ANNUAL REPORT

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

Proposal #: 2012-1398

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

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 **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 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

Because of the funding cycle, notification occurring after budbreak, our intention was not to begin the bulk of the work until the 2013 season. However, we still made substantial progress on objective 1. The Graduate student, Lindsey Thiessen, began work June, 2012, and we tested the sensitivity and specificity of the qLAMP procedure under commercial conditions.

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

Beginning in April, 2012, 22 spore traps were deployed in 17 commercial vineyards and monitored biweekly using the qLAMP procedure. Positive detection from these traps first occurred on May 22, 2012, with only 36 positive detections in commercial fields between April 27 and July 3. 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 with qLAMP and qPCR. Using the qPCR as our standard, the qLAMP procedure was accurate 84% of the time (Table 2), with the qLAMP tending to have false negatives; thus, a low specificity (58%). The biweekly samples had a higher degree of accuracy (94.6%) and specificity (87.5%) (Table 2). While these data indicate that the qLAMP procedure is highly reliable for the bi-weekly sampling proposed to be used for inoculum detection, it also indicated that there was the potential of inhibition of the reaction or insensitivity at very low spore levels associated with the daily sampling interval. Thus, we explored improvements in both the DNA extraction buffer and the qLAMP master mix to improve the accuracy/sensitivity of qLAMP assay. A new master mix was developed that improved reaction speed (positive detection of one spore in less than 15min), and an improved

DNA extraction buffer that is better at removing PCR inhibitors was developed. Combined, these improvements increased the sensitivity of the qLAMP for detection; however, they negatively impacted the accuracy of the assay and our ability to accurately quantify spore number. Thus, the assay it is still only semi-quantitative (e.g. able to distinguish orders of magnitude difference). Currently, it appears that we will be able to categorize samples into having no detection, <10, <100, <1000, and >1000 spores. This relative quantification will still allow us to achieve the goals set forth in this proposal. We are working to alter the qLAMP primer set to slow the reaction down and improve resolution and ability to quantify.

We also evaluated two beta versions of the Smart-DART device with promising results. The devices are easy to use, very compact, and suitable for pathogen detection. However, their current configuration makes them somewhat inconsistent for quantification. Since we were successful in obtaining an SBRI grant to work on design improvements to the Smart-DART device, these issues should soon be, if not already, resolved. The Sart-DART device is scheduled to be on the market in 2014 at ~$1500 per unit. While this is more expensive than prior estimates, we are working to improve our DNA extraction procedure 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.

Table 1. Botany and Plant Pathology Farm contingency table comparing daily qLAMP and daily qPCR samples with regards to accuracy, sensitivity, and specificity.

		BPP Daily qPCR	
		Positive	Negative
BPP Daily qLAMP	Positive	72	1
	Negative	16	22

Accuracy	84.7
Sensitivity	98.6
Specificity	57.9

Table 2. Botany and Plant Pathology Farm contingency table comparing bi-weekly qLAMP and biweekly qPCR samples with regards to accuracy, sensitivity, and specificity.

		BPP Bi-weekly qPCR	
		Positive	Negative
BPP Bi-weekly qLAMP	Positive	28	1
	Negative	1	7

Accuracy	94.6
Sensitivity	96.6
Specificity	87.5

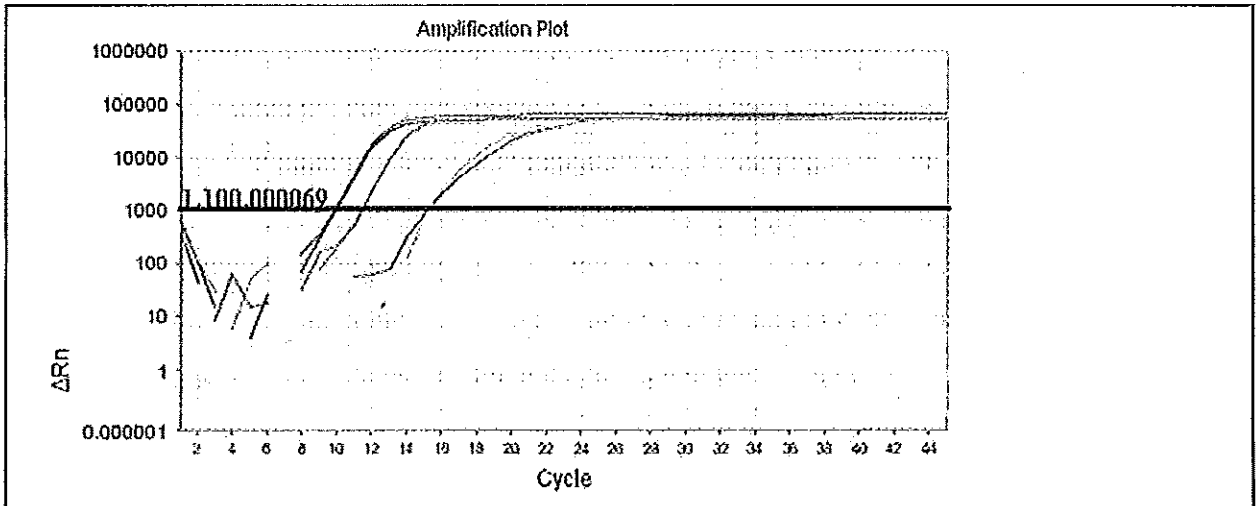

Figure 1. Amplification plot showing 1000 (purple), 100 (green), and 10 (yellow/red) spores.

