Evaluation of Bactericidal Chemicals for Control of *Xanthomonas* on Citrus

RAYMOND G. McGUIRE, Assistant Research Scientist, University of Florida, IFAS, Citrus Research and Education Center, Lake Alfred 33850

ABSTRACT

McGuire, R. G. 1988. Evaluation of bactericidal chemicals for control of *Xanthomonas* on citrus. Plant Disease 72:1016-1020.

Thirteen bactericidal chemicals were evaluated over three seasons on three citrus species to determine their ability to control *Xanthomonas campestris* pv. *citri*. In field trials conducted in Argentina, copper-containing products were compared with other types of bactericides and were generally superior in reducing epiphytic populations of *Xanthomonas* in nursery plantings of sweet orange cv. Pineapple and rough lemon and on mature grapefruit trees. Lesion numbers on leaves and fruit were also reduced with these products. Of the copper-containing chemicals, copper ammonium carbonate (Copper-Count-N) with 8% metallic copper was consistently comparable or superior in its control of *Xanthomonas* to other products averaging 50% copper. Agrimycin, one of seven noncopper products, was similarly effective in four of six trials in which it was tested.

An outbreak of citrus canker in 1984 in Florida citrus nurseries led to an immediate search for chemical compounds with bactericidal properties. State eradication procedures, however, initially required that all seedlings in these nurseries exposed to the previously undescribed strain of *Xanthomonas campestris* pv. *citri* (Hasse) Dye be destroyed by burning (10). Exposed citrus that had left the nurseries between the dates of estimated infestation and canker detection were also destroyed in their new sites whether or not symptoms were present. Within 15 mo, and after more than 19 million seedlings had been burned, this policy evolved into an attempt to achieve eradication by minimal destruction. The Florida strain of *X. c.* pv. *citri* appeared to be less aggressive than the Asiatic strain encountered in much of the world. Subsequently, only symptomatic plants were destroyed, and copper-containing bactericides were sprayed onto adjacent plants at some sites. This latter policy was continued when surveys disclosed the presence in 1986 of a potentially more devastating infestation of the Asiatic strain of the bacterium in orchards and on trees on home properties along the west central coast of the state (10). During this period, other xanthomonads, not pathogenic to citrus, were being isolated from citrus materials (4). The possibility that the pathogen had become endemic within the state and that therefore eradication might not succeed gave further impetus to the search for chemical control.

Various levels of control of bacterial canker of citrus by means of copper-containing compounds have been reported (11,13). The success of the compounds, however, must be weighed against any harm caused by copper to the plant and the accumulation of the metal in the soil. This increase in the soil alters its ecology and can lead to a decline in tree vigor and inhibit the development of beneficial mycorrhizae, especially in acid soils (5). Thus, finding alternatives to copper for control of bacterial pathogens would be advantageous.

This research was an attempt to determine the level of control of citrus canker offered by a number of traditional and experimental chemicals. Recommendations of possibly useful bactericides were received from the chemical and citrus industries, and new compounds were tested in vitro and, on a small scale, in vivo by the Florida Division of Plant Industry prior to field trials. Because quarantine regulations prohibited field work on this disease within Florida, these field trials were conducted in the citrus-producing state of Entre Rios in Argentina, where citrus canker has become endemic (3).

MATERIALS AND METHODS

Three field plots were established in 1985 in Concordia, Entre Rios, at an experiment station under the auspices of the Instituto Nacional de Tecnologia Agropecuaria (INTA). Sweet orange (*Citrus sinensis* (L.) Osbeck 'Pineapple') and rough lemon (*C. jambhiri* Lush.) were planted as nursery plots because they are relatively susceptible to citrus canker and are used as rootstocks. Each nursery plot contained 300 seedlings arranged into 30 groups of 10 plants each; groups were separated by 1 m and plants within a group, by 30 cm. When well established and flush with young leaves, the plants were spray-inoculated with a dilute suspension (1.5×10^4 cfu/ml) of a mixture of local Asiatic

Florida Agricultural Experiment Station Journal Series No. 8518.

