FINAL REPORT

Washington State Grape and Wine Research Program

by email to: ARCGrants@wsu.edu

PROJECT TITLE: Assessing Smoke Taint Risk Based on the Composition of Smoke Exposed Grape Berries and the Resulting Wines

Project Duration: ***(2016-2019)***
WRAC Project No.: 2017-3.EN.CP.CT1

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Cooperator Name:	**Jim Harbertson, PhD**
Organization	WSU, Viticulture and Enology Program
Description of participation:	Dr. Harbertson's team made the wines from the smoke taint trial, following agreed upon winemaking practices.

FINAL REPORT

BUDGET AND OTHER FUNDING SOURCES

FINAL FINANCIAL REPORTING

BUDGET *(3075-7102; 3075-8000)*

	Year 1 FY 17	**Year 2 FY 18**	**Year 3 FY 19**
	Jul 2016 – Jun 2017	Jul 2017 – Jun 2018	Jul 2018-Jun 2019
Total	$45,000	$51,005	$85,090[1]
Footnotes: [1]Includes $5800.00 supplemental funds to support the survey of Washington grape samples following the smoke exposure event in mid-August 2018.			

Total Project Funding: 181,095.00

Project Budget Status: As of 27 June 2019, all of the funds awarded for this project have been spent in support of the project. The majority of the funds were used to support Garrett Lattanzio, an M.S. graduate student in the School of Food Science; Garrett will defend his M.S. thesis on July 26th, 2019. Grant funds also support Ioan Gitsov, a second M.S. graduate student for the spring semester of 2019; Ioan graduated in May of 2019.

The remainder of the funds were used to build and maintain the hoop-house system used for the deliberate smoking of grapevines at the WSU IAREC Prosser Roza research vineyard, as well as for the analysis of grape berry and leaf samples and for making wines at the Wine Science Center (WSC) on the Tri-Cities campus. The return on the investment in the hoop-house and associated smoking equipment in the first year of this grant will continue to be realized in the new proposal related to smoke taint which was approved for FY2020.

A one-time request for supplemental funds of $5,800.00 was made in 2018 to support a survey of grapes from the major growing regions in eastern Washington after the smoke exposure episode which took place during 18-20 August 2018. These funds were used to support the chemical analysis of approximately 90 grape samples collected in the ten day period following the smoke exposure.

<u>Project Summary:</u>

The exposure of vineyards to smoke from wildfires can result in wines which are described as smoke tainted or smoke affected. Affected wines exhibit off-aromas described as medicinal, smoked salmon, cold ash and similar, while descriptors of the aftertaste of affected wines include smoky and ashy, along with increased harshness in the finish (Parker, et al. 2012; Whiting and Krstic, 2007) Evaluations of forestry and other wildland management practices as well as climatic conditions suggest that wildfire outbreaks will continue to occur in close proximity to areas of viticultural importance and may be expect to increase in intensity as well (Westerling, et al. 2006). As smoke affected wines at best require additional processing to ameliorate the effects and in many cases, cannot be used even with additional processing (Fudge, et al. 2011; Fudge et al. 2012), methods for the assessment of the risk associated with smoke exposure for the resulting wines are needed. The proposed research will address that need by the deliberate exposure of research vineyards to smoke of similar intensity and duration as seen in recent wildfire episodes in Washington, along with the analysis of the chemical composition of the smoke exposed fruit and the wines made from that fruit. An evaluation of winemaking practices including minimization of skin contact and the use of enzymatic hydrolysis and reverse osmosis (RO) for smoke taint reduction will be an integral part of the proposed research. This proposal addresses the industry priority for smoke taint analysis and removal through careful characterization of both the affected fruit and the resulting wines.

