FINAL PROJECT REPORT

Project Title: Factors affecting the fruit phase of cherry mildew

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Total Project Funding: $ 200,147

Budget History: WSU-IAREC

Item	Year 1: 2013	Year 2: 2014	Year 3: 2015
Salaries	33,504	34,844	36,238
Benefits	17,087	17,770	18,481
Wages	7,075	7,075	7,075
Benefits	667	667	667
Supplies	3,600	1,000	1,000
Travel	1,000	1,000	1,000
Total	62,933	62,286	$64,461

Budget History: OSU-MCARES

Item	Year 1: 2013	Year 2: 2014	Year 3: 2015
Wages	2,686	2,767	2850
Benefits	215	221	228
Supplies	500	500	500
Miscellaneous			
Total	3,401	3,488	3,578

Original objectives

1) Determine the inoculum concentration threshold for infection of cherry fruit at different developmental stages.

2) Determine the effects of temperature and relative humidity (40% - 99%) on infection and spore production (conidia) of *P. clandestina* on infected cherry fruit.

3) Conduct in-depth studies on the temporary susceptibility of several cultivars of cherry fruit to infection by *P. clandestina* in orchard studies.

4) Evaluate quinoxyfen as a key management component of the fruit phase of powdery mildew, overall maintenance of fruit quality, and prevention of postharvest diseases.

5) Investigate the susceptibility of cherry flowers to infection by *P. clandestina* and the potential relationship between blossom and fruit infection.

DISEASE INCIDENCE
Proportion of diseased leaves/ fruit out of the total number of leaves/ fruit observed

DISEASE SEVERITY
Proportion of infected leaf/ fruit surface area

Significant Findings

- Cherry fruit infection is a subtle process. Infection stays unnoticeable during the growing season, making predictions about disease incidence and severity at harvest very difficult.
- Incidence and severity of fruit disease depends on inoculum concentrations. In general, the more spores are deposited on fruit surfaces; the more disease can be expected.
- Interaction between inoculation dates and inoculum concentrations revealed a dependency of disease development and fruit developmental stages (time of spore deposition).
- Neither humidity nor temperature promoted disease on developing cherry fruit at any time before June.
- Fruit transitions from resistant to susceptible sometime in June (BBCH scale 85 to 87). Phenological development or time of year may be important.
- During the resistance phase powdery mildew spores remain quiescent on fruit surfaces
- Ontogenic resistance (disease resistance increases with maturity) is true for leaves but not for fruit
- Quinoxyfen (Quintec™) alone does not reduce powdery mildew on fruit
- Quinoxyfen (Quintec™) in rotation with Fontelis did reduce incidence and severity of foliar and fruit infection. The reduction was not always statistically significant but perhaps economically important
- Quinoxyfen (Quintec™) in rotation with Procure did increase incidence and severity of foliar infection in 2013

- Fungicide rotations (Quintec, Pristine, Fontelis, Topguard, and Procure) did not affect fruit quality or pitting susceptibility
- Pristine® reduced fruit disease (statistically significant) consistently in 2014 and 2015
- Prebloom through fruit set is NOT a critical period for the establishment of powdery mildew infection and does not relate to fruit infection at harvest, unlike the grape: *E. necator* pathosystem.

