Progress in Understanding and Controlling Postharvest Decays of Apples

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Empire fruit in CA storage become decayed when *Penicillium expansum* grows into the apple through the stem. Fruit with high boron levels appear more susceptible to decay. Because *P. expansum* is resistant to existing fungicides, improved sanitation is critical for controlling postharvest decays.

Most postharvest decays of apples in New York are attributable to *Penicillium expansum*, the cause of blue mold, and *Botrytis cinerea*, the cause of gray mold. Fruit with blue mold are very soft, watery, and have a musty or earthy odor. Empire fruit with gray mold emerge from CA storage looking like baked apples. They have a uniformly light tan skin, fairly firm flesh, and a cider-like odor.

When first introduced, thiabendazole (TBZ) and the other benzimidazole fungicides were very effective against *P. expansum* and *B. cinerea*. Strains of these pathogens developed resistance to the benzimidazole fungicides soon after the fungicides were introduced, but postharvest treatments continued to provide decay control throughout the 1980s. Postharvest treatments remained effective because strains of *P. expansum* and *B. cinerea* with resistance to benzimidazole fungicides showed increased sensitivity to diphenylamine (DPA)(Rosenberger & Meyer, 1985; Sharom & Edgington, 1985). In the northeast, DPA was always applied with a benzimidazole fungicide, and it served to control the benzimidazole resistant strains *P. expansum* and *B. cinerea.* As a result, the benzimidazole/DPA combination remained effective long after benzimidazole fungicides were no longer effective for controlling other diseases such as apple scab, apple powdery mildew, and brown rot of stone fruits. The combination of DPA and thiabendazole (TBZ) is still effective for controlling *B. cinerea* in stored apples in New York.

Figure 1. Empire apple with blue mold decay caused by *P. expansum* that invaded fruit through the stem during CA storage.

Repeated exposure of *P. expansum* to the benzimidazole/DPA combination gradually selected for strains of this pathogen that were resistant to both DPA and benzimidazole fungicides. Today, most isolates of *P. expansum* in New York apple storages are resistant to the DPA/benzimidazole combination. These strains of *P. expansum* produce prodigious quantities of inoculum in decayed fruit because they are unaffected by postharvest treatments.

Most of the published literature on blue mold indicates that *P. expansum* is primarily a wound pathogen. However, Empire fruit that develop blue mold decay during CA storage often have no wounds or skin abrasions that would provide an entrance site for *P. expansum.* How does *P. expansum* gain access to non-injured fruit? What are the major sources of inoculum for infecting fruit with *P. expansum*? Are there new fungicides under development that might be suitable for postharvest treatment of apples? Postharvest research initiated in 1997 was formulated to answer these questions.

When and How Do Fruit Become Infected?

In a previous report in the *NY Fruit Quarterly*, Rosenberger (1998) described an experiment that proves that Empire apples can become infected via invasion through the stem during long-term CA storage. That experiment was repeated with fruit harvested in 1998 and held in CA storage until April 1999. In both the 1997-1998 and the 1998-1999 storage trials, a high incidence of decay was observed in CA-stored Empire fruit that had been inoculated by placing 500 spores of *P. expansum* on the ends of intact stems (Fig. 1). Apples inoculated the same way but held in air storage instead of CA storage developed very little decay when held for similarly long storage intervals. Thus, Empire apples held in cold air were able to stop the invasion of *P. expansum* through their stems whereas apples held in CA storage were unable to resist the pathogen.

The amount of decay that resulted from stem inoculations varied depending

on the orchard in which the fruit was grown. Empire apples used for stem inoculation studies were collected from the same six orchards in both years of the study. In both years, some orchards had significantly more decay than other orchards, but differences among the six orchards were not consistent between years (Table 1). Orchards L-331 and OD had the highest incidence of decay in both years whereas orchards RO and KI had moderate levels of decay in both years. Orchards KA and L-236 showed the greatest variation from year to year.

