Effectiveness of Selected Chemicals in Inhibiting *Pseudomonas syringae* pv. *tomato* in vitro and in Controlling Bacterial Speck

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ABSTRACT

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In laboratory, growth chamber, and field studies, streptomycin sulfate and certain fixed coppers alone and in combination with recommended fungicides (mancozeb and chlorothalonil) were toxic to *Pseudomonas syringae* pv. *tomato* (PST) in vitro and significantly reduced severity of bacterial speck on foliage and fruit of Chico III tomato plants. These chemicals also reduced epiphytic populations of PST on field-grown plants. Of 30 fungicides tested in vitro, only the organic sulfur compounds, especially those containing manganese ethylenebisdithiocarbamate (Mn EBDC), were toxic to PST. In vitro tests suggested a synergistic action between EBDC fungicides and several copper compounds although combined treatments did not always give better disease control than the fixed coppers alone. Mn EBDC compounds (maneb, maneb plus $ZnSO_4$, and mancozeb) alone at field rates were highly toxic to PST in vitro. Mancozeb alone significantly reduced lesion counts in the greenhouse but gave poor control in the field. Our results suggest that both streptomycin and cupric hydroxide are useful in a preventive control program for bacterial speck on tomato transplants grown in southern Georgia.

Bacterial speck of tomato (*Lycopersicon esculentum* Mill.) caused by *Pseudomonas syringae* pv. *tomato* (Okabe) Young et al (hereafter referred to as PST) has been a threat to the southern transplant industry since 1978, when the disease resulted in rejection of 160 ha of tomato transplants by the Georgia Department of Agriculture. Bacterial speck also occurred widely throughout the eastern and midwestern tomato-producing areas of the United States where transplants were shipped in 1978 (6) and has been of concern since. It has also caused losses in California (22) and Florida (19) and has increased in importance outside the United States (5) in recent years. Because bacterial speck was not considered important before 1978 (1,18,20), chemical control measures were not used in transplant fields. The limited information available (2,22) at that time from other areas suggested that fixed copper sprays would provide significant control. More recently, copper compounds were effective in some tests (12,14,26) but not others (4,13). Because of the limited and conflicting information available on chemical efficacy, we conducted laboratory, greenhouse, and field tests from 1979 to 1982 to evaluate various bactericide and bactericide plus fungicide combinations for their inhibition of PST in vitro and control of bacterial speck.

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MATERIALS AND METHODS

The strain of PST used in all studies was obtained from a diseased tomato seedling collected in 1978 from a transplant field near Tifton, GA. Inoculum was prepared by suspending cells from 24- to 48-hr cultures in sterile water at a concentration of 10^8 colony-forming units per milliliter. Bacteria were grown at 25 C on King's medium B (KMB) (7). Unless otherwise stated, the Chico III tomato cultivar was used in all tests.

Greenhouse and growth chamber tests. Tomato plants were grown for 6 wk (16–18 cm tall) on a greenhouse bench in 10-cm pots filled with a methyl bromide-fumigated soil mix (soil:sand:vermiculite, 3:1:1, v/v). Chemicals were applied individually or in combination to runoff to all plant surfaces with a paint sprayer held 30–35 cm from the leaf surface to minimize leaf infiltration. Plants treated with chemicals were allowed to dry and were subsequently inoculated with a suspension of PST applied to runoff with the sprayer as described. Inoculated plants were placed at high humidity (enclosed separately in polyethylene bags) for 36 hr in growth chambers (alternate 12 hr dark and 12 hr light with intensity of 400 μEinsteins/m^2/sec) at 18–20 C and were held in the same chambers for an additional 10–12 days to allow symptom development. Disease severity was measured by counting lesions. Studies were repeated two or more times and 10–15 replicates were used.

Efficacy of various chemicals and combinations. Fourteen treatments involving four bactericides and two fungicides alone and in combination were evaluated (Table 1). The bactericides and rates per liter were cupric hydroxide (Kocide 101, 2.4 and 4.8 g), streptomycin sulfate (Agri-Mycin 17, 1.2 g), copper ammonium carbonate (Copper-Count-N, 5.0 ml), and sulfur (50%)-copper (4.4%) (Top Cop, 5.0 ml). The fungicides and rates per liter were mancozeb (Manzate 200, 2.4 g) and chlorothalonil (Bravo 6F or Bravo 500, 2.5 ml). The rates used are those recommended for field use.

