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USE OF CHLORINE AND CHLORINE DIOXIDE IN FLORIDA CITRUS PACKINGHOUSES TO REDUCE INOCULUM OF DECAY PATHOGENS

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Abstract. **Water and packinghouse machinery used to handle citrus fruit frequently become contaminated with inoculum of** Penicillium digitatum **Sacc. and** Geotrichum candidum **Lk. ex. Pers., 2 major decay organisms of Florida citrus fruit. To reduce decay caused by these fungi, chlorine has been used more extensively the past 2 seasons than in previous years by the Florida industry. A survey of various commercial chlorine applications showed that, in many instances, proper control of pH and chlorine concentration was not maintained for maximum biocidal activity and minimum corrosion of equipment. Spray applications of free chlorine (200 μg/ml) near pH 7 for 15 sec killed spores of** P. digitatum **on surfaces of healthy fruit, but not spores present in a sporulating lesion. Under similar conditions,** G. candidum **was not affected until chlorine was used at 1000 μg/ml. Studies with chlorine dioxide (ClO_2) showed that stability of this material decreased as storage temperatures were increased. Inoculum of** P. digitatum **and** G. candidum **in a commercial soak tank was reduced by maintaining 5-10 μg/ml of available ClO_2 in the tank. Effectiveness of ClO_2 was affected less by pH than chlorine. Demand for ClO_2 was related to the dump rate and cleanliness of the fruit.**

Penicillium digitatum and *Geotrichum candidum,* fungi causing green mold and sour rot, respectively, are major decay pathogens of Florida fresh citrus fruits. Inoculum of these fungi, in the form of spores and hyphae, is present on fruit surfaces at the time of harvest. Injuries to the fruit rind during picking and handling provide natural openings for infection. Fungal inoculum accumulates in water used to handle fruit, such as in fungicide drenchers or soak tanks and on the surfaces of packinghouse equipment, thus adding to the infection and decay of injured fruit.

Chlorine is an effective and economical biocide which has been used extensively to reduce decay initiated during the washing, treating and hydrocooling of fruits and vegetables (9). Chlorine is available as elemental Cl in the compressed gas state, as a liquid in the form of sodium hypochlorite (liquid bleach), and in the dry form as calcium hypochlorite (8, 16). Regardless of form, when chlorine is added to water the active biocidal ingredient formed is hypochlorous acid. Hypochlorous acid is pH sensitive and above pH 8.5 it will dissociate into hypochlorite ion which has much less biocidal activity than hypochlorous acid (6, 8, 16, 17). For the most effective kill, pH of chlorine solutions should be maintained on the acid side of neutrality. Chlorine dioxide (ClO_2) is another biocidal chlorine compound. It is usually generated and used on site (15, 17). Chlorine dioxide does not dissociate in water but remains as a true dissolved gas. It is less sensitive to pH than hypochlorous acid, less reactive with many organic compounds, and is less corrosive to metal (15).

Increased interest in the use of chlorine to reduce green mold and sour rot has been shown by the Florida fresh fruit industry during the past 2 seasons. Various types of chlorine applications have been utilized. This paper reports on the use of chlorine in various installations and the results of studies on efficacy of chlorine during short exposure times in aqueous sprays. Experiences with ClO_2 are also presented with regard to stability at different temperatures, efficacy at varying pH, and use in soak tanks under commercial conditions.

Materials and Methods

Chlorine for laboratory studies was prepared from sodium hypochlorite and adjusted to pH 7 with citric or hydrochloric acid immediately prior to use. Total chlorine was measured iodometrically using a high range chlorine test kit (Model CN-21P, Hach Company, Loveland, CO 80539). Chlorine dioxide was formed by reacting sodium chlorite with muriatic acid in a ClO_2 generating system (Fig. 1) (Clow Corporation, Water management *Division,* Jacksonville, FL 32203). Solutions of total ClO_2 were measured using iodometric titration at pH 2 (13). Dilute solutions of available ClO_2 were measured spectrophotometrically at 360 nm (4). Levels of available ClO_2 in a commercial soak tank were measured using a DPD (N,N-diethyl-p-phenylenediamine) test kit (Model LP-19-Code 6802, Lamotte Chemical Products Company, Chestertown, MD 21620).

