OWB Final Report 2015

I. PROJECT TITLE: Understanding Pinot noir grape and wine aroma composition as a result of changes in vine balance

II. PRINCIPAL INVESTIGATOR:

Michael Qian, Professor of flavor chemistry, Department of Food Science and Technology, Oregon State University, Corvallis, OR 97331. Phone: 541-737-9114, fax: 541-737-1877, email: Michael.qian@oregonstate.edu

Patricia A. Skinkis, Ph.D., Assistant Professor & Viticulture Extension Specialist, Oregon State University, Oregon Wine Research Institute, Department of Horticulture, 4017 ALS Bldg., Corvallis, OR 97331-7304; ph: 541-737-1411, fax: 541-737-3479, email: skinkisp@hort.oregonstate.edu

Graduate Students: Fang Yuan

Cooperators:
Rob Schultz, Vineyard Manager, Stoller Family Estate Vineyards, 15151 NE Stoller Road, Dayton, OR 97114. ph: 503-583-1794, email: Robert.Schultz@StollerFamily Estate.com. Role: Rob will maintain vineyard plots for experiments.

III. SUMMARY

Canopy management and crop thinning are widely accepted practices in wine grape production worldwide. Studies conducted on canopy management and crop thinning in the past 30 years or more have been instrumental in determining the canopy leaf area needed to ripen fruit adequately in warmer production regions in California (Kliewer and Dokoozlian 2005). Because of differences in growing regions and cultivars, ranges of optimized crop load (reproductive growth to vegetative growth or vine balance) need to be carefully evaluated. Although there have been many researches reported crop levels and fruit quality over the past several decades, many of those studies have yielded conflicting results. While some studies have demonstrated crop thinning increases soluble solids (Reynolds, Edwards et al. 1994, Kliewer and Dokoozlian 2005, Kurtural, Dami et al. 2006), anthocyanins (Guidoni, Beaurepaire et al. 2002)(Reynolds, Yerle et al. 1996), and polyphenolic compounds (Prajitna, Dami et al. 2007), some other studies reveal crop thinning has little or no effect on fruit composition (Bravdo, Hepner et al. 1985, Keller, Mills et al. 2005). In addition, most of the researches focus on phenolics, very few studies have reported the crop load on wine flavor.

The relationship of yield with grape composition and wine quality is complicated (Naor, Gal et al. 2002, Chapman, Matthews et al. 2004)(Ough and Nagaoka 1984, Keller, Mills et al. 2005, Reynolds, Schlosser et al. 2007). Sensory implication of yield seems to be dependent on variety, level of crop thinning, and timing of thinning (Diago, Vilanova et al. 2010). Besides crop load, vine vigor also has great impact on fruit quality. A favorable balance between grapevine vegetative and reproductive growth is important in determining fruit and wine quality. The reduced vine vegetative vigor by cover crop treatment has been reported to improve grape quality through reducing berry titratable acidity and increasing the levels of soluble sugar, total

phenol and anthocyanin, therefore enhancing wine color and sensory properties (Tesic, Keller et al. 2007, Celette, Findeling et al. 2009).

The influence of vine balance on wine composition was investigated in three years. Forty compounds were quantified in the wine samples. Principle component analysis (PCA) was performed on the volatile profile of wines. The results showed that different level of vine vigor lead to a very different wine volatile profile in year 2011 and 2012, as low vigor treatment and high vigor treatment of year 2011 and 2012 were well separated on the plot. For the wine of 2011 and 2012, wine from lower vigor vines were closely associated with branched-chain esters such as ethyl isobutyrate, ethyl isovalerate, phenethyl acetate and isoamyl acetate as well as some branched-chain alcohols such as isoamyl alcohol and phenethyl alcohol. However, for year 2013, the impact of vine vigor and crop thinning on the wine volatile profile was limited.

Since C_{13}-norisoprenoids are very important grape derived compounds that contribute to the berry, tobacco, honey, and violet aroma in wine, both the free C_{13}-norisoprenoids and the bound forms were analyzed. Only vine vigor showed impact on the C_{13}-norisoprenoids in grape berries while yield showed very limited impact. Grape berries from high vigor vines consistently have higher total free form C_{13}-norisoprenoids than the grapes from lower vigor vines. However, for the hydrolytically released C_{13}-norisoprenoids, the difference between high vigor and low vigor were not significant. The impact on individual C13-norisprenoid was vintage dependent, and needs further research.

Influence of vine balance on C_{13}-norisoprenoids precursors-carotenoid in grape berries was further studied. Carotenoids analysis showed that β-carotene and neochrome b continuously decreased after véraison, However, the degradations of other carotenoids were not obvious. Our data demonstrated that vine vigor had a greater influence than cluster thinning on carotenoids composition in grapes. Low vigor vines consistently have higher β-carotene and lutein content than high vigor vines regardless the crop load level. The relationship between individual carotenoid and ripening days, vigor level and yield level was investigated by multivariate analysis through linear regression. The results showed that all the carotenoids were significantly correlated with ripening days except for lutein. Neochrome b, lutein and β-carotene were significantly correlated to vine vigor level ($p<0.001$). Neochrome a and β-carotene also correlated to yield level with p value of 0.001 and 0.026 respectively. The result also showed some interactions between time, vigor and yield factors. However, very little has been published regarding carotenoids in wine grapes, weather, sunlight, temperature, nutrient may all affect carotenoid biosynthesis and degradation, much more studies are needed from the whole scientific community.

