Effect of Ozonated Water on Postharvest Pathogens of Pear in Laboratory and Packinghouse Tests

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ABSTRACT

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In treatments with aqueous ozone solutions for 5 min, LD_{95} values for spores of *Botrytis cinerea, Mucor piriformis*, and *Penicillium expansum* were calculated to be 0.99, 0.69, and 0.39 μg of ozone per milliliter of water, respectively. Spore inhibition was directly correlated with ozone concentration in 1- to 5-min exposure times; eight regressions of spore inhibition with concentration were significant at $P = 0.01$, and one was significant at $P = 0.05$. However, in pear fruit (*Pyrus communis* 'd'Anjou') wound-inoculated with *P. expansum* and then treated with water containing up to 5.5 μg of ozone per milliliter for 5 min, levels of decay were similar to those of a control treated with water alone. In a commercial packinghouse test, fewer propagules of *Alternaria* spp. were recovered from chlorinated water than from ozonated water, whereas equal numbers of propagules of both *Cladosporium* and *Penicillium* spp. were recovered. Similar levels of decay were recorded in pear fruit floated through ozonated dump tank water and in fruit treated with chlorinated water, after storage at −1 C for 5 mo.

Pome fruit are inoculated with propagules of *Mucor piriformis* E. Fisch. and *Penicillium expansum* Link as soil and debris on picking bins are removed when the bins are immersed in dump tanks containing water or flotation salt solutions (12,22). Dump tank water was identified as one of the main sites for the accumulation of pathogens as early as 1932, and chlorine (hypochlorite ion and hypochlorous acid from sodium hypochlorite) was found to be effective in reducing the germination of conidia of *P. expansum* (2). The effectiveness of chlorinated water in destroying propagules of *Botrytis cinerea* Pers.:Fr., *M. piriformis*, and *P. expansum* has been repeatedly demonstrated (2,4,18,20,23). A direct relationship between pathogen concentrations in water and decay of pear fruit has also been demonstrated (19). As a result, chlorine is recommended as a disinfestant of dump tank and flume water in Australia (3), Canada (11), South Africa (5), and the United States (10).

Although chlorine kills spores in water and on fruit surfaces, it does not penetrate well into wounds; so fruit that become inoculated during harvest are likely to decay despite treatment with chlorinated water (4,23). Other disadvantages of chlorine include reduced effectiveness in high-pH solutions (21) and corrosion of equipment and production of unpleasant odors (4). Furthermore, chlorine reacts with organic compounds to form trihalomethanes and other chlorinated by-products that regulatory agencies consider hazardous (14).

An alternative to chlorine as a biocide is ozone, a bluish, water-soluble gas. It is a stronger oxidant than hypochlorous acid and has been used to disinfest drinking water since 1893 (16). The effects of ozone on bacteria, viruses, and *Giardia* spp. in water have been reviewed (14). At 1 C, 0.06 μg of ozone per milliliter of water inactivates 99% of *Escherichia coli* within 0.33 min (9). The interactions between gaseous ozone and plant pathogens have been extensively studied (6). Ozone atmospheres retard

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the growth of surface molds on walls of cold-storage rooms containing apples but do not prevent the development of lesions at inoculated apple wounds (17). Moreover, fungal spores suspended in water appear quite resistant to ozone (7,24), at least as compared with bacteria and viruses. For example, the germination of conidia of *Sclerotinia fructicola* is unaffected by exposure to 1.4 μg of ozone per milliliter for 1 hr (24). Recently, Ogawa et al (15) reported that spores of *B. cinerea* on the surface of uninjured tomato fruits were inactivated by exposure to 3.8 μg of ozone per milliliter in 10 min. In contrast, lesions developed when spores were placed in wounds, evidence that at least some of the spores escaped the ozone treatment.

The objectives of this study were to determine the toxicity of aqueous ozone solutions to spores of *B. cinerea, M. piriformis*, and *P. expansum*; to determine the effectiveness of ozonated water in the control of disease in pear fruit wound-inoculated with spores of these fungi; and to compare ozonated with chlorinated dump tank water in a commercial packinghouse with respect to the control of fungal populations in flotation tanks and their effects on pear decay.

