Evaluation of Disinfestant-Flotation Salt-Surfactant Combinations on Decay Fungi of Pear in a Model Dump Tank

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ABSTRACT

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Several disinfestant-flotation salt-surfactant solutions were compared for effect on germination of spores of *Mucor piriformis*, *Penicillium expansum*, and *Phialophora malorum* and decay of pear caused by these fungi after exposure to a 7-hr dynamic circulation and spore addition phase, followed by a 16-hr static phase in a model dump tank. In aqueous systems without soil added to the tank, chlorine at 64 μg/ml inhibited germination from 90 to 100% in all salt solutions. Effectiveness of 4,000 μg sodium o-phenylphenate (SOPP) per milliliter was highest in calcium and sodium lignin sulfonate and lowest in sodium silicate solution. SOPP was less inhibitory to germination than chlorine during the first 1–3 hr of the dynamic phase. In flotation systems with 6.25 mg/ml of soil, chlorine in sodium sulfate and SOPP in sodium lignin sulfonate inhibited germination of spores and reduced decay of fruit more than in sodium silicate. Inhibition of germination of the three fungi was greater at the end of the static phase than during the dynamic phase in several tests with 10 combinations of disinfestant-flotation salt-surfactant.

Control of postharvest decay of pear (*Pyrus communis* L.) fruit involves use of fungicides, sanitation, and proper fruit handling systems (9). A critical component in this integrated approach is the disinfestant-flotation salt solution in the water systems used in packinghouses. In the Pacific Northwest, chlorine and sodium o-phenylphenate (SOPP) are used as disinfestants (2,12). Flotation salts include the sodium forms of carbonate, silicate, sulfate, and lignin sulfonate (16).

Chlorine controls decay by reduction of the viable spore population in packinghouse dump tanks (2,12,14). Chlorine acts on spores in solution and on surfaces of fruit and debris, but has failed to control decay of puncture-inoculated fruit (2,14,17). Fungicidal effectiveness of chlorine is dependent on pH. In alkaline solutions, effectiveness generally is less than in neutral solutions (3,6,8,17). However, chlorine at pH 9.6 was more effective than at 6.8 for control of bacterial soft rot of tomato (1). Several surfactants have been shown to increase the effectiveness of chlorine for control of decay of pear, and improved control may be related to greater penetration of chlorine into wounds, calyx, and stem ends (13,15). SOPP possesses fungistatic properties and is effective against *Botrytis cinerea* Pers.: Nocca and Balbis, *Penicillium expansum* Link: Thom, and *Mucor piriformis* Fischer (2,12). As pH of a SOPP solution increased, the concentration of undissociated phenol decreased, but decay control increased only slightly when the o-phenylphenol concentration doubled (4). Flotation salt solutions altered efficacy of SOPP; spore germination was reduced and decay control increased in sodium lignin sulfonate when compared with sodium salts of carbonate, silicate, or sulfate (16). Sodium lignin sulfonate alone inhibited spore germination of pear decay fungi, but other flotation salts had no antifungal properties (16).

The comparative effectiveness of the many combinations of disinfestants, flotation salts, and surfactants for toxicity to spores of decay fungi and control of decay is not known. Previous research with some combinations was done with static systems and treatment durations less than 1 hr (2,11,14,16). Packinghouse dump tanks represent dynamic environments in which spores are introduced into the system on contaminated bins and infected or infested fruit that enter the system several times each hour. Soil and debris that contain spores also are removed from bins (7) and are distributed through the water by circulation pumps and moving fruit. At night and during nonworking times, the tanks assume a static phase that may last for 16 hr or more.

The objectives of this study were to compare the effectiveness of 16 combinations of disinfestant-flotation salt-surfactant solutions on germination of *P. expansum*, *M. piriformis*, and *Phialophora malorum* (Kidd and Beaum) McColloch and 10 combinations on decay of pear fruit caused by these fungi. Tests were done in a model dump tank and included a 7-hr dynamic phase and a 16-hr static phase. Tests were done twice, once with and once without a standardized concentration of soil in the tank.

MATERIALS AND METHODS

M. piriformis, *P. expansum*, and *P. malorum* were cultured on potato-dextrose agar (Difco) acidified with 1.5 ml of lactic acid per liter (APDA). Cultures 1–2 wk old were flooded with sterile water, and suspensions were adjusted to 1×10^6 conidia per milliliter with the aid of a hemacytometer.

