Control of Bacterial Spot of Pepper Initiated by Strains of *Xanthomonas campestris* pv. *vesicatoria* That Differ in Sensitivity to Copper

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ABSTRACT

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Differences in sensitivity to copper were detected among strains of *Xanthomonas campestris* pv. *vesicatoria*. Sensitivity was judged on the basis of viability of cells after exposure to copper solutions. The solutions were obtained from suspensions of fixed copper in water of pH 7.0–7.5. The amounts of copper in solution in the suspensions were 1–2 mgL^{-1}. Adding mancozeb to a suspension of the fixed copper increased soluble copper to about 13 mgL^{-1}. Strains sensitive to copper were killed by both solutions, but copper-resistant strains were killed only by the higher amount of soluble copper. In the field, sprays of fixed copper controlled sensitive strains only. Sprays of the copper-mancozeb mixture controlled both strains of the bacterium but greater control of the copper-sensitive strains was obtained.

More than 60,000 acres in Florida are planted to pepper and tomato each year. These plants are sprayed with fixed copper compounds to control bacterial spot, which is caused by *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye (8). The relatively low cost and low toxicity to mammals of fixed copper compounds give them an advantage over other chemicals for control of foliar bacterial diseases. The efficacy of fixed copper compounds in control of bacterial diseases, however, has been variable (5). Adding mancozeb to a fixed copper compound improves control (2).

Very little is known about the activity of copper as a bactericide even though copper as a fungicide has received much attention (6). In a recent review, the suggestion was made that copper merely hardens the plant surface, resulting in less penetration by bacterial pathogens (5). However, populations of *X. c.* pv. *citri* at natural concentrations (10^6 cells ml^{-1}) were killed in drops of water placed on copper-sprayed grapefruit leaves placed in a moist chamber (11). Viability of the bacteria was determined by streaking portions of the drop onto nutrient agar.

Sensitivity to copper differed among strains of *X. c.* pv. *vesicatoria* in preliminary tests in which viability of cells in drops of water placed on copper-sprayed pepper leaves was determined. The objective of this work was to determine the sensitivity to copper of cultures of the pepper strain of *X. c.* pv. *vesicatoria* collected over a period of years and to determine the significance of the sensitivity on field control of bacterial spot of pepper.

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MATERIALS AND METHODS

Strains of *X. c.* pv. *vesicatoria* were collected from pepper plants from different locations in Florida during a 13-yr period. One to 10 cultures in each collection were maintained in sterile tap water. Viable cells were recovered by streaking the stock culture onto nutrient agar. For multiplication, a single colony was placed into nutrient broth and the culture shaken continously. The pathogenic race of each strain was determined as described previously (3).

A routine procedure was followed to determine sensitivity of bacteria to copper. The bacteria were removed from nutrient broth by centrifugation and suspended in sterile tap water of pH 7.5. Suspensions were adjusted to a turbidity of 0.3 A at 600 nm in a spectrophotometer (Spectronic 20). Such suspensions were assumed to contain 10^8 cells ml^{-1}. About 5×10^5 cells were transferred to 1 ml of each test solution and the viability of the bacteria at subsequent times was assayed by streaking the suspensions onto nutrient agar. Three replicates of each treatment were included for each test of viability.

Copper solutions were prepared by adding 0.3 g of 77% cupric hydroxide (Kocide 101) to 100 ml of deionized water at pH 7.0 or tap water at pH 7.5. Mancozeb solutions were prepared by suspending 0.15 g of 80% mancozeb (Dithane M-45) in 100 ml of water. The same materials and concentrations were used when copper and mancozeb were combined. Suspensions of chemicals were shaken for 4 hr and the insoluble copper, or mancozeb, was removed by filtration through a membrane filter of 0.2 μm pore diameter. Sterile water was used as a control. Analyses of copper content in the solutions were made by atomic absorption spectrophotometry by the Soils Testing Laboratory of the University of Florida.

