Reduction of *Pseudomonas syringae* pv. *morsprunorum* on Montmorency Sour Cherry with Copper and Dynamics of the Copper Residues

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ABSTRACT

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A rifampicin-resistant strain of *P. syringae* pv. *morsprunorum* (PsmR) was used to study the effectiveness of tribasic copper sulfate (TBS) and 48% copper salts of fatty and rosin acids (Citcop 4E) for reducing populations of PsmR on Montmorency sour cherry trees in spring and early summer. Populations of PsmR were reduced more by 636 and 949 mg of TBS per liter than by 200 mg of Citcop 4E per liter, but several applications were needed to reduce the populations to a low level. Citcop 4E at 200 mg/L was more phytotoxic to cherry foliage than TBS at 636 mg/L. Phytotoxicity was related to the number of applications and was not reduced by adding hydrated lime to the copper treatments. The loss of copper residues from leaves was related by multiple regression analyses to rainfall and the initial level of copper on leaves.

Additional key words: chemical control, *Prunus cerasus*.

Bacterial canker, which is caused by *Pseudomonas syringae* pv. *morsprunorum*, was first observed on *Prunus cerasus* L. cv. *Montmorency* (sour cherry) in Michigan in 1976 (12). No effective control measures have been tested. In Oregon (15), application of copper compounds has been recommended in autumn and just before bud break to control infection of dormant buds on sweet cherry. In England (14), Bordeaux mixture applied to sweet cherry trees at the white bud stage and again at petal fall reduced the leaf spot phase of the disease, but the petal fall spray was highly toxic to the leaves. A single spray of Bordeaux mixture at the white bud stage had no significant effect on the leaf spot phase (7,14), but sprays of streptomycin at full bloom and at 75 and 100% petal fall reduced leaf spot symptoms by 93 to 96% (5). In California (2) and Connecticut (17), blossom blast of pears, caused by *P. syringae* pv. *syringae*, was reduced by a series of streptomycin or fixed copper treatments before, during, and after bloom. These studies suggest that repeated spray treatments in spring may control leaf, blossom, and fruit infections by *P. syringae* pv. *morsprunorum* in Michigan.

The objectives of this study were to investigate the effectiveness of fixed copper compounds in reducing populations of *P. syringae* pv. *morsprunorum* from early bud break through early summer on Montmorency sour cherry and to examine the retention of copper on the foliage. Primarily copper compounds were evaluated because they have activity against some important fungal pathogens of cherry, and registration of these compounds by regulatory agencies was more likely than registration of streptomycin.

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MATERIALS AND METHODS

Field inoculations. The rifampicin-resistant strain of *P. syringae* pv. *morsprunorum* (PsmR) selected by Latorre and Jones (13) was used in all experiments. Inoculum grown on King's medium B (11) for 2 days at 22 C was suspended in 10 ml of 0.01 M phosphate buffer, pH 7.2. Inoculum suspensions containing 10^8 colony-forming units (cfu) per milliliter of phosphate buffer were obtained by adjusting the turbidity of suspensions to 0.04 absorbance units at 625 nm. Montmorency sour cherry trees in East Lansing, MI, were spray-inoculated as indicated in Fig. 1. All trees were inoculated at sunset with a handgun sprayer (28 kg/cm^2) until leaves were lightly wet. The amount of inoculum applied to each tree was 0.2 to 1.5 L depending on tree size.

Spray trials. On inoculated trees, tribasic copper sulfate 53W (TBS) and 48% copper salts of fatty and rosin acids (Citcop 4E; Cities Service Co., Atlanta, GA 30302) were evaluated along with streptomycin sulfate 21.2W (Pfizer Inc., New York, NY 10017). Concentrations of spray solutions containing copper were based on the amount of copper salt per liter. Hydrated lime ($Ca[OH]_2$) was evaluated for reducing phytotoxicity from copper sprays. Because of the persistence of captafol (Difolatan 4F; Chevron Chemical Co., Richmond, CA 94804) on apple trees (16), the possibility that it may improve the retention of copper on cherry leaves was evaluated. All treatments (Tables 1 and 2) were applied to runoff with a handgun sprayer operated at 28 kg/cm^2 on the dates shown in Fig. 1. Treatments were applied to single-tree plots arranged in a randomized complete block design according to tree size. Each treatment was replicated five times.

In 1981, defoliation was rated by visually estimating the percentage of fallen leaves per replication 11 and 13 days, respectively, after the final copper treatment on 25 June. Concentrations of copper on leaves were measured on 8 July, 3 days after the onset of defoliation. Five terminal shoots, each with 12 intact leaves, were selected at random from each replication. Starting from the base of each shoot, leaves at nodes 1, 3, 6, 8, and 11 were harvested and pooled by node number. Leaf areas were measured with an area meter (model LI-3000, LAMBDA Instruments Corp., Lincoln, NE 68504) and copper concentrations were measured with a plasma emission spectrometer, which will be described later. Leaf age was estimated by using a degree-day leaf emergence model developed for Montmorency sour cherry (9). Temperatures were recorded with a hygrothermograph (Bendix Co. Inc., Baltimore, MD 21204) placed in a standard weather instrument shelter 1.5 m above the orchard floor. The number of copper sprays applied to each leaf was estimated based on the approximate date of leaf emergence.

