CONTINUING PROJECT REPORT **YEAR**: 2011 (2 of 3)
WTFRC Project Number: CH-10-105

Project Title: Improved management of powdery mildew of sweet cherry

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Total Project Request: Year 1: 57,280 Year 2: $46,834 **Year 3: $56,905**

Budget
Organization Name: WSU-IAREC **Contract Administrator:** Carrie Johnston
Telephone: 509-335-4564 **Email address:** carriej@wsu.edu

Item	2010	2011	**2012**
Salaries[1]	35,281[1]	36,692	**33,612**[2]
Benefits	14,818	15,411	**17,142**
Wages			
Benefits			
Equipment			
Supplies	5,181[3]	5,188[3]	**4151**[3]
Travel[4]	2,000[4]	2,000[4]	**2,000**[4]
Miscellaneous			
Total	$57,280	$59,291	**$56,905**

Footnotes:
[1]postdoctoral research associate
[2]associate in research
[3]reagents for qPCR; field supplies
[4] travel to The Dalles and Hood River to read T/RH dataloggers and to conduct disease incidence and severity evaluations (objective 4).

OBJECTIVES

1) Determine the presence and regional extent of resistance to QoI fungicides in populations of *Podosphaera clandestina* in Eastern Washington.
2) Investigate "early" cherries as potential sources of inoculum for infection of later cherry fruit. The initial step in the process will be determining whether there is a large inoculum increase in a cv. 'Bing' orchard once fungicide applications are terminated at harvest.
3) Investigate irrigation sets during late dormancy as a means to deplete overwintered inoculum supplies prior to the availability of susceptible host tissue.
4) Investigate various irrigation regimens and nitrogen fertilizer regimens, on the incidence and severity of powdery mildew on cv. 'Lapins' cherries.
5) Investigate full-season fungicide programs for effectiveness in reducing the production of chasmothecia (cleistothecia) and therefore the amount of potential carryover inoculum.

SIGNIFICANT FINDINGS

- A leaf disc bioassay developed in 2010 and 2011 was used to screen isolates of *P. clandestina* for QoI resistance. A total of 18 isolates were collected from the Yakima, Wenatchee, and Columbia Valleys. Most isolates were sensitive to trifloxystrobin at up to 4x labeled rates but several appeared less sensitive at $\geq$ 160 ppm.

- Initial attempts to store powdery mildew over time were successful: *P. clandestina* survived 12 months following freeze drying at -30 C and long-term storage at -80 C (-112 F).

- The new Burkard cyclonic air sampler was compared to rotary impaction traps for the molecular detection of *P. clandestina* in the orchard air. The new sampler was found to be vastly superior to because of improved dependability and the ability to take daily samples with far less labor input and fewer steps in the laboratory components of the assay. PCR Cp values obtained from these air samples were significantly correlated (r = -0.86; $P < 0.001$ in 2010; -0.65; $P < 0.01$ in 2011) with daily spore counts taken by volumetric traps. Use of the cyclonic trap confirmed a large increase in aerial spore populations following harvest.

- Experiments designed to force (using irrigation) an ascospore release the depletion of the overwintered inoculum source were for the second year inconclusive.

- Full-season fungicide programs (standard preharvest programs + postharvest oil applications) were found to have no significant effects on chasmothecia production.

- Irrigation / fertigation experiments were inconclusive due to low mildew incidence and severity but voluminous amounts of microclimatic data were collected.

- Several experimental fungicides were effective against powdery mildew under high disease pressure

METHODS

Objective 1. *(QoI Resistance Survey).* Both conventional and molecular techniques were developed to study the presence and distribution of *P. clandestina* strains resistant to QoI fungicides. Our efforts in 2011 focused on applying the leaf disk bioassay. Isolates were collected from 1-2 orchards in each of The Prosser, Kiona, Benton City, Mattawa, Wenatchee, and Rock Island growing areas. Mass isolates were used. Infected cherry foliage was collected from each orchard site and conidia used to inoculate 'Sweetheart' leaf disks. Leaf disks were treated with 0, 80, 160, 320, 640, and 1280 ppm trifloxystrobin and then inoculated with a known concentration (10,000 / ml) of conidia of various isolates of *P. clandestina*. Disks were transferred to petri plates containing 1.5% water agar and incubated 14 days at 20 C. Seven leaf disks are inoculated in each of four single-plate replications.