Analysis of fruit stem characteristics and seasonal fungicide spray records from the six orchards did not provide any clues for explaining differences in susceptibility to decay. However, susceptibility to decay was correlated with high boron levels in fruits and leaves. Regression analysis of incidence of decay as affected by foliar boron levels in the orchard provided an R^2 of 0.69, indicating that 69 percent of the variation in levels of decay in the six orchards over the 2 years of the trial could be explained by differences in foliar boron (Fig. 2). A similar relationship was noted for levels of boron in fruit (Fig. 3). None of the other mineral levels in leaves or fruit had any significant effect on fruit susceptibility to decay. A follow-up experiment is under way to verify the relationship between boron levels and susceptibility to decay in Empire fruit.

Additional stem inoculation tests were conducted during the 1998-1999 storage season to determine what other variables might affect fruit susceptibility to stem invasion by *P. expansum*. One experiment showed that the incidence of decay following CA storage was similar for apples inoculated with 50 spores per stem and for those inoculated with 500 spores per stem. Other tests showed that the incidence of decay was not significantly affected by holding fruit at ambient temperatures for up to 40 hours between harvest and inoculation. Decay incidence was not affected either by a 24-hour delay between inoculation and cooling of the fruit or by holding inoculated fruit at 34°F in air for up to 14 days before putting them into CA storage. Thus, resistance of fruit to decay could not be enhanced by allowing more time between harvest and inoculation, between inoculation and fruit cooling, or between inoculation and establishment of CA atmosphere.

TABLE 1

Effect of orchard source on percent of Empire fruit that developed decay during CA storage following inoculation of uncut stems.

	Inoculated 4-6 hr after harvest			Inoculated 20-24 hr after harvest		
Fruit source	1997 -98	1998 -99	Grand means for 2 yr.	1997 -98	1998 -99	Grand means for 2 yr.
L-331	65 b[1]	65 c	65 c	35 a	56 b	46 b
OD	44 ab	61 bc	52 bc	44 a	52 b	48 b
RO	24 a	43 bc	33 ab	10 a	19 ab	14 a
KI	44 ab	23 ab	33 ab	23 a	8 a	15 a
KA	69 b	3 a	30 ab	34 a	13 a	22 ab
L-236	35 ab	5 a	18 a	20 a	52 b	35 ab

[1] Means for each year are from observations of 25 fruit. Data was subjected to the angular transformation for statistical analyses. Means separations were determined using LSD to compare means from the factorial analysis of 6 farms X 2 years of observations. Means followed by the same letter are not significantly different ($P \leq 0.05$).

TABLE 2

Estimated number of *Penicillium* spores per liter of air in CA rooms and packinghouses as determined using a Burkard volumetric air sampler.

	1998-1999*	Winter/Spring 2000**
Packinghouses		
Near water flotation tank in the packing line	49.6	118.5
Bagger end of packing line	27.7	40.8
Closed CA rooms or cold storage rooms	3.4	1.3

*Means from single sampling dates at 9 packinghouses and 6 closed CA storage rooms.
**Means from four sampling dates at each of three packinghouses and cold storage rooms.