MATERIALS AND METHODS

Effect of ozone on spore germination. Ozone was produced by passing pure oxygen through a corona discharge ozone generator (Stay-fresh Food, Inc., Ontario, OR). The ozone was mixed with circulating water at 10 C to produce concentrations of 0.1–4.0 μg of ozone per milliliter. The pH of the solutions was 7.5 and was not affected by the ozone concentration. The concentrations were measured with the neutral buffered potassium iodide method (8). Spores of *B. cinerea, M. piriformis*, and *P. expansum* were washed from 7-day-old plates of acidified potato dextrose agar (APDA, acidified with 1.5 ml of 85% lactic acid per liter) and suspended in sterile distilled water. This stock suspension was added to the ozonated water to produce a final concentration of 4×10^3 spores per milliliter at the stated ozone concentration. After exposure times of 0 (control), 1, 3, and 5 min, a 10-ml sample was removed, the liquid phase was removed with a 0.45-μm millipore filter, and the spores retained on the filter were washed with 5 ml of sterile distilled water. Next, the spores were blotted onto the surface of an APDA plate. Plates with spores of *B. cinerea* and *M. piriformis* were incubated at 10 C, and those with *P. expansum* at 15 C, for 24 hr. Germination of 100 spores was examined microscopically at 400×. A spore was considered germinated if the length of the germ tube was at least half the diameter of the spore. The experiment was done three times for each fungus. The percentage of inhibition of germination caused by the ozone was calculated from the germination of spores in water. Data were analyzed by regression of $\log_{10}$ ozone concentration, with the ozone concentrations averaged over the exposure intervals.

Effect of ozone on control of decay of wound-inoculated d'Anjou pear fruit. D'Anjou pear fruit were surface-sterilized with 95% ethanol and then punctured to produce wounds 6 mm in diameter and 4 mm deep at four locations per fruit. Spores were washed from 7-day-old APDA cultures of *P. expansum* and suspended at a concentration adjusted to 2×10^3 spores per milliliter, and 50 μl of the suspension was placed in each wound. Two methods were used to treat inoculated fruit with ozonated water. In the first method, fruit were placed on a packing line and moved under four flat fan-pattern spray nozzles, each delivering 795 ml of ozonated water per minute (ozone concentration 3.1 μg/ml), with an exposure time of about 5 sec. Control fruits were treated with non-ozonated water. Control and ozone treatments each were applied to 22 single-fruit replicates. In the second method, inoculated fruit were immersed in ozonated water in a 3-L tank connected to the main tank of the ozone generator. Ozonated water was circulated through the tank to maintain an ozone concentration of 4.2 μg/ml in one test and 5.5 μg/ml in a second test. Four single-fruit replicates were immersed in the ozonated water for 0 (control), 0.5, 1, 3, and 5 min in each test. Fruit treated with either method were packed in cardboard boxes lined with perforated polyethylene bags. The percentage of wounds infected and lesion diameter were determined after 7 days at 20 C.

Effects of ozone and chlorine on concentration of fungal propagules in dump water and on pear decay in a commercial packinghouse. D'Anjou pear fruit from three grower lots were divided into two subgroups per lot. One subgroup from each lot was floated through a dump tank containing 18,900 L of an aqueous solution of sodium silicate (used as a flotation agent) in which sodium hypochlorite was dissolved. The sodium hypochlorite concentration was adjusted so that the total available chlorine was 54 ± 16 μg/ml, as determined by sodium thiosulfate titration (1). The specific gravity of the sodium silicate solution was 1.026, the pH was 11.6, and the temperature was 5 C. Individual fruit were in the tank about 5 min. These subgroups were processed on 24 and 25 October 1990. On 31 October and 1 November 1990, the remaining subgroups were processed in the same sequential order, but sodium hypochlorite was replaced with ozone produced by a commercial generator (TechOzone, Inc., Corona del Mar, CA) at 0.31 ± 0.09 μg/ml. The pH of the solution was 11.2. Ozone concentrations were determined by an ozone test kit based on the indigo method (Hach Co., Loveland, CO). The water was sampled three times each day at 2-hr intervals from three locations in the tank. The samples were diluted 1:99 with sterile distilled water, and 0.2-ml aliquots were spread over the surface of five APDA petri plates per sample. Fungi that developed in the plates were identified and enumerated after 5 days of incubation at 22 C. At each of the sampling times, about 40 kg of fruit was removed from the cull bin, enough to fill two polyethylene-lined cardboard boxes. Most of these cull fruit contained natural punctures and were culled after the dump tank but prior to a fungicide line spray. One box of each pair was stored at −1 C and examined monthly for 5 mo. The second box was held at 20 C and examined at 7 and 14 days. At each examination, decay incidence was assessed and categorized on the basis of visual symptoms.

