FINAL REPORT

TITLE: Epidemiology and control of cherry powdery mildew (CPM)

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OBJECTIVES:

* Determine if irrigation management can be used to delay the onset of cherry mildew epidemics and/or reduce disease severity.

* Develop oil-based conventional and weather driven cherry powdery mildew management programs.

* Continue evaluating various sprayer technologies and spray volumes

* Investigate the influence of weather variables and current irrigation practices on disease prgoression and aerial conidia populations of conidia of the cherry fungus.

* Continue field evaluations of various "soft" fungicides for efficacy against stone fruit mildews and as components of antiresistance strategies for maintaining the effectiveness of "at risk" fungicides.

SIGNIFICANT FINDINGS:

* Modeling studies. Controlled environment studies. Infection of cherry foliage occurred between 10 and 25 C at relative humidities of 90-100% (Figure 2). *There was no infection at 5 or 30 C.*

Field Studies. Primary infection resulted from natural precipitation in three orchards and the initial irrigation set in 3 orchards. Delayed irrigation resulted in lower disease severity at harvest (Figure 1).

* CPM epidemics at 3 sites intensified during the first two weeks of June (Figure 3). Although more data needs to be collected, it appears that epidemics intensified after several consecutive days with average temperatures > 60 F.

• Irrigation delays of 2-4 weeks had no effects of fruit size, weight, or soluble solids. Some effects were noted after a 6 week delay (Table 2).

• In an orchard where irrigation was delayed in 2001 and 2002, but received normal irrigation in 2003, there were no significant differences in fruit weight (with the exception of the 6 week delay), firmness, and soluble solids. Vegetative growth was also normal (Table 3).

* Qualitative air sampling/early detection studies. Powdery mildews were detected in the field using Innovatek Bioguardian and Rotorod rotary impaction air samplers, and identified using PCR.

-Molecular identification methods. Primers for cherry powdery mildew (*developed by R.A. Spotts*) were tested for cross-reactivity to apple, hop, peach/nectarine, Cosmos, lilac, pea, and rose powdery

mildew. No cross-reaction occurred. The primer is currently being tested for cross reaction with DNA collected from the powdery mildews from 47 disparate plant hosts from 25 different vascular plant families.

* Organic fungicides applied at 7- and 14- day intervals failed to provide statistically significant control of CPM in a high disease pressure cv. 'Bing' orchard (Table 4).

* Oil programs were compared with conventional fungicide programs. The former programs provided savings of $30-60 per acre without compromising disease control (Table 5).

* Quinoxyfen, petroleum oils, neem oil, applied alone or in combination, provided mildew controls equal to that obtained using conventional fungicides.

• Two new compounds (Pristine and a numbered compound from Valent) provided excellent control of CPM in the orchard.

METHODS:

Effect of temperature and relative humidity on colony expansion and foliar disease severity. Cherry leaf disks (cv. 'Bing') were inoculated using a suspension of *P. clandestina* conidia and incubated 21 days at relative humidities of 90-100% at temperatures of 40-95 F (5-35 C). The proportion of disk surface area colonized by powdery mildew was determined 7, 14, and 21 days after inoculation.

Verifying pathogen presence and activity. Air sampling studies. Two air sampling methods were evaluated in preliminary field studies. The first study was conducted using rotary-impaction air samplers.
In a second study, a custom-built air sampler (manufactured by Innovatek of Richland, WA) 50-100 times more efficient than other types currently available was tested through the summer of 2003.

Use of molecular tools for the timely detection of propagules of the cherry mildew fungus. DNA extractions were using a Bio 101 System FastDNA kit. PCR amplification with universal primers was performed with Pfu polymerase according the recommended instructions. Amplifications were performed in a total volume of 25µl using three-step cycling. Amplification products were run on 1% agarose gel at 120 V for one hour, stained with ethidium bromide, and photographed under UV light. Amplification fragment of expected size is interpreted as a positive result. More detailed information about extraction procedures has been published (Falacy et al 2003).

Various fungicide programs were evaluated using efficacy and relative input cost as measures of usefulness. Various combinations and rotations of DMI, quinoline, strobilurin, SAR, oil, whey, and sulfur compounds were applied to Bing and Rainier cherries and evaluated for efficacy and phytotoxicity. Compounds were applied in calendar and weather based management programs. Disease incidence and severity was determined by randomly selecting five terminal shoots from each plot, and picking five leaves from each terminal starting with the last fully open leaf and working down the shoot for a total of 25 leaves per plot. The percentage of the surface area of the underside of each leaf infected by mildew was estimated and recorded. Data were subjected to analysis of variance and means separated according to Fisher's PLSD at $P < 0.05$.

