14-001

FY16 Application for Nursery Research Funding
Washington State Department of Agriculture - Nursery License Surcharge

(Please use one application packet, including the Progress Report page for each proposal. You must use our form - *failure to do so may result in not funding your project.*)

Project Title: Studies on Detection and Spread of Grapevine Viruses in Grower Vineyards and Certified Nurseries

Project Leader: Naidu Rayapati

Institution (if any): Washington State University

Mailing Address: IAREC, 24106 N. Bunn Road, Prosser, WA 99350

Email: naidu.rayapati@wsu.edu **Project Phone Number:** (509)786-9215 **Cell Number:** (509) 788-5350

Note: Project leader or his/her designee must be available at above project phone number on **February 27, 2015** between the hours of 10:00-12:00 and 1:00-3:00.

(Check One) New Project _____ ***Continuing X***

Start Date: July 1, 2015 **Completion Date:** June 30, 2016

Amount Requested for (FY16) July 1, 2015 to June 30, 2016: $23,470

If this is a multiple year project, please estimate and list the following information for each future July 1 - June 30 period listed below through project completion:

Fiscal Years (FY)	July 1, 2016 to June 30, 2017	July 1, 2017 to June 30, 2018	July 1, 2018 to June 30, 2019	July 1, 2019 to June 30, 2020	July 1, 2020 to June 30, 2021
$ Amount Needed					

If you are increasing the above amounts since your last application, please explain why:

*Please list **all** other sources and amounts of funding for this project for the current year only: **(Please notify us by February 15 if other funding has been approved and from where.)**

Source	$ Amount Applied For	Approved	Pending Date of Notification
Washington State Commission on Pesticide Registration	**23,062**		**December 31, 2014**

Total Amount Needed to Fund Project (include all sources*) $ 46,532

If total amount from all sources is not granted, will you be able to complete the project? Partially
Explain:
The number of proposed activities will be scaled down. This will lead to slow progress of research and impacts research-based information reaching growers and nurseries in a timely manner.

Please indicate which sector(s) of the nursery industry stand to benefit from the results of your research: (Letters of support from the industry are encouraged.)

Grapevine certified nurseries are the principal beneficiaries with spill over benefit to wine grape growers

Submit 16 copies of this proposal to:

Tom Wessels, Plant Services Program Manager, P.O. Box 42560, Olympia, WA 98504-2560, twessels@agr.wa.gov, or fax (360) 902-2094
All applications must be postmarked by December 31, 2014.

Please summarize the purpose of this research: (you may attach additional sheets if necessary or submit this summary in your own format)

Background:

Washington State has been recognized as one of the premium wine grape-growing regions in the world. A recent economic impact study estimated that Washington's grape and wine industry contributes nearly $8.6 billion to the state's economy and nearly $14.9 billion to the national economy ("The Economic Impact of Washington State Wine and Grapes" by Stonebridge Research Group™ LLC. [www.stonebridgeresearch.com], April 2012). Wine grape growers have harvested an estimated 230,000 tons (or more) of wine grapes of all varieties in 2014 compared to 210,000 tons harvested in 2013. The National Agricultural Statistics Service report (http://www.nass.usda.gov/Statistics_by_State/Washington/Publications/Fruit/winegrape14.pdf) posted online on March 3, 2014, reported that growers received an average of $1,110 per ton for all varieties harvested during 2013 crop season. Among all varieties, Grenache received the highest price of $1,889 per ton. Compared to 2012 statistics, the overall production of red and white grape varieties in 2013 season has increased by 13 and 10 percent, respectively. Among the top four wine grape varieties, Cabernet Sauvignon produced 42,600 tons (20 percent of the total) followed by Chardonnay with 40,500 tons (19 percent of the total), White Riesling with 40,100 tons (19 percent of the total) and Merlot with 36,000 tons (17 percent of the total). Among them, Cabernet Sauvignon showed highest increase in production of 19 percent compared to 2012 production. Of the total wine grape acreage, nearly 51 percent are red varieties and the rest are white varieties. As of 2014, total wine grape acreage in Washington State was estimated to be around 50,000 acres, including new plantings (www.washingtonwine.org; www.wawgg.org). Overall, prospects are increasingly brighter for further expansion of vineyard acreage to produce high quality grapes for making premium wines at affordable price.

Virus diseases cause a wide range of negative impacts to the production of wine grapes (*Vitis vinifera*). Consequently, the grape and wine industry's research taskforce has identified management of virus diseases impacting fruit quality and vine health as one of the high research priorities. The Wine Advisory Committee of the Washington Wine Commission has also recognized virus diseases as one of the highest viticulture research priorities in 2014 for sustainability of the grape and wine industry. Of the 60 plus viruses currently documented in grapevines worldwide, grapevine leafroll disease (GLD) is rated as the most severe and damaging (http://wine.wsu.edu/virology/images/virus-ext-bull.pdf). In recent years, a disease with symptoms mimicking GLD, designated as grapevine red blotch disease (GRBD), is emerging in many vineyards. We have been studying this emerging disease for the past few years and generating scientific resources for practical applications in vineyards to minimize the spread and mitigate negative impacts of the disease. Activities described in this proposal will be carried out in a complementary and synergistic manner with funding support from other resources, such as WSDA-Nursery License Surcharge and Washington State Grape and Wine Research Program. The research-based information generated from this project will help implementing strategies for the management of GRBD and GLD, two apparently disparate diseases, in a comprehensive manner to advance sustainable growth of the grape and wine industry in Washington State.

The main objectives of the proposal are to (i) Elucidate genetic diversity of virus(es) present in wine grape cultivars showing red blotch symptoms, (ii) Optimize methods for simultaneous detection of viruses to differentiate red blotch from grapevine leafroll disease, (iii) Monitor viruses in different parts of grapevines showing red blotch symptoms.