In East Lansing, we studied the loss of copper from leaves on Montmorency sour cherry trees treated once with 3.2 g of TBS with and without 12 g of hydrated lime per liter and from trees treated four times with 1.3 g of TBS per liter + 4.8 g of hydrated lime per liter. Treatments were applied to 30-yr-old trees and each treatment was replicated four times in a randomized complete block design. Treatments were applied as described earlier. Each replicate contained two or three trees. The high rate of TBS was applied on 18 and 28 September, and 8 and 19 October 1981.

Monitoring bacterial populations. Recovery of bacteria from plant material sprayed with copper compounds can be difficult when using standard isolation procedures. In 1978, Young (20) reported that the bactericidal activity of copper was inactivated in phosphate buffer. To verify this report, we determined the survival of PsmR in distilled water and phosphate buffer with and without TBS + hydrated lime. Treatment solutions of phosphate buffer (13.6 mg of KH_2PO_4 + 14.2 mg of Na_2HPO_4 per milliliter) and distilled water were amended with 9.9 mg TBS + 39.6 mg of hydrated lime per milliliter and then diluted 10- and 100-fold with phosphate buffer and distilled water, respectively. A stock solution containing approximately 10^4 cfu PsmR per milliliter of distilled water was divided into 100-ml aliquots. Aliquots of stock solution were mixed with 10-ml aliquots of treatment solutions to give the treatments listed in Table 3. After 10 min, duplicate 0.1-ml subsamples from each replication were plated onto King's medium

