

ANNUAL REPORT

Proposal Title: Grower Implemented Quantitative LAMP for Initiating and Adjusting Fungicide Program

Proposal #: 2014-1398

Principal Investigators:

Walt Mahaffee, USDA-ARS-HCRL, 3420 NW Orchard Ave Corvallis, OR 97330

Lindsey Thiessen, Dept Botany and Plant Pathology, Cordley Hall, Corvallis, OR 97331

Cooperators: Daniel Jenkins, Associate Professor, University of Hawaii at Manoa, Manoa, HI. Phone: 808-956-9917; Fax: 808-956-3542; danielje@hawaii.edu. Ryo Kubota, Diagenetix, LLC, Honolulu, HI 96822. Phone: 808-956-9917; Fax: 808-956-3542; ryokubot@hawaii.edu

SPECIFIC OBJECTIVES OF PROPOSED RESEARCH:

The mission of this research is to increase the economic sustainability of grape production by providing decision support tools to aid in management of grape powdery mildew. In this project we propose to test the utility of a quantitative Loop mediated isothermal AMPlification assay (LAMP) and handheld device for detection and quantification of airborne inoculum; thereby extending our research on the use of inoculum detection as decision support tool for managing grape powdery mildew. The specific objectives are:

1. Test implementation of a grower preformed quantitative LAMP assay.
2. Examine the effectiveness of adjusting fungicide interval based inoculum density.
3. Assessment of quantitative LAMP (qLAMP) for estimating amount of fruit infection

Results

1. Test implementation of a grower preformed quantitative LAMP assay.

Beginning in March, 23 spore traps were deployed in 7 commercial vineyards and monitored biweekly using quantitative LAMP procedures. Positive detection from these traps first occurred on May 5, 2014 with 22 positive detections in commercial fields between April 21 and August 12, 2013. Intensive disease scouting of numerous vineyards indicated that the powdery mildew disease pressure was very low except at locations that had early detection. This is also in agreement with data from our research vineyard.

At the research vineyard, spore trap samples were collected on a biweekly and daily basis and processed for qLAMP and qPCR. Using the qPCR as our standard, the qLAMP procedure was accurate 70% of the time (Table 1) with the qLAMP tending to have false negatives, thus a low specificity (37%). The low accuracy may be attributed to PCR inhibitors that were present in the early growing season. The early portion of the growing season was relatively dry, and inhibitors, such as pollen and insects, were highly prevalent in DNA extracts utilized in the qLAMP process. Despite amplification inhibition, a significant association was still seen between the qPCR and LAMP assays ($P < 0.0001$) early and late in the season.

Due to the issue with inhibitor prevalence in DNA extractions, spore quantities obtained from 2014 qLAMP assay underestimated spore quantity compared to the qPCR assay (Fig. 1). However, the later season estimates were better correlated. This process was further optimized by utilizing a mastermix that is more tolerant to inhibitors. Loop primers were also removed from the reaction to create a more consistent standard curve (Figure 2).

We continued to evaluate beta versions of the SMART-DART device throughout 2014. Six devices were distributed to growers to conduct the qLAMP assay. The devices are easy to use, very compact, and suitable for pathogen detection. The commercial version will be battery operated with a slimmer casing. The devices provided positive/negative results for DNA amplification, with high accuracy of results (82%) compared to the qPCR assay. Complications in developing software rendered the quantification aspect of the SMART-DART device unusable during the 2014 growing season. However, further optimization of the software is continuing to develop the capability to quantify outputs. The SMART-DART device is currently on the market at \$2490 per unit at <http://diagenetix.com>. While this is more expensive than prior estimates, the unit is now battery powered and easily portable, improving its versatility.

