Effects of the Nonionic Surfactant Ag-98 on Three Decay Fungi of Anjou Pear

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ABSTRACT

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In potato-dextrose agar (PDA), the nonionic surfactant Ag-98 inhibited spore germination, germ tube growth, and mycelial growth of *Botrytis cinerea, Mucor piriformis,* and *Penicillium expansum*, but germination of conidia suspended in aqueous solutions of Ag-98 was not affected after conidia were removed from the Ag-98, washed, and transferred to PDA. Control of decay in both laboratory and packinghouse was greater when Ag-98 was combined with chlorine than when chlorine was used alone. The fungistatic and antifoaming properties of Ag-98 make it suitable for commercial use for addition to chlorine to control decay of pear fruits.

Several fungi, including *Botrytis cinerea* Pers.: Nocca & Balbis, *Mucor piriformis* Fischer, and *Penicillium expansum* Link ex Thom., cause postharvest decay of pear fruits (*Pyrus communis* L.). These fungi are found in dump-tank water in packinghouses (8) and are carried into the water in soil on the undersides of bins (5). Positive relationships between incidence of pear fruit decay and the concentration of spores of these fungi in water have been determined (7). Chlorine often is added to dump-tank water to reduce the concentration of spores of decay fungi (1), but viable spores frequently are present in water containing chlorine (8). Addition of several surfactants to chlorine solutions improved the effectiveness of chlorine and reduced decay of pear fruits (9). Surfactants have been used to control several plant diseases, including apple scab (2), apple powdery mildew (3), and some market diseases of tomato fruit (4).

This paper reports the effects of the nonionic surfactant Ag-98 (80% octylphenoxypolyethoxyethanol, Rohm & Haas Co., Philadelphia, PA) on germination and growth in culture of *B. cinerea, M. piriformis,* and *P. expansum*. Also, laboratory and commercial packinghouse studies are presented on the efficacy of Ag-98 alone and in chlorine solutions for postharvest control of decay diseases of Anjou pear fruits. An abstract of this study has been published (10).

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MATERIALS AND METHODS

Effects of Ag-98 on spore germination. *B. cinerea, M. piriformis,* and *P. expansum* were isolated from decayed pear fruits and cultured on potato-dextrose agar (PDA) (Difco) acidified with 1.5 ml of lactic acid per liter (APDA). Cultures 1–2 wk old were flooded with sterile distilled water, and frequently agitated suspensions were adjusted to obtain 2.5×10^4 conidia per milliliter. Two 40-μl drops of spore suspension were placed on each PDA plate amended with Ag-98 after autoclaving to obtain concentrations of 0–5,000 μg of Ag-98 per milliliter (Table 1). Four replicate plates were made for each fungus at each concentration of Ag-98. Plates with conidia of *B. cinerea* were incubated at 12 C for 22 hr, *M. piriformis* at 20 C for 22 hr, and *P. expansum* at 15 C for 28 hr. Germination of 100 conidia per plate (50 per drop) was determined microscopically, and spores were considered germinated when germ tube length equaled or exceeded the length of the spore. Germ tube length of 50–80 spores was measured at each concentration of Ag-98 with an ocular micrometer. Data were analyzed with linear regression using logarithms of both Ag-98 concentration and percent inhibition of germination or germ tube length relative to germination or germ tube length on unamended PDA controls.

The effect of Ag-98 on spore germination also was studied in aqueous solutions. Conidia of *B. cinerea, M. piriformis,* and *P. expansum* were added to solutions of 0, 1,000, 3,000, and 5,000 μg of Ag-98 per milliliter to obtain a final concentration of 5×10^4 spores per milliliter. Solution temperature was 10 ± 0.1 C. After 0.5, 24, 48, and 72 hr, 1 ml of conidial suspension was removed and filtered through a 0.45-μm Millipore filter. Spores were washed with 30 ml of distilled water to remove residual Ag-98 and were transferred by blotting the filter on the surface of APDA. Germination of 100 spores per plate was determined after 22 hr at 10 C for *M. piriformis* or 15 C for *B. cinerea* and *P. expansum*.

