Annual Progress Report 2011-1182

1.Summary:

In 2010, the influence of altered nitrogen (N) content, caused by cover crop, on Pinot Noir berry composition was studied. Treatments included two levels of cover cropping (solid and alternate) and clean cultivation. Berry N-content was reduced by perennial grass cover crop competition and was found to be the most reduced at the early stage of berry formation and at harvest stage. Two growth regulators, *trans*-zeatin and indole-3-acetic acid, and the berry growth were influenced by changes in N-status at different stages of the berry formation. More significant was the improvement of proline to arginine ratio and the assimilable amino acid content of harvest berries in cover crop treated vines compared to clean cultivated. On the other hand, sugar and pigment contents of the same berries were not negatively influenced by cover crop. Although perennial grass covers were found to significantly reduce vine vigor without affecting berry quality it is likely that cover crop treated grapevines may require over time some adjustment in N supply to overcome potential deficiency levels. In this context, timing and intensity of N-supply will require precise indicators of the vine N-status to manage its application ahead in the vineyard. The preliminary data mentioned above defines berry as targeted organ of the N-limitation due to cover crop. Whether these results are important for berry quality purpose, evidence in the literature shows that plant responses to N-supply is mainly regulated through a root to shoot hormone signals suggesting a prominent role of the shoot as sensor of the N-status. Considering that expanding shoot tips remain a largest sink for carbon in grapes and enters in competition with fruits during the berry formation, the integration of shoots to the experimental design this year will provide the industry with a more comprehensive picture of the contribution of the N-limitation to the two major sink organs of grapes through the analytical measurement of regulators determining the plant growth. Hormones but also assimilates contribute to the sink strength of these two organs. The goal of this project is to evaluate the impact of N-limitation, which reduces vine vigor, on the hormone dynamics of expanding shoot and developing berries during the berry formation along with the resulting influence on gene expression and plant physiology. This approach will reveal some insights into the impact of N on vine balance and will clarify, through metabolic, genetic and physiological measurements, the complex relationships between reproductive and vegetative growth during the grape berry formation.
We will address this question through three objectives.

1) Determine the hormone concentration of four classes of hormones (auxin, cytokinin, gibberellic acid, and abscisic acid) from fruit set to *véraison* in expanding shoots and developing berries of grapevines exhibiting two vine vigor levels (high and low).
2) Compare the gene expression of candidates associated with nitrate assimilation and hormone signaling in vegetative and reproductive organs during the berry formation of grapevines having high and low levels of vine vigor.

3) Determine nitrogen status and photosynthetic assimilation in newly expanding leaves and developing fruit throughout the first phase of berry development in high and low vine vigor grapevines.

2. Annual Report from January 2011 to January 2012.

This report is an annual report that describes preliminary works obtained from the research project 2011-967 and extended this year to the research project 2011-1182. The rationale of the transfer of the research site was mainly due to the integration of the shoot data to the project, which required a more controlled experimental design as opposed to a commercial vineyard. Shoot tipping before and during the growing season, which is commonly practiced in a commercial vineyard, could have altered the hormone profiles of berries and may have prevented from shoot samples. Therefore, our first objective this year was to establish the cover crop competition in Woodhall by seeding an aggressive rye grass in the alleyways, which is supposed to enter rapidly into competition with the grapevines. The first expected signs of differential of vine vigor area expected this growing season. In the meantime, because we have not reached yet the two levels of vine vigor at the experimental station, most research efforts this year were focused on optimizing the objectives 1 and 2 by i) testing an extraction protocol for shoot samples (Chiwocha et al., 2003) and ii) integrating gibberellic acids (GAs) to the existing analytical method, iii) designing primers of candidate genes for the real-time PCR experiment for the time course studies.

3. Project title and UGMVE proposal number:

- Effects of vine vigor reduction on the hormone metabolism in expanding shoots and developing berries throughout the first phase of berry development
- 2011-1182.

