. In the first three experiments, all plants were placed in an air-conditioned mist chamber and held at 20 C at night and 24 C during the day until symptoms developed. Mist was applied for 20 sec every 30 min so leaves stayed continually wet. In the last two tests, inoculated plants were placed in closed plastic bags on a laboratory bench for 4 days, then removed to a greenhouse bench until symptoms developed.

Tomato plants of the susceptible fresh-market cultivar Pik Red (Joseph Harris Co. Inc., Rochester, NY) were grown in the greenhouse in 10-cm clay pots containing Sunshine Mix No. 1 (J. Mollema and Son. Inc., Grand Rapids, MI). A 20-20-20 (NPK) soluble fertilizer (Peters Fertilizer Products, Allentown, PA) was applied biweekly. Chemicals were applied with a hand-held pneumatic sprayer at a height of 25–35 cm. A volume of about 9 ml was applied to each plant to simulate field spraying (100 gal/acre). Four treatments including an SDW control, streptomycin sulfate (Agrimycin 17, 1.2 g/L), oxytetracycline (Mycoshield, 1.2 g/L), and an experimental copper hydroxide + mancozeb combination (KCC-FMX, 5 ml/L) were used. Chemicals were applied 6, 5, 4, 3, 2, or 1 day before inoculation; just before inoculation; immediately after innoculation; and 1, 2, or 4 days after inoculation. Preinoculation chemical treatments were applied to the plants and allowed to dry a minimum of 1 hr or until no visible moisture could be detected. Plants were then placed in the mist chamber until inoculation. Postinoculation sprays were made by removing the plants from the mist chamber, allowing them to dry for 1 hr, and applying the chemical. The plants were again allowed to dry for 1 hr or until no visible moisture was detected and then placed back in the mist chamber. In the final two tests, plants were sprayed at the prescribed times and left on the greenhouse bench until inoculation. After inoculation, plants were placed in plastic bags. Post-inoculation sprays were made by removing the plants from their bags, applying the chemical, and returning the plants to the bags. The experiments were set up in a completely randomized design with three replicates per treatment.

Disease incidence was calculated by counting the diseased leaflets on each plant and reporting it as a percentage of the total number of leaflets per plant. An arc sine square-root transformation was performed on all data to stabilize the variances.

Field tests. In 1982, tests were conducted at the Michigan State University Sodus Horticultural Experiment Farm in southwestern Michigan. The 1983 and 1984 tests were conducted at the Michigan State University Botany and Plant Pathology Research Farm near East Lansing. Plants were grown by a commercial greenhouse operator in southwestern Michigan. Six-week-old tomato plants (cultivar Pik Red) were inoculated in their flats by the methods described for the greenhouse studies. After inoculation, the flats were placed in mist chambers for 7 days until symptoms developed. In each of the 3 yr, chemical efficacy tests were conducted in conjunction with the validation of a predictive forecast model used to time the application of chemical sprays (6). In 1983 and 1984, plants were transplanted in rows 4.9 m long with 1.5 m between rows and an in-row spacing of 0.6 m. There were two rows per plot. In 1984, a single row 6.0 m long was used with a guard row placed between each plot. The experiment was a split-plot design with four replicates. Main plot treatments in 1982 and 1983 were a routine 7-day spray schedule vs. sprays based on a predictive model forecast of bacterial population levels. In 1984, a 4-day spray schedule was used vs. model predictions. Subplot treatments consisted of various chemical controls. In 1982, copper hydroxide + mancozeb at 4.7 L/ha was compared with an unsprayed control. In 1983, streptomycin (200 ppm) and oxytetracycline (200 ppm) were added to the test. In 1984, copper hydroxide (2.2 kg/ha) alone was also included with the 1983 treatments.

Transplanting in each year was done by hand. Plots were cared for according to the standard commercial practices of the area. Carbaryl and chlorothalonil were used as needed for foliar insect and fungal disease control. Chlorathalonil has previously been shown to have no effect on *P. s.* pv. *tomato* (8). Disease incidence in all experiments was determined by counting the speck-infected fruit and reporting it as a percentage of the total number of fruits. In 1984, yields were also determined.