A model dump tank system was designed with a tank that was 117.7 cm long, 8.5 cm wide, and 6 cm deep. The tank was filled with an aqueous solution of flotation salt alone or combined with chlorine as sodium hypochlorite (NaOCl) or SOPP. Chlorine concentrations during the first 7 hr of each test (dynamic phase with addition of spores and circulation of solution) were 64 ± 9 and 83 ± 20 μg of total available chlorine per milliliter (ppm) for experiments with and without soil in the tank (described below), respectively. Many commercial pear packinghouses use between 50–100 μg of available chlorine per milliliter (Spotts, unpublished). During the dynamic phase, chlorine was added with a peristaltic pump to maintain a constant concentration.

Chlorine concentrations at the end of the 16-hr static phase (no addition of chlorine or spores and no circulation) were about 80 and 20% less than during the dynamic phase for systems with and without soil, respectively. SOPP concentration remained stable throughout the experiment at $0.40 \pm 0.05\%$. Chlorine and SOPP concentrations were determined by titration with sodium

thiosulfate by using potassium iodide as an indicator as described previously (12,14). The analytical method for SOPP was obtained from the manufacturer (Steri-Seal, Wentachee, WA). The pH of each solution was measured with a pH meter (Corning Model 7, Corning Glass Works, Corning, NY). Solutions of the sodium form of carbonate, silicate, or sulfate, and calcium or sodium lignin sulfonate were adjusted to a specific gravity of 1.05 as determined with a hydrometer. Because chlorine reacts with lignin sulfonate, this combination was not included in the experiment. Each flotation salt-chlorine combination was tested with and without 3,000 μg per milliliter of the surfactant Ag 98 (13).

Disinfestant-salt-surfactant combinations were evaluated twice for effects on germination of spores of each of the three fungi listed above. In the first of the two series of tests, no soil was included. In the second series, Hood River sandy loam soil from the top 2.5 cm in a pear orchard was mixed for 90 min in a twinshell blender (Patterson-Kelley Co., E. Stroudsburg, PA), passed through a 5-mm screen, and heated at 80 C for 10 min in a microwave oven with a temperature probe (Spacemaker III, General Electric Co., Appliance Park, Louisville, KY). Soil was added to the test solution at 6.25 mg/ml. Average solids concentration in dump water of several packinghouses was 4.97 mg/ml and ranged from 0.37 to 15.13 mg/ml (Spotts, unpublished). Total volume of solution plus additives in the tank was 4 L.

The solution was circulated by removal from one end of the tank and injection into the opposite end with a peristaltic pump operated at 300 ml/min. Inoculum was added by injecting 1.0 ml of a suspension containing 1×10^6 spores of *M. piriformis*, *P. expansum*, or *P. malorum* per milliliter every 4 min into the stream of solution circulated into the tank. Addition of field bins containing pear fruit to commercial dump tanks in Hood River occurs about every 4 min (Spotts, unpublished). Addition of spores and circulation of solution were stopped at 7 hr (end of dynamic phase). Solution was sampled hourly through the dynamic phase and once at the end of the 16-hr static phase for tests without soil and 1, 3, 5, 7, and 23 hr after the first addition of spores for tests with soil in the tank. Spores were removed from the solution by millipore filtration of 3–10 ml of solution, washed, and transferred to APDA as described previously (14). Two replicate samples were removed for germination tests at each of the above times. Spore germination of 100 spores per replicate was determined after incubation at 10, 15, and 20 C for 24 hr for *M. piriformis*, *P. expansum*, and *P. malorum*, respectively. Spores were considered germinated if germ tube length was equal to or greater than the diameter or length of the spore. In tests with soil in the tank, 40 Anjou pear fruit that were surface-sterilized in 0.525% NaOCl, rinsed with tap water, and puncture-wounded (6 mm diameter and 4 mm deep) at four locations per fruit were immersed in the solution for 4 min at 2 hr after the first spore addition. Fruit then were incubated at 20 C for 5 days and the percentage of wounds with decay determined.

Percent inhibition of germination was plotted over time and treatments compared with LSD at $P = 0.01$ (NWA Statpak 4.1, Northwest Analytical, Inc., Portland, OR). Inhibition of

