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THE EFFECT OF SELECTED COPPER BACTERICIDES ON POPULATION DYNAMICS OF *XANTHOMONAS CAMPESTRIS* PV. *VESICATORIA* ON TOMATO LEAFLETS

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Abstract. **Two Cu bactericides (cupric hydroxide and copper ammonium carbonate) and mancozeb were applied in combination or alone to field grown tomato (*Lycopersicon esculentum* Mill.) plants to determine their effects on populations of the bacterial spot pathogen (*Xanthomonas campestris* pv. *vesicatoria* (Xcv)) on tomato foliage. Populations were determined on individually sampled leaflets at weekly intervals for 7 weeks. Compared with unsprayed tomatoes, populations of *X. c. vesicatoria* were significantly reduced over the sampling period on leaflets of all treatments except for those receiving copper ammonium carbonate alone. Populations of *X. c. vesicatoria* on leaflets treated with either Cu bactericide applied with mancozeb did not differ significantly from those on leaflets treated with mancozeb alone. Defoliation followed similar trends, with treatments having higher populations also having greater defoliation.**

Xanthomonas campestris pv. *vesicatoria*, causal agent of bacterial spot of tomato, is a concern to tomato growers in Florida when weather conditions are conducive for disease development (Jones and Jones, 1983). This is true especially when high temperatures and high precipitation are present. For many years, copper bactericides have been the chemical control method of choice for reducing disease severity. However, copper-resistant (Cu^r) strains of the bacterium have predominated in Florida for many years (Marco and Stall, 1983). The combination of copper and mancozeb has been shown to be more effective than copper alone for controlling the bacterial spot pathogen (Conover and Gerhold, 1981). Marco and Stall (1983) demonstrated that Cu^r strains were eliminated when suspended in the combination of copper and mancozeb, but not copper alone. Thus, recommendations to apply copper compounds in combination with mancozeb have been used for many years and have been shown to effectively reduce disease severity on tomato plants. However, it has been our contention that copper alone often provides control equal to copper-mancozeb (Jones et al., 1991a; Jones et al., 1991b; Jones and Jones, 1985).

There are numerous methods for determining the efficacy of bactericides for controlling bacterially incited diseases. One way is to quantify epiphytic populations on the leaf surface to determine if populations of the target bacterium are effectively reduced (Olson and Jones, 1983). Lindemann et al. (1984) observed a relationship between threshold populations of *P. syringae* pv. *syringae* on bean leaves and the occurrence of disease. Thus, an intensive study on population dynamics of *X. c. vesicatoria* was undertaken in the author's laboratory to determine if epiphytic populations of the bacterium were affected similarly as disease by bactericide treatments (Jones et al., 1991a). It was clearly shown that populations of Cu^r strains of *X. c. vesicatoria* were significantly reduced on plants treated with Cu alone or in combination with mancozeb. There was also a reduction in disease severity on plants treated with Cu alone or in combination with mancozeb with a positive correlation between populations of X. c. *vesicatoria* on the surface of tomato leaflets and disease severity. It was apparent that measuring populations of the bacterium on leaf surfaces over an extended time period can provide a fair estimate of the potential of a bactericide.

There has been a plethora of Cu bactericides available over the years for bacterial spot control (Jones and Jones, 1983; Jones and Jones, 1985). Some Cu bactericides when applied without mancozeb have been less effective than others (Jones and Jones, 1985). The objective of this study was to compare the efficacy of two Cu bactericides, cupric hydroxide and copper ammonium carbonate, on leaflets treated with either Cu compound applied alone or in combination with mancozeb by quantifying epiphytic populations on treated leaflets and by assessing disease severity on treated plots.

Materials and Methods

Bacterial strains and inoculum. Four Cu^r strains were selected using the method of Stall et al., (1987) and were used in this study. These strains were grown on medium B of King et al., (1954) for 48 h at 28C. Bacteria were gently removed from the agar surface and suspended in 0.01 M $MgSO_4*7H_2O$. Bacterial concentration was adjusted to 10^8 colony-forming units (cfu)/ml by adjusting the suspension to an optical density of 0.05 at 600 nm with a spectrophotometer. Plants in the field were inoculated early in the morning when dew was still present on the foliage by misting inoculum on the top of all plants with a backpack sprayer (Solo Kleinmotoren GMBH, Sindelfingen, Germany).

Field plots. 'Sunny' tomato transplants grown by Speedling Inc. (Sun City, FL) were set in the field onto raised black polyethylene mulch covered beds. Beds were 71.1 cm wide and spaced 139.5 cm apart from center to center. Plants were set 45.7 cm apart in the rows. Each plot consisted of 10 plants with plots separated from adjacent plots in the same row by 121.9 cm. The experiment was set up as a randomized complete block design.