Effects of Ag-98 on mycelial growth. PDA was amended with 40–200 μg of Ag-98 per milliliter after autoclaving. Eight-millimeter-diameter disks of *P. expansum* and 4-mm disks of *B. cinerea* and *M. piriformis* were transferred from the margins of 1- to 2-wk-old cultures onto the Ag-98-amended PDA. Colony diameters of four replicate plates of each concentration were measured after 3, 6, and 8 days at 20 C for *M. piriformis, B. cinerea,* and *P. expansum*, respectively. Data were analyzed with linear regression using logarithms of Ag-98 concentration and percent inhibition of growth relative to growth on unamended PDA.

Decay control with Ag-98 and chlorine. Pear fruits (cultivar Anjou) were surface-sterilized for 2 min in 0.525% sodium hypochlorite, rinsed with tap water, and puncture-wounded with a blunt metal instrument (3 mm diameter, 4 mm deep) at four locations per fruit. Wounded fruits were immersed for 5 min at 10 ± 2 C in solutions containing 1,000–5,000 μg of Ag-98 and 2×10^3 spores of *B. cinerea, M. piriformis,* or *P. expansum* per milliliter. Ten fruits were treated at each concentration, and the experiment was replicated four times. After treatment, fruits were rinsed with tap water and placed in polyethylene-lined boxes at 20 ± 2 C. Decay incidence was evaluated after 6 days. Data were analyzed with linear regression of the logarithms of percent decay on percent Ag-98 concentration.

In a second series of experiments, the effects of Ag-98 in chlorine solutions on decay control were studied. Anjou pear fruits were surface-sterilized, rinsed, and wounded as described. Solutions containing 0, 1,000, 3,000, and 5,000 μg of Ag-98 and 71 ± 7 μg of total available chlorine per milliliter were prepared and cooled to 10 ± 2 C. Spores of *B. cinerea, M. piriformis,* or *P. expansum* were added to obtain a final concentration of 2

Table 1. Effects of Ag-98 on spore germination and germ tube length of *Botrytis cinerea, Mucor piriformis,* and *Penicillium expansum*

Ag-98 concentration[a] (μg/ml)	Percent inhibition of					
	B. cinerea		*M. piriformis*		*P. expansum*	
	Germination[b,c]	Germ tube length[d,e]	Germination[b,c]	Germ tube length[d,e]	Germination[b,c]	Germ tube length[d,e]
1,000	7.6	58.6	5.1	0.0	1.0	37.9
2,000	13.9	69.4	10.8	53.3	1.8	50.4
3,000	18.3	82.6	16.4	59.4	2.1	64.3
4,000	20.0	73.1	19.1	77.7	3.6	66.1
5,000	19.4	84.0	27.4	82.5	4.1	66.5

[a] Potato-dextrose agar amended with Ag-98 after autoclaving.
[b] Germination of 100 spores per plate on four replicate plates determined after 22 hr at 12 C (*B. cinerea* and *M. piriformis*) or 28 hr at 15 C (*P. expansum*).
[c] Regression equations of log percent inhibition of germination (Y) on log Ag-98 concentration (X) are as follows: *B. cinerea*—$Y=-0.92+0.61X$, $r=0.962$; *M. piriformis*—$Y=-2.32+1.01X$, $r=0.996$; *P. expansum*—$Y=-2.60+0.86X$, $r=0.980$. All correlation coefficients significant at $P=0.01$.
[d] Each value represents the average of 50–80 spores. Germ tubes measured when germination counts made.
[e] Regression equations of log percent inhibition of germ tube length (Y) on log Ag-98 concentration (X) are as follows: *B. cinerea*—$Y=1.16+0.21X$, $r=0.895$; *M. piriformis*—$Y=0.024+0.51X$, $r=0.960$; *P. expansum*—$Y=0.29+0.43X$, $r=0.987$. All correlation coefficients significant at $P=0.05$.

