

# Chlorine and Chlorine Dioxide for Control of d'Anjou Pear Decay

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## ABSTRACT

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The effects of chlorine and chlorine dioxide on germination of *Botrytis cinerea*, *Mucor piriformis*, and *Penicillium expansum* and on d'Anjou pear decay were studied under laboratory and commercial packinghouse conditions. Chlorine at 50  $\mu\text{g/ml}$  significantly reduced conidial germination of all decay fungi after 0.5-min treatment and at 2.5 and 5.0  $\mu\text{g/ml}$  reduced *M. piriformis* and *P. expansum* germination after 5 min. A 0.5-min treatment with chlorine dioxide at 10  $\mu\text{g/ml}$  significantly reduced germination of all decay fungi but did not affect conidial germination at 0.1, 0.5, and 1.0  $\mu\text{g/ml}$ . Treatment with chlorine (50  $\mu\text{g/ml}$ ) or chlorine dioxide (10  $\mu\text{g/ml}$ ) significantly reduced fruit decay, but decay was not controlled when conidia were treated with chlorine dioxide at 0.1, 0.5, or 1.0  $\mu\text{g/ml}$  or chlorine at 0.5, 2.5, or 5.0  $\mu\text{g/ml}$ . Immersion of inoculated fruits in chlorine or chlorine dioxide in commercial packinghouse flumes did not reduce decay. Use of chlorine dioxide for control of pear fruit decay does not presently appear economically feasible.

Additional key word: *Pyrus*

Use of chlorine for control of apple decay was studied as early as 1932 (1) and found to effectively reduce germination of conidia of *Penicillium expansum* Lk. ex. Thom. A chlorine rinse of fruit markedly reduced decay (2), and 4,000  $\mu\text{g/ml}$  available chlorine for apple decay control was recommended for commercial use (2,4). Recent studies showed that pear decay was controlled when punctured fruits were immersed in a 200  $\mu\text{g/ml}$  chlorine solution containing *Mucor piriformis* Fischer or *P. expansum* spores (3). Decay control was poor when pears were inoculated and dried before chlorine treatment. Sodium sulfate, commonly used for pear flotation, did not affect chlorine activity, but sodium silicate raised the pH and decreased the biological activity of the chlorine

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solution. Several disadvantages limiting the use of chlorine include its unpleasant odor, corrosiveness, and rapid breakdown in the presence of organic materials (3).

Chlorine dioxide ( $\text{ClO}_2$ ) has been used commercially for bacterial control in pea, corn (7), tomato, and potato (5) processing plants.  $\text{ClO}_2$  does not react with ammonia-nitrogen compounds, biocidal properties are little affected by pH, and corrosive properties are minimal (5). However, no data are available concerning the effect of  $\text{ClO}_2$  on pear decay fungi.

This study evaluated the effects of low concentrations of chlorine and  $\text{ClO}_2$  on spore germination of *Botrytis cinerea* Pers. ex Fr., *M. piriformis*, and *P. expansum* and determined decay control under laboratory and commercial conditions.

## MATERIALS AND METHODS

*B. cinerea*, *M. piriformis*, and *P. expansum* were grown on potato-dextrose agar acidified with 1.5 ml of 85% lactic acid per liter. Seven-day-old cultures were flooded with sterile distilled water, and suspensions were adjusted to obtain  $11,000 \pm 1,000$  conidia per

milliliter after addition to the chlorine or  $\text{ClO}_2$  solutions.

Chlorine solutions from  $0.5 \pm 0.025$  to  $50 \pm 2.5$   $\mu\text{g/ml}$  were prepared from commercial bleach containing 5.25% sodium hypochlorite. Total available chlorine was determined by sodium thiosulfate titration (6).  $\text{ClO}_2$  was obtained from a commercial generator (Air Temp Control Systems, Inc., Richland, WA 99352), and concentrations from  $0.1 \pm 0.005$  to  $10 \pm 0.5$   $\mu\text{g/ml}$  were determined with phenylarsine oxide by using a  $\text{ClO}_2$  titration kit (Bio-cide Chemical Co., Inc., Norman, OK 73069). All chlorine and  $\text{ClO}_2$  solutions contained 5.0% sodium sulfate, commonly used as a flotation agent for pears. Preliminary studies showed that 5.0% sodium sulfate did not affect spore germination or decay.