Bactericide application and field inoculation. Cupric hydroxide (Kocide 101), copper ammonium carbonate (Copper Count N) and mancozeb (Dithane M-45) were applied alone or Cu compounds were applied in combination with mancozeb. Cupric hydroxide, copper ammonium carbonate, and mancozeb were applied at 0.9 kg, 1.9 L and 0.68 kg, respectively, per 378 L water. Control plots received

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no bactericide application. Field plots were inoculated 3-4 days after the first bactericide application.

Leaf sampling and disease severity ratings. Five asymptomatic leaflets were collected every 7 days from recently matured leaves and placed into individual plastic bags. Samples were processed within an hour of sampling. Leaflets were weighed, placed into separate 125-ml Erlenmeyer flasks containing 10 ml of peptone buffer (McGuire et al., 1986) per g of tissue. Flasks were shaken on a rotary shaker at 150 rpm for 30 min. Serial 10-fold dilutions were made in 5 mM phosphate buffer containing 0.1% NaCl. A 100 μl aliquot of each dilution of three selected dilutions was plated onto each of three plates of Tween A medium (McGuire et al., 1986). After incubation at 28C, colonies typical of *X. c. vesicatoria* were enumerated. Statistical analyses were performed on data without transformation or after transformation of individual leaflet populations by the Log_{10}. Population differences were determined over time by analysis of variance using orthogonal contrast comparisons.

Disease severity assessments were determined two ways. In the first, lesion number was determined on ten individual leaflets in each plot. Prior to analysis, lesion numbers on leaflets were adjusted using the Log_{10} transformation. In the second, percent defoliation was assessed in each plot. Prior to analysis, the percent defoliation data were adjusted using the arc sine transformation.

Results and Discussion

The copper-mancozeb combinations reduced epiphytic populations significantly more than all treatments with the exception of the cupric hydroxide treatment (Table 1, Fig. 1 A, B, and D). On the other hand, copper ammonium carbonate applied alone was the only Cu treatment which did not reduce surface populations significantly compared to the control (Table 1 and Fig 1 C). The fact that a Cu bactericide applied without mancozeb reduced populations of Cu^r strains of *X. c. vesicatoria* was not surprising as this was observed in a previous study (Jones et al., 1991a). However, it is interesting that the copper ammonium carbonate compound was less effective than cupric hydroxide. One reason for the difference, other than chemical composition of the Cu compound, is that the total available Cu was approximately 0.16 kg of Cu as compared to 0.45 kg of Cu in the cupric hydroxide treatment. With the rapid loss of Cu that occurs on the leaf surface (Jones et al., 1991b) and the low level of Cu on copper ammonium carbonate treated leaves to begin with, there was greater likelihood for copper ammonium carbonate treated leaves than for cupric hydroxide treated leaves reaching a lower Cu content conducive for growth of the bacterium. Whatever the explanation for the ineffectiveness of the copper ammonium carbonate treatment, addition of mancozeb to the tank mixture resulted in a much more efficacious treatment (Table 1 and Fig. 1B).

A second interesting aspect concerning the populations of *X. c. vesicatoria* on the leaf surface was that mancozeb applied alone reduced those populations compared to the control (Table 1). Although copper-mancozeb combinations have been shown to reduce Cu^r strains more effectively than Cu alone, the reduction in surface populations by mancozeb would indicate that in the field mancozeb has bactericidal activity. This observation substantiates previous work in which a similar affect in a greenhouse study was noted (Jones et al., 1991a).

Both copper-mancozeb treatments significantly reduced disease severity compared to the control on all three rating dates (Table 2). Cupric hydroxide reduced disease severity significantly compared to the control for two of the rating dates whereas copper ammonium carbonate was effective for one of the dates. Mancozeb did not reduce disease severity in any of the ratings, although populations were significantly reduced. With disease severity determinations using lesion counts per leaflet, both Cu compounds applied in combination with mancozeb and cupric hydroxide alone reduced leaf spots significantly compared to the control (Table 3). Copper ammonium carbonate applied alone or

Table 1. The effects of bactericide treatments on epiphytic populations of *Xanthomonas campestris* pv. *vesicatoria* over a six week sampling period on tomato as determined by contrast comparisons.