IV. OBJECTIVES AND EXPERIMENTS CONDUCTED TO MEET STATED OBJECTIVES:

Grape growers will benefit financially from high yields. Unfortunately, as yields go up, quality of the grapes and wine made from them may goes down. Therefore it is of great interest to manipulate the canopy for both high yield and quality. However, the link of vine vigor, crop thinning and fruit quality is complex and not well understood. The aim of this study is to investigate the influence of different vine vigor and crop level on fruit and wine aroma composition.

Chemicals

Standards of the volatile compounds were purchased from commercial sources: Sigma-Aldrich (Milwaukee, WI), TCI America (Portland, OR), K & K Laboratories (Jamaica, NY), Alfa Aesar (Ward Hill, MA), Firmenich (Princeton, NJ), and J & T Baker (Phillipsburg, NJ), with purity higher than 98% in all case. GC grade of methanol was obtained from EMD (Gibbstown, NJ) and ethanol was purchased from Aaper Alcohol and Chemical Co. (Shelbyville, KY). Tartaric acid was purchased from Mallinckrodt Inc. (Paris, KY). A synthetic wine solution was made by dissolving 3.5 g of L-tartaric acid in 1 L of 12% ethanol solution, and adjusting pH to 3.5 with 1 M NaOH.

Grape samples

Commercial vineyard blocks with high, moderate, and moderate-high vine vegetative vigor (achieved by different cover crop management) were used. The vines of varying vine vigor were spatially replicated in a completely randomized design. There were five replicates of each treatment with each replicate consisting of 16 vines. The vines were Pinot Noir (clone 115 grafted to 101-14), planted in 1998 and trained to a bilateral VSP system. Two crop levels were imposed within each main plot, including a non-thinned treatment (full crop) applied to 8 vines and 1 cluster/shoot (~half crop) in the other 8 vines. Fruits were thinned during the BB size stage of berry development. Grape samples from 2011, 2012 were stored at -80°C until analysis. Fruit collected for analysis at harvest in 2013 were sampled from a set of 4 vines per each treatment replicate in the field, beginning at véraison and continuing weekly until harvest. Four clusters of grapes were collected from each treatment every week. The phenology of grapes at different sampling time was shown in Table 3. Wines from this trial were produced from year 2011, 2012 and 2013 by the Stoller Family Estate staff.

In this study, we didn't include the middle vigor level treatments, and thus there were four different treatments: GCON, grass between vine rows (lower vigor) and full crop (no crop thinning occurred, higher yield); TCON, tilled between vine rows (higher vigor) and full crop (no crop thinning occurred, higher yield); GMT, grass between vine rows (lower vigor) and moderate thinning (lower yield); TMT, tilled between vine rows (higher vigor) and moderate thinning (lower yield).

Quantitative analysis of grape berries

Total soluble solids (TSS)

Approximately 50 gram of fresh grape berries were placed in a clean zip-lock bag and squeezed manually to collect the juice. TSS (°Brix) was measured at room temperature using a PAL-1 pocket refractometer (Atago USA, Inc., Bellevue, WA).

Major volatile analysis in grapes

A 50/30 μm DVB/CAR/PDMS fiber (Supelco Inc., Bellefonte, PA) was used for volatile extraction. Approximately 30 g of grape berries were blended with liquid nitrogen, and 1 g berry

powder was weighed into a 20 mL autosampler vial. Four mL of citric acid/saturate saline buffer (0.2 M, pH 3.2) and 20 μL of internal standard (109 mg/L of 4-octanol) were added. The sample was equilibrated at 50 °C in a thermostatic bath for 15 min and extracted for 30 min at the same temperature under stirring (500 rpm). After extraction, the fiber was inserted into the injection port of GC (250 °C) to desorb the analytes. The extraction and injection were conducted by GERSTEL MPS autosampler (Linthicum, MD).

An Agilent 6890 gas chromatograph equipped with an Agilent 5973 mass selective detector (Agilent, Santa Clara, CA) was used. Compound separation was achieved with a ZB-WAX column (30 m × 0.25 mm i.d., 0.5 μm film thickness, Phenomenex, Torrance, CA). A constant helium column flow rate of 2.5 mL/min was used. The chromatographic program was set at 35 °C for 4 min, raised to 150 °C at 20 °C/min, and then raised to 230 °C at 4 °C/min, hold for 10 min. MS transfer line and ion source temperatures were 280 and 230 °C, respectively. Electron ionization mass spectrometric data from *m/z* 35~350 were collected using a scan model with an ionization voltage of 70 eV.

Hydrolytically liberated C_{13}-norisoprenoids in grapes

One gram of powdered berry was diluted with 4 mL of citric acid/saturate saline buffer (0.2 M, pH 2.5) in 20 mL autosampler vial. And the vial was tightly capped and kept in water bath (99 °C) for 1 hour. After cooling down to room temperature in cold water, 20μl of internal standard was added. After acid hydrolysis, the samples were followed same SPME extraction and MS detection procedures as described above.

