Progress Report

This is an ongoing project

For the Washington Grape & Wine Research Program

Date: April 2013-October 2013.

Project Title: Structural and compositional characterization towards unlocking the unknowns of sour shrivel to sustain optimum fruit composition and wine quality

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Project Budget Number: 13B-3075-5519

Project Summary:

Commercial vineyards were monitored before *veraison* for the incidence of SOUR shrivel disorder in red and white cultivars. In addition to Cabernet sauvignon, SOUR shrivel was observed in Barbera and Riesling, which showed great losses in both yield and quality. Fruit organoleptic attributes coupled with morphology and structure, and tissue organization in various organs of healthy and afflicted grapevines were examined using a range of microscopy techniques. Regardless of grape types, the SOUR shrivel symptoms were identical in all cultivars. As opposed to healthy berries, SOUR shrivel berries were flaccid due to a collapsed nonviable mesocarp, which seriously compromised its fruit quality attributes such as hexoses, nutrients, and various phenolic compounds including anthocyanins. Despite such extreme quality losses, the spatial architecture and integrity of various tissues in organs such as canes, inflorescence framework of afflicted vines were remarkably similar to healthy grapevines with notable exceptions. The cluster framework that assembled SOUR shrivel berries developed red coloration but showed no relation with the flaccidity of the berries as healthy vines that bore entirely SOUR shrivel free clusters, too had the same feature. Furthermore, although the phloem sieve tubes in both grapevines were plugged with callose, a carbohydrate generally implicated in impeding translocation in the phloem, the afflicted grapevines exhibited relatively more plugged sieve tubes. The study revealed that the spatio-temporal pattern of various tissues determining the structure-function relationship in afflicted vines remains intact throughout the growing season; however, the functionality, especially of flows in vascular tissues (sieve tubes) started to slow down and eventually ceased during the course of ripening as evident from dramatic reductions in fruit quality attributes. Hence, in future studies a broader analysis of phloem sieve tubes entailing its flows and ultrastructure is needed. More specifically, grapevines that have been phloem-girdled to simulate the development of SOUR shrivel berries would provide clues about what perturbs the normal progression of ripening process succumbing the clusters to such disorder.

Materials, Methods and Experiments Conducted to Meet Stated Objective(s):

Objective 1. Determine the mechanistic basis of Berry Shrivel by comparing the structure and functionality of vascular pathways (xylem and phloem) of the whole bunch stem

(peduncle, rachis and the pedicel) between healthy vines and vines afflicted with Berry Shrivel.
We monitored red and white cultivars in commercial vineyards for the incidence of SOUR shrivel. About 100 spurs were randomly chosen and tagged two clusters per shoot around veraison and these clusters were periodically observed for the initiation of SOUR shrivel. Once clusters displayed SOUR shrivel symptoms, they were harvested. The berries from healthy and SOUR shrivel clusters underwent routine physical and biochemical analysis. The rachis and peduncle were prepared for microscopy analysis to examine the functionality of vascular pathways in both health and SOUR shrivel clusters. For light microscopy, small pieces of tissues were dehydrated in graded ethanol series, infiltrated with paraffin and finally embedded in paraffin. Thin sections (10-15 µm) were cut using a microtome, the sections were double-stained (fastgreen and safranin) and Aniline Blue and observed with light microscope. For scanning electron microscopy, small pieces of tissues were cut using a sharp razor blade and the tissues were fixed in 3% glutaraldehyde solution overnight. The tissues were dehydrated in a graded ethanol series, critical point-dried and gold coated for observing with scanning electron microscope.

Objective 2. Simulate SOUR shrivel disorder employing various girdling techniques and compare anatomy, morphology and fruit composition among girdled, healthy, and SOUR shrivel clusters. Clusters for objective two were tagged as described above in objective one. Tagged clusters were monitored for the development of SOUR shrivel. Also, healthy vines in the same block or adjacent block with no prior SOUR shrivel history were girdled before *veraison* by means of standard girdling scissors or heat /cold girdling resulting in complete breakage of the bark/phloem tissue. Shoots were girdled in the internode region just above and below the peduncle. The girdled shoots were monitored for the development of SOUR shrivel disorder. At harvest, clusters from tagged vines were excised. These clusters were analyzed physically, compositionally and anatomically to determine whether or not phloem-girdling induced SOUR shrivel.

Major Research Accomplishments and Results (by Objective and including a timeline):

Objective 1: SOUR shrivel is the focus of an intensive scientific investigation owing to its deleterious impact on fruit quality and since the causal factors thus far eluded viticulturists, they refer it to like a mystery story in major grape growing regions worldwide. One of the big stumbling blocks in probing the mystery behind SOUR shrivel is that it is unpredictable varying from season to season both spatially and temporally in the vineyard and on vines. Hence pinning down the underlying mechanisms becomes quite a challenge. Its elusive nature calls for persistent research efforts to generate a continuous flow of information so that grape growers can be provided with remedial measures to minimize its incidences in future. In order to achieve this goal, a deep insight into its symptomatology pertaining to structural modifications of the vascular pathways that deliver sugar, water, and nutrients to berries during their development, ripening, and maturity is indispensable. Anatomical analysis of the shoot system revealed matching spatial architecture and integrity of various tissues in organs such as canes, inflorescence framework between healthy and afflicted vines with notable exceptions (Fig 1). The cluster framework that assembled SOUR shrivel berries developed red coloration but showed no relation with the flaccidity of the berries as healthy

vines that bore entirely SOUR shrivel free clusters, too had the same feature. Furthermore, although the phloem sieve tubes in both grapevines were plugged with callose, a carbohydrate generally implicated in impeding translocation in the phloem, the afflicted grapevines exhibited relatively more plugged sieve tubes. The study revealed that the spatio-temporal pattern of various tissues determining the structure-function relationship in afflicted vines remains intact throughout the growing season; however, the functionality, especially of flows in vascular tissues (sieve tubes) started to slow down and eventually ceased during the course of ripening as evident from dramatic reductions in fruit quality attributes. Hence, in future studies a broader analysis of phloem sieve tubes entailing its flows and ultrastructure is needed. More specifically, grapevines that have been phloem-girdled to simulate the development of SOUR shrivel berries would provide clues about what perturbs the normal progression of ripening process succumbing the clusters to such disorder.