Effect of interval between chemical application and inoculation on chemical efficacy. Cupric hydroxide, cupric hydroxide plus mancozeb, cupric hydroxide plus chlorothalonil, streptomycin plus mancozeb, and streptomycin plus chlorothalonil at rates used in earlier studies were sprayed to cover all surfaces of 30 plants, and 30 plants were sprayed with distilled water (controls). One hour later, 10 plants from each treatment were inoculated. Plants were arranged in a randomized complete block design in a growth chamber at 18–20 C. At 3.5 and 7 days after chemical application, 10 plants from each treatment were removed, inoculated as before, and placed back into the growth chamber.

Effect of sprinkler irrigation on chemical efficacy. The chemical treatments were the same as those in the residual activity study. Plants were sprayed with each chemical or distilled water, allowed to dry, and then placed on a greenhouse bench in a randomized complete block design. Three days after the chemical application, half of the plants of each treatment were placed outside on a polyethylene sheet spread on the ground and exposed to 2.5 cm of overhead irrigation applied over a 3.5-hr period with an oscillating lawn sprinkler. The irrigation procedure was repeated 3 days later. One day later, all the plants were inoculated and placed in a growth chamber as described before. Plants were watered from the bottom to prevent repeated wetting of foliage.

Effect of aging mancozeb plus copper mixtures on efficacy. Mancozeb plus cupric hydroxide mixtures (2.4 and 4.8 g/L, respectively) were prepared and allowed to stand 24, 7, 4, and 0 hr on a laboratory bench before use. The four mixtures were applied separately but simultaneously to plants. The plants were inoculated when dry with a suspension of PST.

Laboratory sensitivity tests. A wide range of chemical compounds were tested for their toxicity to PST. All tests were run at least twice with two to four replicate plates or flasks.

Fungicide toxicity tests. Fungicides and rates per liter tested included: inorganic sulfur (Nutronex 94% W, 4.8 g); organic sulfur compounds: maneb (Manzate 80W, 2.4 g), maneb-$ZnSO_4$ (Manzate D 80W, 2.4 g), mancozeb (Manzate 200 80W, 2.4 g), zineb (Security Zineb Spray 75W, 3.6 g), thiram (Arasan 70S, 1.8 g), ferbam (Fermate 76W, 2.4 g), and zinc metiram (Polyram 80W, 2.4 g); benzene compounds: PCNB (Terraclor 75W, 4.8 g), dichloran (Botran 75W, 2.4 g), dinocap (Karathane WD, 2.4 g), diazoben (Dexon 35W, 1.0 g), and chlorothalonil (Bravo 500, 2.5 ml); heterocyclic compounds: captan (Orthocide 50W, 6.0 g), folpet (Phaltan 50W, 4.8 g), captafol (Difolatan 4F, 5.0 ml), and anilazine (Dyrene 50W, 4.8 g); systemic fungicides: carboxin (Vitavax 75W, 1.4 g), benomyl (Benlate 50W, 1.4 g), thiabendazole (Mertect 340-F, 0.8 ml), thiophanate methyl (Topsin M 70W, 0.6 g), chloroneb (Demosan 65W, 2.4 g), ethazole (Truban 25 EC, 0.6 ml), and triadimefon (Bayleton 50W, 1.2 g); miscellaneous compounds: dodine (Cyprex 65W, 1.8 g) and fentin hydroxide (Du-Ter, 0.6 g); and the antibiotic compound cycloheximide (Actidione PM, 1.2 g). Fungicide mixtures tested included ethazole plus thiophanate methyl (Banrot 40WP, 0.6 g), PCNB plus terrazole (Terr-Coat SD 205, 1.8 g), and thiophanate methyl plus EBDC (Zyban, 1.2 g).

Bactericide toxicity tests. Compounds used primarily as bactericides on tomato transplants tested included copper compounds: cupric hydroxide (Kocide 101, 4.8 g), copper ammonium carbonate (Copper-Count-N, 5.0 ml), and sulfur (50%)-copper (4.4%) (Top Cop, 5.0 ml); and streptomycin sulfate (Agri-Mycin 17, 1.2 g).

Bactericide plus fungicide mixture toxicity tests. Initially, mixtures tested included cupric hydroxide combined with either maneb, maneb-$ZnSO_4$, mancozeb, zineb, ferbam, thiophanate methyl plus EBDC (Zyban), or chlorothalonil; copper ammonium carbonate combined with mancozeb; sulfur (50%)-copper (4.4%) combined with mancozeb; and streptomycin sulfate combined with either mancozeb or chlorothalonil. Rates for the combined treatments were the same as those used when the chemicals were applied separately and were given before. In later tests, other copper compounds (cupric acetate, cupric chloride, cupric nitrate, cupric oxide, and cupric sulfate) were tested alone and in combination with mancozeb. Rates were 4.8 and 2.4 g/L for the copper compounds and mancozeb, respectively.