Carotenoid Analysis in grapes

Since C13-norisoprenoids were generated from carotenoids, we developed analytical method to quantitate carotenoid concentration in the grapes. Extraction of carotenoids was performed following published literature with some modifications (Mendes-Pinto, Ferreira, Caris-Veyrat, & de Pinho, 2005; Oliveira, Barbosa, Ferreira, Guerra, & Guedes, 2006; Oliveira, Ferreira, Pinto, Hogg, Alves, & de Pinho, 2003). Approximately 100 g of fresh berries were blend with liquid nitrogen. Thirty gram of homogenized sample was spiked with 100 μL of internal standard (β-apo-8'-carotenal). Extraction was carried out with 100 mL of ethyl acetate contain 0.1% BHT, agitating for 30 min. After centrifuge at 1500 rpm for 5 min, the resulting upper layer was collected. Then, the extraction procedure was repeated once by 50 mL of ethyl acetate contains 0.1% BHT. The final combined upper layer extracts were concentrated to dryness at 30°C (rotavapor, BÜCHI R205, Labortechnik AG), resuspended in 1 mL of acetone/hexane (1:1, v/v, w/0.1% BHT) and centrifuged at 11000 rpm for 5min. Clear extract were injected to HPLC. Each sample was extracted in triplicates. Sample handling, homogenization, and extraction were carried out under dim light and kept cold to minimize light-induced isomerization and oxidation of carotenoids.

The identification of carotenoids was conducted on a Hewlett-Packard 1090 series HPLC with a model 1090 series diode array detector and HP Chemstation software (Hewlett-Packard Inc, Palo Alto, CA) with a Prodigy C18 column (100 Å, 5 μm, 250×4.6 mm, Phenomenex); The eluents were 100% ethyl acetate (solvent A) and 90% acetonitrile in milli-Q water (v/v) (solvent B).

Total flow rate was 1 mL/min. The following binary gradient system was employed: 0-1 min (100% B); 1-30 min (to 40% B); 31-40 min (40% B); 41-45 min (to 0% B); 46-55 min (0% B). Diode array detection was between 300 and 600 nm. Sample injection volume was 20 μL, and absorbance was recorded at 447 nm.

Positions of absorption maxima (λ_{max}) were used for qualitative analysis. β-carotene was identified by comparison with retention time and UV spectra of commercial β-carotene standard (95% purity, Sigma, St. Louis, MO). Identification of the other carotenoids was performed by comparison retention time and UV visible photodiode array spectra (Mendes-Pinto, Ferreira, Caris-Veyrat, & de Pinho, 2005; Oliveira, Ferreira, Costa, Guerra, & de Pinho, 2004; Oliveira, Ferreira, Pinto, Hogg, Alves, & de Pinho, 2003). All the compounds were run in triplicate and calculated as β-carotene equivalent. The concentration of carotenoids was determined on a Shimazu HPLC system.

Quantitative analysis of aroma compounds in wine

(a) Major Compounds (HS-SPME-GC-MS)

Two mL of wine was diluted with 8 mL of citrate/saturated salt buffer (pH 3.5). The diluted samples were spiked with internal standard (96 mg/L of 3-heptanone, 109 mg/L of 4-octanol) and extracted with DVB/CAR/PDMS fiber (Supelco, Bellefonte, PA). For analysis of hydrolytically-released compounds, 2 mL of wine were diluted with 8 mL of citrate/saturated salt buffer (pH 2.5) and heated to 100 °C for 1 h. After hydrolysis, 20μL of internal standard solution (96 mg/L of 3-heptanone, 109 mg/L of 4-octanol) was added. The GC was an HP 6890 series gas chromatograph with automatic sampler. The column was a ZB-Wax from Phenomenex (30 m × 0.32 mm i.d., and 0.5 μm film thickness). The carrier gas was helium at flow rate of 1 mL/min and the injection was performed in the splitless mode. Injector and detector were held at 250 °C. The column initial temperature was 35 °C, which was held for 4 min and then raised to 230 °C at 5 °C/min, and held at 230 °C for 10 min. MS transfer line and ion source temperature were 280 and 230 °C, respectively. Electron ionization mass spectrometric data from m/z 35~350 were collected using a scan model with an ionization voltage of 70 eV. Compound identification was achieved by comparing mass spectral data from the Wiley 275.L (G1035) database (Agilent Technologies, Santa Clara, CA).

Volatile compounds were identified by compare their mass spectrum with the Wiley-275 library and the authentic standards. Standard calibration curves were developed using pure chemical standards in synthetic wine matrix for wine analysis, and calculated through Chemstation software (Agilent Technologies, Santa Clara, CA).

(b) Highly volatile compounds (HS-GC-FID)

Since the concentrations of acetaldehyde, ethyl acetate, isoamyl acetate, propanol, isobutyl alcohol and isoamyl alcohol in wine were relatively high, they cannot be reliably quantified by SPME method. Therefore HS-GC-FID method was used for the quantification of these compounds. Half mL of wine and 0.5 mL of mili-Q water was pipetted into a 20-mL auto-sampler vial and tightly capped with Teflon-faced silicone septa. An aliquot 20 μl of the internal standard working solution (1 mg/L methyl propionate) were added to each vial. Headspace Auto-sampler was equipped with 1.0 mL syringe and the syringe temperature was kept at 70 °C.

