Annual Progress Report

This is year __3__ of a _3___year proposed project.

For the Washington State Grape & Wine Research Program

Date: January 31, 2014

Project Title: Phenolic Management: Investigation of deficit irrigation severity, UV-Vis modeling and tannin protein binding capacity.

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Cooperators:
Markus Keller, Ph.D., WSU Prosser, Dr. Keller will provide consultation and advisement on measuring and recording vineyard parameters described below.
Russell Smithyman, Ph.D., Ste. Michelle Estates, Dr. Smithyman will provide access to the vineyards, vineyard data (irrigation logs, climate data).
William Riley, Ph.D., Ste. Michelle Estates, Dr. Riley will provide access to the vineyards (for both experiments), vineyard data (irrigation logs, climate data).
Thomas Henick-Kling, WSU Tri-Cities, Dr. Henick-Kling will provide expertise for sensory evaluation of wines.
John Thorngate, Ph.D., Applications Chemist, Constellation Wines U.S. will responsible for modeling of phenolic analysis.
Mark Downey, Ph.D., Group Leader, Plant Production Sciences, Senior Research Scientist, Viticulture & Oenology, Mildura Australia
Hildegarde Heymann, Ph.D. Professor, UC Davis, Dr. Heymann's laboratory will provide consultation for wine produced sensory analysis of fruit maturity trial

Project Budget Number: 13C-3357-5531

I. Project Summary(s): Objective 1: Impact of deficit irrigation severity on grape and wine composition. Situation: All vineyards in Eastern Washington are irrigated due to its arid climate, which in some areas receives less than 10 inches of rainfall annually. Due to the expense of water usage many vineyards are evaluating the use of regulated deficit irrigation to either improve the wine or alter the wine style. Both timing and severity of regulated deficit irrigation are unknown areas of research with regards to altering wine composition and quality. Objective 2: The assessment of phenolics during the winemaking process has proven itself to be a worthwhile endeavor. Wineries around the world have been using phenolic analysis during winemaking to help make winemaking decisions. Several analytical techniques exist however my laboratory's work has been instrumental in the development of the so- called "Harbertson-Adams" assays for

important classes of phenolic compounds. The Harbertson-Adams assays measure functional classes of phenolic compounds; however, the methodology is both labor- and time-intensive. Objective 3: Seed tannins are largely believed to be problematic by winemakers who believe that longer ripening and higher ethanol concentrations in the must contribute to altering the extraction of seed tannins during red wine production. Winemakers believe that seed tannins give red wines a harsh astringency whereas tannins from the skin are more palatable. In model studies it has been shown that seed tannin extraction declines during grape ripening whereas the same model studies demonstrate that greater ethanol concentrations increase extraction of seed tannins. Thus winemakers believe if they wait longer for grapes to ripen they can reduce seed tannin extraction. However as the more ripe fruit will potentially yield a greater alcohol concentration and potentially more seed tannin extraction there remains a conundrum.

2.Although there are several different objectives here we maintain the same approach for extension related activities. Our work is disseminated through talks with local wine and winegrape grower related meetings by oral presentations, posters or discussions with winemakers or grape growers. The work is also presented at the national level by presenting at the American Society of Enology and Viticulture National Conference. Our work is also summarized in trade magazines, extension briefs or through a newsletter edited by my viticulture extension colleague. We use basic extension surveys for our events and include learning based objectives and questions to determine the value of our work.

3. Ultimately the project was designed to provide better information to winemakers and growers about the impacts of deficit irrigation, and grape maturity on grape and wine phenolic composition. The project was also designed to create a model that could be used to predict the phenolic composition of a sample by gathering spectra in the ultra violet and visible wavelength region without having to carry out the entire wet chemistry method.

