1. Project Title

Determine the impact of cluster thinning and cluster zone leaf removal on the hormone content of Pinot noir grape berry.

2. Principal Investigator
Laurent Deluc, OSU
4121 ALS Building
97331 Corvallis
(541) 737-4356
delucl@hort.oregonstate.edu

Co-Principal Investigator
Elizabeth Tomasino, OSU
220A Wiegand Hall
97331 Corvallis
(541) 737-4866
elizabeth.tomasino@oregonstate.edu

3. Cooperator:
Scoot Robbins, OSU – robbinss@hort.oregonstate.edu
Research Farm Manager ALS Building- 97331 Corvallis - (541) 737-5483

4. Objectives of Proposed Research

1) Determine the hormone dynamics of grape berry from flowering to harvest.
2) Estimate the effects of cluster thinning and leaf removal on the hormone profiles during the ripening phase.

5. Justification and Importance of Proposed Research

5.1 Rationale

Understanding the mechanisms involved in the control of grape berry development is a major objective of modern viticulture to produce optimally ripe crop with desirable characteristics. Grape berry development is seen as a genetically programmed process encompassing three different stages (fruit set, berry development and berry ripening). Each important stage and transition require the action of multiple hormones that orchestrate a complex network of interacting genes, proteins, and metabolites, which are responsible for the establishment of the different grape berry tissues. In grape, field studies have demonstrated the influence of hormones in promoting berry growth (auxin, cytokinin, gibberellins) and berry ripening (ABA, brassinosteroids, ethylene) (Bottcher et al., 2010, Cantin et al., 2007). The most recents studies even suggest the implication of hormones late in the season during the fruit maturity (Sun et al., 2010; Bottcher et al., 2013a). Interaction of hormones in grape berry development can vary from synergistic, with overlapping mode of action, to antagonistic effects. Auxin and cytokinin are known to counterbalance the promoting effects of ABA on ripening and are often used in viticultural practices to delay the ripening (Bottcher et al., 2013b). Recent reports suggest the likely role of ethylene accumulation from 7 to 8 weeks after bloom in promoting gene expression of biosynthetic genes involved in ABA and auxin biosynthesis (Sun et al., 2010; and Bottcher et al., 2013), which may explain the presence of a small peak of ethylene during pre*véraison* (Chervin et al., 2004). Apart from a developmental process, plant growth regulators are also responsible for the integration of cues like light, temperature, and plant water status enabling the plant to adaptively respond to environmental changes. Owing to the importance of hormones in many aspect of fruit growth and stress responses, several reports have surveyed individually the dynamics of these compounds in grape berry but few ave attempted to follow simultaneously their stage- and tissue-specific distribution, which is a critical component to determine plant growth regulators mutual roles during grape berry development (Gouthu et al., 2012, Bottcher et al., 2013).

For years, technology limitations have hampered our progress to completely determine the role of plant growth regulators in berry development. The lack of efficient extraction methods in berry tissues, known to contain interfering compounds, has made the quantification of this class of analytes difficult. In addition, these methods were not sensitive enough to quantify theses compounds accumulating at very low level (pg to µg/g dry weight). Liquid Chromatography coupled to Tandem Mass Spectrometry under selective reaction monitoring mode has emerged as prevailing method to map several classes of hormones from the same analytical run (Chiwocha et al., 2003). Our group has recently developed an analytical platform for grape berry to quantify five main classes of hormones that are auxin, cytokinin, GAs, BRs and Abscisic Acid (Gouthu et al., 2012). This method will facilitate the analysis of plant hormones during grape berry development to understand their influence on fruit development.

Another issue that has limited our understanding of hormone dynamics during grape berry development is the natural biological variability of a grape cluster. Individual berries are believed to follow similar developmental progression in which hormones follow same dynamics. However, these dynamics may be shifted in time from one berry to another because of the unequal development of a grape berry presumably caused by uneven flowering events at bloom. Traditionally grape berries from a cluster are collected and extracted together, so that an "average" measurement is taken, which eliminates developmental variation within the berries. This lack of appropriate sampling methodology has made it difficult to drawn definite conclusions regarding the actual contribution of hormones to a specific developmental stage. For the past three years, our group has developed a system to overcome this problem by following similarly developed berries from flowering time to harvest. This method is critical in the expected outcomes of the two proposed aims.