Preliminary baseline sensitivity studies for resistance-prone fungicides (eg. Flint, Cabrio, Quintec) were conducted using the methods of Ypema and Gubler (1997). Detached, symptomless, and untreated cherry leaves were collected from 'Bing' cherry liners. Leaves were disinfested for 30s in a 50% ethanol solution and rinsed using sterile, distilled water. Leaves were placed between autoclaved paper towels to dry. Leaves were dipped in each fungicide treatment and allowed to dry,

ventral side-up, on paper towels. Leaf discs were obtained using a 15 mm cork-borer and four discs placed in a 60x20 mm petri dish prepared with one layer absorbent pad (Gelman 47 mm) wetted with 1 ml sterile distilled water and two layers of Miracloth. The discs were inoculated with *P. clandestina* using an inoculation tower. Disks were placed in Rubbermaid crispers lined with moist paper towels and incubated 10 days at 28.5C in a 12-hour photoperiod.Inhibition of fungal growth was determined by assessing the percentage of leaf disc surface covered with sporulating powdery mildew colonies.

Results and Discussion:

Irrigation timing was compared in two neighboring Yakima Valley orchards with histories of powdery mildew epidemics. The initial irrigation set was applied April 28 and May 10 in the two respective orchards. The first powdery mildew symptoms and signs were observed on May 12 and 20, respectively, *indicating that the beginning of the epidemic was delayed about 2 weeks when irrigation was delayed*. Disease severity at harvest in the "normal" and "delayed" irrigation orchards was a 47.4 and 10.7% indicating that the "intensification" phase of the CPM epidemics was delayed by the irrigation delay (Figure 1; Table 2). The implications of this shift on fruit infection require further investigation. The results of the 2000-2003 studies have improved our understanding of the primary infection process in sweet cherries. We have documented two moisture events that can result in ascospore release and primary infection: 1) impact sprinkler irrigation and 2) natural precipitation (0.1" or greater). In all test orchards, the appearance of primary mildew required about 50 cumulative degree-days > 50 F after a primary infection period.

Irrigation delays were shown to have little effect on tree and fruit horticultural characteristics in 2001-2003 studies. For the third year, irrigation delays of 2-4 weeks had no significant effects on fruit size, soluble solids, and weight (Table 2). This years' studies also included fruit and tree analyses in an orchard where delays were practiced in 2001 and 2003, but irrigated normally this year. Irrigation delays in 2001 and 2002 had no residual effects on fruit size, weight (with the exception of the 6 week delay in 2002), firmness, or soluble solids at harvest in 2003 (Table 3). Average leaf area, shoot number, and shoot length were also not affected. The conclusion drawn from our irrigation studies is that in the absence of natural (precipitation induced) primary infection periods, *irrigation delay offers a safe means of delaying the onset of cherry powdery mildew epidemics.*

The development of a technique for determining the effects of temperature and relative humidity on powdery mildew infection of, and sporulation on, foliar tissue will expedite the development of a risk assessment model. The general high- and low- temperature limits were found to be between 26-30 C (79-86 F) and 5-9 C (41-48.2 F). The optimum temperature for tissue colonization was 25 C (Figure 2). In preliminary stepwise regression analyses, infection of cherry foliage was best predicted using a model containing temperature and temperature*relative humidity interaction terms (R^2 = 0.85) The expansion and continuation of these studies is discussed in the new project proposal. Disease progression studies in the field indicated that foliar incidence and severity increased significantly with the onset of warmer weather in early June (Figure 3).

Although extensive further testing is required, the PCR primer developed by R.A. Spotts did not cross-react with the powdery mildews of apple, hop, and peach. Early indications are that this primer is as specific as the grape, hop, and peach powdery mildew primers developed in our laboratory. The latter routinely amplified *U. necator* DNA, but did not amplify DNA from powdery mildew species collected from 47 disparate plant hosts from 25 different families. The cherry primer is being tested against the DNA of this same group of powdery mildews is in progress and should be complete by March 2004.