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

   This objective will be started in 2013.

3. **Assessment of quantitative LAMP (qLAMP) for estimating amount of fruit infection.**
   Initial experiments indicated that there was a high degree of inhibition by the phenolics present in the juice that could not be overcome for the powdery mildew primer set. However, experiments conducted using primers sets for *Botrytis cinerea* indicate that an appropriate DNA extraction procedure can be developed.

Major Research Accomplishments

1) We demonstrated that the qLAMP procedure is highly specific and sensitive and preforms equivalent to qPCR for detection *E. necator* in the air. Thus, it is suitable for testing in grower trials.
2) Improved the speed of the reaction and robustness of the qLAMP reaction.
3) Developed an improved DNA extraction procedure.

Presentations of Research

Publication

No publications on this project were submitted this year.

Formal Presentations in 2012:

Oregon Wine Symposium (2/21/2012)
Napa Valley Grape Growers Meeting ((2/7/2012)
Eastern Grape Grower Workshop (5/8/2012)
University of Hawaii Manoa (7/19/2012)
Federal Laboratory Consortium for Technology Transfer (9/7/2012)
CAPCA conference (10/22/2012)

Numerous informal presentations at grower meetings in Oregon.

Research Success Statements

We have demonstrated that a quantitative LAMP procedure is highly specific and robust for detecting grape powdery mildew inoculum across 17 commercial vineyards. This procedure takes approximately 10 min for DNA extraction and reaction setup and another 30 min to run the reaction using equipment that costs less than $2000 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. Continuation of this project will test whether this utility can be expanded to adjust fungicide application intervals based on inoculum quantity in vineyard air.

Funds Status

To date, 7 months of funding for the graduate student and about 50% of the supply and travel budget have been spent.

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

Project Year: 2013
Project Duration: Year Two of three year project

SUBMITTED TO: AGRICULTURAL RESEARCH FOUNDATION FOR:
Unified Grant Management for viticulture and enology
American Vineyard Foundation
California Raisin Marketing Board
California Table Grape Commission
Oregon Wine Board
Washington State Grape & Wine Research

SUBMITTED BY:

_____ Date: _____
Walter Mahaffee

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

Cooperators:
Daniel Jenkins, Associate Professor, University of Hawaii at Manoa, Manoa, HI
Ryo Kubota, Diagenetix, LLC, Honolulu, HI

SIGNATURES

APPROVED BY:

_____ _____
Lynda Ciuffetti, Chair Date
Department of Botany and Plant Pathology
Oregon State University

_____ Date: _____
College of Agriculture

_____ Date: _____
Agricultural Research Foundation

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

JUSTIFICATION AND IMPORTANCE OF PROPOSED RESEARCH:
This project addresses National Grape & Wine Initiative Priority 3 (Production Efficiency) and 4 (Sustainable Practices). The use of information regarding inoculum availability and concentration to initiate and adjust fungicide application intervals will improve environmental and economic sustainability (3.1) by targeting applications to periods of highest risk.

Over the past few years viticulturists have faced extreme economic pressure in addition to persistent and escalating political pressure to reduce fungicide use while facing increasing demand from wine makers to produce higher quality fruit with reduced incidence of grape powdery mildew and sulfur residues. These concepts appear incompatible due to the nature of grape powdery mildew epidemics and the fungicides available for management. This is particularly true for viticulturists using low input viticulture practices (e.g. Organic, Biodynamic, LIVE) where there are few fungicide options and those that are available require frequent applications. However, there are still opportunities to optimize fungicide applications.

Many growers have used disease forecasting models, particularly the Gubler/Thomas model (Gubler et al., 1999), to adjust fungicide application intervals in an attempt to apply fungicides at optimal times. There have also been numerous efforts to improve disease forecasting (Moyer et al., 2010; Choudhury, McRoberts and Gubler, personal communication; Calonnec et al., 2008; Calonnec et al., 2009; Rossi et al., 2010; Caffi et al., 2011). However, these approaches assume that overwintering inoculum is present in the vineyard and that once ascospore release has been predicted to occur, inoculum is always available. This assumption is in disagreement with the spore trapping data we accumulated over the past 7 years, and the recent results of Moyer et al. (2011). We have demonstrated that overwintering inoculum is not always available in all vineyards (Falacy et al., 2007; Mahaffee et al., 2011; Sutherland et al., 2012) (e.g. Fig. 1). We have also demonstrated that knowledge of when airborne inoculum is available can be used to delay initiation of the fungicide program in commercial vineyards. Since 2007 in western Oregon, vineyards (N=43) managed utilizing inoculum detection to initiate fungicide

applications have reduced the total number of fungicide applications by 2.3 applications/year without any loss of disease control (e.g. Fig. 2). In 2011 and 2012, Coastal Viticulture Consultants independently demonstrated that quantitative assessment of inoculum availability resulted in fewer fungicide applications with no increase in disease development at most locations tested. However, at one location more and earlier applications were made than in previous years due to early detection. This resulted in improved disease control compared to