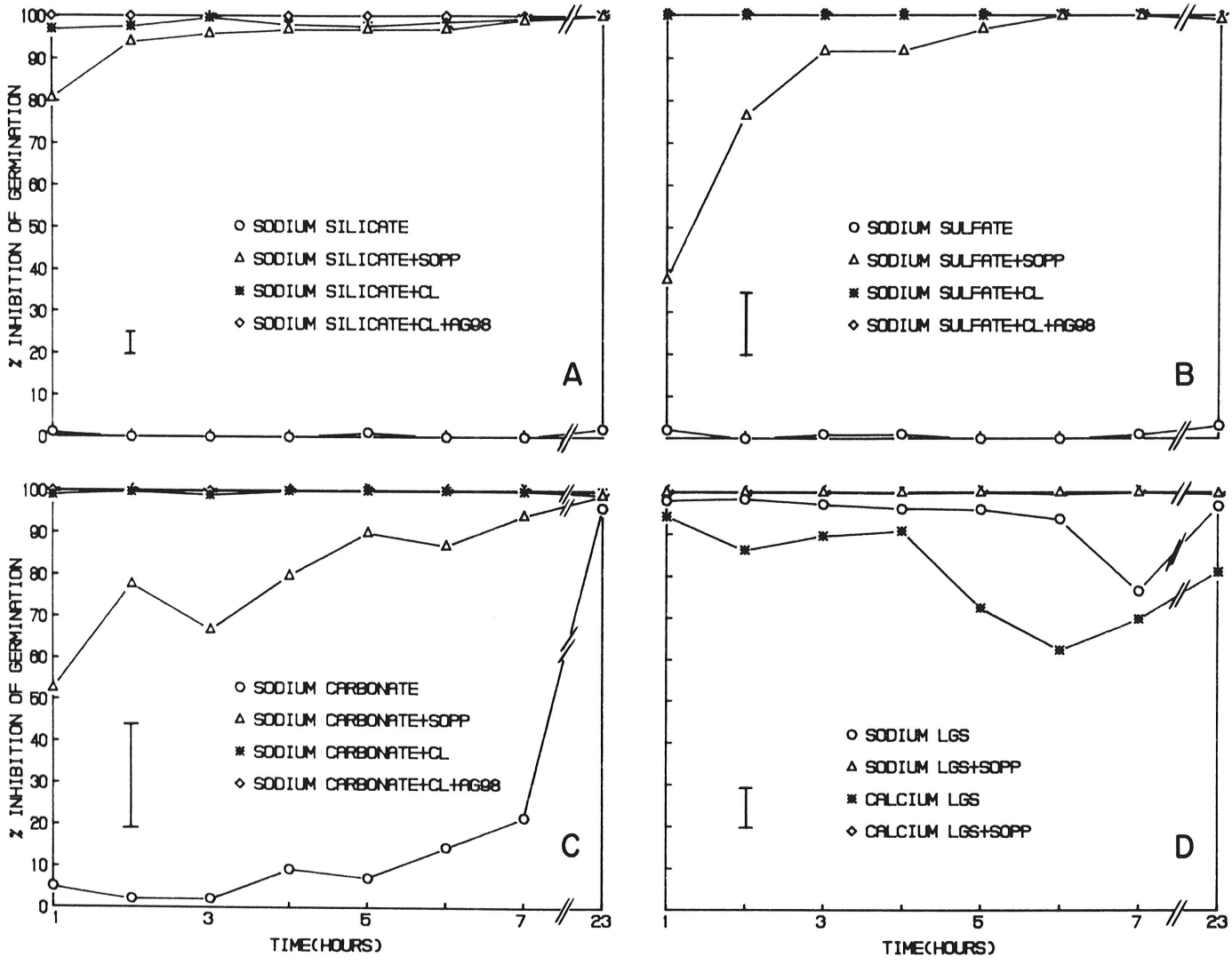

Fig. 1. Inhibition of germination of sporangiospores of *Mucor piriformis* in several disinfestant-flotation salt-surfactant solutions. Flotation salts were: sodium silicate (**A**); sodium sulfate (**B**); sodium carbonate (**C**); and sodium and calcium ligninsulfonate (**D**). Bars indicte LSD at $P = 0.01$.

germination was calculated during the dynamic and static phases for tests with soil. These values, along with incidence of pear decay, were transformed to the arc sine-square root and assessed with analysis of variance and Newman-Keul's multiple-range test by using the NWA Statpak.

RESULTS

Spore germination in systems without soil. Sodium salts of carbonate, silicate, and sulfate without disinfestant had little effect on germination of *M. piriformis* or *P. expansum* during the 7-hr dynamic phase (Figs. 1 and 2). *P. malorum* was sensitive to sodium carbonate and silicate during this phase (Fig. 3). Calcium and sodium lignin sulfonate reduced germination of all three fungi. Calcium lignin sulfonate was more effective than sodium lignin sulfonate for inhibition of germination of *P. expansum* (Fig. 2) and *P. malorum* (Fig. 3), but the reverse was observed for *M. piriformis* (Fig. 1). At the end of the 16-hr static phase, sodium carbonate inhibited germination of *M. piriformis* (Fig. 1) and *P. expansum* (Fig. 2), and all salts except sodium lignin sulfonate were inhibitory to *P. malorum* (Fig. 3). Inhibition caused by sodium lignin sulfonate decreased when compared with shorter exposure times.

