Annual Progress Report
This is year 3 of a 3 year proposed project.
For the Washington State Grape & Wine Research Program

Date: 20 February 2014
Project Title: Systems-based Management of Powdery Mildew and Botrytis Bunch Rot of Wine Grapes (continuation from 2011 and 2012)
Principal Investigator(s): Gary Grove (objectives 1-5) and Michelle Moyer (objectives 1-2)
Collaborator(s):
Project Budget Number: 3361-5524

I. **Project Summary: Non-technical, 1 - 3 succinct paragraphs that cover the following points:** This work is focused on the effect of duration of berry wetness and temperature on the infection of wine grapes by *B. cinerea* in Eastern Washington. Field studies were conducted to evaluate whether the current Washington State University AgWeatherNet Bunch Rot model is compatible with typical weather conditions in Eastern Washington. Tests were performed post-veraison 2011-2013 and during bloom in 2013. The data suggests the currently available predictive model may be under predictive, especially in vineyards with diffuse powdery mildew infections. Four fungicides were also evaluated post-infectively to determine how much time post wetting period/inoculation could elapse and still allow for good protection of flowers and clusters. Results showed that some fungicides might effectively control Botrytis 48-hours post infection of berries. The economic viability of this approach is unclear.

II. **Materials, Methods and Experiments Conducted to Meet Stated Objective(s):**

1. Develop bio rational and sustainable PM (PM) fungicide programs through the judicious application of various fungicide modes of action in conventional and organic, bloom-centered fungicide programs. Efforts were focused on the period before berries develop ontogenic (age related) resistance to PM. Some disease management approaches were evaluated for efficacy against PM and effects on insect populations.

2. Evaluate organic and conventional programs for control of PM on clusters and develop cost-effective leaf removal/fungicide programs for disease management. The latter portion of this objective is part of proposal "Horticultural Impacts of Fruit Zone Leaf Removal in Wine Grapes and Mitigating Botrytis Bunch Rot in the Winery" (M. Moyer, lead PI). Synthetic and organic trials were conducted to evaluate the efficacy multiple fungicide regimens for management of grape powdery mildew. Fungicide applications were made to mature 'Chardonnay' or 'White Riesling' vines located at the Washington State University Irrigated Agriculture Research and Extension Center, Prosser, WA. Powdery mildew incidence and severity were rated on fruit clusters by visually determining the percent cluster area with powdery mildew on 24 arbitrarily selected clusters per plot (12 from the west and 12 from the east side of each plot). On 17 Aug disease incidence and severity was rated on leaves by visually determining the percent leaf area with signs of the disease on 80 arbitrarily selected leaves per plot (40 from the west and 40 from the east side of each plot).

Organic Programs. Sulfur-based disease management programs were evaluated through the period from bloom to fruit set. Disease pressure was relatively low.

Synthetic, bloom-centered mode of action studies. Bloom centered quinoxyfen/myclobutanil regimens were applied to determine the best time to include quinoxyfen for managing powdery mildew on grape bunches. Initial quinoxyfen applications were made at 6-12" shoots, prebloom, bloom, and 14 and 28 days post bloom. Myclobutanil was applied at key stages that did not include quinoxyfen.

3. *Evaluate the Broome Botrytis bunch rot model for use in Eastern Washington.* A total of 21 field inoculations were made between late September and mid-October. The inoculations were made over a broad range of temperature and wetting regimes and many were successful.

4. *Evaluate various PM (QoI/strobilurin) fungicide programs for management of Botrytis bunch rot (BBR)* **a.** Evaluate bloom to pre closure and veraison QoI fungicide applications (targeted for PM) for efficacy against Botrytis bunch rot BBR after veraison. **b.** Evaluate bloom applications of QoI fungicides for late-season BBR control.

Bloom centered quinoxyfen/myclobutanil regimens were applied to determine the best time to include quinoxyfen for managing powdery mildew on grape bunches and determine whether benefits extended to management of BBR. Quinoxyfen applications were made at 6-12" shoots, prebloom, bloom, or 14 and 28 days post bloom. Myclobutanil was applied at key stages that did not include quinoxyfen. Fruit were harvested in mid-September and placed in humidity chambers and incubated 14 d at about 20 C in a 12-hour photoperiod. Disease severity was determined by estimating the percent of bunch area infected by *Botrytis cinerea*.

