Effect of Copper Bactericides on Copper-Resistant and -Sensitive Strains of *Pseudomonas syringae* pv. *syringae*

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ABSTRACT

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Fourteen formulations of copper-based bactericides were evaluated for their efficacy in reducing populations of copper-resistant and -sensitive strains of *Pseudomonas syringae* pv. *syringae* growing on tissue-cultured lilac and of copper-sensitive strains of this pathogen on field-grown lilac. The amount of free cupric ions (Cu^{2+}) in solution was the only predictor of formulation efficacy, but this variable could not be estimated from the metallic copper content of the product. Relative to nontreated controls, all copper-based bactericides reduced the population size of copper-sensitive strains by 50%, but only cupric hydroxide mixed with mancozeb or ferric chloride reduced the population size of copper-resistant strains by an equivalent amount. Several noncopper bactericides, including streptomycin-sulfate, caused only small reductions in bacterial populations on tissue-cultured or field-grown lilacs. In the field, two applications of cupric hydroxide (wetttable powder) when plant growth stages were at dormant (mid-February) and delayed dormant (late February) provided better control than either one or no treatments.

Economically damaging diseases caused by *Pseudomonas syringae* pv. *syringae* occur regularly on ornamental plants grown in nurseries in the Pacific Northwest (5,28,32). Lilac (*Syringa vulgaris* L.) is one of the most susceptible shrubs, and commercial nurseries experience large economic losses from *P. syringae* pv. *syringae*. Maximum symptom expression on lilac, including dead dormant buds, leaf spots, blossom blast, and shoot dieback, occurs in spring, soon after plants have been shipped to retailers. Severe spring epidemics most often follow periods of cool wet weather punctuated with late frosts (28). Since the late 1980s, the severity and frequency of *Pseudomonas*-incited diseases in Oregon nurseries has increased, and annual losses from this pathogen have been estimated at \$8 million for ornamentals (38).

Copper-based bactericides such as Bordeaux mixture, cupric hydroxide, cuprous oxide, copper salts of fatty acids, ammo-

niacal copper, tribasic copper sulfate, and mixtures of cupric hydroxide and ethylene bis-dithiocarbamates (EBDC) are registered for use on woody plants and have been used to control *Pseudomonas*-incited diseases with varying degrees of success (9,16,24,30,43). Other pesticides, including fungicides and disinfectants (6), have bactericidal action but have not been rigorously tested against *P. syringae* pv. *syringae* on woody plants. Present extension publications (32) and chemical labels do not provide enough detailed information on the quantity, timing, or rotation of these products for adequate disease control. Local woody plant nurseries have developed their own chemical disease control programs, usually involving application of copper-based bactericides and/or streptomycin sulfate in fall, winter, and spring. The applicability of these chemical regimens to other nurseries in the region on the wide variety of ornamental crops affected by *P. syringae* pv. *syringae* is unknown.

Copper sprays applied to leaves exist predominantly as insoluble deposits of copper salts (27). Copper salts can be dissolved by water and by forming complexes with organic compounds that leach from leaves (3). These processes on leaf surfaces remove a very small quantity of free cupric ions (Cu^{2+}) from the particulates. The concentration of Cu^{2+} on leaves depends on the equilibrium established with the complexed and soluble forms of copper. Cu^{2+} was shown to be the only form of copper toxic to *P. syringae* pv. *syringae*, either in vitro or on leaves, and no strong relationship was found between the total amount of copper spray applied and the concentra-

tion of Cu^{2+} on leaf surfaces (27). Formulated copper-based bactericides list their active ingredient in percent metallic copper, a value based on the chemistry of the insoluble copper salts. Studies with melanose disease on grapefruit showed that the amount of metallic copper explained only 37 to 60% of the variability in efficacy between copper-based fungicides, with higher rates not always providing proportionally higher levels of disease control (42).

Many strains of *P. syringae* pv. *syringae* isolated from woody ornamentals and fruit trees in Washington, Oregon, and California exhibit high levels of copper resistance in culture (2,38,41). In greenhouse and field trials with fruit trees, the growth rate and population sizes of copper-resistant strains of *P. syringae* pv. *syringae* were not decreased when the amount of cupric hydroxide exceeded four times the registered rate (2). *P. syringae* pv. *syringae* strains isolated from diseased woody ornamentals in a survey of Pacific Northwest nurseries showed that resistance to both copper and streptomycin is widespread in the pathogen population, and the level of resistance to copper increased fourfold between 1982 and 1993 (38). Even though strains are resistant to a given concentration of copper, increases in the quantity of available Cu^{2+} in solution can reduce bacterial population sizes (16,27). There is evidence that some copper-based bactericides are superior to others in controlling bacterial diseases, but results have not been consistent among pathogens or crops (1,9,11-13,22-24,30,33,34,39,43). Tank mixes of copper-based bactericides with fungicides, such as EBDCs (8,31) or heavy metals including iron (16) and zinc (4), have been shown to improve disease control by increasing the quantity of Cu^{2+} in solution. Superior copper-based bactericides or noncopper-based bactericides for control of copper-resistant strains of *P. syringae* pv. *syringae* on nursery crops are greatly needed.

The purpose of this study was to evaluate the efficacy of copper-based bactericides and alternative products for controlling copper- and streptomycin-resistant and -sensitive strains of *P. syringae* pv. *syringae* on tissue-cultured lilacs and copper- and streptomycin-sensitive strains of *P. syringae* pv. *syringae* on field-grown lilac. Additional trials were designed to standardize the number and timing of spray

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applications for field-grown lilac. Chemical analyses of Cu^{2+} concentrations and total dissolved copper in copper-based bactericides were used to relate the quantity of copper in different chemical forms to efficacy.

MATERIALS AND METHODS

Measurement of cupric ion concentrations and total dissolved copper. Cupric ion measurements of copper-based bacteri-

cides were made with an Orion model 94-29 Cu^{2+} specific electrode (Orion Research, Cambridge, MA) connected to an Orion model 601 pH/mV meter. An Orion model 09-01 reference electrode was used in conjunction with the cupric ion electrode. $\text{Cu}(\text{NO}_3)_2$ solutions yielding Cu^{2+} concentrations in 10-fold dilutions between 1,000 and 0.001 $\mu\text{g}/\text{ml}$ were used to plot a calibration curve that related millivolt potentials produced by the electrode with

Cu^{2+} concentrations. The electrode slope was constant, and curves relating millivolt potential and the logarithm of molar Cu^{2+} were linear ($r = 0.97$) for the concentration range of the standards. Standards and copper-based bactericides mixed at label rates with glass-distilled, deionized water were adjusted to pH 7.0 using 1 N NaOH, and the electrical potentials of 100-ml samples were measured to ± 1 mV under uniform temperature and illumination. All copper-based bactericides were tested three times, and results were averaged.

