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Development of grower preformed LAMP PCR for detection-based management programs for grapevine powdery mildew in vineyards

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The objectives of the three year project are:

1. Develop LAMP PCR primers that are specific to *Erysiphe necator* (grape powdery mildew).
2. Develop a rapid and cost-effective DNA extraction protocol for LAMP PCR in the field.
3. Examine LAMP PCR for detection and quantification of *Erysiphe* in vineyard air.
4. Test implementation of LAMP PCR conducted by vineyard managers in commercial vineyards.

1) Work on this project began June 12, 2009 and has incurred expenditures of \$41619.22 for salary (\$2123.43/ 2 weeks) and \$4,600 in supplies as of May 1, 2010.

2) We completed objective 2. A simple DNA extraction protocol that is suitable for consistently detecting <5 conidia of *E. necator* in background of DNA (derived from the airbiota and 25 mg of soil) using LAMP PCR by an unskilled operator was developed. The DNA extraction procedure consists of placing the sample rods into 100 µl of extraction buffer, centrifuging for 1 min, then boiling for 1-5 min, vortexing for 10 sec, and then taking 5 µl and placing the LAMP PCR tube with master mix. The procedure requires a microcentrifuge, 100°C heating block or boiling water, vortex and pipettors (~\$1200 in equipment) and takes less than 10 min to complete. The actual LAMP PCR reaction consists of mixing the Mastermix and DNA, placing it 65°C for 1 h, then 80°C for 5 min, before centrifuging for 5 sec and holding it up to a window or placing against a black light to visualize a green (*E. necator* present) or orange fluorescence (*E. necator* absent). This procedure is simple, requires little training, and minimal capital investment. Based on these results alone, >30 vineyard managers volunteered for the next phase of the project with many offering to purchase the equipment themselves. Current estimates are that it would require \$2500 in capital equipment, \$60 in reagents and 25 minutes in labor (\$50/h), 1.5 h in time to process 10 samples. Traps currently cost \$150 to build or \$600-\$2000 to purchase. Return on investment will depend on the number of applications reduced and the duration of the sampling program but could be recovered in 3 years if one spray is saved on a 40 A block with sampling until flowering. Economy of scale will improve the return on labor and capital investment since the recommended lab equipment will have the capacity to scale up to 48 samples/2h with no increased capital cost.

3) A set of LAMP primers was developed to the ITS2 segment of the ribosomal DNA (rDNA) region of *E. necator* that resulted in detection of >10 copies of the ITS DNA from *E. necator* cloned into TOPO (Invitrogen) or purified DNA from 5 conidia when spiked into a background of DNA extracted from the airbiota and 25mg of soil. Specificity is not clear at this time due to the extreme sensitivity of the LAMP PCR and

contamination of our Erysiphales DNA Library. Samples collected from within vineyards or in riparian areas around vineyards all contained DNA of *E. necator* that was detected using LAMP PCR but not using qPCR or standard PCR. Contamination of *E. necator* DNA was demonstrated by cloning PCR bands and sequencing. In all cases DNA of both *E. necator* and the Erysiphales species was present and the *E. necator* LAMP primers did not amplify the cloned ITS region of other Erysiphales species.

- 4) Refinements in the PCR master mix, PCR conditions, primer design improved visual assessment of the white precipitate to reduce reaction costs. These are currently being tested with a cooperating grower.

Currently we estimate that the protocol will require \$6.00 in supplies/sample and 15-30 minutes in labor (1.5 -2 hours time elapse) to collect, run, and visually assess the reaction of 5 to 10 samples.

We have just begun preliminary grower trials with one grower that has lab experience to look at refinement of protocol for less controlled conditions than a laboratory setting. The grower is being provided with positive and negative samples and processing them.

During the 2010 field season, we will work with 6 growers in testing the performance of the LAMP Protocols by placing three traps at each vineyard location. One trap will be monitored by the grower using LAMP PCR, and we will monitor the other using LAMP-PCR and the protocols used in the previous two years of testing. In addition the growers will be given periodically positive and negative controls to further test performance of the LAMP protocols.

Our budget request for 2010 was reduced from the original projections due to labor savings and reduced reagent costs associated with developed protocols, and obtaining of additional funding through USDA-ARS to purchase 5 sets of equipment for the grower collaborators. We will be requesting \$63,794 instead of the projected \$82,843.