RESULTS

Effect of ozone on spore germination. The relationship between the $\log_{10}$ ozone concentration in water and the $\log_{10}$ percent inhibition of spore germination

Table 1. Regression equations for concentration of ozone in distilled water and inhibition of spore germination of three fungi pathogenic on pear fruit

Fungus	Exposure time (min)	Regression equation[a]	Correlation coefficient[b]
Botrytis cinerea	1	$\log Y = 1.843 + 0.344 \log X$	0.869
	3	$\log Y = 1.955 + 0.094 \log X$	0.888
	5	$\log Y = 1.978 + 0.061 \log X$	0.850
Mucor piriformis	1	$\log Y = 1.815 + 0.815 \log X$	0.924
	3	$\log Y = 1.972 + 0.231 \log X$	0.895
	5	$\log Y = 1.987 + 0.058 \log X$	0.867
Penicillium expansum	1	$\log Y = 1.822 + 0.718 \log X$	0.813
	3	$\log Y = 1.971 + 0.233 \log X$	0.893
	5	$\log Y = 2.000 + 0.051 \log X$	0.785

[a] Y = percent inhibition of germination; X = micrograms of ozone per milliliter of distilled water.

[b] Significant at $P = 0.01$ (except for *P. expansum* in the 5-min exposure, significant at $P = 0.05$).

