Effects of Flotation Salt Solutions on Spore Germination of Four Decay Fungi and on Side Rot of Pear

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ABSTRACT

Sugar, D., and Spotts, R. A. 1986. Effects of flotation salt solutions on spore germination of four decay fungi and on side rot of pear. Plant Disease 70:1110-1112.

Germination of conidia of *Penicillium expansum, Botrytis cinerea,* and *Phialophora malorum* and sporangiospores of *Mucor piriformis* was significantly reduced by 40 min of exposure to sodium lignin sulfonate solutions compared with solutions of sodium carbonate, sodium silicate, sodium sulfate, and water. No spores germinated after 10 min of exposure to solutions of sodium lignin sulfonate + sodium ortho phenylphenate (SOPP). Decay of wounded pear fruit by *Phialophora malorum* increased after flotation in sodium silicate + SOPP solution but decreased after treatment with the sodium salts of lignin sulfonate, carbonate, or sulfate compared with the water control. In a commercial packinghouse, postharvest decay caused by *P. malorum* was less in Bosc pears floated in sodium lignin sulfonate + SOPP than in fruit floated in either sodium carbonate + SOPP or sodium silicate + SOPP.

Postharvest decay of pears in the Pacific Northwest is caused primarily by *Penicillium expansum* Lk. ex Thom. (blue mold), *Botrytis cinerea* Pers. ex Fr. (gray mold), *Phialophora malorum* (Kidd & Beaum.) McColloch (side rot), and *Mucor piriformis* Fischer (Mucor rot) (4,7). Blue mold and gray mold are generally controlled by postharvest applications of benomyl, but no fungicides are registered for control of side rot or Mucor rot (2,4,12).

To minimize injury to fruit, field bins containing pears are submerged in a salt solution of sufficient specific gravity (1.02–1.05) to float the pears (3). Sodium carbonate (soda ash), sodium silicate, and sodium sulfate are the salts most often used for pear flotation. Recently, sodium lignin sulfonate (Orzan SL-50, ITT Rayonnier, Stamford, CT; Lignosite 458, Georgia-Pacific, Bellingham, WA), a by-product of paper manufacture in the Pacific Northwest, has become available for pear flotation. Sodium ortho phenylphenate (SOPP) or chlorine is mixed into the salt solution to reduce populations of spores of decay fungi (3,10,11).

This study was done to determine the effects of the four commercial flotation salts on the germination of spores of pear decay fungi in the presence and absence of SOPP. The effects of these salts in SOPP solutions on decay of pear fruit by *Phialophora malorum* were also evaluated. In addition, the effects of sodium carbonate and sodium silicate compared with sodium lignin sulfonate on incidence of decay were studied during two seasons in a commercial packinghouse.

Oregon Agricultural Experiment Station Technical Paper 7818.

This project is supported in part by cooperative agreement 58-0401-5-00036 between the USDA and Oregon State University for pome fruit disease research, by the Winter Pear Control Committee, and the Fruit Growers' League of Jackson County, Oregon.

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Accepted for publication 22 July 1986 (submitted for electronic processing).

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MATERIALS AND METHODS

Spore germination. Solutions of the sodium salts of carbonate, silicate, sulfate, and lignin sulfonate were prepared to obtain a final specific gravity of 1.05 as measured with a hydrometer. In one series of experiments, the solutions also contained 0.35% SOPP (Steri-seal Inc., Wenatchee, WA); in a second series, no SOPP was used. Solutions were adjusted to 10 ± 1 C before inoculum was added.

Penicillium expansum, B. cinerea, Phialophora malorum, and *M. piriformis* were isolated from decayed pear tissue on potato-dextrose agar (Difco) acidified with 1.5 ml of 85% lactic acid per liter (APDA). Spore suspensions were prepared by washing spores from the surfaces of 7- to 14-day-old cultures and added to salt or salt + SOPP solutions to give a final concentration of 1×10^5 spores per milliliter.

After 10, 20, or 40 min (40 min only in solutions without SOPP), three replicate samples of the solution were removed and filtered through 0.45-μm Millipore filters. Spores on each filter were rinsed with 20 ml of distilled water and transferred to APDA plates. Plates with spores of *Penicillium expansum* were incubated 19 hr at 10 C, then 7 hr at 20 C; plates with *Phialophora malorum* were incubated 20 hr at 20 C; and plates with *B. cinerea* and *M. piriformis* were incubated 22 hr at 10 C. After incubation, 100 spores per plate were examined for germination. Conidia were considered germinated if germ tube length was equal to or greater than maximum spore length.

Postharvest decay. Bosc pear fruit were surface-sterilized in NaOCl at 25 mg of chlorine per liter, rinsed, and puncture-wounded (3 mm diameter, 4 mm deep) at four locations per fruit. Wounded fruit were immersed for 5 min at 10 ± 2 C in 0.40 ± 0.03% SOPP in

solutions of the sodium salts of carbonate, silicate, sulfate, and lignin sulfonate at specific gravity 1.05. Water, with and without SOPP, was used as the control. Conidia of *P. malorum* were added to all solutions just before immersion of the fruit to achieve a final concentration of 2.5×10^4 spores per milliliter. Forty pears were immersed in each salt solution, and the experiment was done twice. After treatments, pears were rinsed with water, stored at −1.1 C in polyethylene-lined boxes, and evaluated for decay incidence on each wound after 2–3 mo.

