Feasibility of Sanitizing Apple Field Bins to Eliminate Postharvest Pathogens

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Objectives:

1. Evaluate effectiveness of sodium hypochlorite and quaternary ammonia compounds for eliminating *P. expansum* from apple field bins.
2. Determine if temperature of the treatment solution affects efficacy of sanitizing solutions.
3. Compare wood and plastic field bins to determine if (a) one type of bin carries more inoculum than the other and (b) if one type of bin can be more easily sanitized than the other.

Non-technical Abstract:

New York State produces approximately 26 million bushels of apples each year. Apples harvested in autumn are held in low-oxygen storage for up to 10 months to allow orderly marketing of the crop and to provide consumers with a year-round supply of high-quality fruit. However, several fungal pathogens can cause apples to decay during storage. In the early 1970's, fungicides became available that prevented postharvest decays in apples. By the mid-1990's, these fungicides had lost effectiveness because the fungi had become resistant to fungicides. No new fungicides have been approved for controlling postharvest decays. In some cases, as much as 15% of the apples held in long-term storage are decayed when apples are removed from storage. Furthermore, spores from decayed fruit spread to sound fruit during the packing process. These spores can cause decays in packed fruit after the fruit is shipped to retail stores. In a survey during winter/spring of 2000, decayed Empire apples were evident in bagged apple displays in nearly 40% of retail stores surveyed.

Previous research has shown that fungal spores can be carried from season to season on the large bins that are used to hold fruit during storage. Sanitizing bins after they are emptied might break the disease cycle, thereby reducing both losses in apple storages and the incidence of decays in bagged apples at the retail level. Research conducted under this project has shown that newer plastic bins harbor large numbers of spores even though the contamination may be less visible than on wooden bins. Therefore, simply switching to plastic storage bins will not resolve the problem. As part of this project, procedures have been developed for comparing commercial sanitizers for effectiveness using small, uniformly-contaminated pieces of wood and plastic bin materials. The next step is to identify the most effective sanitizers and application methods. The final step will be implementing bin sanitation in commercial packinghouses.

Background:

Postharvest decays caused by *Penicillium expansum* cause significant losses of Empire fruit held in long-term storage and sporadic losses of other apple varieties (Rosenberger *et al*. 2000). Fruit held in controlled atmosphere (CA) storages have routinely been treated with a postharvest fungicide. However, fungicide treatments are no longer effective or feasible because *P. expansum* has developed resistance to thiabendazole and the only alternative, captan, is not acceptable in some export markets and is under FQPA review. Packinghouses are heavily contaminated with air-borne spores of *P. expansum*, and this inoculum recycles from one season to the next on contaminated field bins. Effective sanitizing of field bins should make it feasible to store Empire fruit without any postharvest treatment and will reduce inoculum levels that contribute to decays of other apple cultivars during storage. Reducing inoculum levels on bins and in packinghouses should also help to reduce the incidence of decays that appear in consumer packages after apples are packed.

The problem posed by decayed apples in consumer packages was documented during winter and spring of 2000. A survey was conducted during winter of 2000 to determine if and how often rotten

apples occurred in consumer packages presented for sale in chain stores. Data collectors for this survey visited 17 to 20 chain stores in the mid-Hudson Valley (Newburgh, New Paltz, Kingston, Poughkeepsie) on each of four dates. Stores were not notified of the visits. The data collectors were instructed to peruse the displays of bagged apples as if they intended to make a purchase. They recorded the varieties of bagged apples on display and noted whether or not the bags contained any visibly decayed fruit. If decayed fruit were evident in one or more bags, the data collectors counted the number of bags on display and recorded how many of those bags contained decayed fruit. To avoid being conspicuous, data collectors did not examine every bag in a multi-layered display. Instead they attempted to record what consumers would see if they looked at the display with the intention of making a purchase. This method of checking for decays probably under estimated the actual levels of decay in bagged fruit because some decayed fruit may have been hidden within the bags.

For the stores surveyed, the proportion of Empire displays that contained decayed fruit varied from a low of 20% on February 3 to a high of 47% on April 11 (Fig. 1). Among those displays that contained decayed Empire fruit, the proportion of individual bags that contained decays ranged from 18% to 27%. For McIntosh, the proportion of stores with decayed fruit on display ranged from 10-28%, and 12-27% of the individual bags in those displays contained decays. In some cases, juice from decayed fruit had leaked through the bags, leaving the surface of the plastic bags sticky with residue.

The survey reported here was conducted in the Hudson Valley, but the decay problem is not limited to any single region. Labels on bags that contained decayed fruit showed that fruit originated from all of the various production areas within New York as well as from Massachusetts, Vermont, Pennsylvania, Washington State, and Ontario.