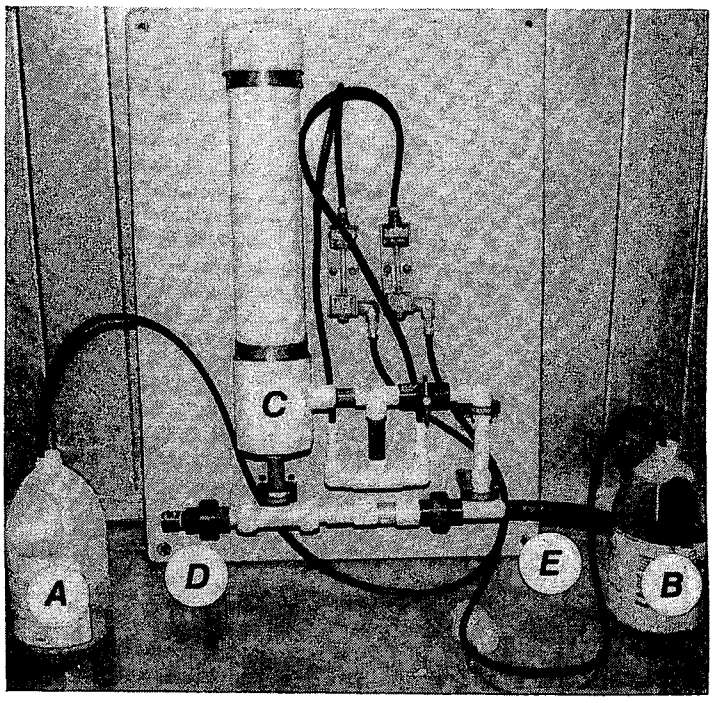

Fig. 1. Chlorine dioxide generator equipped with 2 siphon hoses which mixed sodium chlorite (A) and muriatic acid (B) in a chamber (C) to produce chlorine dioxide which was dissolved in a water stream introduced by a garden hose attached at (D) and discharged at (E).

To study the effect of chlorine and ClO_2 on germination of *P. digitatum* and *G. candidum,* spores were removed in sterile deionized water containing a surfactant (Triton X-100) from cultures growing on Difco potato dextrose agar (PDA). The suspension was filtered through 4 layers of cheesecloth and the spores were washed twice in deionized

water and collected each time by centrifugation. Spore suspensions were adjusted to a concentration of 10^7/ml using a spectrophotometer (14). Concentrations of ClO_2 were prepared at pH 4, 7, or 10 in a boric-citric acid, tertiary sodium phosphate buffer. One ml of the spore suspension was added to 9 ml of the chlorine or ClO_2 solution. Samples of 1 ml were removed after various times of exposure to chlorine or ClO_2 solutions and added to 0.1 ml of 0.1 M sodium thiosulfate to inactivate the chlorine or ClO_2. Drops of the spore suspension were placed at 4 locations on a PDA plate and dispersed with a sterile loop. Spores germinating overnight were killed with lactophenol-aniline blue, and 100 spores were counted at each of the 4 locations/treatment.

Eradication of natural populations of *P. digitatum* and *G. candidum* from fruit surfaces by chlorine was tested by spraying unwashed 'Valencia' oranges [*Citrus sinensis* (L.) Osb.] with chlorine solutions. Fruit were dipped after 15 sec in 200 ml of water containing sodium thiosulfate to inactivate the chlorine. Five fruit were treated similarly and all were washed with a single 200 ml sample of water. A 40 ml aliquot of water was removed, centrifuged, and the contents suspended in 5 ml of sterile water. One ml of this water was plated on each of 4 plates/treatment. Acidified PDA was used to isolate *P. digitatum* and a selective medium (5) was used for *G. candidum*. The selective medium was modified by using only 5 μg/ml of dicloran, and by acidifying after sterilization with 3 drops of 75% lactic acid/100 ml of media. Plates were incubated at 25°C and 28°C for *P. digitatum* and *G. candidum*, respectively.

Eradication of *G. candidum* from soil with ClO_2 was evaluated by collecting surface soil from the grove floor beneath the canopy of 'Valencia' orange trees. The soil was thoroughly mixed and sieved through a 18 x 16 mesh screen. A portion of the sample was used to determine dry weight. Soil (0.3 g) was placed in a test tube and mixed with 9 ml of ClO_2 at varying pH and periods of exposure terminated by inactivation with sodium thiosulfate. The soil was filtered, air dried, and distributed evenly over the surface of petri dishes (100 x 15 mm) containing the selective medium. Colonies of *G. candidum* were counted after incubating plates at 28°C for 3 days and recorded as colonies/g dry weight of soil.

