Populations of propagules of *Penicillium* spp. during immersion dumping of apples

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Population levels of propagules of Penicillium spp. in apple dump-tank water were related to apple cultivar and number of bins emptied into the water. When McIntosh apples were immersed in the dump-tank, the water contained 15,555 propagules/ml compared to water used to handle Spartan with 5,000 and Golden Delicious with 2,200 after 650 bins of each cultivar had been emptied. Propagule populations increased quickly during processing of the first 200 bins of fruit but stabilized and remained relatively constant thereafter. Filtration of immersion water reduced propagule concentrations in water used for McIntosh, Spartan and Golden Delicious when compared to nonfiltered water. Red Delicious dump-tank water averaged 22.8 propagules/ml of Penicillium spp. when treated with 50-100 μg/ml of available chlorine and 7.0 propagules/ml when the water was filtered in addition to chlorination.

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Les niveaux de population de propagules de Penicillium spp. dans l'eau des reservoirs d'immersion des pommes dependaient du cultivar de ce fruit et du nombre de contenants vides dans l'eau. Apres vidage de 650 contenants de chaque cultivar, l'eau contenait 15 555 propagules/ml dans le cas des pommes McIntosh comparativement a 5 000 pour les Spartan et 2 200 pour les Golden Delicious. Le nombre de propagules a augmente rapidement durant le traitement des 200 premiers contenants, mais s'est stabilise et est demeure relativement constant par la suite. La filtration de l'eau d'immersion a reduit la concentration de propagules dans le cas des McIntosh, des Spartan et des Golden Delicious. L'eau des reservoirs de Red Delicious comptait en moyenne 22.8 propagules/ml lorsqu'elle etait traitee avec 50-100 μg/ml de chlore disponible et 7,0 propagules/ml quand elle etait filtree en plus d'être purifiée au chlore.

Introduction

Blue mold rot caused by *Penicillium expansum* is the most common and usually the most destructive of all the rots found on apple, pear, and quince in transit, in storage, and on the market (3). In a study on disorders of apples shipped to the New York market from 1972-1984 blue mold rot was the most damaging parasitic disease (2). Blanpied and Purnasiri (1) found that in water tanks used to handle apples in packing houses levels of 1,000 to 7,000 germinated *Penicillium expansum* propagules per ml of water were common. Spotts and Cervantes (5) showed that propagules of *Penicillium* spp. in dump-tank water increased from 189 propagules/ml in October to 4,167 in March, 1983 suggesting that decaying fruit stored in bins and processed later increased propagule levels. Presently at least one packinghouse in British Columbia uses chlorine to lower dump-tank water spore loads. In a study done in packinghouses in Oregon the average *Penicillium* spp. populations were 543 and 1,423 per ml in 1981-82 and 162 and 1,729 per ml in 1982-83 for dump-tank water containing chlorine, and no chemical, respectively (5).

It was the object of this study to count the number of propagules of *Penicillium* spp. in the immersion water of a packinghouse in British Columbia to determine if chlorination was necessary. We also decided to evaluate the effectiveness of both filtration and chlorination for removing propagules of *Penicillium* in the dump-tank water.

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Materials and methods

Sampling.

Dump-tank water at a commercial packinghouse was sampled daily during emptying of bins (360 kg/bin) of apples from controlled atmosphere (CA) storage. The apples from CA had been placed directly in storage from the field except for the Red Delicious variety which had been drenched with diphenylamine to prevent scald. Dump-tank water containing Golden Delicious was sampled in 1987 on January 19 to 26; Spartans, February 2 to 9; and McIntosh, February 11 to 18. The water was sampled by immersing a 250 ml sample bottle into a flume approximately 3 meters from the dump-tank where apples were immersed to remove them from the bins.

Populations of propagules of *Penicillium* spp. were monitored in the dump-tank water by taking 0.1 ml of the sampled water and spreading it over a petri plate 50 mm in diameter containing 10 ml of potato dextrose agar (Difco, Detroit, MI) acidified with 1.5 ml of 85% lactic acid per liter (APDA) and incubating at 25°C for 3-7 days (5). If the dump-tank water contained chlorine the APDA plates were immediately inoculated at the packinghouse because the effect of chlorine increased with time of exposure. At least 9 plates were inoculated for each sample of dump-tank water taken. After incubation, at 25°C, *Penicillium* spp. colonies were counted and again checked 6 days later for typical blue-green sporulation. Dump-tank water was diluted, up to 10^5 times when it was found that individual *Penicillium* colonies could not be counted because of high numbers.

Filtration.

