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Citrus Leafminer Control and Copper Sprays for Management of Citrus Canker on Lemon in Tucumán, Argentina

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Abamectin and copper bactericides applied alone or in combination with other products for control of citrus leafminer (*Phyllocnistis citrella*; CLM) and citrus canker (*Xanthomonas axonopodis* pv. *citri*) in lemons were evaluated for three seasons in a commercial lemon orchard in Tucumán, Argentina. Citrus canker control treatments were applied on six dates from petal fall to February each year at 28-day intervals with a high volume, air blast sprayer. CLM control treatments with abamectin were applied every 15 days during the period of high CLM pressure. Incidence of citrus canker on fruits was reduced significantly by all spray treatments compared with the non-sprayed control. Control of citrus canker by different sources of copper oxychloride and copper hydroxide was not significantly different. Higher application rates of copper hydroxide (0.20%) were more effective in controlling the disease than lower rates (0.15%). The combination of copper oxychloride with spray oil as an adherent or with disinfectants (quaternary ammonium, peracetic acid) did not improve disease control on fruit. Citrus canker on fruit was reduced 19% by abamectin treatment for CLM control. Addition of abamectin to six copper sprays did not provide greater control than the copper sprays alone.

Citrus canker, caused by *Xanthomonas axonopodis* pv. *citri* (Xac), is a bacterial disease distributed widely in subtropical citrus growing areas of the world (Gottwald et al., 2002). Citrus canker reduces fruit quality and yield, and is a limiting factor for international trade because of its status as a quarantine disease in some countries (EPPO, 2003). Disease control increases the production costs due to the need for more chemical sprays per season and the reduction in marketable yields of fresh fruit.

Citrus is grown in Argentina in two different regions: the northeast and northwest. Oranges and tangerines are cultivated principally in the northeast and lemons and grapefruit in the northwest. These two regions are separated by a wide expanse of arid forest and pastureland. The B-strain of citrus canker was found in northeastern Argentina in 1927 (Betancourt, 1957) and the A-strain was discovered in 1974 and has largely replaced the B-strain (Stall and Civerolo, 1991). In 2002, A-strain citrus canker was reported in the northwestern region in Tucumán and Salta provinces. Citrus canker in Tucumán province is still unevenly distributed with disease-free areas and areas of low incidence of disease.

The citrus area in Tucumán is hot and humid in the summer and with average annual temperature of 19 °C and rainfall totaling from 800 to 1500 mm that occurs mostly in summer. Under these conditions, lemon trees are vigorous, high yielding, and have an extended flowering season. Lemon fruits of different ages are present on the tree all year round and, therefore, fruit susceptible to citrus canker occur during much of the year.

Citrus leafminer (*Phyllocnistis citrella* Stainton; CLM) was reported in Tucumán 7 years before citrus canker (Willink et al., 1996). These insects feed by tunneling beneath the epidermal cell layer, forming galleries. Wounding of the cuticle results in direct exposure of the mesophyll tissue to infection by Xac and massive lesions can result (Gottwald et al., 2002). This increase of the infected foliar area generates many times the amount of inoculum as compared to Xac infection where the leafminer is not present (Gottwald et al., 1997). Leaf flushes on lemon trees in Tucumán are severely affected by CLM from December to March and nearly 80% of young summer flushes are damaged (Salas and Goane, 2001; Salas et al., 2002). Several reports address the interaction of CLM with citrus canker on foliage (Canteros and Caceres, 2002; Gottwald et al., 2007; Leite and Mohan, 1990; Peña et al., 2000), but to our knowledge none of these studies have quantified the effect of control of CLM infestation on the incidence of citrus canker on fruit.

Citrus-producing countries where the disease is present use different strategies for control of citrus canker, such as eradication, suppression, windbreaks, and chemical control (Gottwald et al., 2002). Chemical control has been practiced in citrus areas of Japan, Paraná State in Brazil, Argentina, and recently Florida (J.H. Graham, unpublished data). Since citrus canker was first identified in Tucumán, an integrated management program was defined based primarily on information obtained from research

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studies carried out in northeastern Argentina (Canteros, 2004; Stall et al., 1980, 1982), as well as in Brazil (Leite, 1990; Leite et al., 1987).

