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EFFECT OF MAGNESIUM ON THE TOXICITY OF COPPER TO PEPPER/TOMATO LEAF SPOT BACTERIA, *XANTHOMONAS CAMPESTRIX* PV. *VESICATORIA*

S. S. WOLTZ, J. B. JONES AND J. P. JONES
IFAS, University of Florida
Gulf Coast Research & Education Center
5007-60th Street East
Bradenton, FL 34203

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Abstract. **Copper-sensitive and resistant strains of *Xanthomonas campestris* pv. *vesicatoria* (XCV) were grown in specially purified nutrient solutions to study interacting effects of Cu and Mg on the *in vitro* grown of XCV. The Cu-sensitive strain was inhibited more by selected Cu levels than the resistant strain. Toxic Cu levels (1.0 and 3.17 ppm Cu) supported more growth of XCV when higher levels of Mg were furnished (25 versus 2.5 ppm Mg). The highest Cu level was uniformly inhibitory to Cu-sensitive XCV at 0.25, 2.5, and 25 ppm Mg, whereas the Cu-resistant XCV was capable of significant growth at these Mg levels. A reverse relationship also existed whereby growth of XCV at the lowest Cu levels (0 and 0.317 ppm added Cu) was adversely affected by high (25 ppm) versus low (2.5 ppm) Mg. Thus, Mg can apparently induce a "Cu deficiency" or correct a copper toxicity according to the levels of Cu and Mg under study.**

The causal agent of the bacterial leaf spot diseases of pepper and tomato is usually sensitive to Cu toxicity (1, 2). Most control procedures caused by this species include Cu compounds (1, 5, 6). New strains of *Xanthomonas campestris* pv. *vesicatoria* (XCV) in Florida are more resistant to Cu-associated control procedures (2). The success of Cu control of XCV is closely controlled by environmental factors including levels of Mg (1, 3, 6, 8, 9, 10). A review of the literature (8) suggested that Mg might competitively reduce the toxicity of Cu. Experiments were therefore carried out to clarify the potential effects of Mg on Cu toxicity to XCV.

Materials and Methods

Two strains of XCV, pathogenic to both tomato and pepper, were used to test for ameliorating action of Mg in reducing Cu toxicity. One of the strains (80-5) was Cu-susceptible while the other (81-18) was Cu-resistant (5). Inoculum was prepared from 48 hour nutrient yeast dextrose agar Petri plates using a suspension of 1 x 10^8 cfu/ml. Three drops of inoculum (ca. 0.15 ml) were added to each culture vessel, a 125 ml Erlenmeyer flask containing 20 ml of autoclaved (15 min at 1.05 kg/cm^2) liquid medium.

Macro components of the liquid medium, g/liter included: glucose, 5; L-methionine, 1; glutamic acid, 2; $NH_4H_2PO_4$, 1; and K_2HPO_4, 2. Micro components, mg/liter included: $CuSO_4 \cdot 5H_2O$, 0.04; Fe $(NH_4)_2SO_4)_2 \cdot 6H_2O$, 3.5; $MnSO_4 \cdot H_2O$, 1.5; $Na_2MoO_4 \cdot 2H_2O$, 0.03; and ZN $SO_4 \cdot 7H_2O$, 2.2. Media were freed of most of the Mg and Cu impurities by precipitating and filtering them off as ammonium phosphates. The purification procedure employed was based on the analytical chemistry methodology of Kolthoff and Sandell 1948 (4) and included the following steps:

1) Dissolve components for 2 liter nutrient solution in 0.5 liter, adjust pH to 7 with NH_4OH and add 24 ml of concentrated NH_4OH.

2) Stir and add 4 ml of magnesium phosphate crystal "seeding" suspension prepared by dissolving 0.1 g $MgSO_4 \cdot 7H_2O$ and 0.1 g $NH_4H_2PO_4$ in 10 ml of water, then add while stirring 2 ml NH_4OH diluted 1:3 with water.

3) Mix, refrigerate overnight and filter.