$\times 10^3$/ml, and 10 wounded fruits were immersed in each solution for 5 min. Additional spores then were added to increase the concentration by 1.5×10^3/ml, and a second set of 10 fruits was treated. Two additional sets of fruits also were treated in this manner. After treatment, the 40 fruits at each concentration were rinsed and placed in polyethylene-lined boxes at 20 ± 2 C. Decay incidence was evaluated after 4–6 days. Data were analyzed with linear regression as described.

Decay control with Ag-98 and chlorine in a commercial packinghouse. Anjou pear fruits in wooden field bins each containing about 454 kg of fruit were immersed in a dump tank containing 23,850 L of aqueous solution of sodium sulfate flotation salt at 1.025 specific gravity, 5 C, and 68 ± 8 μg of total available chlorine per milliliter derived from sodium hypochlorite. After about 150 bins of fruit were processed, Ag-98 was added to the dump tank to obtain 2,500 μg/ml, chlorine was readjusted to 68 ± 8 μg/ml, and immersion dumping of Anjou pears continued. Fruits were in the solution at 5 C for about 4 min, then floated through flumes containing 60 μg of chlorine per milliliter without Ag-98. After rinsing with tap water, fruits were treated with benomyl at 300 μl/ml, dried in a heat tunnel at 57 C, and stored in wooden bins at −1 C in a commercial, controlled-atmosphere (CA) room. Oxygen concentration in the storage was 2.5 ± 0.2% and CO_2 was 1 ± 0.2%.

Cull fruits were removed by packinghouse personnel before benomyl application, and three replicate boxes of 100 fruits per box were taken from bins of cull fruits before and after addition of Ag-98 to the dump tank. Fruits were kept at 20 C in polyethylene-lined boxes, and the number of decayed fruits was evaluated after 8 days. The effect of Ag-98 on decay was analyzed with a t test after arc sine $\sqrt{\text{percent decay}}$ transformation of data.

Fruits from CA storage were removed after 7 mo and floated in a sodium sulfate plus chlorine solution similar to that described before. Fruits were rinsed, dried, sorted, and packed by packinghouse personnel. Decayed fruits were removed before packing, and weights of decayed fruits in 43,584 and 98,972 kg of fruits that were processed in the chlorine and chlorine plus Ag-98 treatments, respectively, were determined.

Table 2. Effects of Ag-98 on mycelial growth of *Botrytis cinerea, Mucor piriformis,* and *Penicillium expansum* on potato-dextrose agar (PDA)

Ag-98 concentration (μg/ml)	Percent inhibition of mycelial growth[a] of		
	B. cinerea	*M. piriformis*	*P. expansum*
40	36.3	49.9	30.0
80	68.7	71.5	39.4
120	80.0	84.4	51.7
160	82.5	87.5	58.3
200	82.5	91.7	59.3

[a] Mycelial growth of *B. cinerea, M. piriformis,* and *P. expansum* measured 6, 3, and 8 days, respectively, at 20 C after mycelial disks were placed on Ag-98-amended PDA. Regression equations of log inhibition of mycelial growth (Y) on log Ag-98 concentration (X) are as follows: *B. cinerea*—$Y=0.614+0.612X$, $r=0.958$*; *M. piriformis*—$Y=1.109+0.381X$, $r=0.979$**; *P. expansum*—$Y=0.763+0.448X$, $r=0.991$**. Significance of r at $P=0.01$ indicated by ** and at $P=0.05$ by *.

RESULTS

Effects of Ag-98 on spore germination. Ag-98 incorporated into PDA inhibited germination of *B. cinerea* and *M. piriformis* more than that of *P. expansum*, but regressions of inhibition of germination on Ag-98 concentration were highly significant ($P=0.01$) for all three fungi (Table 1). Similarly, Ag-98 significantly ($P=0.05$) inhibited germ tube growth of all fungi, and inhibition at 5,000 μg of Ag-98 per milliliter ranged from 66.5% for *P. expansum* to 84% for *B. cinerea* (Table 1).

Germination of spores suspended in aqueous solutions of Ag-98 for up to 72 hr before removal of Ag-98 was not affected by treatments, and germination of all fungi was 98–100% of the water control.

