

08-009

FY08 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: Robust sampling strategies and high throughput methods for the detection of grapevine viruses

Project Leader: Naidu A Rayapati

Institution (if any): Washington State University

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

Project Phone Number: (509) 786-9215 **FAX Number:** (509) 786-9370 **Cellular/Pager Number:** (509) 781-0665

Note: Project leader or his/her designee must be available at above project phone number on March 2, 2007 between the hours of 10:00-12:00 and 1:00-3:00.

Amount Requested for (FY08) July 1, 2007 to June 30, 2008: 10,974

Start Date: July 1, 2007 **Completion Date:** June 30, 2008

(Check One) New Project **Continuing**

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, 2008 to June 30, 2009	July 1, 2009 to June 30, 2010	July 1, 2010 to June 30, 2011	July 1, 2011 to June 30, 2012	July 1, 2012 to June 30, 2013
\$ Amount Needed	11,180	11,393			

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	\$19,087	Yes	

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Total Amount Needed to Fund Project (Include all sources) \$ 30,061

If total amount from all sources is not granted, will you be able to complete the project? Partially
 Explain:

The number of proposed activities of the project will be scaled down. This will lead to slow progress of research and impact research outputs reaching the Nurseries in a timely manner. Support from Nursery Research Funding will help to solicit matching funds from Washington State Commission on Pesticide Registration during 2007/2008 cycle.

Submit 15 copies of this proposal to: Tom Wessels, Plant Services Program Manager, P.O. Box 42560, Olympia, WA 98504-2560. All applications must be postmarked by December 31, 2006.

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

The Washington State grape industry has blossomed in recent decades from 'obscurity' to one of growth and prosperity. Consequently, the State has gained recognition as a premier viticultural region around the world and is emerging as one of the world's premium wine-making regions. Currently, Washington State has the largest grape wine industry, ranking second only behind California in vineyard acreage and grape and wine production, and contributes nearly 3 billion dollars to the state's economy. Since viruses can destroy or greatly decrease the economic success of a vineyard, the Pest Management Strategic Plan for Washington State Wine Grape Production (2004) has identified virus diseases of the grapevine as one of the high priorities to be addressed for the long-term viability and economic sustainability of the wine industry.

Of all perennial woody crop species, grapevine seems to be infected by a large number of viruses. At present, the total number of viruses infecting grapevines is an unprecedented 55, belonging to 20 different genera of plant viruses. The rapid expansion of wine grape industry within the past two decades has predisposed the viticultural enterprise to many of the debilitating virus diseases. Since grapevines are vegetatively propagated, it is vital to prevent the spread of viruses through cuttings distributed within Washington State and block exotic/emerging viruses slipping into the state vineyards through cuttings imported from outside the state. Unfortunately, there are no economically feasible chemical agents similar to fungicides and bactericides that are effective against plant viruses. Thus, strategies aimed at the management of grapevine viruses are largely directed at preventing virus spread by utilizing virus-free planting material. Certified Nurseries are the key players in this endeavor because they provide 'clean' planting materials for growers and commercial vineyards.

The sanitary status of a vine can not be determined based exclusively on symptom expression, since typical symptoms of a particular virus disease may not be obvious, even though the vine is infected, and sometimes symptoms can resemble abnormalities caused by mechanical damage to the vine, other diseases and arthropod damage, thereby complicating visual diagnosis. One of the challenges with viruses infecting grapevines is that they are found only in the phloem, occur in low quantities and distributed unevenly in infected plants. Thus, highly sensitive and specific diagnostic tests are essential for the discrimination of viruses infecting grapevines as part of assessing the sanitary status of grapevine cultivars grown by the Certified Nurseries. The two most common lab-based diagnostic tests used for virus detection are enzyme-linked immunosorbent assay (ELISA) and reverse transcription-polymerase chain reaction (RT-PCR). Although ELISA has been a preferred detection method due to its simplicity and effectiveness, it has failed on occasion due to low virus titer, or inhibitory effects of various compounds present in tissue extracts of woody plants. Molecular testing by RT-PCR is often able to detect viruses at much lower concentrations than ELISA. In addition, RT-PCR has been a preferred method where virus-specific antiserum for use in ELISA-based detection is not available or suitable.

The objective of the proposal is to develop robust sampling strategies and high throughput diagnostic assays for the detection of debilitating viruses in Certified Nurseries.