Inhibition of germination of *M. piriformis*, *P. expansum*, and *P. malorum* was from 90 to 100% with chlorine in sodium carbonate, silicate, and sulfate, except inhibition of *P. expansum* in chlorine plus sodium carbonate (Fig. 2), which was 80 and 78% after 7 and 23 hr, respectively. These differences were not significant ($P = 0.01$), nor were there any differences in the percentage of germination of spores exposed to salt solutions of chlorine versus chlorine plus Ag 98.

Effectiveness of SOPP on germination varied with flotation salt and sensitivity of spores of each fungus. In calcium and sodium lignin sulfonate, inhibition of germination of *M. piriformis*, *P. expansum*, and *P. malorum* was 98–100%. In sodium sulfate, inhibition of germination of *P. expansum* and *P. malorum* was 96–100% throughout the experiment and increased in a quadratic manner to this level for *M. piriformis* after 5 hr (Fig. 1). In sodium carbonate, germination of *P. malorum* was inhibited 96–100% and increased from 30 and 52% for *P. expansum* (Fig. 2) and *M. piriformis* (Fig. 1), respectively, after 1 hr to more than 90% by 7 hr. In sodium silicate, inhibition of germination initially was 78–81% for *P. malorum* and *M. piriformis*, respectively, and increased in 1–2 hr to more than 90%. Inhibition of germination of *P. expansum* in sodium silicate initially was 37% and increased in a quadratic manner (Fig. 2). SOPP was less inhibitory to germination than chlorine during the first 1–3 hr of the dynamic phase to all fungi in sodium silicate, to *M. piriformis* in sodium sulfate (Fig. 1), and to *M. piriformis* and *P. expansum* (Figs. 1 and 2) in sodium carbonate.

Germination in systems with soil. In most tests with soil, percentage of germination was similar throughout the dynamic phase, and the patterns observed in the tests without soil did not occur. Therefore, the samples removed every 2 hr during the dynamic phase were analyzed as replicates. During the dynamic phase, the sodium salts of silicate and sulfate, without disinfestant, did not inhibit germination of spores of decay fungi, but sodium