Results and Discussion

Objective 1

In the orchard studies, differing inoculum concentrations and inoculation dates caused variation in disease incidence and disease severity. Significant variations *(P<0.05)* were observed in mean disease incidence and disease severity among fruits inoculated with different conidia concentrations (Table 1). Generally, disease incidence and disease severity increased with increasing inoculum concentrations. Interaction between inoculation dates and inoculum concentrations revealed dependency of disease development on growth stages of fruits (Fig.1a and b). As age of inoculated fruits increased the percentage of infected fruits and the percentage of fruit area covered by powdery mildew increased. The minimum conidial concentration needed to cause both significant disease incidence and significant disease severity varied depending on the inoculation dates or growth stage of fruits. A minimum inoculum concentration of 500 conidia/ml was needed for significant fruit infection (disease incidence and severity) in fruits inoculated in June and a minimum inoculum concentration of 1000 conidia/ml was needed for significant fruit infection on relatively young fruits inoculated in May (Table 1).
In studies on detached fruit in small environmental chambers, only matured fruits inoculated the end of June showed significant disease development. On those fruits, conidial concentration had significant effects *(P<0.05)* on disease severity (Table 2). Disease severity increased with increasing conidial concentration. The conidial concentration of 5000/ml was minimum for 1% disease severity in mature detached fruits in the lab. Interaction between inoculation date and inoculum concentrations indicated dependency of disease development on growth stages of fruits besides conidial concentrations (Fig. 2).

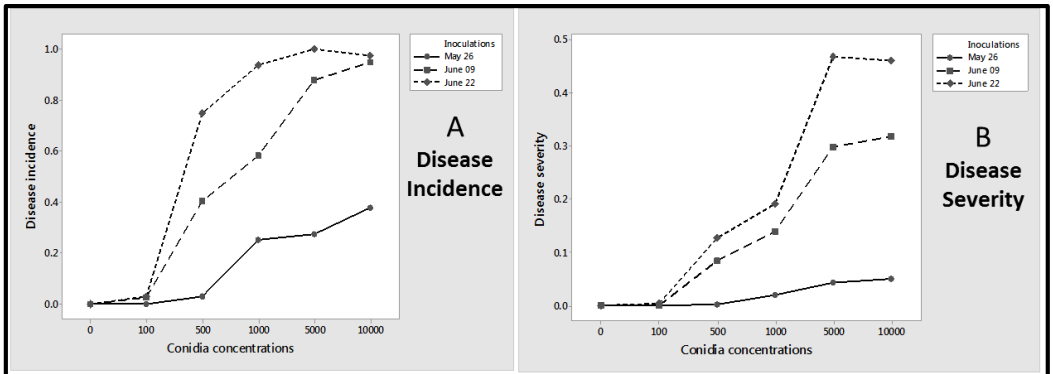

Fig 1 Interaction plot of conidia concentrations (ml^{-1}) and inoculation dates for disease incidence (A) and severity (B) in orchard experiments

Table 1 Powdery mildew disease incidence and severity on cherry fruits caused by inoculation of different suspensions of conidia of *Podosphaera clandestina* on different inoculation dates in orchard experiments - 2015

Inoculation dates	*May 26*		*June 09*		*June 22*	
Conidia concentration	**Powdery Mildew Disease**					
	Incidence	**Severity**	**Incidence**	**Severity**	**Incidence**	**Severity**
10000/ml	0.38 a*	0.050 a	0.95 a	0.32 a	1.00 a	0.46 a
5000/ml	0.27 a	0.044 ab	0.88 a	0.30 a	0.97 a	0.47 a
1000/ml	0.25 a	0.020 bc	0.59 b	0.14 b	0.94 a	0.19 b
500/ml	0.03 b	0.003 c	0.40 b	0.08 c	0.75 b	0.13 c
100/ml	0.00 b	0.00 c	0.02 c	0.0007 d	0.029 c	0.004 d
0/ml	0.00 b	0.00 c	0.00 c	0.00 d	0.00 c	0.00 d

* Results are averages of three replicates. Values for a variable within a column followed by a common letter are not significantly different based on Fisher least significant difference (LSD, *P=0.05*).

