Final Project Report
WTFRC Project Number: CH-10-105

Project Title: Improved management of powdery mildew of sweet cherry

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Other Funding sources: Washington State Commission on Pesticide Registration $37,500 (QoI resistance objective)

Total Project Funding: $161,019 **Year 1:** 57,280 **Year 2:** $46,834 **Year 3:** $56,905

Item	2010	2011	2012
Salaries1	35,281[1]	36,692	33,612[2]
Benefits	14,818	15,411	17,142
Supplies	5,181[3]	5,188[3]	4151[3]
Travel[4]	2,000[4]	2,000[4]	2,000[4]
Total	**$57,280**	**$59,291**	**$56,905**

Objectives

1) Determine the presence and regional extent of resistance to QoI fungicides in populations of *Podosphaera clandestina* in Eastern Washington.
2) Investigate early or mid-season 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 ascocarps (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 and 30 isolates were collected from the Yakima, Wenatchee, and Columbia Valleys in 2011 and 2012, respectively. Most isolates were sensitive to trifloxystrobin at up to 4x labeled rates but several appeared less sensitive at $\geq$ 160 ppm. This technique also resulted in the development new technique to study the effects of temperature and relative humidity on infection and pathogen sporulation.

- 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) ascospore (in order to deplete the overwintered inoculum source) were more promising in 2012 than in 2010 and 2011, probably due to improvements in the molecular air sampling technique. PCR signals increased immediately after a period when water was applied for frost control. The assay most likely detected an irrigation-induced ascospore release.

- Standard fungicide programs were applied from shuck fall to harvest in 50% of a cv. 'Bing' orchard at WSU-IAREC. The other 50% was left untreated for the entire season. Disease severity and airborne inoculum rapidly increased in the treated section after fungicide applications were discontinued at harvest. Levels eventually exceeded those in the orchard section where no fungicides were applied. These results indicate that (once fungicide applications cease) early- to mid-season cherries may serve as a potential source of inoculum for late-maturing cherries in the vicinity. The postharvest increase in disease severity may also serve to significantly increase the production of ascocarps, the overwinter survival propagule. These results indictate the full-season disease management, similar to approaches

used for grapevine powdery mildew, may serve to mitigate problems with cherry mildew over time and that an areawide approach to mildew management should be considered.

- Full-season fungicide programs (standard preharvest programs + postharvest oil applications) were found to have no significant effects on ascocarp (cleistothecia, chasmothecia) production in the orchard. Oil applications had a significant effect on chasmothecia production in the nursery, indicating that they timing of oil applications may be critical and the narrow window (10-14 days in the nursery) may have been missed in previous orchard studies.

- Irrigation / fertigation experiments were inconclusive in 2010 and 2011 due to low mildew incidence and severity but voluminous amounts of microclimatic data were collected. Mildew incidence and severity was again low in 2012, but additional microclimatic data were collected.

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.

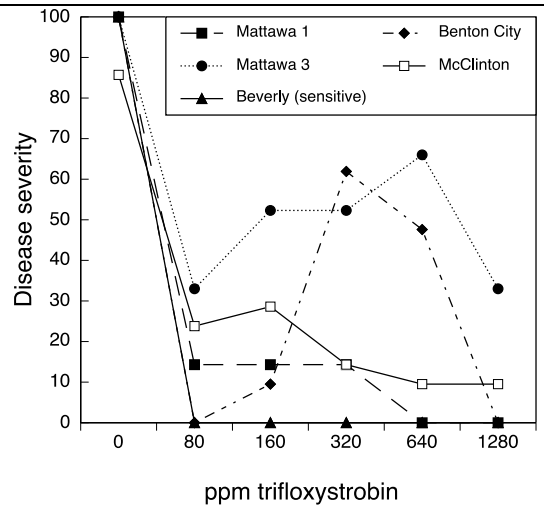

Figure 1. Growth of putative resistant isolates of *P. clandestina* on leaf disks of sweet cherry treated with 0-1280 ppm trifloxystrobin (Flint or Gem) in 2011. A sensitive isolate (Beverly) is included for comparison.