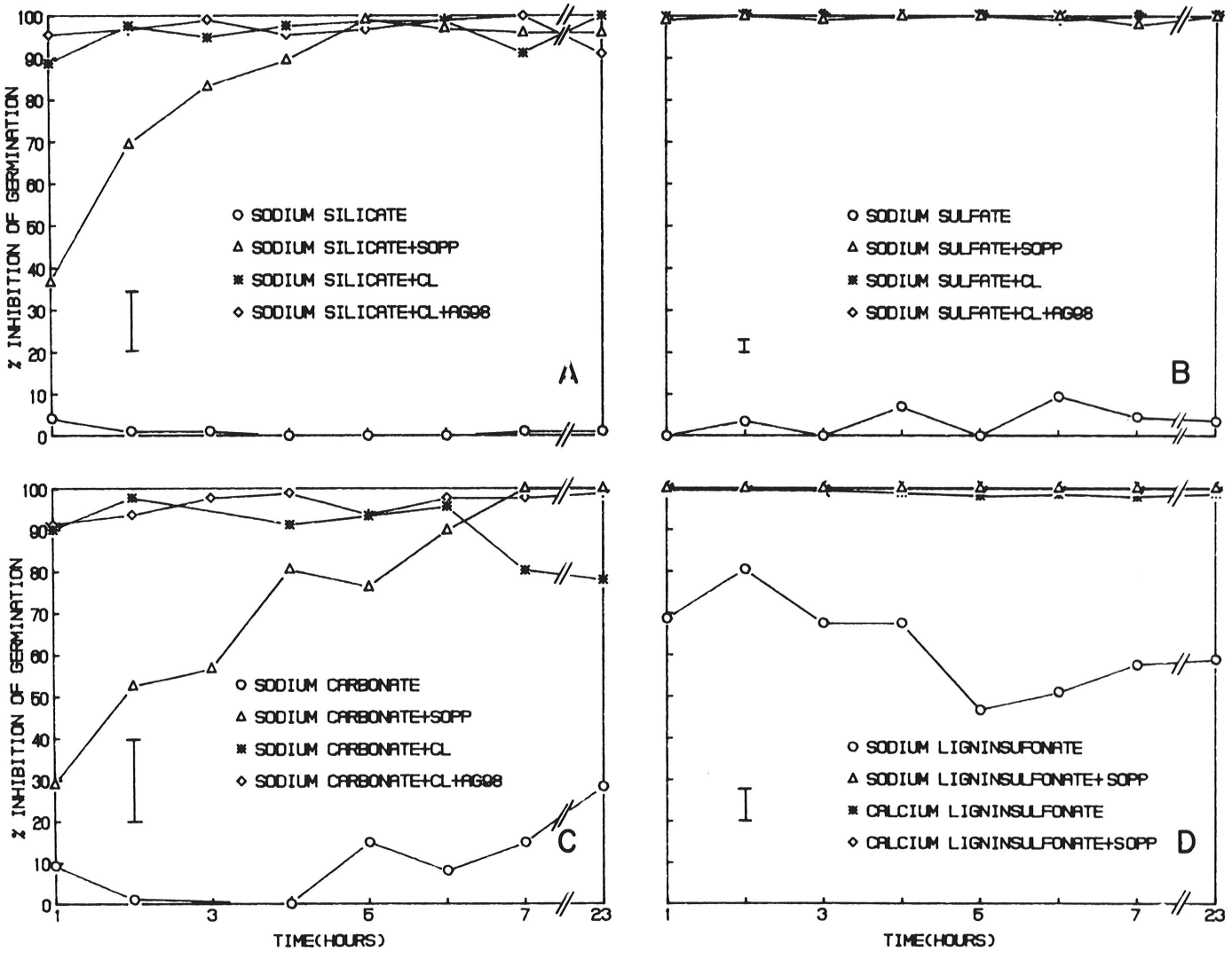

Fig. 2. Inhibition of germination of conidia of *Penicillium expansum* in several disinfestant-flotation salt-surfactant solutions. Flotation salts were: sodium silicate (**A**); sodium sulfate (**B**); sodium carbonate (**C**); and sodium and calcium ligninsulfonate (**D**). Bars indicate LSD at $P = 0.01$.

lignin sulfonate was inhibitory to *M. piriformis*, *P. expansum*, and *P. malorum* (Table 1). At the end of the static phase, inhibition of germination of *P. malorum* increased significantly ($P = 0.05$) in sodium silicate, sulfate, and lignin sulfonate and inhibition of *P. expansum* increased in sodium lignin sulfonate compared with the dynamic phase (Table 1).

Chlorine inhibited the germination of spores of all three fungi more in sodium sulfate than in sodium silicate in the dynamic phase, but the difference was not significant ($P = 0.05$) for *P. malorum*. Addition of Ag 98 to chlorine increased the effectiveness of chlorine for inhibition of *P. malorum* in sodium silicate, but other increases were not significant (Table 1). Inhibition of germination in solutions containing chlorine ranged from 92.4 to 100% in the dynamic phase and from 95.9 to 100% in the static phase.

SOPP effectively inhibited germination in all salt solutions during the dynamic phase, but was most effective in sodium lignin sulfonate and least effective in sodium silicate (Table 1). During the static phase, inhibition of germination in solutions containing SOPP ranged from 94.5 to 100% and was not significantly different ($P = 0.05$) in any comparison except in sodium silicate, where a reduction in effectiveness against *P. expansum* was observed compared with sodium sulfate and lignin sulfonate (Table 1).

Chlorine was significantly ($P = 0.05$) more effective than SOPP in the dynamic phase for inhibition of germination of *M. piriformis* in sodium silicate and sulfate and inhibition of *P. expansum* and *P. malorum* in sodium silicate (Table 1). Inhibition of *P. expansum* in sodium silicate was greater in solutions containing chlorine than SOPP in the static phase.

Decay of pear fruits in systems with soil. In solutions without disinfestant, incidence of decay of wounded pear fruits exceeded 50%, and no differences in incidence of any decay were observed between solutions of sodium silicate, sulfate, and lignin sulfonate (Table 2). Chlorine was significantly ($P = 0.05$) more effective in sodium sulfate than in sodium silicate solution for control of decay caused by *M. piriformis* and *P. expansum* but not *P. malorum* (Table 2). Addition of Ag 98 to chlorine appeared to decrease decay in most comparisons, but only the decrease in decay caused by *P. expansum* is sodium silicate was significant ($P = 0.05$).

SOPP controlled decay caused by *P. malorum* in all salt solutions, but was less effective in sodium silicate than in sodium sulfate or lignin sulfonate for control of decay caused by *M. piriformis* or *P. expansum* (Table 2.) Chlorine and SOPP were equally effective in sodium sulfate, but in sodium silicate, chlorine plus Ag 98 controlled decay caused by *M. piriformis*, and both chlorine and chlorine plus Ag 98 reduced decay caused by *P. expansum* better than SOPP ($P = 0.05$) (Table 2).