Table 2 Powdery mildew disease severity on cherry fruits caused by inoculation of different suspensions of conidia of *Podosphaera clandestina* on different inoculation dates in lab experiments – 2015

Inoculation dates	*June 04*	*June 14*	*June 24*
Conidia concentration	**Powdery Mildew Disease Severity**		
10000/ml	0.000 a*	0.00045 a	0.0300 a
5000/ml	0.000 a	0.0030 a	0.0300 a
1000/ml	0.000 a	0.0010 a	0.0065 b
500/ml	0.000 a	0.000 a	0.0000 b
100/ml	0.000 a	0.000 a	0.0000 b
0/ml	0.000 a	0.000 a	0.0000 b

* Results are averages of three replicates. Values for a variable within a column followed by a common letter are not significantly different based on Fisher least significant difference (LSD, *P=0.05*).

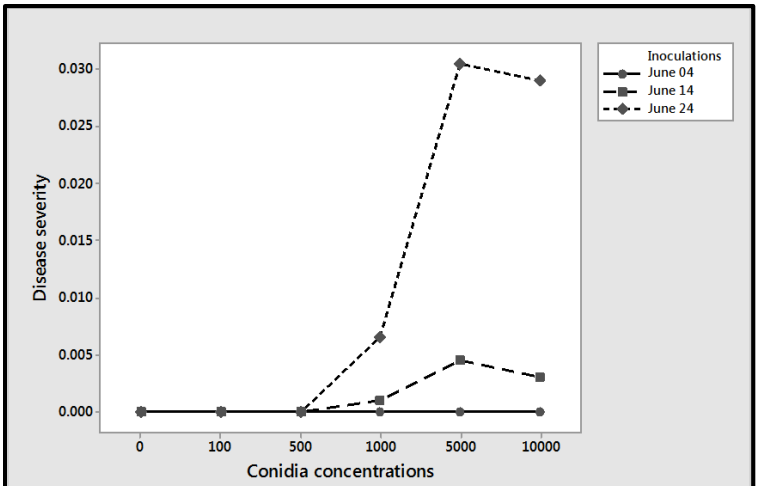

Fig 2 Interaction plot of conidia concentrations (ml^{-1}) and inoculation dates for disease severity in lab experiments.

Objective 2

Cherries (cultivars Bing and Sweetheart) at various developmental stages were used for temperature/ humidity studies. Surface sterilized, detached fruit were inoculated with 5000 spores/ml and incubated at 15^0C (59F), 20^0C (68F), and 25^0C (77F) in small controlled environment chambers (Fig 3). Relative humidity ranged from 10% to 99%. Disease incidence and severity was recorded every 7 days. Four replicates per temperature/humidity combination were assessed and experiments were continuously repeated throughout the season (Fruit set to harvest). No disease could be initiated on cherries picked before the middle of June independent of temperature and humidity regime. The same was true for laboratory fruit inoculation studies discussed in Objective 1. Cherries inoculated 70 days after full bloom showed signs and symptoms of disease in *vitro*. A significant increase in disease incidence was recorded at 25^0C (77F) independent of humidity. In general, disease incidence increased with rising humidity levels. However, the increase was not statistically significant ($P = 0.005$). In general, environmental factors do not seem to play a major role in the initiation of fruit disease. Even under ideal environmental conditions (as determined by our study: 25^0C and above 90% RH) fruit had an 'unexplained' resistance to powdery mildew until the middle of June. This is contrary to observations from leaves or other agricultural commodities (grapes, strawberries, hops) where young tissue is most susceptible to infection and ontogenic resistance develops with increasing maturity levels of the respective host tissue. The inability to produce disease on immature fruit directly reflects the inability of the fungus to produce disease on same aged fruit in the orchard.