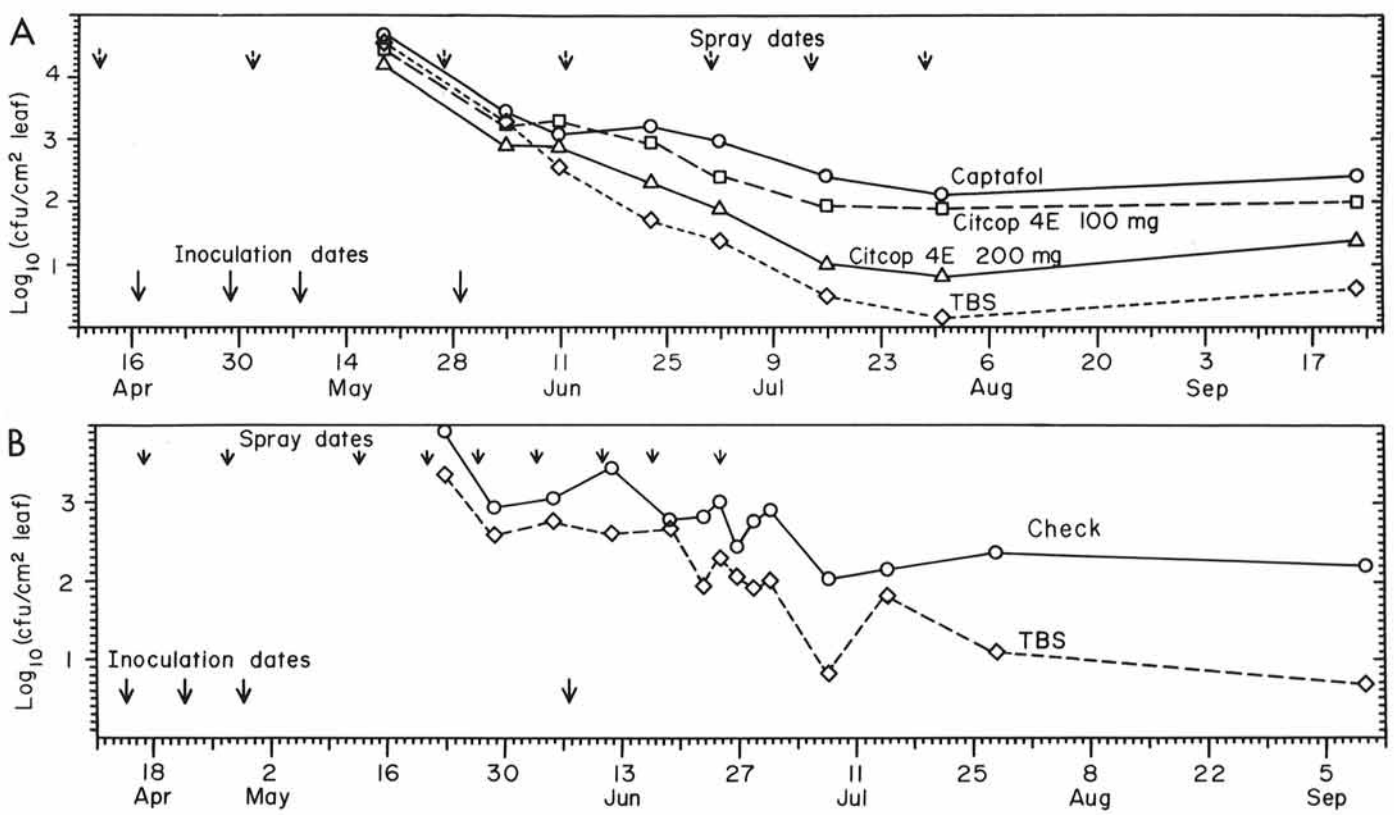

Fig. 1. Colony-forming units (cfu) of rifampicin-resistant *Pseudomonas syringae* pv. *morsprunorum* on Montmorency sour cherry leaves sprayed with copper treatments. **A,** In 1980, treatments were 986 mg of captafol, 949 mg of tribasic copper sulfate (TBS), and 100 and 200 mg of Citcop 4E per liter. **B,** In 1981, treatments were 636 mg TBS per liter and check treatments (nonsprayed and 491 mg captafol per liter combined).

B. Colonies were counted after 5 days of incubation at 22 C. Each treatment was replicated five times.

Populations of PsmR were monitored on each inoculated single-tree replicate. Quadrants were designated in the canopy of each tree and 10 fully-expanded leaves were randomly chosen from each quadrant. Leaf areas were measured with an area meter before each 40-leaf sample was homogenized for 2 min with 300 ml of phosphate buffer in a blender (Waring Products Inc., New Hartford, CT 06057). The homogenate was serially diluted in phosphate buffer and duplicate 0.1-ml subsamples were pipetted onto a modified King's medium B amended with 50 μg of rifampicin (Calbiochem-Behring Corp., La Jolla, CA 92037) and 25 μg of cycloheximide (Sigma Chemical Co., St. Louis, MO 63178) per milliliter. Colonies were counted after 5 days of incubation at 22 C and the cfu of PsmR per square centimeter of leaf were determined.

Monitoring copper deposits on leaves. To determine the rate of copper loss from Montmorency cherry leaves, leaf samples collected after treatments were applied. In 1980, samples were taken on 2 and 10 June; 1, 7, 14, and 29 July; 6 and 12 August; and in 1981 on 2 June, 8 July, and 10 September. In the autumn of 1981, samples were collected on 18, 23, and 28 September and on 3, 8, 13, 19, 24, and 29 October.

To determine the concentration of copper on leaves, quadrants were established in the canopy of each replicate tree and 10 leaves per quadrant were collected randomly. The quantity of copper per square centimeter of leaf area was determined for each replication by cutting 1.5-cm^2 leaf disks from the center of all 40 leaves with a cork borer. The disks were dried at 72 C for at least 18 hr, weighed, and ground to a fine powder with a mortar and pestle. For each replication, 100 mg of leaf powder was digested for 18 hr at 72 C with 2 ml of "Baker Instra-Analyzed" nitric acid (J. T. Baker Chemical Co., Philipsburg, NJ 08865) in a 10-ml Teflon-gasketed screw-cap glass vial. By using a volumetric flask, each digested replication was increased to 10 ml with double-glass-distilled water filtered through a Milli-Q water purification system (Millipore

TABLE 1. Effect of copper treatments on populations of rifampicin-resistant *Pseudomonas syringae* pv. *morsprunorum* (PsmR) on Montmorency sour cherry leaves and the analysis of variance for a set of planned paired comparisons in 1980

Treatments and rates per liter	PsmR recovered on KBrc[u] (log_{10}[cfu + 1/cm^2 leaf])	Reduction in bacteria (%)[v]
Captafol, 986 mg	3.05 ± 1.26	0
Tribasic copper sulfate,		
949 mg + lime[w], 3.59 g	1.84 ± 1.54	94
Citcop 4E[x],		
100 mg	2.69 ± 1.23	56
100 mg + lime, 1.19 g	2.83 ± 1.05	40
100 mg + captafol, 986 mg	2.77 ± 0.98	48
200 mg	2.06 ± 1.39	90
200 mg + lime, 1.19 g	2.02 ± 1.22	91
200 mg + captafol, 986 mg	2.46 ± 1.29	74

Planned paired comparisons between treatments[y]	df	ms	F[z]
Captafol versus copper	1	15.6	10.6**
Tribasic copper sulfate versus Citcop 4E	1	13.6	9.4**
Citcop 4E, 100 mg versus Citcop 4E, 200 mg (I)	1	20.0	13.8**
Citcop 4E alone versus Citcop 4E + lime and Citcop 4E + captafol (II)	1	1.1	0.8
Citcop 4E + lime versus Citcop 4E + captafol (III)	1	1.4	1.0
Interaction between comparisons I and II	1	0.1	0.0
Interaction between comparisons I and III	1	2.5	1.7
Blocks	4	7.2	5.2*
Error	28	1.5	

[u] KBrc = A modified King's medium B amended with 50 μg of rifampicin per milliliter and 25 μg of cycloheximide per milliliter. Each value is the mean of five replications measured on eight sampling dates followed by the standard error of the mean.