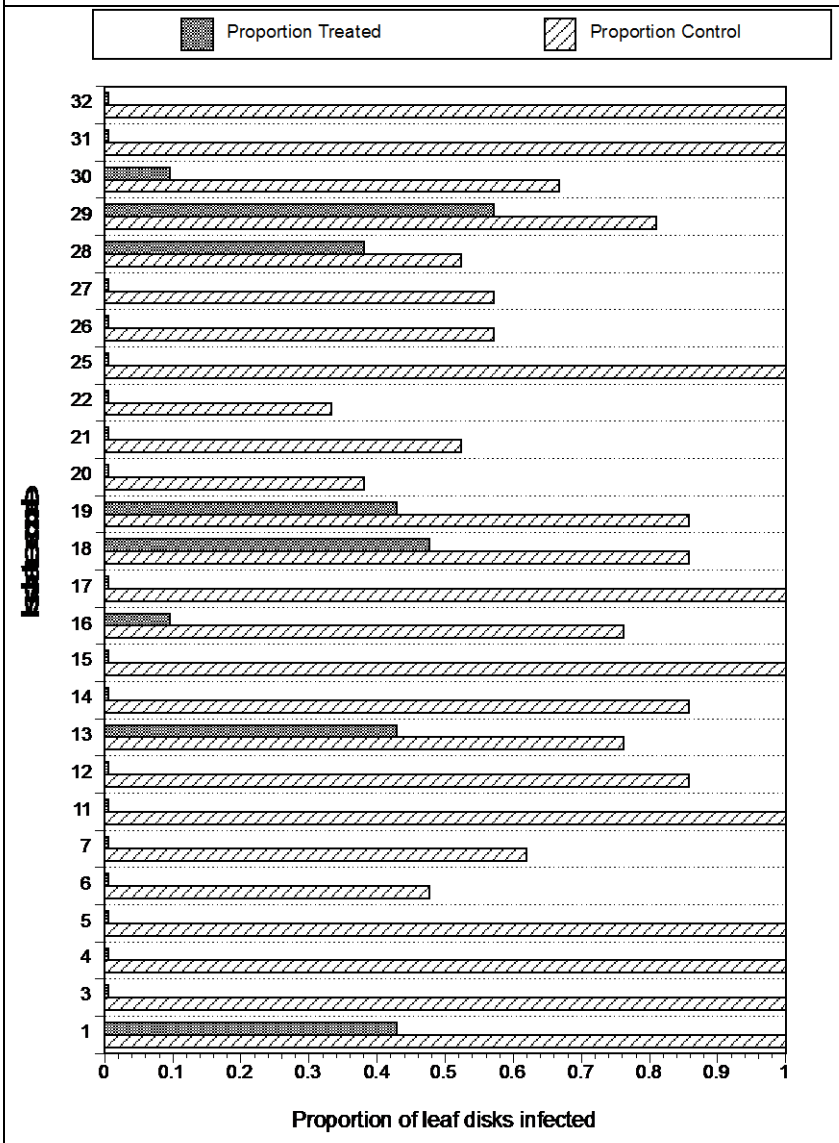

Figure 2. Proportion of leaf disks infected following inoculation with a suspension of spores of *P. clandestina* after 14 days incubation. Values "0" in the treated were given values of 0.05 (instead of 0) to provide contrast. Data shown are from 1280 ppm and untreated control treatments applied to foliage subsequently inoculated with *P. clandestina* from various orchards. Note growth of isolates 1,13,16,18,19, 28-30 on leaf disks treated with trifloxystrobin.