Before injection, samples were incubated at 70 °C for 15 min with 250 rpm agitation. The sample volume was 0.5 mL and injection mode was split mode. Split ratio was 1:10. A J&W DB-WAX (30m X 0.25mm, 0.5 μm) column was used and the initial oven temperature was 35°C, hold for 4 minutes, then 10 °C / min to 150°C, hold for 5 minutes. Carrier gas Flow was 2mL/min. Inlet temperature was 200°C and detector temperature was 250°C.

Statistical analysis

Volatile compounds that significantly changed ($p < 0.05$) during berry development were determined using one-way analysis of variance (ANOVA) with post hypothesis of Tukey's HSD. ANOVA, Multivariate tests (MANOVA) were carried out using SPSS 20.0 (IBM, Armonk, NY, USA). Principal component analysis (PCA) was carried out using Canoco 4.5 (Microcomputer Power, NY, USA).

V. SUMMARY OF MAJOR RESEARCH ACCOMPLISHMENTS AND RESULTS:

Influence of vine balance on wine composition in three years

The quantification results of volatile composition of wine samples are showed in Table 1. Forty compounds were quantified in the wine samples, including 13 esters, 14 alcohols, 5 terpenes, 4 C13-norisoprenoids, 1 aldehyde and 3 volatile acids. Among these compounds, C_{13}-norisoprenoids are very important grape derived compounds that contribute to the berry, tobacco, honey, and violet aroma in wine. C_{13}-norisoprenoids presented in wine as both free and bound form, the bound forms could be released by slow acid hydrolysis during aging. So in this study, in addition to the analysis of free form, acid hydrolysis was also performed to estimate the bound form C_{13}-norisoprenoids in the wines (Table 2).

In order to get a general idea of the impact of different treatments on wine volatile composition during three years, principle component analysis (PCA) was performed based on the volatile profile of wines. The two-dimensional loadings and score plots (bi-plots) for the variables and objects derived from the standardized data set describing the different characteristics of the wine volatiles from different treatments. When all the volatile compounds were included, the two principal components accounted for more than 99% of the total variance in the data. Variables 2011TCON (high vigor-full crop load), 2012TCON, 2011TMT (high vigor-medium crop thinning) and 2012TMT were clustered together on positive axis of dimension 2 of the loading plot. While variables 2011GCON (low vigor-full crop load), 2012GCON, 2011GMT (low vigor-medium crop thinning) and 2012GMT were clustered together on left of negative axis of dimension 2. Variables 2013GCON, 2013GMT, 2013TCON and 2013TMT were clustered together on right side of negative axis of dimension 2. The results showed that different level of vine vigor lead to a very different wine volatile profile in year 2011 and 2012, as "G"(low vigor) treatment and "T" (high vigor) treatment of year 2011 and 2012 were well separated on the plot. However, for year 2013, the impact of treatment on the wine volatile profile was limited. For the wine of 2011 and 2012, wine from lower vigor vines were closely associated with branched-chain esters such as ethyl isobutyrate, ethyl isovalerate, phenethyl acetate and isoamyl acetate as well as some branched-chain alcohols such as isoamyl alcohol and phenethyl alcohol. But for year 2013, the impact of different level of vine vigor and yield was very small, which was very likely associated with the weather. Further works are needed to link the weather and field data with the flavor results.

Influence of vine balance on carotenoid degradation and C_{13}-norisoprenoids synthesis in grape berries

Previous study showed that some of C_{13}-norisoprenoids with trace amount in grape berries could play important roles in the nonfloral grape varieties (Fang and Qian 2006, Loscos, Hernandez-Orte et al. 2007, Crupi, Coletta et al. 2010). The flavorless precursors of C_{13}-norisoprenoids include both glycoconjugates and non-glycosidic compounds (carotenoids and its degradation products) (Winterhalter, Sefton et al. 1990). These precursors could transform to free volatile compounds in wine by enzyme hydrolysis, slow acid hydrolysis and complex chemical rearrangements (Strauss, Dimitriadis et al. 1986, Humpf, Winterhalter et al. 1991, Baumes, Wirth et al. 2002). There is also strong evidence that formation of C_{13}-norisoprenoids in grape

42 berry is corresponding to the carotenoid breakdown (Salinas, Zalacain et al. 2004, Coelho, Rocha
43 et al. 2006).

44 In this study, we specifically monitored the carotenoid degradation and C_{13}-norisoprenoid
45 synthesis during berry development with different vine balance during the grow season of year
46 2013. The phenology of grapes at different sampling time was listed in Table 3. Brix, pH and
47 berry weight was also measured (Table 4). Carotenoids analysis results showed that β-carotene
48 and neochrome b continuously decreased after véraison (Table 5). However, the degradations of
49 other carotenoids were not obvious. Previous results showed that the degradations of these
50 carotenoids occurred mainly before véraison and the concentration after véraison were relatively
51 consistent.