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

Objective 1: Regulated deficit irrigation was applied to a 33-year old own-rooted Cabernet Sauvignon vineyard in Mattawa, Washington in attempt to evaluate the effect of altering the timing and severity of deficit irrigation on grape and wine phenolic composition. In 2011 and 2012 wines were produced from the fruit from the project and included an extended maceration treatment (10 days control & 30 days treatments) to explore the interaction between fruit composition and winemaking techniques whereas in 2013 we included saignée and juice addition to determine if the berry size impacts observed in the vineyard treatment could be compensated for in the winery. Objective 2: We proposed to create a simple to use version of the assay by comparing spectra of a sample to reference data of the assay and modeling it using complex mathematics. We varied both the way we gathered the spectra (4 different ways) and the reference methodology (4 tannin assay types) and measured 5 phenolic variables (anthocyanins, tannins, total iron reactive phenolics, large polymeric pigments, small polymeric

pigments). About 200 Syrah and 200 Cabernet Sauvignon samples have each been assayed in triplicate as well as the spectra taken in triplicate. Partial least square regression is being applied in an attempt to create a mathematical model. Objective 3: In this work we sought to explore the impact of fruit maturity on tannin extraction in winemaking conditions as opposed to model wines extractions. We utilized ethanol concentration and extended maceration at different grape maturity to evaluate their impact on seed tannin extraction. Selected winemaking conditions were applied to fruit with ~20.3 and ~24.9 Brix over two seasons. Merlot grapes were harvested 33 (2011) and 34 (2012) days apart. At each harvest, half of the must was adjusted to emulate the other harvest's soluble solids content to evaluate the effect of ethanol on phenolic extraction at different fruit maturities. Additionally, two maceration lengths of 10 days (control) and 30 days (extended maceration) were tested. We have shown in the past that greater maceration lengths during winemaking increase extraction of seed tannins. In 2013 we changed the experiment slightly and added a third harvest date to the experiment picking at 20 Brix, 24 Brix, and 28 Brix (day 248, 268 and 305 respectively) to determine if ethanol at 16 % (v/v) would alter tannin extraction. However in this case we did not apply extended maceration but did take a portion of the fruit and chaptalize or bleed off and add water to the other target maturities. In each case the juice to solids ratio was carefully monitored so that it was similar within a given harvest date. So we had three harvest dates with 2 different treatments and a control per harvest date.

III. Major Research Accomplishments

As there are three separate objectives I will keep them separated. Objective 1 Impact of deficit irrigation: We have conducted a three-year deficit irrigation project, with the same vineyard being irrigated with the same strategy for three seasons in a row. Berry size was significantly impacted by deficit irrigation as expected. The treatment effect was follows 25%ET<25%-100% ET<70% ET≤100% ET. There was a significant vintage effect but no interaction between vintage and treatment. Significantly greater concentrations of anthocyanins were found in the fruit and wine of the 25% ET treatments. Enhancements in the fruit tannins were only observed on fresh weight basis and thus were as a result of the berry size change and were no. In 2011 and 2012 the extended maceration treatments showed that the 25% ET and 25%-100% had significantly greater tannin concentrations than the control and 70%ET which was due to greater seed tannin on a fresh weight basis. Evaluation of tannin polymer distribution showed that winemaking practices (in this case extended maceration) had an effect on tannin size and composition while viticulture practice had no impact. Sensory evaluation showed that both deficit irrigation and extended maceration separated the sensory composition of the wines. In 2013 when attempts to compensate for berry size in the winery by either juice addition or saignée we found that it was not possible to create the same pigment concentration as the 25% ET wines by using 10%, 18 or 20% saignée on the other treatments (25-100%, 70% and 100% ET treatments respectively). The results of this study suggest that tannins are largely unaffected (structurally or concentration basis) by the severe deficits imposed in the vineyard and the only changes are observed as a result of berry size changes. Further winemaking techniques that enhance extraction of seed tannins like the extended maceration treatment will extract greater amounts of seed tannins and actually change the type of tannins found in the wine (something the vineyard treatment does not do). Thus

severe deficit irrigation only impacts berry size and grape and wine pigmentation. The impacts are quite significant and cannot be compensated for in the winery by bleeding off juice prior to fermentation.

Objective 2. Modeling of phenolic analysis. We proposed to create a simple to use version of the assay by comparing spectra of a sample to reference data of the assay and modeling it using complex mathematics. We varied both the way we gathered the spectra (4 different ways) and the reference methodology (4 tannin assay types) and measured 5 phenolic variables (anthocyanins, tannins, total iron reactive phenolics, large polymeric pigments, small polymeric pigments). About 200 Syrah and 200 Cabernet Sauvignon samples have each been assayed in triplicate as well as the spectra taken in triplicate. Partial least square regression is being applied in an attempt to create a mathematical model. Thus far we are still creating the model, as our industry collaborator has been unable to finish the mathematics. Currently we have found a new collaborator who has extensive experience as he was an author on the paper we based our work (Dr. Roger Boulton UC Davis). We expect to have the model created in the last 6-month of the granting period.

