Pressure Inoculation as a Technique for Postharvest Studies on Fungal Pathogens of Pome Fruits

P. SHOLBERG and M. MEHERIUK, Research Scientists, Agriculture Canada Research Station, Summerland, British Columbia V0H 1Z0, Canada, and W. McPHEE, Okanagan Similkameen Cooperative Growers Association, Oliver, British Columbia V0H 1T0, Canada

ABSTRACT

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The total number of freshly harvested apples that decayed when pressure inoculated with a suspension of 10^3 conidia per milliliter of *Penicillium expansum* depended on the cultivar: 85% decayed for Spartan, 77% for McIntosh, 75% for Golden Delicious, and only 33% for Red Delicious. Puncture and dip inoculation provided 100% infection with all four cultivars. Pressure inoculation of McIntosh with 0, 10^3, and 10^6 conidia per milliliter of *P. expansum* showed a highly significant linear relationship between conidial concentration and total number of fruit decayed. The incidence of rot in Bosc pears pressure inoculated with 10^4 conidia per milliliter of *P. expansum*, *Botrytis cinerea*, or *Phialophora malorum* was 62, 43, and 30%, respectively. Addition of 4% $CaCl_2$ to a suspension of conidia of *P. expansum* at 10^4/ml or greater reduced the number of decayed Golden Delicious apples from 63 to 50%. When 350 ppm of benomyl was added to 4% $CaCl_2$ in a suspension of 10^5 conidia of *P. expansum* per milliliter, the percentages of decayed fruit of McIntosh, Golden Delicious, and Spartan were futher reduced from 39, 37, and 23% to 8, 1, and 0%, respectively. Pressure inoculation disrupts lenticels and allows an avenue of entry for infection by hyphae. Pressure inoculation permits large quantities of fruit to be inoculated at one time and can be useful in determining the efficacy of current and new fungicides.

The relationship of decay area to flesh calcium content in stored apples has been investigated by Conway (2) and Conway and Sams (3,4). Higher flesh calcium contents achieved through vacuum or pressure infiltration of apples with $CaCl_2$ solutions reduced the area of decay caused by *Penicillium expansum* Link. Pressure infiltration has also been used to increase flesh calcium in Spartan apples in order to prevent internal breakdown (Meheriuk and McPhee, *unpublished data*). It became apparent during the latter studies that pressure infiltration could be an effective tool for the inoculation of fruit with conidia. This paper discusses the applicability of pressure inoculation in experiments on control of postharvest pathogens in pome fruits. In this study, wherever pressure infiltration is used as a technique for infecting fruit it is referred to as pressure inoculation.

MATERIALS AND METHODS

Pressure inoculation, experiment 1 (1979). Golden Delicious apples, picked at commercial maturity from the Summerland Research Station orchard, were inoculated under pressure within 10 days of harvest with three concentrations of suspensions of conidia of *P. expansum*, with or without 4% $CaCl_2$. The suspensions of 10^4, 10^5, or 10^6 conidia per milliliter were applied at 414 kPa for 3 min in a 1,125-L retort cooker at 20 C. Four replicates of 15 fruit each were used in all treatments. Treated and control fruit were held in air at 0 C for 95 days and then evaluated for total number of decayed fruit.

Pressure inoculation with fungicide and $CaCl_2$, experiment 2 (1980). Golden Delicious, Spartan, and McIntosh apples picked at commercial maturity from the Summerland Research Station orchard were pressure inoculated within 10 days of harvest with a suspension of 10^5 conidia of *P. expansum* per milliliter in 4% $CaCl_2$. Inoculation pressures were 69, 138, and 207 kPa for 3 min. Benomyl at 350 ppm also was added to the solutions for some of the treatments in order to control decay fungi. Each treatment contained two replicates of 50–60 apples each. All fruit were stored in air at 0 C for 90 days and then evaluated for total number of decayed fruit.

Bosc pears, obtained from Hood River, OR, were pressure inoculated (69 kPa for 3 min) within 14 days of harvest with suspensions of 10^4 conidia per milliliter of *P. expansum*, *Botrytis cinerea* Pers. ex Fr., or *Phialophora malorum* (Kidd & Beaumont) McColloch. The solutions contained either 4% $CaCl_2$ or 0.3% sodium orthophenylphenate. For the second part of the study, Anjou pears, obtained from the Summerland Research Station, were dipped or pressure inoculated within 10 days of harvest in four concentrations of conidia of *P. expansum* (0, 10^2, 10^4, and 10^6 conidia per milliliter). Another set of treatments included two levels of conidia (10^2 and 10^6) and four concentrations of benomyl (44, 88, 175, and 350 ppm) applied as Benlate 50WP. All pears were stored in air at 0 C (120 days for Bosc and 150 days for Anjou) and then evaluated

Table 1. Effect of pressure versus dip inoculation of *Penicillium expansum* on the percent of decayed Golden Delicious apples

Treatment	Decayed fruit (%)
Pressure	86
Dip	28
Analysis of variance	
Pressure vs. dip	***[a]

[a]*** = Significant at the $P = 0.001$ level.