The levels of decay discovered in consumer packages during this survey are very likely contributing to lost sales and consumer dissatisfaction with apples. Further analysis of this problem, along with suggested solutions, has been published elsewhere (Rosenberger, 2000).

Progress to date:

As this is a postharvest project, most of the research is scheduled between November and March. Work completed to date involves development of methods for creating uniformly contaminated bin surfaces and for assessing inoculum density on such surfaces after treatments are applied. Comparisons of sanitizers and effects of temperatures on sanitizer efficacy will be determined in experiments that will be conducted during January, February, and March.

Two methods were developed for creating uniformly contaminated bin surfaces that can then be used to determine the effectiveness of sodium hypochlorite and quaternary ammonia sanitizers. For both methods, small squares of bin material (57 mm on a side) were cut from old oak bins or from plastic bins that had been used to hold apples. The squares were taken from used bins because of concerns that new oak bin wood might release tannins that could suppress spore germination in these studies. New plastic bins might have a smoother surface than older bins, and the latter would therefore be likely to harbor more spores.

Small squares were used as surrogates for bin surfaces because there is no cost-effective way to use full-size bins as experimental units for screening sanitizers. Attempting to evaluate sanitizers on small sections of full-sized bins was also impractical because of problems in applying the sanitizer and assessing spores loads on the "attached" surfaces. The inherent variability of naturally occurring inoculum would also confound experiments involving full-size bins. Therefore, the uniformly sized bin material squares (BMS) used for this study provided functional experimental units for evaluating a variety of factors relating to bin sanitation.

Methods for working with airborne spores deposited on bin material: The first evaluation method involved exposing bin materials to dry, airborne spores of *P. expansum* as would occur when empty bins are left in a packinghouse where there is a high concentration of airborne spores. Covered petri plates containing sterile BMS were distributed in a grid pattern on the floor of an 8x10 room that had an air-tight door and no heating or cooling ducts. With the door closed, spores were released in front of a small fan

by opening petri plates of *P. expansum* growing on acidified PDA agar. Using a piece of rubber tubing attached to a wooden handle, the surfaces of the fungal lawns on these plates were rubbed gently to enhance removal of inoculum from plates. The fan was allowed to run for five minutes after the spores had been released to ensure uniform dispersal of the airborne spores. Shortly after the fan was turned off, the BMS were exposed to spore settling by removing the covers from their petri plate containers. A greased microscope slide contained in a Petri dish was similarly uncovered adjacent to each of the BMS replicates. The exposed BMS and greased slides were allowed to collect settling spores for 16-18 hours (overnight) in still air before the Petri plates were closed again and removed from the room. Spore density on the exposed surfaces was determined by using a microscope to measure spore density per unit area on the microscope slides. Six locations were counted on each greased slide, and numbers were converted to spores per square millimeter.

Each of the BMS were individually placed in 250 ml beakers containing 200 ml of sterile distilled water plus 0.01% Tween 20. Short sections of sterile PVC pipe that just fit inside the beakers were placed on top of the BMS to prevent them from floating, and the beakers with the BMS were then suspended in a sonicator. The sonicator was run for 2 minutes to dislodge deposited spores from the BMS. Sonication also served to break up short chains of conidia, thereby ensuring that most colonies that resulted from dilution plating represented single conidia. After sonication, the BMS were removed from the water, and a 0.1 ml sub-sample of the water was removed from each beaker, placed on acidified PDA, and spread evenly over the plates with a glass rod. (If spore concentrations on the glass slides indicated that 100% recovery from the BMS would result in more than 1000 cfu/ml, then the sonicated solution was subjected to a 1;10 dilution prior to plating on PDA.) Colonies on the PDA plates were counted after incubation at room temperature for 5 days. Data from the plate counts was used to calculate the numbers of spores per square mm that were recovered from BMS. The experiment was run with nine replicates in the first trial and 5 replicates thereafter.

The mean number of spores recovered per square mm of BMS varied from 313 to 33 in the three trials. (Table 1). Significantly more spores were recovered from plastic than from wood in the first trial, but recovery rates were similar for plastic and wood in trials 2 and 3.

Variations on the above procedures were included in the second and third trials to answer specific questions. In the second repetition, the BMS were replaced in their petri plate containers following the first sonication, were allowed to dry, and were then subjected to a second round of sonication to determine if a single sonication was sufficient for recovering most of the spores deposited on the BMS. Results of this trial showed that roughly 98% of all the spores that were recovered in two sonication cycles were released during the first sonication. The proportion of spores released during the first sonication did not differ between plastic and wood.