Chemical control of canker is based on several applications of copper products per season to achieve satisfactory control of the disease (Stall et al., 1980). Copper products are applied to protect young foliage from infection to reduce inoculum available for fruit infection (Stall et al., 1980, 1982). Limited research has been conducted on the efficacy of different rates and formulations of copper and on the combined effects of copper with adjuvants such as spray oil and disinfectants such as quaternary ammonium.

The purpose of this study was to develop information on the control of citrus canker with different rates and formulations of copper and on the contribution that control of CLM may provide in an integrated disease management program.

Materials and Methods

Experiments were conducted during the 2002–03, 2003–04, and 2004–05 seasons in a commercial lemon orchard located in Alderetes, Tucumán province, Argentina. The scion/rootstock combination was Limoneira 8 A Lisbon lemon [*Citrus limon* (L.) Burm.f.] on Swingle citrumelo rootstock [*Poncirus trifoliata* (L.) Raf. x *Citrus paradisi* Macf.]. The grove was planted in 1997 and tree spacing was 8 × 4 m (approximately 300 trees per hectare). Trees were 5 years old at the beginning of the trial.

There were 19 treatments with the compounds listed in Table 1, divided into three groups as follows: 1) the insecticide abamectin applied alone and combination with other compounds; 2) formulations and sources of copper oxychloride (COC) and copper hydroxide (CH) applied alone or in combination with mancozeb (a coordination product of zinc ion and manganese ethylenebisdithiocarbamate) or citrus spray oil as adherent; 3) copper oxychloride combined with quaternary ammonium or peracetic acid in the November, December, and January sprays; and 4) unsprayed control. In view of the results obtained each year and the availability of chemical products, some treatments were not applied in all three seasons (Table 2). Treatments were kept in the same location in the orchard during the three seasons in order to evaluate possible cumulative effects of bacterial inoculum control. Treatments were applied on six dates each year at 28-d intervals with a high volume, air blast sprayer (FMC, Cordoba, Argentina) that delivered approximately 25 to 30 L of spray per tree to incipient runoff. Spray applications started at three-quarters petal fall (the end of September) and ended in February.

CLM populations were monitored weekly in unsprayed areas and abamectin sprays initiated each year in November–December when 25% of leaves were infested and the sprays ended in March–April when parasitism by *Ageniaspis citricola* reached levels of 60% to 70%. In order to control CLM during this period, abamectin was applied every 15 d each season.

Treatments were arranged in a randomized block design with four replications. The block size was three rows of 20 trees each. The central row was the treated row used for sampling and the side rows were buffers against spray drift between blocks. Evaluations were performed on all fruits exceeding size 138 (60 mm in diameter) from three trees of the center row of each plot during April, June, and August each year. Incidence of canker on all harvested fruit was assessed visually and the percent incidence was calculated as the total number of fruit with canker lesions divided by the total number of fruit assessed multiplied by 100.

Canker incidence on fruit for each trial in each season was subjected to a one-way ANOVA. Means were separated using LSD Fisher Alfa test at $P < 0.05$. Comparison of groups of treatments was conducted with orthogonal contrast analysis.

Results

Rainfall during the trial was variable compared with the mean rainfall for the last 20 years (Table 3). Citrus canker incidence on lemon fruits declined during the 3 years of the trial. The highest incidence of the disease on fruit was 76.6% for the unsprayed control in the first year of trial when trees were 6 years old and rainfall during the growing season was 929 mm (Table 3). The following year, there was a severe drought and 60% of the fruit had citrus canker in the non-sprayed control. The lowest canker incidence in the unsprayed control (32.7%) occurred in the last year of the trial in spite of higher rainfall than the year before (Table 3).

Citrus canker incidence on fruits was reduced significantly by all spray treatments compared with unsprayed control (Table 2). Disease control attained with rates and sources of COC and CH was similar and in general there were no significant differences among these treatments (Tables 2 and 4). Higher copper rates were significantly more effective only in the 2003–04 season, the second year of trial (Table 4).

Table 1. Spray materials and sources used in this study.