4. Principal Investigator/Co-Principal Investigators:

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Cooperators: Alex Moeller (Vineyard Manager at the OSU Woodhall experimental Station)

5. Objectives (s) and Experiments Conducted to meet Stated Objectives:

5.1. Determine the hormone dynamics of three classes of hormones (auxin, cytokinins and gibberellic acids) from fruit set to véraison (berry formation phase), in expanding shoots and developing berries from grapevines under high and low level of vine vigor. (Supervisor: Deluc)

The approach to reach this objective will be to quantify the hormone-derived compounds of four hormone families (auxin, cytokinin, GA and ABA) of expanding shoots and developing berries

using Liquid Chromatography-Mass Tandem Spectrometry under Multiple reaction Monitoring Mode. The analytical method was recently optimized at the Mass Spectrometry facilities at OSU and a paper describing data on berry samples was recently accepted (Gouthu et al., 2012).

5.2. Compare the gene expression of candidates associated with nitrate assimilation and hormone signaling in vegetative and reproductive organs during the berry formation of grapevines having high and low levels of vine vigor. (Supervisor: Deluc)

Using the same tissue samples collected for the objective 1, time course studies of candidate genes involved in the plant response to N-status, and in the hormone biosynthesis will be performed. Real Time-Polymerase Chain Reactions will be prepared using an iTaq SYBR Green supermix with ROX dye (BioRad, USA) and will be performed on an ABI PRISM 7500 sequence Detection System at the Center for Genome Research and Biocomputing (OSU).

5.3. Determine nitrogen status and photosynthetic assimilation in newly expanding leaves and developing fruit throughout the first phase of berry development in high and low vine vigor grapevines. (Supervisors: Deluc and Skinkis)

A third split of the berry and shoot tissues samples will be submitted for analysis to a CNS analyzer at the OSU Central Analytical Laboratory to determine the carbon and nitrogen content. In the field, phenological measurements associated with plant growth along with chlorophyll content and photosynthetic assimilation measurements will be performed using a SPAD-502 meter (Konica-Minolta, Ramsey, NJ) and a LICOR 6400 XT (LI-COR Biosciences, USA) respectively.

6. **Summary of Major Research Accomplishments and Results by objective.**

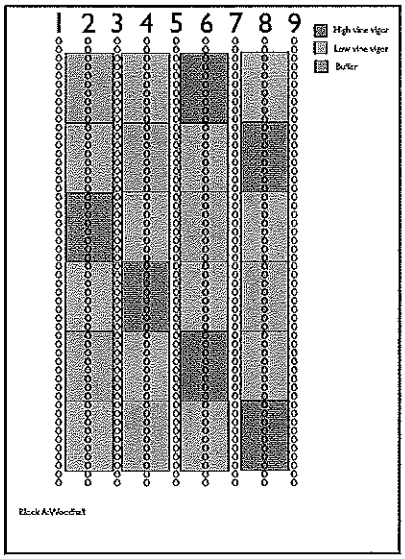

Figure 3: Design of the experimental trial in the 2006 Pinot Noir block A of Woodhall experimental station (OSU).

6.1 Experimental design:

This year, our goal was to establish the cover crop in the block A of the 2006 Pinot Noir at the experimental station (Wodhall, OSU) in a reasonable short window of time to see the effects on plant nitrogen status for the next growing season. An annual rye grass was seeded on the block A at a seeding rate of 51 lbs/acre. Considering that only 50% of N-stored reserves is remobilized the following season, each year N-stored reserves will increase in mature Pinot Noir vines (Schreiner and Scagel, 2006). In this context, we have chosen to work for this project with younger Pinot Noir vines, in which the amount of N-stored reserves from the root, trunk and cane systems is expected not to completely compensate the drastic limitation of N-supply expected to be caused by the cover crop over the time of the experiment. Once the cover established, alleyways of blocks designed high vine vigor will be tilled and will be fertilized with Nitrogen supplies (20 lbs/acre). Treatments will be applied in a

randomized complete block design (**Figure 3**) with 8 vines per each of six replicates.

6.2 Objective 1: Determine the hormone concentration of four classes of hormones (auxin, cytokinin, gibberellic acid, and abscisic acid) from fruit set to *véraison* in expanding shoots and developing berries of grapevines exhibiting two vine vigor levels (high and low) (Deluc).