Concentration of total dissolved copper in the copper-based bactericides was measured with a Perkin-Elmer model 4000 atomic absorption spectrophotometer (Perkin-Elmer Corp., Norwalk, CT) with an air-acetylene flame. Samples were diluted 1,000-fold in glass-distilled deionized water and acidified to pH 3.0 with 10 ml of 5% HNO_3 to ensure the copper salts were completely dissolved. Copper standards for calibration were 10-fold dilutions of a $\text{Cu}(\text{NO}_3)_2$ stock solution acidified with 5% HNO_3 yielding between 1,000 and 0.001 μg Cu/ml. All copper-based bactericides were tested twice, and results were averaged.

Tissue-cultured lilacs. A lilac tissue-culture system, developed for pathogenicity testing of *P. syringae* pv. *syringae* strains isolated from woody ornamentals (37), was used to compare the efficacy of copper-based and non-copper-based bactericides for reducing populations of *P. syringae* pv. *syringae* on plants. *S. vulgaris* cv. Sensation stock plants were obtained from a commercial tissue culture nursery (Microplant Nurseries, Gervais, OR). Plantlets were axenically cultured in 200-ml glass jars with plastic lids on 35 ml of solid lilac tissue culture medium (Murashige and Skoog salts 4.3 g, 6-benzylamino-purine 2 mg, thiamine 0.4 mg, myo-Inositol 100 mg, sucrose 30 g, agar 6.5 g, distilled water 1 liter, pH 5.2; all chemicals from Sigma Chemical Co., St. Louis, MO). Plantlets were grown at 25°C with a 16-h photoperiod under 40W fluorescent bulbs in a constant-temperature room and subcultured every 3 to 4 weeks. Random stock plantlets were sampled periodically for the presence of contaminating bacteria, yeast, and fungi by macerating plantlets in sterile distilled water (SDW) and streaking loopfuls of the resulting aqueous solution onto King's Medium B (KB) (14). Plates were incubated at 28°C for 7 days.

Bacterial strains. Isolation and characterization of *P. syringae* pv. *syringae* strains from diseased lilacs in commercial woody plant nurseries has been described (38). *P. syringae* pv. *syringae* strains used in this study were fluorescent on KB under UV (350 nm), negative for Gram stain (36), ice nucleation active (19), pathogenic on lilac tissue-cultured plantlets, and hybridized with the *syr* genes for syringomy-

Table 1. Effect of copper-based and non-copper-based bactericides, and a systemic acquired resistance product, on populations of copper-sensitive (Cu^s) and copper-resistant (Cu^r) *Pseudomonas syringae* pv. *syringae* strains grown on tissue-cultured lilac (cv. Sensation)

Trade name Active ingredient	Rate per liter		AUPPC ^w	
	Recommended	Applied	Cu^s ^x	Cu^r
Untreated control	NA ^y	NA	538 a ^z	543 a
Copper-based bactericides				
Bordeaux				
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	4.8 g	1.2 g	25 j	353 i
+ lime	4.8 g	1.2 g		
C-O-C-S				
$\text{CuCl}_2 \cdot 3\text{Cu}(\text{OH})_2$ & $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	4.8 g	1.2 g	106 g	398 fg
Kocide 101				
$\text{Cu}(\text{OH})_2$	1.2 g	0.6 g	155 f	423 e
Kocide 101				
$\text{Cu}(\text{OH})_2$	1.2 g	0.6 g	40 ij	256 j
+ $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	NA	50 μg		
Kocide 101				
$\text{Cu}(\text{OH})_2$	1.2 g	0.6 g	133 f	420 e
+ $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	NA	50 μg		
Kocide DF				
$\text{Cu}(\text{OH})_2$	1.2 g	0.6 g	227 d	471 d
Kocide LF				
$\text{Cu}(\text{OH})_2$	1.2 g	0.6 g	334 c	524 ab
Kop-R-Spray				
$\text{Cu}(\text{NH}_3)_2 + \text{Cu}_2\text{CO}_3$	5.0 ml	1.2 ml	34 ij	380 h
ManKocide				
$\text{Cu}(\text{OH})_2 + \text{Zn, Mn \& EBDC}$	1.2 g	0.6 g	54 hi	251 j
Microcop				
$\text{CuSO}_4 \cdot 3\text{Cu}(\text{OH})_2$	4.8 g	1.2 g	53 hi	408 f
Nordox WP				
Cu_2O	6.0 g	1.5 g	133 f	429 e
Phyton 27				
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ & tannic & picric acids	1.6 ml	0.8 ml	146 f	507 b
MAC 570				
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	5.0 ml	2.5 ml	73 h	395 g
Non-copper-based bactericides				
Agribrom				
$\text{C}_5\text{H}_6\text{BrClN}_2\text{O}_2$	1.0 g	1.0 g	191 e	209 k
Agri-mycin 17				
Streptomycin sulfate	1.8 g	1.8 g	407 b	406 f
Ferric chloride				
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	NA	50 μg	507 a	508 b
Kasumin				
Kasugamycin	1.5 ml	1.5 ml	521 a	520 ab
Physan 20				
Quaternary ammonium	1.3 ml	1.3 ml	534 a	509 b
Zinc sulfate				
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	NA	50 mg	518 a	515 b
Systemic acquired resistance product				
Actiguard				
$\text{C}_8\text{H}_6\text{N}_2\text{O}_2$	0.2 g	0.2 g	530 a	530 ab

^w AUPPC = $\Sigma[(Y_{i+1} + Y)/2][X_{i+1} - X_i]$ in which Y_i = bacterial population at the i th observation, X_i = time (hours) of the i th observation.

^x Cu^s = copper-sensitive, strains unable to grow on Casitone-yeast-extract (CYE) medium containing 0.16 mM CuSO_4 , Cu^r = copper-resistant, strains able to grow on CYE medium containing 0.16 mM CuSO_4 .

^y Data not applicable.

^z Treatment means within a column followed by the same letter do not significantly differ according to the Student-Newman-Keuls test ($P = 0.05$).

cin (37). In addition, strains conformed with all LOPAT (17) and GATTa (15) characters for *P. syringae* pv. *syringae*. Three strains matching each of the following phenotypes, isolated from diseased lilacs in the Willamette Valley, Oregon, were selected for these experiments: (i) copper- and streptomycin-sensitive (strains 8A, 29B, 62F), (ii) resistant to 0.16 mM CuSO₄ in Casitone-yeast-extract (CYE) medium (44) and streptomycin-sensitive (strains 4A, 11D, 13F), (iii) resistant to 0.48 mM CuSO₄ in CYE and streptomycin-sensitive (strains 11E, 22A, 58D), and (iv) resistant to 0.48 mM CuSO₄ in CYE and resistant to 100 µg of streptomycin per ml in KB (strains 19C, 20B, 49B). Bacteria were preserved at -80°C in sterile Luria-Bertani broth (35) amended with 20% sterile glycerol.