The majority of isolates were sensitive to trifloxystrobin at 1280 ppm, but 6 isolates appeared less sensitive (Figure 2). The isolates came from orchards in the Prosser (2), Mattawa (3), and Brewster-Omak area (2). We are confirming resistance with the qPCR assay developed in 2010-2011 but the assay requires a significant increase of inoculum of each isolate.

Objective 2. There were large increases in inoculum load in the orchard air during the 6-week period following harvest and the cessation of fungicide applications. In 2012, a more large-scale experiment was conducted. Orchard D-39 at WSU-Roza was separated into two equal segments. The north half received fungicide applications until harvest using standard grower practices. The south half remained untreated throughout the growing season. Disease severity in the *treated* section increased rapidly after harvest and eventually exceeded levels in the untreated section (Figure 3). PCR Cp values also showed a gradual downward trend in the treated section indicating the concentration of *P. clandestina* conidia in the orchard area increased following the cessation of fungicide applications (Figure 4). Results indicate that an areawide approach to mildew management should be considered and that season long disease management may serve to lower inoculum loads that could pose a risk to later cherries in the immediate vicinity and also serve to lower the amount of survival inoculum going into the following growing season.

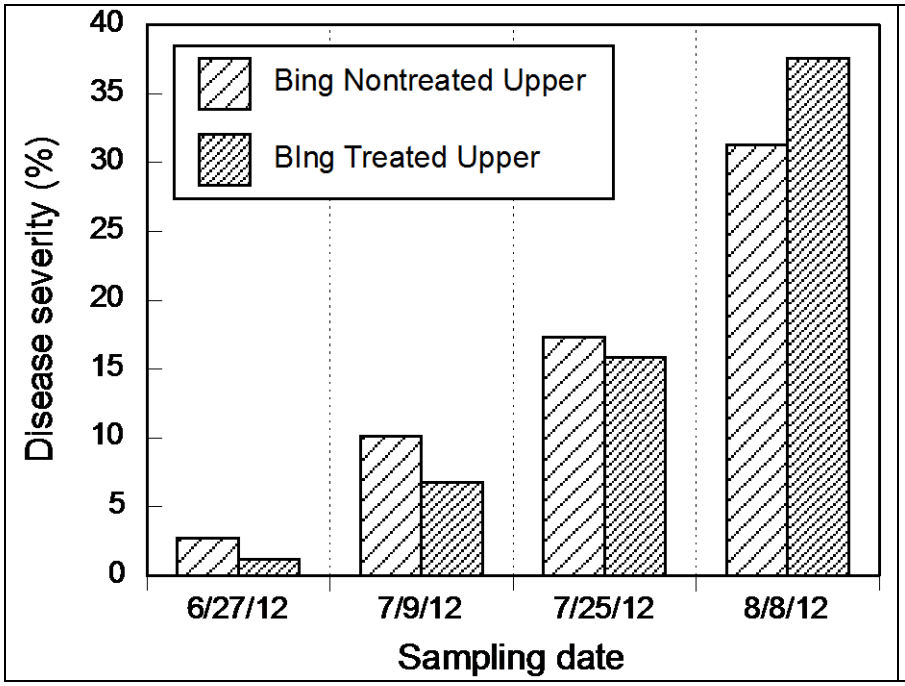

Figure 3. Powdery mildew disease severity progression following harvest. Bing nontreated upper received no preharvest fungicide applications while treated upper received standard grower regime. Note that disease severity in the preharvest treated actually surpasses that in the untreated sections of the orchard.