Project Major Accomplishments:

The primary objectives for the first year of this study were as follows:

1) *Develop the methodology for the application of moderate levels of smoke over extended (2-10 day) periods.*
2) *Analyze grape leaves and fruit for the presence of free and bound smoke related compounds in grapevines exposed to smoke in Objective 1.*
3) *Monitor the extraction of smoke taint related compounds and their glycosides during and following the fermentation of fruit from grapevines exposed to smoke in Objective 1.*
4) *Treat a subset of the smoke affected wines using reverse osmosis to reduce the concentration of smoke taint relate compounds and their glycosides. Monitor the treated wines for the release of glycosidically bound smoke taint related compounds post-treatment.*

During the 2016 growing season, two modular portable hoop-houses large enough to cover 60 vines (2 rows by 30 vines) were designed and built (Figure 1). These houses were used to conduct three separate smoking trials in the WSU Roza experimental vineyard. One trial was done with Riesling, followed by two trials in Cabernet sauvignon. Each trial was 18 hours in duration and included both control and smoked rows. The hoop-house for the control was placed up wind of the smoke house to minimize any issues with smoke drifting from the smoke house. For each trial, a mix of hemlock, red cedar and redwood bark and small chunks of Douglas fir wood were used as fuel for the smoker. Smoke intensity was monitored using PM 1.0, PM 2.5 and PM 10.0 particle counters. Passive air samplers were also used to collect smoke particles during the trials. A PM 2.5 monitor was used in the control hoop-house to ensure that smoke from other hoop-house was not being carried to the control. The hoop-houses were covered with 80% shade cloth, which was sufficient to contain greater than 90% of the smoke inside the house, while keeping the temperature inside the house to no more than 10°F greater than ambient. The houses could be assembled, disassembled and moved using 3-4 people, and once in place required two people to manage the smoke generation, monitor the test equipment and

Figure 1. Modular portable hoop-house for conducting controlled smoke exposures

collect samples. While these trials were only 18 hours in duration, it would be possible to continue the smoking trial for up to several days with a team of 4-6 people working in shifts.

Grape leaves and clusters were collected at two-hour intervals during the smoking trials. Additional samples were collected at the end of each trial to ensure sufficient material to develop extraction protocols and to test analytical methods. These samples were held in coolers during the trials and then transferred to -80°C freezers upon completion of the field trial.

The Riesling was harvested on 04 October 2016 and transported to the Wine Science Center. The fruit was crushed and destemmed, then held overnight at 40°F before pressing the following morning. The juices were then processed using standard white wine making protocols. Samples were collected as the fruit was destemmed, following the overnight cold soak, after pressing and then at the end of fermentation. Additional samples have been collected at weekly intervals during the aging period.

The Cabernet sauvignon was harvest on 09 October 2016 and transported to the WSC. The fruit was destemmed and crushed, then fermented per a standard research red winemaking protocol. Samples were collected daily during fermentation and weekly post-fermentation. UHPLC-QTOF/MS analysis of the wines has found glycosides and other conjugates of guaiacol, 4-methyl guaiacol, cresol, dihydroxy-benzaldehyde and cresotinic acid in the wines made from smoked fruit. In some cases, these compounds are extracted as early as the second day of fermentations, while some do not appear in the samples until 4-5 days into the fermentation.

The primary objectives for the second year of the study were as follows:

1) *Using the methodology developed during year one, conduct extended smoking trials for a period of 2-4 days in Cabernet, Merlot and Chardonnay.*
2) *Conduct one or more of these trials using local sage-lands plant material as a fuel source for the smoking.*

3) *Using methodology developed during year one, monitor leaves, fruit and wines for the presence of smoke related compounds and their glycosides and other conjugates*
4) *Evaluate enzymatic hydrolysis of smoke related glycosides and their conjugates in smoke affected wines*

In the second year of this study, we continued to develop the systems necessary to expose field planted grapevines to moderate levels of smoke for extended durations, with exposures ranging from 26 to 48 hours. As in the first year of the study, a total of 60 vines were covered by each hoop-house for each trial, with one house for the smoke treatments and the second serving as the unsmoked control. Chardonnay vines were smoked for 26 hours, with a mix of rangeland plants