Objective 2 *(inoculum sources)***.** A cv. 'Bing' orchard was used for this study. Rotorod and Burkard cyclonic spore traps were placed within and about 0.1 km downwind of the orchard. Traps were operated continuously beginning at bud burst and continuing through harvest of later varieties in the area. The concentration of inoculum of *P. clandestina* in the air at each sampling location was determined using quantitative PCR and primers developed in the WSU-IAREC pathology laboratory. Daily qPCR signal strengths were compared with actual daily spore counts.

Objective 3 *(inoculum depletion)***.** A 3 acre cv. 'Bing' orchard at WSU-IAREC was used for this portion of the study. The formation and dispersal large numbers of chasmothecia was documented at the site during the late summer and early autumn of 2010. Water was applied by handgun on about April 1, April 8, April 19, and May 10, 2011. At this time chasmothecia/ascospores were mature but there was not yet (aside from April 19 and May 5) much cherry foliage available for infection. The orchard was divided into 4 quadrants. Two quadrants were watered while two were left dry. The air of all quadrants was monitored using Rotorod air samplers. The presence and concentration of *P. clandestina* was determined using the quantitative PCR. The sampling periods compared were 1) 4 hours prior to watering 2) 4 hours after wetting 3) 15 hours during the subsequent evening and 4) 12 hours the following day.

Objective 4 (*irrigation and fertilizer influences*). Studies were conducted in a cv. 'Lapins' orchard near The Dalles, OR. Three fertigation treatments (#1 100 lbs N/acre delivered weekly via injection into irrigation lines, #2 100 lbs N/acre delivered to the ground in a spring split application, #3 60 lbs N/acre delivered weekly via injection into irrigation lines) were superimposed over (microsprinkler) irrigation treatments. Percentage water (irrigation treatments) are based on irrigating the 100% treatment at one acre-inch of water per set (one set per week). Other irrigation treatments include 80%, 60%, and regulated deficit. Treatments were arranged in a randomized complete block design with 5 replications. Each replication consisted of four trees with the center two serving as experimental units. Trees received normal powdery mildew treatments applied by the grower. However, polyethylene bags were used to cover selected branches during fungicide applications and removed immediately afterwards. "Bagged" branches served as untreated foliage and fruit. The incidence and severity of powdery mildew on fruit and foliage was *not* determined at harvest due to insufficient levels of powdery mildew. Temperature and relative humidity was monitored in selected irrigation treatments treatments using Hobo Pro U23-001 data loggers.

Objective 5 (*reduction of carryover inoculum*). A cv. 'Bing' orchard near The Dalles, OR was used for this portion of the study. Treatments were arranged in a randomized complete block design with 4 replications. Each replication consisted of 35 cv. 'Bing' trees. Four treatments were compared: 1) non treated control 2) standard preharvest fungicide program 3) standard preharvest program + a single 2% oil application applied two weeks after harvest 4) standard preharvest program + 2% oil

application applied four weeks after harvest and 4) standard preharvest program + two oil treatments applied two and four weeks postharvest. Several weeks after the cessation of fungicide programs 25 leaves were selected at random from each of 5 trees in the center row of each plot, air dried, comminuted in a blender, and the number of cleistothecia per cm^2 of leaf tissue determined using a dissection microscope.

