BIN AND STORAGE ROOM SANITATION

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THEORETICAL IMPORTANCE OF SANITATION

Sanitation is often described using different terminology. In this article it is defined as follows: sterilization referees to the destruction and removal of all living organisms; disinfection refers to the destruction of all vegetative cells (not spores); and sanitation refers to the reduction of microorganisms on surfaces to levels that do not pose a risk of decay. The principal of sanitation as it relates to plant disease epidemiology requires that the initial inoculum be reduced to a sufficiently low level that the normal development of disease would not reach a high enough level to cause appreciable yield loss. Postharvest disease progress may follow a monomolecular or simple interest curve, a linear curve, an exponential curve, or an asymmetrical sigmoidal curve. Visualizing disease progress in this way helps in quantifying it and designing effective control strategies. If the disease progress is like a simple interest curve, the primary strategy of control would be to reduce or eliminate the initial inoculum (Berger 1984). This will delay the appearance of disease, slow its progress, and lower the total amount of decay. The best strategy to control postharvest diseases is to combine measures that reduce the growth rate (e.g., cold storage, temperature, and resistance) with those strategies that sharply reduce the inoculum (sanitation).

SOURCES OF FUNGAL INOCULUM

Botrytis cinerea, Mucor piriformis, and *Penicillium* spp. cause serious postharvest losses to apples and pears in the Pacific Northwest (PNW). Gray mold caused by *B. cinerea* is common in fruit stored for 1 to 2 months although sporulation on the fruit is minimal and number of spores in the dump water is low throughout the packing season (Spotts and Cervantes 1986). Furthermore the majority of packinghouse air and orchard soil samples that were collected in Oregon yielded no *B. cinerea* inoculum (Lennox et al. 2003) (Table 1). It appears that most of the inoculum for gray mold comes either from fruit infected in the field or in litter contaminating fruit at harvest. On the other hand *Penicillium* spp. and *M. piriformis* spore levels increased as the packing season progressed. Before December, most fungal propagules probably originated from orchard debris and soil carried on bins (Table 1). Densities of *Penicillium* spp. were relatively high in packinghouse air near the packingline and in cold storage as well as in orchard soil and litter (Lennox et al. 2003). From December to the end of the packing season the fungal spores probably come from decaying fruit in bins that are passed through the dump water during the packing operation. There was an extremely high correlation between fruit contamination and blue mold decay. Infested soil and debris are the major sources of inoculum for the infection of fruit by *M. piriformis*. The fruit are inoculated as the soil and debris on picking bins are removed when the bins are immersed in dump tanks or stacked in storage. Bins are also contaminated with spores of *Penicillium* spp. in a similar manner. On bins in some orchards, an average of 3.3×10^6

spores of *P. expansum* were recovered per bin (Sanderson 2000). At these levels, if only 50% of the spores were washed from the bin, after 30 bins were treated per 100 gal, there would be 130 spores/mL in the drench water. It has long been recognized that spores of *Penicillium* accumulate in water systems. Recently the water from the various components in the packing line including the bin dump, flumes and bin fillers were monitored for mold primarily *Penicillium* spp. (Figure 1). The bin dump and the bin fillers were heavily contaminated with mold spores. It is important to reduce the contamination level on fruit because as shown in Table 1, decay is directly correlated to *Penicillium* spp. levels on fruit. Furthermore, contaminated bins appear to be reservoirs of thiabendazole (TBZ) resistant isolates of *Penicillium* spp. and could be the source of TBZ resistant isolates in the field when bins are returned (P. Sanderson, unpublished data).

Table 1. Source of inoculum for *B. cinerea* and *Penicillium* spp. on d'Anjou pear

Inoculum source	*B. cinerea* (CFU/mL)	Correlation to Decay	*Penicillium* spp. (CFU/mL)	Correlation to Decay
Orchard air	0 to 3.1	0.245	0 to 3.1	0.201
Orchard soil	0	---	38 to 431	0.255
Orchard litter	0 to 1,167	0.443	131 to 1,128	0.568
Fruit	0 to 1.4	0.514	0 to 2.7	0.904
Packinghouse air	0	---	0.1 to 11.8	---
Cold storage air	0	---	0 to 3.9	---
Decay in storage	1.2 to 9.1%	1.0	0.2 to 8.4%	1.0

From Lennox et al. (2003).