52 Our data demonstrated that vine vigor regulated through cover crop management had a greater
53 influence than cluster thinning on carotenoids composition in grapes. The most abundant
54 carotenoids in Pinot noir grapes were β-carotene and lutein. Our result showed that low vigor (G)
55 vines consistently have higher β-carotene and lutein content than high vigor (T) vines regardless
56 the crop load level (Table 6 and Figure 2). The relationship between individual carotenoid and
57 ripening days, vigor level and yield level was investigated by multivariate analysis through linear
58 regression. The results (Table 7) showed that all the carotenoids were significantly correlated
59 with ripening days except for lutein. Neochrome b, lutein and β-carotene were significantly
60 correlated to vine vigor level ($p<0.001$). Neochrome a and β-carotene also correlated to yield
61 level with p value of 0.001 and 0.026 respectively. The result also showed some interactions
62 between time, vigor and yield factors. Previously, field data from this trial showed that the G
63 vines have fewer canopies and more sunlight infiltration than the T vines, which might influence
64 the carotenoid biosynthesis. However, very little has been published regarding carotenoids in
65 wine grapes, and there is no report on Pinot noir grapes. Since the planted grass could have
66 nitrogen competition with grapevines, the nitrogen level may affect carotenoid biosynthesis and
67 degradation, much more studies are needed from the whole scientific community. Other minor
68 carotenoid showed more complicated change during ripening.

69 As the breakdown product of carotenoids, free form C_{13}-norisoprenoids in grape berries showed
70 very similar trend with carotenoids. Figure 3 showed total C_{13}-norisoprenoids change during
71 berry development. Only different level of vigor showed impact on the C_{13}-norisoprenoids in
72 grape berries while different level of yield showed very limited impact. Grape berries from high
73 vigor vines consistently have higher total free form C_{13}-norisoprenoids than the grapes from
74 lower vigor vines. However, for the hydrolytically released C_{13}-norisoprenoids, the difference
75 between high vigor and low vigor were not significant. These results were also corresponding to
76 the higher concentration of C_{13}-norisoprenoids in wine from G vines than T vines in year 2013
77 (Table 1 & 2).

78 Table 8 showed multivariate analysis of the relationship between individual C_{13}-norisoprenoid
79 (hydrolytically released) and ripening days, vigor level and yield level through linear regression.
80 The result showed that yield had very limited impact on C_{13}-norisoprenoids in grape during berry
81 development. Most of the C_{13}-norisoprenoids were closely associated with the level of vine vigor
82 and interaction between time and vigor.

Table 1 Free volatile composition of Pinot noir wine produced from different treatments (mg/L)

Year	2011				2012				2013			
Treatment [a]	GCON	GMT	TCON	TMT	GCON	GMT	TCON	TMT	GCON	GMT	TCON	TMT
Esters												
ethyl acetate [b]	12.6±1.4	14.5±3.1	9.80±1.31	11.7±0.8	10.8±0.2	12.2±0.4	11.0±0.3	10.7±0.4	10.0±0.1	10.6±0.1	10.2±0.2	10.4±0.1
isoamyl acetate	453±13	484±3	356±16	351±20	429±16	395±22	233±7	283±10	373±11	323±15	311±10	246±13
ethyl isobutyrate	32.4±2.1	41.1±0.5	29.1±3.6	19.4±0.3	60.4±3.1	39.9±1.8	22.1±0.1	24.6±3.4	8.71±0.79	13.2±2.1	7.25±2.07	6.6±1.41
ethyl butanoate	21.8±0.7	24.7±0.7	15.5±3.0	25.4±0.9	14.3±0.3	10.1±7.2	21.2±0.7	19.7±1.9	21.0±1.1	25.5±3.7	34.4±3.3	31.6±4.1
ethyl isovalerate	6.27±0.10	6.77±1.14	5.54±0.84	3.65±0.35	4.72±0.16	4.98±0.02	2.70±0.21	3.68±0.63	1.81±0.28	3.16±0.16	1.78±0.29	2.34±0.05
ethyl hexanoate	85.6±8.3	69.8±0.6	70.8±0.5	85.8±2.2	48.0±2.0	68.6±2.2	75.7±4.5	63.4±2.6	133±2	104±1	127±24	117±13
hexyl acetate	1.04±0.08	0.89±0.03	0.75±0.01	0.74±0.02	0.59±0.07	0.64±0.01	0.51±0.03	0.65±0.05	2.08±0.23	1.53±0.16	2.04±0.29	1.71±0.20
octyl acetate	0.69±0.01	0.66±0.04	0.64±0.04	0.69±0.01	0.68±0.04	0.75±0.01	0.62±0.03	0.66±0.04	0.52±0.06	0.61±0.08	0.58±0.02	0.57±0.06
ethyl octanoate	73.8±2.2	69.7±2.0	75.0±1.3	77.9±1.8	50.5±0.7	67.5±1.6	66.5±1.1	62.7±5.7	81.5±7.8	97.2±3.3	87.5±8.5	90.5±12.7
octyl butyrate	0.33±0.03	0.46±0.02	0.34±0.03	0.31±0.02	0.36±0.02	0.35±0.02	0.29±0.01	0.24±0.04	0.39±0.05	0.57±0.05	0.46±0.07	0.54±0.11
diethyl succinate	584±14	736±24	553±33	539±12	592±43	628±10	642±4	702±10	221±8	255±12	216±26	214±20
ethyl phenylacetate	0.87±0.09	1.27±0.06	0.74±0.05	0.62±0.00	0.98±0.15	0.67±0.01	0.43±0.04	0.42±0.14	0.52±0.02	0.68±0.02	0.74±0.14	1.12±0.15
phenethyl acetate	5.48±0.01	7.33±0.44	3.94±0.45	3.67±0.04	6.21±0.46	5.75±0.14	3.08±0.07	3.84±0.54	2.89±0.13	3.10±0.07	2.32±0.51	2.44±0.44
Alcohols												
1-propanol [b]	66.1±1.3	73.4±1.7	131±3	157±3	40.6±0.7	53.2±1.4	105±1	115±6	45.6±1.6	65.6±0.7	129±3	143±2
isobutyl alcohol [b]	176±3	169±4	198±3	169±3	297±5	231±7	173±2	166±8	120±3	124±2	122±3	111±1
isoamyl alcohol [b]	451±9	483±22	380±29	399±7	493±14	482±3	346±4	340±19	371±11	377±6	330±13	313±5
1-hexanol	1142±34	1091±7	1035±22	1086±10	842±2	901±16	885±30	904±6	2345±54	2232±3	2809±46	2640±42
trans-2-hexenol	22.4±2.5	20.1±1.7	18.4±0.4	22.6±1.9	24.9±0.6	22.7±0.5	24.1±1.0	17.8±2.6	27.2±2.0	19.4±5.3	23.5±1.1	20.8±0.4
trans-3-hexenol	8.75±0.11	12.6±0.9	9.17±0.93	9.31±0.37	10.5±0.5	10.7±1.1	9.07±0.01	10.3±1.6	19.7±0.71	26.3±1.6	20.8±2.8	16.5±2.4
1-octen-3-ol	15.7±0.6	18.6±3.6	18.0±2.4	16.8±0.1	10.4±0.3	14.4±0.2	14.3±0.1	13.4±0.9	17.9±1.9	23.7±2.9	20.5±2.2	19.7±2.8
2-ethyl-1-hexanol	1.56±0.32	2.21±0.20	3.17±0.17	1.65±0.32	3.56±0.12	3.54±0.02	2.79±0.01	3.27±0.05	2.52±0.01	2.65±0.15	2.34±0.18	2.25±0.14
1-octanol	349±13	328±3	349±2	369±4	325±7	338±9	350±2	340±4	264±18	278±21	293±7	310±7
1-nonanol	2.95±0.22	2.87±0.07	3.00±0.04	2.83±0.11	3.19±0.08	3.32±0.06	3.43±0.05	3.20±0.26	2.11±0.18	2.31±0.14	2.69±0.01	3.04±0.05

