Evaluation of the Fungicides CGA 64251, Guazatine, Sodium *o*-Phenylphenate, and Imazalil for Control of Sour Rot on Lemon Fruits

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ABSTRACT

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The experimental fungicides CGA 64251 and guazatine, respectively, at 0.5 g/L, significantly prevented the expansion of sour-rotted areas on lemon fruits when applied within 8 hr after inoculation. The incidence of sour rot in inoculated lemons was reduced from 90 to 10–15% under the same conditions. Imazalil and sodium *o*-phenylphenate were ineffective. In vitro experiments indicated that guazatine exhibits significantly higher fungicidal and fungistatic effects on *Geotrichum candidum* as compared with CGA 64251.

Sour rot, incited by *Geotrichum candidum* Lk. ex Pers., is an important decay of citrus fruits (6). The disease is widely distributed in citrus groves, particularly in lemons and grapefruits, which must be stored for several weeks or months before marketing (3,4,6). *G. candidum* invades the peel of the fruit through injuries made by mechanical damage or insects during harvest (6). The fungus is commonly found in soils planted to citrus (2,5) and can accumulate with dust and debris in dip tanks or drenchers where injured fruits may become readily infected. In Israel, the decay is most prevalent during the second half of the harvest season as fruits reach maturity and high temperature and humidity provide favorable conditions for infection (3).

In contrast with the substantial progress over the past decade in chemical control of blue and green mold and stem-end rot (6), effective control of sour rot has not yet been achieved. This is because *G. candidum* is remarkably resistant to most fungicides. Therefore, major efforts to obtain control have been directed towards improved sanitation, use of proper handling methods, and storage at low temperatures (5).

This paper reports results with two experimental fungicides that might be used for chemical control of sour rot.

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MATERIALS AND METHODS

A pathogenic strain of *G. candidum* was isolated from infected lemon (*Citrus limon* (L.) Burm.) fruit and maintained on potato-dextrose agar (PDA). Inoculum was prepared by grinding petri dish cultures with a Waring Blendor in a low volume of sterile distilled water. The thickened inoculum was composed of arthrospores and nutrients from the agar medium.

Green Eureka lemons (size 10, 5.5 cm in diameter) were selected at a local packinghouse. The fruits were washed thoroughly with water and placed wet in closed polyethylene film bags to establish a humid environment around the fruit. The bags were held for 24 hr at 25 C. The lemons were artificially inoculated with arthrospores of *G. candidum* by puncturing the rind at two sites with pins that protruded 3 mm from a cork. The pins had been dipped previously in the inoculum. The inoculated fruits were placed back inside the polyethylene bags and incubated at 25 C until used for chemical treatments.

Treatment was administered by dipping the fruits for 1 min in an aqueous solution or a suspension of the test fungicide. In the case of the sodium *o*-phenylphenate treatment, the fruits were dipped for 3 min in the chemical solution and immediately washed with water to avoid a phytotoxic effect. The fruits were then wrapped in plain wrappers, packed in net baskets made of wire, and incubated in a room at 29–30 C and 95–98% relative humidity. Decay development was determined each day by measuring the advancement of the elliptic rot area on the fruit surface. Fifty fruits were used for each treatment. Disease development in each treatment was expressed by plotting the rot area versus time. Results were analyzed by linear regression and considered significant at a probability of 0.05.

The fungicidal effect of the tested chemicals was determined using arthrospores obtained by growing the fungus on yeast extract-glucose medium (1). Five milliliters of spore suspension (0.5 mg of dry spores per milliliter) was added to 50-ml Erlenmeyer flasks containing 5 ml of the test chemical. The spore suspension was shaken with the chemical for 1 min at 25 C followed by a rapid filtration under

Table 1. Effect of chemical treatments on the incidence of sour rot in inoculated lemon fruits

Treatment[a]	Concentration (g/L)	Infected fruits (%)[b]
Control (water)	...	90
Guazatine	0.5	15
	1.0	12
	2.0	10
CGA 64251	0.5	11
	1.0	10
	2.0	10
Imazalil	3.0	75
SOPP[c]	20.0	90

[a] Experimental conditions were as described in Figure 1. Chemical treatments were applied 7 hr after inoculation.