The utilization of these air sampling devices to detect airborne *P. clandestina* conidia and ascospores coupled with molecular identification techniques could possibly be utilized to pinpoint the onset or intensification of CPM epidemics and to study the long-distance dispersal of *P.clandestina*. However, because of the presence of numerous other powdery mildews (e.g. hop, apple, grape, peach, nectarine, apricot) that may be in orchards and hop yards in close proximity to Eastern Washington orchards the discrimination of *P. clandestina* in air samples containing mixed fungal populations is imperative. Identification of *P. clandestina* using designed species-specific PCR primers could be utilized to differentiate Erysiphaceous fungi that are difficult to distinguish using conventional labor-intensive and time-consuming microscopic techniques.

Early detection and identification of spores in low concentrations could provide a safe means to associate the initial fungicide applications with the initial occurrence of airborne pathogen propagules, rather than assumed or predicted activity. Conversely, verification of inoculum availability could enhance a CPM risk assessment model. These approaches could be extremely useful in reducing fungicide applications in the absence of the pathogen or during years of late epidemic onset and thereby enhance our current approach to CPM management.

Several new fungicides were registered, and a new approach (utilizing the fungicidal properties of petroleum oils) for disease management has resulted from this program (Grove, 2001). The efficacy packages for Procure, Flint, Cabrio, Pristine, and Quintec were developed as a portion of this grower-funded research program. The benefits of the oil program are evident in Table 5. The spray regimes containing oil provided the same level of disease control as other approaches, conformed to the APS Fungicide Resistance Action Committee resistance management guidelines, were free of any phytotoxicity, and provided a monetary savings of $31-62 per acre (based on a 4 application program).

Publications

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Tables and Figures

Table 1. Primary infection, disease progression, and disease severity at harvest in orchards where natural and irrigation-induced primary infection events occurred. Pasco trees were treated with fungicides every 14 days.

Location	Primary Infection	Moisture Source	First Symptoms	Disease severity at harvest
Halstead	28 April	Irrigation	12 May	47.4
Pasco*	30 April	Precipitation	12 May	18.0
Zillah	10 May	Irrigation	20 May	10.7

Table 2. Effect of 2001 and 2002 irrigation delays on selected fruit characteristics at harvest, 2003.

Irrigation Delay (2002)	Fruit Weight (2003)	Fruit Firmness (2003)	Soluble Solids (2003)
0	11.48A	297.6A	16.9A
2	10.9AB	295.7A	16.7A
4	10.8AB	303.6A	16.8A
6	10.2B	300.3A	16.9A

Table 3. Effect of 2001 and 2002 irrigation delays on selected vegetative characteristics of 'Bing' cherries at harvest, 2003.

Irrigation Delay (2002)	Number of sucker shoots per limb	Sucker shoot length (inches)	Leaf Area (cm^2)
0	2.3A	26.5A	715.4A
2	1.9A	24.6A	697.2A
4	2.5A	23.3A	629.5A
6	1.3A	21.0A	638.7A

Table 4. Effect of organic fungicides on powdery mildew severity on cv. 'Bing' cherries, Orondo, WA. Disease pressure was extremely high.

Treatment	Program/ Interval	Disease severity
Untreated		89.9 a
Oil Kaligreen	first symptoms 7 day interval following oil	70.7 ab
Oil CalSup	first symptoms 7 day interval following oil	73.9 ab
Oil Serenade	first symptoms 7 day interval following oil	66.1 ab
Oil Microthiol	shuck fall-pit hardening 7 day interval after pit hardening	82.1 a
Oil Kaligreen	shuck fall-pit hardening 7 day interval followingoil	73.0 ab
Oil Kaligreen/CalSup	shuck fall-pit hardening 7 day interval following oil	75.9 ab
Oil Kaligreen/Serenade	shuck fall-pit hardening 7 day interval following oil	61.1 abc
Oil Serenade/CalSup	shuck fall-pit hardening 7 day interval following oil	66.0 ab
Oil Flint	shuck fall-pit hardening 14 day intervals following oil	32.2 c
CalSup	shuck fall-harvest (7 day intervals)	72.3 ab

Table 5. Comparison of oil-based and conventional fungicide programs on powdery mildew of sweet cherry.