The dump-tank used for the filtration experiments held 20,000 L of water which was filtered by using a circulating pump (Model No. RPF 700, Pac. Fab. Inc., Sanford, NC) delivering 250 Umin at 69 kPa through two Jacuzzi sand filter units (Model 24 FM-6, Jacuzzi Canada Ltd., Rexdale, Ont.) containing #16 silica sand connected in parallel for a surface area of 0.5574 m^2. A pressure switch automatically turned on a red signal light and shut off the circulating pump when the pressure between the intake and outlet varied by more than 69 kPa. The sand filter was backwashed manually with an external source of pressurized domestic water. Small amounts of water (20-50 L) were occasionally added to the dump-tank to keep the level adequate for moving fruit. The dump-tank water was usually changed and the tank cleaned every two weeks after approximately 1,000 bins of fruit had been emptied.

Dump-tank water was filtered and sampled for the following varieties on the following dates: McIntosh, January 5 to 12, 1987; Spartans, April 13 to 24; Golden Delicious, April 2 to 9 and May 19 to 27, 1987; Red Delicious, February 23 to March 3, March 23 to 30, and May 5 to 15, 1987.

Chlorination.
Dump-tank water was chlorinated by adding 12% commercial grade sodium hypochlorite to the dump-tank to maintain a concentration of 50-100 µg/ml of available chlorine in the water. This was accomplished by injecting the sodium hypochlorite in the dump-tank with an injector pump (Chem-Feed Model No. C 6125P, Blue-White Industries, Westminster, CA) which was operated when the dump-tank circulating pump was running. The pH of the dump-water was monitored daily with indicator paper at the packinghouse for a rough estimate of pH and again at the laboratory with a pH meter (Fisher Accumet pH Meter, Fisher Scientific Co., USA) to determine if buffer needed to be added to the dump-tank. An increase in pH substantially decreases the biocidal activity of chlorine, and a decrease in pH increases this activity. Available chlorine of the dump-tank water was measured several times each day with a colorometric test kit (Pennwalt Corp., Monrovia, CA 91016).

Dump-tank water was chlorinated for the following varieties on the following dates: Golden Delicious, May 19 to 27; and Red Delicious, March 23 to 30, April 27 to May 4, and May 5 to 15, 1987.

Results

Population levels of *Penicillium* spp. in apple dump-tank water.
Populations of *Penicillium* spp. in the immersion water varied with the apple cultivar. For example, McIntosh dump-tank water contained 15,555 propagules/ml compared to Spartan with 5,000 propagulesIml and Golden Delicious with 2,200 propagules/ml after approximately 650 bins of each variety had been emptied (Fig. 1). Populations of *Penicillium* spp. generally increased with the number of bins dumped. Initially the propagule population rose quickly with the immersion of the first 150-200 bins which also coincided with the first sampling and then rose much more slowly remaining relatively constant for the emptying of the remaining bins.

Effect of filtration on *Penicillium* spp. propagule populations.
Filtered dump-tank water from McIntosh apples contained fewer propagules/ml than nonfiltered water (Table 1). Propagule levels averaged 13,536 ± 2,654 in nonfiltered water compared to 5,535 ± 2,853 in filtered water.

The filter lowered the population of propagules in water used for dumping Spartan apples from controlled atmosphere storage. When the Spartans were dumped with the filter

Table 1. Effect of water treatment on the average number of propagules of *Penicillium* spp./ml in the dump-tank water.

Treatment*	Variety			
	McIntosh	Spartan	Golden Delicious	Red Delicicus
Filtered	5,535 ± 3,853**	967 ± 868	360 ± 716	10,538 ± 5,566
Chlorinated	-	-		22.8 ± 4.0
Chlorinated and Filtered	-	-	0.6 ± 0.7	7.0 ± 3.0
None	13,536 ± 2,654	4,400 ± 841	3,106 ± 835	-

* The dump-tank was emptied and cleaned between treatments.

** The average number of propagules of *Penicillium* spp./ml was determined by counting propagules each day in the immersion water over a period of several days and averaging these values for the whole period.

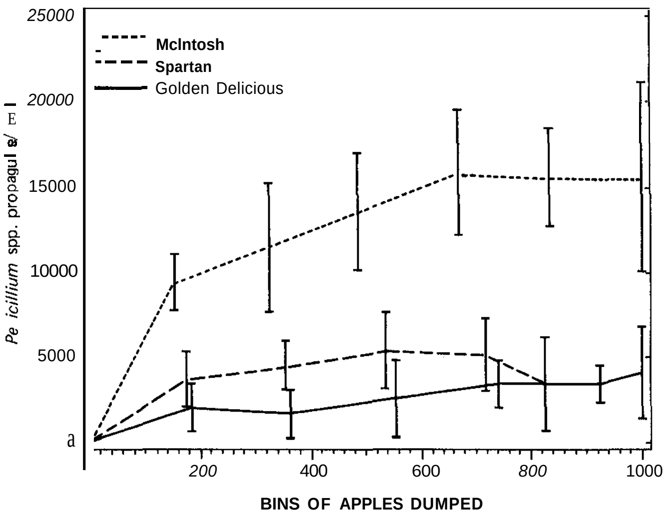

Figure 1. Contamination of dump-tank water by bins of Golden Delicious, McIntosh and Spartan apples after removal from controlled atmosphere storage in 1987 on January 19-26, February 11-18 and February 2-9, respectively. The dump-tank water was sampled each day after the bins had been emptied for that day. Each point represents the mean of nine replications and each bar represents the standard error of the mean.

