Unified Grant Management for Viticulture and Enology
PROPOSAL FORMAT FOR THE 2014-2015 FUNDING CYCLE

1. Project Title: Insect vector distribution and disease progression studies to better describe field epidemiology of grapevine red blotch-associated and vine leafroll virus in Oregon.

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4. Objectives:

1. Follow insect vector distribution and disease progression in established vineyard plots to collect preliminary data on field epidemiology of grape red blotch-associated virus and grape leafroll-associated virus in Oregon.
2. Extension of information on the importance of vectors, leafroll and red blotch disease in Oregon vineyards.

5. Justification and Importance of Proposed Research:
Tissue samples of leafroll-symptomatic plant materials were systematically collected during 2009-2012 in vineyards by OSU Research and Extension Faculty Rick Hilton, Daniel Dalton, Vaughn Walton and Clive Kaiser (see list of scientists above). These collections were conducted in Southern Oregon (2 vineyards), the Willamette Valley (1 vineyard) and Eastern Oregon (3 vineyards). Genomic test results for some of the symptomatic plant materials were negative for grapevine leafroll-associated viruses (GLRaVs) up to 2012. These results were unexpected, as symptoms of affected vines were very similar to leafroll. Our collaborating growers in Talent, Southern Oregon, and Milton-Freewater, Eastern Oregon noticed these 'red blotch' symptoms and consistently reported longer ripening times and lower brix levels of negatively-tested symptomatic grapevines for several consecutive seasons. Many of the vines displayed symptoms that were similar to leafroll virus, but with discontinuous red colorations in regions of the leaf blade. The name 'red blotch' later became associated with these symptoms.

During our survey period for leafroll in Oregon, plant pathologists at Cornell University and UC Davis isolated and identified a similar circular DNA virus in winegrapes (Krenz et al. 2012). PCR primers specific to the new virus (Al Rwahnih et al. 2012) have now been developed. This virus is now known as 'grapevine red blotch-associated virus (GRBaV)'.

Subsequent to the availability of this test, tissues originating from symptomatic Oregon vines were simultaneously tested for both leafroll and red blotch virus at UC Berkeley laboratories for samples collected during 2012 and 2013. Positive identifications of red blotch and leafroll were made from 2012 and 2013 petiole tissue collections in Oregon. Current red blotch levels of the tested vineyards average 1.8% and for leafroll, infection averages 4%. These results, however, still do not account for the sum of symptomatic vines.

Our 2009-2012 surveys were conducted in order to allow spatial analysis of virus-symptomatic vines and mealybug distribution. We employed and continue to maintain sticky cards, which contain mealybugs, leafhoppers and other scale insects (Walton et al. 2013). Our spatial surveys have allowed us to keep track of the spread of visual symptoms since 2009. The new genomic tools have allowed us to describe the spatial distribution of both viruses in all survey vineyards. In addition, we have reported insect vector presence, seasonal phenology, and spatial distribution of leafroll vectors from 2009-2012 (Fig. 1, Walton et al. 2013, Dalton et al. in prep.). These resources put us in a unique position to better understand the possible role of vectors in the spread of these viruses in Oregon vineyards and possibly other viticultural regions of the USA. This spatial information describes statistically significant patching and gaps of both virus types, and the vector insect distribution (Fig. 1; Perry & Dixon 2002, Dalton et al. in prep,) in the affected Oregon vineyards. We believe that this information will be valuable to better understand the epidemiological role of all organisms in question. Our analysis can in addition describe the association or disassociation of symptomatic vines with that of potential viral vectors. We believe that this information will support a better understanding of the role of insects as possible vectors of both vine leafroll and red blotch viruses.

We submit this proposal in coordination with the California grant proposal entitled, 'Evaluating the potential of insect vectors to transmit grapevine red blotch-associated virus (GRBaV)' (Kent Daane, Principle Investigator) as a complementary proposal. Our studies will create a better understanding of the regional epidemiology and importance of red blotch, vine leafroll and its potential vector insects in Oregon. Our California collaborators will continue important work on the most probable insect species transmitting the Geminiviridae (Ghanim et al. 2007, Chen and Gilbertson 2009, Cilia et al. 2012) including leafhoppers that may vector GRBaV (Poojari et al. 2013). Our goal is to build on the current information that we have on the potential vector insects and their seasonal presence and spatial distribution in Oregon vineyards. In addition, we will supply our California collaborators with newly collected insects during 2014-2015.