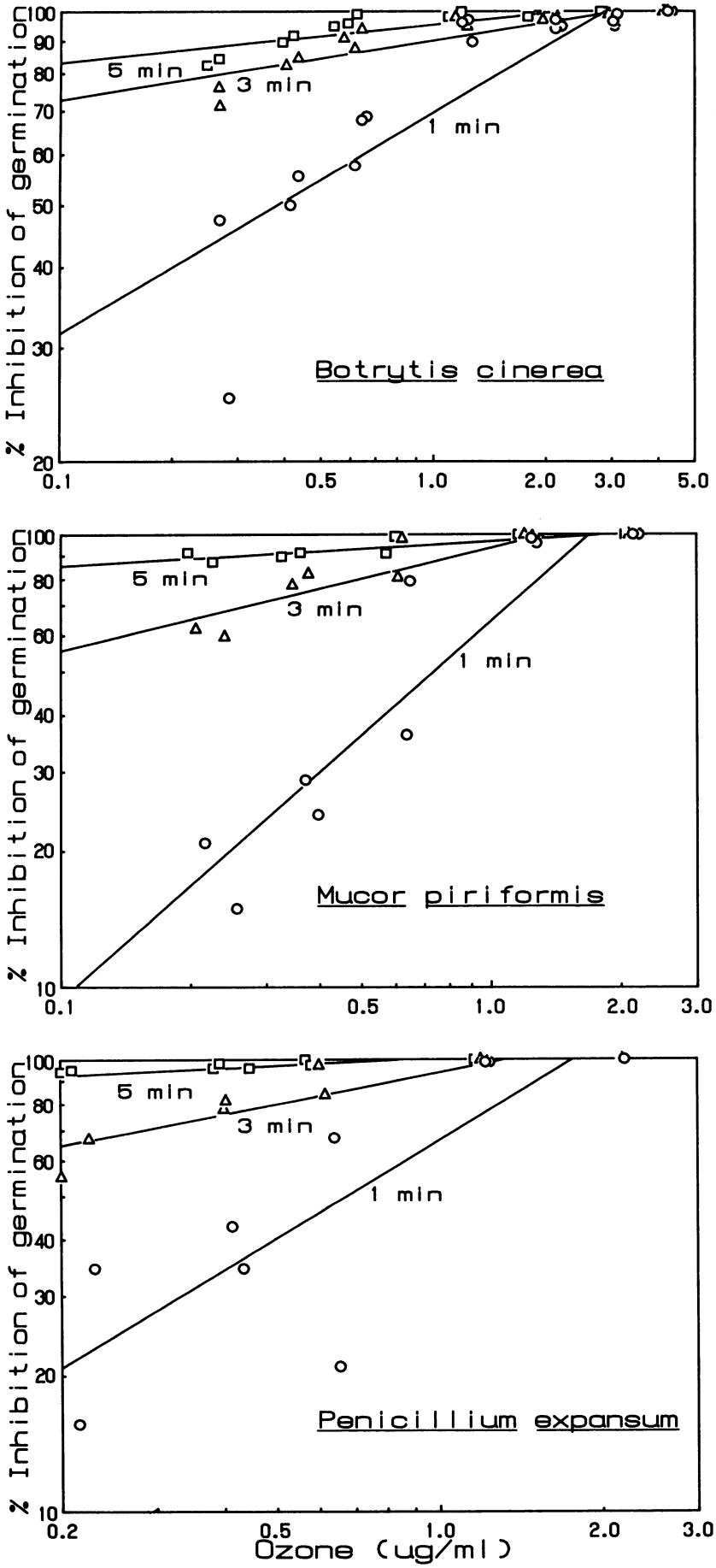

Fig. 1. Inhibition of spore germination of three decay fungi after treatment with 1-, 3-, and 5-min exposures to ozone in various concentrations in water. Regression equations for all lines are given in Table 1.

was linear for all three fungi (Table 1 and Fig. 1). As determined by these regressions, the LD_{95} for all fungi was an ozone concentration of less than 1 μg/ml in a 5-min exposure or between about 1.5 and 2.5 μg/ml in a 1-min exposure. Similarly, if spores were exposed to ozone at 0.1 and 0.5 μg/ml for 1 min, generally less than 50% of germination was inhibited, whereas with a 5-min exposure, 83–96% was inhibited. *M. piriformis* and *P. expansum* were similarly sensitive to ozone, but *B. cinerea* was more sensitive than these fungi to ozone concentrations less than about 1.0 μg/ml for up to 3 min. At higher ozone concentrations or longer exposure times (5 min), *B. cinerea* was less sensitive than the other fungi (Fig. 1).

Effect of ozone on control of decay of wound-inoculated d'Anjou pear fruit. In wound-inoculated fruit treated with ozonated water applied as a line spray, 100% of the wounds became diseased, as in the control fruit. However, lesion diameters in the ozone-treated fruit were significantly smaller ($P = 0.01$) than those in the control fruit (16.3 vs. 17.8 mm, respectively). In fruit that were immersed in water with an ozone concentration of 4.2 or 5.5 μg/ml, 81–100% of the wounds became infected regardless of exposure time from 0.5 to 5.0 min. Lesion diameters in fruit treated with ozone generally appeared smaller than those in control fruit, but the differences were not significant. Ozone did not cause any phytotoxicity in treated fruit.

Effects of ozone and chlorine on concentration of fungal propagules in dump water and on pear decay in a commercial packinghouse. The three most common fungi isolated from the dump tank water were *Alternaria* spp., *Cladosporium* spp., and *Penicillium* spp. No consistent differences were observed in fungal populations in the three sample locations in the tank, and the values were averaged at each sampling time. The number of viable propagules of *Alternaria* spp. in chlorinated water in the packinghouse dump tanks was about one-tenth the number isolated from ozonated water (50 vs. 488 cfu/ml). In contrast, the numbers of propagules of *Cladosporium* spp. (1,317 and 2,308 cfu/ml) and *Penicillium* spp. (305 and 439 cfu/ml) in the two treatments were not significantly different at $P = 0.05$.

