FINAL PROJECT REPORT
WTFRC Project Number: CH-04-406

Project Title: Modeling and Managing Powdery Mildew of Sweet Cherry

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Budget History:

Item	**Year 1:**	**Year 2:**	**Year 3:**
Salaries	17,520	17,409	19,167
Benefits	5,606	5,426	6,708
Wages	12,000	12,200	10,688
Benefits	1,952	1.952	1,176
Equipment			
Supplies	6,700	6,700	1,700
Travel	4,700	4,700	3,700
Miscellaneous			
Total	48,678 (funded 41,000)	47,487 (funded 41,000)	43,139

Objectives:

I. Develop a risk index model (utilizing rainfall, irrigation, temperature, relative humidity, and pathogen presence/activity) for initiating fungicide spray programs and adjusting subsequent spray intervals.

II. Develop means of detecting, identifying, and quantifying airborne propagules of *P. clandestina* early in epidemic progress.

III. Develop and refine economically viable conventional and organic powdery mildew management programs.

IV. Determine the effects of temperature and wetness on acute petroleum oil phytotoxicity. Determine the chronic effects of oils on tree health (reported in 2005).

Objective de-emphasized in 2005 and 2006 due to insufficient funding:

V. Develop baseline sensitivities for resistance-prone compounds. Preliminary studies focused on the DMI fungicides. Future studies will concentrate on Qol and quinoline fungicides.

Significant Findings:

- Cleistothecia (the primary inoculum supply) viability declined from 58% at bud burst to 0% about 1 week after pit hardening. For the third year, the degradation of the ascospore supply required slightly less than 200 cumulative degree-days > 10 C (50 F).

- Investigations on the temperature and humidity *ranges* over which the cherry mildew fungus colonizes (grows on) cherry foliage were completed in 2006. Disease developed at 10 (50 F) -28 C (82.4 F) but did not develop at 7.5 (45.5 F) and 28.5-35 C (86-95 F). The effect of relative humidity (between 80% and 100%) alone was insignificant but there were significant temperature/humidity interactions (equation 1). Multiple regression analyses indicated that disease development on cherry foliage was best described by the equation:

Disease severity = $38.9 + 1.3\ T + -0.052\ T^2*RH + 0.008\ T^{3*}RH$ (equation 1)

where T = temperature and RH = relative humidity. The equation accounted for about 82% of the variability in the raw data ($R^2 = 0.82$). The most significant aspects of these findings are the identification of the temperatures above and below which the fungus does not actively colonize cherry foliage. The temperature algorithm for the secondary infection risk index was partially derived from this equation and previously published information on the latent period. The optimum temperature for colonization was 20.5 C (68.9 F).

- The results of our controlled-environment studies on spore production commenced in 2005 and were completed in 2006 (Figure 1). Sporulation occurred at 12.5 C (54.5 F) -27.5 C (81.5 F) at relative humidities of 80-100% Multiple regression analyses of the raw data indicated that sporulation on cherry foliage was described by the equation:

(log) $Y = -0.003 + 0.05T + 0.09\ T*RH + 0.0001\ T^2 + -0.0004T^3RH$ (equation 2)

with an R^2 of 0.74. The optimum temperature for sporulation was 21.5 C (70.7 F).

• Studies to ascertain the effects of high temperature on the viability of powdery mildew spores were initiated in 2006. It was found that 24 hours of exposure at 40 C was required to kill spores. Exposure times of 0, 4, 8, and 24 hours at 40 C (104 F) resulted in germination levels of 28.1%, 20.9%, 17.0%, and 0%, respectively. Temperatures of 30-39 C (86-102 F) were not lethal regardless of incubation time.

• The PCR assay (using primers developed by the R.A. Spotts group) was found to be extremely sensitive. More thorough sensitivity testing was accomplished in 2006. The assay was found to be sufficiently sensitive to amplify DNA from 100-500 conidia placed directly on glass air sampling medium. Regression analysis revealed a significant ($F = 47.27$; $P = 0.0054$) relationship [y=1.6*exp(-exp(-(x-74.1)/75.4))] (Figure 2) between the numbers of conidia placed on glass sampling rods and successful PCR amplifications with a coefficient of determination (R^2) of 0.93. A new non-phenol extraction procedure developed in 2006 further improved sensitivity to 5-10 conidia placed on glass rods. Intensive testing indicated that the primers did not amplify the DNA of other powdery mildew fungi common in the region.