Our work is additionally aimed to provide timely updates and management tools for winegrape growers regarding management of red blotch and leafroll viruses. This work must be completed to direct a future control program for red blotch and vine leafroll virus. At this time, there is no accurate information on the epidemiology of GRBaV in vineyards. It is unknown if insects spread this virus or whether it originated from infested plant materials. Materials collected in Oregon will aid UC Berkeley studies, which are aimed at testing the importance of possible virus vectors (see list of species, Table 1 below), and this work is currently ongoing.

Researchers at UC Davis and Cornell are studying the pathogen's impact on fruit quality and yield. We will coordinate and complement the work by the virologists and entomologists on these teams. This proposal will focus only on the historical and future field distribution of insect vectors and spread of the virus. We will continue our field collection of potential vector insects and supply information from Oregon to the entomology groups in California who are conducting the transmission studies of insect vectors.

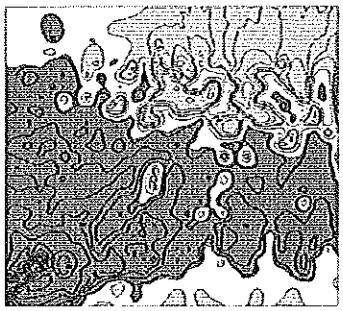

Fig. 1. Spatial distribution of mealybugs in a vineyard in Eastern Oregon using Spatial Analysis by Distance Indices (Perry 1995, Perry 1996, Perry & Dixon 2002, Dalton et al. in prep.). Red areas show significant patching, white areas are non-significant and blue areas show significant gaps. Spatial distribution of virus-infected plants not shown.

6. Procedures to Accomplish Objectives:

Objective 1. Follow insect vector distribution and disease progression in established vineyard plots to collect preliminary data on field epidemiology of grape red blotch-associated virus and grape leafroll-associated virus in Oregon.

By coordinating with our California collaborators, we have identified potential vineyard insect vector species (Almeida and Purcell 2003a, b) that commonly occur in Oregon vineyards by means of our surveys and trap counts, as well as other potential vectors (Table 1).

The studied vineyards in Southern and Eastern Oregon now have known infestation of red blotch. For all sites, we have data of leafhopper and mealybug seasonal presence starting in 2010, and yearly spatial symptomatic vine data since 2009. For 2013, we have the spatial distribution of both vine leafroll infected and red blotch infected vines in each of the studied vineyards. With improved primers developed by our UC Berkeley colleagues, we plan to continue to map the progression of red blotch and leafroll at all sites for 2014. Additional mapping information created from 2009-2012 may be useful to show the importance of mealybugs and help determine interaction between the viruses.

We propose to continue spatial surveys of vineyards and surrounding vegetation for insect vector species. Mapping data will be compared using both ANCOVA (treatment and location as variables) and correlation analyses to determine whether there is a pattern to GRBaV spread – especially from a potential insect vector source (e.g., bordering vegetation to the north, east, south, or west).

Table 1. Potential insect vectors of grapevine diseases in Oregon (see comparative California distribution in complementary proposal).

Common name	**Scientific name**	**Oregon distribution**
Grape phylloxera	*Daktulosphaira vitifoliae*	Willamette Valley and Western Columbia Basin
Potato leafhopper	*Empoasca* spp.	Unknown
Western grape leafhopper	*Erythroneura elegantula*	Southern and Eastern Oregon
Variegated leafhopper	*Erythroneura variabilis*	Unknown
Virginia creeper leafhopper	*Erythroneura ziczac*	All regions surveyed
Blue-green sharpshooter	*Graphocephala atropunctata*	All regions surveyed
European fruit lecanium scale	*Parthenolecanium corni*	All regions surveyed
Grape mealybug	*Pseudococcus maritimus*	All regions surveyed, higher in the South and the East
Grape bud mite	*Tetranychus* spp.	All regions surveyed
Grape whitefly	*Trialeurodes vittatas*	All regions surveyed

i. Survey and spatial analysis of potential insect vectors:
The six vineyards will be surveyed by using six yellow sticky traps placed along the border (4 traps/vineyard) and center (2 traps/vineyard). Trap collections will be conducted once per month starting in June and continuing until September (5 sampling dates), typically the period when most of the listed candidate insect vector species are present at higher population levels. Intense insect vector sampling will in addition be conducted using sweep netting during July of 2014. Here we will use a systematic sampling pattern in a minimum of 40 locations of each study vineyard. This methodology will allow spatial analysis of vectors in each of the vineyards using Spatial Analysis by Distance Indices (Perry 1995, Perry 1996, Perry & Dixon 2002).

