FINAL PROJECT REPORT

Project Title: Biology and control of powdery mildew on sweet cherry.

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Budget History:

Item	**Year 1:** 2003	**Year 2:** 2004	**Year 3:** 2005	**Year 4: 2006**
Salaries	15000	15675	16560	16560
Benefits	524	548	1418	1418
Wages				
Benefits				
Equipment				
Supplies	500	500	500	500
Travel	300	300	300	300
Miscellaneous				
Total	16324	17023	18778	18778

Project Objectives:

1. Determine when fruit infection occurs in relation to maturity.
2. Examine the effect of temperature and relative humidity on fruit infection.
3. Develop an early detection method for PM on fruit.
4. Establish a baseline for powdery mildew (PM) resistance to demethylation inhibitors (DMI's) fungicides.
5. Evaluate foliar mildew levels under various management regimes.
6. Study the relationship between powdery mildew infection and pitting.

Significant Findings:

- ✓ Fruit remain susceptible to PM throughout the growing season, potentially gaining some resistance after reaching 15 ºBrix.
- ✓ Powdery mildew is able to effectively infect fruit under a range of temperature and relative humidity combinations.
- ✓ qPCR - quantification
- ✓ PM isolates resistant to DMI's have been found.
- ✓ Orchard management practices impact the amount of PM infection. Rootstock selection, training system, and cultivar selection all influence the development of PM.
- ✓ Pitting responses to injury vary by cultivar and are related to the temperature at which the injury occurred. PM worsens pitting.

Results and Discussion:

<u>Objective 1: Determine when fruit infection occurs in relation to maturity. Figures 1, 2, & 3.</u>

The timing of cherry fruit infection is a key piece of information that can be invaluable in control and management strategies of PM. A study designed to answer this question was initiated in 2003 and was repeated in subsequent years of this project. Bags were removed from each fruit cluster for a one-week period throughout the growing season so that fruit were exposed to PM spores and infection. Fruit were assessed for mildew incidence upon harvest, and a sampler monitored the daily number of conidia in the orchard air. Three cultivars, Bing, Lapins, and Sweetheart, were included. In 2003, bags were made of Typar material that was not conducive to fruit development. Sensors confirmed that relative humidity within the Typar bags was extremely elevated compared with ambient conditions (data not presented). In the three remaining years of the project, bags were made of a nylon fabric that allowed gas and moisture exchange, yet excluded PM conidia. Sensors confirmed that the relative humidity and temperature within the nylon bags was similar to ambient condition (data not presented).

Among the cultivars, Sweetheart consistently had the highest incidence of PM infection and Bing the lowest. The 2004 growing season had the highest infection level of the three years, and the 2006 season had the lowest for each cultivar. The air sampler indicated similar amounts of conidia present in the orchard for both 2004 and 2006 (maximum values of 531.31 and 553.06 conidia/m^3, respectively) and much lower conidia in 2005 (maximum value of 293.11 conidia/m^3). Data were collected for each of the three cultivars in season 2004, but only cultivar Sweetheart in 2005, and Bing and Sweetheart in 2006. The cool, wet weather conditions in spring 2005 and 2006 were such that pollination was poor and/or flowers suffered frost damage, resulting in loss of fruit for this study in cultivars Bing and Lapins in 2005 and only Lapins in 2006.

In the case of Lapins and Sweetheart, the incidence of powdery mildew was significantly greater on fruit never covered with a bag (positive control) than fruit always covered with the bag (negative control). With Bing, the two controls were not statistically different. This may be due to lower infection rates observed in Bing. Other studies have indicated that cultivar Bing is less susceptible to foliar infection by PM when compared with cultivars Sweetheart and Lapins (report below). Fruit infection was not statistically different among the treatments in which bags were removed for a one week period. In each of the three cultivars, fruit remained susceptible to PM throughout the growing season. Fruit infection declined somewhat near the point at which fruit reached 15 °Brix, about 1 ½ weeks before harvest. Perhaps some slight resistance is gained once 15 °Brix is reached, but these studies show evidence to the contrary of this theory. Powdery mildew incidence was statistically the same in the weeks before and after 15 °Brix was reached consistently.

Objective 2: Examine the effect of temperature and relative humidity on fruit infection. Tables 1 & 2.