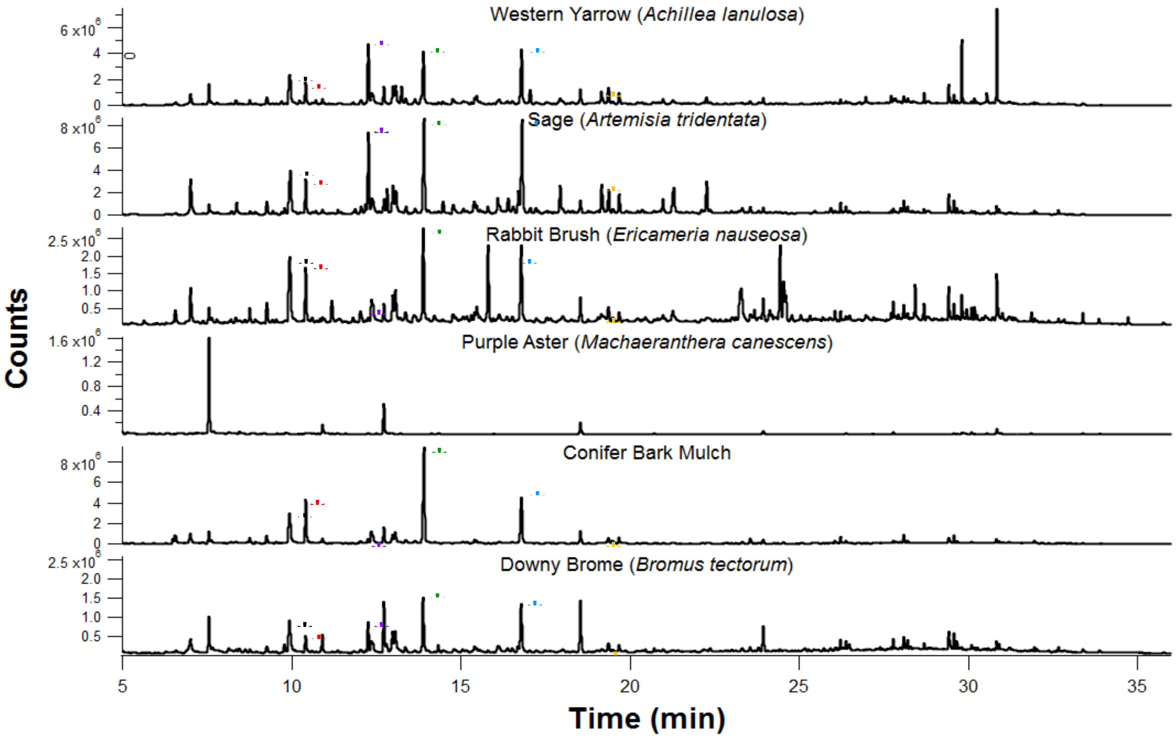

Figure 2. GC/MS "fingerprints" of smoke from several plant species

common to eastern Washington as fuel for the smoke, to mimic the effects of a rangeland wildfire. Similarly, Merlot vines were smoked with the same mixture of rangeland plants, for a duration of 48 hours. Prior to the vineyard smoking trials with rangeland plants, a series of small-scale smoking trials was done at the Wine Science Center with the individual species used in the rangeland mix. Smoke was collected for each of the species and analyzed using GC/MS to develop a smoke “fingerprint” for each plant type (Figure 2). Cabernet Sauvignon was also smoked in 2017, using a similar mix of conifer bark mulch as was used for smoking Cabernet Sauvignon in 2016 to allow for a comparison of Cabernet Sauvignon smoked with similar fuel sources across two vintages. The Cabernet Sauvignon was smoked for a total of 38 hours; the intention was to conduct a 48-hour smoking trial as was done for the Merlot, but during the second night of the trial, high winds threatened to damage the hoop-houses, so the trial was terminated early.

Much of the Columbia Basin, including the Roza vineyard, was affected by smoke from actual wildfires, including smoke from fires in British Columbia during the period of 1-12 August, as well as a second episode from 4-7 September from the fires in the Columbia Gorge and in the Cascades. In both instances, we monitored the density and duration of the smoke using air quality monitors to quantify the potential impact of these smoke episodes on our smoking trials.