Compound (source)	Active ingredient (%)	Product name	Manufacturer	Location
Copper oxychloride	50	Caurifix	BASF SA	Buenos Aires, Argentina
Copper hydroxide (DP)	37.5	ChampDP	Nufarm SA	Buenos Aires, Argentina
Copper hydroxide (Ch)	50	Champion	Nufarm SA	Buenos Aires, Argentina
Copper hydroxide (K1)	50	Kocide 101	Dupont SA	Rosario, Argentina
Copper hydroxide (K2)	35	Kocide 2000	Dupont SA	Rosario, Argentina
Copper hydroxide (H)	50	Hidrocob	Brometan SRL	Burzaco, Argentina
Mancozeb	80	Dithane M-80	Dow Agrosciences	Buenos Aires, Argentina
Abamectin	1.8	Vertimec	Syngenta Agro SA	Buenos Aires, Argentina
Peracetic acid	4.3	Vortexx	Ecolab SA	Buenos Aires, Argentina
Quaternary ammonium (Quat T)	35	Triquart	Ecolab SA	Buenos Aires, Argentina
Didecyldimethylammonium (Quat S)	12	Sporekill	Cotrade SRL	Buenos Aires, Argentina
Spray oil	99.5	EFT	Texaco Argentina	Buenos Aires, Argentina

Table 2. Effect of 19 spray treatments on incidence of citrus canker on lemon fruit for three seasons (2002–05) in Tucumán, Argentina.

Treatment no.	Compounds (source)[z]	Rate (%)	Incidence (%) of fruit with canker each season 2002–03	2003–04	2004–05
1	Copper hydroxide (H) + oil[y]	0.2	NT[x]	NT	2.2 a
2	Copper hydroxide (K1) + oil	0.2	25.5 a[w]	16.9 a	---
3	Copper hydroxide (Ch) + oil	0.2	NT	17.0 a	2.6 ab
4	Copper hydroxide (K2) + oil	0.15	27.4 ab	22.0 abc	4.0 ab
5	Copper oxychloride	0.3	30.1 abc	17.9 a	3.9 ab
6	Copper oxychloride + mancozeb (Mz) + oil	0.3 + 0.2	33.8 abcd	20.7 ab	2.0 a
7	Copper oxychloride + Mz + abamectin + oil	0.3 + 0.2 + 0.02	35.8 bcde	17.8 a	2.3 a
8	Copper oxychloride + peracetic acid	0.3 + 0.1	36.0 bcde	24.4 bcd	4.0 ab
9	Copper oxychloride + oil	0.2	36.2 bcde	30.5 e	6.2 b
10	Copper oxychloride + oil	0.3	37.4 cde	27.1 cde	3.4 ab
11	Copper oxychloride + Quat T	0.3 + 0.1	38.8 cde	---	---
12	Copper oxychloride + Mz + oil	0.2 + 0.1	38.9 de	20.5 ab	3.6 ab
13	Copper oxychloride + Quat S	0.3 + 0.1	43.5 e	NT	NT
14	Copper oxychloride + Quat S + Mz	0.3 + 0.1 + 0.2	43.9 e	NT	NT
15	Copper hydroxide (DP)	0.15	44.2 e	27.1 cde	4.5 ab
16	Quat S	0.1	69.8 f	NT	NT
17	Copper oxychloride	0.2	NT	27.9 de	4.9 ab
18	Abamectin + oil	0.02	NT	43.1 f	11.0 c
19	Unsprayed control	---	76.6 f	60.0 g	32.7 d

[z]See Table 1 for source of the product.
[y]Oil at 0.1% as an adherent.
[x]NT = no treatment in the indicated year of the study.
[w]Means followed by the same letter are not significantly different, least squares means *t* test ($P = 0.05$).

The combination of COC with spray oil as an adherent or quaternary ammonium and peracetic acid as disinfectants did not improve disease control on fruit. The mixture of COC with mancozeb was more effective for disease control than COC alone only in the 2003–04 season (Table 2). The application of quaternary ammonium alone had no effect on disease incidence on fruits compared to the unsprayed control (Table 2).

CLM control with abamectin in combination with a mixture of COC and mancozeb did not improve control of citrus canker on fruit during three seasons of the trial (Fig. 1). In contrast, CLM control with abamectin alone significantly reduced incidence of citrus canker on fruits in two seasons when compared with unsprayed control.

Table 3. Rainfall during the growing season (September to March) in 2002–03, 2003–04, 2004–05, and the average of the last 20 years, Tucumán, Argentina.

Month	Rainfall (mm) 2002–03	2003–04	2004–05	Avg 20 yrs
September	5	9	11	22.2
October	165.5	76	0	73.6
November	166	0	241	101
December	282	118	76	179.5
January	152	145	162	233.1
February	60	112	143	184
March	99	77	66	184.7
Total	929.5	537	699	978.1
Fruit with canker (%)[z]	76.6	60.0	32.7	56.4

[z]In the non-sprayed control.