6.2.1 Reduction of vine vigor, through cover crops, alters the auxin and cytokinin dynamics of berry without affecting berry quality.

In 2010, the altered nitrogen content, caused by cover crop growing in a commercial vineyard, was found to alter the accumulation of the three main hormones during the berry formation (3-indole acetic acid, *trans*-zeatin, and abscisic acid). In conjunction with a natural trend of accumulation across the berry formation phase, the concentration of the three hormones were reduced in the solid cover during the early stages of the berry formation phase (20 to 28 days after bloom) compared to clean cultivated, which corresponds to the developmental period of highest reduction for N berry content (**Figure 1A,B,C,D**), and the highest reduction in berry diameter and weight in solid treated vines. On the other hand, the three hormones were found to be at similar or greater concentration in solid treated plants compared to clean cultivated plants before *véraison* (56 days after bloom) whereas N content was still reduced in solid (**Figure 1D**). This results may suggest a complex relationship between N berry status and the hormones. It is important to note that the quantification of catabolites and storage forms of cytokinins (isopentenyl adenine [iP], ipentenyladenosine [iPR], dihydrozeatin [dZ], dihydrozeatin riboside [dZR]), auxin (IAA-Aspartate, IAA-Alanine, IAA-Methylester) and Abscisic acids (ABA-GE, phaseic acid, dehydrophaseic acid, ABA glucosyl ester,

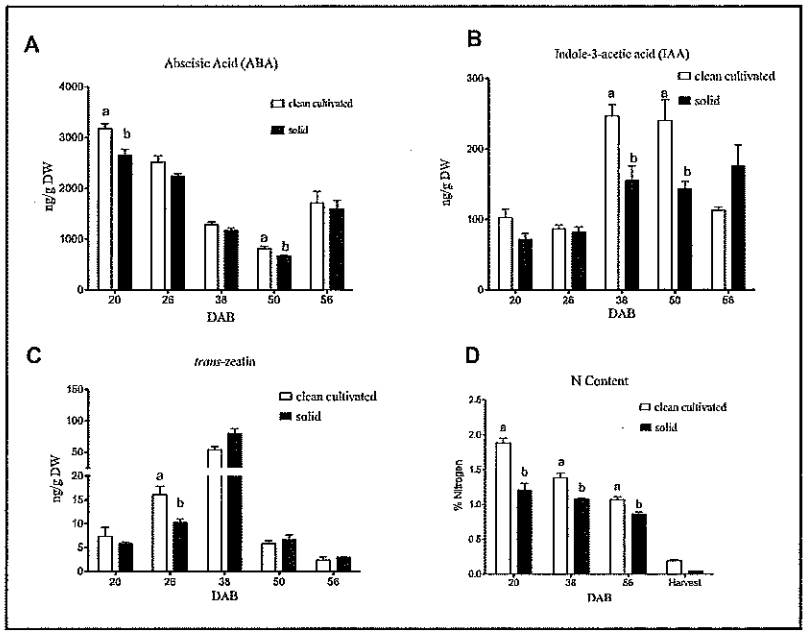

Figure 1: Concentration of three active forms of hormones in two regimes of vineyard floor managments (clean cultivated and solid) during the berry formation phase. A) abscisic acid, B) 3-indole-acetic acid, C) trans-zeatin. Clean cultivated - no cover crop; Solid - grass cover crop in both alleyways; DAB - Days after Bloom; a, b values indicated by different letters (a, b) are significantly different (Tukey's HSD).

Pigment	Clean cultivated	Solid
Delphinidin	35.5 ± 6.4	30.2 ± 9.1
Cyanidin	20.0 ± 2.4	23.8 ± 3.2
Petunidin	7.3 ± 1.7	7.9 ± 2.8
Peonidin	16.1 ± 1.4	21.0 ± 2.9
Malvidin	1,130 ± 181	1,265 ± 261

Table 1: Mean (± standard deviation) of anthocyanidin-3-O-monoglucosides in Pinot Noir berry skin at harvest from vines under low vigor (solid) and high vine vigor (clean cultivated).