The ultimate purpose of this research was to compare the effectiveness of sanitizers that would be applied by dipping contaminated BMS into sanitizing solutions, then measuring the reductions in the numbers of spores that could be recovered. Would simply dipping the BMS into sanitizers remove most of the deposited spores or would the spores remain attached to the BMS even when dipped into a non-agitated solution? To answer that question, spore recovery was compared for BMS that were sonicated as described above as opposed to BMS that were first dipped into sterile water for one minute prior to sonication. In trial runs two and three, the water dip prior to sonication involved submerging the BMS for one minute in 200 ml of sterile water containing 0.01% Tween 20. Results showed that although 50 to 65% of inoculum was removed by the pre-sonication dipping, there was still enough spores retained to allow an assessment of sanitizer effectiveness (Table 1). The proportion of spores removed by dipping was similar for both plastic and wood BMS.

Methods for working with bin material colonized by *P. expansum*: Passive deposition of dry fungal spores represents only one kind of contamination on apple bins. A different kind of contamination occurs when *P. expansum* grows and sporulates directly on the bin surface as a result of having decayed apples in contact with the bin. This kind of contamination generally results in visible blue stains that presumably contain a higher density of inoculum than bin material this is subjected only to passive deposition of dry

fungal spores. The inoculum in stain areas is presumably more difficult to remove because of the hydrophobicity of masses of conidia of *P. expansum*.

To duplicate stain areas on BMS, sterile wood and plastic BMS were submerged in sterile apple juice in plastic petri plates. A small piece of cork was placed on top of the BMS in each plate, and the lids of the stacked petri plates contacted the corks, which in turn served to keep the BMS from floating. The center area of each BMS was inoculated with conidia of *P. expansum,* and within a week the entire surface of the BMS was colonized with sporulating *P. expansum.* At the same time, the apple juice in the petri plates gradually evaporated, leaving a damp, uniformly contaminated BMS that can be used for sanitizer experiments. The BMS with actively growing *P. expansum* will be sonicated and evaluated in the same way as for passively-deposited dry spores.

Assessment of inoculum retention on whole wood and plastic bins: To determine how many spores can be carried on bins, empty bins at several commercial packinghouses were "washed" using a portable postharvest drencher. Water samples were collected after each set of five bins had been washed, and inoculum levels in the water samples were assessed by dilution plating. The number of spores recovered per bin was calculated by taking into account the number of bins washed, the total volume of the wash water in the drencher, and the number of colonies per milliliter of water placed on dilution plates.

The bin-washing experiment showed that a single contaminated bin can carry more than two billion spores (Table 2). Plastic bins carried only about one-fourth as many spores as the wooden bins from the same CA room, but the plastic bins still harbored more spores than their relatively "clean" appearance would have suggested. Thus, switching to plastic bins will not eliminate the need for sanitizing field bins.

Inoculum levels detected on bins are sufficient to account for high levels of decay observed in stored fruit. Fungicide-resistant spores can accumulate in water flumes or postharvest drenches as contaminated bins are processed. A postharvest drencher containing 1000 gallons of solution could accumulate sufficient inoculum from 40 badly contaminated bins to raise inoculum concentrations in the solution to more than 20,000 spores/ml. For many postharvest experiments with Empire fruit, spore concentrations of 20,000 spores/ml are enough to cause 100% decay in wounded fruit. Thus, inoculum carried on wooden bins in clearly sufficient to account for carry-over of inoculum from year to year, and sanitizing bins may be critical for breaking the disease cycle.

Summary:

Work completed to date has shown that dry spores can be uniformly deposited on and recovered from both wood and plastic BMS. Approximately 98% of spores on BMS can be removed with a single 2-minute sonication, and spore recovery can be measure by dilution plating. Dipping BMS in water solutions prior to sonication removes only about half of the inoculum deposited, so sanitizer treatments can be compared by dipping BMS prior to sonication and dilution plating. Wood and plastic bin materials retained dry-deposited spores equally well in lab experiments. Plastic bins from a commercial storage released only about one-fifth as many spores as wood bins from the same storage room, but the wooden bins might have contained more decayed fruit, thereby adding uncontrolled variables to that comparison.

The work completed as part of this project will result in reduced use of postharvest fungicides. In other work that was not part of this project we have shown that postharvest fungicides still control decays caused by *Botrytis* whereas *P. expansum* is resistant to the fungicides. In this project, we provided further evidence that bins are carrying huge quantities of inoculum that is released when the bins are passed through postharvest drenching systems. Until this work was done, no one realized how many spores are cycling from year to year on bins. Many packinghouse operators will henceforth avoid postharvest fungicide treatment because the treatment process distributes fungicide-resistant spores from bins to the fruit and greatly increases the incidence of decays caused by *P. expansum*. Eliminating postharvest fungicides will allow *Botrytis* to grow unchecked, but *Botrytis* has caused relatively few losses compared to those caused by the fungicide-resistant strains of *P. expansum*. Eliminating postharvest treatment could result in a significant reduction in detectable pesticide residues at the consumer level because up to

47% of detectable residues in apples have been associated with postharvest treatments (Kuchler *et al*.. 1997)

Surveys of chain stores confirmed that postharvest apple decays are prevalent on consumer packages at the retail level. Presumably this is reducing return sales and profitability of the apple industry. Improved methods of decay control via sanitation should result in reductions in postharvest losses both before and after fruit are packed.