In **aim 1**, using the developed analytical method mentioned above and the adapted flower-to-berry tracking system mentioned above, we propose to map the grape berry development phase by plant hormones. Of the five commonly measured (Auxin, ABA, Cytokinins, GA, BRs), we will also quantify two new hormones (Jasmonate and salicylate) to provide a detailed survey of the hormone dynamics during grape berry development. In addition to this hormone analysis, ethylene production using Gas Chromatography fitted with a Flame Ionization Detector will also be mesured. Given the importance of hormones in the integration of changing developmental and environmental cues, we also propose to develop **a second aim** to examine the impact of two common viticultural practices (cluster thinning and leaf removal) on the profiles of hormones during grape berry development. Both cluster thinning and leaf removal have been found to have an important impact on the expression of genes that are controlled by hormones (Pastore et al., 2011; Pastore et al., 2013). To collect meaningful data, a minimum of two years data will be collected.

5.2 Background and preliminary data

The rationale of the proposed research is based upon our preliminary data that indicate that **1)** hormone dynamics are spatially and temporally controlled during critical phases of the fruit development, **2)** quantification of storage forms and/or catabolites of a given hormone informs with respect to its regulation, and **3)** hormone dynamics can differ between developmentally different berry classes coexisting within a cluster.

5.2a Improved analytical method to measure simultaneously several classes of hormones.

To dissect the complex hormonal interaction system during grape berry development, an extraction protocol and a LC-MS based analytical method including simultaneous detection of three classes of hormones (auxin, cytokinin, ABA) were optimized in the PI's lab. Resulting quantifications of the analytes were consistent with the overall trend previously described for these hormones but revealed new patterns not previously reported in grape (Gouthu et al., 2012). Since the publication, two other classes of hormones (Brassinosteroids and Gibberellins) were integrated in the analytical method (data not shown). Dynamics of two major hormones influencing the ripening process in several tissues and developmentally different berry classes will be given as proof of concept to estimate the influence of berry variability on the interpretation of the hormone profiles.

5.2b Spatio-temporal distribution of two ripening-related hormones (auxin and ABA) is critical to understand their influence at the ripening onset.

ABA (abscisic acid) is regarded as the main promoter of berry ripening. Alternative possible pathways for ABA exist, so the examination of synthesis intermediates can be useful in determining which pathway(s) is (are) active in a particular tissue. The regulation of the biological active form of ABA relies on the activity of its catabolism and its modification into a storage form by the addition of a sugar (Nambara et al., 2005). Concentrations of ABA its main catabolite (diphaseic acid [DPA]), and its storage form (ABA-Glucose Ester) were measured during the ripening onset the concentration in skin, pulp, and seed. At mid*véraison*, three berry classes (Green Soft [GS], Pink Soft [PS], and Red Soft [RS]) were measured separately to estimate any potential variation among developmentally different berries (**Figures 1 and 2**). ABA dynamics were found to consistently increase across the three tissues from pre*véraison* (PV) to mid*véraison* stage but its dynamics varies depending on the tissue and among the three mid*véraison* berry classes (**Fig.1**). The increase in ABA from PV to GS stage was found to be mainly consistent with a decrease of its catabolite (DPA). Varying ABA concentrations from green to red berries stage s was found to be inversely correlated to that of ABA-GE, especially in pulp (**Fig.1B**). This last information would not have been observed by the traditional approach of aggregating berries at *véraison*. With respect to auxin, the major form of storage for IAA results from its conjugation with one amino acid (aspartic acid). Like ABA, the regulation of auxin (IAA) concentration is mainly correlated to the level of the storage form (IAA-Asp) but the correlation is tissue specific. In pulp, an increase