Accepted for publication 6 June 1988.

strains of *X. c.* pv. *citri*. Strains LTv3 and LTv5 had been isolated from lesions on lemon leaves and identified serologically by M. Messina of INTA, and his identifications were confirmed by fatty acid analyses (3). The day after inoculation, chemical treatments were applied, followed 1 wk later by spray application of a second bacterial suspension (2.5×10^6 cfu/ml). Populations of bacteria were maintained during dry periods by simulating rainfall by means of overhead irrigation every second day. Three tests were conducted over 2 yr: for 10 wk in summer/fall 1986 and for 16 wk in both spring/summer 1986/1987 and fall/winter 1987. Between tests, old foliage was pruned away, and the new flush of leaves was reinoculated at the beginning of subsequent trials.

Chemical treatments, replicated three times, were applied monthly during the trial periods. Over the 2 yr, chemicals tested included the four antibiotics ABG-8000WP (Abbott Laboratories; 50% spectinomycin), Agrimycin (Pfizer; 15% streptomycin + 1.5% tetracycline), and Kasumin Liquid and Kasuran WP (PBI/Gordon Corp.; 2% kasugamycin and 5% kasugamycin + copper oxychloride [45% metallic copper], respectively); Agrishield (Dow Corning 5772; a silicone quaternary ammonium compound, 83% a.i., applied with wetting agent Tween 80 [Sigma Chemical Co.] or X77-Spreader [Ortho Chemicals; 72% a.i.]); GLC-719 (Great Lakes Chemical Corp.; a bromine-releasing compound); benzoyl peroxide (ABCO Industries, Inc.; 40% a.i.); Aliette (Rhône-Poulenc, Inc.; fosetyl-Al, 80% a.i.); tribasic copper sulfate (Quimica Argentina; 47% metallic copper); Copper-Count-N (Mineral Research & Development Corp.; copper ammonium carbonate, 8% metallic copper); Kocide 101 (Kocide Chem. Corp.; cupric hydroxide, 50% metallic copper); and Kocide + maneb (DuPont; 80% a.i.). Water was the control treatment.

Epiphytic populations of *X. c.* pv. *citri* were monitored weekly by random collection of two newly expanded leaves from each group of 10 plants in a treatment. These leaves were washed 1 hr in 20 ml of 0.075 M phosphate buffer (pH 6.95) containing, per liter, 4.50 g of KH_2PO_4, 5.95 g of Na_2HPO_4, and 1 g of peptone. Then, 0.2 ml of the wash and dilutions of the wash were plated onto a semiselective Tween culture medium (6). This medium, at pH 7.4, was modified to contain, per liter, 10 g each of Bacto peptone and KBr, 0.25 g of anhydrous $CaCl_2$, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.05 g of boric acid, and 8 ml of Tween 80. It was amended after autoclaving with the antibiotics (Sigma) cycloheximide at 100 mg/L, cephalexin at 40 mg/L, and 5-fluorouracil at 6 mg/L and occasionally, when saprophytes were especially numerous, with crystal violet at 3 mg/L. Compared with King's medium B, the efficiency of recovery of *X. c.* pv. *citri* on this Tween medium was between 93 and 120%. After 1 wk of incubation at 30 C, colonies characteristic of *Xanthomonas* were counted and related to the previously recorded weight of the leaves washed earlier.

Additionally, disease expression was recorded once in early summer and once

Table 1. Comparison of epiphytic populations of *Xanthomonas campestris* pv. *citri* associated with chemical treatments on Pineapple sweet orange and rough lemon seedlings and on grapefruit trees