[b] Fifty fruits were used per treatment. Results are the mean of three different experiments.

[c] Sodium *o*-phenylphenate.

reduced pressure on Ederol filter paper (J. C. Binzer, West Germany). After extensive washing with sterile distilled water, the spores were tested for viability. The same procedure was also performed with spores germinated for 1 hr.

Viability tests were performed by placing 12.7 mm of Schleicher and Schull filter paper disks on sterilized blotting paper in a petri dish. A drop of fungicide-treated spore suspension was then placed on each disk, and the seeded paper disks were transferred aseptically to PDA plates. The plates were incubated at 25 C and observed each day for 5 days for fungal growth. Results were expressed as the minimal inhibition concentration (MIC) observed after 5 days.

The paper disk procedure described above was also used for bioassay of the fungistatic effect. The paper disk was impregnated with a given concentration of the test chemical and allowed to dry before adding a drop of untreated spore suspension. The disks were transferred to PDA plates and observed for fungal growth as described earlier. The fungistatic effect was also tested by adding the chemical to the PDA at 45 C before pouring the agar plates. The untreated spores were streaked on the PDA plates and examined each day for 5 days for growth.

The following chemicals were tested for sour rot control: CGA 64251 (1-[[2-(2,4-dichlorophenyl)-4-ethyl-1,3-dioxolan-2-yl]methyl]1*H*-1,2,4-triazole) (Sonax WP 10%; Ciba-Geigy, Greensboro, NC 27409); sodium *o*-phenylphenate (SOPP, 20%; Dow Chemical, Midland, MI 48604); guazatine (9-aza-1,17-diguanidinoheptadecane acetate) (Panoctine 40%; KenoGard, Stockholm, Sweden); and imazalil (1-[2-(2,4-dichlorphenyl)-2-(2-propenyl-oxy)ethyl]-1*H*-imidazol) (60% EC; Janssen Pharmaceutica, Beerse, Belgium). Concentration of fungicides was expressed according to the active ingredients in the formulation.