	Treatment					
Treatment	CuOH	CAC	CuAC + EBDC	CuOH + EBDC	EBDC	Control
CuOH[z]	—	NS[y]	NS	NS	NS	**
CuAC	NS	—	**	**	NS	NS
CuAC + EBDC	NS	**	—	NS	*	**
CuOH + EBDC	NS	**	NS	—	*	**
EBDC	NS	NS	*	*	—	*
Control	*	NS	*	*	*	—

[z]CuOH = cupric hydroxide; CuAC = copper ammonium carbonate; EBDC = ethylene bis dithiocarbamate (mancozeb).
[y]NS = not significant; * = significant at p = 0.05 level; ** = significant at p = 0.01 level according to LSD.

Table 2. The effect of various bactericides on control of bacterial spot as measured by percent defoliation.

	Defoliation (%)[y]		
Treatment	Week 1	Week 2	Week 3
CuOH[z]	15.3 bc[x]	7.2 bc	8.8 ab
CuOH + EBDC	8.3 c	5.3 bc	5.3 b
CuAC	23.6 ab	6.3 bc	13.8 ab
CuAC + EBDC	9.4 c	4.5 c	7.2 b
EBDC	21.2 ab	8.4 bc	9.4 ab
Control	25.7 a	14.8 a	17.7 a

[z]CuAC = copper ammonium carbonate; CuOH =copper hydroxide; EBDC = ethylene bis dithiocarbamate (mancozeb).
[y]Values were transformed using the arc sine transformation prior to analysis.
[x]Values in the same column followed by the same letter are not significantly different at p = 0.05 according to LSD.

Table 3. The effect of bactericides on control of bacterial spot as measured by number of lesions per leaflet.

	Lesions per leaflet (no.)[y]		
Treatment	Week 1	Week 2	Week 3
CuOH[z]	3.5 abc[x]	4.7 cd	2.2 bcd
CuOH + EBDC	2.5 bc	3.0 e	2.0 cd
CuAC	3.2 abc	5.7 abc	4.2 a
CuAC + EBDC	2.6 abc	3.8 de	1.6 d
EBDC	4.3 ab	7.7 a	3.2 abc
Control	4.6 a	7.0 ab	3.9 ab

[z]CuAC = copper ammonium carbonate; CuOH = copper hydroxide; EBDC = ethylene bis dithiocarbamate (mancozeb).
[y]Values in the same column followed by the same letter are not significantly different at p = 0.05 according to LSD.
[x]Values were transformed using Log_{10} transformation prior to analysis.