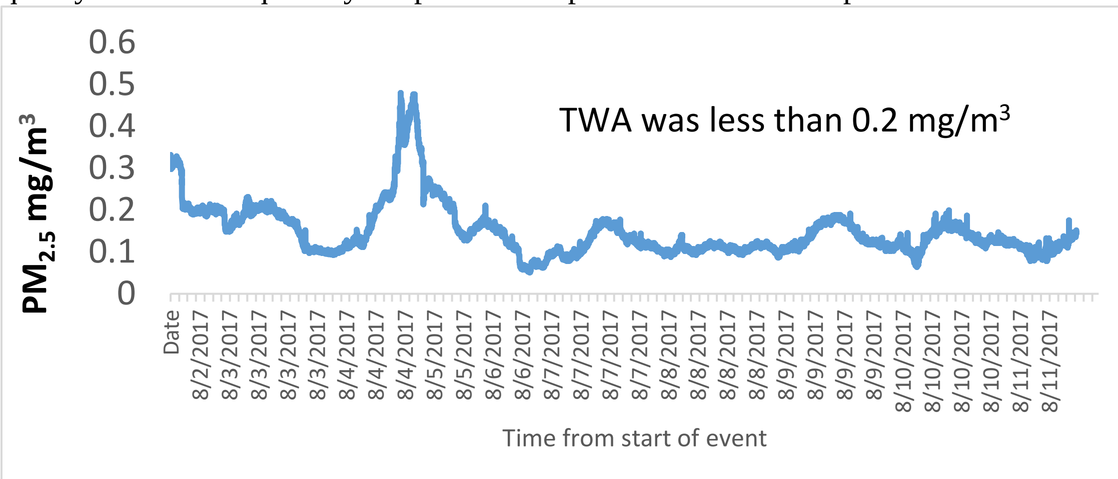

Figure 3. $PM_{2.5}$ (mg/m^3) in the Roza vineyard during the period August 2nd-12th, 2017

The smoke intensity during the August episode was low, ranging from 0.1 to 0.2 mg per cubic meter, while the September smoke density was higher, ranging from 0.6-1.0 mg/m^3. The low smoke density coupled with the pre-véraison timing suggests that the risk of exposure from the August episode was relatively low, but the smoke density during the second episode was high enough that there was significant risk of smoke taint. While the smoke exposure from these episodes complicates the analysis of the results from our trial, the smoke treatments applied to the trial vineyards were sufficiently intense that it will still be possible to determine the effects of the deliberate exposure.

Wines were made from the affected fruit at the WSU Ste Michelle Wine Science Center, using an extended skin contact white wine protocol and a standard red winemaking protocol. The Chardonnay grapes were crushed then soaked on the skins overnight to allow sufficient opportunity for smoke related compounds to be extracted prior to pressing. For the red fermentations samples were collected throughout the fermentation processes for determination of smoke related compounds and their associated glycosides. Figure 4 shows peak area for 4-methylguaiacol di-pentoside, a glycoside of 4-methylguaiacol (4MG), which present in the fermentations of the fruit exposed to smoke, but not in the control. In the smoked fermentation, 4MG-dipentoside is extracted early in the fermentation, as the sugars make the compound soluble in water. After peaking on day 3, the concentrations decrease as the fermentation progresses. All the wines from the 2017 smoking trials have perceptible smoke taint.