Table 1. Contingency table representing LAMP assay and quantitative PCR (qPCR) results for the presence of *Erysiphe necator* sampled from custom made impaction spore traps from both commercial vineyards and research plots at the Oregon State University Botany and Plant Pathology Field Lab in 2014.

		qPCR ^a		Fisher's Exact Test ^b	Accuracy	Sensitivity	Specificity
		Positive	Negative				
G-LAMP	Positive	2	4	0.22	82%	94%	18%
	Negative	9	58				
L-LAMP	Positive	36	8	< 0.0001*	70%	94%	37%
	Negative	61	123				

^aqPCR results based on TaqMan® probe with minor groove binder for detecting *E. necator* DNA. "Positive" and "Negative" indicate the number of samples for which *E. necator* DNA was detected and not detected, respectively.

^bFisher's exact test probability was used to assess the null hypothesis that the LAMP assay was not significantly different from the qPCR assay.

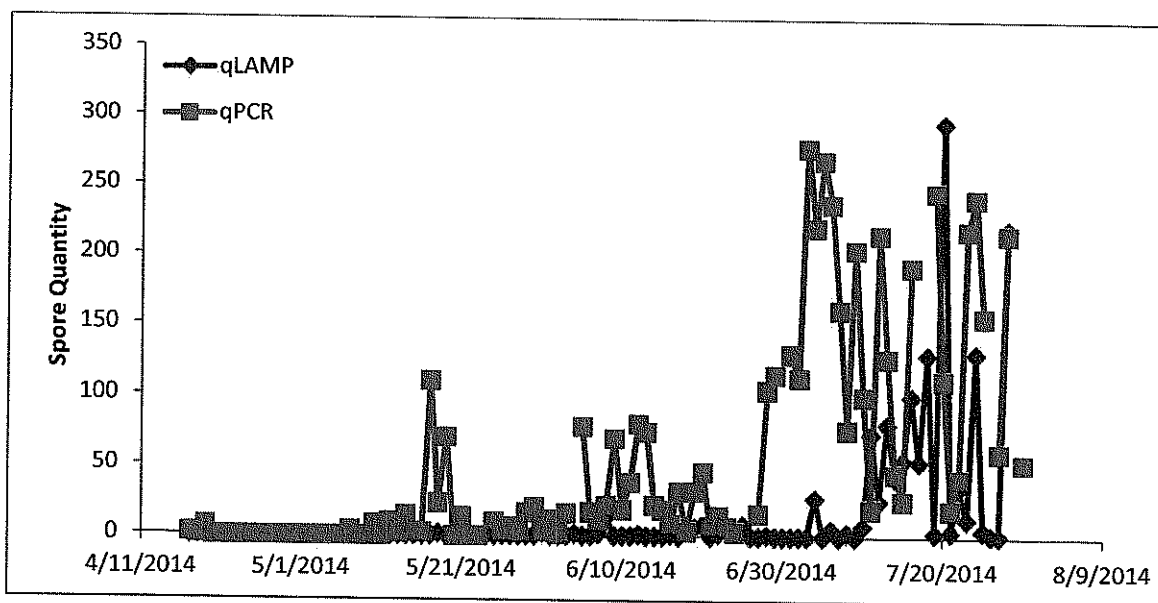


Figure 1. Airborne *E. necator* inoculum concentrations as estimated using quantitative PCR (Red line) and quantitative LAMP (blue line). Values represent the number of *E. necator* conidia impinged over 3 or 4 days on sample rods mounted on an impaction spore trap. Approximately 100,000 liters of air were sampled per day.