Discussion

Irrespective of citrus canker and CLM treatments, incidence of citrus canker on lemon fruit was most directly related to rainfall and inversely associated with tree age. Rainfall is the most important factor driving canker epidemics because bacteria are released from lesions by moisture and dispersed from lesions by water droplets and windblown rain (Gottwald et al., 2002). Immature leaves and fruit are the most susceptible tissues to water penetration of stomatal openings and mesophyll infection (Graham et al., 1992a, 1992b.). Under subtropical conditions in Tucumán, lemon trees are highly vigorous with numerous leaf flushes and periods of flowering and fruit set during the growing season . At the beginning of the study trees were juvenile in their behavior with nearly continuous activity. In the third season of the study, periods of flushing and flowering activity were fewer and more uniform. The influence of tree age on citrus canker incidence and severity has been reported from previous studies on disease control in Brazil and Argentina (Leite and Santos, 1988; Stall and Civerolo, 1991). In the current study, rainfall was below average in the second and third seasons and older trees became less susceptible to bacterial infection and CLM infestation. Therefore, fruit disease incidence decreased overall and disease control by best treatments increased to a high level.

In these trials, copper-based products tested either alone or in combination with other products and applied six times during the growing period of fruits gave a moderate to high level of control of canker disease on fruit. This was previously demonstrated in Brazil and Argentina for oranges and grapefruit (Graham et al., 1982; 2006; Leite et al., 1987; Stall et al., 1980). Higher applica-

Table 4. Orthogonal contrast comparisons of the incidence of citrus canker on lemon fruit for treatments grouped according to copper formulation, and combinations with mancozeb, oil, and disinfectants used.[z]

Contrast groups	Treatment no.	Mean[y] (citrus canker incidence[x]) 2002–03	2003–04	2004–05
Copper oxychloride 0.2% vs.	9,12,17	37.6 a	23.3 a	2.8 a
Copper oxychloride 0.3%	5,6,7,10	34.3 a	20.8 b	4.9 a
Probability of a greater F value[w]		0.27	0.0007	0.07
Copper hydroxide 1.5% vs.	4,15	35.8 a	24. 6 a	4.2 a
Copper hydroxide 2.0%	1,2,3	25.4 a	17.2 b	4.8 a
Probability of a greater F value		0.017	0.0005	0.19
Copper oxychloride with oil vs.	6,7,9,10,12	36.4 a	23.3 a	3.5a
Copper oxychloride without oil	5,17	30.1 a	22.8 a	4.2 a
Probability of a greater F value		0.39	0.72	0.47
Copper oxychloride (all treatments) vs.	5,6,7,9,10,12,17	35.4 a	23.2 a	3.7 a
Copper hydroxide	1,2,3,4,15	32.3 a	20.9 a	3.3 a
Probability of a greater F value		0.16	0.053	0.63
Copper oxychloride vs.	5,9, 10,17	34.6 a	25.8a	4.5 a
Copper oxychloride with mancozeb	6,7,12	36.2 a	19.7 b	2.6 a
Probability of a greater F value		0.80	0.0002	0.078
All copper oxychloride (all treatments) vs.	5,6,9,10,12	35.8 a	24.6 a	3.9 a
Copper oxychloride, mancozeb, abamectin	7	35.9 a	17.8 b	2.3 b
Probability of a greater F value		0.99	0.022	0.03
Copper hydroxides vs.	3,4,15	NT[v]	21.9 a	3.3 a
Copper oxychloride, mancozeb, abamectin	7	NT	17.8 a	2.3 a
Probability of a greater F value			0.13	0.10
Copper oxychloride vs.	5,9,10	34.6 a	NT	NT
Copper oxychloride + peracetic acid Quat S/T	8,11,13	39.4 a	NT	NT
Probability of a greater F value		0.17	---	---
Copper oxychloride vs.	5,6,9,10, 12,17	NT	24.1 a	3.9 a
Abamectin	18	NT	43.1 b	11.0 b
Probability of a greater F value			<0.0001	<0.001
Copper hydroxide vs.	1,2,3,4,15	NT	20. 9 a	3.3 a
Abamectin	18	NT	43.1 b	11.0 b
Probability of a greater F value			<0.001	<0.0001

[z]Disinfectants: peracetic acid, quaternary ammonium (Quat T, Quat S).
[y]Means for contrasted treatment groups. Means within contrasts that are followed by different letters are significantly different at the indicated level of probability.
[x]Incidence (%) of fruit with citrus canker.
[w]*F* statistic for the two treatment groups listed above.
[v]NT = no comparison in the indicated year of the study.