Efficacy of ClO_2 in eradicating *P. digitatum* and *G. candidum* from a commercial soak tank was evaluated by collecting water samples periodically from it during the packinghouse operation. Water was collected in brown, 250 ml plastic bottles containing 5 ml of 0.1 M sodium thiosulfate. Samples were refrigerated overnight and plated the following day on acidified PDA and selective media. Aliquots of 0.5 ml of the water sample were spread and dried on the surface of each of 5 plates used for each treatment.

Results

Commercial packinghouses used various systems of chlorination. These included commercial service company installations and standard systems designed for swimming pools that were modified for packinghouse use. We observed that concentrations of chlorine were not always effectively controlled and pH levels were not always maintained near neutrality in commercial packinghouses (Table 1). Installations utilizing gas for the chlorine source generally contained solutions with pH in the acid range, indicating a lack of buffering to counteract the acid formed when the chlorine gas reacted with water. In contrast, liquid chlorine applications utilizing bleach stabilized with alkali lacked sufficient neutralization with acid. These chlorine solutions tended to be more alkaline. Time of exposure of fruit to the chlorine solutions varied, but applications by spray usually were applied for a shorter period of time than solutions applied in soak tanks.

Table 1. Variation in conditions of chlorine application in commercial packinghouses during the 1983-84 season.

Source of chlorine	Number of samples[y]	Total chlorine (μg/ml)[z] Range	Total chlorine (μg/ml)[z] Mean	pH Range	pH Mean
Gas	12	20-280	108	2.8- 8.0	4.8
Sodium hypochlorite	19	20-1080	246	6.5-12.4	9.1
Calcium hypochlorite	4	50-370	153	6.3- 8.3	7.0

Exposure times (sec.)[x]			
Spray		Soak tank	
Range	Mean	Range	Mean
2-65	19	24-40	33

[z]Concentration of total chlorine at the point of discharge from spray nozzles or in the soak tank.
[y]Samples were collected during the 1983-84 season from 20 commercial packinghouses.
[x]Seconds between application of chlorine to fruit and the time treated fruit reached the washer brushes.

The importance of pH to the killing power of chlorine solutions is illustrated by the results presented in Table 2. Chlorine concentrations of 100 μg/ml at a pH near 7 killed spores of *P. digitatum* and *G. candidum* within 10 sec. At a pH of 9, spores were not killed until the concentration was doubled. At pH 11, spores were not even killed after being exposed to 100 μg of chlorine/ml of water for 30 sec. These observations indicated that in many commercial installations, chlorine was not being utilized effectively for killing propagules of these pathogens.

The effectiveness of brief spray applications of chlorine to dirty fruit to eradicate surface populations of the decay fungi is shown in Table 3. Natural spore populations of *P. digitatum* removed from chlorine treated noninfected fruit were mostly inactivated with 200 μg/ml of chlorine left on the fruit for 15 sec. However, similar treatments of chlorine did not kill many of the spores if the treated fruit contained a sporulating lesion of green mold. Viability of propagules of *G. candidum* removed from fruit after a 15-sec chlorine treatment was not substantially reduced until the concentration of chlorine was increased to 1000 μg/ml (Table 3).

Table 2. Effect of pH on fungicidal activity of chlorine solutions against spores of *Penicillum digitatum* and *Geotrichum candidum*.

Chlorine (μg/ml)	Time (sec.)	pH 7	pH 9	pH 11
0	10	+	+	+
	30	+	+	+
100	10	−	+	+
	30	−	+	+
200	10	−	−	+
	30	−	−	+
300	10	−	−	+
	30	−	−	+
1000	10	−	−	+
	30	−	−	+

+ = Germinated spores.
− = No germination.

Table 3. Percentage kill of propagules of *P. digitatum* and *G. candidum* on the surface of 'Valencia' oranges following 15-sec. exposure to chlorine.

Total chlorine[z] (μg/ml)	Percentage kill	
	P. digitatum	*G. candidum*
50	62	—
100	85	—
200	98	17
500	—	3
1000	—	57

[z]Chlorine was adjusted to a pH of 6.8-7.0 immediately before treating fruit.