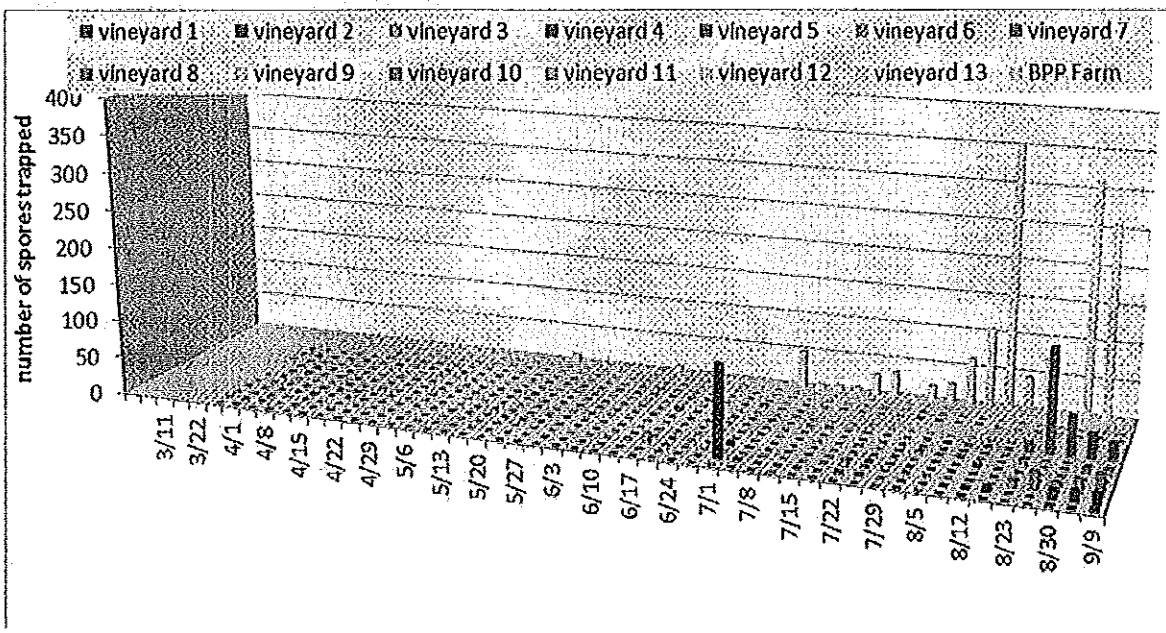

Fig. 1. Airborne *E. necator* inoculum concentrations as estimated using quantitative PCR. Values represent the number of *E. necator* conidia impinged over 3 or 4 days on sample rods mounted on an impaction spore trap. Approximately 100,000 liters of air were sampled per day.