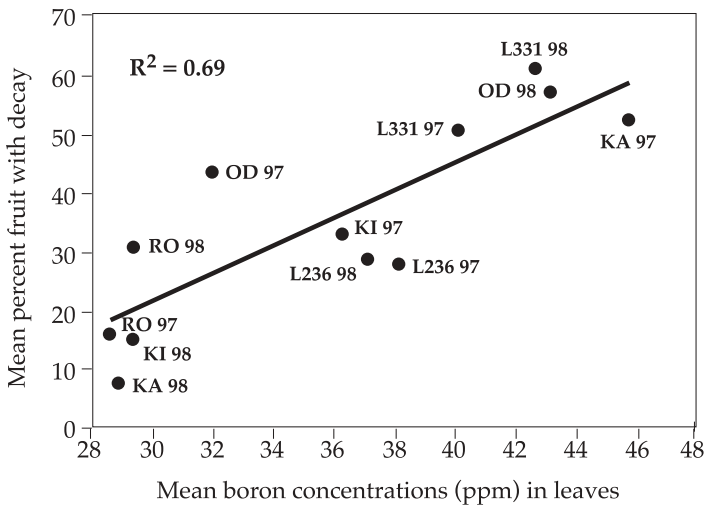

Figure 2. Results of regression analysis of the effect of boron concentration levels in leaves on suseptibility of fruit to postharvest decay, with data taken from six orchards over two successive years (1997 & 1998).

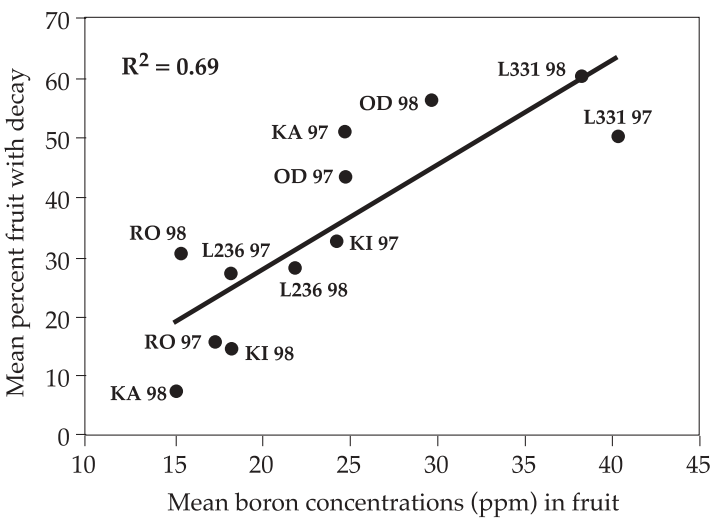

Figure 3. Results of regression analysis of the effect of boron concentration levels in fruit on suseptibility of fruit to postharvest decay, with data taken from six orchards over two successive years (1997 & 1998).

TABLE 3

Results from washing four different lots of empty apple bins, 1999.

	No. of Penicillium spores per bin as estimated from wash water samples	% reduction in spore load on sanitized bins compared to similar non-sanitized bins
Dirty bins from same lot as sanitized bins	835,244,000	
Bins sanitized with fresh sanitizer solution	1,538,000	99.82 %
Bins sanitized just at end of sanitizer usefulness	7,435,000	99.11 %
2nd set of dirty bins	424,542,000	

TABLE 4

Effectiveness of various fungicides applied to wounded Empire fruit for the prevention of blue mold decay caused by P. expansum.

Materials and rate of formulated product per 100 gal	% fruit with decay at various intervals following treatment*			
	8 wk.	10 wk.	12 wk.	14 wk.
Control	62 c**	91 c	96 c	97.0 c
Mertect 340-F 8 fl oz	<1 a	2 a	6 b	5.6 ab
Mertect 340-F 16 fl oz	<1 a	1 a	2 ab	9.3 b
Captan 50W 2.5 lb	46 b	75 b	84 c	88.0 c
Scholar 50W 8 oz	0 a	<1 a	<1 ab	0.2 a
Scholar 50W 16 oz	0 a	0 a	0 a	0.2 a

*Fruit were inoculated and treated on 11 December, then held at 34 F in air storage and evaluated at the indicated intervals after treatment.

**Means followed by the same letter are not significantly different (Fisher's Protected LSD, P≤0.05).

What are the Major Sources of Inoculum for *P. expansum*?