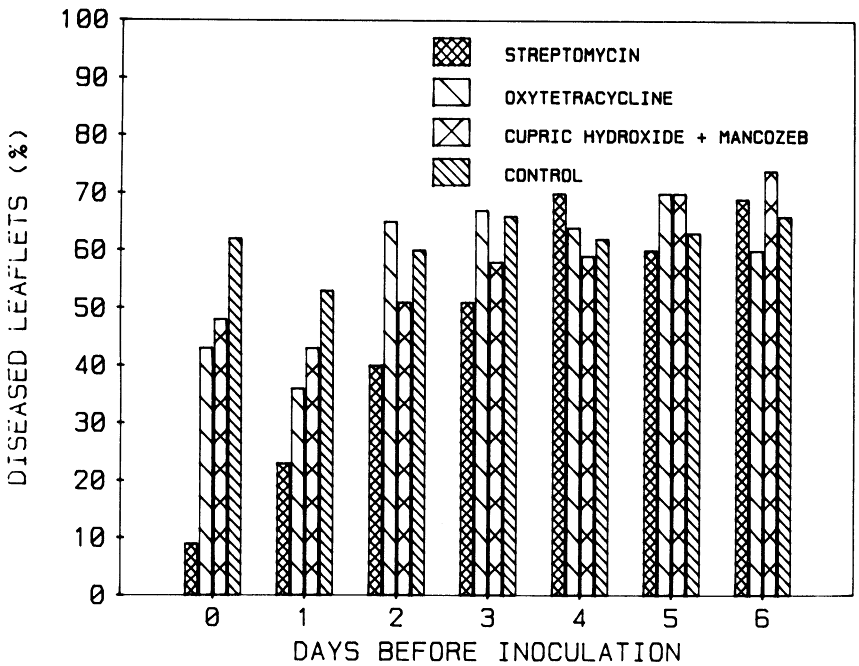

Fig. 1. Effects of chemical and timing of application before inoculation on the incidence of bacterial speck on greenhouse-grown Pik Red tomato plants.

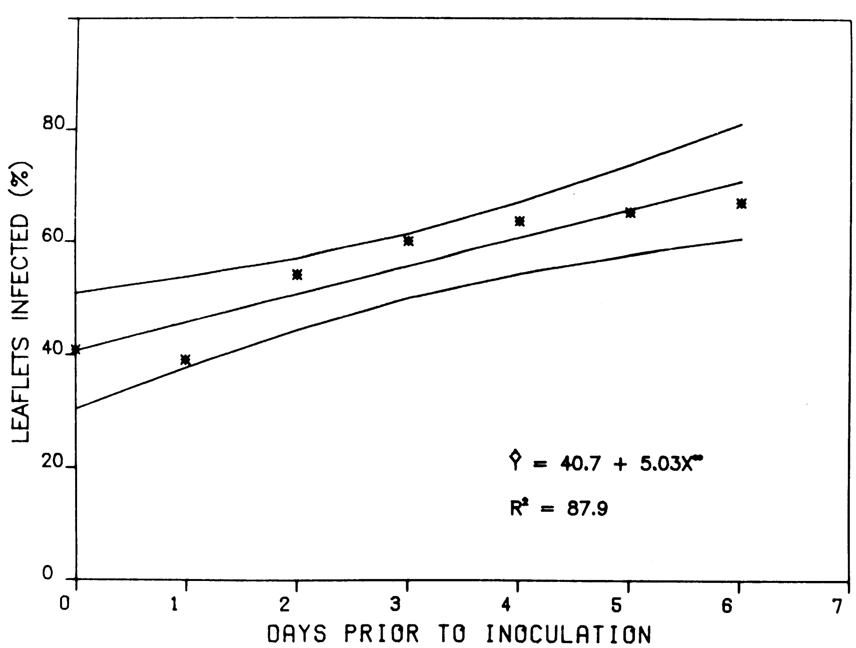

Fig. 2. Fitted regression line and 95% confidence limits relating incidence of bacterial speck infection to the time of application of control chemicals. Individual values are the means of four treatments including a streptomycin (200 ppm), oxytetracycline (200 ppm), copper hydroxide + mancozeb (KCC-FMX, 5 ml/L), and a water control.