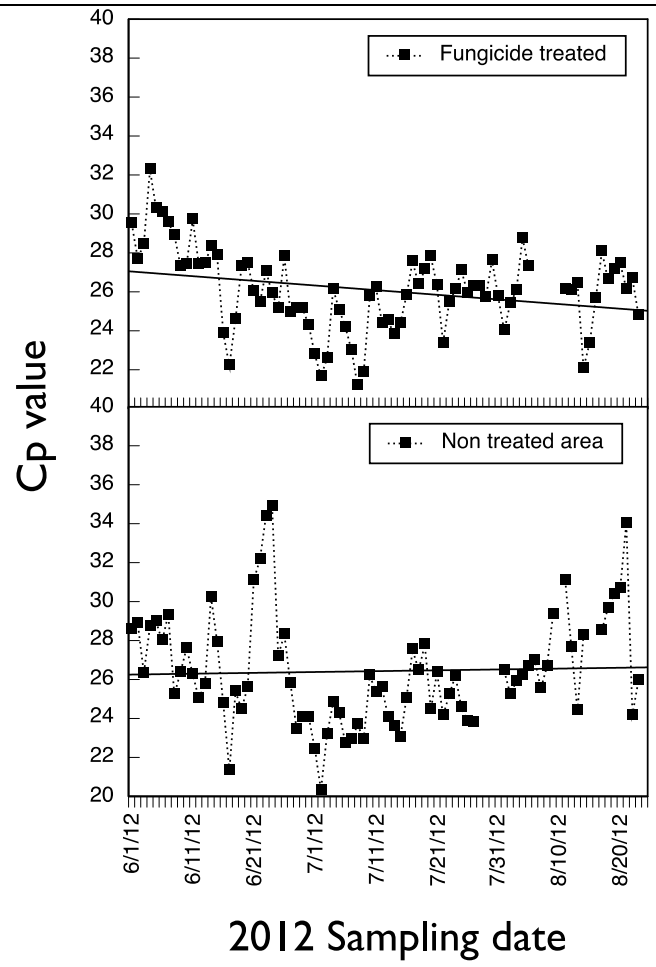

Figure 4. DNA signal strength in fungicide-treated (preharvest) and nontreated portions of a cv. 'Bing' orchard. The decreasing Cp value in the portion treated with preharvest fungicides is indicative of increases in airborne conidia concentrations following the cessation of fungicide applications.

Objective 3. Studies in 2012 were conducted using a modified (more efficient) Burkard cyclonic air sampler and improved qPCR techniques. Samplers were operated immediately prior to and through the only period of water-based frost protection in two (D-51 and D-39) mildew research orchards at WSU-Roza. Cp values decreased significantly during the 8 hours immediately following the irrigation water (Figure 5). Lower Cp values indicate stronger DNA signals during that period. We conclude that water used for frost protection increases the amount of *P. clandestina* DNA in the orchard air. Considering the overwintering inoculum load and the response to wetting, we conclude that the decreased Cp values reflect the presence of ascospores (primary inoculum) in the orchard air. Further studies should include successive applications of water in order to determine whether the practice can be used in conjunction with timely fungicide applications to deplete the amount of primary inoculum and therefore delay the onset of powdery mildew epidemics.