Conidia were added to freshly prepared chlorine or  $\text{ClO}_2$  solutions at  $10 \pm 2$  and  $20 \pm 2$  C, mixed; after 0.5, 5, and 10 min, 3 ml were filtered through a 0.45- $\mu\text{m}$  millipore filter (Schleicher & Schuell, Inc., Keene, NH 03431). Conidia were immediately washed with 20 ml of sterile distilled water. In addition to the above treatments, 1  $\mu\text{g/ml}$   $\text{ClO}_2$  and 5  $\mu\text{g/ml}$  chlorine at 10 C and 5-min exposure to conidia were also tested with the surfactant Potato Kleen 211 (Pennwalt Corp., Monrovia, CA 91016) at 0.1% (v/v).

Conidia were removed from filters by agitation in 3 ml of sterile distilled water. Spore recoveries from filters were 86, 74, and 62% for *M. piriformis*, *B. cinerea*, and *P. expansum*, respectively. Treated conidia were placed on potato-dextrose agar and germinated at 12 C for 24 hr. Germination of 100–200 conidia was determined for each temperature-time treatment combination.

D'Anjou pear fruits were surface-sterilized with 95% ethanol and inoculated with treated conidia of *B. cinerea* or *P. expansum* by needle puncture through drops of inoculum and by placing *M. piriformis* inoculum into nail punctures

**Table 1.** Effect of exposure time to chlorine or chlorine dioxide on germination of *Botrytis cinerea*, *Mucor piriformis*, and *Penicillium expansum* conidia

Treatment ( $\mu\text{g/ml}$ )	Percent germination <sup>a</sup> of								
	<i>B. cinerea</i> exposed (min)			<i>M. piriformis</i> exposed (min)			<i>P. expansum</i> exposed (min)		
	0.5	5	10	0.5	5	10	0.5	5	10
Chlorine									
50.0	0 a	0 a	0 a	0 a	0 a	0 a	1 a	0 a	0 a
5.0	100 c	99 b	97 b	57 b	1 a	0 a	36 b	13 b	10 a
2.5	93 b	95 b	98 b	97 c	47 b	12 b	70 c	43 c	10 a
0.5	94 bc	99 b	99 b	100 c	93 c	97 d	92 cd	92 d	83 b
Chlorine dioxide									
10.0	1 a	0 a	0 a	1 a	0 a	0 a	32 b	0 a	0 a
1.0	98 bc	98 b	99 b	89 c	92 c	88 cd	87 cd	90 d	90 bc
0.5	99 bc	99 b	95 b	77 bc	89 c	94 d	95 d	91 d	83 b
0.1	96 bc	97 b	99 b	85 c	75 c	81 c	89 cd	87 d	93 bc
Distilled water	99 bc	98 b	99 b	91 c	88 c	97 d	95 d	93 d	97 c

<sup>a</sup> Each value based on 100–200 conidia examined 24 hr after treatment. Numbers followed by the same letter within columns are not significantly different at  $P=0.01$  according to Duncan's new multiple range test.

**Table 2.** Effect of exposure time to chlorine or chlorine dioxide treatment of *Botrytis cinerea*, *Mucor piriformis*, and *Penicillium expansum* conidia on decay of d'Anjou pear