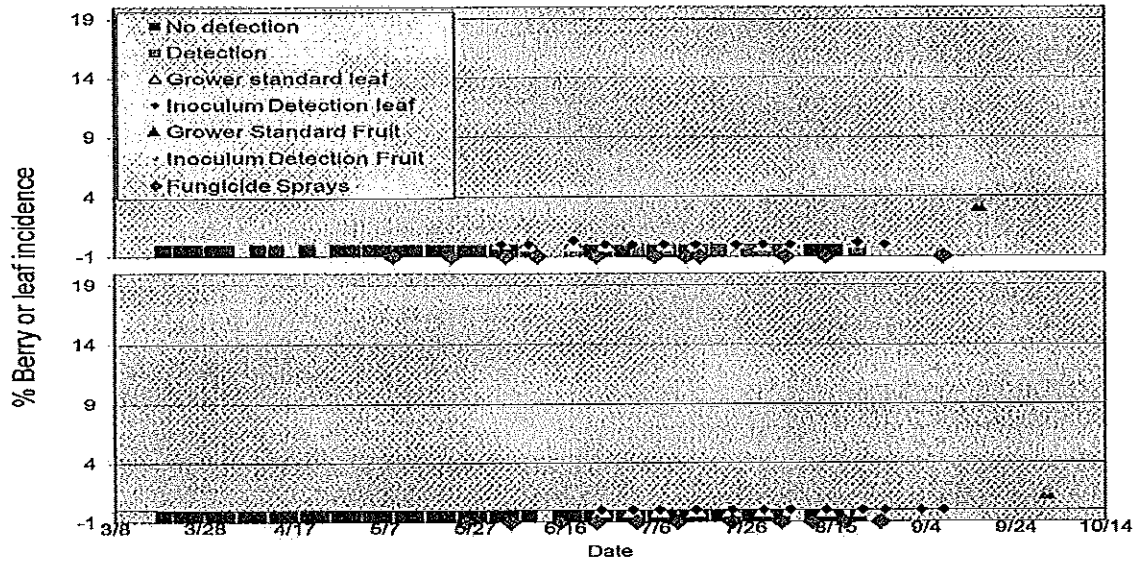

Fig. 2. Representative vineyards utilizing inoculum detection to initiate fungicide program. Squares indicate 3 to 4 day periods were detection did (green) or did not (red) occur. Leaf (yellow) and fruit disease (purple) from the grower standard fungicide program (triangles) verses delayed fungicide application (diamonds) leaf (blue) and fruit (orange) disease. The pink diamonds indicate fungicide applications that were made in the grower standard. Applications were withheld in the inoculum detection block until detection (green square) or bloom occurred (approximately mid-June).

previous years (Seth Schwebs, Personal Communication). Further, Doug Gubler's group has shown that inoculum detection using our qPCR assay detects powdery mildew incidence prior to visual symptoms and insect detection (Sutherland et al., 2012). These results indicate that inoculum detection is a useful decision support tool for managing grape powdery mildew.

However, the qualitative and quantitative PCR methods require expensive lab equipment and technical skill that limit implementation by many viticulturists. We, therefore, developed a LAMP assay (http://loopamp.eiken.co.jp/e/lamp/anim.html) for implementation by nontechnical users. The assay proved to be extremely sensitive and robust when conducted using reagents and materials under proper conditions (Fig. 3 and Table 1). The lab performed LAMP was accurate 89% of time when compared to the qPCR data with a sensitivity of 78%, indicating that false negatives were more common than false positives. For this analysis, we assumed that the qPCR was accurate. Some of the discrepancy between assays may be due to differences in spore numbers collected on the qPCR and LAMP traps since many of the disagreements occurred at or near detection limits (<10 spores).

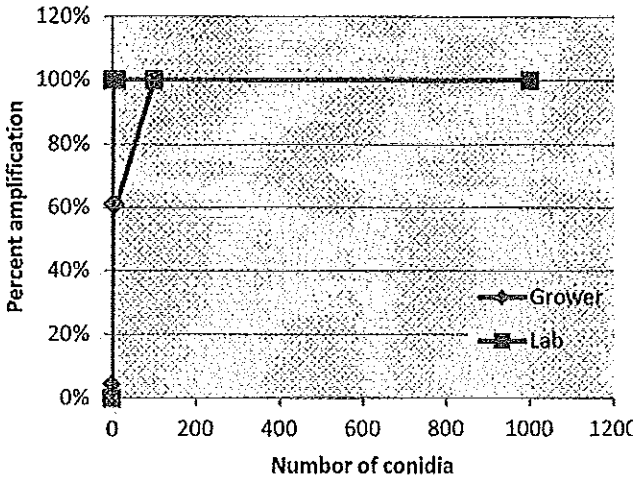

Fig. 3. Sensitivity of grower and lab performed LAMP-PCR for 2010 and 2011. Each point represents the mean of ≥21 replications.

Table 1. Summary of lab performed LAMP-PCR results in comparison to qPCR results from 8 and 4 vineyards in 2010 and 2011, respectively.

		Lab LAMP PCR	
		Positive	**Negative**
qPCR	**Positive**	**133**	**37**
	Negative	**14**	**262**

All samples were processed in the lab within 6 h of collection from the field.

Table 2. Summary of grower performed LAMP-PCR results in comparison to qPCR results from 8 and 4 vineyards in 2010 and 2011, respectively.

		Grower LAMP PCR	
		Positive	**Negative**
qPCR	**Positive**	**17**	**12**
	Negative	**20**	**44**

All samples were processed by growers at their location on same day as the qPCR.

Implementation by growers in 2011 was not as successful as in 2010 with an accuracy of 66% (Table 2) compared to 74% in 2010. We were able to determine that there were three reasons for the reduced performance. First, due to significantly delayed budbreak, reagents were stored far longer than in previous years. Second, the master mix storage conditions were less than optimal, which caused the master mix to suffer some degradation that resulted in nonspecific white precipitation. The degradation was a result of freeze-thaw cycles, which reduced the longevity of reagents compared to those stored in the lab. This issue was addressed by storing all reagents in an insulated cryobox, which reduced temperature fluctuations in various freezers. Third, the concentration of the stock magnesium sulfate provided with the enzyme changed, reducing the buffering capacity of the master mix (e.g. pH was not optimal for DNA extension), which resulted in less white precipitant. Thus, the

growers were required to make subjective inferences on whether or not amplification occurred. These results clearly indicated that the proposed LAMP assay was not appropriate for implementation. For these reasons, we pursued the development of a more robust detection system.