Inoculation and chemical treatments.

Before each experiment, strains were recovered from frozen storage by streaking onto KB and incubating for 48 h at 28°C. Bacteria were harvested from KB plates and suspended in SDW to a concentration of approximately 1×10^4 CFU/ml (OD₅₉₀ = 0.20). Plantlets were submerged and gently swirled in the bacterial suspension for 30 s and returned to their jars. Inoculated plantlets in the jars were exposed to a temperature of -5°C in a chest freezer for 10 min to simulate a frost event.

Where copper-based bactericides were labeled for ornamentals, the minimum dosage recommended by the manufacturer was selected. At these recommended rates, most bactericides were phytotoxic to the tissue-cultured lilacs and the rates were reduced by 50 or 75% (Table 1). The following copper-based bactericides were tested: Bordeaux mixture 4-4-100 (copper sulfate, 50% metallic Cu, plus hydrated lime [21]), C-O-C-S WDG (copper oxychloride and basic copper sulfate, 50% metallic Cu; Clean Crop, Platte Chemical Co., Fremont, NE), Kocide 101 (cupric hydroxide, 50% metallic Cu), Kocide 101 plus ferric chloride (FeCl₃·6H₂O, Sigma), Kocide 101 plus zinc sulfate (ZnSO₄·7H₂O, Sigma), Kocide 2000 (cupric hydroxide, 35% metallic Cu), Kocide DF (cupric hydroxide, 40% metallic Cu), Kocide LF (cupric hydroxide, 15% metallic Cu), ManKocide (cupric hydroxide, 30% metallic Cu, plus mancozeb; Griffin Corp., Valdosta, GA), Kop-R-Spray (copper ammonium complex, 8% metallic Cu; Cooke Laboratory Products, Portland, OR), Microcop (tribasic copper sulfate, 50% metallic Cu; The Chas. H. Lilly Co., Portland, OR), Nordox WP (cuprous oxide, 50% metallic Cu; Monterey Chemical Co., Fresno, CA), Phyton 27 (copper sulfate plus tannic and picric acids, 5.5% metallic Cu; Source Technology Biologicals, Minneapolis, MN), and MAC 570 (copper sulfate, 15% metallic Cu; Nufarm Ltd., St. Joseph, MO).

Several additional products are labeled for plant disease control and were tested at

labeled rates as potential substitutes for copper-based bactericides (Table 1): Agri-brom (1-bromo-3-chloro-5,5-dimethyl-2,3-imidazolidinedione; Great Lakes Chemical, West Lafayette, IN), Agri-mycin 17

(streptomycin sulfate; MSDAGVET, Renway, NJ), Kasumin (kasugamycin; United Agri-Products, Greeley, CO), Manzate 200 DF (mancozeb; E. I. du Pont de Nemours, Wilmington, DE), Physan 20 (quaternary

Table 2. Effect of bactericide application on blight of field-grown lilac (cv. Ellen Willmott) caused by copper- and streptomycin-sensitive *Pseudomonas syringae* pv. *syringae* strains in the Willamette Valley, Oregon, in 1995, 1996, and 1997

Trade name Active ingredient	Rate/ha	AUDPC ^w		
		1995	1996	1997
Untreated control	NA ^x	1,623 a ^y	783 a	791 a
Copper-based formulations				
Bordeaux	1.8 kg	435 c	223 g	254 d
CuSO ₄ ·5H ₂ O + lime				
Kocide 101	0.45 kg	492 c	324 ef	333 d
Cu(OH) ₂				
Kocide 101	0.45 kg	ND ^z	ND	272 d
Cu(OH) ₂ + FeCl ₃ ·6H ₂ O	0.005 kg			
Kocide 101	0.45 kg	ND	256 fg	273 d
Cu(OH) ₂ + Manzate	0.45 kg			
Kocide 2000	0.45 kg	ND	ND	480 c
Cu(OH) ₂				
Kocide DF	0.45 kg	ND	581 cd	551 c
Cu(OH) ₂				
Kocide LF	0.63 liter	ND	775 a	684 b
Cu(OH) ₂				
ManKocide	0.45 kg	ND	ND	258 d
Cu(OH) ₂ + EBDC, Mn, Zn				
Phyton 27	0.63 liter	728 b	ND	ND
CuSO ₄ ·5H ₂ O + tannic & picric acids				
Non-copper-based formulations				
Agribrom	0.45 kg	ND	637 bc	ND
C ₅ H ₆ BrClN ₂ O ₂				
Agri-mycin 17	0.45 kg	ND	355 e	303 d
Streptomycin sulfate				
Kasumin	0.12 liter	ND	706 ab	ND
Kasugamycin				
Manzate	0.45 kg	ND	519 d	472 c
Mancozeb				
Physan 20	1.40 liter	ND	744 a	ND
Quaternary ammonium				
Zinc sulfate	1.8 kg	761 b	ND	ND
ZnSO ₄ ·7H ₂ O				
Plastic shelters	NA	ND	235 fg	194 d

^w AUPPC = $\Sigma[(Y_{i+1} + Y_i)/2][X_{i+1} - X_i]$ in which Y_i = bacterial population at the i th observation, X_i = time (hours) of the i th observations.

^x Not applicable.

^y Treatment means within a column followed by the same letter do not significantly differ according to the Student-Newman-Keuls test ($P = 0.05$).

^z Not determined.

Table 3. Percent metallic copper as specified by manufacturer and measured free Cu²⁺ and dissolved copper in 14 formulations of copper-based bactericides

Copper-based bactericide	Percent metallic copper	Free Cu ²⁺ (µg/ml) ^y	Dissolved copper (µg/ml) ^z
Bordeaux	25	2.50 ± 0.04	155 ± 5
C-O-C-S	50	1.50 ± 0.04	359 ± 16
Kocide 101	50	0.50 ± 0.03	139 ± 4
Kocide 101 + FeCl ₃ ·7H ₂ O	50	22.00 ± 0.05	143 ± 11
Kocide 101 + ZnSO ₄ ·6H ₂ O	50	0.80 ± 0.08	150 ± 13
Kocide 2000	35	0.70 ± 0.01	121 ± 13
Kocide DF	40	0.10 ± 0.03	132 ± 18
Kocide LF	15	0.01 ± 0.002	76 ± 1
Kop-R-Spray	8	9.40 ± 0.20	126 ± 2
ManKocide	30	6.40 ± 0.04	138 ± 23
Microcop	50	13.00 ± 0.50	345 ± 21
Nordox WP	50	0.04 ± 0.001	579 ± 22
Phyton 27	6	5.40 ± 0.40	83 ± 5
MAC 570	15	3.70 ± 0.50	375 ± 21

^y Cu²⁺ liberated in aqueous solution (pH 7.0) measured by cupric ion electrode.