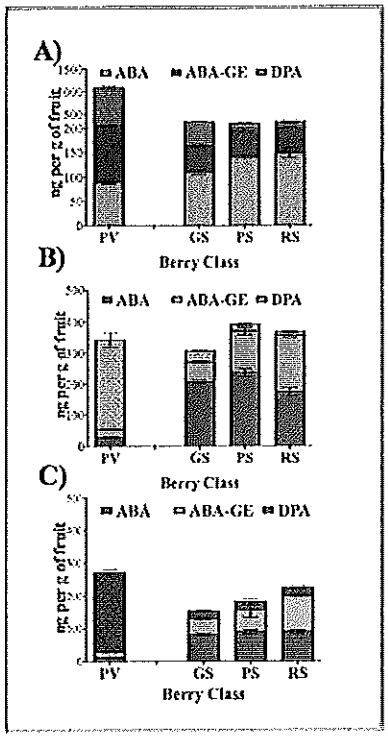

Figure 1: Stage and tissue specific accumulation of ABA-related compounds during the ripening transition (nanogram per gram of fruit). **A)** in Seed, **B)** in Pulp, **C)** in Skin. **ABA**: Abscisic acid; **ABA-GE**: ABA-glucose ester; **DPA**: diphaseic acid; PV: pre*véraison* stage (10 days before mid*véraison*); **GS**: Green Soft stage; **PS**: Pink Soft; **RS**: Red Soft stage. **Remark:** The GS, PS, and RS berry classes were collected at the same time on clusters at mid*véraison* stage. LC-MS/MS quantification were performed using four biological replicates.

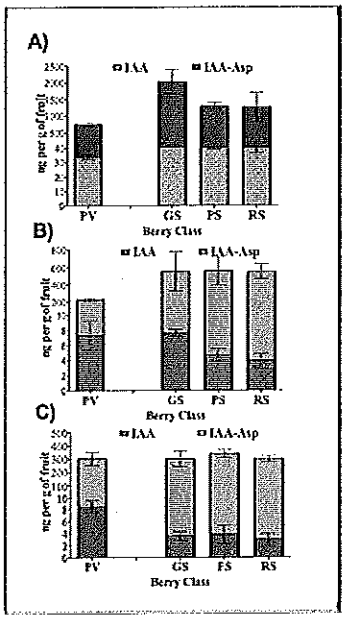

Figure 2: Stage and tissue specific accumulation of IAA-related compounds during the ripening transition (nanogram per gram of fruit). **A)** in Seed, **B)** in Pulp, **C)** in Skin. **ABA**: Abscisic acid; **ABA-GE**: ABA-glucose ester; **DPA**: diphaseic acid; **PV**: pre*véraison* stage (10 days before mid*véraison*); **GS**: Green Soft stage; **PS**: Pink Soft; **RS**: Red Soft stage.

of IAA-Asp during the ripening onset will result in a decrease or a maintain of IAA concentration (**Fig.2B,C**). Overall, this suggests a stage and tissue specific regulation of these two ripening-related hormones during a short developmental window. Similar results were observed for cytokinin and brassinosteroid-related compounds (data not shown). In this context, to reflect the stage-wise linear progression of hormone accumulation there is need to monitor individual berries or berry classes that follow a similar developmental program rather than aggregated berries. Using a berry tracking system (section **6.2**), this issue will be addressed in **aims 1 and 2** and is critical to understand the effects of hormones on grape berry development.

5.2c Inverse dynamics of IAA and ABA at the onset of ripening correlate with the developmental state of grape berry.

To date, there is growing evidence that an increasing ratio [ABA]/[IAA], along with sugar accumulation, is one of the major developmental cues responsible for the ripening transition in grape berry (Bottcher et al., 2010). This ratio was compared at pre*véraison* stage with those observed within GS, PS and RS mid*véraison* berry classes (**Fig.3**). During the ripening transition, we observed a significant increase of this ratio regardless of the type of tissue between the three berry classes. This suggests that the balance between hormones rather than the concentration itself may be more critical to initiate the ripening process. *Through the **aim 1**, by simultaneously these hormones from the same run, the dynamics of these ratios will be compared. This will provide valuable information with respect to their hormone synergistic or antagonistic effects on fruit development.*

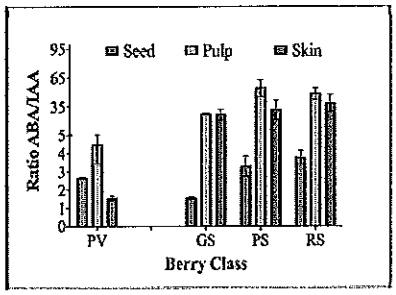

Figure 3: ABA/IAA in seed, pulp and skin during the ripening transition. **PV:** pre*véraison* stage (10 days before mid*véraison*); **GS**: Green Soft stage; **PS**: Pink Soft; **RS**: Red Soft stage. **Remark:** The GS, PS, and RS stages were collected at the same time on clusters at mid*véraison* stage.

5.2.d Hormones mediate environmental and nutrient changes.