• For three consecutive years (Figure 3), the PCR assay facilitated the detection of low levels of *P. clandestina* inoculum in air samples within hours of collection in field studies *prior to disease onset*. Air sampling results also confirmed the presence of ascospores in the orchard when their presence was predicted by the temperature/rainfall (primary infection) component of the model. Parallel studies using a Burkard volumetric spore trap indicated the initial detections were a result of ascospore releases during all years of the study.

• *Model and detection based disease management.* The basic code for the model is nearly complete and the current version is in beta on 2007 iteration of the AgWeatherNet web site. The client enters the date of bud burst to activate the model. At this point the model begins degree-day calculations to determine the point of exhaustion/degradation of the primary inoculum supply (model component A; Figure 5). The supply is (conservatively) considered exhausted at $\geq$ 250 cumulative degree-days after bud burst. The model looks for 0.1" precipitation at 50 F or greater prior to the exhaustion of the primary inoculum supply (model component B, Figure 5). When primary infection occurs, the secondary infection/risk index component of the model is activated (model component C, Figure 5). When a significant epidemiological event occurs model output is hyperlinked to pertinent management recommendations.

The beta version of the model and/or the results of detection studies were used to initiate and schedule orchard fungicide applications in 2006. Spray programs were applied according to tree phenology or as specified by 1) the primary infection component of the model or 2) the initial detection using the molecular air sampling techniques. In cases 1 and 2, the secondary infection risk index was initiated at primary infection or first detection and subsequent spray intervals adjusted accordingly. The initiation of the model- and sampling-driven regimes began at least two weeks after the initiation of the phenology-based program and resulted in an elimination of 2 fungicide applications without compromising disease control. For example (Figure 4), a program initiated at the first primary infection identified by the model resulted in disease incidence and severity values of 2.2% and 16.0%, while programs initiated at first detection were 0.4% and 6.8%, respectively. Disease incidence and severity in the industry standard and untreated controls was 3.9% and 32.3% and 48.9% and 78.3%, respectively. Disease incidence and severity values in the model- and detection-based programs were not statistically different from the phenology/calendar program. However, six fungicide applications were made using the standard (phenology/calendar) approach, while four applications were made using the model-driven and detection approaches.

• Five new (significantly less expensive) formulations of tebuconazole were tested for efficacy against powdery mildew and compared to Elite, the commercially available form of the chemical. All formulations provided mildew control equal to that attained using Elite. Different fungicide regimes that conform to FRAC recommendations for resistance management were evaluated in a second trial. There were no significant differences between programs (Table 1).

Results and Discussion:

Our vision for improved management of powdery mildew of cherries involves the use of a forecasting model that incorporates components to predict the exhaustion of the overwintered inoculum supply (model component A), primary infection (component B, i.e. ≥ 0.1" of precipitation at ≥ 50 F), disease pressure once primary infection has occurred (secondary infection risk, model component C), and eventually the initial presence of *P. clandestina* in the orchard air (component D).

Ascocarp degradation model (component A) and primary infection. This model component identifies the period of time over which primary infection (from ascospores) can occur provided adequate moisture and conducive temperatures. Model component "B" was validated using the air sampling technique described below. Ascospores of *P. clandestina* were detected only when predicted to be by present by the rules of model component B.