In one study, different rates of mancozeb and cupric hydroxide were used in mixtures. Treatments included mancozeb (Manzate 200) at 2.4 g/L mixed with cupric hydroxide (Kocide 101) at either 4.8, 2.4, 1.2, or 0.3 g/L and cupric hydroxide at 4.8 g/L mixed with mancozeb at either 2.4, 1.2, 0.6, or 0.3 g/L. Other studies were run to determine if aging of the mixture influenced effectiveness. Mancozeb plus cupric hydroxide were mixed 24, 7, 4, and 0 hr before being tested for toxicity.

Sensitivity of PST to the various chemicals was determined as follows: 1) Chemicals were incorporated in KMB poured into petri dishes, bacteria streaked (three separate streaks with a turbid suspension), and growth visually compared with controls (no chemical added) after 48 hr; 2) chemicals were placed in wells (7 mm) cut with a sterile cork borer in plates of KMB, lawns of bacteria established, and zones of inhibition measured after 48–72 hr; or 3) chemicals were incorporated in nutrient broth, shake cultures established, and changes in bacterial populations measured at appropriate intervals by dilution platings on KMB. The chemicals were used without sterilization and were added aseptically after the medium was autoclaved.

Field evaluations. Field tests to determine the efficacy of selected bactericides, fungicides, and bactericide plus fungicide combinations were conducted in 1979 and 1981. Treatments in 1979 included cupric hydroxide, cupric hydroxide plus mancozeb, cupric hydroxide plus chlorothalonil, streptomycin plus mancozeb, streptomycin plus chlorothalonil, mancozeb, chlorothalonil, and a control (water). Chlorothalonil was applied to the control plots beginning 4 wk after transplanting, when early blight caused by *Alternaria solani* became a serious threat. Treatments in 1981 included those used in 1979 plus streptomycin alone, sulfur (50%)-copper (4.4%) alone and in combination with mancozeb, and copper ammonium carbonate alone and in combination with mancozeb. Also, cupric hydroxide was used at 2.2 and 4.4 kg/378 L in 1981, whereas only the high rate was used in 1979. Rates for all other chemicals used in 1979 and 1981 are given in the sections that describe greenhouse and laboratory experiments. All chemical treatments were applied to runoff with a back-mounted mistblower at 7-day intervals.

Plots were established in April of each year on the University of Georgia Plant Sciences Farm near Athens. Standard commercial practices for land preparation, fertilization, and weed and insect control were used. Sprinkler irrigation was used as needed. In 1979, two-row plots 9.8 × 0.97 m and 1.9 m apart were established and each was split in half to accommodate two tomato cultivars. Twenty Chico III plants were transplanted 45 cm apart in half of each plot and 16 Marion plants 60 cm apart in the other. In 1981, three-row plots separated 3.7 m with rows spaced 0.97 m apart were used. Each row had 15 Chico III plants spaced 45 cm apart. Treatments were replicated four times in a randomized complete block design. Disease was initiated by transplanting infected plants (40% in 1979 and 27% in 1981) among healthy plants in the plots to provide even distribution of inoculum.

Table 1. Lesion counts on Chico III tomato plants sprayed with various chemicals and inoculated when dry with a suspension of *Pseudomonas syringae* pv. *tomato*

Chemical[a]	Mean number of lesions per plant[b]		
	Test no. 1	Test no. 2	Test no. 3
Cupric hydroxide (Kocide 101)	62	62	38
Cupric hydroxide + mancozeb (Manzate 200)	25	81	38
Cupric hydroxide + chlorothalonil (Bravo 6F)	33	149	40
Copper ammonium carbonate (Copper-Count-N)	44	...	...
Copper ammonium carbonate + mancozeb	31	...	...
Copper ammonium carbonate + chlorothalonil	23	...	...
Sulfur (50.0%)-copper (4.4%) (Top Cop)	167	...	...
Sulfur (50.0%)-copper (4.4%) + mancozeb	68	...	...
Sulfur (50.0%)-copper (4.4%) + chlorothalonil	181	...	...
Streptomycin (Agri-Mycin 17)	13	29	20
Streptomycin + mancozeb	3	25	41
Streptomycin + chlorothalonil	3	...	18
Mancozeb	102	144	...
Chlorothalonil	448	259	...
Control (no chemical)	518	222	325
FLSD ($P = 0.05$)	44	50	43

[a] Rates were those recommended for field use and expressed as amount per liter: cupric hydroxide (4.8 g), copper ammonium carbonate (5 ml), sulfur (50%)-copper (4.4%) (5 ml), streptomycin sulfate (1.2 g), mancozeb (2.4 g), and chlorothalonil (2.5 ml).