The effects of flotation salt solutions on postharvest decay of commercially packed and stored Bosc pears were studied in a cooperating packinghouse. Two dump tanks of 7,570- and 8,705-L capacity were filled with aqueous solutions of either of two flotation salts to a final specific gravity of 1.05. In 1983 tests, sodium carbonate and sodium lignin sulfonate were compared; in 1984, sodium silicate and sodium lignin sulfonate were compared. SOPP was added in each tank to an initial concentration of 0.45% as determined by sodium thiosulfate titration, and additional SOPP was added to each tank periodically during the experiment to maintain SOPP levels of 0.40–0.45%. Solution temperatures during experiments were 10 ± 2 C. Field bins containing Bosc pears (about 454 kg per bin) from an orchard with a recent history of side rot were immersed in either solution. Pears were floated 1–2 min, then lifted from the solution onto elevating belts. To avoid injury, salt solutions were removed from the fruit surface with a fresh-water rinse. Subsequently, benomyl (0.3 g a.i./L) was applied to the pears as a line spray. Pears were sorted, graded, and sized according to industry standards, then individually wrapped in paper and packed in polyethylene-lined fiberboard cartons (20 kg per carton). Over a 2-hr period, 25 cartons containing 100 size U.S. no. 1 grade fruits each were randomly selected from packing lines in which each type of flotation salt had been used. The selected cartons of fruit were stored at −1.1 C. After 5 mo of storage, pears showing decay lesions were counted. Infected fruit were surface-sterilized in NaOCl (25 mg of chlorine per liter); tissue from lesion margins was placed on PDA plates, which were incubated at 20 C for 1–2 wk, and fungal colonies were identified.

RESULTS

Spore germination. Sodium lignin sulfonate solutions without SOPP significantly ($P = 0.01$) reduced germination of spores of all decay fungi tested after 40 min of exposure (Table 1). Sodium carbonate and sodium silicate significantly reduced germination of *Penicillium expansum*. No germination was observed after exposure to sodium lignin sulfonate + SOPP (Table 2). After 20 min of exposure, all flotation salts + SOPP inhibited germination of *Phialophora malorum* to a greater extent than SOPP alone. Sodium silicate reduced germination of conidia of *B. cinerea* after 10 and 20 min, and sodium carbonate after 20 min, compared with SOPP alone (Table 2).

Table 1. Effects of flotation salts on germination of spores of *Botrytis cinerea, Mucor piriformis, Penicillium expansum*, and *Phialophora malorum* after 40 min of exposure

Flotation salt[y]	Percent germination[z] of			
	B. cinerea	*M. piriformis*	*P. expansum*	*P. malorum*
Sodium lignin sulfonate	19 a	10 a	17 a	5 a
Sodium carbonate	95 b	92 b	61 b	50 ab
Sodium silicate	99 b	93 b	69 b	61 ab
Sodium sulfate	97 b	90 b	96 c	83 b
None	99 b	90 b	96 c	87 b

[y] Salt concentration of solutions adjusted to specific gravity of 1.05.
[z] Each value represents the mean of 100 spores per replicate, each treatment replicated twice. Numbers followed by the same letter within columns are not significantly different according to Duncan's new multiple range test ($P = 0.01$).

Table 2. Effects of flotation salts and exposure time on spore germination of *Botrytis cinerea, Mucor piriformis, Penicillium expansum* and *Phialophora malorum* in sodium ortho phenylphenate (SOPP) solution

Flotation salt[y]	Percent germination[z]							
	B. cinerea exposed (min)		*M. piriformis* exposed (min)		*P. expansum* exposed (min)		*P. malorum* exposed (min)	
	10	20	10	20	10	20	10	20
Sodium lignin sulfonate	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a
Sodium carbonate	91 bc	78 b	94 b	88 b	70 b	68 b	68 b	16 ab
Sodium silicate	86 b	76 b	93 b	93 b	64 b	70 b	68 b	43 b
Sodium sulfate	97 bc	90 c	91 b	85 b	75 b	73 b	87 bc	47 b
None	98 c	97 c	95 b	87 b	90 b	88 b	97 c	94 c

[y] Salt concentration of solutions adjusted to specific gravity of 1.05. All solutions contained 0.35% SOPP.
[z] Each value represents the mean of 100 spores per replicate, each treatment replicated three times. Numbers followed by the same letter within columns are not significantly different according to Duncan's new multiple range test ($P = 0.05$).

Postharvest decay. Incidence of decay of Bosc pear fruit from *P. malorum* was significantly ($P = 0.05$) less after treatment in SOPP solutions of sodium sulfate, sodium lignin sulfonate, and sodium carbonate than that in the water control (Table 3). Decay of fruit immersed in SOPP + sodium silicate solution was significantly ($P = 0.05$) greater than in all other treatments.