Secondary infection risk index (component C). The results of studies on the effects of temperature on foliar infection and latent period were used to develop the basic rules for secondary infection risk index. The index is initiated (following primary infection) when

1) there are four consecutive days with ≥ 6 consecutive hours at 15-28.5 C (59-83.3 F). When these conditions are met the index is initiated by adding the first 20 index points

2) on each day when there are ≥ 6 consecutive hours between 15-28.5 C (59-83.3 F), add 20 index points

3) index decreases10 points each day with < 6 consecutive hours between 15-28.5 °C (59-83.3 F)

4) Index decreases 10 points on any day with ≥ 6 hours ≥ 28.6 °C (83.3 F)

5) If none of the above are true, then no change

The index, which ranges between 0 and 100, will be used to adjust spray intervals at low (indices of 0-40), moderate (indices of 40-50), and high (indices of 60-100) disease pressures. At this juncture the powdery mildew model is in the experimental or "beta" stage ready for extensive field-testing and the development of cherry-specific spray intervals for various fungicide classes. Further improvements will result from in-depth studies on the effects of relative humidity and temperature on spore production and high temperatures on colony and spore survival. We need to emphasize that the results used to develop the basic model rules were obtained in controlled environments and that adjustments to algorithms may need to be made after extensive field studies and further controlled-environment research.

PCR techniques and air sampling studies. The primers developed by R.A. Spotts were tested for sensitivity for detection of powdery mildew in reaction mixtures and on glass rods used in orchard air

sampling studies. The PCR assay was demonstrated to be extremely sensitive, e.g. DNA extracted from 1 and 5 spores placed directly into reaction mixtures was detected 83% and 100% of the time, respectively. The PCR assay consistent detected DNA extracted from 100-500 conidia placed directly on glass rods used for air sampling. The air sampling technique described herein shows promise as a research and disease management tool. The method utilizing a Rotorod air sampler operated continuously was the only assay that detected *P. clandestina* in the orchard air early enough to be of practical significance during all years of the study. During 2004-2006, *P. clandestina* was not detected in the orchard air during March and early- to mid- April, indicating that "background" DNA from previous epidemics should not result in "false positives". The initial detection of the fungus in the orchard air in 2005 occurred during a rain event in late-April, while in 2006 this occurred during a rain event in late May. The presence of ascospores in the orchard air (which was predicted using component B of the predictive model) during these rain events was confirmed using a Burkard volumetric air sampler. Positives did not occur for the following 5-10 days after the initial detection. The resumption of "positives" preceded the appearance of visible symptoms by 3-5 days. The air sampling/PCR technique confirmed the presence of the fungus in the orchard throughout the fruiting season. Results of this study should represent the initial step in the incorporation of an inoculum availability component into a cherry powdery mildew risk assessment model. The significance of this component has several potential benefits. The plant disease triangle dictates that any plant disease results from the interaction between host, pathogen, and environment. If the pathogen were absent, even the most disease-conducive weather conditions would not result in disease. Results of the fungicide program initiated upon initial detection of the pathogen in the orchard indicate the potential value of this air sampling technique: control measures are instituted only upon *actual* pathogen presence rather than *predicted* presence. The new and more sensitive non-phenol extraction should make the technique significantly more sensitive in the orchard.

Treatment and rate/A[z]	Spray Timing[y]	% Mildew Severity[w,x]	% Mildew Incidence[v,w]
Non-treated		13.3 a	89.3 a
Stylet oil 97% (conc. 1%)	1,2		
Pristine 38WG 14.5 oz + Sylgard 309 0.03% v/v	4		
Quintec 250SC 7 fl oz	6	3.2 b	39.8 bc
Pristine 38WG 14.5 oz + Sylgard 309 0.03% v/v	1,4,7,8		
Rally 40W 5 oz	2,6	2.7 b	36.3 bc
Rally 40W 5 oz	1,4		
Pristine 38WG 14.5 oz + Sylgard 309 0.03% v/v	2,6,7,8	1.5 b	24.0 c
Procure 480SC 12 fl oz	1,2,4,6	2.4 b	31.8 bc
Procure 480SC 16 fl oz	2,6		
Flint 50WG 3 oz	4,8	3.5 b	42.3 bc
Rally 40W 5 oz	1,2,		
Quintec 250SC 7 fl oz	4,6	2.1 b	28.8 bc
Rally 40W 4 oz	1,4,		
Quintec 250SC 7 fl oz	2,6	2.0 b	32.5 bc
Elite 45WP 6 oz + Induce 0.06%	3,5,7	4.2 b	46.8 bc
Flint Max 50WG 6 oz	3,5,7	3.5 b	54.3 abc
Gem 500SC 3 fl oz	3,5,7	3.9 b	63.3 ab

[z]Formulated rate per acre, percent spray mix or volume per volume.
[y]Dates for spray applications: 1 = 9 May, 2 = 23 May, 3 = 1 Jun, 4 = 6 Jun, 5 = 15 Jun, 6 = 20 Jun, 7 = 27 Jun, 8 = 5 Jul.
[x]Percentage of leaf area affected.
[w]Means within a column followed by the same letter are not significantly different according to Tukey-Kramer HSD P=0.05.
[v]Percentage of leaves with mildew symptoms.