In one test, several concentrations of copper were obtained from copper sulfate in deionized water. After filter sterilization, solutions were diluted sequentially 1:1 with sterile deionized water from 128 mgL^{-1} to 1 mgL^{-1} of copper. Two strains, 80-5 (sensitive) and 80-6 (resistant), were tested for viability in the solutions after 3 hr of exposure.

Field test. The importance of copper sensitivity of strains of *X. c.* pv. *vesicatoria* on control was assessed in a field trial. Six treatments (Table 1) were arranged in a complete randomized block with four replicates. Each plot contained 12 pepper plants that were set when about 10 cm tall. The transplants were placed about 30 cm apart in rows that were 1 m apart. Each treatment row was separated by a buffer row of plants.

The strains of the bacterium were established in the plots by inoculation of the plants. A suspension containing 10^5 cells/ml^{-1} was injected into an area of 1 cm^2 of one leaf per plant one day before transplanting. A mixture of four different

Table 1. Slope and correlation coefficients[a] for bacterial spot progress in plots of pepper

Spray treatment	Copper resistance of inoculum	Slope	Corr. coef.
Control	+	0.1	0.95
Control	−	0.1	0.99
Copper	+	0.07	0.83
Copper	−	0.03	0.90
Copper + mancozeb	+	0.05	0.98
Copper + mancozeb	−	0.01	0.81

[a] Disease severity was estimated by the Barratt-Horsfall system and converted to percent disease by Elanco tables. The percent disease was transformed by gompits $Y = -\ln(-\ln[y])$. Slopes and correlation coefficients of disease over time were determined from five rating dates.

Table 2. Viability of strains of *Xanthomonas campestris* pv. *vesicatoria* from pepper in Florida after exposure to copper

Strain designation[a]	Race of pepper strain	Hours of exposure to copper		
		0	4	24
68-1	1	+[b]	+	+
69-1	1	+	−	−
69-20	2	+	+	+
70-7	2	+	+	+
71-21	1	+	−	−
71-34	2	+	+	+
72-6	2	+	+	+
76-1	2	+	+	+
76-2	2	+	+	+
77-3	1	+	+	+
80-5	1	+	−	−
80-6	2	+	+	+
81-18	2	+	+	+

[a] First two numbers refer to year of isolation.
[b] + = Growth on nutrient agar (NA); − = no growth on NA. Each + or − represents three replicates. Source of copper was Kocide 101 at a concentration of 3 gL^{-1}.

Table 3. Viability of two strains of *Xanthomonas campestris* pv. *vesicatoria* after exposure to various amounts of soluble copper from copper sulfate for 3 hr

Copper (mgL^{-1})	Copper-sensitive strain	Copper-resistant strain
0	+[a]	+
1	−	+
2	−	+
4	−	+
8	−	+
16	−	±
32	−	±
64	−	−
128	−	−

[a] + = Growth on nutrient agar (NA); ± = growth on NA, but fewer colonies; and − = no growth on NA. Each + or − represents three replicates.

Table 4. Viability of a copper-resistant strain of *Xanthomonas campestris* pv. *vesicatoria* after exposure to solutions from bactericides

Exposure (hr)	Copper[a]	Copper + mancozeb	Mancozeb[a]	Control
0.0	+[b]	+	+	+
0.25	+	+	+	+
0.5	+	+	+	+
1.0	+	±	+	+
2.0	+	−	+	+
4.0	+	−	+	+
24.0	+	−	−	+

[a] Copper was obtained from Kocide 101, and mancozeb was obtained from Dithane M-45. Kocide was used at 3 gL^{-1} and Dithane M-45 at 1.5 gL^{-1}. Insoluble material was filtered from suspensions 4 hr after materials were added to water.
[b] + = Growth on nutrient agar (NA); ± = growth on NA, but fewer colonies; and − = no growth on NA. Each + or − represents three replicates.