Objective 3

Developing cherries were covered with Nitex bags around 4-27 in each years. Applying Nitex mesh covers by the end of April protected the developing cherries from airborne spores. No signs or symptoms were detected in the negative control in 2014 and 2015. In 2013, some disease was observed in some replicates of the negative control caused by an insufficient closure of the bags. Cherry development was not negatively impacted by the Nitex cover. First

Fig 3 Detached fruit assays using small humidity-controlled environment chambers

signs of foliar infection were observed 25 days after full bloom (DAFB) in 2014 and 32 DAFB in 2015. First signs of fruit infection were observed in the middle of June in both years. The time lag between earliest spore deposition and symptom development was 56 days (2014) and 49 days (2015). This is the longest time period of powdery mildew spore quiescence on host tissue ever reported. No consistent and significant relationships between date of inoculation or inoculation type (natural exposure to airborne spores versus spray inoculations with 5000 spores/ml) were observed. This indicates that spores deposited on fruit as early as the onset of disease on leaves will translate to fruit infection at harvest.

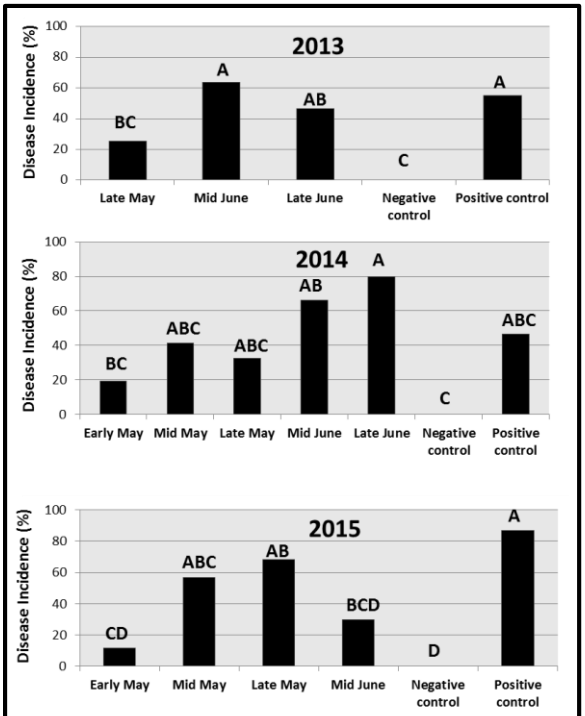

Fig 4 Powdery mildew disease incidence resulting from a two week exposure of fruit to airborne spores. Means with different letters are significantly different (P = 0.05) based on Tukey's HSD test. Data is from cultivar Bing, Prosser, 2013-2015

Objective 4

2013 was a mild powdery mildew year at the WSU experimental orchard, with an average disease incidence of 42% and severity of 2% in the untreated control. No significant effect of fungicide application on leaf disease incidence was found when compared to the untreated control (Table 3). However, a triple application of Quintec reduced disease severity by 15% (Table 3). This was the only significant reduction seen in 2013. In 2014, Procure and Topguard were replaced with Fontelis and Pristine. Additionally, stylet oil was applied in some treatments as a post-harvest control measure. The same fungicides rotations were applied in 2015. In 2014 and 2015, severe powdery mildew outbreaks were observed at the WSU experimental orchard, with an average disease incidence of 85.5 and 90.5 %, respectively. Leaf disease severity peaked at 54 and 32%, respectively, (Table 4 and 5). At harvest, 54% (2014) and 64% (2015) of all inspected fruit (n=400 per treatment) showed signs of infection. Disease severity reached 65% (2014) and 71% (2015) (Table 3). In 2014, one fungicide application (Treatment 4, Fontelis-Quintec rotation) reduced disease incidence significantly by 15.8% and severity by 48%. The same effect was not observed in 2015 (Table 5). However, treatments 2, 8 and 11 did reduce leaf disease severity significantly (P = 0.005) compared to the untreated control in both years (Table 4 and 5). However, none of these treatments reduced the total amount of leaves infected (incidence). Fruit disease was also significantly reduced by Fontelis®-Quintec™ rotations (Treatment 1, 3, and 4) in 2014 but not 2015. On fruit, application of Pristine® (Treatment 10 and 11) significantly reduced (P = 0.005) disease incidence and severity in both years. Pristine also significantly reduced leaf severity but not incidence (Tables 4-6). In this study, Pristine® was the only fungicide able to minimize leaf infection and reduce fruit disease consistently over time. The sole application of Quintec™ (quinoxyfen) is not a key management component for reduction of powdery mildew disease on leaves or fruit. Only in one year and under low disease pressure,

Quintec™ had a significant effect on leaf disease severity (Table 3). Under high disease pressure, disease reduction was only achieved in combination with Fontelis®. Even though not always statistically significant, reduction was great enough to have real life importance.