We hypothesized that a fluorescent dye or probe would be useful in detecting DNA amplification. Based on our experience using intercalating dyes to monitor LAMP assays, we did not pursue them because of cost and difficulty in implementing. Instead, we formed a collaboration with Daniel Jenkins at the University of Hawaii at Manoa and Ryo Kubota (Diagenetix, LLC) to pursue the development of a quantitative LAMP assay (Jenkins et al., 2011; Kubota et al., 2011; Kubota et al., 2011) using their Gene-DART and Smart-DART technology. This system is based on an assimilating probe that is specific for the loop portion of the LAMP amplicon (Fig. 4) and fluoresces upon amplification. The Gene-DART is a two part fluorescent probe master mix consisting of the assimilating probe, LAMP primers, and the Optigene Isothermal Master Mix, no dye (Optigene, Ltd. West Sussex, United Kingdom). The Optigene master mix is stable at room temperature for several weeks, making it highly suitable for field applications. Smart-DART (Fig. 5) is the handheld device that runs the LAMP reaction and records fluorescence over time using an android based smart phone or tablet computer. We have already developed and tested probes for both the forward and reverse loop of the *E. necator* LAMP amplicon. Using this approach, we were able to detect one conidium with no ambiguity in less than 30 min (Fig. 6). Testing to date indicates that both the forward and reverse probes are specific with equal fluorescence being detected as long as both loop primers are

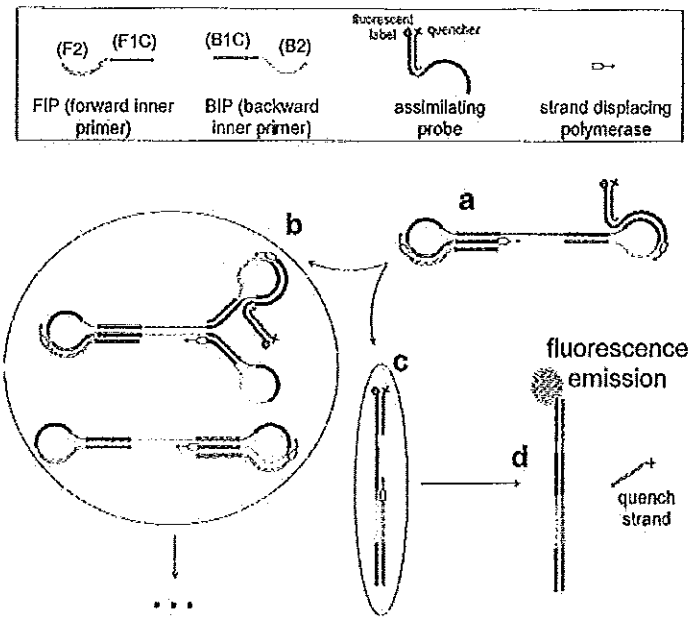

Fig. 4. Principle of assimilating probes for sequence-specific, real-time detection of the LAMP reaction. Hybridization of the assimilating probe to the loop of the LAMP amplicon (a) initiates priming, followed by extension (b,c) which ultimately results in fluorescence emission when the probe quencher strand is displaced (d) from the amplicon.

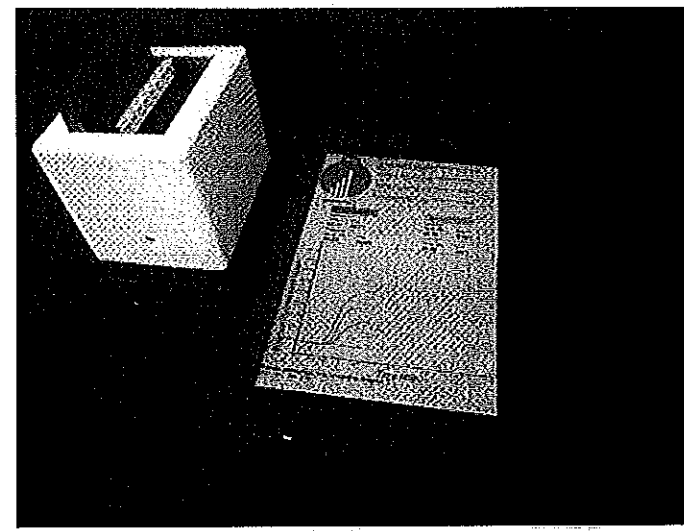

Fig. 5. Diagenetix Smart-DART handheld LAMP unit with tablet (or smartphone) interface. Capable of processing 8 samples in 30 min.