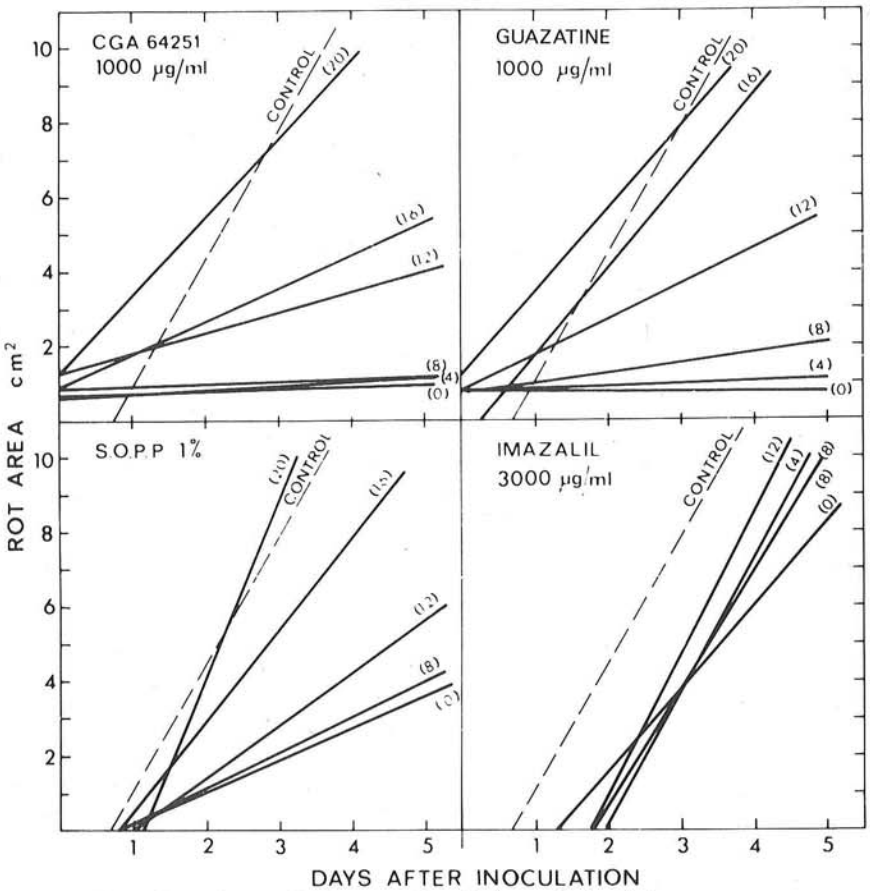

Fig. 1. Effect of the time period between inoculation and chemical treatment on sour rot development. Numbers in parenthesis indicate hours after inoculation when chemical treatment was carried out. Each treatment was applied to 50 fruits. Regression lines were calculated from three different experiments. Treatments with 95% confidence limits for insignificant decay advancement show regression coefficients of 1 or less.

Table 2. Fungicidal and fungistatic effects of four fungicides on *Geotrichum candidum* in vitro

	Minimal inhibiting concentrations (mg/L)[a]			
	Fungicidal effect[b]		Fungistatic effect[c]	
Chemical	Test I	Test II	Test III	Test IV
Guazatine	4	10	1	1
CGA 64251	200	100	3	20
SOPP[d]	>1,000	500	>100	>100
Imazalil	>1,000	750	60	>60

[a] Results are averages of five different experiments.
[b] Tested with (I) nongerminating and (II) germinating spores.
[c] Tested (III) by introducing the chemical into the potato-dextrose agar and (IV) by paper disk bioassay.
[d] Sodium *o*-phenylphenate.

RESULTS AND DISCUSSION

Guazatine and CGA 64251 prevented sour rot development at 1 g/L when applied immediately after inoculation (Fig. 1). No significant advancement of lesions could be detected when treatments with CGA 64251 were performed within 8 hr of inoculation. A very limited rot development was measured when guazatine was applied after 8 hr. A significant development of sour rot occurred when the chemical treatment was delayed for 12 hr after inoculation. At a delay of 16 hr, the development of sour rot in the guazatine-treated fruits was similar to that in the control fruits but still considerably lower in the lemons treated with CGA 64251. Treatments with SOPP and imazalil applied at concentrations of 10 and 3 g/L, respectively, could not prevent the development of sour rot regardless of the application time. However, a significant retardation in the development rate of lesion size was observed with SOPP when applied up to 12 hr after inoculation (Fig. 1).

Both guazatine and CGA 64251 reduced the incidence of sour rot from 96 to 15% and significantly inhibited the expansion of the lesion at a concentration as low as 0.5 mg/L (Table 1). Imazalil and SOPP were ineffective. Preliminary experiments on the control of sour rot with guazatine have previously been reported in Australia (7) and Florida (2).

In vitro studies indicated that the most active fungicide was guazatine, followed by CGA 64251 (Table 2). SOPP and imazalil were least active. It is noteworthy that the MIC values for the fungistatic effects of guazatine and CGA 64251 varied considerably when determined by different procedures. Moreover, when the fungicides were tested for inhibition of spore germination in yeast extract-glucose medium (1), an MIC of 50 mg/L was obtained for guazatine and >100 mg/L for CGA 64251.

The fungicidal and fungistatic activity of CGA 64251 was significantly lower than the activity of guazatine (Table 2). These results contradict Brown's observation (2) that CGA 64251 was more active than guazatine. The MIC values obtained for the two fungicides by Brown (2) were also considerably higher than in our

study. It would be interesting to determine whether the different results were caused by different isolates used in the two studies.

In spite of the relatively lower activity of CGA 64251 under in vitro conditions, its performance under in vivo conditions was similar or even slightly better than guazatine. It is possible that the effectiveness of CGA 64251 is augmented by factors present under in vivo conditions. Studies are now in progress to develop commercial chemical control of sour rot with the two fungicides.

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