The work reported here represents the initial stages of a multi-year research effort. By March 2001, we should have enough information on effectiveness of various sanitizers against dry-deposited spores to allow for commercial-level testing with full-size bins during the next summer. Additional lab research will be needed to determine if sanitizers that are effective against dry-deposited spores will be equally effective for bin material that has been colonized by *P. expansum*. Even if an effective sanitizer can be identified, packinghouse operators may need to spend more than $75,000 to equip packinghouses with the appropriate bin-washing equipment that would be needed to apply sanitizers to bins as they are emptied. Although this sounds cost-prohibitive, the absence of alternatives may leave packinghouses with little choice. The risks of losing up to 15% of fruit to decay during long-term storage plus the sales-depressing effects of decayed fruit in consumer packages will provide a strong incentive for investing in sanitation equipment if the value of sanitizing bins can be thoroughly documented.

Publications:

Kuchler, F., R. Chandran and K. Ralston. 1997. The linkage between pesticide use and pesticide residues. Am. J. Alternative Agric. 11:161-167.

Rosenberger, D. A. 2000. Postharvest pathogens create new problems for apple storages. *In:* 2000 Cornell Fruit Handling and Storage Newsletter (C. B. Watkins and D. A. Rosenberger, eds.). (In press).

Rosenberger, D., Meyer, F., and Ahlers, C. 2000. Progress in understanding and controlling postharvest decays of apples. N.Y. Fruit Quarterly 8(3):24-28.

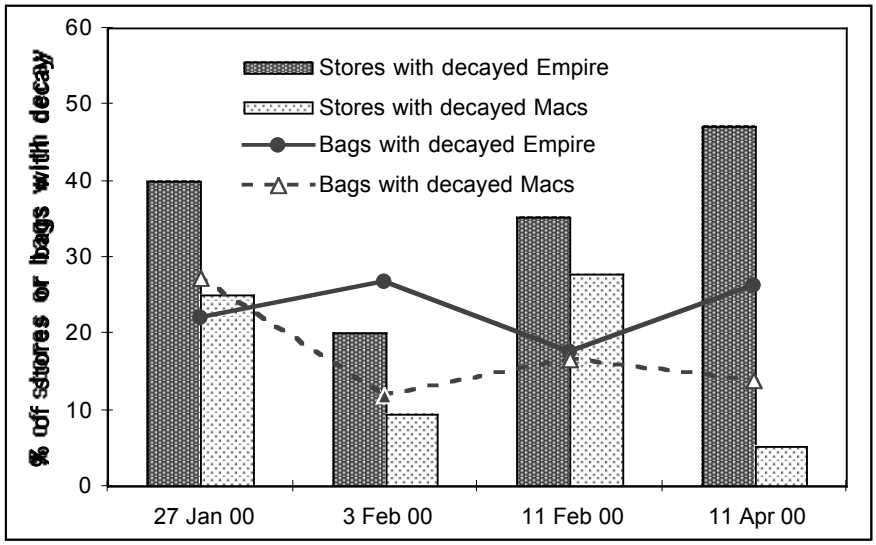

Figure 1. Percentage of bagged apple displays in retail stores that contained decayed fruit, and estimated proportions of bags in those displays that contained decays. Results are based on surveys of 17-20 stores on each of four dates.

Table 1: Numbers of spores recovered from squares of wood or plastic bin materials exposed to spores in a settling chamber, then washed in a sonicator.

	No. of spores/square mm recovered from bin materials using a single sonication	% of total spores recovered in two sonications that were released in the first sonication	% of total spores deposited that were removed by dipping in water for one minute prior to sonication
Trial #1:			
Wood bin	174 a	n.d.	n.d.
Plastic bin	224 b	n.d.	n.d.
P-value	0.03		
Trial #2:			
Wood bin	313	98.2	66
Plastic bin	313	97.9	58
P-value	0.97	0.79	0.21
Trial #3:			
Wood bin	33	n.d.	47
Plastic bin	54	n.d.	49
P-value	0.35		0.86

Table 1. Numbers of *Penicillium* spores per bin as determined by washing bins with a portable drencher and dilution-plating sub-samples from the wash water.

	Spores/bin
Summer 1999	
Wooden bins, set #1	835,244,000
Wooden bins, set #2	424,542,000
Summer 2000	
Wooden bins	2,233,499,427
Plastic bins	481,918,410

Means for washing 25 bins/set in 1999 and 20 bins/set in 2000.