Along with a contribution to developmental processes, hormones are known to integrate signal from environmental changes. Water deficit irrigation is a widely used viticulture practice that enhances the accumulation of pigments through a greater accumulation of ABA (Deluc et al., 2009). ABA was also found to accumulate in response to increased temperature on grape berry clusters (Carbonell-Bejerano et al., 2013). Defoliation treatment was found to disrupt the Auxin and ABA signaling at the gene expression level suggesting an alteration of the hormonal content in this particular condition (Pastore et al., 2013). Likewise, changes

in the source/sink relationships , through cluster thinning, results in an extensive reprograming of gene expression during the ripening phase of grape berry (Pastore et al., 2011). In **Aim 2**, we propose to study the changes in hormone dynamics of grape berries growing under two viticulture practices (cluster zone leaf removal and moderate cluster thinning) known to impact the close environment of a cluster and its nutrient status. *The outcome of **Aim 2*** will identify the nature and the dynamics of hormones affected by these practices.

5.2e. Flowering events and seed-to-berry weight ratio determines ripening stages at véraison

To overcome the problem of grape berry variability, we developed a flower tagging system (**Fig.4A**), which allowed us to identify individual berries that develop similarly during the berry development. A correlation between day of flowering and berry ripening states of berries was observed. Early flowering events (day 1 to 4) resulted in more advanced berries at mid*véraison* and late flowering events (from day 8 to 12) showed less advanced berries at mid*véraison* (**Fig.4B**). However, 20% of early flowering events yielded lagging berries at mid*véraison,* which suggests the existence of another controlling factor to determine a ripening stage at *véraison*. The seed-to-berry weight ratio was measured from the same mid*véraison* berries and found to be an appropriate indicator of the ripening states of these berries. Most of the advanced berries (Red soft) at mid*véraison* had an average seed-to-berry ratio ranging from 5 to 6% while pink and green berries were found to have ratio greater than 7 and 10%, respectively (**Fig.4C**). *For the two aims, these two parameters (flowering time and seed-to-berry weight ratios) will be critical to select individual berries following a similar developmental program. This will allow us to overcome the problem of intra-cluster berry variability in the interpretation of the hormone profiles.*

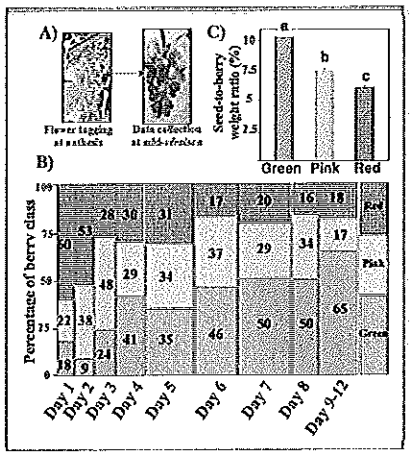

Figure 4: Relationship between flowering time, seed-to-berry weight ratio and grape berry ripening states at mid*véraison* (year 2013). **A)** Procedure of tagging based on color-coded strings relative to the day of flowering; **B)** Contingency table describing the percentage of three berry classes (green, pink and red) per day of flowering in the **x** axis; **C)** Seed-to berry weight ration in percent on each berry class collected at mid*véraison*. Letters in **B)** denote significance difference (Tukey's mean comparison test - α=0.001).

Remark: A subset of 2,500 flowers tagged from 30 Pinot noir clusters was in year 2013. Experiments conducted in 2011 and 2012 resulted in similar trends (data not shown).

6. Procedures to accomplish the objectives.

These preliminary observations indicated that despite of our current knowledge, there is still a need to better delineate the dynamics of hormones at the stage and tissue levels in order to understand their respective roles not only as part of a physiological process, but also in the adaptive responses of grape berry to changing environmental conditions. To achieve this tack, **Aim 1** focuses on the stage- and tissue-specific distribution of eight plant hormones during grape berry development. **Aim 2** investigates changes in the hormone profiles caused by environmental changes (cluster zone leaf removal) and by altered source/sink relationship (moderate cluster thinning).

6.1. Overall management of the vine, viticulture parameters and practices, flower tagging.
The experiment will be conducted at the OSU Woodhall Experimental Station (Alpine, OR) on seven year old Pinot noir vines with North-South orientation (clone Pommard: rootstock 101-14) that are moderately vigorous. Canopy size will be controlled by shoot thinning to 16 shoots per vine that will be vertically shoot positioned. The three sets of vines (n=12 per treatment-control, leaf removed, and cluster thinned) will be under the same irrigation regime. Midday leaf water potential will be monitored across the season in the three sets of vines. Additional thinning of newly formed shoots will be conducted during May and June. With respect to the flower tagging procedure, all the flowers from 60 clusters of 36 vines (12 per treatment) will be tagged using a color-coded strings during the bloom season.