^z Total dissolved copper measured by atomic absorption spectrophotometry.

ammonium; Maril Products, Tustin, CA), and Actiguard (benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester; Novartis Limited, Basel, Switzerland). Ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) and zinc sulfate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) (Sigma) were tested independently of cupric hydroxide for efficacy.

Bactericides were mixed in 50 ml of SDW, and the lilac plantlets were treated immediately after inoculation and the simulated frost event. The plantlets were submerged and gently swirled in the bactericide solution for 30 s, then returned to their jars and incubated at 25°C with a 16-h photoperiod. An exception to this protocol was made for Actiguard, which functions through systemically activating plant resistance; it was applied to the plantlets 4 days prior to bacterial inoculation and freezing.

At 0, 12, 24, 48, 72, and 96 h postbactericide treatment, plantlets were aseptically transferred to 15-ml plastic tubes containing 5 ml of SDW. Tubes were sonicated for 2 min in an ultrasonic cleaner (Model 3210, Branson Sonic Power Co., Danbury, CT) to liberate bacteria. Plantlets were blotted dry and weighed. Serial dilutions of

the sonicate were made to 10^{-8} , spread on KB plates, and incubated for 48 h at 28°C. Water-treated plantlets were used as controls. All bactericides were tested three times with all 12 *P. syringae* pv. *syringae* strains.

Data collection. *P. syringae* pv. *syringae* populations were estimated after 48-h incubation at 28°C from dilution plates with densities between five and 300 colonies. Populations were expressed as the logarithm (base 10) of bacteria per gram of fresh tissue.

Field-grown lilacs. In 1995, 1996, and 1997, treatments with copper-based and non-copper-based bactericides for bacterial blight control were arranged in completely randomized designs in a block of lilac (*S. vulgaris* cv. Ellen Willmott) planted in 1993 on a 1.5 × 4.5 m spacing. A cultural technique to protect shrubs from frost and rain was used in 1996 and 1997. Translucent plastic (6 mil), supported by a tripod of metal stakes 1.5 m tall, was used to cover shrubs assigned to this treatment. Shrubs were covered from mid-January through the end of April. The plastic tents completely surrounded the shrubs, and duct

tubing (15 cm diameter) was incorporated into the top of the tent to increase ventilation.

Inoculation and chemical treatments.

All shrubs were inoculated with equal parts of three copper- and streptomycin-sensitive *P. syringae* pv. *syringae* strains isolated in the Willamette Valley from diseased lilacs (38). Inoculum was applied each year in the last week of January or the first week of February when the plants were fully dormant and in mid-February at delayed dormant, just prior to bud break. Inoculum was applied using a Solo backpack sprayer (Model 435, Solo, Newport News, VA) at a concentration of 10^8 CFU/ml and a rate of 200 ml per shrub, providing a final inoculum dose of 2×10^{10} CFU per shrub.

Rates for all chemical treatments were the minimum dosage for ornamentals recommended by the manufacturer (Table 2). When a product was not labeled for use on ornamentals, the minimum rate for control of bacterial pathogens on other woody plants was selected. These copper-based bactericides were tested: Bordeaux mixture 4-4-100, Kocide 101 alone, Kocide 101 plus ferric chloride, Kocide 2000, Kocide DF, Kocide LF, ManKocide, and Phyton 27. Several additional products were tested as substitutes for copper-based bactericides: Agribrom, Agri-mycin 17, Kasumin, Manzate 200 DF, Physan 20, and zinc sulfate.

Bactericides were applied twice in 1995, 1996, and 1997, 1 week after each inoculation. The first application was made in early February while the shrubs were still fully dormant; the second was made in early March when the shrubs were at delayed dormant, just prior to bud break. In the 1995 trial, which also evaluated the number and timing of Kocide 101 sprays, a third application was made to selected shrubs in the last week of March, immediately prebloom. Well water, used for mixing bactericides, had a pH of 6.4. Each treatment was applied to eight single shrub replicates; approximately 1 liter of spray solution was used per shrub.

Data collection. Incidence of bacterial blight was evaluated once per week for 6 weeks, from the middle of March through the end of April. Disease incidence was recorded by counting the number of green shoots with more than 50% of the leaf or flower tissue blighted, as shoots with this level of disease usually dropped.

Data analysis, tissue-cultured and field-grown lilac. For the studies on tissue-cultured lilac, the log-transformed counts of *P. syringae* pv. *syringae* were plotted as a progress curve of bacterial population increase over time. The area under the population progress curve ($\text{AUPPC} = \sum [(Y_{i+1} - Y_i)/2] [X_{i+1} - X_i]$ in which Y_i = bacterial population at the i th observation, X_i = time [hours] of the i th observation) was calculated for each bactericide treatment (40). Data collected in the

Fig. 1. Population progress curves for copper-resistant *Pseudomonas syringae* pv. *syringae* strains grown on tissue-cultured lilac (cv. Sensation) treated with $\text{Cu}(\text{OH})_2$ bactericides.

lilac field trials were plotted as a disease progress curve of disease incidence over time (AUDPC). The SAS general linear models procedure (Statistical Analysis Systems, Release 6.08 ed., SAS Institute, Cary, NC) was used to perform analysis of variance on the AUPPC and AUDPC means, which were separated by the Student-Newman-Keuls test ($P = 0.05$).

RESULTS

Measurement of cupric ion concentrations and total dissolved copper. Significant differences existed between the amount of Cu^{2+} and total dissolved copper for the 14 copper-based bactericides (Table 3). The amount of Cu^{2+} was not significantly correlated with the percent copper as metallic ($y = -0.09\ln(x) + 34$, $R^2 = 0.0002$) or to the total dissolved copper ($y = -5.0\ln(x) + 204$, $R^2 = 0.0063$). Metallic copper and total dissolved copper concentrations were not significantly correlated with each other ($y = 3.6x + 83$, $R^2 = 0.17$).

Disease development in tissue-cultured lilacs. Bacterial blight epidemics take 4 to 6 weeks on lilacs in the field; however, epidemics take only 4 to 6 days on the tissue-cultured lilac plantlets. All copper-based bactericides reduced populations of copper-resistant (Figs. 1 and 2) and -sensitive (Figs. 3 and 4) *P. syringae* pv. *syringae* strains on plantlets immediately after treatment (time = 0) relative to the water-treated controls. Bacteria that were able to escape or tolerate the chemicals became established and multiplied on the plantlets, increasing as much as 1,000 $\times$ in the first 24 h. Populations continued to increase an additional 10 $\times$ in each subsequent 24-h period. After 96 h, plantlets with *P. syringae* pv. *syringae* populations in excess of 1×10^6 CFU/g showed visible symptoms of bacterial blight, including water-soaked lesions on leaves, vein and petiole necrosis, and tip dieback. Water-treated, noninoculated control plants showed no effect from the simulated frost event.