Relatively little is known about the sources of inoculum that contribute to postharvest decays caused by *P. expansum*. Recirculating solutions in postharvest treatment tanks and water flotation tanks have been suggested as the primary sources of inoculum. However, airborne inoculum in packinghouses may contaminate bins as they are moved through and out of the packinghouse. That airborne inoculum landing on bins, along with decayed fruit left in bins coming off the packing lines, may provide inoculum for next year's crop. Furthermore, inoculum that recycles from year to year on bins or as contaminants in packinghouses and storages would receive repeated exposure to postharvest fungicide treatments and therefore are the most likely sources for strains with resistance to DPA.

Experiments were conducted to measure levels of inoculum in packinghouses, storage rooms, and empty bulk bins. A portable air sampler for agar plates (Burkard Manufacturing Co.) was used to measure concentrations of airborne spores of *P. expansum* in various storage and packinghouse environments. Spore trapping was conducted in eight packinghouses and six closed CA rooms during April 1998 and in one additional packinghouse in June 1999. Additional spore trapping was conducted at three packinghouses and their associated cold storage rooms on February 1 and 22, March 14, and April 4, 2000. In packinghouses, air was sampled close to the water flotation tank where apples are floated out of the bins and also at the far end of the packing line where automatic baggers are usually located. Air in CA rooms was measured by inserting the spore trap through portholes in the CA room doors. Each time that spore trapping was done in the winter and spring of 2000, agar plates were also left exposed to the air for one minute near the water flotation tanks to determine how many spores would be captured just by settling of spores from the air.

Airborne inoculum levels ranged from 50 to more than 100 spores per liter of air near the water flotation tanks. Spore levels in air were roughly half as great at the far end of packing lines, and there were very few airborne spores in closed CA rooms and in cold storage rooms (Table 2). Spores of *P. expansum* landed on exposed agar plates at the rate of two spores per square inch per minute when plates were exposed near the water flotation tanks in packinghouses.

The levels of airborne inoculum detected in packinghouses suggest that anything coming out of the packinghouse during the packing season will probably be contaminated with spores of *P. expansum*. Even if water flotation tanks are chlorinated, empty bins may become recontaminated by the time they are bundled and removed from the packinghouse. If the empty bins are stored in empty CA rooms in the same building as the packing operation, then inoculum generated during the packing operation will continually drift into the rooms used to store bins.

A bin sanitation experiment was conducted during July 1999 to determine levels of inoculum residing in empty bins and effectiveness of sanitizing bins with a quaternary ammonia solution. Four separate lots of 25 empty bins were "washed" using a portable postharvest drencher. The four lots included: (a) dirty bins that had been bundled and stacked outdoors for about two months after they were emptied, (b) a comparable group of bins from the same lot that had been sanitized by the packinghouse operator using a fresh charge of Deccosan quaternary ammonia sanitizer, (c) another group from the same lot that was sanitized with Deccosan after several hundred bins had already been processed, and (d) a set of dirty bins from a different CA room. The sanitizer was applied by the packinghouse operator using a modified postharvest drencher that directed cascades of water against all sides of the bins after they had been unbundled and all decayed fruit had been manually removed.

To assess spore levels on non-sanitized and sanitized bins, water samples were collected after sets of five bins had been washed in the portable drencher. This approach provided five replicate samples from each test lot of 25 bins. Inoculum levels in the water samples were assessed by dilution plating. Inoculated agar plates were incubated for 35 days at 34°F to inhibit growth of contaminants while *P. expansum* spores germinated and formed visible colonies on the plates. Colonies of *P. expansum* were counted, and the number of spores recovered per bin was calculated by taking into account the number of bins washed, the total vol

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Postharvest decay in Empire has been increasing in recent years.

ume of the wash water in the drencher, and the number of colonies per milliliter of water plated on dilution plates.

The bin-washing experiment showed that a single contaminated bin could carry more than 800 million spores (Table 3). Although sanitizing bins provided a 99 percent reduction in the number of spores per bin, some sanitized bins still carried nearly 8 million spores and the economic benefit from 99 percent control is unknown. The difference between spore numbers remaining on the bins treated at the beginning compared to those treated at the end of the solution cycle was not significant and showed that the sanitizer had reasonable longevity in the recycling wash water.