Treatment ( $\mu\text{g/ml}$ )	Percent decay <sup>a</sup> caused by								
	<i>B. cinerea</i> conidia treated (min)			<i>M. piriformis</i> conidia treated (min)			<i>P. expansum</i> conidia treated (min)		
	0.5	5	10	0.5	5	10	0.5	5	10
Chlorine									
50.0	0 a	0 a	0 a	13 a	0 a	0 a	27 a	0 a	0 a
5.0	97 b	100 b	100 b	100 c	67 bc	73 b	100 b	83 b	77 b
2.5	100 b	97 b	100 b	93 c	100 c	73 b	97 b	93 b	87 b
0.5	100 b	97 b	93 b	100 c	87 c	97 b	93 b	97 b	93 b
Chlorine dioxide									
10.0	0 a	0 a	0 a	63 b	23 ab	0 a	60 ab	3 a	0 a
1.0	100 b	100 b	100 b	97 c	100 c	100 b	73 b	90 b	90 b
0.5	87 b	100 b	93 b	100 c	90 c	100 b	83 b	97 b	100 b
0.1	93 b	93 b	80 b	93 c	100 c	97 b	93 b	97 b	83 b
Distilled water	100 b	100 b	100 b	90 bc	100 c	100 b	90 b	97 b	93 b

<sup>a</sup> Each value represents the mean of three fruits, each inoculated five times. Numbers followed by the same letter within columns are not significantly different at  $P=0.01$  according to Duncan's new multiple range test.

(3 mm diameter, 4 mm deep). Fruit were held at  $18 \pm 2$  C and 100% relative humidity for 3–7 days, when visible decay was evaluated.

**Packinghouse studies.** D'Anjou pear fruits were wiped with 95% ethanol, punctured with a nail, and inoculated with 2,000 conidia of *B. cinerea*, *M. piriformis*, or *P. expansum* per milliliter. Fruits were immersed for 130 sec in commercial packinghouse flumes containing chlorine at  $130 \pm 10$   $\mu\text{g/ml}$  or  $\text{ClO}_2$  at 0.35 (24 October 1979) or 0.5  $\mu\text{g/ml}$  (26 November 1979). On 26 November, Potato Kleen 211 at approximately 0.1% was added to the  $\text{ClO}_2$  flume. Control fruit were immersed in tap water at  $7 \pm 1$  C. After treatment, fruits were held at  $18 \pm 2$  C and 100% relative humidity for 7 days, when decay was evaluated.

## RESULTS

Chlorine at 50  $\mu\text{g/ml}$  and  $\text{ClO}_2$  at 10  $\mu\text{g/ml}$  significantly ( $P=0.01$ ) reduced conidial germination of all decay fungi after treatment for 0.5 min (Table 1). At 2.5 and 5.0  $\mu\text{g/ml}$ , chlorine did not affect

germination of *B. cinerea* conidia but effectively reduced *M. piriformis* and *P. expansum* germination after 5 min (Table 1). At these concentrations, germination decreased significantly ( $P=0.01$ ) as treatment time increased.  $\text{ClO}_2$  at 0.1, 0.5, and 1.0  $\mu\text{g/ml}$  did not affect conidial germination of *B. cinerea*, *M. piriformis*, or *P. expansum* even after a 10-min treatment (Table 1). Although chlorine and  $\text{ClO}_2$  appeared less effective at 10 than 20 C, differences were significant ( $P=0.01$ ) in only 4% of the comparisons. Addition of surfactant to 5  $\mu\text{g/ml}$  chlorine or 1  $\mu\text{g/ml}$   $\text{ClO}_2$  did not affect conidial germination.

No decay occurred when fruits were inoculated with conidia of *B. cinerea*, *M. piriformis*, or *P. expansum* treated with 50  $\mu\text{g/ml}$  chlorine for 5 min or 10  $\mu\text{g/ml}$   $\text{ClO}_2$  for 10 min (Table 2). Fruit inoculated with conidia treated with 50  $\mu\text{g/ml}$  chlorine for 0.5 min or 10  $\mu\text{g/ml}$   $\text{ClO}_2$  for 5 min had significantly ( $P=0.01$ ) less decay than control fruit (Table 2). Conidial treatment with chlorine at 0.5, 2.5, and 5.0  $\mu\text{g/ml}$  or  $\text{ClO}_2$  at 0.1, 0.5,

and 1.0  $\mu\text{g/ml}$  for 10 min or less did not significantly ( $P=0.01$ ) affect resultant fruit decay (Table 2). In a few treatments, such as *M. piriformis* conidia treated with 5  $\mu\text{g/ml}$  chlorine for 10 min, decay occurred when no germination was observed after 24 hr. When germination of treated *M. piriformis* conidia was measured daily, 2 and 5% germinated in 48 and 72 hr, respectively.