(Table 1 continued)

1-decanol	0.35±0.02	0.38±0.01	0.37±0.05	0.32±0.02	0.31±0.01	0.31±0.02	0.29±0.01	0.34±0.06	0.29±0.00	0.38±0.03	0.32±0.05	0.35±0.07
1-undecanol	0.41±0.03	0.45±0.04	0.30±0.18	0.39±0.01	0.32±0.00	0.37±0.02	0.28±0.01	0.28±0.01	0.79±0.17	1.04±0.18	0.30±0.09	0.34±0.07
benzyl alcohol	278±3	337±15	295±5	316±3	239±8	222±6	333±3	283±17	164±6	135±0	138±16	108±11
phenethyl alcohol	3800±134	4928±149	3285±215	2962±18	4565±342	4432±49	2896±21	3261±436	2386±41	2823±60	2091±390	2132±299
Terpenes												
linalool	1.95±0.22	2.05±0.10	2.50±0.30	2.12±0.09	2.01±0.01	2.13±0.08	2.54±0.08	2.38±0.34	1.45±0.07	1.87±0.08	1.86±0.24	2.02±0.40
α-terpineol	1.01±0.04	1.05±0.09	0.90±0.03	0.88±0.01	1.05±0.04	0.80±0.02	0.79±0.02	0.74±0.06	0.69±0.00	0.66±0.04	0.57±0.03	0.60±0.08
β-citronellol	1.12±0.01	1.59±0.06	0.78±0.07	1.01±0.05	1.45±0.16	1.67±0.05	1.17±0.04	1.38±0.17	1.94±0.03	2.12±0.13	1.75±0.27	1.90±0.23
nerol	4.84±0.15	4.75±0.09	4.13±0.92	3.78±0.55	2.81±0.03	2.16±0.04	2.51±0.06	1.96±0.03	1.05±0.10	1.13±0.02	1.06±0.19	1.02±0.06
geraniol	0.14±0.01	nd	0.22±0.01	0.29±0.02	0.20±0.03	0.27±0.09	0.11±0.01	0.25±0.02	0.49±0.01	0.38±0.07	nd	0.25±0.01
C_{13}-norisoprenoids												
β-damascenone	0.07±0.00	0.14±0.02	0.05±0.01	0.05±0.01	0.12±0.02	0.14±0.01	0.11±0.00	0.09±0.02	0.67±0.01	1.04±0.03	0.44±0.11	0.51±0.11
β-ionone [c]	114±0	33.2±1.2	97.6±6.4	84.6±1.7	45.4±6.7	39.5±2.4	39.7±2.3	33.9±7.7	47.2±0.3	69.0±0.5	63.7±17.9	64.3±15.6
vitispirane	5.38±0.29	5.28±0.03	4.68±0.12	4.39±0.05	5.04±0.17	4.61±0.02	4.26±0.04	4.28±0.15	4.00±0.01	4.13±0.00	3.99±0.10	3.96±0.10
TDN	3.89±0.01	3.90±0.05	3.88±0.02	3.83±0.00	3.94±0.02	3.89±0.01	3.83±0.01	3.85±0.03	3.83±0.01	3.89±0.00	3.84±0.04	3.84±0.04
Aldehydes												
Acetaldehyde [b]	2.38±0.41	5.53±0.44	3.41±0.52	3.71±0.09	3.06±0.24	5.16±0.04	3.12±0.10	3.53±0.11	8.15±0.31	4.17±0.09	10.1±0.2	7.47±0.14
Acids												
hexanoic acid	229±10	291±24	275±15	229±1	133±6	162±19	135±5	137±23	363±14	494±4	345±65	379±80
octanoic acid	104±2	147±20	135±11	119±3	51.0±0.4	66.6±10.0	74.2±4.8	63.6±10.2	134±6	183±5	141±20	157±35
decanoic acid	9.81±0.54	15.2±2.7	13.7±0.07	11.2±0.09	6.61±0.10	8.64±2.00	9.27±1.14	7.01±0.89	12.2±0.6	16.7±0.2	12.3±1.0	14.4±3.7