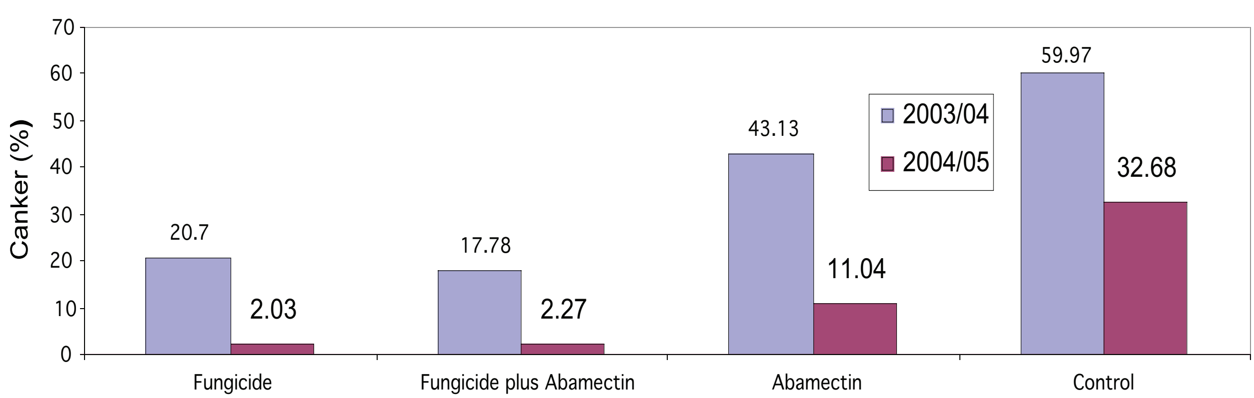

Fig. 1. Effect of abamectin spray alone and in combination with fungicide (copper oxychloride 0.3% + mancozeb 0.2% + oil 0.1%) for control of canker on lemon fruit in trials conducted in Tucumán, Argentina, 2003–05.

tion rates of COC and CH were more effective for controlling the disease than lower rates. However, higher copper doses reduced the number of marketable fruit due to a stippling damage and darkening of existing blemishes (unpublished observations).

CLM damage had a direct effect on the canopy architecture, size and production of lemon trees in Tucumán. Our study showed for the first time that controlling CLM damage with abamectin treatment reduced incidence of citrus canker on fruits irrespective of copper treatment. There was a similar effect of CLM control on disease incidence on leaves (G. Gastaminza, personal communication). In contrast, disease incidence on fruits was not reduced by CLM control when combined with six copper sprays compared to copper sprays alone. We observed that copper protected CLM galleries from bacterial infection. Copper may also promote more rapid healing of the wounds caused by feeding, such that infection of galleries is reduced (Gottwald et al., 2002). Further evaluations of lower copper rates and reduced number of copper sprays in combination with CLM control on disease control are underway. Reduction in copper use that does not compromise disease control would be beneficial to reduce the risk of stippling of the fruit and to lower copper accumulation in the soil.

During the trials, fruit drop caused by canker was counted and evaluated. Citrus canker had no effect on fruit drop (data not shown) of lemons in contrast to oranges in Brazil (Graham et al., 2006). Three years of trials demonstrated that citrus canker in lemon in Tucumán can be controlled effectively. During the trial, an average of up to 80% canker-free fruit was obtained. This level of control allows citrus growers to maintain fresh fruit production for domestic and overseas markets.

Chemical control of citrus canker is only a part of an integrated program for disease prevention and control in countries like Argentina where the disease is endemic (Leite, 1990). In Tucumán, where regions of the state are still canker-free, this program relies on prevention of introduction of the canker into citrus orchards by restricting access to properties with fences and establishing new plantings with disease-free trees. To reduce disease spread once the pathogen is introduced into an area, disinfection of tools, machinery, harvest equipment, and decontamination of personnel is practiced; and strict protocols for survey to monitor the disease occur throughout the process of fruit production in the orchard. Packinghouses are the last step in the process of fresh fruit production where sanitization of the fruit and intense inspection for blemishes is conducted.

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