4) Put on heated magnetic stirrer, heat to 23-27°C and drive off excess ammonia for 2 hours. Avoid overheating to prevent discoloration of the alkaline solution.

5) Cool, add 1 ml 20% NaOH while stirring, and stir 1 hour more.

6) Adjust pH to 5.8 with H_2SO_4, add micronutrients, make to 2 liters and deliver 20 ml into each 125 ml Erlenmeyer.

Autoclave standardized copper sulfate and magnesium sulfate solution at the same time the culture vessels are autoclaved. Aseptically deliver the variable amounts of Cu and Mg in small, standard volume to establish the experimental variables. This procedure has 2 important purposes: 1) to remove background Cu and Mg, and 2) to insure that the added elements are present in a soluble condition at the outset of the experiment eliminating the risk of loss of nutrient as compounds precipitated during autoclaving. A pH of 5.8 is used as a hydrogen ion concentration that essentially eliminates precipitation of Cu and Mg as phosphates, hydroxides, carbonates or other compounds.

Flasks were cultured in the laboratory (randomized block design in triplicate) on a reciprocal shaker at 72 oscillations per minute at 25-26°C. Low volume in the flasks permitted a gentle back and forth motion of liquid to effect gas exchange and maintain cells in suspension. Cell growth was evaluated by the technique of Starr (7) using optical density through a 1 cm path of 72 hour cultures at 625 nm. Magnesium levels (0.25, 2.5, and 25 ppm Mg) were combined factorially with Cu levels of 0, 0.317, 1.0, 3.17, and 10.0 ppm.

Results and Discussion

The Cu-sensitive strain did not grow as well as the resistant strain in liquid shake culture (Table 1). Growth response for both strains appears to be affected by Cu-mediated Mg responses as well as the more prominent Mg-mediated Cu response. Since none of the treatments in Table 1 has been selected as a control, simple comparisons were made among the indicated treatments.

The Cu-sensitive strain receiving 0.25 ppm Mg had the best growth at 0 and 0.317 ppm Cu and was inhibited by the higher levels. With 2.5 ppm Mg, 0.317 ppm Cu gave the best growth of XCV; the 0 ppm Cu treatment was

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Table 1. Effect of combinations of magnesium and copper on the growth[z] of *Xanthomonas campestris* pv. *vesicatoria in vitro*.

Copper (ppm)	Copper sensitive strain			Copper resistant strain		
	Magnesium (ppm)					
	0.25	2.5	25	0.25	2.5	25
0.0	61[y]	49	25	49	181	285
0.317	58	104	186	44	173	262
1.0	20	51	112	55	114	324
3.17	9	7	37	54	132	351
10.0	8	7	9	30	90	266

[z]Expressed in optical density units, O.D. x 10^3, 1 cm light path at 625 nm.
[y]LSD, 5% level for within table means: copper sensitive strain = 32; copper resistant strain = 74.

inferior due to a Cu deficiency while 1, 3.17 and 10 ppm Cu were inhibitory to XCV growth. With 25 ppm Mg, 0 ppm Cu was again inferior in XCV growth support to 0.317 ppm Cu, the best Cu level; higher Cu levels inhibited growth.

The Cu resistant XCV did not respond differentially to Cu levels at the 0.25 ppm Mg level which was below the optimum for Mg nutrition. With 2.5 ppm Mg, a growth inhibition to Cu was expressed at 10 ppm Cu relative to 0, 0.317 ppm Cu but not at the other levels. With 25 ppm Mg, the 3.17 ppm Cu level produced the most growth of XCV while the lower levels of Cu appeared to be too low for XCV response; 10 ppm Cu was slightly inhibitory.

The Cu-sensitive XCV strain apparently represents a biotype that is more responsive to Cu than the resistant strain since the sensitive strain was affected more by very low as well as moderate and high levels of Cu. The Cu-resistant strain was little affected over a relatively wide range of Cu. The sensitive strain, due to its greater requirement for Cu could also be termed Cu-inefficient.