Optimal conditions for fruit infection, including temperature and relative humidity, have not been well studied. Studies by Grove found that leaf infection is optimal at 20 and 25 °C, and that infection could occur under a range of temperature and relative humidity combinations. Conidia produced from leaf infections most likely initiates infection on cherry fruit. Thus, knowledge of the optimal temperature and relative humidity for fruit infection will aid in the development of a PM prediction model for fruit infection.

A laboratory assay using detached fruit of cultivar Bing was developed to evaluate temperature and relative humidity combinations. Preliminary studies in 2004 led to the successful inoculation of detached cherry fruit with PM. In 2005, this assay was done with twelve combinations of temperature and relative humidity chosen to represent common environmental conditions during four key stages of fruit development, full bloom, initiation of pit hardening, completion of pit hardening, and harvest. In 2006, this assay was repeated but modified to include only one temperature, 18 °C, and six levels of relative humidity. In each year, immature fruit were inoculated by touching a sporulating leaf to the fruit surface. After being held at the appropriate temperature and relative humidity treatment for six days, fifty spores per fruit were assessed for germination. A conidium was deemed germinated if it had at least one hypha twice the length of the conidium. Extensive colonization of the fruit surface and any sporulation were also noted.

In both 2005 and 2006, the differences between treatments on percent germination were not statistically significant, indicating that PM has excellent fitness for infecting sweet cherry fruit under the wide range of temperature and relative humidity conditions tested. Germination was quite low in 2006 in comparison with 2005. An average of 6.8% of the evaluated conidia germinated, while 14.2% germinated in 2005. The highest percentage of spore germination in 2005 occurred at 18.6 °C, which corresponds to average day time high temperature during pit hardening, usually the last week of May at MCAREC. Analysis revealed that the number of fruit with extensive hyphae was not independent of the relative humidity at which fruit were incubated. Thus, humidity had an effect on the growth capabilities of PM. This temperature was then used for the 2006 studies. About 10% of the inoculated fruit developed secondary hyphae and/or an extensive colony of hyphae at the point of inoculation but did not sporulate in 2005. In 2006, this percentage was 5.6%. The majority of fruit in this category were incubated at 18.6 °C regardless of relative humidity. In 2006, fruit held at 92% relative humidity had the highest germination rate. Of all the fruit inoculated in both studies, only one sporulated; it was incubated at 15.5 °C and 68% relative humidity for six days.

These results contrast a similar study by G. Grove, where spore germination increased with increasing relative humidity. This study found no such relationship. Grove’s study, however, defined spore germination as when the germ tube length exceeded the width of the spore, only included one temperature (20 °C), and held the detached fruit for a maximum of 24 hours. These results indicate that PM is capable of infecting cherry fruit under a wide range of environmental conditions.

Objective 3: Develop an early detection method for PM on fruit.

A polymerase chain reaction (PCR) technique was successfully developed to identify cherry PM in both fruit and leaf tissue. PCR uses certain primers designed specially for cherry PM that are specific to enough to delineate cherry PM from other common PM fungi. PCR is a molecular tool that clones cherry PM DNA so that it can be quickly detected on plant tissue, even before it can be seen with the naked eye. Techniques for using both regular and quantitative PCR, which allows cherry PM DNA to be quantified, have been worked out. Future diagnostic, population, and genetic studies will benefit from this.

Objective 4: Establish a baseline for powdery mildew (PM) resistance to demethylation inhibitors (DMI's) fungicides. Tables 3 & 4.

Several orchardists in the PNW have expressed concern about losing effectiveness of certain fungicides used to control PM, particularly in the class of DMI's. Studies on several other crops have positively identified resistance of PM to certain DMI's; so, a preliminary study was undertaken in 2005 to assess DMI resistance in cherry PM.

A leaf disk assay was used to test five commercially available DMI's: Elite (tebuconazole), Orbit (propiconazole), Procure (triflumizole), Rally (myclobutanil), and Rubigan (fenarimol). Of the ten orchards evaluated, five were suspected as having PM resistance to one or more fungicides and were located in Hood River, The Dalles, and Prosser. Procure was the only fungicide that seemed to retain its effectiveness in all of the orchards. In 2006, techniques were refined and the orchard area expanded to best determine whether or not an orchard has DMI resistant PM.