When inoculated fruit were immersed in commercial packinghouse flumes containing chlorine or  $\text{ClO}_2$ , no reduction in decay was measured. When surfactant was added to the  $\text{ClO}_2$  flume, decay control was not improved.

## DISCUSSION

In early decay control research with chlorine, concentrations of 2,500–4,000  $\mu\text{g/ml}$  were common (1,2,4). Presently, many fruit packinghouses use 100  $\mu\text{g/ml}$  available chlorine to lower dump tank spore loads and reduce decay. In this study, 50  $\mu\text{g/ml}$  available chlorine in distilled water almost completely prevented conidial germination of *B. cinerea*,

*M. piriformis*, and *P. expansum*. Because high chlorine levels are expensive to maintain and corrosive to packinghouse equipment, lower concentrations are desirable. However, additional studies with packinghouse water are necessary before current recommendations can be changed. In addition, use of less chlorine would require frequent monitoring to compensate for fruit lots high in organic debris that rapidly inactivates chlorine. Chlorine in a packinghouse flume did not control decay of previously inoculated fruit. This agrees with previous studies showing that chlorine gave poor decay control when used after inoculation (3). Because low chlorine levels effectively reduced conidial germination in this study, methods that improve chlorine penetration into wounds appear necessary to increase decay control.

$\text{ClO}_2$  is less reactive with organic material (5) and may penetrate wounds more effectively than chlorine.  $\text{ClO}_2$  has been used successfully as a bactericide at residual concentrations of  $1 \mu\text{g}/\text{ml}$  or less (5,7). In this study,  $\text{ClO}_2$  effectively reduced *B. cinerea*, *M. piriformis*, and *P. expansum* conidial germination and resultant decay in the laboratory at  $10 \mu\text{g}/\text{ml}$  but not at  $0.5$  or  $1.0 \mu\text{g}/\text{ml}$ . When used in a packinghouse flume at  $0.5 \mu\text{g}/\text{ml}$ ,  $\text{ClO}_2$  did not reduce decay of inoculated fruit. Although  $\text{ClO}_2$  is not corrosive to equipment and is effective over a wide pH range (5), the  $10 \mu\text{g}/\text{ml}$  level necessary in our study to obtain significant fungicidal activity greatly exceeds the  $0.5$ – $1.0 \mu\text{g}/\text{ml}$  levels used for commercial control of bacteria and appears too costly for use in Oregon apple and pear packinghouses.

#### LITERATURE CITED

1. BAKER, K. F., and F. D. HEALD. 1932. Some problems concerning blue mold in relation to cleaning and packing of apples. *Phytopathology* 22:879-898.
2. BAKER, K. F., and F. D. HEALD. 1934. Investigations on methods of control of the blue-mold decay of apples. *State Coll. Wash. Agric. Exp. Stn. Bull.* 304. 32 pp.
3. BERTRAND, P., and J. SAULIE-CARTER. 1979. Postharvest decay control of apples and pears after immersion dumping. *Oreg. State Univ. Exp. Stn. Spec. Rep.* 545. 9 pp.
4. HEALD, F. D. 1939. Control of rots of apples. *State Coll. Wash. Agric. Exp. Stn. Bull.* 158. 8 pp.
5. RAUH, J. S., and T. TAYLOR. 1978. Chlorine dioxide use in food processing applications. 10th Annu. Symp. on Food Sci. IFT-Intermountain Section, Sun Valley, ID.
6. Standard Methods for Examination of Water and Waste Water. 1975. New York. 14th ed., pp. 316-318. American Public Health Assoc.
7. WELCH, J. L., and J. F. FOLINAZZO. 1959. Use of chlorine dioxide for cannery sanitation and water conservation. *Food Technol.* 13:179-182.