There were no significant differences in fruit firmness (FF), fruit size, soluble solid content (SSC), and titratable acidity (TA) of 'Bing' fruit at commercial harvest among the 11 treatments (Fig 5). Pitting susceptibility was not affected by the different fungicide treatments. After 2 weeks at 32°F, there were no differences in FF (increased ~20% compared to initial), TA (reduced ~15% compared to initial), fruit color, and stem browning among the fungicide treatments.

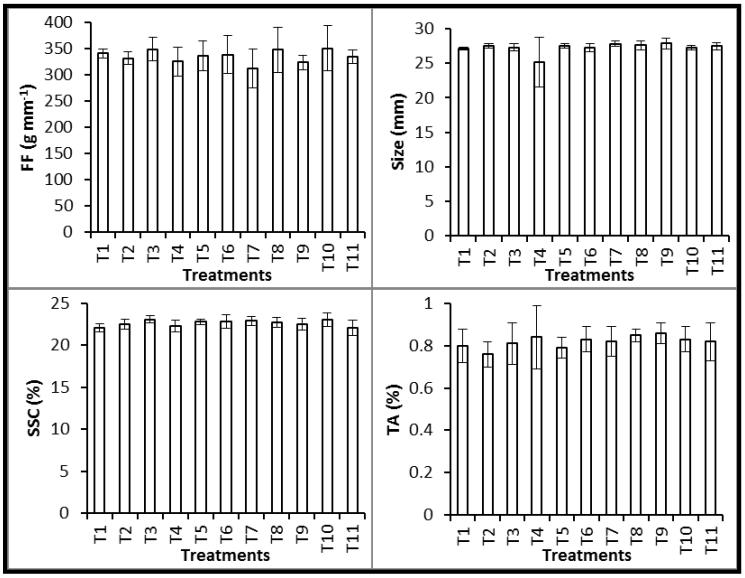

Fig 5 Effect of fungicide treatments (T1-T11) on fruit quality of 'Bing' cherries at harvest in 2015.

Table 3 Effect of fungicide rotations on sweet cherry powdery mildew incidence and severity on **LEAVES** - 2013

2013 TM T #	Pre-harvest Fungicide rotation*	Powdery mildew Incidence (%)**						Powdery mildew Severity (%)**					
		Upper canopy		Lower canopy		Combined		Upper canopy		Lower canopy		Combined	
1	Q-Q-PR	77	AB	35	ABCD	56	AB	2.9	BC	0.4	CD	1.7	BC
2	PR-Q-Q	83	AB	44	ABC	64	AB	2.9	BC	1.1	ABCD	2.0	B
3	PR-PR-Q	87	A	52	AB	70	A	2.6	BC	1.0	ABCD	1.8	BC
4	PR-PR-PR	83	AB	38	ABCD	61	AB	5.8	A	2.0	AB	3.9	A
5	PR-PR-PR	90	A	40	ABCD	65	AB	5.7	A	2.2	A	3.9	A
6	PR-PR-PR	65	ABC	21	BCD	43	BC	3.9	AB	1.2	ABCD	2.5	AB
7	Q-Q-Q	25	D	6	D	16	D	0.5	C	0.1	D	0.3	C
9	T^{14}-Q-T^{14}	46	CD	10	CD	28	CD	2.0	BC	0.2	D	1.1	BC
10	T^{14}- T^{14}- T^{14}	78	AB	35	A	57	A	2.6	BC	0.8	BCD	1.7	BC
11	T^{7}-T^{7}-T^{7}	71	ABC	63	A	67	AB	2.3	BC	1.4	ABC	1.9	BC
8	None	53	BCD	30	ABCD	42	BCD	2.8	BC	1.2	ABCD	2.0	B