One of the changes in 2006 was to use monoclonal isolates as the inoculum source as opposed to the mix of isolates from one orchard used in 2005. To obtain a monoclonal isolate of PM, a single conidium is transferred to a leaf using a hair. Furthermore, inoculation was done by transferring a single chain of conidia with a hair to each leaf disk in the assay following the application of the fungicide. PM isolates were collected from orchards in Wenatchee, Yakima, Parkdale, Mosier, Hood River, and The Dalles, and at least ten monoclonal isolates per orchard were attempted. Sweet cherry PM was found to be difficult to culture, and many monoclonal isolates did not survive or did not produce sufficient conidia to carry out the leaf disk assay. In total, thirteen monoclonal isolates were successfully evaluated. Procure again held up remarkably well in that none of the isolates showed any loss of sensitivity to it. A great variability existed among isolates, even when collected from the same orchard. For example, one isolate collected from an Orchard 8 was resistant to Orbit, Rally, and Rubigan, but another isolate from Orchard 8 was not resistant to any of the DMI's. Two isolates from two different orchards, Orchard 6 and 8, were sensitive to all of the DMI's tested and showed no signs of resistance. One of these, Orchard 6, is a certified organic operation.

DMI fungicides have a single mode of action and target only one gene to control fungi. Resistance develops much more quickly in single mode of action fungicides, because a simple mutation in the fungus can decrease the effectiveness of the fungicide. The life cycle of PM ensures great genetic variability among this group of fungi, so that resistance to DMI's is a valid concern. These results confirm that resistance to DMI's does exist among populations of cherry PM in the PNW. However, this study was not thorough enough to determine the prevalence of these isolates in orchards. A great effort should be made to educate growers about the importance of engaging strategies to preserve the effectiveness of the DMI's currently available, such as using the maximum labeled rate and rotating DMI's with fungicides from other classes.

Objective 5: Evaluate foliar mildew levels under various management regimes. Figures 4 - 7.

Management practices, such as pruning, cultivar selection, and rootstock selection, influence the development of PM in an orchard. In 2003 and 2004, ten shoots of current year's growth were collected per tree, and the incidence of PM was recorded for the outer most ten leaves. No more than ten trees per management practice were surveyed. Three training systems (steep leader, central leader, and Spanish bush), four rootstocks (Edabriz, Maxima 14, Pontileb, and Mazzard), and five cultivars (Bing, Lapins, Regina, Staccato, and Sweetheart) were compared. All of the trees were part of other, ongoing studies.

In both years, PM incidence was significantly greatest in trees trained with the Spanish bush system. Spanish bush pruning strategies promote heavy branching and dense foliage that would diminish air movement through the canopy, thereby creating a more favorable environment for PM. Both central and steep leader are more conducive to encouraging air flow through the canopy. With rootstocks, Mazzard consistently had the significantly highest PM incidence and Edabriz the least. This is also likely related to air flow in the canopy, as trees with Mazzard rootstock have a much larger, denser canopy. Edabriz is a more dwarfing rootstock, which enables greater air circulation.

A range of PM resistance was evident among the five cultivars. In both 2003 and 2004, cultivar Regina had the lowest incidence of PM. In 2003, Sweetheart had the highest incidence, and Staccato had the highest incidence in 2004. Bing was always the cultivar with the second lowest incidence, and Lapins was the third.

These results illustrate the importance of PM consideration when selecting a training system, rootstock, and cultivar, particularly for new orchards. If the orchard is known or has the potential to have high PM infection levels, then cultivar selection, training system, and/or rootstock should be carefully chosen. Selecting a cultivar such as Regina and/or using a central leader training system can lower or delay a PM epidemic and ultimately dependence on fungicides.