Five different decays were identified among the fruit from the chlorine or ozone water treatments (Table 2). After 14 days at 20 C, the decays caused by *M. piriformis* and *Penicillium* spp. were the most common. Total decay and decay caused by *Penicillium* spp. were less in the chlorine treatment than in the ozone treatment. In 5 mo of storage at −1 C, the most common diseases in fruit were caused by *Alternaria* spp. and *Penicillium* spp. No differences in disease incidence were observed between fruit

Table 2. Decay of d'Anjou pear fruit after ozonated and chlorinated water treatments in a commercial packinghouse

Pathogen	Percentage of fruit decayed[a] at storage temperature of:			
	20 C[b]		−1 C[c]	
	Chlorine[d]	Ozone[e]	Chlorine	Ozone
Alternaria spp.	0.3	0.7	7.1	6.3
Botrytis spp.	0.4	0.3	3.1	3.0
Mucor piriformis[f]	10.0	9.5	3.9	2.0
Penicillium spp.	7.8*	20.8	5.1	11.0
Pezicula malicorticis	0.0	0.0	1.6	0.8
Total decay	18.5**	31.3	20.8	23.3

[a] Significant differences between chlorine and ozone treatments according to the unpaired t test at $P = 0.05$ and 0.01 are indicated by * and **, respectively. Each value is based on six boxes of d'Anjou pear fruit, each box containing about 20 kg.
[b] Evaluated after 7 and 17 days.
[c] Evaluated monthly for 5 mo.
[d] Sodium hypochlorite in sodium silicate flotation solution, with total available chlorine adjusted to 54 ± 16 μg/ml, pH 11.6, at 5 C.
[e] Ozone in sodium silicate flotation solution, with ozone concentration adjusted to 0.31 ± 0.09 μg/ml, pH 11.2, at 5 C.
[f] Includes fruit infected with *Rhizopus stolonifer* at 20 C.

from the chlorine treatment and fruit from the ozone treatment at this storage temperature.

DISCUSSION

The germination of spores of three common decay fungi was inhibited by treatment with ozonated water. However, from our results and those of previous studies (7,15,24), the concentration of ozone required to kill fungal spores is considerably higher than the levels used to inactivate bacteria, viruses, and *Giardia* (9,14). For example, we calculated that the LD_{95} for conidia of *B. cinerea* with a 5-min exposure was 0.99 μg/ml. In contrast, the LD_{99} for *E. coli* with a 0.33-min exposure is only 0.06 μg/ml (9).

Ozonated water did not control decay in wound-inoculated pear fruit. Ozone in air does not reduce infection in inoculated wounds in apple (17). Also, ozonated water fails to inactivate spores of *B. cinerea* placed in surface injuries of tomato fruit (15). Similarly, a lack of decay control has been observed in inoculated fruit treated with chlorine (4,23). Apparently, strong oxidants such as ozone and chlorine react with plant tissue and extracellular biochemicals at wound sites and often fail to inactivate microbes attached to or embedded in plant tissue. Both chlorine and ozone react rapidly with organic matter (14). The activity of both chlorine (14) and ozone (13) is affected by the pH of the solution. Chlorine is more effective for decay control in a sodium sulfate solution at pH 7.8 than in sodium silicate at pH 11.2 (21). Similar information relating the pH of ozone solutions to decay control is not available.

At the commercial storage temperature of −1 C, similar decay levels developed in fruit floated in chlorinated dump solutions and fruit in ozonated dump solutions in a packinghouse. We were not permitted to include a water control in the commercial packinghouse trials and had to compare ozone with chlorine as the industry standard. We have shown in this study that ozone inhibits the germination of spores of *B. cinerea, M. piriformis*, and *P. expansum*. If a similar effect occurred in dump tank water, we would expect an indirect mode of decay control to occur as the concentration of viable fungal propagules was lowered. A positive relationship has been established between decay of d'Anjou pear fruit and the concentration of viable spores of *B. cinerea, M. piriformis*, and *P. expansum* in water (19).

For spores of *B. cinerea, M. piriformis*, and *P. expansum* the LD_{99-100} of chlorine at pH 9 in a 5-min exposure was about 50 μg/ml (23). To achieve this level of inhibition of these fungi with ozone solutions in 5 min, concentrations of 1.9, 1.4, and 0.8 μg/ml, respectively, were required. These are considerably higher concentrations than the 0.31 μg/ml used in our packinghouse tests and may explain the higher recovery of fungal propagules from dump tank water treated with ozone rather than chlorine. After one test, the ozone concentration was increased, and slight off-gassing occurred. Additional research is needed to determine the maximum amount of ozone that can be dissolved safely in packinghouse dump tank water and to relate that concentration to efficacy against the accumulation of spores of fungal pathogens in the water.