RESULTS AND DISCUSSION

Objective 1. A leaf disk bioassay was developed to study practical resistance of orchard isolates. The technique involves inoculation of 'Sweetheart' leaf disks with a known quantity of conidia of *P. clandestina*. Leaf disks are incubated 10-14 days in petri plates containing 1.5% water agar. Fourteen of 18 isolates tested were sensitive to trifloxystrobin at all concentrations tested. However, isolates from 4 locations (2 from Mattawa and 2 from Benton City) grew on leaf disks treated with up to 320 ppm of fungicide. Two isolates grew at 1280 ppm trifloxystrobin. The obligate parasitic (needs to be cultured on an actively growing cherry plant) nature of *P. clandestina* makes studies of this nature inherently difficult. Methods to facilitate long-term storage of isolates were investigated during 2010-2011. Initial attempts to store powdery mildew over time were successful: *P. clandestina* survived 12 months following freeze drying (at -30 C) followed by storage at -80 C (-112 F). Further development of this technique should accelerate fungicide resistance studies and breeding efforts.

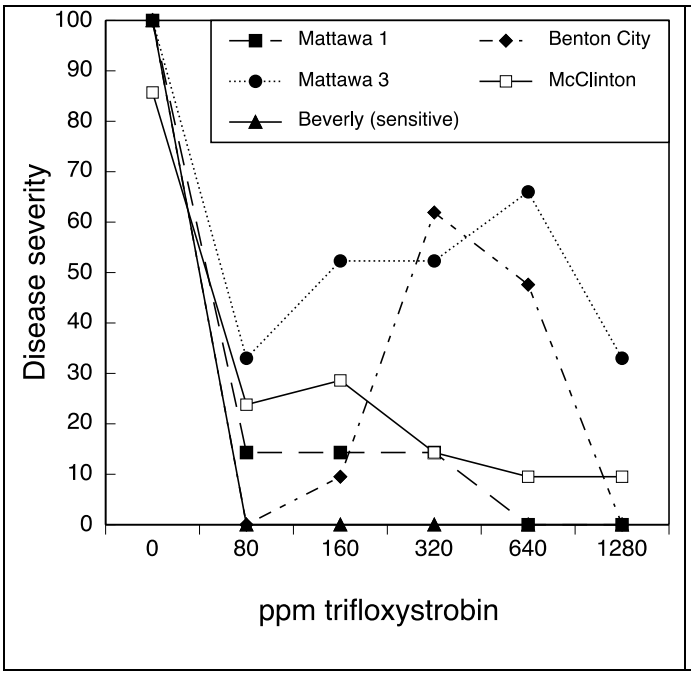

Figure 1. Growth of putative resistant isolates of *P. clandestina* on leaf disks of sweet cherry treated with 0-1280 ppm trifloxystrobin (Flint or Gem). A sensitive isolate (Beverly) is included for comparison.

Objective 2. A new Burkard cyclonic air sampler was evaluated in tandem with rotary impaction (Rotorod) traps for the molecular detection of *P. clandestina* in the orchard air. The new sampler was found to be vastly superior to Rotorod devices because of improved dependability and the ability to take daily samples with far less labor input and fewer steps in the laboratory components of the assay. PCR Cp values obtained from these air samples were correlated (r = -0.65; $P < 0.01$; Figure 2) with daily spore counts taken by volumetric traps. Use of the both volumetric and cyclonic traps confirmed a large increase in aerial spore populations following harvest. Series of these traps will be

used during 2012 in further studies on this objective.