Sweep net-collected insects for each sampling location will be directly placed into separate vials containing 95% ethyl alcohol. These samples will be investigated under a stereomicroscope to determine species composition of the most likely vector insects. A subsample of vector insects will be shipped to UC Berkeley for viral detection. All sampled vineyards will contain at least 32 rows and 22 pole-to-pole "bays" (Charles et al. 2009) containing 3-6 vines per bay. Data collected during 2014 will be combined with historically collected data of all potential vector insects and analyzed using standard ANOVA in order to describe the presence of potential insect vectors similar to that described in Walton et al. (2013).

ii. Spatial mapping of virus spread:
During September and October of 2014, 200 vines in each plot will be systematically sampled and plant materials placed in a -80°C freezer within 24 hours of collection. Duplicates of petioles of leaves of each of the sampled vines will be annotated and genomically secured for shipment. All plant materials will be shipped to UC Berkeley for viral detection. Data from analyzed plant materials will be analyzed using Spatial Analysis by Distance Indices (Perry 1995, Perry 1996, Perry & Dixon 2002) in order to document spread over time. In addition, spatial data and analysis will allow us to determine if there is significant spatial association or disassociation between potential vector insects and virus-infected plants.

Objective 2. Extension of information on the importance of vectors, leafroll and red blotch disease in Oregon vineyards.

Results will be provided to grape industry and OSU Cooperative Extension personnel through grower reports and seminars. In addition, we plan to organize a regional vineyard workshop for growers and industry during 2014 to highlight the importance of the organisms involved in vector transmission in vineyards. Vaughn Walton, Clive Kaiser and Rick Hilton are the statewide and regional extension agents of the important affected regions. They have given numerous presentations on mealybugs and grape insect pests at grower and research symposia. Walton, Kaiser, Hilton and personnel from Walton's lab have presented on this aspect since 2008. Results will also be published in popular and scientific journals (see report for the list of grape publications 2008-2013). Walton, Kaiser, and Hilton are strongly committed to the grape industry and have a good relationship with growers, consultants and industry personnel that will aid in research extension.

7. Timetable for Project:

May-Sep 2014: Conduct seasonal survey of potential vectors at six sites that have been surveyed since 2009.
Sep-Oct 2014: Follow disease progression in six established vineyard plots in three winegrape growing regions of Oregon.
June 2014-May 2015: Extension of new and current information on insect vectors, virus status and spread in Oregon vineyards.

8. Present outlook and estimated success in accomplishing objectives
This work is based on similar approaches conducted to evaluate vector transmission of GLRaV-3. This work complements other proposals (mainly from UC Davis) to study grapevine red blotch disease. However, this project is unique as it focuses on the regional vineyard vectors and disease epidemiology. Information on grapevine red blotch disease epidemiology is critical to inform management decisions; grape growers must determine whether infected vines threaten adjacent healthy vineyards and take timely and appropriate action.

9. Outreach and Education:
See objective 2.

10. Budget Support Summary by Objectives:

Personnel: All personnel will work on both objectives; it is estimated that 90% of the funding will be directed towards Objective 1 and 10% towards Objective 2. We request continued funding for our experienced team. Faculty Research Assistant (Daniel Dalton) will coordinate insect collections and plant material collections; Rick Hilton and Clive Kaiser will conduct regional collections through the season, as well as outreach activities for each region. Vaughn Walton will conduct and coordinate data collection, statistical analysis, reporting, paper publication and extension.

Benefits: Benefits are based on OSU Sponsored Projects Office rates and 62% for Staff scale (FY 2014-2015), and 0% for student assistants.

Travel: The travel was based on the calculation for field site visits in Oregon ($3000). Domestic travel for field research has been estimated using a $0.56 per mile reimbursement.

Permanent Equipment (>$5,000): None

Supplies and Expenses (Disposable Supplies $7,000): Resources are projected annually for laboratory consumables and disposables (chemicals, plastic/glass ware, sample bags, pipets and pipet tips, micro-centrifuge tubes, protective clothing and materials for sample collections, sample processing, material costs for traps and attractants, and costs for publications (journal articles, Extension bulletins, posters, fact sheets, etc.) at $2,000. The added costs are for molecular supplies and contracting with personnel at UC Berkeley who will conduct the genomic analysis (e.g., primers) for $5,000.

Publication costs: We estimate one publication in the first year, set at $600 per publication, based on recent charges (page charges and reprints) for journals in the Entomological Society of America.

11. Total Budget Request:

	% Time on Project	Requested 2014-2015
Personnel:		
Faculty Research Assistant (Daniel Dalton)	25%	10,000
Student Assistant (One each in Southern and eastern Oregon)	20%	8,748
Employee Benefits (62%)		6,200
Supplies and Expenses		7,000
Travel		3,000
Computer Time/Supplies/Publications		600
TOTAL REQUEST		35,548

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