Table 2. Influence of cultivar and mode of inoculation[a] on total number of apples decayed by *Penicillium expansum*

Cultivar	Inoculum	Decayed fruit (%)	
		Puncture + dip	Pressure inoculation
Golden Delicious	Water	88 ± 8[b]	12 ± 3
	Conidia	100 ± 0	75 ± 3
McIntosh	Water	92 ± 10	18 ± 16
	Conidia	100 ± 0	77 ± 14
Spartan	Water	100 ± 0	35 ± 15
	Conidia	100 ± 0	85 ± 6
Red Delicious	Water	97 ± 4	2 ± 4
	Conidia	100 ± 0	33 ± 14

[a]Apples were either dipped or pressure inoculated (207 kPa for 3 min) with a suspension of 10^3 conidia per ml.

[b]All treatments were replicated four times, except pressure-inoculated water for Red Delicious, which was replicated three times.

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for total number of decayed fruit.

Pressure inoculation, experiment 3 (1986). McIntosh, Golden Delicious, Spartan, and Red Delicious apples from the Summerland Research Station, picked at commercial maturity, were subjected within 10 days of harvest to the following treatments: 1) puncture plus dip in tap water; 2) puncture plus dip in conidial suspension; 3) pressure inoculation with tap water; and 4) pressure inoculation with conidial suspension.

Fruit was punctured to a depth of 5 mm with three finishing-nail heads imbedded on a board. The nail heads were arranged in a triangular fashion with 5 mm between nails. The conidial suspension contained 10^3 conidia per milliliter of *P. expansum*. Inoculation pressure was 207 kPa for 3 min. Treated fruit were stored for 90 days in air at 0 C and then evaluated for decay after an incubation period of 7 days at 20 C.

McIntosh apples were subjected to a factorial set of treatments involving three pressures (69, 138, and 207 kPa), three infiltration times (1, 3, and 5 min), and three inoculum concentrations (0, 10^3, and 10^6 *P. expansum* conidia per milliliter). Treated fruit were stored in 0 C air for 90 days and then evaluated for total number of decayed fruit after an incubation period of 7 days at 20 C.

Fruit in the above experiments were visually examined for decay and if an individual fruit had one or more decay areas of 1 cm or more, it was considered a rot. Degree of decay was not considered in this study, but in many cases, especially where pressure inoculation was used, several circular areas of decay coalesced into a large area of decay.

The data from the above experiments were subjected to an analysis of variance and the degrees of freedom and sum of squares for treatments were partitioned into component comparisons.

Pressure apparatus. A 1,125-L retort cooker connected to an air pump (620 kPa) was used for the pressure infiltration of all solutions. Inside the cooker was a mobile stainless steel tank capable of holding eight boxes of fruit (approximately 70–80 fruit per box). Sufficient conidial suspension was placed in the tank to cover the fruit and weighted covers were used to keep the fruit submerged during treatment. An external pressure gauge monitored pressure within the retort cooker.

Electron microscopy. Apple lenticels were examined by scanning electron microscopy (5) to observe the effect of pressure infiltration, at 207 kPa, upon them.

RESULTS AND DISCUSSION

Method of inoculation. Inoculation by pressure was more effective than dipping on the number of apples decayed by *P. expansum* when Golden Delicious apples were inoculated in 1979 (Table 1). Puncture of apples followed by a dip in a conidial suspension resulted in a 100% incidence of decay in the four cultivars tested in 1986 (Table 2). Pressure inoculation without puncture was quite effective in establishing infection by *P. expansum* in Golden Delicious, Spartan, and McIntosh, but only fair in Red Delicious (Table 2). The surprising observation in the above study was the high incidence of decay with punctured apples dipped in tap water, possibly because of contamination by conidia of *P. expansum* from the fruit, holding sacks, dip tank, and storage environment. Pressure inoculation of unwounded apples with the same tap water and stored in the same environment resulted in a much lower percentage of decayed apples.

Electron microscopy of pressure-treated fruit revealed injury to lenticel tissue. The lenticel cavity had been widened and tissue lining the cavity was disrupted to expose parenchyma beneath. Examination of many injured lenticels did not uncover any conidia within a lenticel, but hyphae were found inside the lenticels from *P. expansum* conidia that had germinated nearby.

Effect of conidial concentration. No effect of conidial concentration was observed with Golden Delicious in treatments that included $CaCl_2$, but when each method of inoculation was analyzed separately the number of apples decayed following both pressure inoculation and dipping showed linear relationships with conidial concentration (Table 3). The percentage of dipped fruit that decayed increased with the higher conidial concentration but decreased for pressure-inoculated fruit. Results in 1986 showed a highly significant linear and quadratic relationship between conidial concentration and number of decayed fruit of pressure-inoculated McIntosh apples (Table 4). Infection rate was high at 10^3 conidia per milliliter, but only a small increase in number of decayed fruit was noted at 10^6 conidia per milliliter. The latter result may explain the lack of a linear trend in the earlier trial (Table 3) where levels of 10^4, 10^5, and 10^6 conidia per milliliter were used.