7'hydroxyABA, neophaseic acid) did not reveal a clear trend regarding the effects of cover crop on their respective accumulation. This suggests that the alteration of the concentration observed for the active forms mentioned above are mainly due to either a *de novo* synthesis or an enhanced transport to the berry from another part the plants. Sugar and pigment accumulations (**Table 1**) were not affected by cover crop. On the other hand, the amino acid composition at harvest was found to be 40% lower in solid compared to clean cultivated but the content of assimilable amino acids as percentage of total amino acids was higher in berries from cover crop treated vines (**Figure 2**). Indeed, the proline to arginine ratio was reduced from 1.31 to 0.65 in solid treated vines, which explains the increase in assimilable amino acids. This result is of significant importance for enology purpose and must need to be confirmed this year.

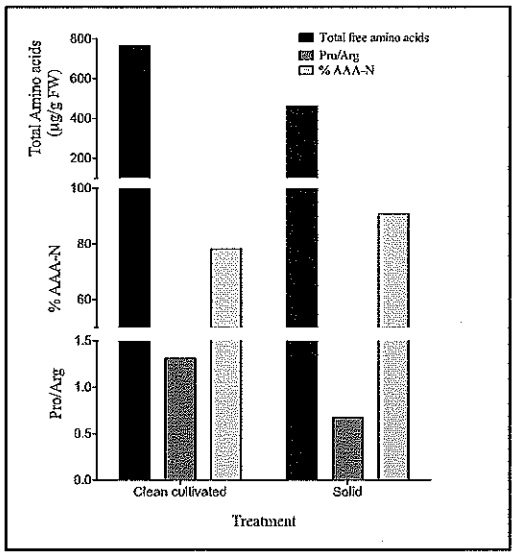

Figure 2: Comparison of total free amino acid content, percentage of assimilable amino acids, and proline to arginine ratio of the berries

6.2.2. Integration of GAs in the analytical method and recovery of analytes using an adapted extraction protocol for shoot tissues.

As previously mentioned in the report, shoot samples collected this year were used to validate a new extraction protocol for green tissues. Prior the assessment of the extraction protocol, each single GA analytes was run through the Mass Spectrometry instrumentation to identify the molecular transition that serves as molecular signature for each GA analytes (**Table 2**). An extraction protocol used for lettuce was used to extract the four classes of hormones from shoot samples collected the last growing season. The extraction protocol adapted from Chiwocha et al. 2003. The recovery of GAs, which are expected to be the lowest concentrated hormone in the tissues among the four classes quantified ranges from 16% to 74% depending on the analytes.

The four bioactive forms had from 43% to 73% of recovery from our extraction samples, which is consistent with data in literature (Chiwocha et al., 2003, Kojima et al., 2009). Same calculation for IAA, cytokinin and ABA-derived compounds ranges from 36% to 82% of recovery.

6.3 Objective 2: Quantify by real-time PCR experiments the expression of genes associated with nitrogen assimilation in both organs, response to N-status and hormone biosynthesis (Deluc).

RNA from shoot samples and berry samples were extracted this year using an extraction protocol optimized in the PI's laboratory. Reverse transcriptase were performed using to synthesize a first strand complementary that will be used as template for the real time PCR. Genetic sequences for each annotated candidate gene were retrieved through the grape genome browser to design the primers for PCR amplification (http://gbrowse.cribi.unipd.it/private/gbrowse/vitis_vinifera/). The design of the primers was performed using Primer3 software by following the default parameters of the software except for the length of the PCR product that was assigned to a maximum of 150 base pair (http://frodo.wi.mit.edu/). Efficiency of the primers for PCR amplification is currently assessed through semi-quantitive Reverse transcriptase PCR reactions.

Analyte	Molecular Mass at the quadrupole 1	Molecular Mass at the quadrupole 2*
GA_1	349.135	185.1, 157.1
GA_3	345.3	221.1, 227.1,
GA_4	331.3	213.1, 257
GA_7	329.101	223.1, 211
GA_8	363.1	275.1, 257.1
GA_9	315.108	271.1, 253.1
GA_{19}	361.104	317, 273.1
GA_{20}	331.131	287.1, 225.1
GA_{24}	345.135	283.2, 213
GA_{29}	347.093	303.1, 241

Table 2: Acquisition of the transition for each GA member. In green: bioactive forms, in orange: catabolytes, *: The molecular mass at the third quadrupole can be represented by different fragments of the molecule.