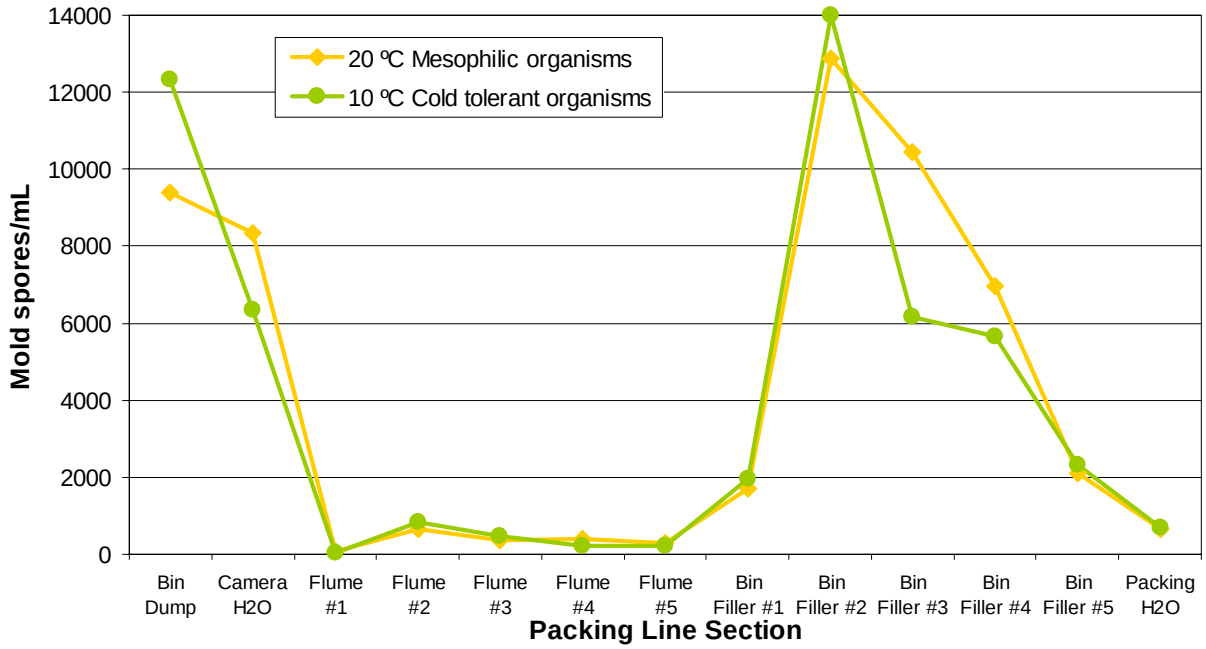

Figure 1. Number of cold tolerant and mesophilic mold spores found in water from each section of the packingline at a commercial packinghouse.

FACTORS AFFECTING BIN SANITATION

Physical, chemical, and biological factors influence the efficiency of sanitizers (Table 2). Prior to sanitization the bin or wall surface must be clean to ensure effective removal of mold propagules. If colonized pieces of fruit or leaves are left behind, decay fungi may regrow from within these tissues and continue to contaminate the bin or wall. Exposure to the sanitizer is important and usually the longer the surface is in contact with the sanitizer the better will be the control. Temperature of the sanitizer should be as high as possible but should not be allowed to go over 131 °F in order to avoid excessive corrosiveness. The sanitizer should only be used at the recommended concentration because too little sanitizer will not be effective and too much sanitizer could be corrosive. The effect of pH is very important especially in the use of chlorine sanitizers which are almost ineffective at pH levels above 7.5.

Table 2. Factors that affect a sanitizer's ability to reduce microorganisms

Factor	Characteristic	Comment
Physical	Surface features	Clean thoroughly
	Exposure time	As long as possible
	Temperature of solution	As high as possible but not over 131°F
	Concentration of sanitizer	Use at label rate
	Soil	Remove as much as possible
Chemical	pH	Buffer water
	Water properties	Use water conditioners
	Inactivators	Rinse with water if present
Biological	Microbial load	Sanitizers vary in effectiveness against fungi, bacteria, yeast, and viruses.

From Schmidt (1997), University of Florida, IFAS Extension Online Publication FS14.

TRADITIONAL SANITIZERS

The chemical sanitizers fall into seven classes according to Schmidt (1997) (Table 3). For completeness all the classes are listed but only the most common ones will be discussed in this article. Chlorine compounds are relatively cheap and leave no residue. Included in this group are liquid chlorine, sodium and calcium hypochlorite and inorganic and organic chloramines. In general hypochlorites are effective at 50 ppm at 75 °F but require doubling of exposure time for each 18 °F drop in temperature. Chlorine dioxide (ClO_2) has 2.5 times the oxidizing power of chlorine and is used at concentrations ranging from 1 to 10 ppm. Roberts and Reymond (1994) evaluated it for packinghouse use and concluded that it had desirable properties for postharvest decay management. The quaternary ammonium compounds (QACs) are a highly diverse class of products that all have positively charged cations in common with each other. There activity can be improved by the use of ethylenediaminetetraacetic acid (EDTA) as a chelator similar to chlorine with the addition of surfactants. Recently products containing peroxyacetic acid have been promoted as potential replacements for chlorine. Important properties of these sanitizers are their low corrosiveness, tolerance to hard water, and favorable biodegradability.