*Q = Quintec 250SC, 7 fl oz/A, PR = Procure, 14.5 oz/A, T^{7}= Topguard 1.04SC, 7 fl oz/ A, T^{14} = Topguard 1.04SC 14 fl. Oz / A. Applied to run-off (400 gal/A = 1.5 gal per tree). Fungicide application dates: 5/7/2013, 5/23/2013, 6/6/2013

** Disease evaluation date: 7/21/13. Results are averages of four single tree replicates. Values for a variable within a column followed by a common letter are not significantly different based on Tukey's HSD test (*P=0.05*).

Table 4 Effect of fungicide rotations on sweet cherry powdery mildew incidence and severity on **LEAVES** - 2014

2014 TMT #	Pre-harvest	Post-harvest	Powdery mildew Incidence (%)**						Powdery mildew Severity (%)**					
	Fungicide rotation*	Oil^	Upper canopy^^		Lower canopy^^		Combined^^		Upper canopy^^		Lower canopy^^		Combined^^	
1	Q-Q-F-F	2x	95	A	77	AB	86	AB	65	AB	14	BC	40	AB
2	F-Q-Q-F	2x	100	A	74	AB	87	AB	47	AB	13	C	30	B
3	F-F-Q-Q	2x	100	A	69	AB	84.5	AB	55	AB	18	BC	37	AB
4	F-F-F-Q	2x	89	A	55	B	72	B	40	B	15	B	28	B
5	Q-Q-Q-Q	2x	95	A	66	AB	80.5	AB	44	AB	31	AB	38	AB
6	Q-Q-Q-Q	none	99	A	72	AB	85.5	AB	53	AB	27	ABC	40	AB
8	F-F-F-F	2x	100	A	87	AB	93.5	AB	49	AB	20	BC	35	B
9	F-F-F-F	none	100	A	78	AB	89	AB	53	AB	27	ABC	40	AB
10	P-P-P-P	2x	100	A	71	AB	85.5	AB	60	AB	26	ABC	43	AB
11	P-P-P-P	none	95	A	62	AB	78.5	AB	45	AB	11	C	28	B
7	None	none	95	A	76	A	85.5	A	68	A	40	A	54	A

Table 5 Effect of fungicide rotations on sweet cherry powdery mildew incidence and severity on **LEAVES** - 2015

2015 TMT #	Pre-harvest	Post-harvest	Powdery mildew Incidence (%)**						Powdery mildew Severity (%)**					
	Fungicide rotation*	Oil^	Upper canopy^^		Lower canopy^^		Combined^^		Upper canopy^^		Lower canopy^^		Combined^^	
1	Q-Q-F-F	2x	99	A	80	A	89.5	A	22	C	18	A	20	AB
2	F-Q-Q-F	2x	97	A	65	A	81	A	21	C	10	A	15	B
3	F-F-Q-Q	2x	100	A	65	A	82.5	A	21	C	5	A	13	B
4	F-F-F-Q	2x	100	A	60	A	80	A	39	AB	8	A	23	AB
5	Q-Q-Q-Q	2x	98	A	75	A	86.5	A	29	BC	10	A	19	AB
6	Q-Q-Q-Q	none	100	A	74	A	87	A	32	BC	6	A	19	AB
8	F-F-F-F	2x	100	A	69	A	84.5	A	32	BC	4	A	18	B
9	F-F-F-F	none	100	A	71	A	85.5	A	24	BC	14	A	19	AB
10	P-P-P-P	2x	98	A	85	A	91.5	A	19	C	12	A	16	B
11	P-P-P-P	none	97	A	68	A	82.5	A	22	C	8	A	15	B
7	None	none	100	A	81	A	90.5	A	50	A	14	A	32	A

Table 6 and 7:

*Q = Quintec 250SC, 7 fl oz/A, F = Fontelis, 20 oz/A, P = Pristine, 14.5 oz/A. Applied to run-off (400 gal/A = 1.5 gal per tree). Fungicide application dates: 4-30, 5-14, 5-28, 6-10-2014. Oil application dates: 7-1-14, 7-15-14.