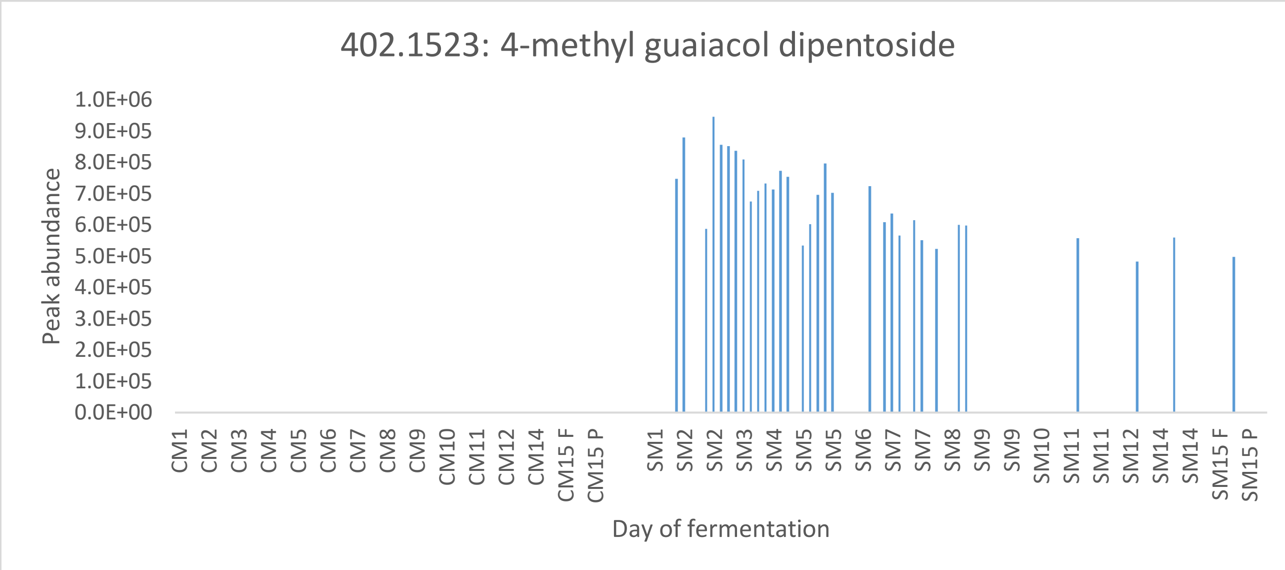

Figure 4. 4-methylguaiacol di-pentoside in control and smoked Merlot fermentations. This glycoside is extracted early in the fermentation of must from smoke-exposed fruit, but concentrations diminish as the fermentation progress. The compound is not present in fermentations of the control fruit.

A portion of each of the resulting wines has been treated after primary and malolactic fermentations with reverse osmosis to reduce the concentrations of smoke related compounds. In addition to the typical R.O. membrane used to remove the free volatile phenols, a second R.O. membrane with a large enough molecular weight cut-off (MWCO) to allow removal of the glycosides of smoke related compounds was also trialed. While our focus is on the removal of smoke related glycosides, this membrane will also remove glycosides of other compounds as well, including anthocyanin glycosides. Never-the-less, this approach could lead to the ability to use enzymatic or other treatments to hydrolyze the glycosides of smoke related compounds in a simpler matrix than the wine itself, while allowing the return of beneficial glycosides to the affected wines.

The primary objectives for the third year of the trial were as follows:

1) *Using the methodology developed during year one, conduct extended smoking trials for periods of 2-4 days in Cabernet Sauvignon and Merlot to provide smoke exposed fruit to support ongoing mitigation trials*
2) *In Cabernet, conduct smoking trials at 50% véraison, 14 days later and 28 days later to evaluate how risk associated with smoke exposure varies during the ripening period.*
3) *Using materials generate in Objectives 1 & 2, continue to evaluate reverse osmosis and enzymatic approaches for mitigation of smoke taint in wines made from exposed fruit.*
4) *Provide analytical support using GC/MS, LC/QTOF and other approaches as required for the following projects:*
    a. *Markus Keller—riesling irrigation, cabernet cooling*
    b. *Jim Harbertson--maturation trials*
    c. *Thomas Henick Kling--acid adjustment trial*

During the third year of this study, the hoop-house smoking system developed in year one was used to conduct a timing of exposure trial in Merlot. Objective 1 was modified to include a pre-véraison smoke exposure, a smoke exposure at 50% véraison, a three-week post véraison exposure and a six-week post véraison exposure. The pre- véraison exposure, which took place on July 18th-20th, was done four weeks prior to the 50% véraison exposure (August 15th-17th). The 3-week post véraison exposure was conducted September 5th-7th and the 6-week post véraison smoking was done September 26th-28th. The pre-véraison and 3-weeks post véraison exposures were both 48 hours duration, but the véraison exposure (38 hours) and the 6-week post veraison (42 hours) were both cut short by weather events (a severe thunderstorm and high winds, respectively). Additional improvements were made to the smoking system, including repositioning the smoke delivery pipes from the row middles (Figure 5) to deliver the smoke directly to the fruiting zone. Preliminary trials were also done in the final smoking of the year to subdivide the hoop-houses into zones to which differing amounts of smoke could be delivered.

Figure 5. Repositioning of smoke delivery pipe from row middle to into each row.

Berry samples collected during the smoking trials were extracted and analyzed for their content of smoke taint related glycosides. Several glycosides were found, including the di-pentosides and di-glucosides of guaiacol and 4-methyl guaiacol. It was noted that the color intensity of the extracts made from smoke fruit was reduced compared the extracts from control fruit and that the hue was different as well (Figure 6A). An untargeted analysis of the UHPLC/QTOF-MS data for the extracts found decreased concentrations of anthocyanin and flavonoid glycosides in the smoked skin extracts relative to the control skin extracts (6B).