6.2 Monitoring similarly developing berries across the growing season.
Based on our preliminary data, the majority of flowering events per cluster occurred in the middle of the bloom (day 4 to 8) (**Fig.5**). Tagged flowers and resulting berries with mid-flowering events (2 to 3 day range) will be collected at eight times during the growing season (**Fig.5**). Six berries per plant will be sampled per collection day. Once collected berries will be flash frozen in liquid nitrogen and stored at -80°C prior to phenological measurements (seed weight, berry weight, brix, color index). After measurements of the distribution of the seed-to-berry weight ratio among the berries collected, berries having the smallest range of seed-to-berry weight ratio will be identified. This will guarantee the appropriate segregation of similarly developing berries that is required for the hormone profiling.

6.3 Aim 1: Determine the hormone dynamics of grape berry from flowering to harvest.
The approach will be to monitor the tissue and stage specific accumulation of seven hormones during grape berry development using Liquid and Gas Chromatography coupled to Mass Tandem Spectrometry. The control vines used for **Aim 1** will be shaded clusters (no leaf removal in the cluster zone) with two clusters per shoot.

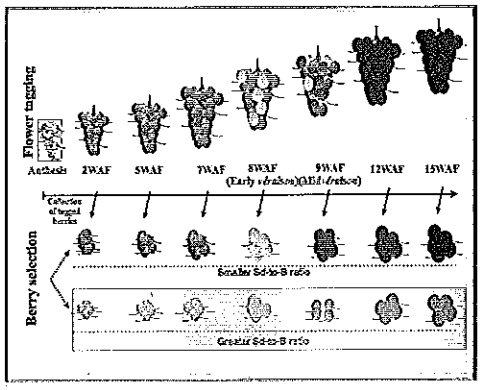

Figure 5: Methodology to monitor developing berries. WAF: weeks after flowering. Remark: After a first screening of berries originating from mid event of flowering seed-to-berry weight (from **WAF5** till harvest) will serve to segregate advanced berries from lagging berries. Grey rectangle represents berries with greater seed-to-berry ratio that will be discarded.

6.2.a Hormone extraction and fractionation.
Fifty milligrams of homogenized and lyophilized berry tissues (skin, pulp, and seed) will be extracted for 18 to 24 hours at 4°C. A solution of internal standards containing deuterated version of each metabolite will be added to the extraction buffer (Gouthu et al., 2012). Several purification and fractionation steps will be conducted to remove interfering compounds (carbohydrate, phenolics, chlorophyll) and to selectively purify the different hormones (Gouthu et al., 2012).

6.2.b Chromatographic conditions and Mass Spectrometry Analysis).
Mass Tandem Spectrometry under Selection Reaction Monitoring mode (positive or negative) will be used to selectively quantify the different classes of plant growth

regulators. Cytokinin, auxin will be identified in the positive mode while ABA, GA, BRs will be identified in the negative mode. Quantification will be expressed in ng of analytes per gram of berry to accurately describe the amount of hormones in a berry.

6.2.c Quantification of ethylene using GC fitted with a Flame Ionization Detector.

For the ethylene quantification, 3 to 5 freshly collected berries per treatment at 5,7,8 and 9 WAF will be tightly enclosed in 50 mL centrifuge tube for 3 hours at 20°C at the research site. Ethylene will be measured using a Gas Chromatograph fitted with a flame ionization detector (Sun et al., 2010).

6.3. Aim 2: Estimate the effects of cluster thinning and leaf removal on the hormone profiles during the ripening phase.

6.3.a Extraction and quantification of the hormones. (See section 6.2.a).

In treated vines, hormone analyses will be performed on basal clusters of four vines.

6.3.b Leaf removal on Pinot noir grapevines and canopy measurement.