** Disease evaluation date: 7/1/2014. Results are averages of four single tree replicates. Values for a variable within a column followed by a common letter are not significantly different based on Tukey's HSD test (*P=0.05*).

^Post-harvest treatment, 2x = applied twice in a 14 day interval, none = no fungicides or oil were applied.

^^ 25 leaves from 5 branches were evaluated on the upper portion of the tree (upper canopy) and the lower portion of the tree (lower canopy) for a total of 50 leaves per tree (combined)

Table 6 Effect of fungicide rotations on sweet cherry powdery mildew incidence and severity on **FRUIT** – 2014 & 2015

TMT	Pre-harvest	Post-harvest	Powdery mildew FRUIT disease (%)**							
			2014				2015			
	Fungicide rotation*	Oil^	Incidence		Severity		Incidence		Severity	
1	Q-Q-F-F	2x	10	B	10	B	41	AB	41	AB
2	F-Q-Q-F	2x	28	AB	33	AB	35	AB	35	AB
3	F-F-Q-Q	2x	15	B	15	B	38	AB	38	AB
4	F-F-F-Q	2x	12	B	13	B	49	AB	48	AB
5	Q-Q-Q-Q	2x	25	AB	28	AB	34	AB	34	AB
6	Q-Q-Q-Q	none	23	AB	25	AB	37	AB	37	AB
8	F-F-F-F	2x	34	AB	40	AB	45	AB	45	AB
9	F-F-F-F	none	34	AB	40	AB	34	AB	34	AB
10	P-P-P-P	2x	15	B	15	B	23	B	23	B
11	P-P-P-P	none	20	B	20	B	27	B	28	B
7	none	none	54	A	65	A	64	A	71	A

*Q = Quintec 250SC, 7 fl oz/A, F = Fontelis, 20 oz/A, P = Pristine, 14.5 oz/A. Applied to run-off (400 gal/A = 1.5 gal per tree). Fungicide application dates: 4/23/2015, 5/7/2015, 5/21/2015, 6/4/2015; Oil application dates: 6/18/2015, 7/2/2015.
**Disease evaluation date: 7/3/2014, 6/15/2015. Results are averages of four single tree replicates. 100 fruit per rep were evaluated. Values for a variable within a column followed by a common letter are not significantly different based on Tukey's HSD test (*P=0.05*).
^Post-harvest treatment, 2x = applied twice in a 14 day interval, none = no fungicides or oil were applied.

Objective 5

Prebloom through fruit set is a critical period for the establishment of powdery mildew infection in many agricultural commodities (e.g. apple, strawberry, hops, and grapes). Infections during this time can directly dictate severity of infection at harvest. The presence of sweet cherry powdery mildew on cherry flowers and its relation to fruit infection was investigated. Aerobiological studies have shown that the incidence of *P. clandestina* in the orchard air is very low during cherry bloom. Additionally, there is a three to four week gap between average blooming time of cherries (beginning of April, principal growth stage 6) and the onset of disease symptoms on leaves (beginning of May, principle growth stage 7). This is in stark contrast to other powdery mildew susceptible commodities, like apple and grape, where bloom occurs at times when powdery mildew is already established on the host. Flower inoculation experiments and species detection using PCR and *P. clandestina* specific primers were conducted during 2013-2015 to investigate the relationship between bloom and fruit infection. Detached cherry flowers inoculated with powdery mildew conidia did abort quickly after inoculation. The fragile petals turned brown and died within two days post inoculation. No spore germination was observed. In orchard studies, developing cherries protected with Nitex bags (applied at Shuck fall) did not develop any disease symptoms. Cherry flowers collected from experimental and commercial orchards (cultivars Bing, Early Robin, Lapin, and Sweetheart) were subjected to PCR assays using species specific primers. Powdery mildew was not detected in any sample during a three year period but the positive control. The low incidence of airborne conidia in combination with the absence of disease on cherries exposed to airborne spores during bloom, the natural absence of spores on flower petals, and the inability of flower petals to sustain infection strongly indicates that the flowering stage is not relevant to powdery mildew disease at harvest.