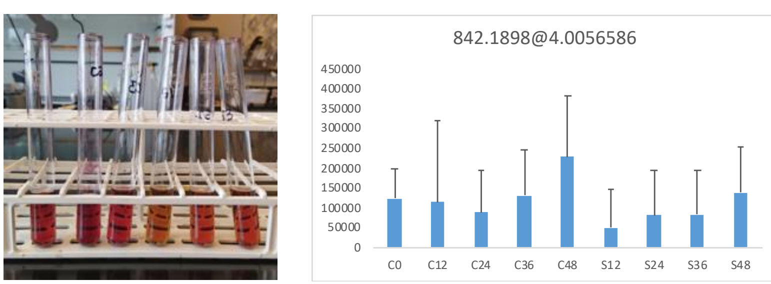

Figure 6A Extraction of skins of smoked grapes on the left and non-smoked on the right (Photo taken by Gabrielle Chalvidal); 6B peak areas for Pelargonidin 3-(6''-malonylglucoside)-7-(6'''-caffeylglucoside) [entity with mass of 842.1989 and retention time of 4.00 minutes] in control and smoked berry extracts; berry samples were collected at regular intervals during the smoke exposure.

The evaluation of reverse osmosis as a technique for mitigation of smoke characters in affected wines shifted from the use of R.O. to remove only the free compounds to the evaluation of membranes with larger molecular weight cut-offs (MWCO >1000). The use of these membranes should allow both the free compounds and their glycosides to migrate to the permeate stream. Samples of permeate streams from affect wines were treated using a range of commercial glycosidase enzyme preparations to hydrolyze the glycosides of smoke related volatile phenols.

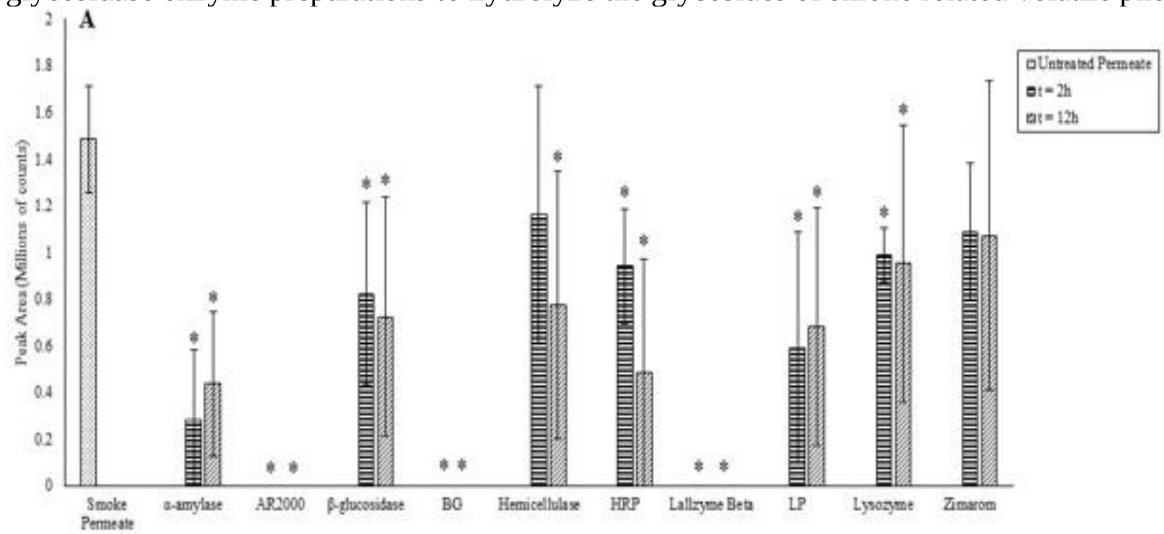

Figure 7 Coumaric acid di-glucoside peak area in permeates treated using several commercial glucosidase preparations.

In Figure 7, the di-glucoside of coumaric acid was reduced relative to the untreated permeate by each of the enzyme preparations. Three of the commercial enzyme preparations (AR2000, BG and Lallzyme B) were able to complete remove the coumaric acid di-glucoside in both the 2 hour and 12 hour incubations. Additional analysis is underway to determine whether the enzymes are removing both glucose moieties from the coumaric acid. The difference in extent of reduction may be due to different amounts and types of enzyme activities in these commercial preparations, which are include different types of enzyme activities in the preparations. Work is ongoing to measure the amounts of different relevant activities in each preparation so that consistent activity levels can be used for each preparation in future trials.