[v] Percent reduction in bacteria is the difference in recovery of PsmR between the captafol (alone) treatment and each copper treatment divided by the captafol treatment and multiplied by 100.

[w] Lime = hydrated lime, $Ca(OH)_2$.

[x] Citcop 4E = 48% copper salts of fatty and rosin acids.

[y] Planned paired comparisons between treatments were determined for PsmR recovered on KBrc.

[z] Asterisks: * = significant, $P = 0.05$; and ** = significant, $P = 0.01$.

TABLE 2. Effect of copper treatments on populations of rifampicin-resistant *Pseudomonas syringae* pv. *morsprunorum* (PsmR) on Montmorency sour cherry leaves and the analysis of variance for a set of planned paired comparisons in 1981

Treatments and rates per liter	PsmR recovered on KBrc[u] (log_{10}[cfu + 1/cm^2 leaf])	Reduction in bacteria (%)[v]
Check (no spray)	2.76 ± 0.72	0
Captafol, 491 mg	2.79 ± 0.93	0
Tribasic copper sulfate		
636 mg	2.16 ± 1.04	76
636 mg + lime[w], 3.59 g	1.91 ± 1.20	86
636 mg + captafol, 491 mg	2.05 ± 1.02	81
Citcop 4E[x]		
200 mg	2.40 ± 0.74	58
200 mg + lime, 3.59 g	2.52 ± 0.91	44
200 mg + captafol, 491 mg	2.27 ± 1.12	69
Streptomycin sulfate, 121 mg	2.03 ± 1.08	82

Planned paired comparisons between treatments[y]	df	ms	F[z]
Check vs captafol alone	1	0.0	0.0
Check and captafol versus copper and streptomycin	1	37.2	28.3**
Copper versus streptomycin	1	2.1	1.6
Tribasic copper sulfate versus Citcop 4E (I)	1	13.1	10.0**
Copper alone versus copper + lime and copper + captafol (II)	1	0.8	0.6
Copper + lime versus copper + captafol (III)	1	0.2	0.2
Interaction between comparisons I and II	1	0.8	0.6
Interaction between comparisons I and III	1	2.5	1.9
Blocks	4	12.5	9.5**
Error	32	1.3	

[u] KBrc = A modified King's medium B amended with 50 μg rifampicin and 25 μg cycloheximide per milliliter. Each value is the mean of five replications measured on 15 sampling dates followed by the standard error of the mean.

[v] Percent reduction in bacteria is the difference in recovery of PsmR between the check treatments (average of the check and captafol treatments) and each copper treatment divided by the check treatments and multiplied by 100.

[w] Lime = hydrated lime, $Ca(OH)_2$.

[x] Citcop 4E = 48% copper salts of fatty and rosin acids.

[y] Planned paired comparisons between treatments were analyzed for PsmR recovered on KBrc.

[z] Asterisks: ** = significant ($P = 0.01$).

TABLE 3. Recovery of rifampicin-resistant *Pseudomonas syringae* pv. *morsprunorum* (PsmR) from distilled water and phosphate buffer each containing tribasic copper sulfate (TBS) + hydrated lime (lime)

Suspending solution	Concentration of amendments		PsmR recovered on King's medium B (colony-forming units/ml)
	TBS (mg/ml)	Lime (mg/ml)	
Distilled water	0.000	0.000	187 ± 83[z]
Distilled water	0.009	0.036	3 ± 1
Distilled water	0.095	0.360	0 ± 0
Distilled water	0.949	3.600	0 ± 0
Phosphate buffer[y]	0.000	0.000	294 ± 80
Phosphate buffer	0.009	0.036	372 ± 72
Phosphate buffer	0.095	0.360	278 ± 56
Phosphate buffer	0.949	3.600	371 ± 71

[y] Phosphate buffer at 0.01 M and pH = 7.2.

[z] Mean of five replications followed by the standard error of the mean. Using a set of planned paired comparisons between treatments, distilled water without TBS + hydrated lime versus all phosphate buffer treatments were significantly ($P = 0.01$) different (ms = 80,712, F = 13.5, error df = 16, and error ms = 5,968).

Corp., Bedford, MA 01730). Each diluted sample was poured into a 16 × 125-mm screw-capped culture tube and held at room temperature for 24 hr until precipitates formed. Each sample was centrifuged at 150 *g* for 30 min and the supernatant was decanted into a clean culture tube.