[b] Each value is a mean of counts collected from two (tests 1 and 3) or three (test 2) similar but separate runs with 10–15 replicates in each run. Counts were made 12–15 days after inoculation.

Plants for the tests were grown until 20–25 cm tall on a greenhouse bench singly in 266-ml paper cups containing the soil mix described before. Diseased plants were produced by inoculating healthy plants with a paint sprayer as described previously 10 days before transplanting into the field.

Disease was estimated by rating lesion severity on foliage and by counting the harvested fruit with speck. Visual ratings were made on individual plants on a 0–10 scale where 0 = no bacterial speck lesions, 1 = a few isolated lesions, and 10 = several to many lesions on most leaves. Six hand harvests were made each year from late June until early August. Ripe and pink fruit were harvested, weighed, and speck-infected fruit counted. In 1979, the effect of the chemical treatments on epiphytic populations of PST was determined by collecting leaf samples and assaying in the laboratory seven times from 28 May to 12 July. Leaflets (20 per cultivar in each plot) free of speck symptoms were collected at random, placed in polyethylene bags on ice, and processed within 3 hr. Each leaf sample was placed in 100 ml of sterile distilled water in a 250-ml flask, shaken vigorously for 10 min with a wrist-action shaker, and appropriate dilutions plated in five plates of KMB. Plates were incubated at 25 C and colonies of PST were counted after 72–96 hr. Comparison of a pure plate culture of PST with the dilution plates under fluorescent light allowed for distinguishing and counting colonies. Colonies were selected at random from the dilution plates and tested for oxidase reaction (10), hypersensitivity on tobacco (8), and for pathogenicity on tomato to confirm that colonies being counted were PST.

RESULTS

Greenhouse and growth chamber tests. *Effect of various chemicals and combinations.* A wide range of chemicals and combinations significantly reduced lesion numbers on Chico III tomato plants (Table 1). Chlorothalonil alone was the only chemical tested that failed to give a response. Except for the poor performance of sulfur (50%)-copper (4.4%) (Top Cop), most bactericide and bactericide-fungicide combinations were effective. Although there was considerable variation in lesion counts among trials, streptomycin was more effective than the copper compounds when all tests were considered. Mancozeb significantly reduced lesion counts when used alone.

Effect of interval between application and inoculation. Cupric hydroxide alone and in combination with mancozeb or chlorothalonil showed excellent residual activity when applied 1 hr, 3.5 days, and 7 days before inoculation (Table 2). Streptomycin combined with either mancozeb or chlorothalonil was more effective than cupric hydroxide or cupric hydroxide plus fungicide combination when applied 1 hr before inoculation but not when applied 3.5 and 7 days before inoculation.

Effect of sprinkler irrigation on residual activity. The application of 5.0 cm of irrigation within 7 days after chemical application had a significant influence on chemical effectiveness in some cases but not in others (Table 3). Plants sprayed with a streptomycin plus chlorothalonil mixture had significantly more lesions when subjected to irrigation than when no irrigation was applied. Lesion counts were usually higher on irrigated plants but were not usually significantly ($P = 0.05$) different because of great variation in counts among plants. Lesion counts on plants treated with the bactericides or bactericide plus fungicide combinations and kept dry were always significantly lower than on untreated checks, suggesting good residual activity after 7 days.

Effect of aging of mancozeb-copper mixture on efficacy. Mean numbers of lesions on plants treated with mancozeb plus cupric hydroxide mixed and held 24, 7, 4, and 0 hr before use were 74, 83, 32, and 67, respectively, whereas the mean count on control plants was 373. Lesion counts among the chemical treatments were not significantly different ($P = 0.05$), but all were significantly different from the control.

Laboratory sensitivity tests. *Toxicity of fungicides to PST.* Few of the 30 fungicides tested were toxic to PST in vitro (Table 4). Fungicides listed in the methods section but omitted from Table 4 did not significantly inhibit growth of PST. Toxicity was generally limited to the organic sulfur compounds (dithiocarbamates). These compounds caused 80–100% growth inhibition when PST was streaked directly on plates of KMB containing field rates (Table 4). Zones of inhibition also occurred when the same chemicals were placed in agar wells and lawns of bacteria were established (Table 4). Maneb was more toxic than either maneb-$ZnSO_4$ or mancozeb, and all three maneb-type compounds were more toxic than zineb in the agar well tests. Thiram caused wide zones of inhibition, but the zones were indistinct with scattered bacterial colonies, suggesting low toxicity.