Summary of key outcomes from this project:

1) The development of a system for applying measured amounts of smoke to field planted grapevines. The use of modular portable hoop-houses covered with shade cloth provides a viable approach for ongoing field trials to better understand smoke exposure in vineyards. Using this system we have been able to conduct as many as four smoke exposures in a season. With additional personal and modules it would be possible to conduct additional exposures as well as to conduct true replicated field studies. Additional work is underway to provide more consistent intensity of smoke over the duration of the exposures.

2) The type of plant material that is burning affects the composition of the smoke resulting in differences in the composition of the affected fruit and wines as well as their sensory characteristics. We developed a system for collecting and analyzing samples of smoke

from various plant species (16 different botanicals to date). The GC/MS "fingerprints" of the smoke can be used to help identify smoke related compounds in the fruit and wine affected by the smoke from individual plant species.

3) Analysis of samples taken during fermentations of affected fruit have shown some patterns for the extraction of smoke related volatiles and their glycosides during fermentation. Work continues for the identification of smoke related compounds and their glycosides. These experiments were not designed to understand the extraction patterns for these compounds, but may be an important area for future investigation.

4) Extracts of the skins of smoke affected berries had reduce color intensity and a different hue (more orange) than extracts from the skins of non-exposed fruit. In evaluation of these extracts using an untargeted analysis of their composition found reduced concentrations of several anthocyanin and flavonoid glycosides.

5) Reverse osmosis using a membrane with a larger MWCO to allow the glycosides of smoke related compounds to migrate into the permeate followed by enzymatic treatment of the permeate to hydrolyze the glycosides was demonstrated during the final year of the grant. Standardization of the enzyme activity of the various enzyme preparations used in the initial trial is underway; after completion, it will be easier to directly compare the efficacy of the various enzymes test.

Factors which affected the conduct of the project

1) Personnel: Two research technicians came and went during the course of this project. Chris Sorensen participated in the first smoking season, but departed in November 2016. Rosemary Veghte participated in the second and third smoking seasons, but departed in November 2018 (November is apparently a difficult month for technician retention).

   A new post-doctoral student will join the lab in July 2019, which should provide more stability to the lab as two MS students finish their degrees. Additionally, a WSDA Specialty Crop Block Grant proposal submitted for the current cycle was approved for funding; assuming USDA confirms the approval, that three year project would begin in September 2019. The focus of that project is to work with Stephen Ficklin's group in Pullman to using neural networks and other multivariate modeling techniques to develop predictive models for smoke taint risk using the data generated in this project and in the new smoke taint project set to begin in July 2019. These two developments should significantly enhance the ability of my team to manage the data arising from these projects.

2) Teaching responsibilities: The evolution of the Viticulture and Enology program over the past four years has involved the development of several new courses, particularly for the Enology curriculum. During the period of this project, I have developed and taught or co-taught four new courses, including VE 435, Grape and Wine Chemistry, VE 440/441, Winery Equipment and Operations, VE 433, Critical Thinking in Vineyard and Winery Management and FS 501, Instrumental Analysis of Grapes, Wines and Spirits/Analysis of

Chromatographic and Mass Spectrometry Data. Each of these class was developed from scratch, adding to the workload associated with teaching these classes.

Now that each of these classes has been taught at least once, the effort required to continue to teach them will be less, involving periodic updates to lecture/lab material which is easier than the initial development of the material. The new post-doc will also provide significant support for the analytical classes. The Horticulture Department has also committed to provide me with Teaching Assistant support for both semesters of the 2019-2020 academic year, in recognition of my teaching load, which is above the requirements for my teaching appointment.

3) The 2017 fire season: While the impact of wildfires on vineyards has been an ongoing issue, the sheer number of fires in 2017, coupled with the tremendous damage wrought by the October fires in Napa and Sonoma counties served to focus attention on the problem. In response to industry requests for information on the effects of smoke exposures in 2017, I have made several presentations in Washington, Oregon and California in 2017 and 2018, including sessions at the ASEV National Conference in Bellevue, WA, in June 2017 and at the Unified Wine and Grape Symposium in Sacramento in January 2018. My students have made poster presentations at the 2018 and 2019 ASEV National Conferences and at the WAWGG meeting in 2018.