New Fungicides for Postharvest Decays?

Novartis has a new fungicide with the trade name of "Scholar" that could prove useful as a postharvest fungicide on apples. The generic name for Scholar is fludioxonil. It is a phenylpyrrole fungicide with a different mode of action than any of the other fungicides currently registered for field or postharvest use on apples. Scholar has received Section 18 registrations for controlling brown rot on stone fruits in several states, but Novartis is still uncertain whether the product can be registered on apples.

An experiment was conducted during the 1998-1999 storage season to compare the effectiveness of Scholar with that of Captan and Mertect 340-F. (Mertect 340-F is a commercial formulation of TBZ). Each treatment was replicated four times using 25 fruit per replicate. Empire apples were harvested September 15 and were held at 34°F until December 11 when the experiment was initiated. Fruit were wounded on a single hemisphere using a large cork fitted with three finishing nails spaced about 1 cm apart in a triangular pattern. Wounds simulated stem punctures, a common entry site for *P. expansum* in apples. Baskets containing 25 wounded fruit were dipped for 20 sec into a spore suspension that contained 2,500 conidia per ml or a benzimidazole-sensitive isolate of *P. expansum*. Fruit were allowed to dry for approximately 1 hr after inoculation and were then submersed for 30 sec in treatment solutions. Treated fruit were then arranged on spring cushion trays, placed in wooden crates, and moved to cold storage at 34°F. Apples were evaluated for decay on four different dates. Fruit were considered decayed if any one of the three wounds was infected.

All of the four treatments that involved Mertect 340-F or Scholar were equally effective for the first 10 weeks following treatment. By 12 weeks after treatment, however, the incidence of decay in fruit treated with Mertect 340-F had begun to increase (Table 4). At the end of 14 weeks, both rates of Scholar provided better control of decay than did the high rate of Mertect 340-F. Results from this experiment are consistent with results from other trials where activity of Mertect 340-F seemed to decline with increasing duration of storage. Scholar exhibited better residual activity in this trial than did Mertect 340-F. Differences between Scholar and Mertect 340-F would have been more dramatic if a Mertect 340-F-resistant strain of the pathogen had been used as inoculum. Captan was ineffective for controlling decay in this test.

What Happens Next?

The appearance of *P. expansum* with resistance to both DPA and TBZ means that packinghouse operators no longer have effective fungicides for preventing postharvest decays in apples. Empire fruit may be uniquely susceptible to invasion via stems. Recognition of this weakness in Empire was delayed by the availability of effective fungicides.

In the absence of effective fungicides, better sanitation will be critical for controlling postharvest decays. Even if a new fungicide such as fludioxonil is eventually registered for apples, resistance to the new fungicide will develop quickly unless inoculum levels are kept to a minimum. The negative cross-resistance between DPA and benzimidazole fungicides was a totally fortuitous and serendipitous event that is not likely to be repeated. In the absence of DPA, the benzimidazoles would have controlled postharvest decays for only about 5 years as compared to the 17 years (1973-1990) of good control the industry enjoyed with the DPA/benzimidazole combination.

Research is needed to determine the most cost-effective method for sanitizing bins and storages and to determine if sanitation measures can be correlated with reductions in the incidence of decays the following year. The area of bin sanitation has not been well investigated for any crop. Developing and proving the effectiveness of a comprehensive sanitation strategy will be a complex and lengthy process, especially since much of the work must necessarily be done in commercial packinghouses where establishment of appropriate controls and replications is difficult.

The relationship between high boron levels and increased susceptibility to decay is being pursued by establishing varying levels of boron in replicated field plots. If the relationship can be verified, then growers may want to reduce boron applications in Empire blocks where foliar boron levels exceed 35 ppm. Alternatively, analysis of foliar boron levels in late summer might be used as a predictor for

postharvest decay problems and fruit from high-boron orchards could be marketed earlier in the season.

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