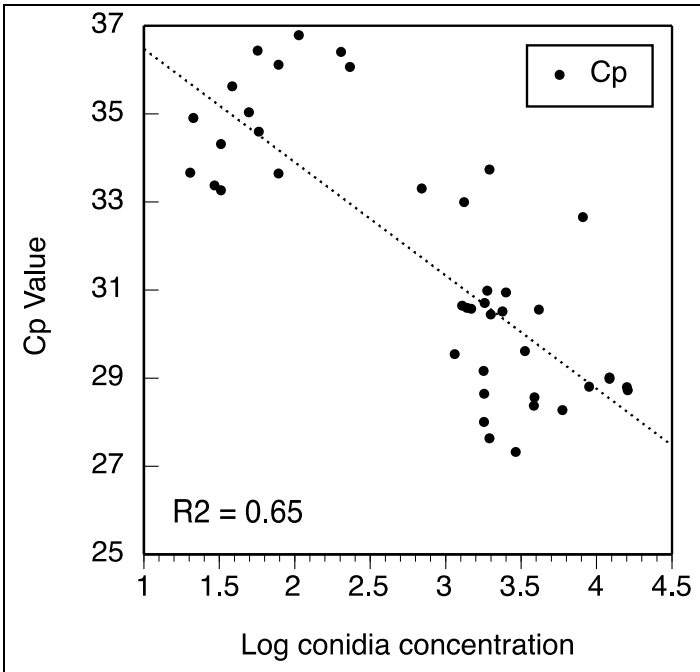

Figure 2. Association of PCR Cp values and daily conidia counts, 2011. Cp values were obtained using species-specific primers to determine the concentration of *P. clandestina* conidia in air samples taken daily. Actual conidia counts were taken using a Burkard volumetric spore trap.

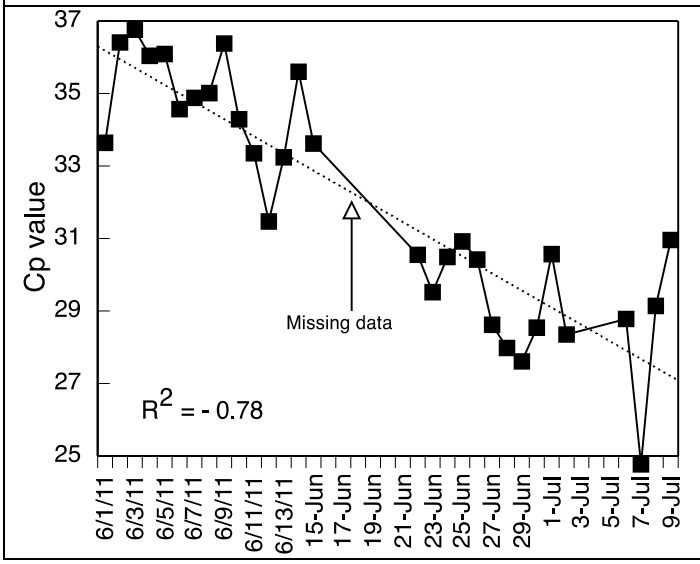

Figure 3. Association of qPCR signal strength and the logarithm of daily conidia during June-early July 2011. Note significant decrease in Cp concurrent with postharvest inoculum increase.

Objective 3. A major component of this research effort is related to the formation, dispersal, and functional or actual eradication/neutralization of chasmothecia, the overwintering propagule of *P. clandestina*. In other pathosystems, the real or functional elimination of such sources has resulted in delayed disease onset and reduced disease severity during the subsequent growing season. The results of early-season irrigation regimes to promote ascospore release were inconclusive (Figure 5). Differences in PCR signal strength were observed but there were no discernible patterns. The experiments will be repeated in 2012 in a larger commercial orchard using the new Burkard cyclonic air sampler.

Cp Values					
Date	**Treatment**	**Period 1**	**Period 2**	**Period 3**	**Comments**
4-1	Wet	35.08/36.71	40.82/na	Na/na	
	No Wet	Na/32.72	Na/33.13	37.4/34.86	
4-8	Wet	Na/na	37.6/37.6	Na/na	
	No Wet	Na/37.14	34.63/36.23	35.49/37.13	
4-19	Wet	35.43/Na	Na/na	40.4/na	
	No Wet	39.68/39.73	35.22/37.54	39.63/35.05	
5-10	Wet	37.64/44.61	36.42/44.19	36.11/37.32	
	No Wet	39.28/39.71	35.85/37.09	34.28/34.5	

Table 1. Quantitative PCR values obtained from air samples taken during the application of water to tree trunks and scaffold branches on April 12. Cp values are inversely proportional to amount of DNA in samples. Consistent differences were not observed during any of the four "watering" periods.