RESULTS AND DISCUSSION

Greenhouse tests. The average amount of disease in each experiment varied according to the method of incubation. In general, higher levels of disease occurred when plants were placed in plastic bags after inoculation. Although mean levels of disease varied from experiment to experiment, the relationship between the various treatments remained constant. Therefore, only the data for one preinoculation and postinoculation experiment are presented.

The effectiveness of all chemical treatments were directly related to time of application. Although the interaction between chemicals and time of application (Fig. 1) was not statistically significant ($P = 0.1$), there was a significant decrease in overall efficacy of the chemicals as the time of application before inoculation was increased (Fig. 2). Streptomycin provided the best control compared with the untreated control but only when it was applied within 1 day of inoculation (Fig. 1). In preinoculation applications, it may be that chemical residues are degraded or diluted to a point where they are no longer effective in controlling the speck organism. Further study needs to be done on the fate of the chemical before inoculation. Postinoculation experiments provided similar results (Fig. 3). Streptomycin reduced bacterial speck by 39% compared with the control but only if applied within 24 hr of inoculation. The 25% reduction in disease incidence by oxytetracycline was significant compared with the control, but overall levels of disease were still high. The copper hydroxide + mancozeb treatment had levels of disease not significantly different from the control. Chemical applications were not made beyond 4 days because symptoms had already begun to develop. Thus it would appear that, after infection, even a chemical with good eradicant properties would do little to reduce the amount of disease after more than 2–3 days unless it could also provide protection to new and as yet uninfected tissue.

Field tests. In 1982, only the copper hydroxide + mancozeb combination was used. Because of cool, wet conditions throughout the summer, conditions for disease development were very favorable. There were no significant differences in the incidence of diseased fruit between control and treatment plots (Table 1). In 1983, conditions for disease development were less favorable and all three chemical treatments significantly decreased speck incidence compared with the control (Table 1). However, only streptomycin and possibly the copper hydroxide + mancozeb reduced speck incidence to levels that would be considered economically acceptable to commercial growers. Based on the greenhouse efficacy results and the fact that symptoms developed within 4 days of inoculation, the spray schedule in 1984 was shortened to 4 days. Anything less than this would probably not be economically feasible for growers, although a cost-benefit study should be done to confirm this. The 1984 growing season was generally hot and dry and unfavorable to disease development. All treatments significantly (P = 0.05) reduced disease incidence compared with the untreated control. There was significantly more bacterial speck on fruit in the oxytetracycline treatment when compared with copper hydroxide alone but not when compared with the other two chemical treatments (Table 1).

In evaluating the 3 yr of field data, it appeared that the most important factor in determining the degree of control of bacterial speck was the presence or absence of conditions favorable for speck development. When environmental conditions were highly favorable such as in the greenhouse experiments and in the field during the 1982 growing season, no chemical provided effective control. When environmental conditions were less favorable for disease development such as in 1983 and 1984, copper compounds as well as antibiotics were effective in reducing fruit infection. The addition of mancozeb to copper hydroxide as been reported to increase disease control. In our experiments, the addition mancozeb did not significantly increase control. Marco and Stall (9) demonstrated that the addition of mancozeb to copper-containing compounds resulted in an increase in the amount of available copper in solution, and this may explain the increased activity of the combined materials that have been reported.

Growers in northern temperate climates probably cannot depend on chemical control to reduce the level of bacterial speck infection. Available copper compounds apparently provide protection only when conditions for disease development are limiting. Antibiotics, which offer an increased level of protection, are currently not registered for field use. If registration occurs, the timing of application is critical. Streptomycin required application within 24–48 hr of infection for maximum effectiveness. Streptomycin or oxytetracycline plus an adjuvant in combination with a predictive forecast system may provide the type of control growers need. However, registration in the near future is unlikely because of human health concerns associated with antibiotic use and because of the potential for antibiotic resistance. The development of streptomycin resistance in *Xanthomonas campestris* pv. *vesicatoria* is well documented (14). It is likely that similar resistance would occur in *P. s.* pv. *tomato* if antibiotics were used on a regular basis. Of greater concern to growers may be the reports of copper tolerance in *X. campestris* pv. *vesicatoria* reported from Florida and Mexico (1,9).