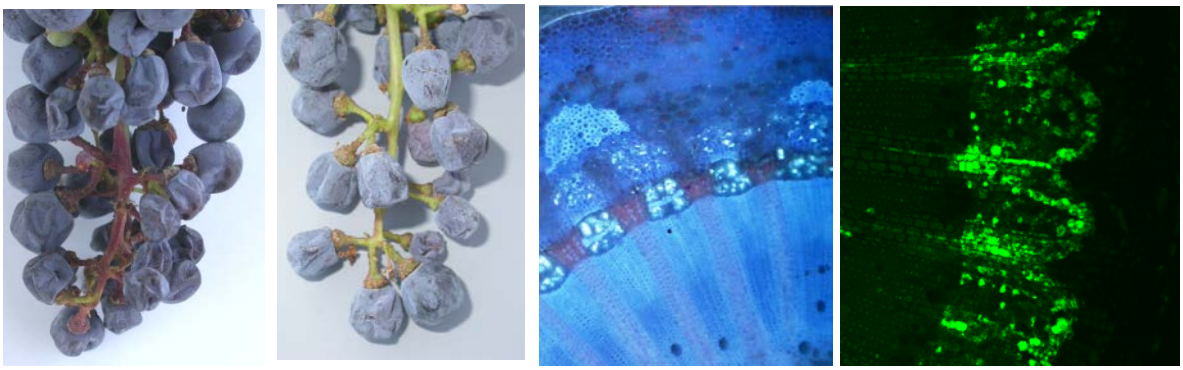

Figure 1. Micrographs showing chlorophyllous and chromated bunch stem, and vascular tissues in SOUR shrivel clusters of grapevines.

Objective 2: Since SOUR shrivel is a moving target, a logical way to go about it is to simulate the SOUR shrivel under greenhouse conditions to capture its inception and progression of the symptoms. To do so, attempts were made to impede sugar translocation by physical means of girdling the phloem tissues. Girdling led to shriveling of the berries. In future, more advanced techniques involving continuous supply of cold treatment to phloem tissues will be employed to simulate SOUR shrivel.

Dissemination of Research:

Publications (Refereed)

1. **Bondada**, B. and M. Keller. 2013. Structural and Fruit Compositional Anomalies Related to Various Shrivels Types Developing During Ripening of Grape Berries. Acta Horticulturae (In Press).

2. **Bondada, B.** and M. Keller. 2012. Not all shrivels are created equal – morpho-anatomical and compositional characteristics vary among different shrivel forms that develop during ripening of grape (*Vitis vinifera* L.) berries. American Journal

of Plant Science 3: 879-898. **(*Cover article, see publications*)**

3. **Bondada, B**. and M. Keller. 2012. Morpho-anatomical symptomatology and osmotic behavior of grape berry shrivel. Journal of the American Society for Horticultural Science 137:20-30.

Extension Publication

Bondada, B. 2013. Berry shrivels – Fruit ripening disorders of grape. 106-107. In: Moyer, M. M. and O'Neal, S. D. (Eds.). Field Guide for Integrated Pest Management in Pacific Northwest Vineyards. A Pacific Northwest Extension Publication PNW 644.

Industry Trade Journals

1. Bondada, B. 2013. An Expensive Disorder. Good Fruit Grower. February 2013, pp. 24-25.

2. Bondada, B. 2013. Ripening disorders – Not all berry shrivels are created equal. Wines and Vines, January 2013.

Presentation at International Meeting

Bondada, B. 2013. Structural and fruit compositional anomalies related to various shrivels types developing during ripening of grape berries. IX International symposium on grapevine physiology and biotechnology, April 21-26, 2013, La Serena, Chile.

Industry Presentation

Bondada, B. and M. Keller. 2013. Structural and compositional characterization towards unlocking the unknowns of sour shrivel. Washington Association of Wine and Grape Growers, Annual Meeting Convention and Trade Show, February 5-8, Kennewick, WA.

International (Invited speaker)
Bondada, B. 2012. Winegrape berry shrivels: are they all the same. South Valley Spring Meeting, February 28th, 2012, Oliver, BC, Canada.

National (Invited speaker)
Bondada, B. 2013. Berry shrivels – ripening disorders of grape berries. Presentation to the Pennsylvania Quality Assurance Group, Philadelphia, August 6th, 2013.

Bondada, B. 2102. Physiological disorders related to ripening of grape berries. Alabama State University, Montgomery, AL, November 1st, 2012.

Regional (Invited speaker)

Bondada, B. 2012. Not all shrivels are created equal. Washington Grape Society Annual

Meeting, Grandview, WA. November 15th, 2012.

Funds Status:
We will spend the whole amount toward the expenses involving analyses of tissues with various microscopes, fruit composition etc. as per the original projections detailed in the budget for this project.

Other Sources of Funding: None