Treatment	Timing[1]	Disease severity[2]	Disease severity [3]	Cost/A	FRAC Guidelines[4]
Untreated		56.4A	52.7A		
Procure	Full season	9.6C	6.6BC	$94.72	No
Procure + Flint	SF, SF+14 1C, 2C	1.5C	2.1C	$126.44	Yes
Oil Flint Procure	SF, SF + 14 1C 2C	13.1BC	5.9BC	$63.98	Yes
Oil Procure Flint	SF, SF + 14 1C 2C	7.9C	9.2BC	$63.98	Yes
Cabrio	Full season	1.0C	0.1C	-	No
Pristine	Full season	0.3C	3.8BC	-	No

[1] = SF (shuck fall), SF +14 (14 days after shuck fall), 1C (first cover), 2C (second cover)
[2,3] = percentage of leaf surface area infested
[4] = APS Fungicide Resistance Action Committee (FRAC) recommends no more than three applications of any single fungicide mode of action per season

Project total (3 years): $112,972

Figure 1. Effect of irrigation delay on disease onset and intensification Yakima Valley cherry orchards. First symptoms were noted 12 and 20 May in Halstead and Zillah orchards, respectively. Note comparative disease severity at harvest.

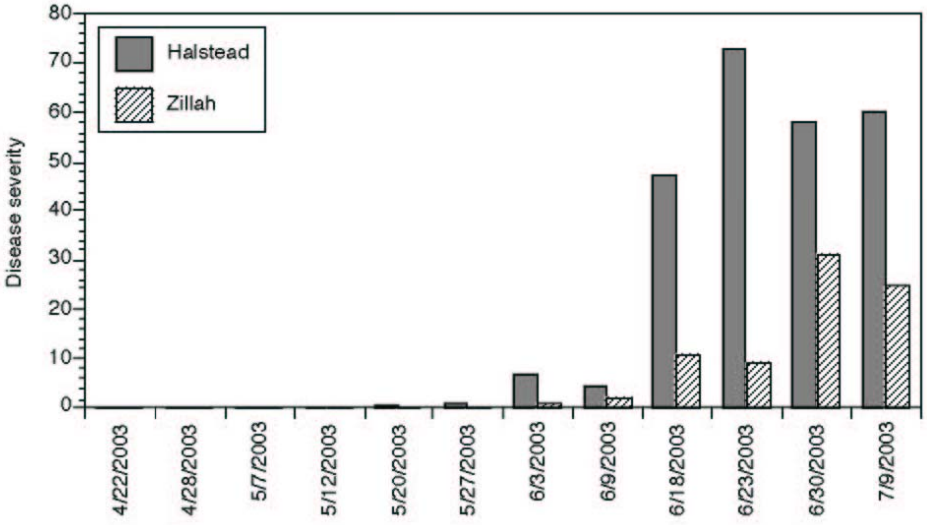

Figure 2. Influence of tempeature and relative humidity on colonization of cherry leaf disks by *Podosphaera clandestina*.

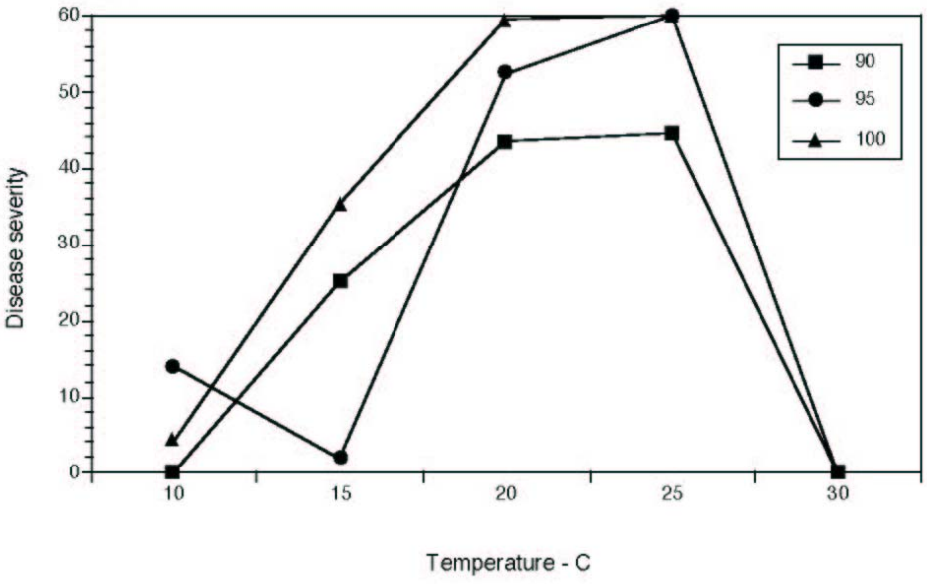

Figure 3. Progression of CPM following primary infection in the Yakima and Columbia Valleys, 2003. Large increase in severity (days 30-40 after primary infection) corresponded with onset of warm weather.