		Log_{10} bacterial population[b]							
		Summer/fall 1986		Spring/summer 1986/1987		Summer/fall 1987		1986	1987
Treatment	**Rate/L**[a]	**Orange**	**Lemon**	**Orange**	**Lemon**	**Orange**	**Lemon**	**Grapefruit**	**Grapefruit**
Water		5.66	4.62	3.88	2.10	4.24	2.48	2.12	2.49
Spectinomycin	0.2 g	5.82	5.08	NT	NT	NT	NT	1.16	NT
Benzoyl peroxide	20.0 ml	NT	NT	3.52	2.09	NT	NT	NT	2.29
GLC-719	0.5 g	NT	NT	NT	NT	3.96	NT	NT	NT
Aliette	6.0 g	NT	NT	NT	NT	3.24	1.79	NT	NT
Agrishield	6.0 ml	5.76	5.29	3.39	1.53	3.35	1.78	0.85	1.23
Agrimycin	1.2 g	5.44	4.88	2.70	0.84	2.34	1.46	NT	NT
Kasumin	0.5 ml	5.46	5.18	3.20	1.73	3.26	2.82	NT	NT
Kasuran	1.0 g	5.41	4.90	3.00	0.74	2.85	0.97	NT	NT
Kocide	2.4 g	5.44	4.27	3.05	0.75	2.49	0.30	NT	NT
Kocide + maneb	2.4 + 1.8 g	4.88	4.65	2.17	0.89	2.26	0.76	0.60	0.64
Tribasic $CuSO_4$	3.0 g	4.44	4.71	2.67	0.94	2.73	1.03	NT	NT
Copper-Count-N	7.5 ml	2.87	1.68	2.12	0.97	1.81	0.96	0.41	0.75
Orthogonal contrast		**Probability that epiphytic populations (log_{10}) are not significantly different**							
Agrimycin vs. water		0.499	0.601	0.011	0.001	0.000	0.017	NT	NT
Agrishield vs. water		0.746	0.183	0.268	0.099	0.036	0.095	0.038	0.048
Aliette vs. water		NT	NT	NT	NT	0.019	0.098	NT	NT
GLC-719 vs. water		NT	NT	NT	NT	0.492	NT	NT	NT
Benzoyl peroxide vs. water		NT	NT	0.417	0.974	NT	NT	NT	0.280
Spectinomycin vs. water		0.617	0.355	NT	NT	NT	NT	0.045	NT
Kasumin vs. water		0.531	0.260	0.133	0.282	0.022	0.412	NT	NT
Kasuran vs. water		0.439	0.649	0.053	0.000	0.002	0.001	NT	NT
Kocide vs. water		0.487	0.474	0.067	0.000	0.000	0.007	NT	NT
Kocide + maneb vs. water		0.022	0.953	0.001	0.001	0.000	0.000	0.034	0.036
Tribasic $CuSO_4$ vs. water		0.001	0.850	0.010	0.002	0.001	0.001	NT	NT
Copper-Count-N vs. water		0.000	0.000	0.000	0.002	0.000	0.001	0.021	0.035
Copper-Count-N vs. Agrimycin		NT	NT	0.193	0.693	0.198	0.231	NT	NT
Copper-Count-N vs. Kasuran		NT	NT	0.053	0.491	0.001	0.978	NT	NT
Copper-Count-N vs. tribasic $CuSO_4$		NT	NT	0.215	0.943	0.031	0.861	NT	NT
Copper-Count-N vs. Kocide + maneb		NT	NT	0.912	0.812	0.279	0.627	0.772	0.665
Kocide + maneb vs. Kocide		0.091	0.439	0.053	0.682	0.574	0.190	NT	NT
Kasuran vs. Kasumin		0.881	0.493	0.643	0.006	0.318	0.000	NT	NT
Noncopper vs. copper		0.000	0.000	0.007	0.000	0.000	0.000	0.093	0.043

[a] During summer/fall 1986, Agrishield was decreased to 1.2 ml of formulation per liter of water and Copper-Count-N was increased to 32.5 ml/L. During summer/fall 1987, Kasumin was increased to 3.95 ml/L and Kasuran to 1.6 g/L.

[b] Seasonal mean, per gram (fresh weight) of leaf. NT = not tested.

in early winter by counting all lesions on three leaves randomly selected from the lower canopy of each plant in the sweet orange and rough lemon plots.