Digested samples were analyzed for copper by using a plasma emission spectrometer (Jarrel-Ash model 955 Plasma Atomcomp; Fisher Scientific, Waltham, MA 02254) with the inductively coupled plasma operated at 1.1-kw forward power, 1.6-mm flame height, 0.84 to 0.98 kg/cm^2 nebulizer pressure, and a sample flow rate of 1.4 ml/min. The spectrometer was standardized with a 10 μg/ml stock solution of copper in 20% "Baker Instra-Analyzed" nitric acid prepared by serially diluting a 1,000 μg/ml copper atomic spectral standard solution ("Baker Instra-Analyzed"; J. T. Baker Chemical Co., Philipsburg, NJ 08865). The results from the spectrometer were used to determine the micrograms of copper per square centimeter of leaf. All vials, glassware, and utensils used in the copper analyses were washed in 24% nitric acid (commercial grade) for 30 min before being rinsed with distilled, and double-glass-distilled, Milli-Q-filtered water. Blank samples were processed repeatedly to monitor for copper contaminants in the reagents and on the glassware.

The relationship between copper loss from the leaves and rainfall was established by using stepwise regression (8). The amount of rainfall and the difference in the level of copper on leaves between two sampling periods were computed. Rainfall was measured with a tipping-bucket rain gauge (WEATHERtronics Inc., West Sacramento, CA 95691) and recorded with an event recorder or a microcomputer-based instrument (10). Citcop 4E and TBS treatments were analyzed separately by using a second degree polynomial of the general form:

$$CL = b_0 + b_1C + b_2R + b_3RC + b_4C^2 + b_5R^2 + b_6(RC)^2 + \epsilon$$

in which CL = the difference in the level of copper (micrograms of Cu^{++} per square centimeter of leaf) on leaves between two sampling periods, R = rainfall in millimeters between sampling periods, C = the level of copper (micrograms per square centimeter of leaf) on leaves for the first of the two sampling periods, b_0 to b_6 = partial regression coefficients, and ϵ = a normally distributed random variable with mean zero and variance (σ^2). Factors included in the final equation were significant, $P = 0.01$. Only the regression models having the best combination of high coefficients of determination and residuals supporting the assumptions that errors were independent and normally distributed were retained (8). The data were plotted by using a computer program designed to plot randomly selected three-dimensional data points (19).

General data analyses. Data collected sequentially during the growing season were analyzed as a split-plot with time (18). Treatment differences in most experiments were analyzed by using planned paired comparisons. All data were analyzed with the Stat 4 statistical program (3) and Cyber 750 computer (Control Data Corp., Minneapolis, MN 55440). Unless stated otherwise differences were significant, $P = 0.01$.

RESULTS

Copper phytotoxicity. In 1980, leaf chlorosis and defoliation were not observed in any copper treatment. In 1981, copper-treated trees had significantly greater defoliation than nonsprayed and streptomycin- and captafol-sprayed trees (Table 4). Trees sprayed with Citcop 4E had significantly more defoliation than trees sprayed with TBS. Hydrated lime and captafol did not reduce the level of defoliation in the TBS and Citcop 4E treatments.

Leaves at the base of terminal and lateral shoots were the first to show injury from copper sprays. Trees sprayed with Citcop 4E had more leaves showing injury than trees sprayed with TBS (Table 5). On 8 July, the amount of copper on leaves sprayed with TBS or Citcop 4E was significantly ($P = 0.05$) higher on leaves that emerged on 11 May than on those that emerged on 26 June (Table 5). The approximate threshold concentration of copper that resulted in phytotoxicity was 8.3 and 1.4 μg Cu^{++}/cm^2 of leaf for TBS and Citcop 4E, respectively. These leaves received seven and four applications of TBS and Citcop 4E, respectively.

Recovery of bacteria from buffered copper solutions. High numbers of PsmR (278 to 372 cfu/ml) were recovered from all phosphate buffer solutions containing TBS (Table 3). Only a few PsmR (0 to 3 cfu/ml) were recovered from distilled water amended with 0.009 mg of TBS + 0.036 mg of hydrated lime per milliliter. Recovery of PsmR from the phosphate buffer + TBS treatments and from phosphate buffer alone were similar and significantly greater than recovery of PsmR from distilled water alone.