Table 5. Amount of copper in solution at various times after adding pesticides to water

Time of sampling (hr)	Copper[a]	Copper + mancozeb[a]	Water
0.0	1.2[b]	2.9	0.1
0.25	1.3	4.1	···
0.5	1.3	4.3	···
1.0	1.2	4.8	···
2.0	1.5	7.8	···
4.0	1.5	10.3	···
8.0	1.5	13.0	···
24.0	1.1	9.5	···

[a] Copper was obtained from Kocide 101, and mancozeb was obtained from Dithane M-45. Kocide was used at 3 gL^{-1} and Dithane M-45 at 1.5 gL^{-1}.
[b] Figures are mgL^{-1} of copper. Mean of three replicates.

copper-resistant cultures of the pepper strain race 2 was inoculated into leaves of the cultivar Early Calwonder (ECW). Plants of the 10-R breeding line, which is near-isogenic to ECW, were inoculated with a mixture of four different copper-sensitive cultures of pepper strain race 1. The two cultivars of pepper were used to restrict movement of the copper-resistant bacteria among plots. Plants of 10-R are hypersensitive to race 2 isolates.

Copper and copper plus mancozeb were sprayed onto plants at concentrations used in the laboratory. Sprays were applied with a hand-operated compressed-air single-nozzle sprayer. Coverage of plants was accomplished by making one to three passes over the plants, depending upon the size of plants. The first spray was applied five days after the plants were set in the field. The sprays were applied on a 3- to 4-day schedule thereafter.

Ratings for disease severity were made on a weekly basis with the Barratt-Horsfall system and were converted to percentages by the Elanco tables (10). The disease proportions were linearized with the Gompertz transformation ($Y = -\ln(-\ln[y])$, where Y equals gompit and y equals proportion of disease (1). The slope of the linear regression of the transformed diseased proportions was the epidemic rate. The regression was calculated from five ratings of disease. Statistical differences of treatment means were determined by analysis of variance and Duncan's multiple range test using the SAS program under the General Linear Model procedure.

RESULTS

Cells of two strains that survived in drops of water placed on copper-sprayed pepper leaves were not killed in the copper solutions obtained by filtering insoluble copper from suspensions of 77% cupric hydroxide. Cells of two other strains that were killed on copper-sprayed pepper leaves were killed in the copper solutions. Thus, screening of strains of *X. c.* pv. *vesicatoria* for sensitivity to copper was possible with copper solutions obtained from the fixed copper.

Variations in copper sensitivity of strains occurred from collection to collection, but the strains within a collection were similar with regard to copper sensitivity. The sensitivity of a representative strain of each collection is listed in Table 2. Sensitivity to copper was not related to the year of isolation. Cultures of race 2 of the pepper strain were always resistant to copper but strains of race 1 were either sensitive or resistant.

One mgL^{-1} of copper in solution killed all cells of the sensitive strain in 3 hr of exposure, but the viability of a resistant strain in 8 mgL^{-1} appeared undiminished when compared with the control. Some reduction in the viability of the resistant strain occurred in 16 mgL^{-1} of copper and only a few cells survived in 32 mgL^{-1}. All cells were killed in 64 mgL^{-1} of copper during 3 hr of exposure (Table 3).

Cells of a copper-resistant strain survived for 24 hr in a solution of copper from 77% cupric hydroxide as well as they did in sterile tap water (Table 4), but the same strain survived only 1 hr in a solution of copper and mancozeb. The cells survived more than 4 hr but less than 24 hr in the solution of mancozeb without copper. The amount of copper in solution did not change during a 24-hr period in a suspension of 77% cupric hydroxide and was between 1 and 2 mgL^{-1} (Table 5), but the amount of copper in solution increased to 13 mgL^{-1} during the first 8 hr after mixing mancozeb and cupric hydroxide. The copper content of the tap water used in the suspensions was about 0.1 mgL^{-1}.