Copper- and streptomycin-sensitive and -resistant strains had similar population growth curves on control plantlets treated with water (Figs. 1 to 4). There was no significant difference between the growth of *P. syringae* pv. *syringae* strains resistant to 0.16 mM and those resistant to 0.48 mM CuSO_4 for any of the copper- or non-copper-based bactericides, and the data were combined. All copper-based bactericides except Kocide LF reduced the AUPPC values of copper-sensitive strains by more than 50% (Table 1). Bordeaux, C-O-C-S, Kocide 101 plus ferric chloride, Kop-R-Spray, MAC 570, ManKocide, and Microcop treatments reduced AUPPCs of copper-sensitive strains by 80% or more. Treatments with Kocide 101, Kocide 101 plus zinc sulfate, Kocide 2000, Kocide DF, Kocide LF, Nordox, and Phyton 27 reduced AUPPCs by 38 to 75%. For copper-

resistant strains, only Kocide 101 plus ferric chloride and ManKocide reduced AUPPCs by 50% (Table 1). Bordeaux, C-O-C-S, Kop-R-Spray, MAC 570, and Microcop treatments reduced AUPPCs by 25 to 35%. Kocide 101, Kocide 101 plus zinc sulfate, Kocide 2000, Kocide DF, Kocide LF, Nordox, and Phyton 27 reduced AUPPCs by only 7 to 22%.

The amount of Cu^{2+} in a formulation was the best predictor of efficacy of copper-based bactericides for reducing populations of copper-sensitive (Fig. 5A) or -resistant strains (Fig. 5B) of *P. syringae* pv. *syringae* on tissue-cultured lilac. Treatments with bactericides that released 1.50 μg Cu^{2+} /ml or more resulted in the largest reductions in AUPPCs. These include Bordeaux (2.50 μg /ml), C-O-C-S (1.5 μg /ml), Kocide 101 plus ferric chloride (22.0 μg /ml), Kop-R-Spray (9.40 μg /ml), Microcop (13.0 μg /ml), and MAC 570 (3.70 μg /ml). Smaller reductions in AUPPCs were observed for bactericides that released 0.80 μg Cu^{2+} /ml. These include Kocide 101 (0.50 μg /ml), Kocide 101 plus zinc sulfate (0.80 μg /ml), Kocide 2000 (0.70 μg /ml), Kocide DF (0.10 μg /ml),

Kocide LF (0.01 μg /ml), and Nordox (0.04 μg /ml). The only exception was Phyton 27, which liberated 5.40 μg Cu^{2+} /ml yet was not in the group of copper-based bactericides causing the largest reductions in AUPPCs.

There was no significant difference between the growth of copper-resistant or -sensitive *P. syringae* pv. *syringae* strains on plantlets treated with non-copper-based bactericides, and the data were combined (Fig. 6). All non-copper-based bactericides reduced AUPPCs significantly over water-treated controls (Table 1). Agribrom was the only non-copper-based formulation that reduced AUPPCs by over 60%. For plantlets treated with ferric chloride, Kasumin, Physan 20, Actiguard, and zinc sulfate, the AUPPCs were reduced by 5% or less relative to the water-treated controls.

Disease development in field-grown lilacs. Climatic variation among years led to large differences in disease epidemics. Noninoculated shrubs in a nearby plot experienced lower levels of disease than did the inoculated shrubs in all three trials. In 1995, conditions were very favorable for

Fig. 2. Population progress curves for copper-resistant *Pseudomonas syringae* pv. *syringae* strains grown on tissue-cultured lilac (cv. Sensation) treated with various copper-based bactericides.

disease development, with late-season frosts and wind-driven rainstorms. The disease epidemic was severe; on average, 80% of green shoots on control shrubs were blighted, and virtually all flowers were blasted. In 1995, Bordeaux and Kocide 101 reduced AUDPCs by more than 70%; whereas Phyton 27 and zinc sulfate reduced AUDPCs by 50% (Table 2). None of these treatments provided a commercially acceptable level of disease control in 1995. The amount of Cu^{2+} in a formulation was again the best predictor of efficacy of copper-based bactericides for controlling copper-sensitive strains of *P. syringae* pv. *syringae* on field-grown lilac ($y = 6.4\ln(x) + 54$, $R^2 = 0.59$).

The disease epidemic in 1996 was much less severe than in 1995; on average, 40% of green shoots and flowers on control shrubs were blighted, and there were significant differences among treatments. Treatments with Kasumin, Kocide LF, and Physan 20 reduced AUDPCs by less than 10% and were not significantly different from the untreated control (Table 2). Agri-brom, Kocide DF, and Manzate treatments resulted in AUDPCs 20 to 35% less than the control; whereas AUDPC reductions

from Agri-mycin 17 and Kocide 101 treatments were 54 and 59%, respectively. Kocide 101 + Manzate and Bordeaux were the most effective treatments in 1996, reducing AUDPCs by 67 and 72%, respectively. The plastic shelters were also very effective, with AUDPCs 70% less than the control.

The disease epidemic in 1997 was very similar to the epidemic in 1996. In 1997, Kocide LF reduced AUDPCs by only 14% (Table 2). Kocide 2000, Manzate, and Kocide DF had AUDPCs 30 to 40% less than the control; whereas AUDPC reductions from Agri-mycin 17, Bordeaux, Kocide 101, Kocide 101 plus ferric chloride, Kocide 101 plus Manzate, and ManKocide were between 57 and 68%. The plastic shelters were also very effective in 1997, with AUDPCs 75% less than the control.

Number and timing of spray applications. Single or multiple applications of Kocide 101 at any time in 1995 significantly reduced AUDPCs compared with the nontreated control. A 70% reduction in AUDPCs relative to the control (AUDPC = 1,623) was measured on shrubs treated with Kocide 101 at both dormant and de-

layed dormant growth stages (AUDPC = 492). A single Kocide 101 spray at dormant (AUDPC = 855) or delayed dormant (AUDPC = 746) was statistically equal, and these treatments had AUDPCs approximately 50% lower than the control. If Kocide 101 was not applied until prebloom (AUDPC = 1,456), a significant epidemic was already in progress and AUDPCs were reduced only 20% relative to the control.

DISCUSSION

The large number of copper-resistant strains of *P. syringae* pv. *syringae* present in commercial ornamental nurseries where copper-based bactericides have been used for many years is probably responsible for increased reports of disease control failure. Because the concentration of cupric ions and not the total amount of copper in a formulation determines toxicity to bacterial cells, it is unlikely that simply increasing the number or rate of copper applications will provide effective control of copper-resistant strains of *P. syringae* pv. *syringae*.