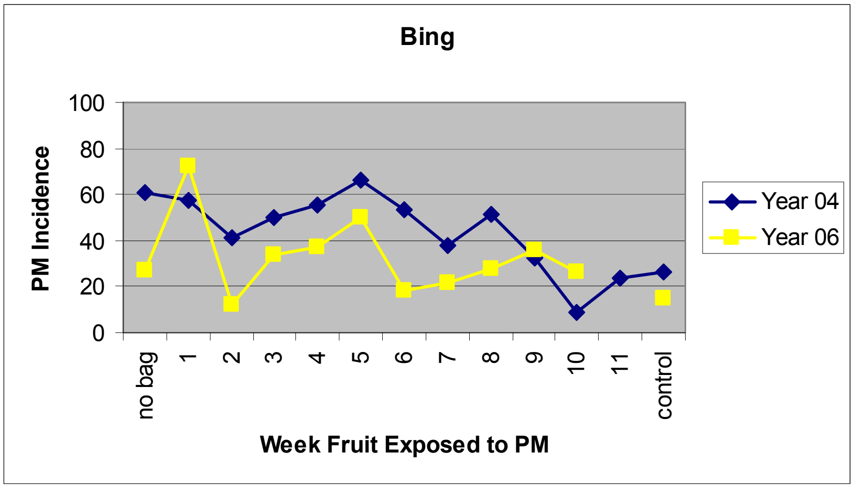
Objective 6: Study the relationship between powdery mildew infection and pitting. Tables 5 - 11.

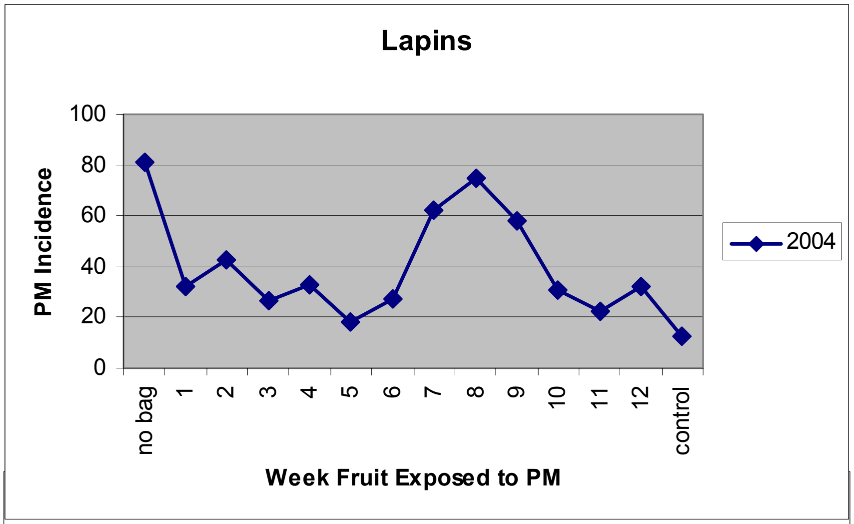
PM has been proposed to worsen the effects of pitting, resulting from handling/injury to the fruit. Studies in 2004, 2005, and 2006 were designed to clarify the relationship between PM and pitting. Infected fruit were collected and categorized based on their percent surface area infected: no PM (0%), slight PM (1-33%), moderate PM (34-66%), or severe (67-100%). Cultivars Bing, Lapins, and Sweetheart received a standard injury at 1, 4, or 20 °C with a special tool. After two weeks of storage at either 1 or 4 °C, pitting was rated on a scale where 1 = no pitting, 2 = slight pitting, 3 = moderate pitting, and 4 = severe pitting. Due to poor fruit set, only cultivar Sweetheart was included in 2005, and only Lapins with no PM or slight PM infection levels were included in 2006.