Effect of copper on bacterial populations. In 1980, trees treated with copper had significantly lower populations of PsmR than trees

TABLE 4. Defoliation of Montmorency sour cherry trees from various copper treatments and the analysis of variance for a set of planned paired comparisons in 1981

Treatments and rates per liter	Defoliation (%)[w]
Check (no spray)	0.1 ± 0.3
Captafol, 491 mg	0.3 ± 0.5
Tribasic copper sulfate	
636 mg	6.5 ± 6.5
636 mg + lime[x], 3.59 g	7.1 ± 8.7
636 mg + captafol, 491 mg	11.7 ± 9.2
Citcop 4E[y]	
200 mg	43.7 ± 30.8
200 mg + lime, 3.59 g	45.4 ± 32.6
200 mg + captafol, 491 mg	37.1 ± 26.6
Streptomycin sulfate, 121 mg	0.1 ± 0.3

Planned paired comparisons between treatments	df	ms	F[z]
Copper versus no copper	1	12,575	38**
Tribasic copper sulfate versus Citcop 4E (I)	1	16,951	51**
Copper alone versus copper + lime and copper + captafol (II)	1	0	0
Copper + lime versus copper + captafol (III)	1	35	0
Interaction between comparisons I and II	1	96	0
Interaction between comparisons I and III	1	419	1
Check versus captafol alone	1	0	0
Captafol versus streptomycin sulfate	1	0	0
Blocks	4	243	1
Error	32	331	

[w] Each value is the mean of five replications followed by the standard error of the mean. Data were recorded on 6 and 8 July.
[x] Lime = hydrated lime, $Ca(OH)_2$.
[y] Citcop 4E = 48% copper salts of fatty and rosin acids.
[z] Asterisks: ** = significant ($P = 0.01$).

TABLE 5. The relationship of the number of copper sprays applied to Montmorency sour cherry leaves and residues of copper on the leaves to phytotoxicity

Leaf emergence (date)[v]	Sprays (no.)[t]	μg Cu^{++}/cm^2 leaf[u]			Phytotoxicity[s]		
		Check[v]	TBS[w]	Citcop 4E[x]	Check	TBS	Citcop 4E
11 May	7	0.4 a[z]	8.3 a	2.3 a	−	+	+
22 May	5	0.5 a	7.2 b	1.5 b	−	−	+
2 June	4	0.5 a	5.5 c	1.4 b	−	−	+
11 June	3	0.4 a	4.6 d	0.9 bc	−	−	−
26 June	1	0.3 a	2.6 e	0.6 c	−	−	−

[s] Knowing the position of the leaf on the terminal, the date of leaf emergence was calculated with a Montmorency sour cherry leaf emergence degree-day model.
[t] Estimated number of sprays applied to each leaf.
[u] Each value is the mean of five replications sampled on 8 July 1981.
[v] Nonsprayed.
[w] TBS = tribasic copper sulfate at 636 mg/L.
[x] Citcop 4E (= 48% copper salts of fatty and rosin acids) at 200 mg/L.
[y] + = chlorotic leaves observed, − = no chlorotic leaves observed.
[z] Values within a column followed by the same letter do not differ significantly according to Duncan's multiple range test, $P = 0.05$.

treated with captafol (Table 1). Among the copper treatments, trees treated with TBS had significantly fewer PsmR than trees treated with Citcop 4E, and trees treated with 200 mg of Citcop 4E per liter had significantly fewer PsmR than trees treated with 100 mg/L. Following the last inoculation on 29 May, populations of PsmR declined steadily as four applications of copper were applied (Fig. 1A). By 31 July, populations of PsmR on trees treated with TBS and Citcop 4E (200 mg/L) were 98.9 and 95.0% less, respectively, than those on captafol-treated trees. The decline in populations of PsmR from 4 to 10 June in the TBS treatment occurred despite the development of leaf symptoms of bacterial canker starting 10 June. However, leaf symptoms were not severe enough to detect differences between treatments.

In 1981, trees treated with copper and streptomycin had significantly fewer PsmR than nontreated and captafol-treated trees (Table 2). Streptomycin-treated trees had populations of PsmR similar to trees treated with TBS or Citcop 4E. Trees treated with TBS had significantly fewer PsmR than trees treated with Citcop 4E. Except on 20 June, populations of PsmR were lower on TBS-treated trees than on nontreated or captafol-treated trees (Fig. 1B). On 29 June and 10 September, populations of PsmR on the TBS-treated trees were 94 and >99%, respectively, less than on the check treatments. Populations of PsmR on trees treated with 200 mg of Citcop 4E per liter were below populations on nonsprayed trees except on 14 July (*unpublished*). Populations of PsmR were not affected by adding hydrated lime or captafol to the copper treatments in 1980 and 1981. No symptoms of bacterial canker developed in 1981.