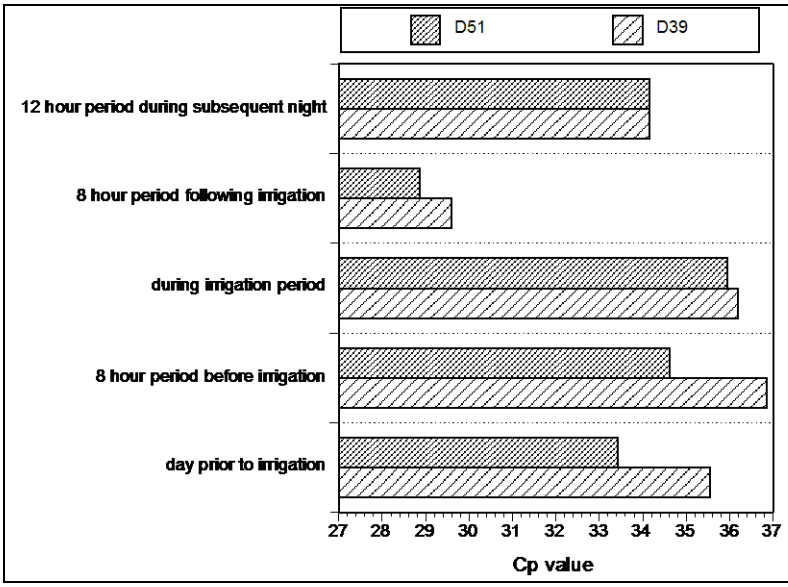

Figure 5. DNA signal strength (inversely proportional to Cp value) in WSU-Roza D51 and D39 when irrigation was used for frost protection. Note the decrease in Cp value (increase in signal strength) during the 8 hours following the application of water.

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 2010 and 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 (Figure 6). Microclimatic data from 2012 will be downloaded during the first week of November.

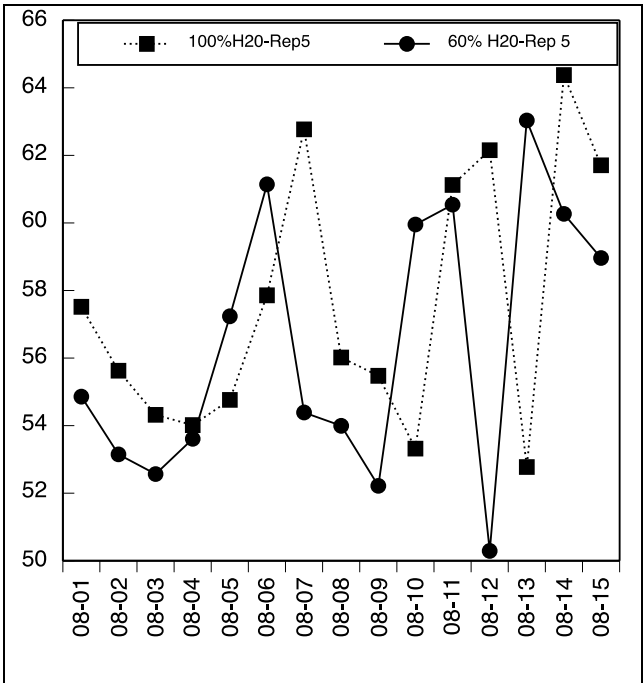

Figure 6. Relative humidity in 100% and 60% irrigation regimes during early August 2011.

Objective 5. Orchard studies 2010-2012 indicated that postharvest oil or Kaligreen applications made after standard fungicide applications were terminated resulted in no significant inhibition of chasmothecia formation. However, in 2012 we conducted parallel studies in Quincy, WA, nursery where we followed specific fungicide modes-of-action with late season oil applications. Although the chasmothecia counting process will not be complete until November 1, a significant oil effect was observed in the untreated controls (Figure 7). Furthermore, the number of chasmothecia increased exponentially between the September 15 and October 1 sampling dates, indicating the existence of a narrow window where late season fungicide applications may be most effective for lowering the amount of potential overwintering inoculum. We plan to present the entire set of data from various fungicide modes-of-action at the research review.