Mean±SD presented (n=3). [a] GCON: grass between vine rows (lower vigor) and full crop (no crop thinning occurred, higher yield); TCON: tilled between vine rows (higher vigor) and full crop (no crop thinning occurred, higher yield); GMT: grass between vine rows (lower vigor) and moderate thinning (lower yield); TMT: tilled between vine rows (higher vigor) and moderate thinning (lower yield). [b] Concentration presented as mg/L. [c] Concentration presented as ng/L.

Table 2 Hydrolytically released C_{13}-norisoprenoids in Pinot noir wines produced from different treatments (mg/L)

Year	2011				2012				2013			
Treatment [a]	GCON	GMT	TCON	TMT	GCON	GMT	TCON	TMT	GCON	GMT	TCON	TMT
vitispirane	16.8±2.0	18.2±1.0	14.1±2.4	14.6±0.1	23.4±2.6	25.5±4.3	22.4±0.5	20.3±1.9	22.5±2.7	22.0±1.9	15.7±2.9	18.1±1.6
TDN	10.1±0.3	9.03±0.31	8.61±0.68	7.54±0.52	11.8±1.1	10.1±0.9	9.31±0.17	7.57±0.44	13.4±2.8	12.3±1.0	8.22±1.09	10.4±0.4
β-damascenone	1.15±0.06	0.96±0.01	1.34±0.21	1.16±0.01	0.95±0.23	0.92±0.13	1.17±0.06	0.86±0.09	2.17±0.36	2.30±0.65	1.53±0.26	1.72±0.10
β-ionone [b]	98.6±2.4	56.3±0.3	103±2	82.8±3.9	51.8±12.9	48.5±3.9	62.3±3.3	36.8±3.0	60.6±3.7	58.2±7.7	63.9±13.2	62.7±3.6

Mean±SD presented (n=3). [a] GCON: grass between vine rows (lower vigor) and full crop (no crop thinning occurred, higher yield); TCON: tilled between vine rows (higher vigor) and full crop (no crop thinning occurred, higher yield); GMT: grass between vine rows (lower vigor) and moderate thinning (lower yield); TMT: tilled between vine rows (higher vigor) and moderate thinning (lower yield). [b] Concentration presented as ng/L.