6.4 Objective 3: Determine the nitrogen status and photosynthetic carbon assimilation in expanding shoots and developing berries during the berry formation in high and moderate vine vigor grapevines. (Deluc and Skinkis)

Last year, N status in berry samples was estimated from high (clean cultivated) and low (Solid) vine vigor grapevines (Stoller commercial Vineyard). The measurements, performed during the berry formation and at harvest, revealed significant differences with a lower nitrogen content in lower vine vigor grapevines, which is consistent with several parameters of plant growth including post-harvest biomass, shoot growth suggesting that shoots are also affected by reduction of nitrogen. The Co-PI Skinkis was recently granted for a LICOR 6400 instrument, which will be used for the photosynthetic measurements that will complement traditional plant growth measurements. SPAD measurements, pruning weight, shoot lengths will be performed. Once the cover grass will be set up, amino acid composition

will be monitored as previously described.

7. Outside Presentations of Research

During the 3-year study, the trial site will be used in workshops with grape growers and winemakers to showcase findings of the project. After completion of analyses and interpretation of data, results will be submitted for peer-review with the intention of publishing in specialized journals like the *American Journal of Enology and Viticulture* or those with a broader impact, like the *Journal of Experimental Botany*. This research will be presented before publication at the Oregon Wine Research Viticulture & Enology Colloquium in February 2014 and eventually at the American Society for Enology and Viticulture Annual Meeting in June 2014.

8. Research Success Statements

The study will provide growers with additional metrics to monitor the reduction of vine vigor in the cool climate Willamette Valley of Oregon. This research can help them better understand how these new metrics can be further exploited to follow the vine balance and how this vine balance is adjusted through hormone metabolism. The analytical measurements of the main hormones responsible for plant and fruit growth will contribute to better understand the complex relationships between fruit and shoot under reduced vine vigor condition and the impact on fruit quality. The time course studies of candidate genes will provide the growers with new field genetic markers that will be proposed as molecular tools in further project (loop-mediated isothermal PCR or LAMP-PCR). Finally, results at this stage of the project show that the reduction of the vine vigor, through cover crop, results in a modification of the berry amino acid composition in favor of a higher assimilation of amino acids for yeast assimilation, which is desirable for enological purpose. The confirmation of this outcome the following years of the trial will position the permanent grass cover crop management as desirable practice to reduce or to maintain low vine vigor and to improve fruit and wine quality.

9. Fund Status

33% of a research assistant salary + OPE were covered by this grant. $708 was spent in renting a 160 L nitrogen tank used to crush the shoot and berry samples. The salary of an undergraduate during the growing season was spent for the data collection and tissue sampling. $660 were spent for trips to Woodhall for data collection and fruit samplings. $2,000 in gas supplies, chemical, column purchase and kit for PCR were spent. $2,000 were spent in the hormone analyses including run of the instrumentation at the Mass Spectrometry Facilities and the purchase of some deuterated internal standard + purchase of Solid Phase Extraction columns (HLB and MCX).

10. Literature cited

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

Gouthu S, Morre J, Maier CS, Deluc LG. (2012). (2012a) An analytical method to quantify three plant hormone-families in grape berry using Liquid Chromatography and Multiple Reaction Monitoring Mass Spectrometry. Book Chapter in Recent Advances in Phytochemistry.

Kojima M, Kamada-Nobusada T, Komatsu H, Takei K, Kuroha T, Mizutani M, Ashikari M, Ueguchi-Tanaka M, Matsuoka M, Suzuki K, Sakakibara H (2009) Highly Sensitive and High-Throughput Analysis of Plant Hormones Using MS-Probe Modification and Liquid Chromatography-Tandem Mass Spectrometry: An Application for Hormone Profiling in Oryza sativa. Plant Cell Physiology **50:** 1201-1214

Schreiner RP, Scagel CF, Baham J. (2006). Nutrient uptake and distribution in a mature'Pinot Noir' vineyard. HortScience 41(2):336-345.