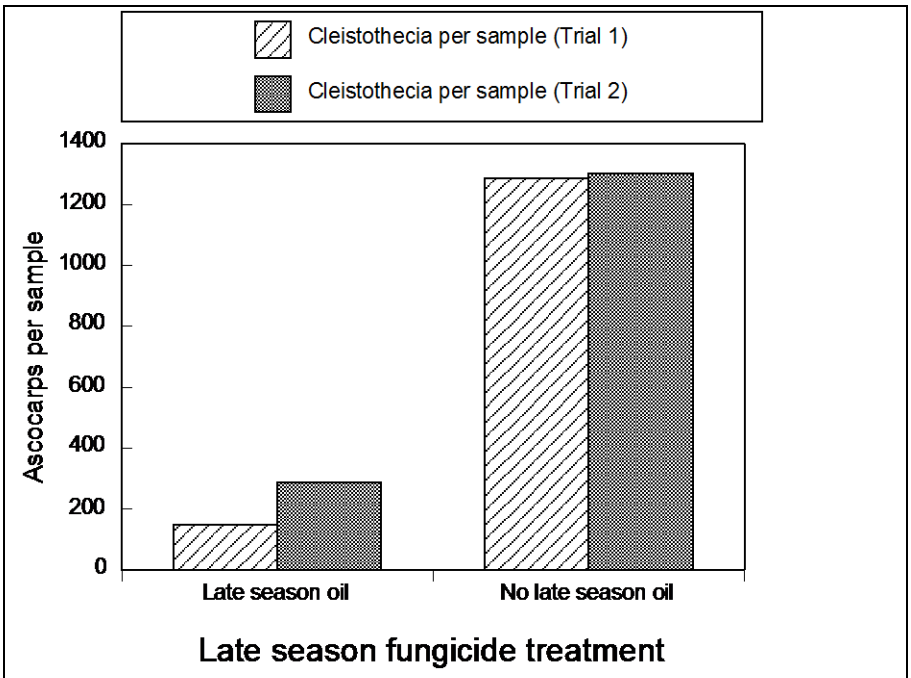

Figure 7. Effect of late season oil applications on ascocarp (chasmothecia or cleistothecia) production in untreated controls in a cherry *nursery*. Oil applications were inadvertently timed during a critical window for ascocarp formation.

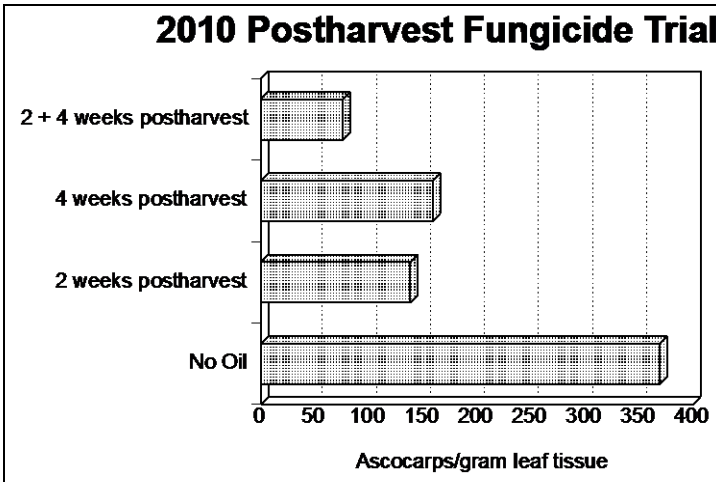

Figure 8. Chasmothecia (ascocarp) production on cv. 'Bing' trees receiving grower-standard preharvest fungicide program versus three full-season programs. There were no significant differences in chasmothecia production at $P < 0.05$