Dynamics of copper deposition. In the autumn of 1981, the amount of copper loss for trees treated with 3.2 g of TBS per liter with and without hydrated lime were 28 and 27%, respectively; therefore, data from these two treatments were combined. Leaves from trees sprayed with 3.2 g of TBS per liter had 9.5 and 6.0 μg more copper per square centimeter of leaf on 18 and 23 September, respectively, than trees sprayed with 1.3 g of TBS per liter (Fig. 2). On 28 September, the concentrations of copper on leaves retreated with 1.3 g of TBS per liter increased from 3 to 10 $\mu g/cm^2$ of leaf, and was 3 μg higher than the concentration remaining on the leaves that had received 3.2 g of TBS per liter. Copper concentrations dropped dramatically between 18 and 23 September and between 28 September and 3 October because of 40- and 89-mm rain on 21 and 30 September, respectively. On 3 October, copper concentrations on leaves that had received either 1.3 or 3.2 g of TBS per liter were 3 $\mu g/cm^2$ of leaf, and concentrations in the latter treatment remained relatively constant. On 8 October, the trees that had received 1.6 g of TBS per liter were sprayed a third time and the concentration of copper increased to 10.2 $\mu g/cm^2$ of leaf, remaining at this concentration through 13 October due to lack of rain. After an additional 15 mm of rain, however, the concentration of copper dropped to 4.8 $\mu g/cm^2$ leaf by 19 October. On this date, the trees that had received 1.6 g of TBS per liter were sprayed a fourth time and concentrations of copper increased to 9.5 $\mu g/cm^2$ leaf, which dropped to 6.1 $\mu g/cm^2$ leaf on 24 October following an 8-mm rain. On 27 October, 5 mm of rain had no effect on the concentration of copper.

Copper residue data for Citcop 4E in 1980 and 1981 were analyzed by using stepwise regression to determine if the loss of copper (CL) from leaves was a function of rainfall (R) and initial concentration of copper (C) on leaves. The data included 225 points and the resulting model was:

$$CL = (-7.6827 \times 10^{-3}) + (1.6186 \times 10^{-1} C) + (1.9529 \times 10^{-2} RC) - (3.080 \times 10^{-4} R^2).$$

The R^2 value was 0.823. A nomogram of the original data shows how copper loss (micrograms of Cu^{++} per square centimeter of leaf) increased as rainfall and concentration of the initial deposits of copper on leaves increased (Fig. 3A).

A similar analysis of residue data for TBS in 1980 and 1981 included 194 points and the resulting model was:

$$CL = -1.6584 + (2.8058 \times 10^{-1} C) + (1.2271 \times 10^{-1} R) + (5.5985 \times 10^{-3} RC) - (1.167 \times 10^{-3} R^2).$$

The R^2 value was 0.9073. As rainfall amounts and the initial concentrations of copper on leaves increased, greater quantities of copper were lost from leaves (Fig. 3B).

There were no differences in copper loss between copper treatments with and without captafol or hydrated lime.

DISCUSSION

In the early 1950s, organic fungicides began to replace copper compounds for the control of cherry leaf spot and brown rot on Montmorency sour cherry in Michigan. By 1970, copper compounds were eliminated from most cherry disease control programs. Bacterial canker developed for the first time on Montmorency sour cherry in Michigan in 1976 (12) and it has continued to be a sporadically significant problem. In this study, repeated application of copper compounds reduced populations of *P. syringae* pv. *morsprunorum*, the cause of bacterial canker, in Michigan (12). We suggest that, prior to 1970, the frequent use of copper to control other diseases also controlled bacterial canker. Now that copper compounds are not used on cherry, the pathogen is no longer being suppressed.

TBS has several advantages over Citcop 4E for use on Montmorency sour cherry. TBS more effectively reduced populations of PsmR, was less phytotoxic, and maintained higher concentrations of copper on leaves than Citcop 4E. Unlike Bordeaux mixture, the addition of hydrated lime to formulations of TBS and Citcop 4E did not reduce the phytotoxic effects of copper.

Our results indicate the following strategy should be used for reducing populations of *P. syringae* pv. *morsprunorum* on Montmorency sour cherry. TBS should be applied every 7 to 10 days starting at the green-tip bud stage to prevent buildup of populations of *P. syringae* pv. *morsprunorum* on emerging leaves and flower parts. Populations of PsmR were probably higher in the experimental orchard than populations of *P. syringae* pv. *morsprunorum* in commercial orchards because we made several artificial inoculations; therefore, populations of *P. syringae* pv.

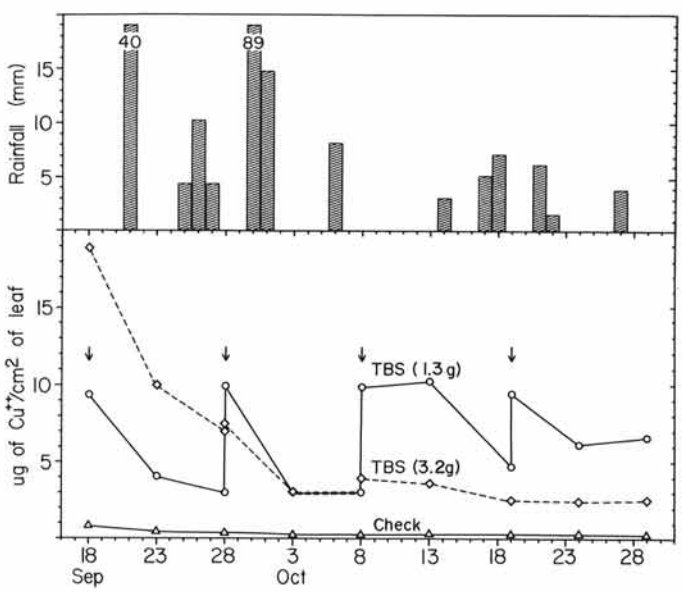

Fig. 2. The relationship between rainfall and the loss of copper from Montmorency sour cherry leaves. Trees were sprayed on 18 September 1981 with 3.2 g of tribasic copper sulfate (TBS) with and without 12 g hydrated lime per liter and on 18 and 28 September and on 8 and 19 October with 1.3 g of TBS per liter + 4.8 g of hydrated lime per liter. Arrows indicate the dates sprays were applied. Check trees were not sprayed.