Table 1. Various fungicide regimes used to manage powdery mildew of cherries. Note that regimes that conform to FRAC resistance management guidelines provide mildew control equal to that obtained using one product and that the oil-based management program (Stylet Oil, Pristine, Quintec) provided control statistically equal to other regimes.

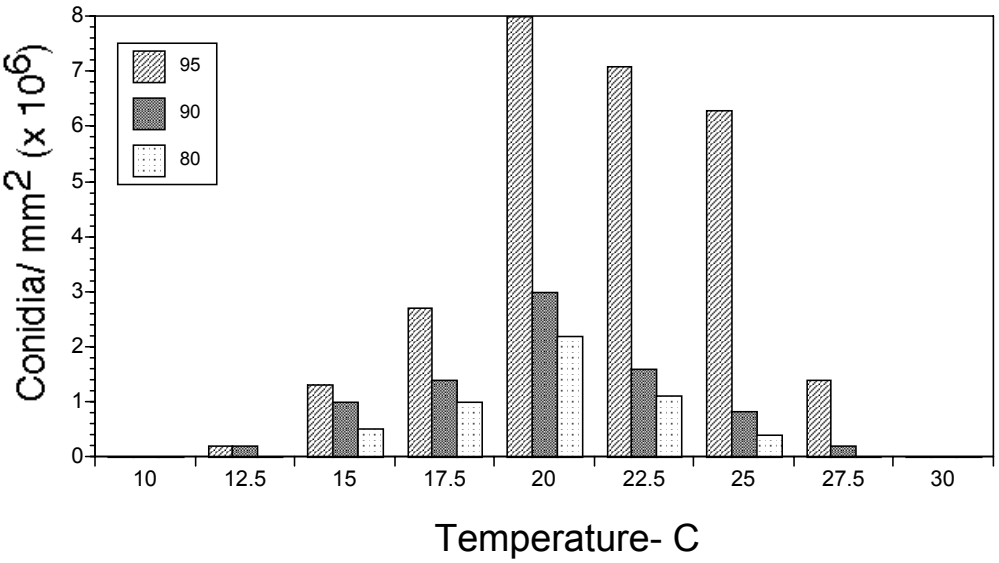

Figure 1. Production of conidia of *Podosphaera clandestina* a 10-30 C under various humidities. Only a trace of sporulation occurred at 70% RH.

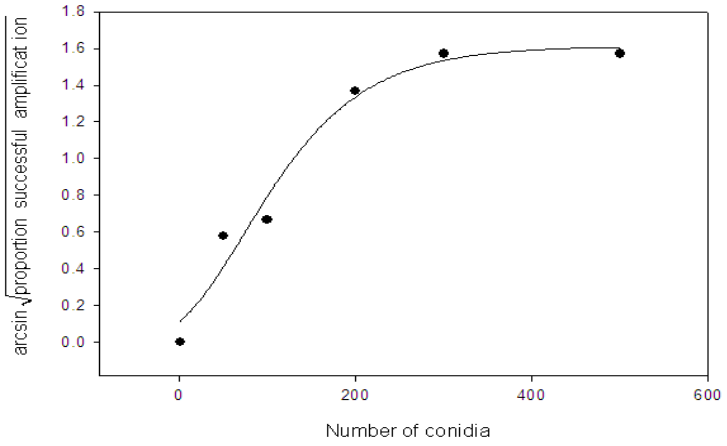

Figure 2**.** Results of PCR assay sensitivity tests: proportion of positive amplifications versus inoculum level. Conidia of *Podosphaera clandestina* were placed directly on glass air sampling rods coated in silicon grease using a human eyelash and extracted using a FastDNA kit and procedure. Specimen DNA was amplified using primers specific to *P. clandestina*.