   The additional focus on the smoke exposure issue has helped to improve funding opportunities, as evidenced by the WSDA SCBG grant approval this year, as well as the request for a full proposal for the USDA SCRI planning grant proposal submitted by me, Elizabeth Tomasino at Oregon State and Anita Oberholster at UC Davis. An earmark for smoke exposure research was also included in a recent USDA funding bill as well.

Information Dissemination, Extension, and Outreach Activities:

Ashmore, P.L., Veghte, R., Thomas S. Collins, 2019: Smoke Exposure and Grape Maturity. Poster presentation, 70th National Conference of the American Society for Enology and Viticulture, Napa, CA, USA, June 18th-20th, 2019

Gitsov, I.P., Thomas S. Collins, 2019: Mitigating Smoke Taint in Wine by Reverse Osmosis. Poster presentation, 70th National Conference of the American Society for Enology and Viticulture, Napa, CA, USA, June 18th-20th, 2019

Collins, T.S. 2018: Quality Impacts of Smoke—Washington Wine. Washington State Tree Fruit Association Annual Meeting. Yakima, WA, USA. December 3rd-5th, 2018.

Collins, T.S. 2018: Smoke Taint Research. Washington Grape Society Meeting. Sunnyside, WA, USA. November 15th, 2018.

Collins, T.S. 2018: Smoke Taint Research Update. Rootstock Symposium, Napa, CA, USA. November 8th, 2018.

Collins, T.S. 2018: Smoke Taint Update. WSU/Washington Wine Commission seminar, Richland, WA, USA. September 15th, 2018. Sent via AMS to IAREC Prosser, WSU TFREC Wenatchee and WSU REC Puyallup.

Lattanzio, G., Thomas S. Collins, 2018: Assessing Smoke Taint Risk Based on the Composition of Smoke Exposed Grape Berries and the Resulting Wines. Poster presentation, 69th National Conference of the American Society for Enology and Viticulture, Monterey, CA, USA, June 19th-21st, 2018.

Veghte, R.M., Higgins, M.F., Booth, P.A., Lattanzio, G.A., Thomas S. Collins, 2018: Compositional Differences in the Combustion Products of High Desert Botanicals in Grapes and Wines. Poster presentation, 69th National Conference of the American Society for Enology and Viticulture, Monterey, CA, USA, June 19th-21st, 2018.

Collins, T.S. 2018: Smoke Taint in the Field and in the Winery. National Viticulture and Enology Extension Leadership Conference (NVEELC). Prosser, WA, USA. May 21st-22nd, 2018.

Collins, T.S. 2018: Smoke Taint Update. Washington Winegrowers Convention. Kennewick WA, USA. February 6th-8th, 2018.

Collins, T.S. 2018: Wildfires and Wine. Unified Wine and Grape Symposium, Sacramento, CA, USA. January 23rd-25th, 2018

Collins, T.S. 2017: From the Smoke-damaged Vineyard to the Winery: What We Know About Remediation. Pete Christensen Wine Grape Short Course, Davis, CA, USA, December 12th-13th, 2017.

Collins, T.S. 2017: The Science of Smoke Taint. Answering Tough Questions: Post-Fire Technical Think Tank, Napa Valley Vintners Association meeting, Napa, CA, USA, October 27th, 2017.

Collins, T.S. 2017: Smoke Taint Outreach Seminar. 68th National Meeting of the American Society for Enology and Viticulture, Bellevue, WA, USA, June 26th-29th, 2017.

Literature Cited:

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Parker, M.; Osidacz, P.; Baldock, G.A.; Hayasaka, Y.; Black, C.A.; Pardon, K.H.; Jeffrey, D.W.; Geue, J.P.; Herderich, M.J.; Francis, I.L. Contribution of Several Volatile Phenols and Their

Glycoconjugates to Smoke-Related Sensory Properties of Red Wine. *J. Ag. Food Chem.* **2012**, *60*, 2629-2637.

Westerling, A.L.; Hidalgo, H.G.; Cayan, D.R.; Swetman, T.W. Warming and Earlier Spring Increase Western U.S. Forest Wildfire Activity. *Science* **2006** *313*, 940-943.

Whiting, J.; Krstic, M.P; Understanding the Sensitivity to Timing and Management Options to Mitigate the Negative Impacts of Bush Fire Smoke on Grape and Wine Quality—Scoping Study. Department of Primary Industries: Knoxfield, Vic., Australia, **2007**