Effect of pressure. No significant effect of pressure was observed over the range of 69–207 kPa (Table 4).

Effect of $CaCl_2$ and fungicides. Addition of $CaCl_2$ to the conidial suspension reduced the total number of Golden Delicious apples decayed by *P. expansum* (Table 5), but not the total number of Bosc pears. Inclusion of 350 ppm of benomyl to a conidial suspension of *P. expansum* almost eliminated decay in McIntosh, Golden Delicious, and Spartan apples (Table 6). Sodium orthophenylphenate was ineffective in reducing the number of Bosc pears decayed by *P. expansum*, *B. cinerea*, or *Phialophora malorum*. Decay incidence

Table 3. Effect of *Penicillium expansum* conidial concentration on the percent of decayed Golden Delicious apples when comparing pressure versus dip inoculation

Concentration (conidia/ml)	Decayed fruit (%)	
	Pressure inoculation	Dip inoculation
10^4	97	19
10^5	89	27
10^6	71	37
Analysis of variance		
Concentration	NS	NS
Linear	NS	NS
Quadratic	NS	NS
Concentration × pressure vs. dip	***[a]	
Linear	***	
Quadratic	NS	

[a]*** = Significant at the $P = 0.001$ level.

Table 4. Effect of *Penicillium expansum* conidial concentration on the percent of decayed fruit of pressure-inoculated (69, 138, and 207 kPa for 1, 3, and 5 min) McIntosh apples

Concentration (conidia/ml)	Decayed fruit (%)
0	23
10^3	79
10^6	82
Analysis of variance	
Concentration	***[a]
Linear	***
Quadratic	***
Pressure	NS
Linear	NS
Quadratic	NS
Time	NS
Linear	NS
Quadratic	NS

[a]*** = Significant at the $P = 0.001$ level.

Table 5. Effect of $CaCl_2$ on the percent of Golden Delicious apples decayed by *Penicillium expansum* when pressure inoculated

Treatment	Decayed fruit (%)
0% $CaCl_2$	63
4% $CaCl_2$	50
Analysis of variance	
$CaCl_2$	***[a]

[a]*** = Significant at the $P = 0.001$ level.

Table 6. Effect of cultivar when pressure inoculated (69, 138, and 207 kPa) with a 4% $CaCl_2$ solution containing 10^5 conidia per milliliter of *Penicillium expansum* and 350 ppm of benomyl on percent of decayed fruit

Cultivar	Decayed fruit (%)	
	Benomyl (0 ppm)	Benomyl (350 ppm)
McIntosh	39	8
Golden Delicious	37	1
Spartan	23	0
Analysis of variance		
Cultivar	***[a]	
Benomyl	***	
Cultivar × benomyl	*[b]	

[a]*** = Significant at the $P = 0.001$ level.
[b]* = Significant at the $P = 0.05$ level.

in the Bosc pears was significantly highest with *P. expansum* at 62% and lowest at 30% with *P. malorum*. In Anjou pears, no decay was observed in any of the dip treatments. The pressure-inoculated fruit had rot incidences of 15, 18, 17, and 48% with *P. expansum* conidial concentrations of 0, 10^2, 10^4, and 10^6 conidia, respectively. Benomyl, at a rate of 44 ppm, prevented decay even at the highest conidial concentration (48% without benomyl and 0% with benomyl).

The beneficial effect of calcium on reducing decay in apples inoculated with fungal conidia has been reported by Conway (2) and Conway and Sams (3,4). Calcium levels in excess of 1,200 ppm (dry weight) in apple tissue would be required to reduce decay significantly in Delicious apples (2). Our results also showed that inclusion of benomyl with the inoculum could either prevent or sharply reduce decay (Table 6). On the other hand, pressure infiltration with calcium leads to increased postharvest decay if the solution is contaminated with conidia of *P. expansum*. Our results showed that when 350 ppm of benomyl was not added to the 4% solution of $CaCl_2$, the incidence of rots increased from 3 to 33%. Pressure infiltration of fungicides would likely produce a higher level of fungicide residue in the fruit than either dipping or spraying, and specific tests to determine that this residue would not infringe on legislated levels would be necessary before this technique could be used commercially.

Pressure inoculation can serve as a useful technique in the inoculation of pome fruits and in the evaluation of fungicide efficacy. The pressure-inoculation method allows large quantities of fruit to be inoculated at one time. Because infection was somewhat dependent upon cultivar, some cultivars, such as Red Delicious, may not be suitable for this method because of their low rate of infection. Red Delicious apples apparently have fewer open lenticels than Golden Delicious or McIntosh (1). Whether susceptibility to inoculation by the pressure technique will remain fairly constant from year to year is unknown.

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