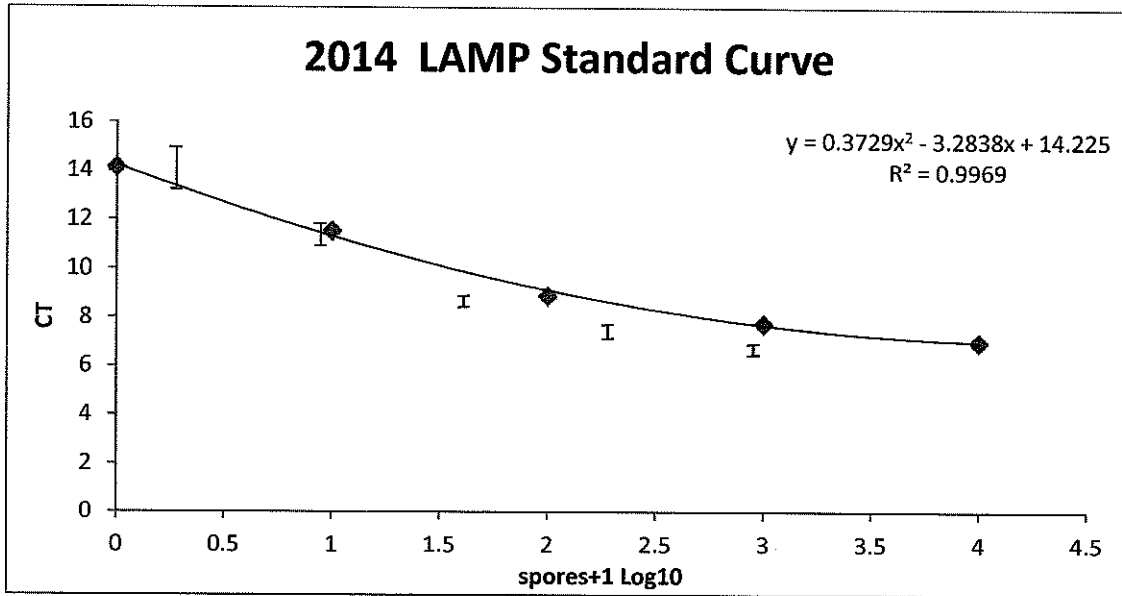


Figure 2. Standard curve based on new qLAMP protocols using thermostable polymerase and four primers (FIP, BIP, F3, and B3) to estimate relative airborne *E. necator* inoculum in vineyards for 2014. Each point represents the mean of 4 replications with 3 subsamples per replication.

2. Examine the effectiveness of adjusting fungicide interval based inoculum density.

Fungicide programs were initiated upon initial detection of grape powdery mildew or at bloom, whichever came first. Subsequent spore detections above 10 spores reduced the spray interval to a short fungicide application interval. Below 10 spore detections, fungicides were maintained at a long interval. Disease scouting was conducted weekly throughout the growing season by obtaining the disease incidence on 10 leaves per 50 vines within grower standard control and modified interval plots. The areas under disease progress curve for the control (0.27 ± 0.73) and modified interval (0.11 ± 0.28) were not significantly different ($P = 0.58$) (Figure 3), which indicates that the modified interval may be as effective at managing grape powdery mildew as the grower standard. The increased disease in Figure 3 at the end of the season is largely attributed to the increase in lateral shoots after hedging that did not receive timely fungicide applications.