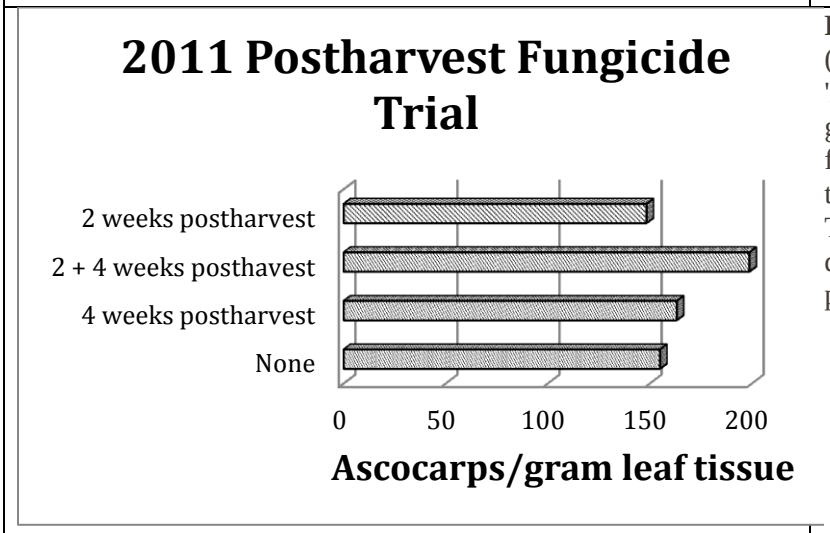

Figure 9. Chasmothecia (ascocarp) production on cv. 'Bing' trees receiving grower-standard preharvest fungicide program versus three full-season programs. There were no significant differences in chasmothecia production at $P < 0.05$

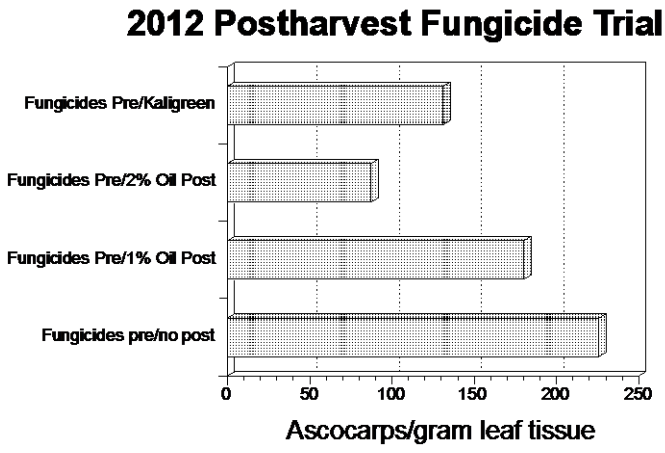

Figure 10. Chasmothecia (ascocarp) production on cv. 'Bing' trees receiving grower-standard preharvest fungicide program versus three full-season fungicide programs (1% and 2% oil applied postharvest.. There were no significant differences in chasmothecia production at $P < 0.05$

Executive Summary

Improved Management of Powdery Mildew of Sweet Cherry

Gary G. Grove, WSU-IAREC

Over 50 orchards in the Yakima and Wenatchee Valleys, Columbia Basin, and Omak/Brewster area were surveyed for resistance to QoI (strobilurin) fungicides. Evidence for resistance was found in a small minority of orchards and, following an inoculum increase, will be confirmed using a PCR assay. Water-based frost protection was shown to increase the amount of *P. clandestina* propagules in the orchard air, but further studies are needed to determine whether sequential irrigation sets can be used to deplete the primary inoculum source. Orchard experiments indicated that large increases in disease severity and airborne inoculum occur during the 6-week period following cessation of fungicide programs at harvest. This implicates early season cherries as an inoculum source for nearby later-developing varieties and intimates the need for full-season disease management in areas where multiple varieties are grown. Late season increases in disease levels were also shown to contribute significantly to the amount of survival inoculum going into the dormant season. Postharvest oil and potassium bicarbonate applications had no significant effect on the production of ascocarps (cleistothecia, chasmothecia) in the orchard but significantly late season oil applications significantly reduced numbers in cherry nurseries. A brief window of ascocarp formation occurred in the nursery indicating that orchard applications may have made outside of this brief window. Orchard experiments designed to identify irrigation and nitrogen factors affecting powdery mildew were unsuccessful due to lack of adequate disease. Our findings indicate possible future areas of research that include: 1) determining whether successive early-season water applications can be used to deplete the overwintered inoculum supply 2) studies to identify the narrow window of ascocarp formation in orchards and determine whether specific fungicide modes of action can be used to interrupt the process 3) investigate an area-wide approach to managing powdery mildew that utilizes full-season disease control without compromising (through resistance) our current fungicide arsenal. It is also apparent that a vigorous extension effort is needed in the area of fungicide resistance and the economics of full-season disease management.