Objective 3. Impact of fruit maturity and winemaking technique: We found that alcohol concentrations in the range of 11.7-14.4% (v/v) ethanol showed no significant effect on seed tannin extraction. Thus counter to expectations greater ethanol concentrations applied to commercially unripe fruit during normal or extended maceration did not result in greater seed tannin extraction. We also found that the amount of seed tannin extracted during extended maceration was independent of maturity but instead varied as a result of vintage. Thus the model studies appeared to be inaccurate and the worries of winemakers can be assuaged as seed tannin extraction is largely unaffected by grape ripening at alcohol concentrations normally achieved using wine yeast 11.7-14.4% ethanol (v/v). In the final year of the project we found that ethanol concentrations unachievable by *Saccharomyces cerevisiae* but instead by *Saccharomyces bayanas* had a significant impact on tannin extraction (16.4% (v/v) which was independent of harvest date. We also found that the wines made from the final harvest date had significantly greater tannins in the wine than the two other harvest dates, which were not different from each other (consistent with our previous results). The difference can be ascribed to the decrease in berry size, which declined by 18% from 24 Brix to 28 Brix. These results are extremely significant and some appear to be in direct contrast to beliefs widely held by winemakers in the industry. We found that seed tannin extraction does not decline as a result of longer ripening periods but instead is altered by ethanol concentrations that are greater than what can be achieved by wine yeast. Secondly waiting for more mature fruit does not result in wines with less tannin, which is widely believed by winemakers. We found no change between 20-25 Brix and once the berry dehydrated between 25-28 Brix there was a concentration effect and the resulting wines had greater tannin concentration. Thus we have concluded that winemakers who want to utilize wine yeast strains such as Lavlin EC-1118 they can achieve both greater ethanol concentrations (16.4% ethanol v/v) and greater tannin concentrations. Within the confines of the ethanol concentrations achieved by *Saccharomyces cerevisiae* there is no risk of greater tannin extraction from 20-28 Brix fruit. Secondarily we found small but significant impact from the high ethanol treatment

on pH (~0.2 increase) and copigmentation that results in wines that are less colorful. Results from sensory showed that color, ethanol and maturity were important factors in the final wines.

IV. Result Dissemination

Published work:

Casassa, L.F., C.W. Beaver, M.S. Mireles, R.C. Larsen, H. Hopfer, H. Heymann, J.F. Harbertson*. 2013. Influence of fruit maturity, maceration length, and ethanol amount on chemical and sensory properties of Merlot wines. Am. J. Enol. Vitic. 64:437-449.

Casassa, L.F., R.C. Larsen, C.W. Beaver, M.S. Mireles, M. Keller, W.R. Riley, R. Smithyman, J.F. Harbertson. 2013. Sensory impact of extended maceration and regulated deficit irrigation in Washington State Cabernet Sauvignon wines. Am. J. Enol. Vitic. 64:505-514.

Casassa, L.F., R.C. Larsen, C.W. Beaver, M.S. Mireles, M. Keller, W.R. Riley, R. Smithyman, J.F. Harbertson. 2013. Impact of extended maceration and regulated deficit irrigation in Cabernet Sauvignon wines: characterization of proanthocyanidin distribution, anthocyanin extraction and chromatic properties. J. Agric. Food Chem. 61: 6446-6457.

Abstracts that were presented as either an oral (O) or poster (P) presentation.

Harbertson, J.F. (2013, July 18) Impact of Winemaking Techniques on Red Wine Phenolics. Advances in Red Wine Production at Am. Soc. of Enol. Vitic. 38th Annual ASEV-Eastern Section Meeting, Winston-Salem North Carolina (O)

Harbertson, J.F. (2013, June 28) Effect of winemaking practices on tannins. Tannin Symposium at Am. Soc. of Enol. Vitic. 64th Annual Meeting, Monterey, CA. (O)