LITERATURE CITED

1. Anonymous. 1975. Standard Methods for Examination of Water and Waste Water. 14th ed., pp. 316-318. American Public Health Association, New York.
2. Baker, K. F., and Heald, F. D. 1932. Some problems concerning blue mold in relation to cleaning and packing of apples. Phytopathology 22:879-898.
3. Beattie, G. J., McGlasson, W. B., and Wade, N. L. 1989. Postharvest Disease of Horticultural Produce. Vol. 1. Commonwealth Scientific and Industrial Research Organisation, Victoria, Australia.
4. Bertrand, P., and Saulie-Carter, J. 1979. Postharvest decay control of apples and pears after immersion dumping. Oreg. State Univ. Agric. Exp. Stn. Spec. Rep. 545.
5. Eksteen, G. J., and Combrink, J. C. 1987. Manual for the Identification of Post-harvest Disorders of Pome and Stone Fruits. Stellenbosch, South Africa.
6. Heagle, A. S. 1973. Interactions between air pollutants and plant parasites. Annu. Rev. Phytopathol. 11:365-388.
7. Hibben, C. R., and Stotzky, G. 1969. Effects of ozone on the germination of fungus spores. Can. J. Microbiol. 15:1187-1196.
8. Intersociety Committee. 1969. Methods for ambient air analysis: Tentative method for the manual analysis of oxidizing substances in the atmosphere. 44101-01-70T. Health Lab. Sci. 7:152-156.
9. Katzenelson, E. B., Kletter, B., and Shuval, H. I. 1974. Inactivation kinetics of viruses and bacteria in water by use of ozone. J. Am. Water Works Assoc. 66:725-729.
10. Kupferman, E., and Waelti, H. 1991. Management of postharvest diseases and disorders. Tree Fruit Postharvest J. 2:1-16.
11. Meheriuk, M., and McPhee, W. J. 1984. Postharvest handling of pome fruits, soft fruits, and grapes. Agric. Can. Publ. 1768E.
12. 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.
13. Nakareseisoon, S., and Gordon, G. 1989. The very slow decomposition of aqueous ozone in highly basic solutions. Ozone Sci. Eng. 11:49-58.
14. National Academy of Sciences. 1987. Drinking Water and Health: Disinfectants and Disinfectant By-Products. National Academy Press, Washington, DC.
15. Ogawa, J. M., Feliciano, A. J., and Manji, B. T. 1990. Evaluation of ozone as a disinfectant in postharvest dump tank treatments for tomato. (Abstr.) Phytopathology 80:1020.
16. Rice, R. G., Robson, C. M., Miller, G. W., and Hill, A. G. 1981. Uses of ozone in drinking water treatment. J. Am. Water Works Assoc. 73:3-16.
17. Schomer, H. A., and McColloch, L. P. 1948. Exposure of apple to ozone atmospheres. U.S. Dep. Agric. Circ. 765.
18. Sholberg, P. L., and Owen, G. R. 1991. Populations of propagules of *Mucor* spp. during immersion dumping of Anjou pears. Can. Plant Dis. Surv. 71:33-35.
19. Spotts, R. A. 1986. Relationships between inoculum concentrations of three decay fungi and pear fruit decay. Plant Dis. 70:386-389.
20. 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.
21. Spotts, R. A., and Cervantes, L. A. 1989. Evaluation of disinfestant-flotation salt-surfactant combinations on decay fungi of pear in a model dump tank. Phytopathology 79:121-126.
22. Spotts, R. A., Holmes, R. J., and Washington, W. S. 1988. Sources of spores and inoculum concentrations related to postharvest decay of apple and pear. Australas. Plant Pathol. 17:48-52.
23. Spotts, R. A., and Peters, B. B. 1980. Chlorine and chlorine dioxide for control of d'Anjou pear decay. Plant Dis. 64:1095-1097.
24. Watson, R. D. 1942. Ozone as a fungicide. Ph.D. thesis, Cornell University, Ithaca, NY.