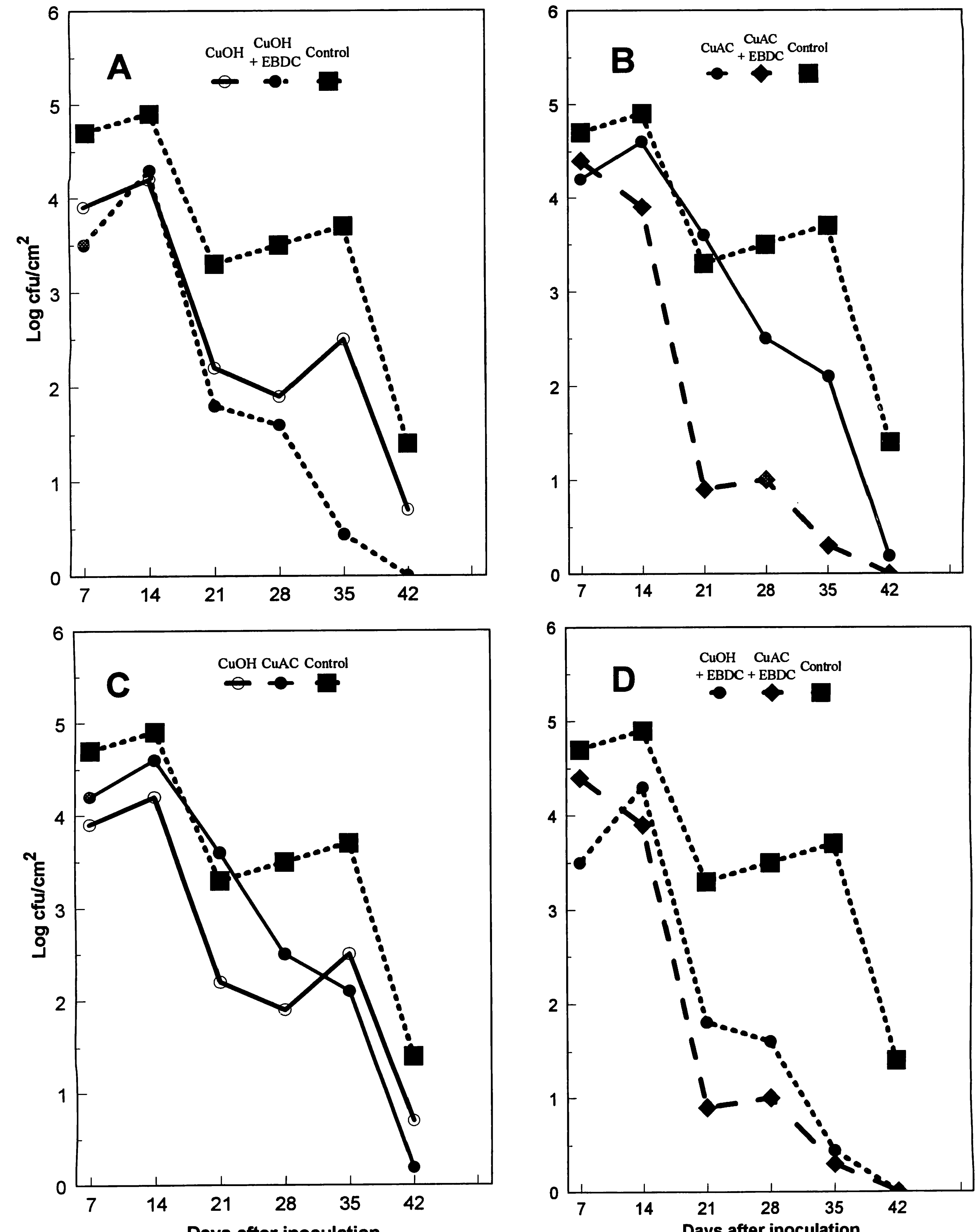

Figure 1 A-D. Effect of bactericides (CuAC = copper ammonium carbonate; CuOH = cupric hydroxide; EBDC = ethylene bis dithiocarbamate (mancozeb)) on epiphytic populations of *Xanthomonas campestris* pv. *vesicatoria* in spring 1987.

the mancozeb treatment did not reduce disease severity compared to the control.

It was apparent from this study that epiphytic populations do play a significant role in disease development. It was also evident that choosing the correct Cu formulation was important when developing control strategies. Finally, suspensions composed of mancozeb copper can greatly improve disease control capabilities.

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IMPROVING YIELD OF CUCUMBERS IN NEMATODE-INFESTED SOIL BY DOUBLE-CROPPING WITH A RESISTANT TOMATO CULTIVAR, USING TRANSPLANTS AND NEMATICIDES

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Abstract. **A study was conducted in 1992 at the Red River Research Station in northwestern Louisiana in a field infested with *Meloidogyne incognita* (Kofoid & White) Chitwood. The influence of a nematode-resistant tomato (*Lycopersicon esculentum* Mill.) cultivar, use of cucumber (*Cucumis sativus* L.) transplants, and pre-plant application of nematicides on growth and yield of trellised cucumbers double-cropped with tomatoes was studied. Plant fresh and dry weight, stem length, number of female flowers, and yield of 'Dasher II' cucumbers planted after the nematode-resistant tomato cultivar 'Celebrity' were significantly higher than after the susceptible tomato cultivar 'Heatwave'. Using cucumber transplants significantly increased cucumber growth and yield in comparison to direct seeded cucumbers. Pre-plant application of the nematicide ethoprop at 2.8 lb/acre through drip irrigation significantly increased the yield and growth of cucumbers. Preceding cucumbers with a nematode-resistant tomato cultivar significantly reduced the number of nematodes in soil samples taken after the last harvest of cucumbers.**

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Root-knot nematodes, *Meloidogyne incognita* (Kofoid & White) Chitwood, cause significant losses in yields of many economic crops throughout the world including tomatoes and cucumbers. Development of tomato cultivars resistant to root-knot nematodes has proven to be an effective, economical, and environmentally safe means of reducing losses from this pest. However, attempts to produce commercial cucumber cultivars that are resistant to root-knot nematodes have not been successful (Deakin et al., 1971).

Trellising cucumbers can increase yield and enhance fruit quality (Baker, 1977; Hanna et al., 1987; Konsler and Strider, 1973; Russo et al., 1991). Recent studies have shown that trellised cucumbers double-cropped with tomatoes is feasible (Hanna, 1993) and can minimize the cost of trellising cucumbers (Hanna et al., 1989). This study was undertaken to determine the influence of nematode-resistant and susceptible tomato cultivars, direct seeding or transplanting cucumbers, and pre-plant application of ethoprop nematicide on nematode population, growth, flowering, and yield of trellised cucumbers double-cropped with tomatoes.

Materials and Methods

Six-week old transplants of 'Celebrity' (nematode-resistant) and 'Heatwave' (nematode-susceptible) tomatoes were