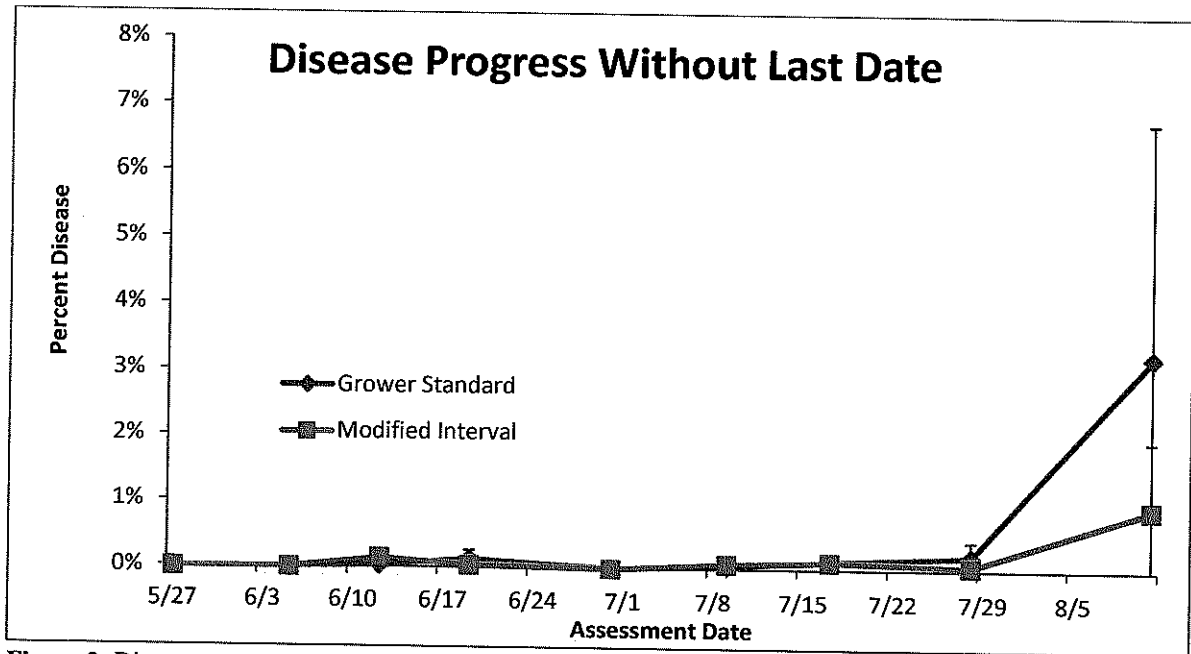


Figure 3. Disease progress curves for field disease incidence without the final assessment at the end of the season determined by field scouting in 2014 for 6 commercial vineyards. A sample of 500 leaves were assessed from a modified interval plot (fungicide program initiation was delayed until disease detection and subsequent applications were adjusted to a shortened interval if airborne inoculum reached a 10 spore threshold) (red line) and a control plot (fungicide initiation followed the grower standard) (blue line). Error bars are based on the standard error for each data point. Area under disease progress curve (AUDPC) values were determined using average disease incidence (%). Detection and control AUDPC values were 0.27 ± 0.73 and 0.11 ± 0.28 , respectively, in 2014. The control plots were not significantly different from the modified interval plots ($P = 0.58$).

Since disease was so very low in 2014, we made one final assessment within 2 weeks of harvest (Figure 4). Significant leaf disease was present in all fields, which indicates that conditions were favorable for disease development once fungicide applications had ceased.