5. *Further evaluate the post infective activity of BBR fungicides for use in conjunction with forecasting model.* Twenty-four, 48, 72, and 96 hours after harvest/wetting inoculated flowers and clusters were dipped in solutions of Elevate, Vangard, Scala, and Pristine and placed in humidity chambers and incubated at 70 F. Disease severity was determined by estimating the percent of bunch area or blossoms infected by Botrytis.

III. Major Research Accomplishments – include the following points:

1. Objectives 1 and 2: results are reported in companion report *Horticultural impacts of fruit-zone leaf removal in wine grapes and mitigating Botrytis bunch rot in the winery* (M.M. Moyer, G.G. Grove, and J. Harbertson).

Objective 3. *Evaluate the Broome Botrytis bunch rot model for use in Eastern Washington.* Field results were subjected to stepwise regression analysis. The best regression models representing field inoculation data were of the form:

$\sqrt{Y} = b_0 + b_1T + b_2W + b_3T{*}W$ *(experiment 1)*

$\sqrt{Y} = b_0 + b_1T + b_2T^2W + b_3T^3W$ (*experiment 2*)

and $\sqrt{Y} = b_0 + b_1T + b_2TW + b_3T^3$ (*experiment 3*). All estimated parameters (regression coefficients) were significant at $P < 0.05$. Coefficients of determination (R^2) for equations 2, 3, and 4 were 0.28, 0.16, and 0.40, respectively. Residuals had a random pattern and were normally distributed.

Portions of the vineyard were severely infested with powdery mildew during both years of the study, specifically the "inoculum row" that was left unsprayed throughout the season to provide powdery mildew inoculum for various lab projects. Even with the presence of a heavily infested powdery mildew row, the vines where clusters were collected had a standard spray regime to control powdery mildew levels and powdery mildew colonies were not visible on tested clusters, implying that any powdery mildew present would be as diffuse colonies. Our tests showed a wetness period of as little as 0.75 hours of wetting was required for *B. cinerea* infections to develop. These wetting durations are shorter than the wetting periods specified by Broome Bunch Rot model currently used on the WSU AgWeatherNet.

A possible reason for these shortened wetness periods required for Botrytis infection in our vineyard is the expected presence of diffuse powdery mildew colonies on the clusters. This is relevant because even a diffuse infection can increase rates of Botrytis infection.

This information shows the inherent flaw in the currently available Bunch rot model being used in Eastern Washington. We postulate than in the presence of diffuse powdery mildew infection, *B. cinerea* can establish an infection with a wetness period of less than three hours, sometimes in less than 1 hour.

The current bunch rot model available on AgWeatherNet requires about 4 hours of wetting in order to hasten warnings of infection. Our results indicate that this wetting period required before recommended spraying for grey mold may be too long and therefore under predictive under medium to high levels of powdery mildew infestation. Use of the Broome Model in vineyards with high levels of powdery mildew infestation may not be sufficiently conservative to prevent catastrophic economic losses under conditions conducive to bunch rot. This information suggests that, rather than utilizing predictive models for Botrytis bunch rot, regular protective spraying should be performed until more research is done. The models do not and cannot predict the effect of powdery mildew infection on Botrytis levels, especially in the presence of diffuse colonies of powdery mildew.

Regression analyses of the 2011-13 field data were significant but a model with consistent terms was not identified. However, various combinations of temperature and wetting were significant during all years of the study. Coefficients of determination ranged from 0.16 to 0.40, indicating that model terms failed to account for a large portion of the variability in the data. Ironically, the highest amount of variability (40%) was accounted for by model components that best described the wetting/uninoculated data. The reason for this is unclear but may possibly be due to differences in background inoculum or varying levels of diffuse powdery mildew infections.

Objective 4. *Evaluate various PM (QoI/strobilurin) fungicide programs for management of Botrytis bunch rot (BBR*). Bunch rot severity ranged from 70.9 to 92.2 and 74.0 to 95.0 in trials 1 and 2, respectively (Tables 1 and 2). None of the "dual-purpose" fungicides provided control of bunch rot when applied according to a powdery mildew application schedule.