Effects of Ag-98 on mycelial growth. Ag-98 inhibited mycelial growth of all three fungi, and inhibition at 200 μg/ml ranged from 59.3 to 91.7% for *P. expansum* and *M. piriformis*, respectively (Table 2). Regressions of inhibition of mycelial growth on Ag-98 concentration were significant at $P=0.01$ for *M. piriformis* and *P. expansum* and at $P=0.05$ for *B. cinerea*. Inhibition of mycelial growth on Ag-98-amended PDA occurred at lower concentrations of Ag-98 than did inhibition of spore germination.

Decay control with Ag-98 and chlorine. Regressions of decay of pear fruits on Ag-98 concentration were significant ($P=0.05$) for all three fungi (Table 3). Decay control with 5,000 μg of Ag-98 per milliliter relative to the water check was 16, 26, and 29% for *B. cinerea, M. piriformis,* and *P. expansum*, respectively.

When Ag-98 was added to chlorine solutions, decay was reduced more than with Ag-98 or chlorine alone. Control of decay caused by *M. piriformis* and *P. expansum* was correlated significantly ($P=0.05$) with Ag-98 concentration (Table 4). Although decay caused by *B. cinerea* was reduced from 42% in chlorine to 8% in chlorine plus 5,000 μg of Ag-98 per milliliter, considerable variability in decay occurred, and the relationship was not significant (Table 4).

In the commercial packinghouse study, decay caused by *P. expansum* in

Table 3. Effects of Ag-98 on decay of Anjou pear

Ag-98 concentration[a] (μl/ml)	Percent decay[b,c] caused by		
	Botrytis cinerea	*Mucor piriformis*	*Penicillium expansum*
0	87	96	89
1,000	82	82	70
2,000	79	80	67
3,000	70	76	71
4,000	77	78	65
5,000	73	71	63

[a] Fruits immersed 5 min in Ag-98 solutions at 10 C.
[b] Each value represents the average of 40 fruits, each wounded four times. Percentage of wounds with decay was evaluated after 6 days at 20 C.
[c] Regression equations of log percent decay (Y) on percent Ag-98 concentration (X) are as follows: *B. cinerea*— $Y = 1.928 - 0.149X$, $r = -0.812$; *M. piriformis*— $Y = 1.957 - 0.212X$, $r = -0.903$; *P. expansum*— $Y = 1.906 - 0.235X$, $r = -0.820$. All correlation coefficients significant at $P = 0.05$.

Table 4. Effects of Ag-98 in chlorine solution on decay of Anjou pear

Ag-98 concentration[a] (μg/ml)	Percent decay[b,c] caused by		
	Botrytis cinerea	*Mucor piriformis*	*Penicillium expansum*
0	42	69	46
1,000	19	51	39
3,000	11	24	29
5,000	8	4	25

[a] Fruits immersed 5 min in solutions containing specified Ag-98 concentration and 71 ± 7 μg/ml total available chlorine.
[b] Each value represents the average of 40 fruits, each wounded four times. Percentage of wounds with decay was evaluated after 4–6 days at 20 C.
[c] Regression equations of log percent decay (Y) on percent Ag-98 concentration (X) are as follows: *B. cinerea*— $Y = 1.51 - 1.33X$, $r = -0.944$ n.s.; *M. piriformis*— $Y = 1.93 - 2.49X$, $r = -0.967$*; *P. expansum*— $Y = 1.65 - 0.54X$, $r = -0.983$*. Significance of r at $P = 0.05$ indicated by *.

cull fruits that were sampled before storage was significantly less ($P = 0.05$) when fruits were treated with Ag-98 plus chlorine than with chlorine alone, resulting in 17 and 44% decay, respectively. Decays caused by other fungi were less than 4% of all fruit, and treatment differences were not significant.

After 7 mo of storage at −1 C, total decay was 3.4 and 3.0 kg per bin (454 kg of fruit) for fruits treated with chlorine and chlorine plus Ag-98, respectively. Individual fungi responsible for decay were not identified.