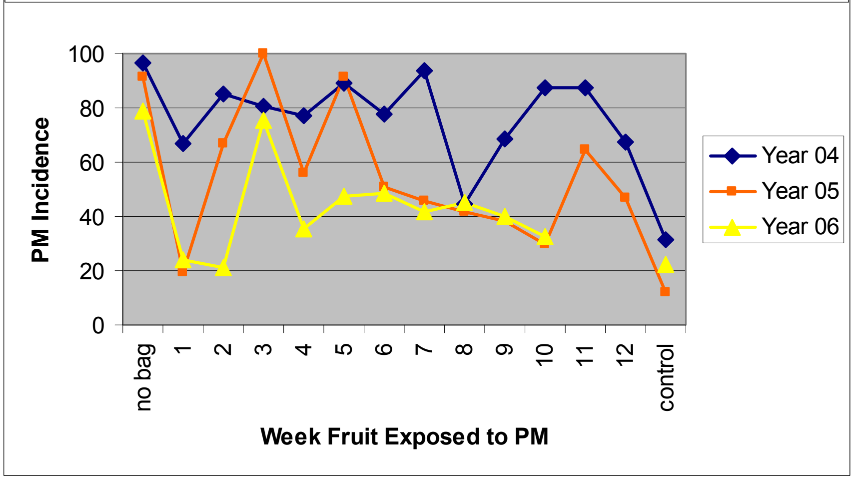
In each of the three years, cultivar Sweetheart had the greatest injury due to pitting, suggesting a possible cultivar effect. Storage temperature had no effect on pitting. With both Bing and Sweetheart, pitting was related to the temperature at which the injury occurred; the impact delivered at 1 °C resulted in significantly greater pitting than those at 20 °C. With Lapins, this effect was inexplicably opposite in 2004 but held true in 2006. The effect of temperature is in accordance with previous reports by Lidster and Tung.

PM worsened pitting in all of the studies except Bing 2006, where PM had no effect on pitting. Whether or not PM is directly responsible for this trend is unknown. In 2004 and 2005, mildewed fruit were less mature in terms of size, color, and °Brix. Previous studies by Facteau and Rowe have shown that immature fruit are more susceptible to pitting than mature fruit. Mildew might be delaying fruit maturity and thus causing an increase in pitting damage, or mildew impacts the integrity of the fruit, somehow rendering it more susceptible to pitting.

Figures 1, 2 & 3. Average percent fruit infected, or PM incidence, in the bagging study for each cultivar. The positive control where fruit were never bagged is indicated by “no bag. ” Subsequent weeks correspond to the period of time when fruit were exposed (bag removed) and vulnerable to PM infection. The negative control where fruit remained covered by a fabric bag the entire season is indicated by “control.” PM incidence ratings were done following harvest.







Tables 1 & 2. Results from the detached fruit inoculation study. The average percent of germinated conidia with hyphae at least twice as long as its conidium, are listed as % Germinated Conidia relative the temperature-relative humidity treatment. The number of fruit with extensive hyphal colonization from the detached fruit inoculation study is presented. A Chi-squared test for homogeneity revealed that extensive, secondary hyphae production is not independent of the temperature – relative humidity treatment in 2005. In 2006, there were no differences among treatments.

2005				
Temperature		Relative Humidity	% Germinated Conidia	# Fruit with Extensive Hyphae
°C	°F			
12.79	55	70	12.25	2
		79	15.17	2
		88	11.42	1
15.46	60	68	9.17	3
		75	6.50	1
		83	14.00	5
18.62	65	68	12.33	6
		74	22.50	4
		80	9.25	2
23.76	75	70	7.25	0
		75	7.50	1
		79	16.58	0