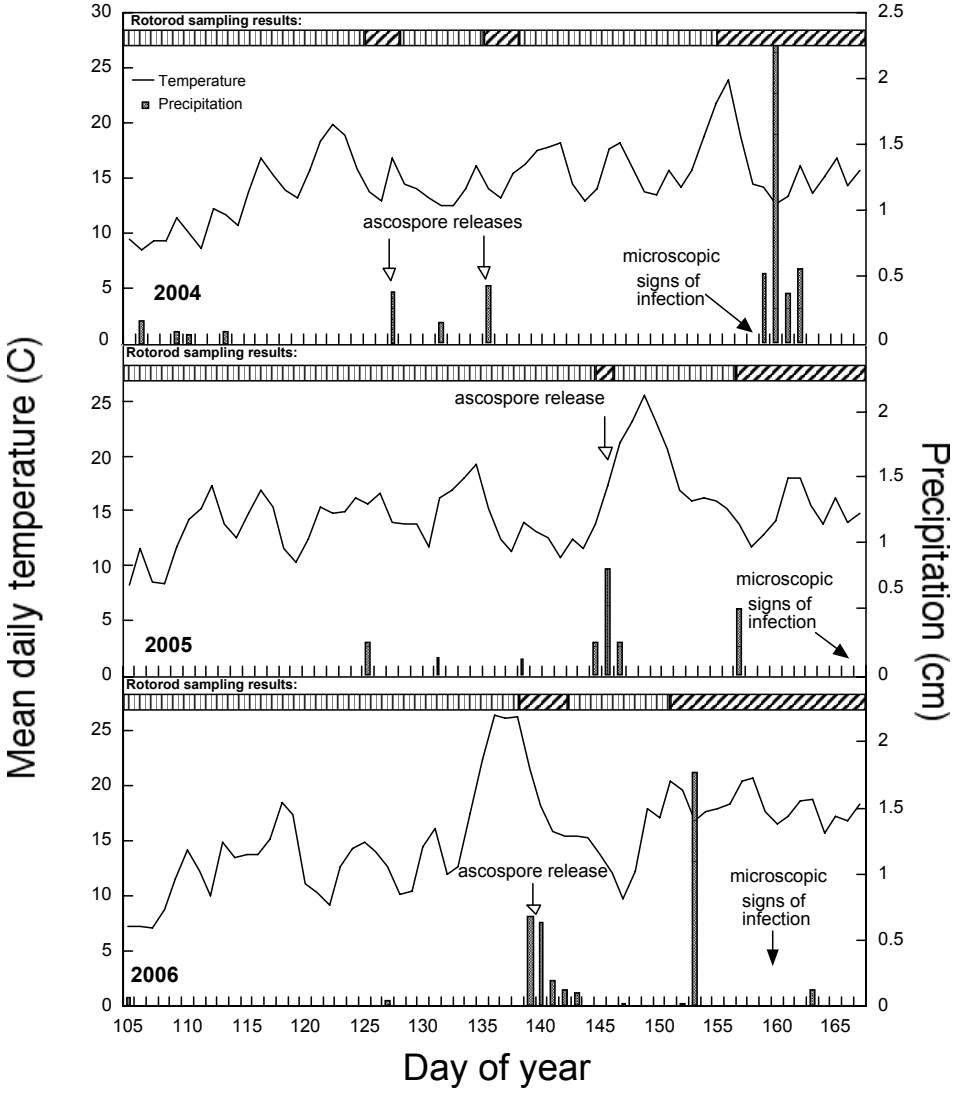

Figure 3. Results of 2004-2006 studies using the PCR-based technique for detection of *Podosphaera clandestina* in orchard air samples. The horizontal bar in the upper portion of each graph indicates the time period over which vineyard air was sampled during the growing season. The segement with vertical lines indicates the period where no amplification of *P. clandestina* DNA occurred. Segments represented by diagonal lines indicate sampling periods where positive PCR amplification indicated the presence of *P. clandestina* in the orchard air. Displayed is daily mean temperature (Celsius (20 C = 68 F; solid line), precipitation (cm; bars), ascospore releases (open arrows) confirmed using a Burkard air sampler and morphological features for propagule identification, and appearance of powdery mildew signs (visible mycelia) over the sampling periods (solid arrows).