Rate/A or percent solution formulated product	Spray timing [z]	**PM Disease severity**		**Bunch Rot Severity**
		% leaf area diseased	% cluster area diseased	
Non-treated		16.0a	57.7a	86.3a
Rally 40WSP 5 oz + Break-Thru 8 fl oz/100 gal Quintec 250SC 4 fl oz + Break-Thru 8 fl oz/10(gal	1,3,5 2,4,6	1.5 b	9.8 b	90.2a
Inspire Super 20 fl oz + Break-Thru 8 fl oz/100 ga Quintec 250SC 4 fl oz + Break-Thru 8 fl oz/10(gal	1,3,5 2,4,6	1.4 b	3.1 b	70.9a
A19334 10.5 fl oz + Break-Thru 8 fl oz/100 gal Quintec 250SC 4 fl oz + Break-Thru 8 fl oz/10(gal	1,3,5 2,4,6	1.8 b	4.1 b	
A19334 13 fl oz + Break-Thru 8 fl oz/100 gal Quintec 250SC 4 fl oz + Break-Thru 8 fl oz/10(gal	1,3,5 2,4,6	1.4 b	3.5 b	
Quadris Top 14 fl oz + Break-Thru 8 fl oz/100 gal Quintec 250SC 4 fl oz + Break-Thru 8 fl oz/10(gal	1,3,5 2,4,6	2.2 b	6.4 b	76.7a
Torino 3.4 fl oz + Break-Thru 8 fl oz/100 gal	1,2,3,4,5 ,6	1.8 b	13.8 b	
GWN-10250 8 fl oz + Break-Thru 8 fl oz/100 gal	1,2,3,4,5 ,6	10.8ab	40.7ab	
GWN-10250 8 fl oz + Break-Thru 12 fl oz/100 ga	1,2,3,4,5 ,6	2.6 b	30.7ab	
GWN-10250 8 fl oz + Break-Thru 24 fl oz/100 ga	1,2,3,4,5 ,6	2.4 b	25.5ab	

Table 1. Powdery mildew and bunch rot severity on foliage and clusters treated with "dual purpose" fungicides during the growing season.

Rate/A or percent solution formulated product	Spray timing [z]	PM Disease severity: % leaf area diseased	PM Disease severity: % cluster area diseased	Bunch Rot Severity
Non-treated..		1.62ab	4.27ab	95.0a
Rally 40WSP 5 oz + Break-Thru 8 fl oz/100 gal Quintec 250SC 4 fl oz + Break-Thru 8 fl oz/100 gal	1,4,6 2,5,8	0.19 b	2.35ab	88.0a
Quintec 250SC 4 fl oz + Break-Thru 8 fl oz/100 gal..	1,2,4,5,6,8	0.31 b	1.63ab	
Luna Experience 8.6 fl oz + Break-Thru 8 fl oz/100 gal Quintec 250SC 4 fl oz + Break-Thru 8 fl oz/100 gal..	1,4,6 2,5,8	0.57 b	0.73 b	92.0a
Pristine 8 oz + Break-Thru 8 fl oz/100 gal Quintec 250SC 4 fl oz + Break-Thru 8 fl oz/100 gal	1,4,6 2,5,8	0.25 b	0.10 b	79.6a
Luna Tranquility 16 fl oz + Break-Thru 8 fl oz/100 gal..	1,2,4,5,6,8	0.04 b	0.14 b	83.0a
Luna Experience 8.6 fl oz + Break-Thru 8 fl oz/100 gal..	1,2,4,5,6,8	0.06 b	0.28 b	74.0a
Tevano 13 fl oz	1,3,5,7	4.68a	15.35a	
Tevano 6.5 fl oz	1,3,5,7	0.61 b	13.90ab	
Pristine 8 oz + Break-Thru 8 fl oz/100 gal ..	1,3,5,7	0.14 b	0.30 b	

Table 2. Powdery mildew and bunch rot severity on foliage and clusters treated with "dual purpose" fungicides during the growing season.