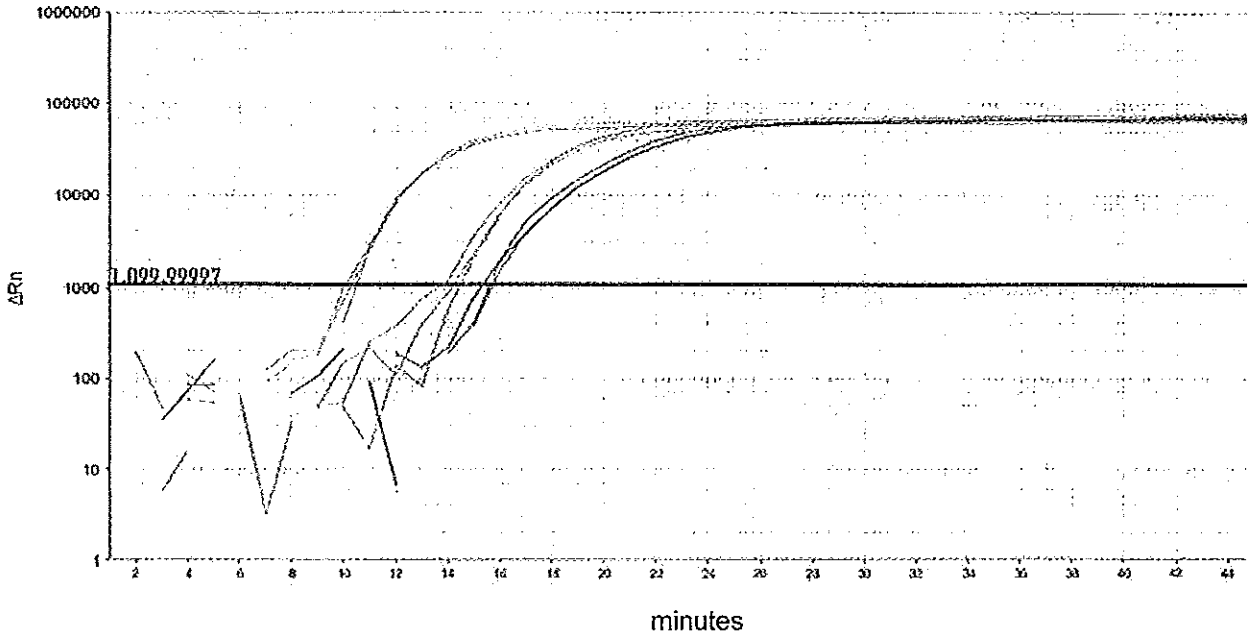

Fig. 6. Amplification curves for positive and negative samples using an assimilating probe in a qLAMP reaction. Reactions run on an ABI StepOnePlus real-time qPCR machine. Green= 1000 spores, orange= 10 spores, and blue=1 spore. Several negative field samples are not visible due to the lack of fluorescence detected.

used. However, the time to detection is significantly reduced (~50%) with the use of both probes with only a minor increase in costs (<$0.25/rxn.).

EXPERIMENTAL PROCEDURES TO ACCOMPLISH SPECIFIC OBJECTIVES:

qPCR Assay. DNA will be extracted from pairs of 1.1mm x 40mm stainless steel rods coated in silicone vacuum grease (Dow Corning, Midland, MI) using the PowerSoil® DNA extraction kit (Mo Bio Laboratories, Inc. Carlsbad, CA) following the manufacturer protocol. A set of control rods containing 500 *E. necator* (GPM) spores will be included in each DNA extraction to ensure extraction efficiency. Samples will be analyzed using quantitative PCR on an ABI StepOnePlus qPCR machine (Life Technologies, Foster City, CA) the same day that they are processed, and then stored at -20°C. Each 15µl qPCR reaction includes 7.5µl Path-ID® qPCR Master Mix (Invitrogen, Life Technologies Corp., Carlsbad, CA), 400nM final concentration of each GPM-specific forward and reverse primer and probe, and 1.5µl extracted field sample DNA. The two-step PCR conditions are 95°C for 10 min, followed by 55 cycles of 95°C for 15 sec and 65°C for 40 sec. The sequence of the GPM-specific primers and probes are: Unc144 forward 5'-CCGCCAGAGACCTCATCCAA-3', Unc511 reverse 5'-TGGCTGATCACGAGCGTCAC-3', GPM Probe 5'-6FAM-ACGTTGTCATGTAGTCTAA-MGBNFQ-3'.

For all qPCR experiments, each sample will be analyzed in triplicate and every reaction plate will contain the 500 spore extraction control as well as $1x10^2$ spore and $1x10^4$ spore positive controls, and a no-template control. Data acquisition and cycle threshold (Ct) analysis will be conducted using ABI StepOne™ software. The average GPM Ct values of the known positive controls from each reaction plate will be compared to the GPM spore standard curve described below to confirm the efficiency of each qPCR reaction plate. The efficiency thus confirmed, spore quantification for each unknown field sample will be determined by comparing the average sample Ct value to the spore standard curve.