A total of five sets of primary clusters per vine (n=12) will be subjected to a leaf removal (100% leaves pulled in the cluster zone) at grain-pea sized stage (BBCH-EL stage 73) and maintained free of leaves during the remainder of the growing season. Two clusters per shoot will be maintained during the growing season. The hormone dynamics during the lag and the ripening phase will be compared between shaded and sun exposed clusters (**Table 1**). To determine difference in sun exposure, the amount of sunlight received in the cluster zone will be measured at solar noon on the clusters of both groups of vines on clear cloudless days at each time collection point using a ceptometer LP80 (Lee and Skinkis, 2013). Berry skin temperature will be monitored from treated and control vines that are not used for the hormone analyses. Berry skin temperature will be monitored in two clusters per vine (n=3) for each treatment group using 24T-type thermocouples positioned in sub-cuticular tissues of the berries. Each probe will be connected to a CRX1000 data logger (Campbell Scientific, USA) from Week 5 to harvest. Correlation between changes in temperature and hormone levels will be evaluated.

Viticultural practice	Control	Sun exposed	Cluster thinned
Leaf Removal	Shaded	Leaf removed	Leaf removed
Cluster Thinning	Two clusters per shoot	Two clusters per shot	One cluster per shoot

Table 1: Description of the different treatments

6.3.c Moderate Cluster thinning on Pinot noir grapevines.

To limit the overall cost of the experiment, sun-exposed treated plants (see **6.3.b**) will be used as control vines in this comparative analysis. Twelve additional vines, with sun-exposed clusters, will be thinned at grain-pea sized stage to one cluster per shoot. Pigment accumulation (Color Index), TSS and titratable acidity will be monitored during the ripening season on both treated and controlled vines to determine the impact of cluster thinning on fruit parameters. Berry diameter will be measured to estimate any berry compensation events in the treated vines. To determine whether cluster thinning has an impact on vegetative growth, primary shoot lengths will be measured on both sets of vines and pruning weight per vine will be measured every winter. Cluster weights will be measured at harvest and yield per vine will be estimated for each treated group.

6.3.d Measurement of flavonoid accumulation in berry skin of treated vines.

To estimate the impact of both viticultural practice on pigment composition, fifty milligrams of homogenized and lyophilized berry skin tissues for each treated group will be used to extract anthocyanins and flavonols from 5 weeks after flowering until harvest (Mattivi et al., 2006).

6.4 Statistical tools.

Statistical analysis of viticulture parameters and hormone quantities will be carried out using the General Linear Model method of JMP statistical package (SAS Institute, USA).

7. Timetable for Project

	2014				2015				2016			
	3rd quarter	4th quarter	1st quarter	2nd quarter	3rd quarter	4th quarter	1st quarter	2nd quarter	3rd quarter	4th quarter	1st quarter	2nd quarter
Berry tagging and collection	X	X			X	X						
Hormone extraction and quantification			X	X			X	X				
Ethylene Analysis	X				X							
Data analyses, outreach and scholarly outputs		X	X	X		X			X	X	X	X

8. Present outlook and estimated Success in accomplishing objective(s)

For the past three years, the PI's team has established expertise in the quantification of plant hormones in grape berries. To date, the group is able to perform accurate quantification on 37 hormone related compounds of five plant growth regulator families (auxin, ABA, cytokinin, GAs, and BRs). The development of a fine-tuned methodology to monitor similarly developing berries will guarantee a clearer picture of the hormone dynamics during important stages of berry development, and in changing environmental conditions. The implementation of an existing method dedicated to the analysis of small molecules and developed at the Mass Spectrometry Facility (http://mass-spec.science.oregonstate.edu) will insure reproducible quantification. In this context, the success to describe authentic profiles of these major regulators during fruit development is very high. This information will also be valuable for industry groups and faculty members conducting hormone trials in the field. Overall, the scope of this proposed research fits with the NGWI research priority "Understanding & Improving Quality" and the OWB Priority "Fruit ripening and Maturity".

9. Outreach and Education

During the three years of the project, results at workshops with grape growers and winemakers. During the first two years, concepts and preliminary results will be presented at the industry and academic conferences of the American Society of Enology and Viticulture and at the "Grape Day" conferences at OSU. After completion of the analyses and interpretation of the data, results will be proposed for oral presentation prior publication at the Oregon Wine Symposium, at the American Society for Enology and Viticulture Annual Meeting, and at the American Society of Plant Biology conference.

10. Budget Support Summary by objective(s):

Aim 1 (Year 1 and Year 2):

Remark: For this aim, only control vines will be used. During Anthesis and WAF2, tissue materials from 4 vines will be treated as one tissue. Separation of the tissue (seed, pulp and skin) will start from week 5 till harvest.