Stability of ClO_2 was dependent upon time of storage and storage temperature (Table 4). Solutions of ClO_2 stored for 5 weeks lost strength most rapidly at the highest storage temperature of 32.2°C. At this temperature, 71% of the ClO_2 was lost within 5 weeks compared to only 18% at 4.4°C. As temperatures were increased in increments of 5.6 degrees from 4.4 to 32.2°C, concentrations of ClO_2 decreased more rapidly.

Table 4. Percentage loss of chlorine dioxide stored in the dark at various temperatures.[z]

Temperature (C)	Weeks		
	1	3	5
4.4	6 ± 0.4[y]	8 ± 0.1	18 ± 0.3
10.0	10 ± 0.6	14 ± 0.2	27 ± 0.1
15.6	13 ± 0.5	18 ± 0.1	32 ± 0.5
21.1	19 ± 0.1	23 ± 0.2	41 ± 0.2
26.7	21 ± 0.2	29 ± 0.1	52 ± 0.3
32.2	26 ± 0.4	42 ± 0.3	71 ± 0.1

[z]Initial solution of chlorine dioxide (1069 μg/ml).
[y]Data are the mean and standard error of 3 analyses.

Effectiveness of ClO_2 in killing *P. digitatum* and *G. candidum* was evaluated using minimal levels which provided a high degree of kill within 120-sec (Table 5). Over a pH range of 4 to 10, ClO_2 did not vary much in its level of biocidal activity, except for some loss in activity against *P. digitatum* with 4μg/ml at pH 10. Propagules of *G. candidum* in naturally infected soil were much more difficult to eradicate than were spores in clean water. Spores were killed within 120 sec at 2 μg/ml, but 35 μg/ml of ClO_2 only eradicated approximately 50% of the propagules in soil during the same exposure period.

Table 5. Percentage kill of *Penicillium digitatum* and *Geotrichum candidum* by chlorine dioxide at three levels of pH.

Organism	Inoculum	Chlorine dioxide (μg/ml)[z]	Time of exposure (sec.)				
			pH	30	60	90	120
P. digitatum	spores	4	4	72	77	91	99
			7	92	100	100	100
			10	30	62	59	64
G. candidum	spores	2	4	96	99	100	100
			7	78	99	100	100
			10	84	95	99	100
G. candidum	soil[y]	35	4	24	24	19	52
			7	27	46	52	50
			10	23	16	23	56

[z]Approximately 35% of the chlorine dioxide was utilized by spores and 44% by the soil inoculum.
[y]Grove soil naturally infested with *Geotrichum candidum*.

Effectiveness of ClO_2 for reducing populations of *P. digitatum* and *G. candidum* in a soak tank was evaluated in a commercial packinghouse processing 'Marsh Seedless' grapefruit. The ClO_2 was generated on site and was added intermittingly to maintain a concentration ranging from 5-10 μg/ml. Since an untreated tank handling a comparable amount of fruit was not available to serve as a control, an initial sample was taken approximately 2 hr after the start of the days run. Propagules within this sample are reported as the control (Table 6). It would be safe to assume that as the day progressed, levels of inoculum in the tank would have greatly exceeded those reported for the control if ClO_2 were not present.

Table 6. Propagules of *Penicillum digitatum* and *Geotrichum candidum* recovered from a commercial soak tank treated with chlorine dioxide.[z]

Sample[y]	Propagules/ml			
	Trial 1		Trial 2	
	P digitatum	*G. candidum*	*P. digitatum*	*G. candidum*
Control[x]	1240	32	10,000	1440
1	0	0	640	36
2	0	0	1,040	101
3	40	39	360	4
4	0	18	1,000	45
5	0	1	520	9
6	0	0	40	0
7	120	0	700	27
8	0	0	360	20
9	0	0	—	—

[z]Concentrations ranged from 5-10 μg/ml of available chlorine dioxide.
[y]Samples were collected in Trial 1 and 2 during a 4- and 5-hr. 2 min period, respectively, after initiation of the experiments on April 12 and April 26, 1984.
[x]Control sample was collected approximately 2 hr after processing was initiated and before chlorine dioxide was added to the tank.