Executive Summary

Powdery mildew infection of cherry fruit is best characterized as a slow and invisible process. Developing fruit displays a long period of resistance to infection and the pathogen remains quiescent and viable on fruit surfaces for an extraordinary period of time. These findings are in contrast to findings related to foliar infections. Foliar infections actively progress during the season starting in the beginning of May (Bing cherries in Prosser: 32 days after full bloom in 2015 and 25 days after full bloom in 2014; BBHC scale 73 to 75) and ending with the production of overwintering structures in September and October (principle growth stage 9, BBCH stage 91 to 97). It has been generally assumed that powdery mildew conidia are ephemeral and do not persist without a susceptible host to thrive and proliferate. Sweet cherry powdery mildew persisted under adverse conditions for an 8 week period. However, inoculation concentration studies intimated that a greater number of spores need to be deposited on very young fruit to result in significant disease incidence at harvest. This indicates that not all spores survive the extended periods of quiescence. There is a biochemical line of communication between host and pathogen that still has to be elucidated. It is unknown what transmits resistance to susceptibility in cherries; a development always observed during the month of June (around 60 to 80 days after full bloom in 'Bing'). The inability of *P. clandestina* to thrive on fruit picked before June is clearly not a result of environmental factors. Even under ideal growth conditions, fruit infection could not be initiated *in vitro*. The only times *in vitro* studies resulted in infection was on fruit picked 60 to 80 days after full bloom (BBCH stages 85 to 87). Additionally, environmental conditions in the orchard already favor infection, as can be observed on actively sporulating powdery mildew colonies on leaves. The connection between time and/or crop phenology and disease onset is a key piece in understanding fruit infection and will be invaluable for the breeding program, in particular for breeding of new, powdery mildew resistant late season varieties.

Both the absence of ontogenic resistance (increasing resistance with maturity) in fruit and the fact that cherry bloom is not a critical period for infection *by P. clandestina* is contrary to what is a commonly observed pattern in many other powdery mildew species (apple, grape, hops, strawberry, etc.). Knowledge about critical periods of infection has been proven useful to direct management strategies. To date no critical infection period has been associated with infection of sweet cherry by *P. clandestina*. The continuous application of fungicides will remain crucial for disease management. Quinoxyfen (Quintec™) has become popular due to its novel mode of action. Rotation trials were conducted to elucidate the best application time during the season. Applications of Quintec™ (FRAC group 13) in rotation with Fontelis® (FRAC group 7) reduced incidence and severity of fruit infection, even though the effect was only statically significant in 2014. Pristine® (FRAC group 7 and 11) reduced fruit disease significantly in 2014 and 2015. It did not reduce the total number of leaves infected but reduced the severity of foliar infection. Protecting developing fruit from airborne spores remains a challenge. Most fungicides are protective and not fungicidal and need to be applied to cover the surfaces of both fruit and foliage. If coverage is incomplete, *P. clandestina* will use the untreated space to initiate infections. Since fruit are constantly and rapidly expanding, the protective fungicide layer becomes interrupted quickly leaving the fruit vulnerable to infection. The level and duration of protection of fruit during these rapid periods of expansion should be investigated.