1) Material, Supplies, and fee for using facilities:

- *Quantification of Auxin, Cytokinin, ABA, GA, BRs, JA and SA:*

For the first two time points (Anthesis and Week 2), flower and emerging berries will be treated as a whole and 8 samples (2 time points X 4 replicates) will be run under two acquisition modes. For remaining time points, 72 samples will be run under two acquisition modes (positive and negative) corresponding overall to 55 hours of run at an hourly rate of $135/hour.

Total= 55 X $135 = **$7,425**

The first year, total of **$3,500** is requested for purchasing deuterated internal standards required for the quantification of the hormones.

- *Quantification of ethylene production at the ripening onset (Week 5,7,8,9):*

For the ethylene quantification, 16 samples are expected to be run using GC fitted with FID at $35 per run.

Total = 16 X $35 = **$560**

2) Domestic travel to research sites (All years):

- Travel funds are requested to travel to the research site (Woodhall Experimental Station, Alpine, OR) for tagging and collecting berry samples. OSU motor pool vehicles will be rented on a rental fee of $21.00 and a mileage charge of $0.30/mile. 10 trips are scheduled (MV, MV+1 Week, MV+2 Weeks, MV+3 weeks, MV+4 Weeks, MV + 5 weeks, estimated harvest) for a total of vehicle expense estimated at (400 miles X 0.3) + (10 X $21) = **$330.** Dry ice at the personal cost of the PI will be used to store the samples during the collection. Three tanks of 160L nitrogen tank ($75/tank) will be rented for tissue separation and crushing. The total will be : $75 X 3 = **$225.**

- Travel to attend scientific meetings: All expenses will be covered by the PI's personal funding.

3) Personnel:

One PhD student under the PI's supervision for the hormone profiling will be hired at 0.49 FTE during the three years of the project. Eight undergraduate students will be hired during the bloom season to participate to the flower tagging at an hourly rate of $9.5/hour over an estimated period of 15 days. Averaged hours per day are expected to be 4 hours.

Total = **$4,050**

Aim 2 (Year 1 and Year 2):

Remark: For this aim, vines for moderate cluster thinning and cluster zone leaf removal will be used. As the two treatments in both will be at grain-pea sized stage, only six time points will be considered (Week 5,7, 8, 9,12,15).

1) Material, Supplies, and fee for using facilities:

- *Quantification of Auxin, Cytokinin, ABA, GA, BRs, JA and SA in treated vines:*

144 samples are estimated to be run under two acquisition modes corresponding to a total of 96 hours at an hourly rate of $135/hour.

Total = 96 X 135 = **$12,960.**

- *Quantification of ethylene production at the ripening onset (Week 5,7,8,9):*

For the ethylene quantification, 32 samples (4 time points X 4 replicates X 2 treatments)are expected to be run using GC fitted with FID at $35 per run.

Total = 32 X $35 = **$1,120.**

The research project is being submitted to AVF, CGRRF, CGTC and OWB.

11. Total budget Request

	% time on project	Request 2014-2015	Projected 2015-2016	Projected 2016-2017
Personnel				
Professional				
SRA/Tech*	49	21,663	22,097	22,538
Lab Assistant				
...Other (Undergraduate students)		4,050	4,050	
Employee Benefits**		3,246	3,803	4,221
Supplies and Expenses		25,565 + 225	22065 + 225	
Items and Cost				
Equipement (Itemize when cost >$1000)				
Travel				
Trips/Purpose/Costs		330	330	
Computer Time				
Overhead (where appropriate)				
Indirect Costs**				
TOTAL REQUEST		$55,079	$52,570	$26,759

*: 2% increase for year salary is proposed in the projected year from 2014 to 2017
**: 11% increase in employee benefits is proposed in the projected years (from 2014 to 2017).
** Indirect Costs: Indirect costs cannot be covered by AVF, CRMB, CTGC, CGRIC, CGRRF, or OWB.

12. Literature cited

Bottcher C., Keyzers RA., Boss PK., and C. Davies (2010) Sequestration of auxin by the indole-3-acetic acid-amido synthetase GH3-1 in grape berry (*Vitis vinifera* L.) and the proposed role of auxin conjugation during ripening. *Journal of Experimental Botany* 61(13):3615-3625.