Objective 5. *Further evaluate the post infective activity of BBR fungicides for use in conjunction with forecasting model.* *Fruit inoculations***.** Based on the results for post infective fungicide studies on fully ripe clusters, it appears that growers may have up to 48 hours after infection to apply most fungicides to reduce the severity of bunch rot. This knowledge can be used to produce a better model for Botrytis control, as it allows for a delay in spraying without a loss of too much control ability. It also suggests a longer establishment period on grapes in the typical climatic conditions experienced in southeastern Washington than found in other viticulture areas.

Fungicide	Treatment	***R***^{2}	***R***^{2} **Adjusted**	***F***	***Prob > F***
cyprodinil	I[1]	0.26	0.23	9.7	0.004
	S[2]	0.20	0.17	6.9	0.01
pyraclostrobin/boscalid	I	0.01	0.01	0.07*	NS**
	S	0.10	0.09	0.09*	NS**
fenhexamid	I	0.31	0.29	12.7	0.001
	S	0.13	0.12	3.8*	NS**
pyrimethanil	I	0.16	0.15	5.4	0.03
	S	0.27	0.25	10.6	0.003

Table 3. Coefficients of determination (R2), R2 adjusted for degrees of freedom, F-rations, and significant levels from regression of bunch rot severity on clusters inoculated with B. cinerea, or not inoculated but wetted, on post infective treatment (hours).

Regression analysis was used to determine the relationship between disease severity and lapsed time between wetting (S) and wetting/inoculation (I). Data from each fungicide were analyzed separately for both runs of each experiment and then on the combined data. Disease severity values obtained from each fungicide treatment were regressed on hours post inoculation.

F-tests were conducted to determine if results from the multiple runs of each fungicide were significantly different. The *F*-tests indicated that results from each experiment were not significantly different, so results of the combined data are shown in Table 3. The effect of time was significant in all treatments except pyraclostrobin/boscalid and wetted but inoculated clusters treated with fenhexamid. In all cases where the relationship between disease severity and time were significant, the regression coefficient for time (b_1) was positive.

_*Flower inoculations.* Average infection rates on grape flower clusters with post infection fungicide applications are presented in Table 4. All inoculations and pesticide dips were performed in June 2013. Pesticide dips were performed 24, 48, and 72 hours post spray with either $2x10^4$ conidia/mL (Inoculated) or sterile water (Control) and allowed to incubate on PDA for 14 days. Percent infection was determined as infected blooms out of ten blooms total, 4 replications per cluster.

	24 hours	**48 hours**	**72 hours**
Cyprodinil Inoculated	7.5	17.5	20.0
Cyprodinil Control	5.0	12.5	12.5
Pyrimethanil Inoculated	12.5	20.0	37.5
Pyrimethanil Control	5.00	15.0	27.5
Fenhexamid Inoculated	37.5	12.5	32.5
Fenhexamid Control	27.5	20.0	27.5
Untreated Control (Inoculated)	50.0	22.5	57.5
Untreated, Not Inoculated	27.5%	37.5%	22.5%

Table 4. Per cent of *V. vinifera* flowers infected following inoculation with *B. cinerea* and then treated with fungicide 24, 48, and 72 hours after inoculation.

IV. How have results been or expected to be disseminated to grape and wine industry stakeholders and audiences of interest?

Newhouse, J., Grove, G., and Moyer, M.M. 2013. Effectiveness of biopesticide-based programs on grape powdery mildew. Washington State Grape Society Annual Meeting (poster).

Grove, G.G. Moyer, M.M., and Schwager, M. Effect of bloom-centered quinoxyfen application on severity of powdery mildew on *Vitis vinifera* 'White Riesling' berries. WAWGG Annual Meeting (poster).

Schwager, M. 2013. Evaluation of predictive models for Botrytis bunch rot infection in wine grapes and their applicability in Eastern Washington. MS Thesis, Washington State University.

V. Project Budget Status:

Project is complete. $285 remains unspent.

VI. Other Sources of Funding:

Mitigation of Fungicide Resistance Risk Using Biopesticides and Leaf Removal in Washington Wine Grapes IR4. $23,911.