The sensitive strain receiving 25 ppm Mg grew better with 0.317 than 0 ppm added Cu, possibly reflecting an induced Cu deficiency. With 0.25 and 2.5 ppm Mg, Cu reduced growth at 3 levels, namely 1.0, 3.17, and 10.0 ppm Cu. At 25 ppm Mg, however, growth was reduced only at 3.17 and 10 ppm Cu. The Cu-resistant strain did not respond very much to Cu at 0.25 ppm Mg because Mg supply limitations produced a relatively standard growth rate; there was, however, a slight suppression at 10 ppm Cu, the highest level. With 2.5 ppm Mg the highest Cu level, 10 ppm, suppressed growth. At 25 ppm Mg there appeared to be interactions that produced best growth at 3.17 ppm Cu with significant growth reductions at 0.317 and 10.0 ppm Cu.

Literature Cited

1. Conover, R. A. 1981. Mixtures of copper and maneb or mancozeb for control of bacterial spot of tomato and their compatibility for control of fungus diseases. Proc. Fla. State Hort. Soc. 94:154-156.
2. Cook, A. A. and R. E. Stall. 1982. Distribution of races of *Xanthomonas vesicatoria* pathogenic on pepper. Plant Dis. 66:388-389.
3. Jones, J. B., S. S. Woltz, and J. P. Jones. 1983. Effect of foliar and soil magnesium application on bacterial leaf spot of peppers. Plant Dis. 67:623-624.
4. Kolthoff, I. M. and E. B. Sandell. 1948. Textbook of quantitative inorganic analysis. The MacMillan Co., New York.
5. Marco, G. M. and R. E. Stall. 1983. Control of bacterial spot of pepper initiated by strains of *Xanthomonas campestris* pv. *vesicatoria* that differ in sensitivity to copper. Plant Dis. 67:779-781.
6. Olson, B. D. and A. L. Jones. 1983. Reduction of *Pseudomonas syringae* pv. *morsprunorum* on Montmorency sour cherry with copper and dynamics of the copper residues. Phytopathology 73:1520-1525.
7. Starr, M. P. 1946. The nutrition of phytopathogenic bacteria. I. Minimal nutritive requirement of the genus Xanthomonas. J. Bacteriol. 51:131-143.
8. Weinberg, E. D. (ed.). 1977. Microorganisms and minerals. Marcel Dekker, Inc. New York.
9. Woltz, S. S. and J. P. Jones. 1979. Effects of magnesium on bacterial spot of pepper and tomato and on inhibition of *Xanthomonas vesicatoria*. Plant Dis. Rptr. 63:182-184.
10. Woltz, S. S., J. B. Jones, and J. P. Jones. 1983. Effects of magnesium on bacterial leaf spot of pepper. Bradenton AREC Res. Rpt. BRA1983-15.

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REDUCTION IN THE CONTROL OF COMMON NIGHTSHADE (*SOLANUM AMERICANUM*) BY PARAQUAT DUE TO COPPER FUNGICIDES

W. M. STALL AND S. R. KOSTEWICZ
Vegetable Crops Department
IFAS, University of Florida
Gainesville

AND

R. L. BROWN
Collier County Extension Department
Florida Cooperative Extension Service
Naples

Abstract. **Common nightshade (*Solanum americanum* Mill.) plants were sprayed with paraquat (1, 1-dimethyl-4, 4-bipyredinium) at 0.0, 0.25, or 0.50 lb./acre after being treated with a cupric hydroxide fungicide 3 times per week for 0, 1, or 2 weeks. Abscission of leaves from plants treated with either rate of paraquat was greater when no Cu fungicide was applied. The nightshade showed increased regrowth when the Cu fungicide was applied for two weeks.**

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Common nightshade is a serious weed problem in Florida tomato production. It is difficult to control with selective herbicides in crops such as tomato (*Lycopersicon esculentum* Mill.), pepper (*Capsicum annuum*, L.), and eggplant (*Solanum melongena* L.) because of similar physiology and close genetic relationship. Yield losses in tomato have been documented due to increasing interference from nightshade species (5). The use of soil fumigation and polyethylene mulch in vegetable production has eliminated most weeds from the surface of raised beds. Weed control in the row middles is still a significant problem.