Objective 4. Experiments to designed to investigate the influence of irrigation and fertilizer regimens on powdery mildew incidence and severity were again unsuccessful due to low levels of disease. However, long-term experiments to determine the influence of various irrigation regimens on orchard microclimate established in 2010and will continue over the course of the three-year study. Weather stations placed in various irrigation regimes collect temperature and relative humidity values at 15-minute intervals. Obvious differences were apparent during a period of summer heat in 2011. Further analysis and mining of this voluminous data set continues.

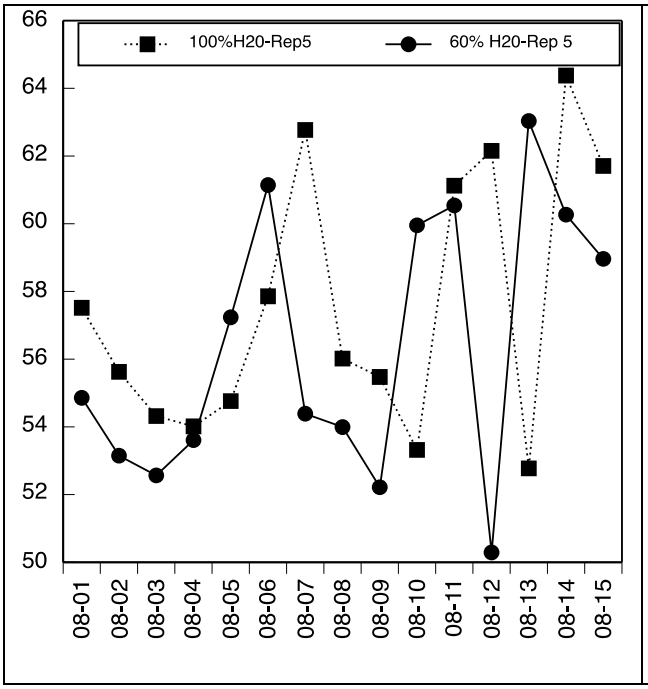

Figure 4. Relative humidity in 100% and 60% irrigation regimes during early August 2011.

Objective 5. The continuation of fungicide programs after harvest could perhaps reduce the number of

chasmothecia and therefore reduce potential carryover inoculum. However, the utilization of synthetic fungicides with high potential for the development (DMI, QoI, or quinoline) of resistant populations poses significant risks to the cherry industry. During 2011 we tested a "hybrid" full-season program: a preharvest alternation of synthetics/oils followed by several postharvest oil programs. This particular program resulted in no significant suppression of chasmothecia populations (Figure 5). We will in 2012 investigate various combinations of synthetic and contact compounds for suppression of chasmothecia formation in nursery studies.

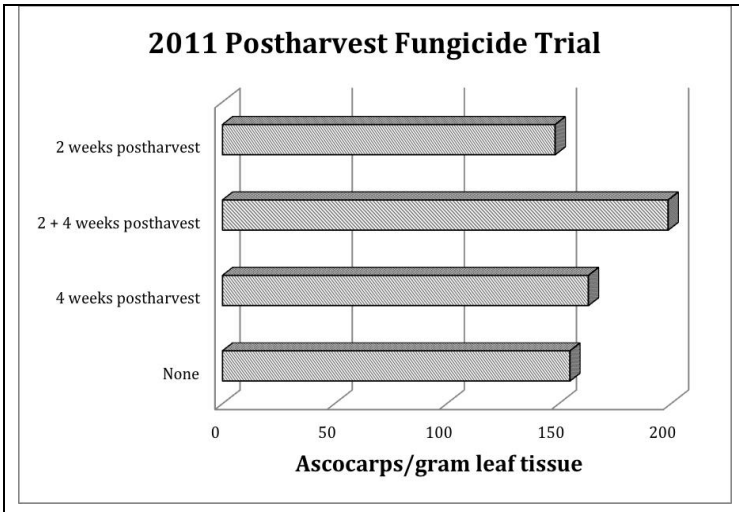

Figure 5 Chasmothecia (ascocarp) production on cv. 'Bing' trees receiving grower-standard preharvest fungicide program versus three full-season programs. There were no significant differences in chasmothecia production at $P < 0.05$