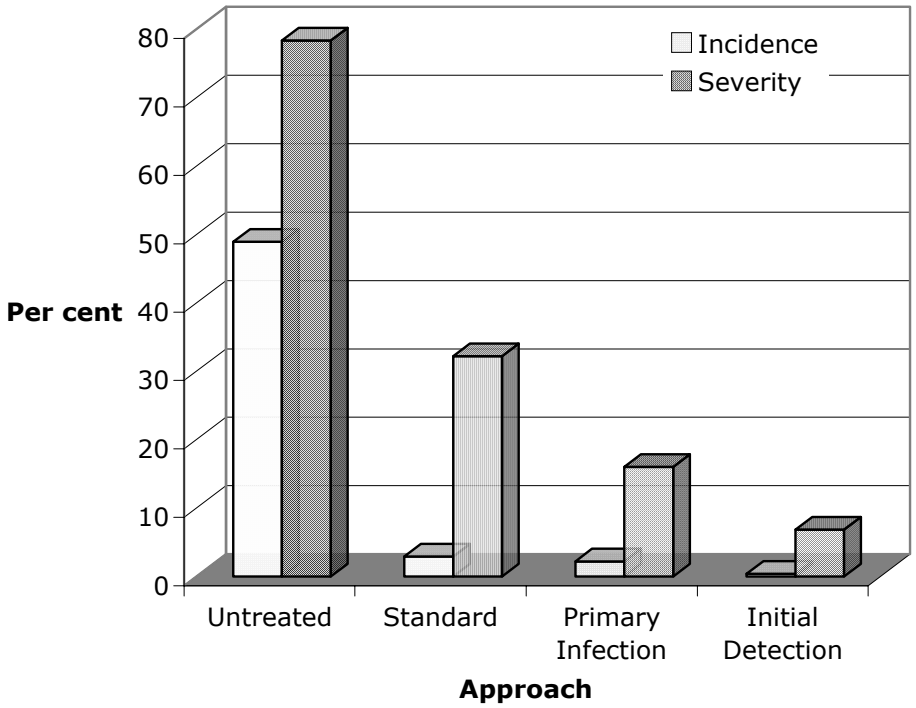

Figure 4. Cherry powdery mildew incidence and severity values attained using different fungicide strategies. Standard program was initiated at shuck fall without regard to weather conditions. "Predicted infection" program was initiated 24 hours after the first post-bud break occurrence of 0.1" of precipitation at 50 F or greater. "Actual detection" treatment regime commenced 24 hours after initial detection of *P. clandestina* in the orchard air using the Rotorod air sampler/PCR identification technique.

2006-05-20	A 224	B 0.17	C 64.5	Primary infection has occurred. Fungicide treatment recommended. Secondary infection/risk Assessment Index initiated.* Powdery Mildew of Cherry Management Options	

Cherry Powdery Mildew Secondary Infection/Risk Assessment Index Summary
For station: Moses Lake

Date	Risk Index	Disease Pressure	Status or Event	Consecutive Hours between 59 and 82 F	Hours Temp > 86 F	Spray Interval	Notes
2006-05-20	n/a		Primary Infection	3	0		
2006-05-23	0	Low	Pathogen present	6	0		
2006-05-24	20	Low	Pathogen present	13	0		
2006-05-25	40	Moderate		8	0		
2006-05-26	60	High	Pathogen reproducing every 5 days	D 7	0	Short	Treatment recommended if fungicide protection has expired Powdery Mildew of Cherry Management Options

Figure 5. Output of model components A, B, and C during a critical period during the season. Events at A (degree-day threshold), B (precipitation), C (temperature) coincided to result in primary infection. Note that 0.17" of precipitation was received at 64.5 F prior to the accumulation of 250 cumulative degree days > 50 F; *at this point primary infection occurs which initiates secondary infection risk index.* Note crossing of high-pressure threshold in the Risk Index Column in D. The epidemiological events a A-C and D are hyperlinked to management recommendations.