The high toxicity of compounds containing ethylenebisdithiocarbamate (EBDC) was further evident when PST was streaked on agar (KMB) containing 50, 25, 10, 5, and 1% recommended field rate of mancozeb. No growth occurred on agar containing the 50 and 25% rates. Growth was inhibited about 70% at the

Table 2. Mean number of lesions per plant on Chico III tomato plants sprayed with selected chemicals and inoculated with *Pseudomonas syringae* pv. *tomato* at three intervals thereafter

Treatment[a]	Time between treatment and inoculation[b]		
	1 hr	3.5 days	7 days
Cupric hydroxide	210	71	83
Cupric hydroxide + mancozeb	229	65	53
Cupric hydroxide + chlorothalonil	137	115	129
Streptomycin + mancozeb	101	88	108
Streptomycin + chlorothalonil	50	80	97
Control (no chemical)	564	913	584
FLSD ($P = 0.05$)	67	60	124

[a] Rates were those recommended for field use and are expressed as amount per liter: cupric hydroxide (4.8 g), streptomycin sulfate (1.2 g), mancozeb (2.4 g), and chlorothalonil (2.5 ml).
[b] Each value is a mean of 10 replicates.

Table 3. Lesion counts on Chico III tomato plants sprayed with various chemicals and subjected to sprinkler irrigation or kept free of moisture before inoculation with *Pseudomonas syringae* pv. *tomato*

Treatment[a]	Irrigation[b]	No. lesions/plant[c]
Cupric hydroxide	No	158
	Yes	257
Cupric hydroxide + mancozeb	No	109
	Yes	158
Cupric hydroxide + chlorothalonil	No	117
	Yes	150
Streptomycin + mancozeb	No	173
	Yes	241
Streptomycin + chlorothalonil	No	122
	Yes	263
Control (no chemical)	No	424
	Yes	336
FLSD ($P = 0.05$)		118

[a] Rates were those recommended for field use and are expressed as amount per liter: cupric hydroxide (4.8 g), streptomycin sulfate (1.2 g), mancozeb (2.4 g), and chlorothalonil (2.5 ml).
[b] A total of 5 cm of irrigation was applied: 2.5 cm 3 and 6 days after chemical application. Plants were inoculated 7 days after chemical application.
[c] Each value given is a mean of 10 replicates; the experiment was repeated once.

10% rate but was normal at the 5 and 1% rates. Growth rate as determined by dilution plating 8 hr after inoculation was inhibited 100, 100, 100, 96, and 48% when PST was grown by shake culture in nutrient broth containing 100, 50, 25, 10, and 5% field rate of mancozeb, respectively. No inhibition of PST occurred when it was grown on KMB or in nutrient broth containing four times the field rate of chlorothalonil.

Toxicity of bactericides to PST. Cupric hydroxide, copper ammonium carbonate, and streptomycin sulfate, when incorporated into agar at field rates, completely inhibited PST (Table 4). Cupric hydroxide in agar at 50, 25, and 10% of field rates inhibited growth on streaked plates by 100, 40, and 0%, respectively. Copper ammonium carbonate was weakly inhibitory (20% growth reduction) when incorporated in agar at 50% field rate but caused no growth retardation at 25 and 10% field rates. The sulfur (50%)-copper (4.4%) compound was naturally contaminated with a fluorescent bacterium tolerant to copper, making testing on agar impossible. Streptomycin caused complete inhibition when incorporated in agar at 50 and 25% field rates. A few isolated colonies grew on agar with the 10% rate. Transfers made from two of these colonies were resistant to normal rates of streptomycin and remained pathogenic to tomato. Streptomycin also caused large zones of inhibition when placed in agar wells (Table 4). All copper compounds caused small inhibition zones when tested similarly.

Toxicity of fungicide plus bactericide mixtures to PST. Some compounds were significantly more toxic to PST when combined than when used separately (Table 4). The increased toxicity of the mixtures, particularly mancozeb plus cupric hydroxide, suggested a synergistic rather than additive effect. Later tests showed that several other copper compounds acted synergistically with mancozeb in inhibiting PST in vitro (Table 5). A mixture rate study with mancozeb plus cupric hydroxide suggested that the rate of cupric hydroxide was more critical than the rate of mancozeb in influencing inhibition of PST in vitro (Table 6). Aging of a mancozeb plus cupric hydroxide mixture had a slightly detrimental effect on its toxicity to PST. Inhibition zones averaged 9.2, 11.7, 12.4, and 13.9 mm when the mixture was prepared 24, 7, 4, and 0 hr before use and placed in agar wells.

There was no synergistic action when the mancozeb plus streptomycin or chlorothalonil plus cupric hydroxide mixtures were tested against PST.