The outputs of this project will allow us to determine appropriate timing of sampling for reliable indexing of viruses and hence will assist the Certified Nurseries wishing to collect tissue samples for virus testing. By working together, the project will develop close linkages with WSDA Plant Protection Division and Certified Nurseries for ensuring the availability of virus-free planting materials to the expanding viticulture industry in Washington State. The results will be disseminated to Certified Nurseries, WSDA and other stakeholders of the grape industry for an increased awareness of the negative impact of virus diseases.

Methods of research:**i. Determine best type of tissue and time of sampling for virus detection.**

The sensitivity and reliability of a diagnostic assay is influenced by many factors like low virus concentration, uneven distribution of virus within the plant, fluctuations in virus titer during the crop season, and presence of inhibitors like polyphenols and polysaccharides in plant extracts, especially in field-grown woody plants. A standardized sampling protocol is therefore required to overcome these shortcomings. To assist Certified Nurseries and WSDA with sampling appropriate grapevine tissue for reliable virus testing by RT-PCR, we will collect leaf samples from two different cultivars (Cabernet sauvignon and Chardonnay) infected with Grapevine leafroll-associated virus-3 (GLRaV-3) at bi-weekly intervals from the beginning of the season (starting two weeks after bud break) and test concurrently for the presence of the virus by both ELISA and RT-PCR. Dormant canes collected during the winter will be tested at monthly intervals. Such a comparative study using both ELISA and RT-PCR will allow us to determine the appropriate timing for reliable indexing and if cultivar-specific 'factors' have an influence on test results.

ii. Simultaneous detection of grapevine viruses.

Mixed infections of different viruses are common in grapevines due to the method of their propagation. Simultaneous amplification and detection of different viruses in a single RT-PCR assay, called multiplex RT-PCR, should allow us to test large numbers of samples for the concurrent detection of different viruses in a cost-effective manner. However, since multiplex RT-PCR is a complicated enzyme-catalyzed reaction, many factors (including a variety of defined and undefined inhibitors in sample materials) could adversely affect the assays, resulting in false negative results. To circumvent these problems, we will initially optimize multiplex RT-PCR assay conditions for GLRaV-3 and a 'house-keeping' RNA sequence of host origin as a co-amplification template in the same tube with the target viral sequence. Since the 'house-keeping' RNA sequence will be amplified in all samples (irrespective of whether the sample is infected or not), this will reveal the specificity of the RT-PCR and assist with accurate interpretation of results for any given virus. In the following year, we will validate the multiplex RT-PCR assay for the concurrent detection of multiple viruses using field-collected samples from Certified Nurseries.

Expenditure Breakdown:

(Please include salaries, supplies, travel, etc.)

Salaries

Research Associate ¹	\$ 3,776
Time slip ¹	\$ 1,900
Supplies²	\$ 3,000
Travel³	\$ 720
Employee benefits	
Research Associate (@36%)	\$ 1,359
Time slip (@11.5%)	\$ 219
Total	\$ 10,974

¹Salary (one month) for a Research Associate to conduct research and coordinate lab and field research activities. Time-slip (200 hrs @9.50/hr) to assist in sample collection in the field and preparation of sample extracts for testing by ELISA and PCR.

²Research consumables and lab disposables for sample collections, detection of different viruses by ELISA and PCR and molecular analyses.

³Vehicle charges (@ \$0.36/mile) for visiting nurseries to collect samples.

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

Note: Funding is not available for general overhead cost.



Inland Desert Nursery, Inc.
Washington State Certified Grapevines

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December 15, 2006

Tom Wessels
Plant Services Program manager
P.O. Box 42560
Olympia, WA 98504.

To Whom It May Concern:

Inland Desert Nursery, Inc. fully supports Naidu Rayapati's proposal for funding to improve our state's grapevine certification program. It is vital that Washington wine-grape growers are provided with the cleanest material available to plant in their vineyards. Without continued program funding, the productive sustainability of Washington vineyards will be at jeopardy.

As Washington nurseries begin to compete with California nurseries for the first time, we must be able to provide a product we are confident with and can stand behind. Our goal should be not to match California's program, but rather to surpass California's program. With clean and frequently tested mother-blocks, the viruses that have devastated wine regions across the world will never be an issue in Washington.

The growth of viticulture in Washington State is exciting for a nurseryman, and as the largest grapevine nursery in the state, it is our goal to deliver the cleanest material available to our customers. For years, we have built a reputation for quality. We not only depend, but insist that Washington State's Certification program parallels what we stand for. Funding for research outlined in Dr. Rayapati's proposal will greatly improve the grape nursery programs in Washington State.

Sincerely,

Tom C. Judkins Jr.
Kevin C. Judkins