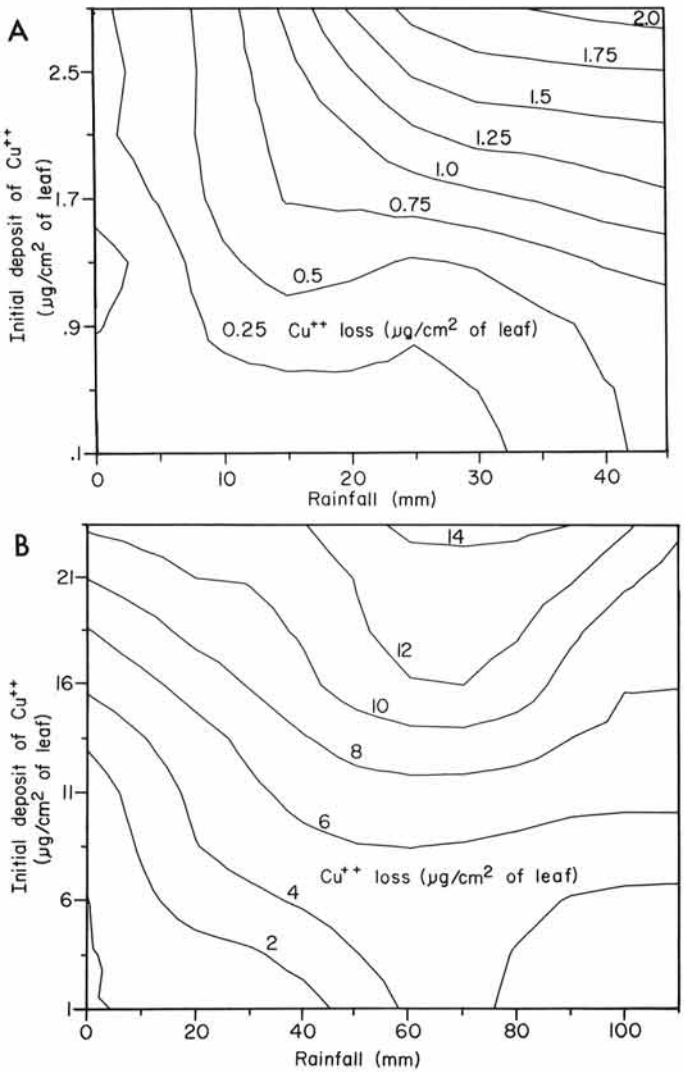

Fig. 3. Nomogram relating the amount of copper lost from Montmorency sour cherry leaves to initial deposits of copper on the leaves and to the amount of rainfall. **A,** Citcop 4E and **B,** tribasic copper sulfate.

morsprunorum in commercial orchards should be reduced to levels below those recorded in this study. As reported by other workers (5,14), repeated applications were more effective than single applications for bacterial canker disease control. However, applications should be discontinued after the shuck-fall bud stage to avoid phytotoxicity.

In England (4), leaf scars of sweet cherry infected with *P. syringae* pv. *morsprunorum* in autumn developed cankers the following spring, and application of Bordeaux mixture to sweet cherry trees in autumn decreased the development of cankers by 75–85% (5). In Michigan, Montmorency sour cherry was susceptible to leaf scar infection by *P. syringae* pv. *morsprunorum* and *P. syringae* pv. *syringae* in September and October (*unpublished*). Therefore, repeated application of the copper pesticides in autumn should help control leaf scar infections, particularly during periods of frequent rainfall. Early defoliation resulting from autumn applications of Bordeaux mixture was a problem on sweet cherry (1); in our study, however, no defoliation resulted from autumn applications of TBS on Montmorency sour cherry. This suggests that autumn applications of TBS may be safer than Bordeaux mixture.

In 1980, PsmR was recovered from Montmorency cherry leaves even after repeated application of coppers in spring and summer. In laboratory studies, survival of PsmR in distilled water was dramatically reduced when amended with TBS + hydrated lime at concentrations similar to those applied to Montmorency sour cherry trees in 1980. This indicates PsmR may survive in or on leaf tissues in areas protected from copper deposited on the leaf surface. It also may be possible that the mucoid substances referred to by Crosse (6) protect the bacteria from copper residues.

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