Methods of research:

Objective i: Elucidate genetic diversity of virus(es) present in wine grape cultivars showing red blotch symptoms.

A comprehensive understanding of the genetic makeup of grapevine red blotch-associated virus (GRBaV) in vineyards showing grapevine red blotch disease (GRBD) symptoms is critical to develop strategies for reducing spread of the disease. For this purpose, we have collected leaf samples during 2014 season from ten wine grape (red- and white-fruited) cultivars from four appellations (AVAs) showing symptoms of or suspected

for GRBD. Extracts prepared from these samples were stored at -80°C freezer for studying genetic diversity of GRBaV. During this year, we will use these stored extracts to test for the presence of GRBaV (and other grapevine viruses) by polymerase chain reaction (PCR) technology. In addition, virus-positive samples from 2015 season will be included in this study. The genome of GRBaV from virus-positive samples will be amplified by PCR and the amplified DNA fragments will be cloned and nucleotide sequence determined. The nucleotide sequence data will be analyzed for genetic diversity of GRBaV using sophisticated bioinformatics programs previously employed by Rayapati for other grapevine viruses. The results will be used to profile the genetic makeup of GRBaV within and between wine grape cultivars and across vineyard blocks showing GRBD symptoms. In addition, PCR results will be analyzed to determine the occurrence of GRBaV in vineyards as single or mixed infections with other viruses.

Objective ii. Develop methods for simultaneous detection of viruses to differentiate red blotch from leafroll disease.

Due to perennial nature of grapevines, mixed infections of different viruses are common rather than exception. Since symptoms produced by GRBD in many wine grape cultivars are similar, though not identical, with grapevine leafroll disease (GLD), visual diagnosis of these two diseases in vineyards is a challenge. Therefore, each symptomatic sample has to be tested for GRBaV and Grapevine leafroll-associated virus 3 (GLRaV-3) using separate PCR assays. To improve this time consuming process, have optimized a multiplex PCR assay, where two viruses can be detected concurrently by the same PCR assay, for the detection of GRBaV and GLRaV-3 present in vines showing GRBD/GLD symptoms. Such a capability should allow us to test large numbers of samples for the detection of these two viruses in a cost-effective and time-sensitive manner. Towards this goal, the multiplex PCR assay optimized last year will be validated for simultaneous detection of GRBaV and GLRaV-3 using field samples collected during 2014 and 2015 seasons. The test results will be analyzed to document the occurrence of single and mixed infections of these two viruses in grapevines showing GRBD or GLD symptoms. Appropriate controls will be included to reveal the specificity of each multiplex PCR assay and assist with reliable interpretation of assay results. Where ever necessary, DNA bands amplified from multiplex PCR assays will be cloned and nucleotide sequence determined to confirm if the PCR-amplified DNA is indeed specific to a particular virus.

Objective iii: Monitor viruses in different parts of grapevines showing red blotch symptoms.

One of the characteristic features of GRBD is that symptoms appear soon after *véraison* (onset of berry ripening) and these symptoms are largely restricted to mature leaves near basal parts of shoots. This raises the question of whether GRBaV and other viruses are confined to leaves showing symptoms or present throughout the vine irrespective of symptoms. Thus, testing samples from symptomatic and non-symptomatic parts of GRBD-affected grapevines will provide information on the distribution of viruses to optimize sampling strategies for reliable detection of viruses in individual grapevines. To conduct this study, we have identified three vineyard blocks affected with GRBD and samples will be collected from symptomatic vines of three cultivars (Merlot, Syrah and Cabernet Sauvignon) in late September when symptoms are apparent. Shoots from six symptomatic vines will be divided into apical, middle, and basal portions and petiole and bark samples from each portion will be tested separately for GRBaV by PCR. Equal number of shoots collected from healthy grapevines will be included as controls. The test results will be analyzed to determine correlation between the presence of virus(es) and symptoms. In addition, samples from berry clusters (pedicle, berry skin, seed, etc.) will be tested for GRBaV. We expect that this activity will provide information for improving sampling methods in the diagnosis of GRBD in vineyards and to help differentiating disease symptoms from those induced by abiotic stress (such as discolorations caused by nutrient deficiency) or caused by mechanical injury during vineyard operations.

Timeline of activities

Field-based research will be carried out during the season between June and November 2015 and lab-based research will be conducted throughout the project period. Presentations will be made at Washington grape industry-sponsored annual meetings in November 2015 and February 2016 and at national scientific meetings during summer 2016.

Expenditure Breakdown:
(Please include salaries, supplies, travel, etc.)

Salaries	
Research Associate[1]	$ 11,031
Hourly wages (Time slip) [2]	$ 1,680
Supplies[3]	$ 6,235
Travel[4]	$ 500
Employee benefits	
Research Associate (@42%)	$ 3,861
Hourly wages	$ 163
Total	**$ 23,470**

[1]Salary (25% FTE) for a Research Associate to carryout sample collections from nurseries and grower vineyards, processing and testing in the lab, conducting research, analyzing results and preparing reports.
[2]Hourly wages @ $12.00/hr for 140 hrs.
[3]Research consumables and lab disposables for sample collections, virus detection by ELISA and PCR, and costs for molecular analyses (DNA cloning and nucleotide sequencing).
[4]Vehicle charges (@¢51/mile) for visiting nurseries and vineyards to collect samples and attend Washington Association of Wine Grape Growers Annual Meeting and Trade Show in February 2016.

The information requested on this page will have a direct bearing on whether your research request is approved or denied. Letters of support from the industry are also encouraged.

Note: Funding is not available for general overhead cost.