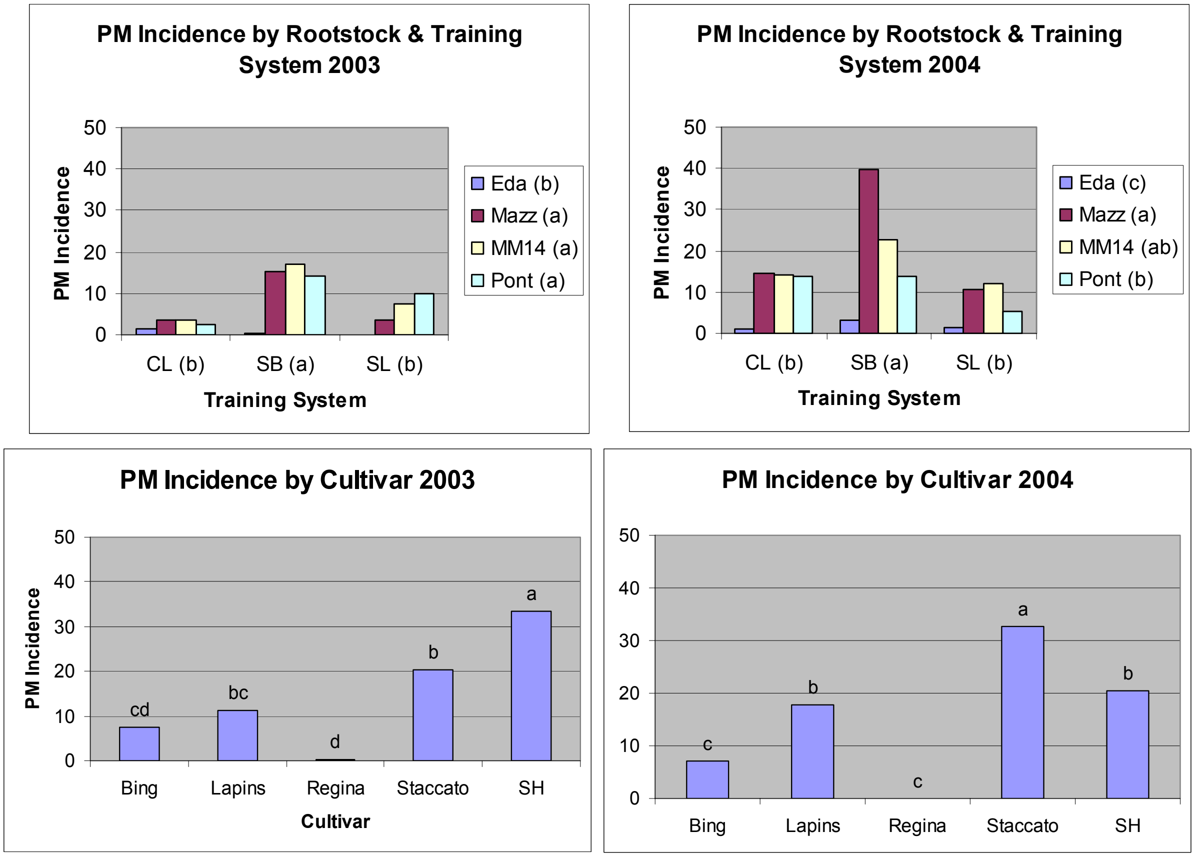
2006; temp = 18.6 °C		
Relative Humidity	% Germinated Conidia	# Fruit with Extensive Hyphae
68	8.08	2
74	4.67	2
80	5.75	0
86	4.42	1
92	11.92	0
98	6.08	3

Table 3 & 4. ED50 values (mg/L) from resistance studies 2005 and 2006.

2005 Orchard	Location	Elite	Orbit	Procure	Rally	Rubigan
1	**The Dalles**	54.20	47.86	15.85	42.66	5128.00
2	**The Dalles**	9.77	3.8×10^{37}	11.75	10.47	1.5×10^{39}
3	**The Dalles**	18.62	12.88	19.05	15.85	1.1×10^{46}
4	**Prosser**	18.62	10.47	12.02	7.94	11.22
5	**Hood River**	12.30	12.59	15.14	14.13	9.12
6	**Hood River**	8.71	7.08	10.96	9.77	17.38
7	**Hood River**	66.68	10.96	20.89	10.23	7.59
8	**Hood River**	8.13	53.70	13.49	21.88	8.71
9	**Hood River**	11.22	7.41	10.96	9.77	13.49
10	**Prosser**	8.71	7.94	12.88	7.41	77.62
	Max Labeled Rate	**67.41**	**43.17**	**149.80**	**59.92**	**62.60**

2006 Orchard	Location	Elite	Orbit	Procure	Rally	Rubigan
1	**Wenatchee**	419.76	79.43	25.18	15.21	97.28
2	**The Dalles**	1570.36	5023.43	31.48	33.65	688.65
3	**Prosser**	74.30	23.55	17.02	21.88	5.12×10^{21}
4	**Hood River**	65.31	47.32	24.49	212.32	35.89
5	**Hood River**	737.90	1559.55	14.39	19.72	1741.81
5	**Hood River**	25.24	12.27	13.84	35.34	100.23
6	**Mosier**	32.66	9.33	14.09	19.06	11.75
7	**Wenatchee**	28.84	82.22	11.78	10.09	11.75
7	**Wenatchee**	633.87	8.47	10.86	11.43	51.40
8	**Yakima**	21.98	3.27×10^{8}	11.35	4560.37	3.1×10^{17}
8	**Yakima**	31.48	17.50	10.94	10.52	42.76
9	**Yakima**	18.62	81.66	12.82	17.91	21.48
9	**Yakima**	112.20	864.97	22.54	31.33	583.45
	Max Labeled Rate	**67.41**	**43.17**	**149.80**	**59.92**	**62.60**