running, it was done over a 14 day period and the filter operated for long periods without fruit being added to the dump water. Under these conditions, the spore population averaged 967 ± 868 compared to 4,400 ± 841 propagules/ml for the nonfiltered water.

Similarly, the filter lowered the number of propagules in the water used for dumping Golden Delicious apples to an average of 1,360 ± 716 compared to 3,106 ± 835 propagules/ml for the nonfiltered water.

Effect of chlorination on propagule populations.
Addition of 12% sodium hypochlorite to maintain 50-100 µg/ml free chlorine in the dump-tank reduced *Penicillium* spp. propagule numbers to extremely low levels. Red Delicious apples dumped into chlorinated nonfiltered water contained 22.8 ± 4.0 propagules/ml on average. Filtered water that was chlorinated into which Red Delicious apples were dumped contained only 7.0 ± 3.0 propagulesIml. Chlorine was also effective for keeping average propagule populations at 0.6 ± 0.7 for Golden Delicious apples dumped between May 19-27, 1987 when using the same filtered water from Red Deliciousthat had been treated with chlorine.

Filtered dump-tank water used less chlorine than nonfiltered water in this trial. For example, when filtered versus nonfiltered dump-tank water were compared for the amount of 12% sodium hypochlorite needed to maintain 50-100 µg/ml available chlorine for 1,000 bins of Red Delicious apples dumped, 109 liters was required for filtered and 131 L for nonfiltered water for fruit dumped between April 27 and May 15, 1987.

The average pH of the water for all runs which used chlorine was between 5.8 and 6.2 and did not require the addition of buffer for effective action.

Discussion

The McIntosh immersion water contained four times more propagules/ml than the Golden Delicious and three times more than the Spartan dump-tank water (Table 1). This indicated that the bins of McIntosh contained more apples with sporulating *Penicillium* spp. on them and were a greater storage risk. For all varieties, propagule concentrations were sufficiently high (greater than 1,500 per ml) in the immersion water after emptying approximately 200 bins that they posed a significant risk of infecting fruit if injury to the fruit should occur. Spotts (4) has shown for decay of pears that infection increases most rapidly with inoculum concentrations under 1,000 conidia/ml and that as concentrations go to 1,500 conidia/ml there is no further increase in infection.

Filtration was partially effective in removing propagules of *Penicillium* spp. from the dump-tank water. For example, with Spartan apples, the filtered water contained 967 propagules/ml compared to 4,400 propagules/ml for nonfiltered water (Table 1). Furthermore, the filtered water was from Spartan apples that were dumped in April compared to nonfiltered water which was from apples that were dumped in early February. Normally, fruit stored for a longer time would be expected to produce more propaguleslml in the dump-tank water (4).

Unfortunately, the filter system did not always work efficiently. A major problem with its operation was the need for frequent backwashing. This was not always practical under commercial operating conditions leading to water only being partially filtered. The filter system was effective at times but too erratic in its present form to provide reliable water sanitation. It was not able to lower propagule concentrations to levels low enough to remove the risk of infection to injured apples. Its main value lies in its ability to prolong the use of the dump-tank water by removing debris and enabling longer packing runs before the tank must be cleaned. Use of the filter also reduces the amount of dump-tank chemicals released into the environment. Filtration must be used in association with a method of disinfection such as chlorination to destroy pathogens in the dump-tank water.

Chlorine was very effective in destroying propagules of *Penicillium* spp. in filtered Red Delicious dump-tank water reducing the average number from 10,538 to 7. Chlorine was not quite as effective in nonfiltered water but nevertheless maintained a concentration of 22.8 propaguleslml.

It is clear from these results that 50-100 µg/ml of free chlorine is very beneficial in reducing the likelihood of infection by *Penicillium* spp. Fruit immersed into nonchlorinated water quickly becomes contaminated with high numbers of *Penicillium* spp. propagules. This fruit is more susceptible to infection because there is a much higher chance that the pathogen will be present at the site **of** an injury and, for this reason, the fruit is at a higher risk to decay as it moves into the marketing channels.

Acknowledgments

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