Many of the copper- and non-copper-based bactericides were very phytotoxic to the tissue-cultured lilacs, and rates had to be reduced. This was not unexpected because the plantlets have a very thin cuticle. The advantages of using a tissue-culture system were that environmental conditions were constant and chemicals did not weather off leaves with rain or dew. Conditions were very favorable for disease development on the tissue-cultured lilacs, and rapid screening of new products for efficacy against bacterial pathogens could be easily done in such a system. Phytotoxicity was not a problem for the field-grown lilacs treated with copper- or non-copper-based bactericides while the shrubs were dormant. However, growers should be cautious about the phytotoxic effects of some copper-based bactericides when applied to green tissues or to plant species more sensitive to copper than lilac.

Populations of *P. syringae* are generally low on young leaves and flowers, but these tissues are colonized by epiphytes over time (10,18,20,29). Bactericide treatments must reduce or suppress bacterial population increases on plant surfaces to reduce or delay disease epidemics. The tissue-cultured lilacs were free of microflora prior to inoculation with low concentrations of *P. syringae* pv. *syringae*. All copper- and non-copper-based bactericides reduced bacterial populations on the lilac plantlets but were never eradicated. Thus the efficacy of each bactericide was dependent on its ability to reduce the initial population size and not the population growth rate. The bacterial cells that remained may have survived the bactericide treatment or colonized areas of the plantlets that were not reached.

Copper-resistant strains of *P. syringae* exhibit a quantitative rather than qualitative resistance to copper in laboratory cul-

Fig. 3. Population progress curves for copper-sensitive *Pseudomonas syringae* pv. *syringae* strains grown on tissue-cultured lilac (cv. Sensation) treated with $\text{Cu}(\text{OH})_2$ bactericides.

ture. Strains of *P. syringae* pv. *syringae* with two discrete levels of copper resistance were used in this study. The growth of strains resistant to a relatively low concentration of copper (0.16 mM CuSO_4) was equivalent to the growth of strains resistant to a much higher concentration of copper (0.48 mM CuSO_4) on plantlets treated with copper-based bactericides. Andersen et al. (2) showed that exposure of copper-resistant *P. syringae* to sublethal concentrations of CuSO_4 in growth medium resulted in an increased resistance to copper in subsequent generations of more than sevenfold. They hypothesized that the low, constitutive level of resistance to cupric ions expressed by these strains of *P. syringae* may be important in the process by which cells are induced to express higher levels of resistance upon sensing higher concentrations of cupric ions. Strains inoculated to lilac plantlets in our experiments were not induced to maximum levels of copper resistance before inoculation; however, the cells that survived treatment with copper-based bactericides may have been induced to express a higher level of resistance by the sublethal concentrations of cupric ions to which they were exposed. Such an induction phenomenon could explain why the bacterial strains with initially different levels of resistance multiplied at an equivalent rate and reached similar maximum populations.

Menkissoglu and Lindow (26) showed that Cu^{2+} is the only form of copper toxic to copper-sensitive and copper-resistant strains of *P. syringae*. They found no evidence for the toxicity of copper complexed with glucose, fructose, sucrose, succinate, or citrate, organic compounds found commonly on leaf surfaces. Soluble but complexed forms of copper, while abundant on orange and bean leaves, had no significant toxicity toward copper-resistant or -sensitive strains of *P. syringae* in their study. In a second paper (27), they showed that the concentrations of Cu^{2+} found on bean leaves treated with either high or low rates of $\text{Cu}(\text{OH})_2$ were very similar, demonstrating that the amount of Cu^{2+} will be largely determined by the equilibrium constants of the organic complexes and leaf surface chemistry, and not by the quantity of insoluble copper salts that are present.

Cu^{2+} concentrations liberated by the copper-based bactericides ranged from 0.04 to 22.0 $\mu\text{g}/\text{ml}$ and could not have been estimated using the metallic copper content provided by the product's label. However, Cu^{2+} concentrations were the best predictor of a bactericide's efficacy against copper-resistant or -sensitive strains of *P. syringae* pv. *syringae* on the tissue-cultured lilac and against copper-sensitive strains on field-grown lilac. Excepting Phyton 27, bactericides that released more than 1.50 μg Cu^{2+}/ml were most effective against *P.*

syringae pv. *syringae*. Phyton 27 is a product used to control *Ophiostoma ulmi* and is formulated to be injected into elm trees rather than applied to leaves. Although it released a moderate concentration of Cu^{2+} in aqueous solution, this did not translate to efficacy against *P. syringae* pv. *syringae* on the phyllosphere.

Significant differences in Cu^{2+} concentration and efficacy against copper-resistant and -sensitive strains of *P. syringae* pv. *syringae* existed between the various formulations of cupric hydroxide. There has been an industry-wide trend to reduce the metallic Cu in bactericides to limit copper contamination of soil and water and reduce production costs for the manufacturer. While Kocide 2000 (35% metallic Cu) was not significantly different from Kocide DF (40% metallic Cu), Kocide LF (15% metallic Cu) was much less effective, and none of these formulations released as much Cu^{2+} or were as effective as Kocide 101 (50% metallic Cu). ManKocide (30% metallic Cu) was very effective, but cupric hydroxide is not the only active ingredient in this product; some bactericidal activity should be attributed to the 15% mancozeb.

Kocide 2000 and DF are both dry flowables, and neither was as effective as Kocide 101, a wettable powder. Timmer and Zitko (42) also found liquid and dry flowable formulations of cupric hydroxide not highly effective against melanose on grapefruit. Their study and one by Schwartz and McMillan (39) found significant differences between cupric hydroxide bactericides produced by different manufacturers, suggesting that inert materials could affect the availability of Cu^{2+} , even at equivalent rates of metallic Cu.

Other researchers have compared the efficacy of different formulations of copper-based bactericides, and results have varied between and among pathosystems. One study of copper-sensitive strains of *P. syringae* pv. *syringae* on apricots found Bordeaux mixture (basic copper sulfate plus hydrated lime) to be superior to cupric hydroxide (43). This was corroborated by another group, which determined tribasic copper sulfate to be more effective than a formulation containing copper salts of fatty and rosin acids at reducing copper-resistant populations of *P. syringae* pv. *morsprunorum* on cherry (30). Copper ammonium

Fig. 4. Population progress curves for copper-sensitive *Pseudomonas syringae* pv. *syringae* strains grown on tissue-cultured lilac (cv. Sensation) treated with various copper-based bactericides.