A third plot of five large trees of grapefruit (*C. paradisi* Macf.), naturally infected with *X. c.* pv. *citri*, was similarly assayed. Each tree was divided into five equal sectors (NE, SE, S, SW, NW), and during the first trial lasting 13 wk in summer/fall 1986, each sector received monthly applications of spectinomycin, Agrishield, Copper-Count-N, Kocide + maneb, or a water control. Chemical drift was minimized with the use of hand-operated backpack sprayers. During a second trial lasting 36 wk over spring/summer/fall 1986 and 1987, spectinomycin was replaced by benzoyl peroxide. Bacterial populations were maintained by means of overhead irrigation every second day, but because the position of a sector relative to the sun greatly affected bacterial populations, treatments were placed such that each occupied a different position on each tree. Besides epiphytic development of *Xanthomonas* sampled weekly from two sites per sector and seasonal lesion expression on 30 leaves per sector, a comparison of lesion numbers on 30 fruit from each treatment of a tree was completed at the end of the second year.

Analyses of variance were performed on the $\log_{10}$ of the number of epiphytic bacteria per gram (fresh weight) of leaf using sample dates as repeated measures (12). Treatments were then compared using orthogonal contrasts. For comparison, the seasonal mean population of epiphytic *Xanthomonas* per gram (fresh weight) of leaf was determined. Treatments were also analyzed according to the numbers of lesions on leaves and fruit.

RESULTS

Epiphytic populations of *X. c.* pv. *citri* frequently were lowest on leaves of Pineapple sweet orange and rough lemon seedlings treated with the copper chemicals tribasic copper sulfate, Kocide, and Copper-Count-N (Table 1). Control of bacteria with these chemicals was statistically significant when contrasted individually with the water control and when contrasted as a group with noncopper bactericides, although they usually could not be differentiated among themselves. In these tests, addition of maneb to Kocide did not significantly increase control by the latter. Of these copper chemicals, Copper-Count-N, with 8% metallic copper, often ranked better at reducing bacterial populations than those compounds containing more than six times as much copper. Agrimycin also provided control not significantly different from that of Copper-Count-N in four of the six trials, but the other antibiotic treatments, spectinomycin and kasugamycin, proved ineffective. The addition of copper to kasugamycin, however, significantly improved control of epiphytic populations in both seedling plots. On rough lemon, especially at the higher concentration of 1.6 g/L, Kasuran gave control equivalent to that of Copper-Count-N. Unlike kasugamycin, spectinomycin could not be tested at a higher concentration because of phytotoxicity at 100 ppm.

Aliette and GLC-719 were tested only one season; control of epiphytic *X. c.* pv. *citri* by Aliette was significant on sweet orange, whereas GLC-719 was ineffective. Agrishield at 0.12% (1,000 ppm a.i.) did not reduce epiphytic populations in the trial of summer/fall 1986, but control was much improved the second year when a higher concentration of 0.6% was used and when the wetting agent X77-Spreader was substituted for Tween 80. Although Agrishield at a concentration above 5,000 ppm a.i. was phytotoxic on the new foliage of these seedlings, no damage occurred when this chemical was applied at 1.2% to the overwintered leaves of grapefruit.

The small plot of mature, naturally infected grapefruit trees provided a useful comparison with the young, more susceptible plants of the seedling trials. Epiphytic populations were similar to those on leaves of rough lemon seedlings but were much lower than populations on the leaves of the sweet orange plants (Table 1). Supporting the data from the seedling trials, Copper-Count-N and Kocide + maneb most effectively reduced bacterial numbers. Agrishield also gave significant control over 2 yr, as did spectinomycin the year it was tested, but benzoyl peroxide did not. Although sampling was sufficiently extensive to allow statistical differentiation among the five grapefruit trees and among the positions of sectors, there was no replication of treatments on a given sector. Because this interaction is potentially important, the results from this set of trials should be considered tentatively.