Getz et al (4) have shown that tomato fruit are susceptible to infection only until they reach 3 cm in diameter; therefore, control efforts should be greatest during the early part of the growing season. Growers would be wise to develop a good preventative control program including buying disease-free seed and transplants. Even these measures have their drawbacks, because hot-water treatment of expensive hybrid seed reduces germination and the organism can survive undetected as an epiphyte on leaf surfaces of the plant (13) and increase to cause an epiphytotic when environmental conditions become favorable.

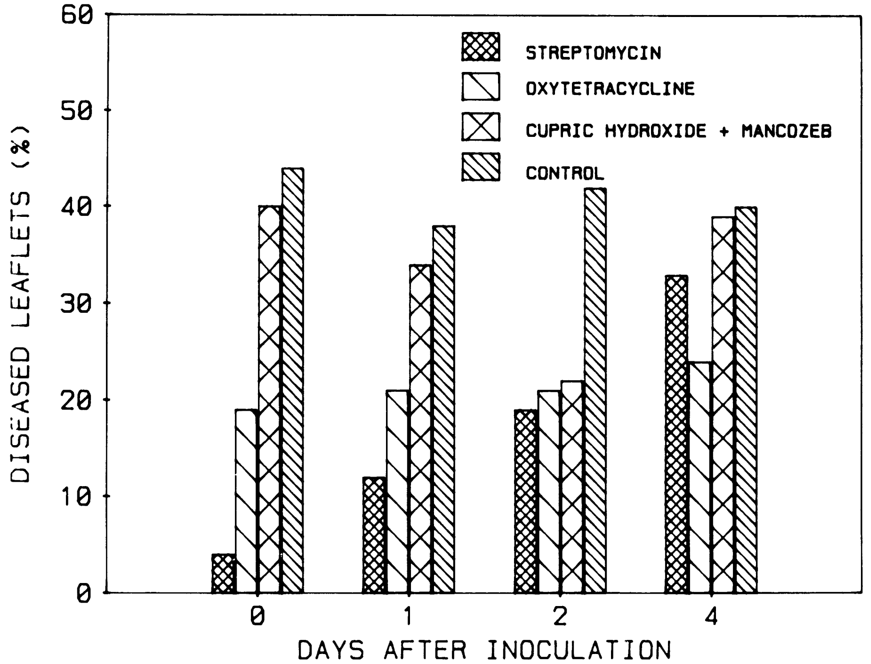

Fig. 3. Effects of chemical and timing of application after inoculation on the incidence of bacterial speck on greenhouse-grown Pik Red tomato plants.

Table 1. Effects of selected antibiotics and fixed copper compounds on infection of tomato fruit by *Pseudomonas syringae* pv. *tomato* at Sodus, MI, in 1982 and East Lansing, MI, in 1983 and 1984

	Percent fruit infection		
Chemical[y]	**1982**	**1983**	**1984**
Copper hydroxide + mancozeb	68.3[z]	5.6 b	0.7 ab
Streptomycin	...	2.8 a	1.0 ab
Oxytetracycline	...	10.3 c	2.5 b
Copper hydroxide	...	...	0.5 a
Control	63.3	20.9 d	7.5 c

[y] Rates were those recommended for field use and expressed as amount per liter: copper hydroxide (2.0 g) + mancozeb (2.0 g), streptomycin (1.2 g), oxytetracycline (1.2 g), and copper hydroxide alone (2.7 g). Chemicals were applied to inoculated Pik Red tomato plants with a CO_2 boom sprayer in 830 L of water per hectare. In 1982 and 1983, a 7-day spray schedule was used beginning 12 June and 28 May, respectively. In 1984, a 4-day spray schedule was used beginning 14 June. Plots received 10, 10, and 12 spray covers in each year, respectively.

[z] Means within columns followed by the same letter are not significantly different (P = 0.05) according to Duncan's multiple range test.

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