Field evaluations. The 1979 growing season was unusually cool and moist for Georgia. The 10.9 cm of rainfall that occurred during May was distributed so that a significant amount was recorded on each of 14 days. High epiphytic populations developed on foliage, and many fruit were infected early in the season (Table 7). Data collected from Chico and Marion plants were similar so only Chico data are presented. Cupric hydroxide alone and in combination with mancozeb or chlorothalonil was effective in reducing epiphytic populations of PST on foliage throughout the growing season (Table 7). Streptomycin in combination with mancozeb or chlorothalonil was also effective but performed more erratically than treatment with cupric hydroxide. Mancozeb reduced populations in some cases but performed erratically on different sampling dates. Bacterial populations on chlorothalonil-treated plants were high throughout the growing

Table 4. In vitro sensitivity of *Pseudomonas syringae* pv. *tomato* to various bactericides and fungicides alone and in combination

Chemical name[a]	Growth inhibition[b]	
	Inhibition on streaked plates with chemical in agar (%)	Clear zones around agar wells filled with chemical (mm)
Maneb	100	5.3
Maneb-$ZnSO_4$	100	2.8
Mancozeb	100	2.9
Zineb	80	t[c]
Thiram	80	11.8[d]
Ferbam	80	1.4
Thiophanate methyl-EBDC (Zyban)	100	t
Cupric hydroxide	100	1.8
Copper ammonium carbonate	100	t
Sulfur (50%)-copper (4.4%)	–[e]	0
Streptomycin sulfate	100	23.5
Maneb + cupric hydroxide	...[f]	14.3
Maneb-$ZnSO_4$ + cupric hydroxide	...	12.8
Mancozeb + cupric hydroxide	...	11.8
Mancozeb + copper ammonium carbonate	...	9.3
Mancozeb + sulfur (50%)-copper (4.4%)	...	1.8
Mancozeb + streptomycin sulfate	...	17.8
Zineb + cupric hydroxide	...	6.5
Ferbam + cupric hydroxide	...	4.8
Zyban + cupric hydroxide	...	13.1
Chlorothalonil + cupric hydroxide	...	2.6
Chlorothalonil + streptomycin sulfate	...	25.1
FLSD ($P = 0.05$)		2.4

[a] Trade names and rates are given in Materials and Methods.
[b] Growth was rated visually and zones were measured after 48 hr at 23-25 C.
[c] t = Trace or inhibition zone too small for measurement.
[d] Zone was indistinct with scattered colonies.
[e] Commercial product was contaminated with a bacterium.
[f] Chemical mixtures were not incorporated into the solid medium.

Table 5. In vitro sensitivity of *Pseudomonas syringae* pv. *tomato* to various copper compounds alone and in combination with mancozeb

Treatment[a]	Zone of inhibition around agar wells filled with chemical (mm)
Cupric acetate	2.2
Cupric acetate + mancozeb	10.9
Cupric chloride	4.3
Copper chloride + mancozeb	12.1
Cupric hydroxide	<1.0
Cupric hydroxide + mancozeb	11.4
Cupric nitrate	2.4
Cupric nitrate + mancozeb	10.6
Cupric oxide	0.0
Cupric oxide + mancozeb	2.7
Cupric sulfate	2.3
Cupric sulfate + mancozeb	11.8
Mancozeb alone	2.4
FLSD ($P = 0.05$)	1.1

[a] Rate for all copper compounds was 4.8 g/L and mancozeb was 2.4 g/L.
[b] Each value is the mean of measurement on inhibition zones around 12 wells.

Table 6. In vitro sensitivity of *Pseudomonas syringae* pv. *tomato* to mancozeb plus cupric hydroxide mixtures containing various rates of each chemical

Treatment[a]		Zone of inhibition around agar wells filled with chemical (mm)[b]
Mancozeb (g)	Cupric hydroxide (g)	
2.4	0.0	2.3
2.4	0.6	0.6
2.4	1.2	2.7
2.4	2.4	6.6
2.4	4.8	11.5
0.0	4.8	<1.0
0.3	4.8	8.9
0.6	4.8	8.3
1.2	4.8	9.9
FLSD ($P = 0.05$)		1.8

[a] Rates shown are Manzate 200 and Kocide 101.
[b] Each value is a mean of measurements of inhibition zones around 12 wells.