Solution pH. The pH of sodium carbonate and silicate solutions with and without soil was 11.2 ± 0.2. The pH of all sodium sulfate solutions was 7.8 ± 0.3, except sodium sulfate plus SOPP, which was 10.75 ± 0.15. The pH of sodium and calcium lignin sulfonate solutions without SOPP was 6.7 ± 0.1 and 6.4 ± 0.1, respectively,

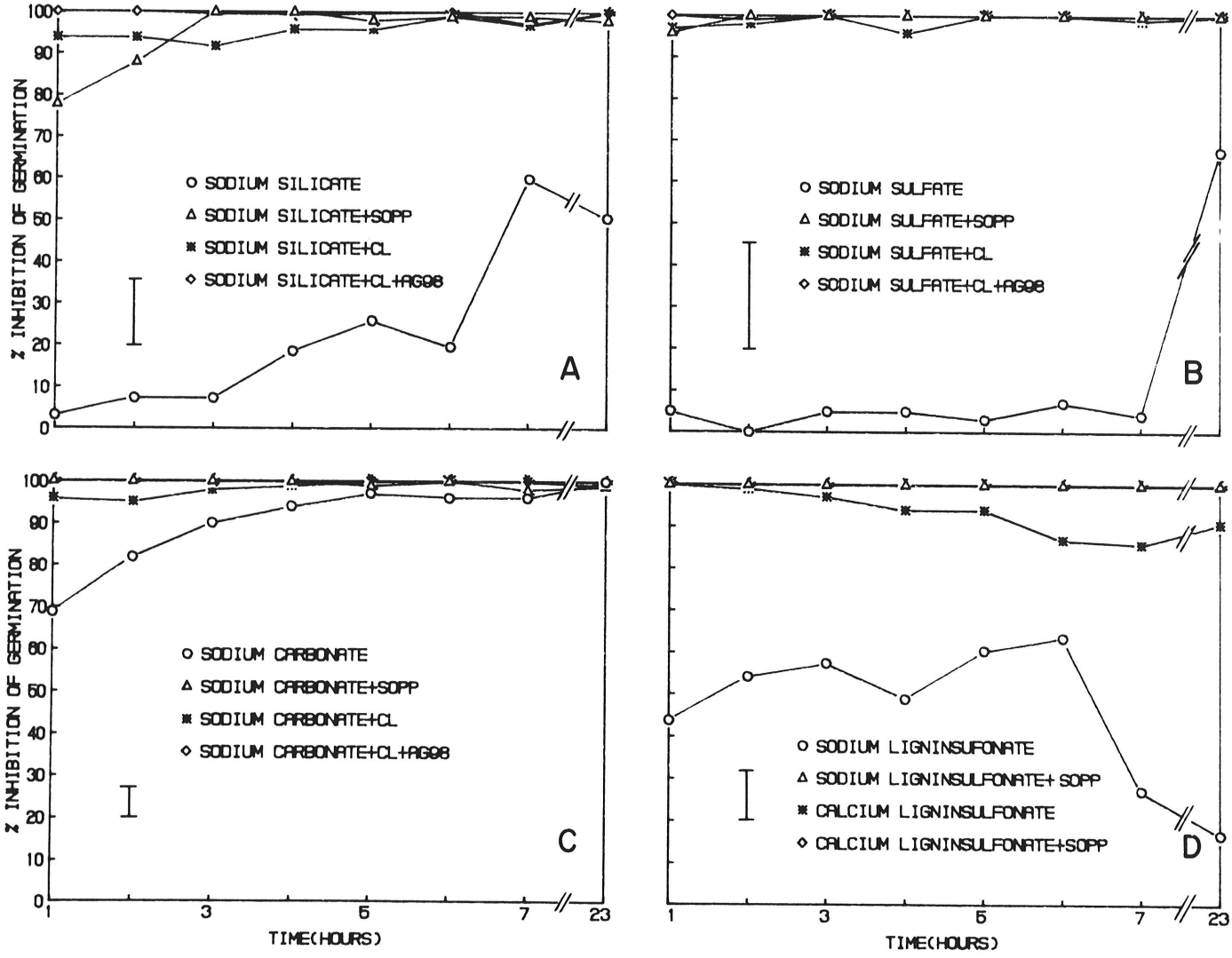

Fig. 3. Inhibition of germination of conidia of *Phialophora malorum* in several disinfestant-flotation salt-surfactant solutions. Flotation salts were: sodium silicate (**A**); sodium sulfate (**B**); sodium carbonate (**C**); and sodium and calcium ligninsulfonate (**D**). Bars indicate LSD at $P = 0.01$.