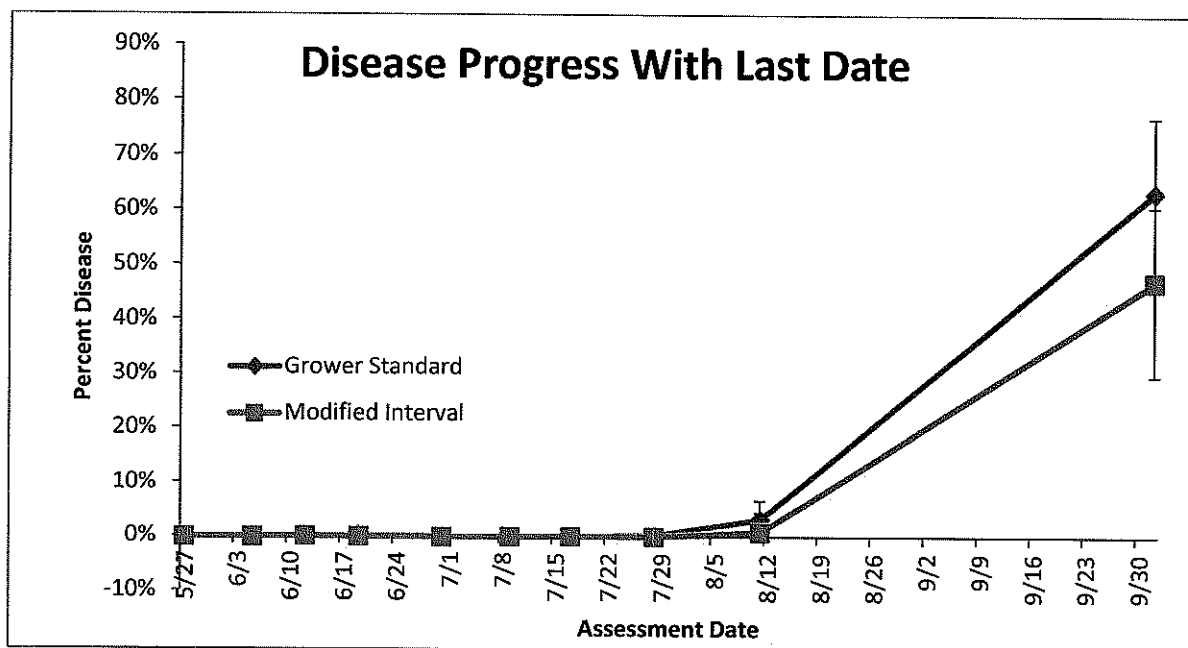


Figure 4. Disease progress curves for field disease incidence including end-of-season assessments determined by field scouting in 2014 for 6 commercial vineyards. A sample of 500 leaves were assessed from a detection plot (fungicide program initiation was delayed until disease detection and subsequent applications were adjusted to a shortened interval if airborne inoculum reached a 10 spore threshold) (red line) and a control plot (fungicide initiation followed the grower standard) (blue line). Error bars are based on the standard error for each data point. Area under disease progress curve (AUDPC) values were determined using average disease incidence (%). Detection and control AUDPC values were 16.8 ± 8.8 and 12.5 ± 12.4 , respectively, in 2014. The control plots were not significantly different from the detection plots ($P = 0.48$).

3. Assessment of quantitative LAMP (qLAMP) for estimating amount of fruit infection

This objective was not pursued in 2013 or 2014 due to the reduced funding (50%) received.

Major Research Accomplishments

- 1) We demonstrated that the qLAMP procedure is highly specific and sensitive and preforms equivalent to qPCR for detection of *E. necator* in the air. Thus, it is suitable for testing in grower trials.
- 2) We improved the speed of the reaction and robustness of the qLAMP reaction, and created a quantitative standard curve for further testing.
- 3) We demonstrated the efficacy of a modified spray interval system utilizing spore quantities to extend spray intervals.

Presentations of Research

Publication

Thiessen, L.D., Keune, J.A. Neill, T.M., Turechek, W.W., Grove, G.G. and Mahaffee, W.M. 2014. The development of a grower performed inoculum detection assay for

management of grape powdery mildew (*Erysiphe necator*) Phytopathology 104: (submitted).

- Mahaffee, W.F. 2014. Use of airborne inoculum detection for disease management decisions. Pp, 39-54. In: Detection and Diagnostics of Plant Pathogens, M. L. Gullino and P. Bonants, Eds. Springer Verlag, NY. (In press)
- Mahaffee, W.F. Schwebs, S., Hand, F., Gubler, D. Baily, B. and Stoll,R. 2014. Improving management of grape powdery mildew with new tools and knowledge. Practical Winery and Viticulture Spring:59-67

Formal Presentations in 2014:

- Oregon Wine Symposium (2/25-26/2014)
- Salem Grape Growers meeting (3/4/2014)
- Oregon State Grape Day (4/1/2014)
- Mid-Valley Oregon Grape Grower Workshop (5/21//2014)
- Annual Meeting of the American Phytopathological Society (8/2/2014)
- Salinas Valley Spinach Growers (11/14/2014)

Numerous informal presentations at grower meetings in Oregon.

Research Success Statements

We have demonstrated that a qLAMP procedure is highly specific and robust for detecting grape powdery mildew inoculum across 6 commercial vineyards. This procedure takes approximately 10 min for the DNA extraction and reaction setup and another 30 min to run the reaction using equipment that costs less than \$3200 to obtain. In cooperation with commercial vendors we have developed a master mix and device that will be suitable for commercial implementation. These results continue to indicate that implementation of inoculum detection for initiation of fungicide applications for grape powdery at the grower level will be technically and commercially feasible in the near future. This process has been made quantitative, and has shown the utility of adjusting fungicide application intervals based on inoculum quantity in vineyard air.

Funds Status

To date, all funds will have been spent by May 1.