Fig. 5. Relationship between the concentration of Cu^{2+} ($\mu\text{g/ml}$) and reduction of populations of (A) copper-sensitive strains of *Pseudomonas syringae* pv. *syringae* ($P = 0.03$), (B) copper-resistant strains of *P. syringae* pv. *syringae* ($P = 0.05$) grown on tissue-cultured lilac (cv. Sensation) relative to the untreated control. Points are the mean observations for each of the 14 copper-based bactericides described in Table 3.

carbonate was found to be superior to Cu plus maneb (EBDC) against *Xanthomonas campestris* pv. *citri* on citrus (24), but the reverse was true for Cu plus mancozeb against *P. syringae* pv. *tomato* on tomatoes (8). In our study, both were very effective against copper-sensitive strains of *P. syringae* pv. *syringae*, but ManKocide (cupric hydroxide plus mancozeb) was markedly better at controlling copper-resistant strains.

Copper plus mancozeb consistently outperformed all other copper-based bactericides in field studies with *P. syringae* pv. *tomato* on tomatoes (8,11,12), *P. syringae* pv. *syringae* on beans (39), and *X. campestris* pv. *vesicatoria* on peppers (22,23,33,34). The increased toxicity of mancozeb (manganese-zinc ethylene bis-dithiocarbamate) mixtures with copper-

based bactericides appears to be associated with the ability of the dithiocarbamate anion to chelate copper and transport Cu^{2+} to copper-susceptible sites within the bacterial cell (25). Lee et al. (16) showed that adding iron in the form of ferric chloride to cupric hydroxide resulted in increased toxicity to copper-resistant strains of *X. campestris* pv. *juglandis* on walnut. The ferric ion apparently has a direct physiological effect on *X. campestris* pv. *juglandis*, resulting in an increased susceptibility to the toxic effect of the cupric ion. Also, the addition of ferric chloride to cupric hydroxide increased the availability of Cu^{2+} on leaf surfaces by lowering the pH and through cation exchange between Cu^{2+} and Fe^{3+} . This phenomenon appears to be Fe^{3+} -specific, as lowering pH with hydrochloric acid or adding MnSO_4 ,

MgCl_2 , MgSO_4 , CaCl_2 , NaCl , and KCl did not increase the availability of Cu^{2+} . In our study, adding ferric chloride to cupric hydroxide increased the Cu^{2+} concentration 40-fold and was very effective against copper-resistant strains of *P. syringae* pv. *syringae*. Zinc, used either as a substitute for copper or in addition to copper, has been effective in controlling copper-resistant strains of *X. campestris* pv. *juglandis* (4). Zinc formulation appears to be important; in our study, cupric hydroxide plus zinc sulfate was not significantly better than cupric hydroxide alone at controlling *P. syringae* pv. *syringae*, while cupric hydroxide plus zinc and maneb, cupric hydroxide plus zinc sulfate-calcium hydroxide, and alternating sprays of cupric hydroxide and zinc sulfate-calcium hydroxide, were effective against *X. campestris* pv. *juglandis*.

Agribrom was the only non-copper-based bactericide as effective as the copper-based bactericides against *P. syringae* pv. *syringae* on tissue-cultured lilac. Unfortunately, the results from the tissue-cultured lilac were not confirmed in the field. The effect of multiple applications of Agribrom is worth investigating, as results were promising early in the field season. Agri-mycin 17 was only moderately effective against streptomycin-sensitive strains of *P. syringae* pv. *syringae* on both tissue-cultured and field-grown lilac, and several copper-based bactericides were more effective. Several studies have rated streptomycin sulfate equal to or better than copper-based bactericides in field trials with *P. syringae* pv. *tomato* on tomatoes (7,8,11), *X. campestris* pv. *vesicatoria* on peppers (23), and *X. campestris* pv. *citri* on citrus (24), especially when applied very frequently and throughout the growing season. Agri-mycin 17 is only registered for use on a few ornamental woody plants in Oregon. Widespread use of streptomycin on ornamentals in the future is unlikely, as streptomycin resistance genes are widespread in strains of *P. syringae* pv. *syringae* isolated from Northwest nurseries (38).

Copper- and non-copper-based bactericides applied to field-grown lilacs have not consistently provided adequate disease control. In 1995 when disease levels were very high, a commercially acceptable level of control was not achieved with any of the copper- or non-copper-based bactericides. Protecting woody plants from late spring frosts and heavy rain is important for preventing epidemics. Disease levels on the sheltered shrubs were equivalent to levels on shrubs treated with the most effective copper-based bactericides. Nurseries in the Pacific Northwest should consider integrating cultural practices into their overall management of *Pseudomonas* diseases. For example, growing lilac and other

Fig. 6. Population progress curves for *Pseudomonas syringae* pv. *syringae* strains grown on tissue-cultured lilac (cv. Sensation) treated with non-copper-based bactericides.

woody plants susceptible to *P. syringae* pv. *syringae* under plastic shelters until the risk of frost injury is over and heavy spring rains have ended is an alternative to sole reliance on chemical disease management.

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LITERATURE CITED

- Andersen, G. L., and Lindow, S. E. 1986. Occurrence and control of copper tolerant strains of *Pseudomonas syringae* on almond and citrus in California. (Abstr.) *Phytopathology* 76:1118.
- Andersen, G. L., Menkissoglou, O., and Lindow, S. E. 1991. Occurrence and properties of copper-tolerant strains of *Pseudomonas syringae* isolated from fruit trees in California. *Phytopathology* 81:648-656.
- Arman, P., and Wain, R. L. 1958. The role of leaf exudates in the solution of copper from Bordeaux mixture. *Ann. Appl. Biol.* 46:366-374.

- Burnham, T. J. 1995. Walnut blight: Promising breakthroughs on control. *Pacific Nut Prod.* 2:4-5.
- Canfield, M. L., Baca, S., and Moore, L. W. 1986. Isolation of *Pseudomonas syringae* from 40 cultivars of diseased woody plants with tip dieback in Pacific Northwest nurseries. *Plant Dis.* 70:647-650.
- Chase, A. R. 1986. Effect of experimental bactericides on three bacterial diseases of foliage plants. *J. Environ. Hortic.* 4:37-41.
- Colin, J. E., and Chafik, Z. 1986. Comparison of biological and chemical treatments for control of bacterial speck of tomato under field conditions in Morocco. *Plant Dis.* 70:1048-1050.
- Conlin, K. C., and McCarter, S. M. 1983. Effectiveness of selected chemicals in inhibiting *Pseudomonas syringae* pv. *tomato* in vitro and in controlling bacterial speck. *Plant Dis.* 67:639-644.
- Gaignard, J. L., and Luisetti, J. 1992. A method using in vitro and in situ grapevine plants to screen chemicals active against *Pseudomonas syringae* pv. *syringae*. *Agronomie* 12:401-409.
- Gross, D. C., Cody, Y. S., Proebsting, E. L., Jr., Rademaker, G. K., and Spotts, R. A. 1983. Distribution, population dynamics and characteristics of ice-nucleation-active bacteria in deciduous fruit tree orchards. *Appl. Environ. Microbiol.* 46:1370-1379.
- Jardine, D. J., and Stephens, C. T. 1987. In-

fluence of timing of application and chemical on control of bacterial speck of tomato. *Plant Dis.* 71:405-408.