Bottcher C., Harvey K., Forde CG., Boss PK., and C. Davies (2011) Auxin treatment of pre-veraison grape (*Vitis vinifera* L.) berries both delays ripening and increases the synchronicity of sugar accumulation. *Australian Journal of Grape and Wine Research* 17: 1-8.

Bottcher C., Boss PK., and C. Davies (2013a) Increase in cytokinin levels during ripening in developing *Vitis vinifera* cv. Shiraz berries. *American Journal of Enology and Viticulture* 64(4):527-531.

Bottcher C., Harvey KE., Boss PK., and C. Davies (2013b) Ripening of grape berries can be advanced or delayed by reagents that either reduce or increase ethylene levels *Functional Plant Biology* 40:566-581.

Bottcher C., Burbidge CA., Boss PK., and C. Davies (2013c) Interactions between ethylene and auxin crucial to the control of grape (*Vitis vinifera* L.) berry ripening. *BMC Plant Biology* 13:222 doi:10.1186/1471-2229-13-222.

Cantin CM., Fidelibus MW., and CH. Crisosto (2007) Application of abscisic acid (ABA) at version advanced red color development and maintained post harvest quality of 'Crimson Seedless' grapes *Postharvest Biology and Technology* 46:237-241.

Carbonell-Bejerano P., Santa Maria E, Torres-Perez R., Royo C., Lijavetzky D., Bravo G., Aguirreola J., Sanchez-Diaz M., Carmen Antolin M., and JM. Martinez-Zapater (2013) Thermotolerance responses in ripening berries of *Vitis vinifera* L., cv Muscat Hamburg *Plant Cell Physiology* 54 (7):1200-1216.

Chiwocha SD., Abrams SR., Ambrose SJ., Cutler AJ., Loewen M., Ross AR, and AR. Kermode (2003) A method for profiling classes of plant hormones and their metabolites using liquid chromatography-electrospray ionization tandem mass spectrometry: an analysis of hormone regulation of thermodormancy of lettuce (*Lactuca saliva* L.) seeds. *Plant Journal* 35(3):405-417.

Chervin C., El-Kereamy A., Roustan JP., Latché A., Lamon J., and M. Bouzayen. (2004) Ethylene seems required for the berry development and ripening in grape, a non-climacteric fruit. *Plant Science* 167:1301-1305.

Deluc LG., Quilici DR., Decendit A., Grimplet J., Wheatley MD., Schlauch KA., Mérillon JM., Cushman JC., and GR Cramer (2009) Water Deficit alters differentially metabolic

pathways affecting important flavor and quality traits in grape berries of Cabernet Sauvignon and Chardonnay. BMC Genomics 8:10:212 doi: 10.1186/1471-2164-10-212.

Gouthu S., Morre J., Maier CS., and LG. Deluc (2012) An Analytical method to quantify three plant hormone families in grape berry using liquid chromatography and multiple reaction monitoring mass spectrometry. *Springer Sciences in Recent Advances in Phytochemistry* (41):17-35.

Lee J., and PA. Skinkis (2013) Oregon 'Pinot Noir, grape anthocyanin enhancement by early leaf removal. *Food Chemistry* 139:893-901.

Nambara E., and A. Marion-Poll (2005) Abscisic acid biosynthesis and catabolism *Annual Review of Plant Biology* 56:165-185.

Mattivi F., Guzzon R., Vrhovsek U., Stefanini M., and R. Velasco (2006) Metabolite profiling of grape: Flavonols and anthocyanins. *Journal of Agricultural and Food Chemistry* 54(20):7692-7702.

Pastore C., Zenoni S., Fasoli M., Pezzotti M., Tornielli GB., and I. Filipetti (2013) Selective defoliation affects plant growth, fruit transcriptional ripening program and flavonoid metabolism in grapevine. *BMC Plant Biology* 13:30 doi:10.1186/1471-2229-13-30.

Pastore C., Zenoni S., Tornielli GB., Allegro G., Dal Santo S., Valentini G., Intrieri C., Pezzotti M., and I. Filipetti (2011) Increasing the source/sink ratio in *Vitis vinifera* (cv Sangiovese) induces extensive transcriptome reprogramming and modifies berry ripening. *BMC Genomics* 12:631 doi:10.1186/1471-2229-13-30.

Sun L., Zhang M., Ren J., Qi J., Zhang Q., and P. Leng (2010) Reciprocity between abscisic acid and ethylene at the onset of berry ripening and after harvest. *BMC Plant Biology* 10:257