Table 7. Epiphytic populations (cfu/ml) of *Pseudomonas syringae* pv. *tomato* on symptomless leaves and percentages of fruit with bacterial speck from Chico III tomato plants sprayed at 7-day intervals with selected chemicals in the field near Athens, GA, in 1979

Treatment[a]	Sample dates[b]								Percent fruit with speck[c]
	28 May	4 June	11 June	18 June	25 June	3 July	12 July	Mean	
Cupric hydroxide	0.0	26.5	2.0	1.7	0.0	6.0	13.1	7.0	25.0
Cupric hydroxide + mancozeb	0.0	6.3	0.0	0.0	0.0	0.0	0.0	0.9	25.6
Cupric hydroxide + chlorothalonil	0.0	14.0	1.3	0.0	0.3	0.0	0.0	2.2	21.2
Streptomycin + mancozeb	9.7	43.4	27.2	8.4	0.2	97.0	1.0	26.7	22.9
Streptomycin + chlorothalonil	12.3	113.8	23.9	25.1	1.8	6.0	11.7	27.8	25.1
Mancozeb	109.5	91.3	177.7	232.3	0.8	150.0	68.7	118.6	30.9
Chlorothalonil	209.3	220.9	171.1	100.5	83.4	225.0	123.7	161.9	30.7
Control[d]	202.9	224.7	137.6	96.4	90.5	200.0	89.6	148.8	34.4
FLSD ($P = 0.05$)								29.2	4.8

[a] Rates of chemicals in kilograms or liters per 378 L of spray were: cupric hydroxide (Kocide 101, 4.4 kg), streptomycin (Agri-Mycin 17, 200 ppm or 1.1 kg), mancozeb (Manzate 200, 2.2 kg), and chlorothalonil (Bravo 6F, 1.2 L).
[b] Each value is a mean of plate counts made on four replicates (plots). Twenty symptomless leaves were collected at random from each plot, placed in 100 ml of distilled water, and samples plated on five plates of KMB.
[c] Mean of four replicates harvested six times.
[d] Chlorothalonil was applied to this plot beginning 25 May to prevent loss of plots to early blight.

season. Fruit infection was more severe in early harvests and ranged from 21 to 34% among the eight treatments tested. All bactericide and bactericide plus fungicide treatments significantly reduced speck infection on fruit, but infection rates were fairly high on fruit from all treatments. Treatments were less effective in reducing fruit infection than in controlling epiphytic populations on leaves. Total yields of fruits were not significantly different among treatments so yield data are not presented.

The 1981 growing season was unfavorable for bacterial speck development except for a brief cool, moist period in late May and early June. Disease that developed during this period allowed evaluation of the 14 treatments tested (Table 8). Streptomycin sulfate alone and in combination with either mancozeb or chlorothalonil significantly reduced lesion counts on foliage and fruit compared with chlorothalonil. Plots receiving streptomycin alone, however, were moderately infected with early blight caused by *Alternaria solani* and heavily infected by leaf mold caused by *Cladosporium fulvum*, whereas all other treatments were mostly free of these diseases. Cupric hydroxide alone and in combination with mancozeb also significantly reduced bacterial speck infection on both foliage and fruit. The 4.4-kg rate of cupric hydroxide, especially in combination with mancozeb, was more effective than 2.2 kg. Copper ammonium carbonate alone or in combination with mancozeb also gave significant control. The sulfur (50%)-copper (4.4%) compound was significantly less effective than other bactericide or combination treatments. Mancozeb alone resulted in slight reductions in bacterial speck on foliage and fruit. Yields of total fruit (healthy plus diseased) were not significantly different among treatments except that plots without diseased plants produced significantly higher yields than plots with diseased plants regardless of treatment (Table 8).

Table 8. Bacterial speck ratings on and yields of Chico III tomato plants receiving various chemical treatments in field tests near Athens during 1981

Chemical treatment[a]	Disease ratings[b]			Yield[c] (metric tons/ha)	Percent fruit with speck[d]
	22 May	29 May	4 June		
Cupric hydroxide (low rate)	0.04	0.18	0.72	77.53	5.0
Cupric hydroxide (high rate)	0.00	0.02	0.45	77.95	5.9
Cupric hydroxide (low rate) + mancozeb	0.00	0.2	0.13	80.28	4.4
Cupric hydroxide (high rate) + mancozeb	0.00	0.01	0.04	71.72	2.1
Streptomycin sulfate	0.04	0.00	<0.01	79.00	2.4
Streptomycin sulfate + mancozeb	0.00	0.01	<0.01	68.95	2.8
Streptomycin sulfate + chlorothalonil	0.00	0.00	<0.01	80.86	5.3
Sulfur (50%)-copper (4.4%)	0.12	1.67	4.90	75.94	20.7
Sulfur (50%)-copper (4.4%) + mancozeb	0.07	1.49	2.84	77.53	18.4
Copper ammonium carbonate	0.01	0.36	0.83	79.86	7.2
Copper ammonium carbonate + mancozeb	0.00	0.04	0.12	72.55	3.3
Mancozeb	0.87	2.40	3.60	81.45	28.4
Chlorothalonil	1.00	3.09	4.83	73.29	32.0
Chlorothalonil (no diseased plants)	0.00	0.30	0.36	97.88	1.5
FLSD ($P = 0.05$)	0.38	0.63	0.90	9.92	3.3