Control of disease severity by the different bactericides closely paralleled their effects on epiphytic populations of *Xanthomonas* (Table 2). Untreated leaves of Pineapple sweet orange seedlings averaged over seven and 14 lesions in the first and second years, respectively, whereas those of rough lemon averaged approximately six. Lesions on leaves of grapefruit, from mature trees not readily comparable in susceptibility to seedling plants, averaged in control sectors nearly 10 and six in

Table 2. Effect of bactericides on the number of canker lesions caused by *Xanthomonas campestris* pv. *citri* on leaves of Pineapple sweet orange and rough lemon seedlings and on grapefruit trees

		Lesions per leaf or fruit[b]							
		Fall 1986	Summer 1987		Fall 1987		1986	1987	
Treatment	Rate/L[a]	Orange leaves	Orange leaves	Lemon leaves	Orange leaves	Lemon leaves	Grapefruit leaves	Grapefruit leaves	Grapefruit fruit
Water		7.3 a	14.3 b	6.4 a	14.7 a	5.4 a	9.5 a	5.9 a	60.4 a
Spectinomycin	0.2 g	4.4 cd	NT	NT	NT	NT	6.7 a	NT	NT
Benzoyl peroxide	20.0 ml	NT	11.7 bc	6.3 a	NT	NT	NT	3.3 b	22.6 b
GLC-719	0.5 g	NT	NT	NT	9.4 abcd	NT	NT	NT	NT
Aliette	6.0 g	NT	NT	NT	10.8 abc	2.5 bcde	NT	NT	NT
Agrishield	6.0 ml	6.6 ab	12.1 bc	5.3 ab	6.6 cde	4.6 ab	0.9 b	0.8 c	11.3 c
Agrimycin	1.2 g	3.8 de	13.2 bc	3.0 c	4.4 de	3.1 abcd	NT	NT	NT
Kasumin	0.5 ml	4.9 bcd	21.7 a	7.3 a	12.2 ab	3.5 abc	NT	NT	NT
Kasuran	1.0 g	5.9 abc	9.9 bcd	2.4 c	7.9 bcde	1.8 cde	NT	NT	NT
Kocide	2.4 g	3.5 de	10.7 bcd	2.2 c	8.7 bcde	2.0 cde	NT	NT	NT
Kocide + maneb	2.4 + 1.8 g	3.0 e	9.8 bcd	1.7 c	5.2 de	0.5 e	0.2 b	0.2 c	3.6 c
Tribasic $CuSO_4$	3.0 g	3.2 de	6.1 cd	3.2 bc	6.1 cde	0.9 de	NT	NT	NT
Copper-Count-N	7.5 ml	0.9 f	5.5 d	1.7 c	3.5 e	1.3 cde	1.3 b	0.7 c	7.9 c

[a] During summer/fall 1986, Agrishield was decreased to 1.2 ml of formulation per liter of water and Copper-Count-N was increased to 32.5 ml/L. During summer/fall 1987, Kasumin was increased to 3.95 ml/L and Kasuran to 1.6 g/L.

[b] Means within a column followed by the same letter are not significantly different ($P > 0.05$) according to the Ryan-Einot-Gabriel-Welsch multiple F test. NT = not tested.

1986 and 1987, respectively. Again, in all plots, the copper chemicals were most effective at reducing lesion numbers. Kasuran outperformed Kasumin in four of the five seedling trials, but the addition of maneb to Kocide did not significantly reduce mean lesion numbers compared with those from leaves treated with Kocide alone. Of the noncopper treatments, Agrimycin often reduced the development of lesions in the seedling trials, whereas Agrishield demonstrated good control of lesions on grapefruit leaves and fruit.

DISCUSSION

Although eradication of *X. c.* pv. *citri* remains the goal of industry and regulatory officials in those regions throughout the world where the pathogen has been introduced, many countries have waited too long to act and now find the bacteria well established. Frequently, a lack of cooperation on one side of an international border dooms a neighboring country to increasingly greater waves of epidemics. In Argentina, where citrus canker moved into the northern states from Brazil in 1976 and from there into the southeastern states of Corrientes and Entre Rios, eradication has proved impossible (7). The disease was rediscovered in Florida in 1984 after an earlier and successful eradication by 1933, and the isolation of the state from foreign sources of the bacteria suggested another successful eradication could be completed (10). More recent investigations, however, have suggested that the weakly aggressive Florida strain may be an endemic mutant and that eradication, therefore, might be impossible (2). In this case, as in those in other countries where growers must learn to live with the threat of citrus canker, some measure of control must be made available.