Field test. Bacterial spot on unsprayed ECW plants inoculated with the copper-

resistant strain progressed at the same rate as on unsprayed 10-R plants inoculated with the copper-sensitive strain (Fig. 1). Progress of disease on plants inoculated with the copper-resistant strain and sprayed with copper alone was not statistically different from the controls. Copper or copper plus mancozeb reduced the progress of bacterial spot on plants inoculated with the copper-sensitive strain. The mixture of copper plus mancozeb was more effective than copper alone in controlling the copper-resistant strain and nearly as effective as copper alone in controlling the copper-sensitive strain. The correlation coefficients and slopes of the regression lines derived from transformed disease proportions over time are presented in Table 1.

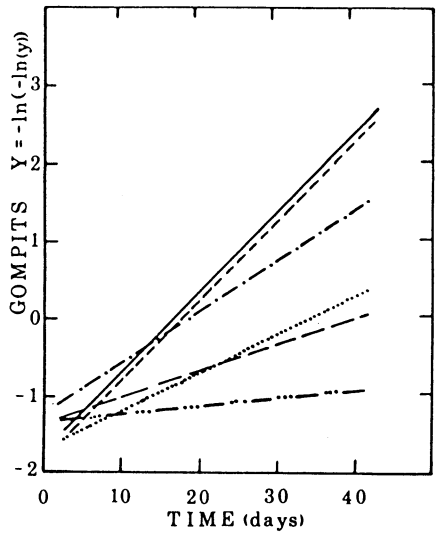

Fig. 1. Regression lines for bacterial spot increase in plots of pepper. Treatments consisted of inoculation with copper-resistant strains of *Xanthomonas campestris* pv. *vesicatoria* and either not sprayed (——), sprayed with copper (·—·), or sprayed with copper plus mancozeb (····); inoculation with copper-sensitive strains and either not sprayed (——), sprayed with copper (– –), or sprayed with copper plus mancozeb (···—). Regression lines were derived from five ratings of proportion of disease that were transformed to gompits. The correlation coefficient and slope of each regression line are given in Table 1.

DISCUSSION

Variation in sensitivity to copper was discovered among strains of *X. c.* pv. *vesicatoria* in laboratory tests. Results of the field test for control of bacterial spot of pepper supported the laboratory determination of copper sensitivity. The relationship of the field tests to the laboratory tests was important because the amount of soluble copper in rainwater on copper-sprayed plants in the field might not be the same as the amount of soluble copper in suspensions of fixed copper in a glass container. Soluble copper increases if water of low pH is used or if certain organic compounds are present (6).

The mixture of mancozeb with fixed copper provided better control of bacterial spot in the field than the fixed copper alone, as reported previously (2). The increase in water-soluble copper in the mixture apparently is important for the increased disease control. Parsons and Edgington found ethylene thiram monosulfide (ETM), an ethylenebis-dithiocarbamate, to be antibacterial but adding copper did not increase the stability of ETM (9).

Resistance to copper in bacteria was not expected because many sites for biocidal action occur in living cells (6). Those sites, however, would not be inactivated if resistance was the result of reduced uptake of the element by the bacterium. Very little study has been directed to uptake of copper by bacteria but a great deal of study has been directed to the uptake of iron (7). With iron, at least three mechanisms of uptake have been identified. Each may be defective, resulting in reduced uptake. Similar uptake systems may occur for copper.

The decreased copper-sensitivity of strains of *X. c.* pv. *vesicatoria* has existed in Florida for many years. Spray materials for control of bacterial spot in Florida were probably screened with the copper-resistant strain present. In fact, the copper-resistant strain may have been an important factor in the development of the copper-mancozeb mixture for control of bacterial spot. Therefore, recommendations for control of bacterial spot with sprays need not be changed based on this work.

The ecology of the strains of *X. c.* pv. *vesicatoria* is of interest. Race 2 of the pepper strain is found very seldom outside Florida, but race 1 occurs in Florida and in other areas of the world (4). This is evidence that race 2, at least, is endemic to Florida. The endemic nature of the bacterium and the judicious use of copper to control bacterial spot would be conducive to selecting and maintaining a copper-resistant strain. The copper-sensitive strains may be imported into Florida on contaminated seeds.

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