DISCUSSION

Spore germination, germ tube growth, and mycelial growth of *B. cinerea, M. piriformis,* and *P. expansum* were all reduced when the fungi were placed on Ag-98-amended PDA. However, when conidia were removed after 72 hr from a solution containing Ag-98 at 5,000 μg/ml, there was no effect of Ag-98 on spore germination. Thus, Ag-98 appears to possess fungistatic rather than fungicidal properties and is effective alone only when fungi remain in continuous contact with the surfactant. In a similar study with the anionic surfactant Nacconol, a fungistatic effect on mycelia and spores was observed in vitro with four decay-causing fungi of tomato fruits (4). As with Ag-98, higher concentrations of Nacconol were required for inhibition of spore germination than for inhibition of mycelial growth.

Previously, we found that several surfactants improved the effect of chlorine for decay control of pear fruits (9). The use of surfactants with fungicides such as benomyl and captan for postharvest decay control lowered residues of fungicides on fruit surfaces and reduced the effectiveness of the fungicides, however (6). Although Ag-98 at 5,000 μg/ml reduced decay by 16–29%, addition of Ag-98 to chlorine reduced decay more than either Ag-98 or chlorine alone. The improved efficacy of chlorine with Ag-98 may be related to improved penetration of chlorine into wounds, calyx, and stem ends. In our preliminary studies, greater decay control with chlorine was obtained with Ag-98 than with previously tested surfactants (9) or Nacconol. Nacconol did not enhance the ability of chlorine solutions to reduce the incidence of *B. cinerea* decay of tomato fruits (4). Ag-98 possesses several advantages over previously tested surfactants that make it suitable for commercial use. First, use of many surfactants resulted in an increase in decay if chlorine was not in the solution (9). Ag-98 provided limited decay control without chlorine and will not become a negative factor in packinghouses that do not carefully monitor and maintain chlorine at proper concentration. Second, the antifoaming properties of Ag-98 are necessary in the dynamic, circulating dump and flume systems of packinghouses. Third, although additional chlorine initially was required with Ag-98 to achieve a desired chlorine concentration, the chlorine level was maintained easily in our commercial test with a variable-speed peristaltic pump.

LITERATURE CITED

1. Bertrand, P., and Saulie-Carter, J. 1979. Postharvest decay control of apples and pears after immersion dumping. Ore. State Univ. Exp. Stn. Spec. Rep. 545. 9 pp.
2. Burchill, R. T., and Swait, A. A. J. 1977. Eradication of the perithecial stage of apple scab with surfactants. Ann. Appl. Biol. 87:229-231.
3. Clifford, D. R., and Hislop, E. C. 1975. Surfactants for the control of apple mildew. Pestic. Sci. 6:409-418.
4. Hoy, M. W., and Ogawa, J. M. 1984. Toxicity of the surfactant Nacconol to four decay-causing fungi of fresh-market tomatoes. Plant Dis. 68:699-703.
5. Michailides, T. J., and Spotts, R. A. 1986. Factors affecting dispersal of *Mucor piriformis* in pear orchards and into the packinghouse. Plant Dis. 70:1060-1063.
6. Spotts, R. A. 1984. Effect of a surfactant on control of decay of Anjou pear with several fungicides. Plant Dis. 68:860-862.
7. Spotts, R. A. 1986. Relationships between inoculum concentration of three decay fungi and pear fruit decay. Plant Dis. 70:386-389.
8. Spotts, R. A., and Cervantes, L. A. 1986. Populations, pathogenicity, and benomyl resistance of *Botrytis* spp., *Penicillium* spp., and *Mucor piriformis* in packinghouses. Plant Dis. 70:106-108.
9. Spotts, R. A., and Peters, B. B. 1982. Use of surfactants with chlorine to improve pear decay control. Plant Dis. 66:725-727.
10. Spotts, R. A., and Cervantes, L. A. 1986. Addition of the surfactant Ag98 to chlorine to improve control of decay of pear fruit. (Abstr.) Phytopathology 76:1065.