[a] Rates of chemicals in kilograms or liters per 378 L of water were: cupric hydroxide (2.3 [low rate] or 4.6 [high rate] kg), streptomycin sulfate (1.1 kg), sulfur (50%)-copper (4.4%) (1.9 L), copper ammonium carbonate (1.9 L), mancozeb (2.2 kg), and chlorothalonil (1.6 L).
[b] Ratings were made on a 0–10 scale where 0 = no disease and 10 = numerous lesions involving most foliage. Ratings made on 22 May did not include the diseased plants established as inoculum sources. The 29 May and 4 June ratings also included originally diseased plants.
[c] Yields resulting from six hand harvests were made from 25 June to 12 August and included healthy and diseased fruit.
[d] Counts made on the first harvest only on 25 June.

DISCUSSION

Historically, transplant growers in southern Georgia have used various formulations of maneb or chlorothalonil in a preventive program to control fungal diseases on tomato transplants. Before 1978, bactericides were used on tomato transplants primarily on an "as needed" basis to control sporadic outbreaks of bacterial spot caused by *Xanthomonas campestris* pv. *vesicatoria* (hereafter designated XCV). The major outbreak of bacterial speck in 1978 suggested the need for a bactericide in the preventive spray program to limit losses to Georgia transplant growers and to minimize introduction of inoculum sources into northern fruit-producing areas where transplants are shipped. Results of our tests suggested that both streptomycin and certain fixed copper compounds provide sufficient control to be included

in the tomato transplant spray program. These compounds reduced bacterial speck lesions on foliage and fruit as well as epiphytic populations of PST on field-grown plants. Elimination of epiphytic populations is important because PST may reside for extended periods in protected sites on plants under adverse environmental conditions to cause infection when conditions again become conducive (21,23). The Georgia Cooperative Extension Service currently recommends a streptomycin application when transplants are in the true-leaf stage to eliminate any seedborne (6) or weed-associated (21) inoculum followed by routine sprays of mancozeb plus fixed copper. Streptomycin is recommended as a replacement for the copper in later sprayings if weather conditions become conducive for bacterial speck development or the disease appears in transplant fields. Although our tests suggest that streptomycin is more effective than fixed coppers against PST, a fixed copper plus mancozeb combination is the best choice for routine use because of resistance (24) to streptomycin in XCV caused by asssociated R plasmids (11). Although streptomycin resistance has not been detected in field populations of PST in Georgia, such resistance has been reported among other pseudomonads (9,25), and resistant strains may appear with widespread use of the antibiotic. Our recovery of a streptomycin-resistant strain of PST in laboratory screening tests suggests this possibility.

Extension personnel in Georgia recommend tank mixtures of mancozeb and fixed copper for bacterial disease control because field observations (*unpublished*) suggest that a combination is more effective than copper alone. Although our greenhouse and field results did not consistently indicate benefits from mixing, in vitro tests suggest a synergistic action between the dithiocarbamate fungicides, especially Mn EBDC compounds, and certain copper compounds. Some earlier field tests also suggest a synergistic response (3,12,14,15,17). In Pennsylvania, tribasic copper sulfate plus mancozeb gave significantly better control of bacterial speck than tribasic copper alone (12,14,15,17). Similar results were obtained in control of bacterial spot in Florida (3). Failure to show more benefit of fungicide plus bactericide mixtures in our tests possibly resulted from the better than normal spray coverage in our tests that gave optimum opportunity for copper activity. Our results and another recent study (16) fail to show increased activity of a mancozeb plus copper mixture after aging as some growers contend.

Toxicity to PST among fungicides in the laboratory tests was limited to the organic sulfur compounds. Maneb compounds alone proved highly toxic to PST in vitro and significantly reduced lesion counts in growth chamber tests. These results were unexpected because EBDC compounds are not recognized for their toxic effect on bacteria. Mancozeb alone gave either poor or no control of bacterial speck in our field tests as has been reported (14). Ways to maintain toxicity of EBDC compounds to PST under field conditions perhaps should be explored in view of their relatively high toxicity in vitro and the limited number of bactericides available.

Despite our generally favorable results, bactericide and bactericide plus fungicide combinations have not always controlled bacterial speck (4,13). Even in our tests, the best chemical treatments were less effective in preventing fruit infection than was expected based on greenhouse results, possibly because of the readily available inoculum sources in the form of diseased plants transplanted among the healthy plants or because of the weathering of the chemicals. PST spreads rapidly from point inoculum sources (23) and is difficult to control once established. Bactericide or bactericide plus fungicide combinations must be strictly preventive if chemical control of bacterial speck is to be successful.

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