Quarantine regulations in Florida from 1984 through 1987 required that all field work with *X. c.* pv. *citri* be conducted outside the state. The experience gained by INTA in Argentina made this country an obvious choice for carrying out further research. Although the soils and climate differ from those of Florida, overhead irrigation of the Argentine plots in summer kept populations active by simulating conditions likely to occur in Florida. Similarly, to be more applicable to the nursery industry in Florida, seedlings of cultivars grown for use as rootstocks were selected. One disadvantage of moving the research to Argentina, however, was that the indigenous Asiatic strain of *X. c.* pv. *citri* was more virulent than the less aggressive Florida strain.

Argentina has not been able to eradicate citrus canker. The disease, however, is kept under control by the substitution of resistant cultivars, by the planting of windbreaks to reduce the force with which driving rains can spread bacteria, by increased inspection and immediate defoliation of infected trees to reduce inoculum, and by the use of copper sprays in regular orchard management.

Copper can build up in soils through the accumulation of runoff after spraying and the decomposition of treated leaves (15). In the surface horizon of neutral or alkaline soils, copper is thought to complex with organic matter and present a lesser danger even at high concentrations (5). Its increased availability in acidic soils, however, can lead to a decline in the vigor of trees (8). Mycorrhizal fungi infecting the roots of citrus are especially susceptible to copper, and their debilitation may exacerbate mineral deficiencies (5). Although Florida citrus soils have some natural capacity to bind copper and remove much of it from further activity (1), a standard solution to the problem of excessive copper in soil has been to add lime to achieve a pH between 6 and 7 (9). Nevertheless, the use of low-copper compounds or noncopper alternatives, if proved as effective and inexpensive as the traditional copper chemicals, would be prudent.

Unfortunately, this and previous research have failed to discover effective and low-cost alternatives to copper for control of *X. c.* pv. *citri* (13). Many products are highly bactericidal in vitro but do not perform well in the field. Benzoyl peroxide appeared to control *Xanthomonas* on tomato as well as Copper-Count-N did in greenhouse experiments conducted by the Florida Division of Plant Industry (T. Schubert, *personal communication*) but gave minimal control on citrus under field conditions. Antibiotics also work well in the laboratory but perhaps lose their activity rapidly or are washed away in a natural environment. Also, resistance to antibiotics can develop quickly, as has been seen in Florida with streptomycin when used for control of *X. c.* pv. *vesicatoria* on tomato (14). Agrishield, which polymerizes over the leaf surface or on other materials and produces a monomolecular film incorporating a quaternary ammonium compound, was most effective on older, fully expanded leaves. At the beginning of the second year of this study, before reapplication of chemicals to grapefruit trees and after 4 mo over winter without treatment, bacteria recovered from leaves of this treatment were not significantly more numerous than those from the copper treatments, all of which were much less than populations on control leaves. The bactericidal activity of Agrishield is reactivating, and the chemical does not wash off; expansion of the leaf, however, ruptures the film, allowing bacteria to enter unprotected areas.

Copper-Count-N provides the best alternative to traditional copper applications for control of *X. c.* pv. *citri*. Its copper content is just 8%, although at label rates, the amount of copper applied in the orchard is 0.62 kg/ha. Nevertheless, control of *X. c.* pv. *citri* is equal to or better than that provided by tribasic copper sulfate or Kocide, with which 1.32 or 1.12 kg/ha of copper, respectively, is regularly applied. Kasuran also appears promising in this respect, offering nearly equivalent control when kasugamycin is combined with copper at a rate of 0.67 kg/ha. No phytotoxicity was observed with either Kasuran or Copper-Count-N at the recommended rates, and perhaps these rates could be reduced and still provide acceptable control.