Figures 4 - 7. Average percent PM infected leaves by different management systems. Rootstock abbreviations are as follows: Eda = Edabriz, Mazz = Mazzard, MM14 = Maxima 14, and Pont = Pontileb. Training system abbreviations are as follows: CL = central leader, SB = Spanish bush, and SL = steep leader. In all figures, statistical differences are indicated by the lower case letter in parentheses.



Tables 5 - 11. The average rating of pit development following a standard impact on fruit with varying degrees of PM infection. A pit rating of 0 = no damage, 1 = slight damage, 2 = moderate damage, and 3 = severe damage. Prior to the injury, fruit were sorted based on their level of PM infection where No PM = no visible PM, Slight PM = 1 – 33% of the fruit surface colonized, Moderate (Mod.) PM = 34 – 66% of the fruit surface colonized, and Severe PM = 67 – 100% fruit surface colonized by PM. Statistical differences in the severity of pitting among the fruit infection levels and temperature at which the fruit were injured are indicated by different letters.

Bing 2004					
Injury Temp C	Storage Temp C	Powdery Mildew Rating			
		No PM (a)	Slight PM (a)	Mod. PM (a)	Severe PM (b)
1 (c)	1	3.08	2.72	2.92	3.20
1 (bc)	4	2.48	2.68	2.48	3.52
4 (b)	1	2.52	2.48	2.88	2.92
20 (a)	1	2.08	2.16	2.20	2.36

Lapins 2004					
Injury Temp C	Storage Temp C	Powdery Mildew Rating			
		No PM (a)	Slight PM (ab)	Mod. PM (bc)	Severe PM (c)
1 (ab)	1	2.28	2.40	2.68	2.96
1 (a)	4	2.32	2.48	2.56	2.40
4 (ab)	1	2.48	2.48	2.68	2.64
20 (b)	1	2.56	2.68	2.72	2.84

Sweetheart 2004					
Injury Temp C	Storage Temp C	Powdery Mildew Rating			
		No PM (a)	Slight PM (b)	Mod. PM (b)	Severe PM (b)
1 (b)	1	2.56	2.88	2.76	2.96
1 (b)	4	2.56	2.88	2.84	2.88
4 (ab)	1	2.44	2.68	2.68	2.80
20 (a)	1	2.32	2.56	2.80	2.68

Sweetheart 2005					
Injury Temp C	Storage Temp C	Powdery Mildew Rating			
		No PM (a)	Slight PM (a)	Mod. PM (b)	Severe PM (b)
1 (a)	1	2.12	2.12	2.64	2.60
1 (a)	4	2.08	2.20	2.44	2.60
4 (a)	1	1.80	1.84	2.52	2.84
20 (a)	1	1.72	1.84	2.56	2.68

Bing 2006					
Injury Temp C	Storage Temp C	Powdery Mildew Rating			
		No PM (a)	Slight PM (a)	Mod. PM (a)	Severe PM (a)
1 (b)	1	2.24	2.04	1.96	1.74
1 (b)	4	1.96	2.28	2.36	2.28
4 (b)	1	2.12	1.80	2.24	2.00
20 (a)	1	1.67	1.36	1.64	1.40

Lapins 2006			
Injury Temp C	Storage Temp C	Mildew Rating	
		No PM (a)	Slight PM (a)
1 (b)	1	2.21	2.17
1 (b)	4	2.24	1.96
4 (b)	1	2.00	1.96
20 (a)	1	1.40	1.30

Sweetheart 2006					
Injury Temp C	Storage Temp C	Powdery Mildew Rating			
		No PM (a)	Slight PM (b)	Mod. PM (b)	Severe PM (ab)
1 (b)	1	2.60	2.72	2.76	2.64
1 (ab)	4	2.24	2.60	2.44	2.56
4 (ab)	1	2.28	2.76	2.88	2.48
20 (a)	1	2.12	2.40	2.60	2.20