Table 3. Some chemical sanitizers

Sanitizer	Product examples	Use notes	Disadvantages
Chlorine compounds	Sodium and calcium hypochlorite	Effective for 1 min at 50 ppm at 75°F	May raise water pH by forming sodium or calcium salts. Sodium levels above 100 ppm will damage sensitive apples
Chlorine dioxide	Stabilized ClO_2	Effective from 1 to 10 ppm. Can be used as a foam	More expensive than hypochlorites and must be generated on site
Iodine	Iodophors	12.5 to 25 ppm for 1 min. Used in hand sanitizing solutions.	May stain porous surfaces and some plastics
Quaternary Ammonium Compounds (QACs)	Environmental fogs and room deodorizers	Good against molds, ineffective with some bacteria	Inactivated in hard water
Acid-Anionic	Include an inorganic acid plus a surfactant	For acid rinse and sanitation	Low activity on molds and yeasts
Fatty acid or carboxylic acid	Phosphoric acids, Organic acids	For rinse and sanitation with low foaming potential	Low activity against yeast and molds
Peroxides	Hydrogen peroxide	Limited application in the food industry	High concentrations of 5% and above can be an eye and skin irritant
Peroxides	Peroxyacetic acid	Possible chlorine replacement	Affected by pH. Any pH increase above 7-8 reduces activity.

From Schmidt (1997), University of Florida, IFAS Extension Online Publication FS14.

Spotts and Cervantes (1994) tested several chemical and physical methods for sanitizing plastic and wood bins by testing the treatments on 2 x 3 cm pieces of plastic and wood inoculated with spores of *P. expansum* in pear juice. The most effective treatment was steam for 5 or 10 seconds on both plastic and wood surfaces (Table 4). Chlorine compounds, sodium orthophenylphenate (SOPP), and QACs were all effective sanitizers although sodium hypochlorite was more effective on plastic than on wood.

Table 4. Effect of sanitizers on spores of *Penicillium* spp. on plastic or wood bin material

Sanitizer	Rate/L	Exposure Time (min.)	Spore Kill (%)	
			Plastic	Wood
ClO_2	3.5 mg	10	---	68.7
ClO_2	3.5 mg	20	---	69.0
Chlorofoam	200 mg	3	---	65.0
Na hypochlorite	100 mg	10	97.0	70.0
Na hypochlorite	200 mg	3	90.7	69.7
Na hypochlorite	200 mg	10	98.3	78.0
SOPP	0.25%	3	80.0	94.0
SOPP	0.50 %	3	92.7	94.0
QAC 1	2.0 mL	10	96.3	87.0
QAC 2	3.9 mL	3	96.7	97.0
QAC 3	7.8 mL	3	96.7	---
Steam	---	5 sec	99.7	99.0
Steam	---	10 sec	100.0	99.0

From Spotts and Cervantes (1994), Acta Horticulturae 367:419-425.

EXPERIMENTAL SANITIZERS

In the last 10 years research has been conducted on a number of sanitizers that could supplement the present sanitizers or replace some of them (Table 5).

Table 5. Some additional sanitizers that have shown promise in experiments

Sanitizer	Possible rate	Application method	Comments
Ozone water[1]	0.31 µg/mL	Corona discharge ozone generator mixed with circulation water	Higher concentrations of ozone are need for fungal spores
Ozone in air[2]	0.3 ppm to 1.0 ppm	Corona discharge ozone generator automatically controlled	Delays sporulation and reduces ethylene concentration in closed containers
Carbon dioxide[3]	13% in air	Fumigation for 21 days at 59°F	Kills codling moth larvae and reduces *Penicillium* spp. spores
Storox	1:30 dilution to 1:50 dilution	Cold fogging	Very effective on molds on walls and bins
Acetic acid	4.5 mL/m^3	Fumigation by vaporizing 4.5 mL/m^3 acetic acid	Very effective but research needed on corrosiveness, rates, and exposure times

[1]See Spotts and Cervantes (1992), Plant Dis. 76:256-259.
[2]See Palou et al. (2001), Plant Dis. 85:632-638.
[3]See Cossentine et al. (2004), HortScience 39:429-432.