Eradication of citrus canker undoubtedly remains the favored choice of growers in those regions of the world where the disease threatens to become established. When elimination of this threat is not economically feasible, however, the experience in Argentina demonstrates that an acceptable level of integrated control is quite possible. Chemical control of *X. c.* pv. *citri*, when necessary, can be fairly easily incorporated into existing disease management programs.

ACKNOWLEDGMENT

Me gustaria agradecer a la gente de la Estación Experimental Agropecuaria Concordia, Entre Rios, por su cooperación en este proyecto, especialmente a los que trabajaron juntos conmigo: Cesar Hurtado, Mario Scheifler, Victor Scheifler, y Victor Figueredo.

This research was funded in part by USDA-ARS Cooperative Agreement 58-43 yk-5-3.

LITERATURE CITED

1. Anderson, C. A. 1986. Distribution of Cu and Zn in soil of an old citrus orchard. Agron. Abstr. 86:27.
2. Gabriel, D. W., Burges, A. R., Lazo, G. R., and Roffey, R. 1986. *Xanthomonas campestris* pvs. *citri, alfalfae*, and *phaseoli* are genetically and pathologically related. (Abstr.) Phytopathology 76:1076.
3. Gottwald, T. R., McGuire, R. G., and Garran, S. 1988. Asiatic citrus canker: Spatial and temporal spread in simulated new planting situations in Argentina. Phytopathology 78:739-745.
4. Graham, J. H., McGuire, R. G., and Miller, J. W. 1987. Survival of *Xanthomonas campestris* pv. *citri* in citrus plant debris and soil in Florida and Argentina. Plant Dis. 71:1094-1098.
5. Graham, J. H., Timmer, L. W., and Fardelmann, D. 1986. Toxicity of fungicidal copper in soil to citrus and vesicular-arbuscular mycorrhizal fungi. Phytopathology 76:66-70.
6. McGuire, R. G., Jones, J. B., and Sasser, M. 1986. Tween media for semiselective isolation of *Xanthomonas campestris* pv. *vesicatoria* from soil and plant material. Plant Dis. 70:887-891.
7. Muraro, R. P. 1986. A review of Argentina's citrus canker control program with cost estimates for a similar program in Florida. Citrus Ind. 67:29-31.
8. Reuther, W., and Smith, P. F. 1952. Iron chlorosis in Florida citrus groves in relation to certain soil constituents. Proc. Fla. State Hortic. Soc. 65:62-69.
9. Reuther, W., Smith, P. F., and Scudder, G. K. 1953. Relation of pH and soil type to toxicity of copper to citrus seedlings. Proc. Fla. State Hortic. Soc. 66:73-80.
10. Schoulties, C. L., Civerolo, E. L., Miller, J. W., Stall, R. E., Krass, C. J., Poe, S. R., and DuCharme, E. P. 1987. Citrus canker in Florida.

Plant Dis. 71:388-395.

11. Serizawa, S., and Inoue, K. 1982. Studies on citrus canker disease (*Xanthomonas campestris* pv. *citri* (Hasse 1915) Dye 1978). V. Influence of application interval of bordeaux mixture and of inorganic copper on the control effect. Bull. Shizuoka Citrus Exp. Stn. 18:37-48.
12. Snedecor, G. W., and Cochran, W. G. 1967. Statistical Methods. Iowa State University Press, Ames. 593 pp.
13. Stall, R. E., Miller, J. W., Marco, G. M., and deEchenique, B. I. C. 1980. Population dynamics of *Xanthomonas citri* causing cancrosis of citrus in Argentina. Proc. Fla. State Hortic. Soc. 93:10-14.
14. Stall, R. E., and Thayer, P. L. 1962. Streptomycin resistance of the bacterial spot pathogen and control with streptomycin. Plant Dis. Rep. 46:389-392.
15. Stevenson, F. J., and Fitch, A. 1981. Reaction with organic matter. Pages 69-96 in: Copper in Soils and Plants. J. F. Loneragan, A. D. Robson, and R. D. Graham, eds. Academic Press, New York. 380 pp.