

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 (ClO_2) has been used commercially for bacterial control in pea, corn (7), tomato, and potato (5) processing plants. 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 ClO_2 on pear decay fungi.

This study evaluated the effects of low concentrations of chlorine and 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 ClO_2 solutions.

Chlorine solutions from 0.5 ± 0.025 to 50 ± 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). ClO_2 was obtained from a commercial generator (Air Temp Control Systems, Inc., Richland, WA 99352), and concentrations from 0.1 ± 0.005 to 10 ± 0.5 $\mu\text{g/ml}$ were determined with phenylarsine oxide by using a ClO_2 titration kit (Bio-cide Chemical Co., Inc., Norman, OK 73069). All chlorine and 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 ClO_2 solutions at 10 ± 2 and 20 ± 2 C, mixed; after 0.5, 5, and 10 min, 3 ml were filtered through a 0.45- μm 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 $\mu\text{g/ml}$ 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 ^a 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

^aEach 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 ^a 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

^aEach 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 ± 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 ± 10 $\mu\text{g/ml}$ or 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 ClO_2 flume. Control fruit were immersed in tap water at 7 ± 1 C. After treatment, fruits were held at 18 ± 2 C and 100% relative humidity for 7 days, when decay was evaluated.

RESULTS

Chlorine at 50 $\mu\text{g/ml}$ and 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. 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 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 $\mu\text{g/ml}$ 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 $\mu\text{g/ml}$ 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 $\mu\text{g/ml}$ 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 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 ClO_2 , no reduction in decay was measured. When surfactant was added to the 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*,