TABLE 1. Inhibition of germination of spores of *Mucor piriformis, Penicillium expansum*, and *Phialophora malorum* in several flotation salt-disinfestant solutions[a] containing soil during dynamic and static phases

		Inhibition of germination (%)[c]					
		M. piriformis		*P. expansum*		*P. malorum*	
Flotation salt	Disinfestant[b]	Dynamic	Static	Dynamic	Static	Dynamic	Static
Sodium silicate	None	0.0 a	0.0 a	3.3 a	0.3 a*	8.0 a	29.0 a*
	SOPP	83.1 d	98.4 c*	59.2 b	94.5 c	88.9 b	99.5 c*
	Chlorine	92.4 e	98.9 c*	93.8 c	99.5 d*	95.5 c	99.0 c
	Chlorine + Ag 98	96.3 e	95.9 c	97.9 cd	100.0 d	99.6 d	100.0 c
Sodium sulfate	None	2.6 b	0.0 a	8.8 a	5.1 a	8.4 a	75.4 b*
	SOPP	92.4 e	100.0 c	99.3 d	100.0 d	97.8 cd	100.0 c
	Chlorine	100.0 f	100.0 c	98.9 d	100.0 d	98.0 cd	100.0 c
	Chlorine + Ag 98	100.0 f	100.0 c	100.0 d	100.0 d	99.9 d	100.0 c
Sodium lignin sulfonate	None	8.6 c	9.4 b	58.8 b	74.7 b*	85.7 b	98.0 c*
	SOPP	100.0 f	100.0 c	99.7 d	100.0 d	99.7 d	100.0 c

[a]Solutions adjusted to specific gravity of 1.05 and contained 6.25 mg of soil per milliliter in a total volume of 4 L.
[b]Total available chlorine was 64 μg/ml, and SOPP (sodium o-phenylphenate) was 0.40% in dynamic phase. Ag 98 concentration was 3,000 μg/ml.
[c]Numbers followed by the same letter within columns are not significantly different at $P = 0.05$ according to Newman-Keul's multiple-range test. An asterisk indicates a significant difference in germination at $P = 0.05$ between dynamic and static phases. Analyses based on arc sine-square root transformed data.

TABLE 2. Effect of flotation salt-disinfestant-surfactant solutions containing soil on incidence of decay of puncture-wounded Anjou pear fruits

		Percent decay[a] caused by		
Flotation salt	Disinfestant	*M. piriformis*	*P. expansum*	*P. malorum*
Sodium silicate	None	94 e	100 e	55 c
	SOPP	61 d	95 e	7 ab
	Chlorine	46 cd	86 d	18 b
	Chlorine + Ag 98	29 c	38 c	5 ab
Sodium sulfate	None	90 e	96 e	74 c
	SOPP	4 ab	5 ab	3 a
	Chlorine	0 a	8 ab	11 ab
	Chlorine + Ag 98	2 a	3 a	2 a
Sodium lignin sulfonate	None	86 e	98 e	64 c
	SOPP	11 b	13 b	3 a

[a]Fruits immersed 4 min in solution. Each value represents the average of 40 fruits, each wounded four times. Wounds were evaluated for decay after 5 days at 20 C. Numbers followed by the same letter within columns are not significantly different at $P = 0.05$ according to Newman-Keul's multiple-range test. Analyses based on arc sine-square root transformed data.

and was 8.7 ± 0.2 and 7.8 ± 0.1 with SOPP, respectively.

DISCUSSION

On a population basis, the percent of spores of all fungi exposed to any of the solutions for 1 hr or more increased quadratically during the dynamic phase. For example, the percentage of spores in the tank for more than 1 hr was 0, 50, 66, and 75% after 1, 2, 3, and 4 hr, respectively. Thus, a disinfestant solution that kills quickly may be more effective initially than a slower acting one but would be similar in effectiveness after a few hours. In a previous study we reported that 50 μg of chlorine per milliliter reduced germination of *M. piriformis* and *P. expansum* to 1% or less after a 30-sec exposure (14). SOPP did not reduce germination of these fungi after 20 min in sodium carbonate, silicate, or sulfate, but spores did not germinate after 10 min in a SOPP-sodium lignin sulfonate solution (16). Thus, in most salt solutions, chlorine appears to kill spores more quickly than SOPP. During the first 1–3 hr of the dynamic phase, chlorine was more inhibitory to germination than SOPP in sodium silicate, sulfate, or carbonate, and these results are consistent with the rapid disinfestant action of chlorine. However, chlorine was not more inhibitory than SOPP during the last half of the dynamic phase or at the end of the static phase. Also, chlorine or chlorine plus Ag 98 reduced the incidence of decay in fruits treated 2 hr after the first spore addition (early dynamic phase) more than did SOPP. Although no germination was observed in several tests, some decay occurred in fruit floated in these solutions. Several explanations are possible and include greater sensitivity of the infectivity test than the germination test, and germination of spores in fruit wounds as a result of a nutrient status more favorable in the wound than on potato-dextrose agar.

Under commercial conditions, spores are continuously added to a dump tank solution and may remain in suspension for several days. Also, fruit is continuously exposed to spores in the solution, and concentration of viable spores in the solution is an important factor in inoculation and decay. The polynomial relationships between inoculum dose of several fungi and disease incidence for decay of pears were established previously (10). The ability of chlorine in sodium sulfate or SOPP in sodium lignin sulfonate to kill spores rapidly may be advantageous for quickly lowering the inoculum dose in the dump tank solution. However, when choosing between chlorine or SOPP as a disinfestant, additional factors such as cost of chemical, corrosiveness, stability in the presence of organic matter, and ease of disposal must be considered (2).