- Jones, J. B., Woltz, S. S., Jones, J. P., and Portier, K. L. 1991. Population dynamics of *Xanthomonas campestris* pv. *vesicatoria* on tomato leaflets treated with copper bactericides. *Phytopathology* 81:714-719.
- Jones, J. P., and Jones, J. B. 1988. Field control of target spot and bacterial speck of tomato. *Proc. Fla. State Hort.* 101:358-361.
- King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. *J. Lab. Clin. Med.* 44:301-307.
- Latorre, B. A., and Jones, A. L. 1979. *Pseudomonas morsprunorum*, the cause of bacterial canker of sour cherry in Michigan, and its epiphytic association with *P. syringae*. *Phytopathology* 69:335-339.
- Lee, Y.-A., Schroth, M. N., Henderson, M., Lindow, S. E., Wang, X.-L., Olson, B., Buchner, R. P., and Teviotdale, B. 1993. Increased toxicity of iron-amended copper-containing bactericides to the walnut blight pathogen *Xanthomonas campestris* pv. *juglandis*. *Phytopathology* 83:1460-1465.
- Lelliott, R. A., and Stead, D. E. 1987. *Methods in Plant Pathology*. Vol. 2, Methods for the Diagnosis of Bacterial Diseases of Plants. Blackwell Scientific Publications, Oxford.
- Lindow, S. E. 1983. Methods of preventing frost injury through control of epiphytic ice-nucleation-active bacteria. *Plant Dis.* 67:327-333.
- Lindow, S. E., Arny, C. C., and Upper, C. D. 1978. Distribution of ice nucleation-active bacteria on plants in nature. *Appl. Environ. Microbiol.* 36:831-838.
- Lindow, S. E., and Connell, J. H. 1984. Reduction of frost injury to almond by control of ice nucleation active bacteria. *J. Am. Soc. Hortic. Sci.* 109:48-53.
- MacSwan, I. C. 1972. Preparation of tank-mix Bordeaux mixture. Oregon State University Co-op. Ext. Fact Sheet 185, Corvallis.
- Marco, G. M., and Stall, R. E. 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.
- McCarter, S. M. 1992. Effects of bactericide treatments on bacteria spot severity and yield of different pepper genotypes and on populations of certain insects. *Plant Dis.* 76:1042-1045.
- McGuire, R. G. 1988. Evaluation of bactericidal chemicals for control of *Xanthomonas* on citrus. *Plant Dis.* 72:1016-1020.
- Medhekar, S., and Boparai, K. S. 1981. Fungicidal bis(1-amidino-*O*-ethylisourea) copper (II) carbamates. *J. Agric. Food Chem.* 29:421-422.
- Menkissoglou, O., and Lindow, S. E. 1991. Relationship of free ionic copper and toxicity to bacteria in solutions of organic compounds. *Phytopathology* 81:1258-1263.
- Menkissoglou, O., and Lindow, S. E. 1991. Chemical forms of copper on leaves in relation to the bactericidal activity of cupric hydroxide deposits on plants. *Phytopathology* 81:1263-1270.
- Moore, L. W. 1988. *Pseudomonas syringae*: Disease and ice nucleation activity. *Ornamentals Northwest* 12:4-16.
- O'Brien, R. D., and Lindow, S. E. 1989. Effect of plant species and environmental conditions on epiphytic population sizes of *Pseudomonas syringae* and other bacteria. *Phytopathology* 79:619-627.
- Olson, B. D., and Jones, A. L. 1983. Reduction of *Pseudomonas syringae* pv. *morsprunorum* on Montmorency sour cherry with cop-

- per and dynamics of the copper residues. *Phytopathology* 73:1520-1525.
31. Parsons, I. M., and Edgington, L. V. 1991. The possible role of fixed coppers in combination with ethylene bis-dithiocarbamate for control of *Pseudomonas syringae* pv. *tomato*. (Abstr.) *Phytopathology* 71:563.
 32. Pscheidt, J. W., ed. 1996. Pacific Northwest Plant Disease Control Handbook. Oregon State University Co-operative Extension, Corvallis.
 33. Ritchie, D. F., and Bennett, M. H. 1990. Evaluation of copper and other materials for control of bacterial spot. *Fungic. Nematicide Tests* 46:142-143.
 34. Ritchie, D. F., and Bennett, M. H. 1991. Impact of copper and additives to copper on pepper yield in the presence of copper-sensitive and -resistant bacterial pathogen strains. *Fungic. Nematicide Tests* 47:105-106.
 35. Sambrook, J., Fritsch, E. F., and Maniatis, T. 1989. *Molecular Cloning: A Laboratory Manual*. 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
 36. Schaad, N. W., ed. 1988. *Laboratory Guide for Identification of Plant Pathogenic Bacteria*. 2nd ed. American Phytopathological Society, St. Paul, MN.
 37. Scheck, H. J., Canfield, M. L., Pscheidt, J. W., and Moore, L. W. 1997. Rapid evaluation of pathogenicity in *Pseudomonas syringae* pv. *syringae* with a lilac tissue culture bioassay and syringomycin DNA probes. *Plant Dis.* 81:905-910.
 38. Scheck, H. J., Pscheidt, J. W., and Moore, L. W. 1996. Copper and streptomycin resistance in strains of *Pseudomonas syringae* from Pacific Northwest nurseries. *Plant Dis.* 80:1034-1039.
 39. Schwartz, H. F., and McMillan, M. S. 1989. Assaying bactericide efficacy against pseudomonads on bean leaf surfaces. *Plant Dis.* 73:822-824.
 40. Shaner, G., and Finney, R. E. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. *Phytopathology* 67:1051-1056.
 41. Spotts, R. A., and Cervantes, L. A. 1995. Copper, oxytetracycline, and streptomycin resistance of *Pseudomonas syringae* pv. *syringae* strains from pear orchards in Oregon and Washington. *Plant Dis.* 79:1132-1135.
 42. Timmer, L. W., and Zitko, S. E. 1996. Evaluation of copper fungicides and rates of metallic copper for control of melanose on grapefruit in Florida. *Plant Dis.* 80:166-169.
 43. Wimalajeewa, D. L. S., Cahill, R., Hepworth, G., Schneider, H. G., and Washbourne, W. 1991. Chemical control of bacterial canker (*Pseudomonas syringae* pv. *syringae*) of apricot and cherry in Victoria. *Aust. J. Exp. Agric.* 31:705-708.
 44. Zevenhuizen, L. P. T. M., Dolfin, J., Eshuis, E. J., and Scholten-Koerselman, J. 1979. Inhibitory effects of copper on bacteria related to the free ion concentration. *Microb. Ecol.* 5:139-164.