Ozone

There has been widespread interest in the use of ozone since 1997 when the triatomic form of oxygen (O_3) was recognized as being generally regarded as safe (GRAS). The current threshold limit value is 0.3 ppm. Ozone can be used as a relatively brief prestorage or storage treatment in air or water, or as a continuous or intermittent atmosphere throughout the storage period (Palou et al. 2001). Ozone does not kill all spores and only delays germination. It does not penetrate into cartons with small vents but penetrated into large plastic bins. It is valuable for reducing inoculum and could also be used to reduce ethylene concentration in an enclosed space which would help prolong shelf life.

Carbon Dioxide

High levels of carbon dioxide (CO_2) can be used as an aid in sanitation of empty bins to reduce *Penicillium* spp. contamination and codling moth larvae (Cossentine et al. 2004). When diapausing codling moth larvae and spores of *P. expansum* and *B. cinerea* were placed in wooden fruit bins and fumigated for 21 days at 13% CO_2, 75% of the codling moths died and 80% of the *P. expansum* spores failed to germinate. Interestingly, CO_2 in modified atmosphere packaging had a similar suppressive effect on *B. cinerea* development especially when additional CO_2 was placed in the bag (D. Sugar, unpublished data).

Storox

Storox, a relatively new broad spectrum sanitizer, containing a mixture of hydrogen peroxide and peroxyacetic acid was tested in several pome fruit cold storage rooms in the PNW. Storox applied by cold fogging at dilution rates of 1:50 or 1:30 in one and 12 rooms respectively, reduced mold contamination to low levels compared to the untreated control (W. McPhee, unpublished data). For example in one room treated with 50 gal Storox (1:50) applied by cold fogging, the average spore count from the four walls and floor was reduced to zero (Figure 2). The Storox treatment was also tested on wooden bins and reduced *P. expansum* populations by 97.9% when used at 2700 ppm (P. Sanderson, unpublished data). In this trial Storox performed better than sodium hypochlorite or QACs used at 500 ppm.

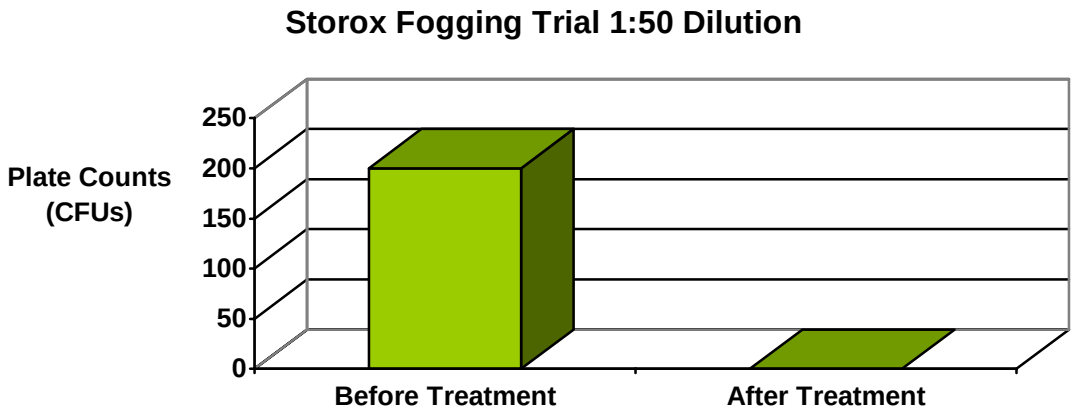

Figure 2. A cold storage room was treated by cold fogging 50 gallons of Storox solution (1:50) on to the four walls and floor. The number of microorganisms on the four walls and floor were monitored before and 72 hours after treatment.

Acetic Acid

Acetic acid vapor is another material that is being evaluated for storage room and bin sanitation. Acetic acid vapor has been shown to destroy spores of *B. cinerea* and *P. expansum* on apples (Sholberg and Gaunce 1995) and pears (Sholberg et al. 2004). Recently the vapor of acetic acid was tested at a packinghouse in British Columbia. An empty cold storage room (1983 m^3) containing six empty wooden apple bins was used in the trial. The object was to determine if acetic acid vapor had any potential as a sanitizing agent. Prior to treatment levels of mold contamination were assessed and areas on the walls and bins were inoculated with fungal spores. Shortly after inoculation the room was fumigated by boiling approximately 9 L of acetic acid into the sealed room. The room was vented 20 hours later and the refrigeration equipment was thoroughly washed with water because of concerns with corrosion. Several problems with monitoring and delivery of acetic acid occurred during this preliminary trial and we do not recommend repeating it until further research is conducted. However the results indicated that acetic acid vapor would reduce contamination by mold on hard wall surfaces to zero and those on foam insulation to lower numbers. The effect on bins was less clear. Mold was eradicated from four of the six bins. Two bins remained contaminated probably because they were located further away from the source of acetic acid vapor.

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