In trial 1, the dump rate averaged 197-236 hl (250-300 boxes)/hour for 2 hr and 433-472 hl (550-600 boxes)/hr for the remaining 2 hr. All samples collected following treatment contained fewer propagules of *P. digitatum* than before treatment and all, except sample 3, contained less *Geotrichum* propagules. Approximately 3735 ml of sodium chlorite and 1261 ml of acid were required to generate sufficient ClO_2 to maintain 5-10 μg/ml in the tank of 946-liter (250-gallon) capacity for 4 hrs. The pH of the ClO_2 solution was reduced because of the acid introduced during the generation of ClO_2, and caustic soda was added to the tank periodically to maintain the pH above 5.

In trial 2, the dump rate averaged 393-472 hl (500-600 boxes)/hr. The fruit and water were extremely dirty and 5500 ml of sodium chlorite and 2,750 ml of acid were required to generate ClO_2 to treat the tank for 3 hr and 20 min. The water contained approximately 10 mg dry matter/100 ml which inactivated 55% of the ClO_2. Inoculum levels of *P. digitatum* and *G. candidum* were much higher in trial 2, but all samples collected after treatment contained less inoculum than before treatment. In both of the trials, levels of ClO_2 greater than 5-10 μg/ml would have been required to eradicate all of the inoculum.

Discussion

According to our observations in commercial packinghouses, many applications of chlorine appear to be performing poorly for decay control due to insufficient control of chlorine concentration and pH. Use of chlorine in initial water rinses applied to fruit after dumping may be beneficial in reducing populations of *P. digitatum*. Chlorine applied at 200 μg/ml and left on fruit for 15 sec eradicates

spores on the surfaces of healthy fruit but it does not eradicate spores in lesions or inhibit the pathogen once it has invaded the fruit rind (9). When mixed with soil, *G. candidum* is more difficult to eradicate because of the association of the organism with soil debris (5). Much or all of the chlorine is tied up by the soil and organic matter which prevents the chlorine from reacting with fungus propagules. When applied as a spray, higher levels of chlorine would be required to kill the propagules. Higher concentrations of chlorine in a spray could cause atmospheric chlorine to exceed OSHA regulations for worker safety (1). Additional studies are needed to determine efficacy, phytotoxicity, corrosiveness, and levels of chlorine in the worker atmosphere.

Since the effectiveness of chlorine is also dependent upon exposure time, longer periods of wetting of the fruit with chlorine should be more effective. Until recently, however, the fruit reached the washer brushes only a few seconds after receiving the chlorine treatment. The alkaline soap or sodium orthophenylphenate formulations used on washer brushes to wash fruit then inactivated the chlorine. Currently, because of the recent outbreak of canker (3), treatment of fruit with chlorine sprays of 200 μg/ml for 2 *min is mandatory* to eradicate any canker bacterial cells on the surface of fruit (2). It will be necessary to determine how this longer period of exposure to chlorine affects decay control.

Chlorine dioxide is another form of chlorine which could be utilized in Florida packinghouses. The material is not as pH dependent as chlorine and is, reportedly, less corrosive and reactive with organic matter than chlorine (15, 17). Though no comparisons were made here, ClO_2 can be more active than chlorine against microorganisms (4, 7). Stability of ClO_2 is such that the material would need to be generated on site. A system for generating ClO_2 for use in commercial packinghouses could be easily devised (13).

Chlorine is not considered as a fungicide but rather as a disinfectant to reduce inoculum levels of decay pathogens (9). A reduction in the level of inoculum reduces the probability of infection and decay (10, 12). Chlorine could not replace the standard packinghouse fungicide treatment. It should, however, be a required treatment for a soak tank installation and be considered for use in aqueous sprays if decay is a problem (11) to supplement postharvest fungicide treatments for decay control.

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SEASONAL AND TEMPERATURE EFFECTS ON SEED GERMINATION IN STORED GRAPEFRUIT

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Abstract. **The effects of grove source, harvest date, storage temperature and polyethylene shrink film on seed germination in stored grapefruit were studied for fruit harvested in March, April and May, 1983 and February, March and April, 1984. Numbers of germinated seeds in fruit at harvest increased as the season progressed and was greater for some groves than others. Seeds did not germinate in fruit stored at 10°C for up to 11 weeks but did germinate in fruit stored at 21° and 30°. Sealing the fruit in polyethylene shrink film did not alter the percentage of seeds germinating during storage. Seeds removed from the fruit germinated faster than those in the fruit stored at the same temperature. Abscisic acid at 10^{-4}M inhibited germination of seeds from fruit harvested early in the season but not late season fruit. Harvesting the fruit prior to the time when seeds start to germinate and storing it at temperatures which inhibit**

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