L.F. Casassa, R.C. Larsen, C.W. Beaver, M.S. Mireles, M. Keller, W.R. Riley, R. Smithyman, J.F. Harbertson 2013. Interactive effect of regulated deficit irrigation and skin contact time in Washington State Cabernet Sauvignon wines. Am. Soc. of Enol. Vitic. 64th Annual Meeting, Monterey, URL: (http://www.asev.org/docs/2013TechnicalAbstracts.pdf) pg 98 (O)

J.F. Harbertson, L.F. Casassa, C.W. Beaver, M.S. Mireles, R.C. Larsen, H. Hopfer, H. Heymann. 2013. The effect of fruit maturity, ethanol concentration and maceration length in Washington State Merlot wines. Am. Soc. of Enol. Vitic. 64th Annual Meeting, Monterey, CA URL: (http://www.asev.org/docs/2013TechnicalAbstracts.pdf) pg 100 (O)

L.F. Casassa, R.C. Larsen, C.W. Beaver, M.S. Mireles, M. Keller, W.R. Riley, R. Smithyman, J.F. Harbertson 2013. Interactive effect of regulated deficit irrigation and skin contact time in Washington State Cabernet Sauvignon wines. Am. Soc. of Enol. Vitic. 38th Annual ASEV-Eastern Section Meeting, Winston-Salem NC, Am. J. Enol. Vitic. 64:421A (O)

Casassa F., P.Romero, M.Mireles, M.Keller, W.Riley, R.Smithyman, J.F.Harbertson. 2012. Timing and severity of regulated deficit irrigation on Cabernet Sauvignon. grapes: agronomical and compositional effects. Am. Soc. of Enol. Vitic. 63rd Annual Meeting, Portland, OR 63:440A. (O)

Casassa F., R.C.Larsen, M.Mireles, J.F.Harbertson. 2012. Timing and severity of regulated deficit irrigation on Cabernet Sauvignon wines: interactive effect of skin contact time. Am. Soc. of Enol. Vitic. 63rd Annual Meeting, Portland, OR 63:448A. (P)*

Harbertson J.F., F.Casassa, R.C.Larsen. 2012. Effect of fruit maturity, ethanol concentration, and maceration length on wine phenolics. Am. Soc. of Enol. Vitic. 63rd Annual Meeting, Portland, OR 63:440A. (O)

Yue Y. J.F.Harbertson. 2012. Improvement in protein precipitation tannin analysis by altering resuspension buffer formulation to neutral pH. Am. Soc. of Enol. Vitic. 63rd Annual Meeting, Portland, OR 63:443A. (O)**

Posters Presented at Washington Association of Grape and Wine Growers Meeting.

L.F. Casassa, R.C. Larsen, C.W. Beaver, M.S. Mireles, M. Keller, W.R. Riley, R. Smithyman, J.F. Harbertson 2013. Interactive effect of regulated deficit irrigation and skin contact time in Washington State Cabernet Sauvignon wines. Washington Association of Grape and Wine Growers, Feb 7-9 2013, Kennewick WA. 2013.

Harbertson, J.F, F. Casassa, and R.C. Larsen. 2012. Effect of Fruit Maturity, Ethanol Concentration, and Maceration Length on Wine Phenolics. Washington Association of Grape and Wine Growers, Feb 7-10 2012, Kennewick WA. 2012.***

Yue, Y., and J.F. Harbertson. 2012. Improvement in protein precipitation tannin analysis by altering resuspension buffer formulation to neutral pH. Washington Association of Grape and Wine Growers, Feb 7-10 2012, Kennewick WA.

Casassa, F., P. Romero, W. Riley, R. Smithyman, M. Keller, and J. F Harbertson. 2012. The effect of timing and severity of deficit irrigation on Columbia Valley Cabernet Sauvignon grape and wine phenolics made with different maceration periods. Washington Association of Grape and Wine Growers, Feb 7-10 2012, Kennewick WA. ****

*Best Student Enology Poster
**Best Student Enology Oral Presentation
*** 1st Place Professional Category Poster Washington Association Wine Grape Growers
****2nd Place Student Category Poster Washington Association Wine Grape Growers

V. Project Budget Status

Our funds will be expended at the end of the funding cycle.

VI. Other Sources of Funding:

Maria M. Mireles funding (100% Tech II) is payed for by the Prosser Agriculture Research Station as part of Harbertson negotiation package. This money will be expended in June 2014. Mrs. Mireles is responsible for wet chemistry, sampling, and data gathering for the project.