A GPM spore standard curve will be prepared by suspending conidia harvested from infected *Vitis vinifera* cv. Chardonnay plants in 0.05% v/v Tween20 solution. Conidial concentrations will be determined using hemocytometer counts, and the suspension carefully transferred to silicone-coated stainless steel rods in volumes corresponding to $1x10^2$, $1x10^3$, $1x10^4$, and $1x10^5$ conidia (1 and 10 spore quantities will be transferred directly using an eyelash brush). The suspensions will be allowed to dry on the stainless steel rods in the laminar flow hood and DNA extractions performed using the PowerSoil method explained above. Five separate GPM conidia dilution series will be prepared in this manner and analyzed using qPCR as described. A standard curve will be generated by averaging the Ct values for each spore quantity from the five independent GPM conidia dilution series.

Field DNA Extraction for qLAMP Assay. Stainless steel sample rods (1.1mm x 40mm) collected from field traps will be placed into 2ml microcentrifuge tubes containing 200 µl of TE buffer (pH 7.5, 10mM Tris, 1mM EDTA), vortexed for 5 minutes, centrifuged for 2 minutes, boiled for 5 minutes, and centrifuged for another 2 minutes. The procedure requires a microcentrifuge ($450), boiling water (hot pot – 20$), vortex ($200) and a 5ul pipettor ($150), plus <$3 in disposables (tips, gloves, etc.), and takes less than 10 minutes to complete. All grower-performed extractions will be frozen following the qLAMP assay and collected for further testing by our lab.

qLAMP Assay. Optigene Isothermal Master Mix, no dye, will be used for the qLAMP assay because of its long shelf life at room temperature (3 to 4 weeks), its robust and rapid amplification, and its commercial availability, making it suitable for immediate implementation. Each 25µl qLAMP reaction will consist of 20µl of the Gene-DART master mix (1X Optigene master mix, ultrapure water, and the primers and probes at the final concentrations listed in Table 3), to which 5µl of the extracted DNA will be added. The reactions will then be placed in the Smart-DART device and incubated at 65°C for 30 minutes. The amount of fluorescent amplification will be recorded every 30 sec using the Smart-DART app on an android device (smartphone or tablet). The spore number in each sample will be determined by comparing the resulting CT to a standard curve derived as described above except using the field DNA extraction method and the qLAMP assay on the Smart-DART system. Single reactions for each field sample will be run on each unit due to equipment restrictions instead of triplicate reactions,

Table 3: Primer and probe sequences and final concentration in *E. necator* qLAMP PCR reaction.

	Nucleotide Sequence (5' → 3')	Final Conc. µM
FIP EN	ACCGCCACTGTCTTTAAGGGCCTTGTGGTGGCTTCGGTG	2.4
BIP EN	GCGTGGGCTCTACGCGTAGTAGGTTCTGGCTGATCACGAG	2.4
F3 EN	TCATAACACCCCCCTCAAGCTGCC	0.24
B3 EN	AACCTGTCAATCCGGATGAC	0.24
Forward Loop EN	AAACTGCGACGAGCCCC	1
Reverse Loop EN	ACTTGTTCCTCGCGACAGAG	1
FL-forward probe	FAM-6-ACGCTGAGGACCCGGATGCGAATGCGGATGCGGATGCCGA**AAACTGCGACGAGCCCC**	0.08
RL-reverse probe	FAM-6-ACGCTGAGGACCCGGATGCGAATGCGGATGCGGATGCCGA**ACTTGTTCCTCGCGACAGAG**	0.08
Qstrand	TCGGC ATCCG CATCC GCATT CGCAT CCGGG TCCTC AGCGT – **BHQ***	0.16

All primers from Sigma-Aldrich Corp., St. Louis, MO.

FAM-6-carboxyfluorescein; BHQ-black hole quencher; bold red text-probe sequence specific for *E. necator;* orange text-complementary sequence, anneals to Qstrand to form assimilation probe (fig.4)

as with the qPCR. During development, each run will consist of the samples from the three traps (discussed below) and positive and negative controls. The positive controls will be used to assure that quantitation is preforming as expected and the negative control will test for

contamination. Each run will cost $3.60 in reagents/per trap and $6.20 in positive and negative controls. In addition, the growers will be given at least 4 sets of blind samples throughout the season consisting of 0, 1, 10, and 100 spores to assess sensitivity and accuracy of quantification. After beta testing this summer and refining the fluorescent detection components, the Smart-DART unit with android device is estimated to cost $1500.

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

Three impaction traps (capable of sampling 145 L/min) will be placed at each collaborating vineyard location. One trap will be maintained and processed by the grower using the field DNA extraction and qLAMP assay procedures, the second trap will be collected and processed by our lab using the same methods, the third trap will be collected and processed by our lab using the Power Soil DNA extraction kit and qPCR assay. Known positive (0, 1, 10, and 100 spores) and negative samples will also be given to growers during the season as quality checks on the extraction and detection procedures. The qLAMP PCR product from all initial positives from each trap will be restriction digested and examined by gel electrophoresis to confirm that *E. necator* DNA was indeed amplified. Since it is not possible to sequence LAMP products or run PCR from LAMP products, bands from the restriction digest will be cloned and sequenced as further confirmation. To further test the qLAMP assay, four traps will be placed at our research vineyard at the OSU Botany and Plant Pathology Field Laboratory, two collected biweekly and two collected daily. One sample from each collection frequency will be processed using the qLAMP and qPCR extraction and assay procedures. This broad approach will allow us to further assess the robustness of the field DNA extraction and qLAMP assay and the success of grower implementation. Response operator characteristic analyses will be used to assess performance of the qLAMP by using the qPCR as the theoretical positive.

