ANNUAL REPORT

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

Proposal #: 2013-1398

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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 **L**oop mediated isothermal **AMP**lification assay (qLAMP) and handheld device for detection and quantification of airborne inoculum; thereby extending our research on the use of inoculum detection as a 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:

Due to reduced funding, Objective 1 had to be reduced and Objective 3 eliminated in the second year of this 3 year project. The extensive cooperation of several participating growers who shuttled samples from the upper Willamette Valley to Corvallis, allowed for us to complete Objective 2 using lab processed samples only.

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

Beginning in March, 2013, twenty spore traps were deployed in 13 commercial vineyards and monitored biweekly using qPCR and qLAMP procedures. To reduce costs, a grower shuttle system was established to transport samples from throughout the Willamette Valley to the lab in Corvallis every Monday and Thursday. Positive detection from these traps first occurred on May 15, 2013, with 137 positive detections in commercial fields between April 18 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. 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 83% of the time (Table 1) with the qLAMP tending to have false negatives, thus a low specificity (76%). The lower than normal accuracy may be attributed to inhibitors discovered on the rods used during May through July of the 2013 growing season. Rods from a new vendor were discovered to release inhibitors over time. A new cleaning process for sterilizing the rods was developed that included a hexane soak step to remove oils and minerals on the steel rods. Improvements were also made in the DNA extraction buffer to improve inhibitor removal. Despite the early-season inhibitor issue, a significant association was

still seen between qPCR and qLAMP assays ($P < 0.0001$) early and late in the season.

The quantitative LAMP protocol was refined and tested in 2013. Due to the issue with rod preparation in May-July, the spore quantities obtained for 2013 qLAMP underestimated spore quantity compared to the qPCR assay (Fig. 1). However, the later season results were better correlated once the new rod cleaning and DNA extraction procedures were implemented.. This process was further optimized by utilizing a mastermix that was more tolerant to PCR inhibitors. Loop primers were also removed from the reaction to create a more consistent standard curve (Figure 2).

Table 1. Contingency table representing LAMP assay and quantitative PCR (qPCR) results for the presence of *Erysiphe necator* sampled using custom made impaction spore traps located in commercial vineyards and research plots at the Oregon State University Botany and Plant Pathology Field Lab in 2013.

		qPCR[a]	
		Positive	Negative
LAMP	Positive	80	11
	Negative	25	95
Fisher's Exact Test (P)[b]		<0.0001***	
Misclassification Rate		17%	
Accuracy		83%	
Sensitivity		79%	
Specificity		76%	

[a]qPCR 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.
[b] Fisher's exact test probability was used to assess the null hypothesis that the LAMP assay was not significantly different from the qPCR assay. *** = significant chi-squared test at P <0.05.

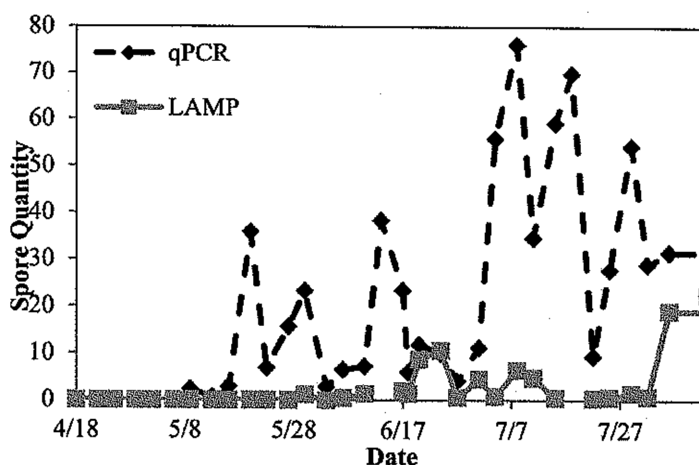

Figure 1. Airborne *E. necator* inoculum concentrations as estimated using quantitative PCR (dashed line) and quantitative LAMP (solid line). Values represent the number of *E. necator* conidia impinged over 3 or 4 days on sample rods mounted on an impaction spore trap. Approximately 100,000 liters of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

We continued to evaluate beta versions of the SMART-DART device and have a commercial version being manufactured in the coming months. We currently have 9 devices for testing with growers in the 2014 growing season. The devices are easy to use, very compact, and suitable for pathogen detection. The commercial version will be battery operated with a slimmer casing. While the funding of the first year of this project contributed to building of these devices, the >97% of the SMART-DART device development was funded through an SBRI grant to work on design improvements to the SMART-DART device and private venture capital. The SMART-DART device is scheduled to be on the market in 2014 at ~$1500 per unit. While this is more expensive than prior estimates,

we have improved the DNA extraction protocol to work with a lower grade/cheaper centrifuge to offset these costs. Therefore, the total capital investment should still be approximately $2,000 per setup.