In tests on fruit stored commercially for 5 mo, significantly ($P = 0.05$) fewer fruit decayed from *P. malorum* after flotation in sodium lignin sulfonate solutions than in sodium carbonate or sodium silicate solutions (Table 4). The predominant type of decay was side rot, with *P. malorum* isolated from 77–94% of the lesions. The second most prevalent fungus was *Cladosporium herbarum* (Pers.) Lk. ex S. F. Gray, which causes a decay lesion visually indistinguishable from that of *P. malorum* (12). In both years of the study, decay from *Penicillium expansum* or *B. cinerea* was less than 1% (Table 4).

Table 3. Incidence of decay of Bosc pear fruit by *Phialophora malorum* after immersion in flotation solutions for 5 min at 10 C and storage for 2–3 mo at −1.1 C

Solution[y]	Wounds infected[z] (%)
Sodium sulfate + SOPP	3.3 a[z]
Sodium lignin sulfonate + SOPP	3.7 a
Sodium carbonate + SOPP	12.0 a
Water + SOPP	16.7 ab
Water	30.4 b
Sodium silicate SOPP	63.9 c

[y] Solutions contained 2.5×10^4 conidia per milliliter of *P. malorum* and were adjusted to a specific gravity of 1.05; sodium ortho phenylphenate (SOPP) concentration was 0.40%.
[z] Each value represents the average of the 80 fruits with four wounds per fruit. Values followed by the same small letter are not significantly different according to Duncan's new multiple range test ($P = 0.05$).

DISCUSSION

The effectiveness of sodium lignin sulfonate solutions in inhibiting spore germination of pear decay fungi indicates antifungal properties not previously ascribed to this material alone. Commercial formulations of sodium lignin sulfonate also contain reducing

Table 4. Effectiveness of flotation salt in sodium ortho phenylphenate (SOPP) solutions in controlling postharvest decay of Bosc pears in a commercial packinghouse after 5 mo of storage at −1.1 C

Flotation solution[y]	Percent decay[z] caused by		
	Phialophora malorum	*Penicillium expansum*	*Botrytis cinerea*
1984–1985			
Sodium silicate + SOPP	3.19 a[z]	0.00 a	0.00 a
Sodium lignin sulfonate + SOPP	0.22 b	0.00 a	0.19 a
1983–1984			
Sodium carbonate + SOPP	21.73 a	0.33 a	0.04 a
Sodium lignin sulfonate + SOPP	6.54 b	0.12 a	0.04 a

[y] Solutions adjusted to a specific gravity of 1.05 and SOPP concentration of 0.45%.
[z] Each value is the mean decay incidence from 25 cartons of Bosc pears. Values in each column in each year followed by the same letter are not significantly different according to Duncan's new multiple range test ($P = 0.05$).

sugars, calcium, magnesium, phosphorus, potassium, iron, aluminum, sulfur, and ammonia (5,6). Sodium benzoate (0.6 g/L) was included in the formulation used in packinghouse tests reported here but was not part of the formulation used in spore germination tests and in experiments on inoculated fruit. The chemical identity of the antifungal component(s) has not been studied.

Complete inhibition of spore germination after exposure to sodium lignin sulfonate + SOPP in vitro suggests that this treatment may be useful for control of decay during flotation of pears and other fruits in aqueous solutions. The dump-tank solution has been implicated as an important site for dispersal of fungal spores that infest the surfaces of stored fruit (1,10), and the relationship between inoculum dosage and disease incidence has been established for several postharvest diseases of pear (8). The reduction of decay in pears in a commercial packinghouse may be a consequence of the decreased population of viable spores after exposure to sodium lignin sulfonate + SOPP. However, because fruit float in the dump-tank solution only 1–2 min, many spores of decay pathogens may not receive adequate exposure to effectively reduce subsequent germination. In previously reported research (9), spores of *M. piriformis* and *P. expansum* were treated for 1 min in solutions at 43.3 C of sodium lignin sulfonate, sodium carbonate, sodium sulfate, and sodium silicate containing SOPP. Germination of spores of these fungi was less after treatment in sodium lignin sulfonate than in the other salt solutions.

Because incidence of decay caused by *Phialophora malorum* was greater after treatment in sodium silicate solution than in sodium sulfate solution but germination of conidia of *P. malorum* in sodium silicate and sodium sulfate solutions was not different, factors other than conidial germination must be involved in decay incidence. The effects of flotation salts on the viscosity and surface tension of SOPP solutions may be involved and may alter penetration of conidia into wounds. These factors remain to be investigated.

Decay of Bosc pears caused by *P. malorum* under commercial conditions was less after flotation in sodium lignin sulfonate than in sodium carbonate or sodium silicate solutions. The low incidence of blue and gray molds in these experiments could be explained by the application of benomyl, which obscures assessment of potential beneficial effects of flotation salts on incidence of these diseases. Previous research (13) has shown that *Penicillium expansum* is more aggressive than *Phialophora malorum* in infecting pear wounds. Consequently, *P. expansum* must be inhibited in order to obtain information about infection by *P. malorum*.

ACKNOWLEDGMENTS

We wish to thank Robert L. Rackham and James M. Witt for their work to make sodium lignin sulfonate available.

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