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

In order to test whether knowledge of inoculum quantity can be used to adjust fungicide application intervals, 3 replicated blocks will be established at 3 vineyards. At each vineyard, individual blocks will be divided in half and each half randomly assigned to either the grower standard program or adjustment of fungicide interval based on inoculum concentration. An impaction trap will be place in each replicate block and used to adjust application intervals for that replication. The adjustment will be done as follows: fungicide application will not be initiated until detection or flower separation/beginning of bloom, and then adjusted to long intervals when no detection is occurring and short intervals when inoculum concentration is >100 spores over a three to four day period.

Disease levels in the field will be determined by visual assessments in which disease severity is rated for 10 leaves from 50 plants in each plot. All field locations will be monitored for rainfall, temperature, humidity, and leaf wetness at 15 minute intervals in order to assess the suitability of environmental conditions for disease development. The utility of the data derived from the development of the qLAMP protocol will be increased by examining the correlations between observed disease levels, the concentration of spores in air samples as determined by qLAMP, and weather parameters. This analysis will help refine existing disease forecasters and to further assess the robustness of the qLAMP assay.

3. **Assessment of qLAMP for estimating amount of fruit infection.**

At both véraison and harvest, 200 clusters will be sampled from at least 15 vineyards. From each group of 200 clusters, 20 clusters will be randomly selected and visually assessed for disease levels by microscopically examining 25 berries per cluster. The remaining clusters will be crushed and juice processed and analyzed using the qLAMP assay. Various methods for concentration of DNA from the juice will be examined.

Research Timetable for Project:

	2012				2013				2014				2015
Objective	Jan	April	July	Oct	Jan	Feb	July	Oct	Jan	April	July	Oct	Jan
Test utility of qLAMP by Growers		field and disease assessment			Grower Training	field and disease assessment			Grower Training	field and disease assessment			
Altering application interval				analyses and reports		field and disease assessment		analyses and reports		field and disease assessment			analyses and reports
qLAMP estimentation of fruit disease													

Present Outlook and Estimated Success in Accomplishing Objectives.

The value of inoculum detection to initiate fungicide applications has clear economic value that has been realized by both Oregon and California viticulturists. Oregon growers using the system have saved on average 2.3 applications/per year over the past 6 years. Several have also commented on the added benefit of having some knowledge of the amount of inoculum present to help adjust their fungicide program all season long. Due to the timing of funding and subsequent hiring of the graduate student working on the project, a different approach to the objective was taken. Beginning in April and prior to notification of funding, 22 traps were distributed to growers throughout the Willamette Valley. Samples were processed biweekly as above using the qLAMP protocol. At the Botany Farm in Corvallis, biweekly and daily samples were process using qLAMP and qPCR. Results indicate that the qLAMP procedure is highly specific and sensitive and preforms equivalent to the qPCR for detection. However, it is still only semi-quantitative (e.g. able to distinguish orders of magnitude difference). This is largely due to modifications that increased both the sensitivity and speed of the reaction, but in turn, reduced the resolution of the data. Currently, it appears that we will be able to categorize samples into having no detection, <10 spores, <100, and <1000 and >1000. This relative quantification will still allow us to achieve the goals set forth in this proposal. We also evaluated two beta versions of the Smart-DART device with promising results. The devices are easy to use, very compact, and suitable for pathogen detection. However, their current configuration makes them somewhat inconsistent for quantification. The good news is, we were successful in obtaining an SBRI grant to work on design improvements to the Smart-DART device. The Smart-DART device is scheduled to be on the market in 2014 at ~$1500 per unit. While this is more expensive that prior estimates, we are working to improve our DNA extraction procedure to employ a lower grade/cheaper centrifuge that will offset this cost increase. Total capital invest should still be approximately $2,000 per setup.

Outreach and Education

Mahaffee presented results at the Oregon Wine Symposium, OSU Viticulture and Enology Research Day, field days at cooperating vineyards, Willamette Valley Tech Group, NAPA Valley Grape Growers meeting (3/7/2012) and at the Association of Applied IPM Ecologist meeting in Oxnard (2/6/2012). USDA travel restriction prevented further travel outside of the state. In 2013, Mahaffee will have a demonstration booth at the Oregon Wine Symposium (2/20-21/13) and present at the Oregon Wine Research Institute Grape day (3/5/2013). If funding and USDA policy allows, Mahaffee will also present at two or more California Viticulture meetings in 2013.