Sodium salts of carbonate, silicate, and sulfate were not fungitoxic in 40-min exposures (16), but germination of *P. malorum* was reduced by these flotation salts after the 16-hr static phase in this study. Studies with dump tank solutions spanning several hours are important because most packinghouses operate one or more 8-hr shifts during the harvest season. Following a single shift, 16 hr may elapse between addition of spores to the tank. Thus, in previous studies a treatment duration of less than 1 hr was inadequate to give a complete understanding of long-term population dynamics of spores in dump tank solutions (1,11,14,16).

The concentration of soil and debris in dump tank water varies greatly. Immediately after tanks are cleaned, spore and soil levels may be very low when fruit is processed that has been presized previously and has a low incidence of decay. However, when bins directly from the field are immersed, soil, debris, and inoculum may build up to high levels. The conditions in our study with and without soil in the solutions simulated two situations that are often encountered commercially. We found that patterns of inhibition of germination were similar with and without soil and were affected less by soil load than by the flotation salt-disinfestant solution. However, chlorine, which reacts rapidly with organic matter (5), was added continously to our system to maintain a constant level.

Chlorine inhibited germination of conidia of *M. piriformis*, *P. expansum*, and *P. malorum* more than 90% in solutions of sodium carbonate, silicate, and sulfate during dynamic and static phases in systems without soil. At these high levels of inhibition, improvement in effectiveness of chlorine by addition of Ag 98 was not detected. However, in systems with soil, addition of Ag 98 to chlorine increased the inhibition of germination and decreased the decay of *P. expansum* in sodium silicate. This is consistent with a previous report in which efficacy of chlorine was increased following addition of Ag 98 and may be related to the improved penetration of chlorine into wounds, as well as the fungistatic

properties of Ag 98 (13).

The fungicidal action of chlorine is pH dependent (3,8). The differences in pH of solutions may account for the increase in inhibition of germination and decrease in decay of chlorinated solutions of sodium sulfate at pH 7.8 compared with sodium silicate at pH 11.2. A pH range of 8.0 to about 8.5 has been recommended to give the best balance between stability and effectiveness of chlorine (6,17).

Previously, we attempted to study population dynamics of decay spores in various flotation systems in commercial dump tanks but encountered problems with variable and unpredictable conditions that were beyond the control of the researcher (Spotts, *unpublished*). Although caution must be used when applying the results of our model system to commercial situations, we believe this highly controlled system provided useful comparisons of the effects of flotation solutions on decay fungi of pear fruit and may serve as a basis for additional studies conducted under commercial conditions.

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HARTMAN'S PLANTS, INC., Sebring, FL

H. J. HEINZ CO., Bowling Green, OH

HOECHST ROUSSEL AGRI. VET. CO., Somerville, NJ

ICI AMERICAS, INC., Western Res. Ct., Richmond, CA

ICI AMERICAS, INC., Mountain View, CA

ILLINOIS CROP IMPROVEMENT ASSOCIATION, Urbana, IL

ILLINOIS FOUNDATION SEEDS, INC., Champaign, IL

ISTITUTO DI FITOVIROLOGIA, Torino, Italy

JANSSEN PHARMACEUTICA, Piscataway, NJ

LANDIS ASSOCIATES, INC., Valdosta, GA

MERCK & CO., INC., Rahway, NJ

MOBAY CORPORATION, Kansas City, MO

MONSANTO AGRICULTURAL CO., St. Louis, MO

NOR-AM CHEMICAL CO., Wilmington, DE

NORTHFIELD LABORATORIES, Dept. of Agriculture, Northfield, Australia

NORTHRUP KING CO., Woodland, CA

PENNWALT CORPORATION, Ag. Chem. Div., Philadelphia, PA

PETOSEED CO., INC., Woodland, CA

PFIZER, INC.–TEKCHEM, New York, NY

PIONEER HI-BRED INTERNATIONAL, INC., Johnston, IA

RHONE-POULENC AG. CO., Research Triangle Park, NC

ROHM & HAAS CO., Philadelphia, PA

ROTHAMSTED EXP. STATION, Herts, England

SAKATA SEED AMERICA, INC., Salinas, CA

SANDOZ CROP PROTECTION CORPORATION, Des Plaines, IL

O. M. SCOTT & SONS, Marysville, OH

UNIROYAL CHEM. CROP PROT. R & D, Bethany, CT

USDA FOREST SERVICE, Ogden, UT

W-L RESEARCH, INC., Evansville, WI