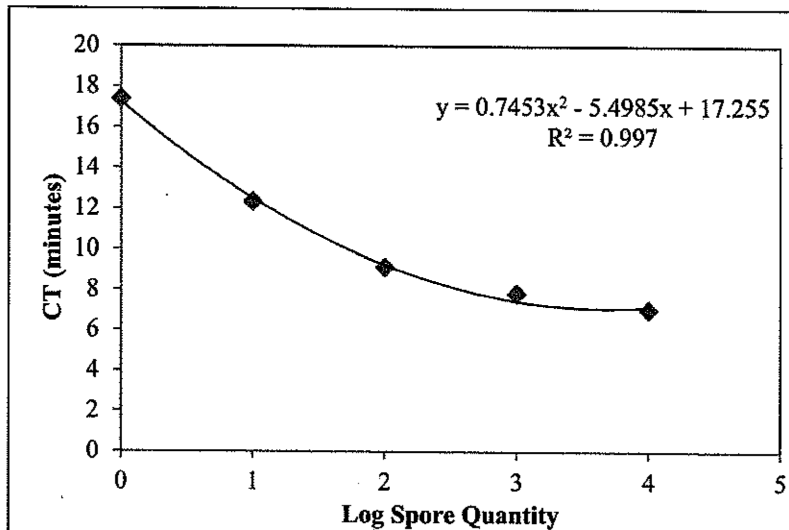

Figure 2. Standard curve based on new qLAMP protocols using thermostable polymerase and four primers (FIP, BIP, F3, and B3) to estimate relative airborne *E. necator* inoculum in vineyards for 2013. Each point represents the mean of 4 replications with 3 subsamples per replication.

2. <u>Examine the effectiveness of adjusting fungicide interval based inoculum density.</u>

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 spores, fungicides were maintained at a long interval. Disease scouting was conducted several times 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 (18.93 ± 9.40) and modified interval (52.81±29.58) were not significantly different (P = 0.3169) (Fig. 3), which indicates that the modified interval may be as effective managing grape powdery mildew as the previous grower standard. The increased disease (Fig. 3) at the end of the season is largely attributed to one grower who did not follow the protocol on trap placement. This specific trap was not placed in the area of the vineyard with the most disease the previous season, and thus, underestimated inoculum presence.

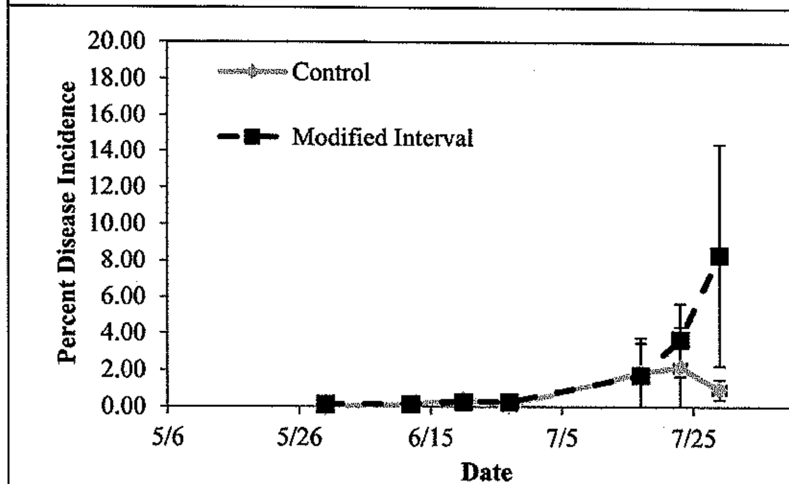

Figure 3. Grape powdery mildew disease progress curves of field disease incidence determined by accessing 500 leaves per plot on each date. Modified interval (fungicide program initiation was delayed until disease detection and subsequent applications were adjusted to a shortened interval if airborne inoculum reached a 10 spore threshold [dashed line]) and a control plot (fungicide initiation followed the grower standard) [solid line]).Points and error represent the mean and the standard error for of 5 commercial

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

This objective was not pursued in 2013 due to the reduced funding 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 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 knowledge of site-specific spore quantities to extend spray intervals.

Presentations of Research:

Publications:

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, XXX-XXX. 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 Viticutlure (In Press)

Formal Presentations in 2013:

Oregon Wine Symposium (2/19/2013)
Oregon Wine Research Institute Grape Day (4/2/2013)
American Vineyard Foundation (4/18/2013)
Salem Grape Growers meeting (6/4/2013)
Eastern Oregon Grape Grower Workshop (6/11//2013)
Napa Valley Organic Grape Growers Meeting (7/25/2013)
International Congress of Plant Pathology (8/28/2013)

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 13 commercial vineyards. This procedure takes approximately 20 min for the DNA extraction and reaction setup and another 30 min to run the reaction using equipment that costs less than $2000. 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 qLAMP assay has been made quantitative, and the continuation of this project will test whether this utility can be expanded to adjust application interval based on inoculum quantity in vineyard air.

Funds Status

To date, all funds will have been spent by May 1 due to the reduced funding in 2013-14.