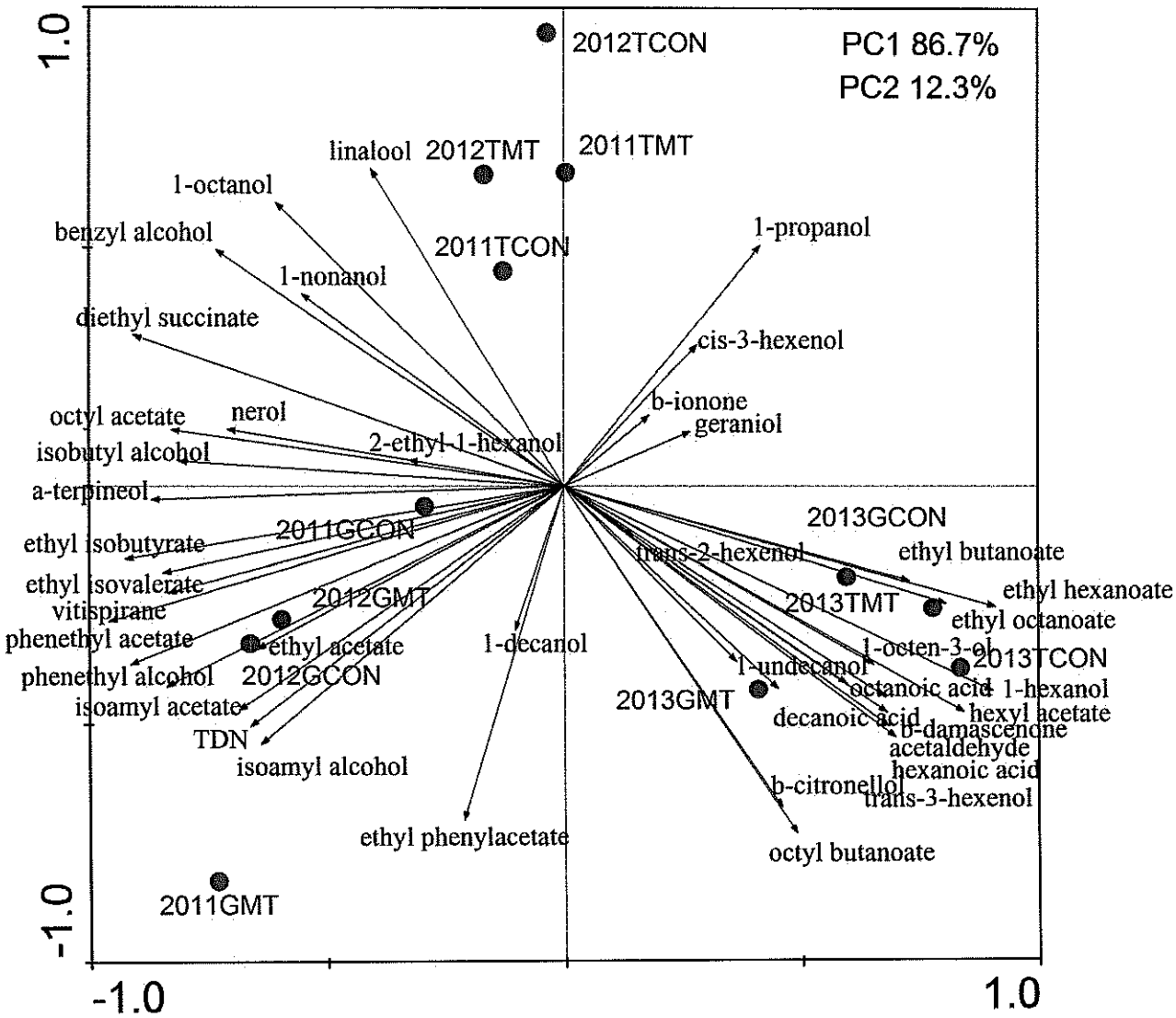

Figure 1 Principal Components Analysis (PCA) bi-plots for wine volatile composition of three years.

Table 3 Phenology of grapes at different sampling time

Date	Days post véraison	Description
12-Aug	0	Beginning of véraison (20 to 25% color change)
19-Aug	7	75% véraison
26-Aug	14	Nearly 100% full color
3-Sep	22	Full color, ripening and ~18-20 degree Brix
10-Sep	29	Full color, ripening, ~21 degree Brix
18-Sep	37	Harvest

Table 4 Brix, pH and berry weight of grape samples in this study

	Treatment [a]	Brix	pH	Berry weight (g/berry)
12-Aug	GCON	9.8 (1.2)	2.36 (0.08)	0.69 (0.10)
	GMT	9.7 (1.7)	2.34 (0.12)	0.71 (0.10)
	TCON	10.7 (1.9)	2.46 (0.14)	0.70 (0.09)
	TMT	11.5 (1.0)	2.50 (0.10)	0.82 (0.06)
	P-value	*ns*	*ns*	*ns*
19-Aug	GCON	13.2 (0.9)	2.66 (0.04)	0.74 (0.13)
	GMT	14.2 (0.8)	2.72 (0.07)	0.77 (0.04)
	TCON	14.1 (1.2)	2.71 (0.05)	0.79 (0.01)
	TMT	15.3 (1.8)	2.79 (0.10)	0.84 (0.08)
	P-value	*ns*	*ns*	*ns*
26-Aug	GCON	15.8 (0.4) b	2.79 (0.09) b	0.98 (0.10)
	GMT	16.8 (0.2) a	2.79 (0.08) b	1.03 (0.10)
	TCON	17.2 (0.6) a	2.83 (0.07) ab	0.98 (0.08)
	TMT	16.7 (0.4) ab	2.96 (0.09) a	0.92 (0.10)
	P-value	*0.002*	*0.024*	*ns*
3-Sep	GCON	19.3 (0.9)	2.79 (0.16)	1.04 (0.06)
	GMT	20.1 (0.7)	2.85 (0.08)	1.04 (0.08)
	TCON	19.7 (1.1)	2.86 (0.09)	1.00 (0.08)
	TMT	20.7 (1.0)	2.89 (0.04)	1.04 (0.07)
	P-value	*ns*	*ns*	*ns*
10-Sep	GCON	19.5 (0.5)	3.27 (0.11)	1.08 (0.09)
	GMT	19.7 (1.1)	3.28 (0.12)	1.04 (0.11)
	TCON	20.1 (1.8)	3.28 (0.12)	1.07 (0.06)
	TMT	20.9 (0.6)	3.37 (0.09)	1.00 (0.06)
	P-value	*ns*	*ns*	*ns*
18-Sep	GCON	21.0 (0.6)	3.43 (0.03)	0.96 (0.08)
	GMT	21.6 (1.2)	3.51 (0.09)	1.00 (0.04)
	TCON	21.6 (1.2)	3.49 (0.16)	1.07 (0.14)
	TMT	22.0 (0.8)	3.52 (0.11)	0.98 (0.10)
	P-value	*ns*	*ns*	*ns*

Mean (SD) presented. Different letters represent significantly (Tukey HSD, $P<0.05$) different in means (n=5 dependent field sample). ns, not significant. [a] GCON: grass between vine rows (lower vigor) and full crop (no crop thinning occurred, higher yield); TCON: tilled between vine rows (higher vigor) and full crop (no crop thinning occurred, higher yield); GMT: grass between vine rows (lower vigor) and moderate thinning (lower yield); TMT: tilled between vine rows (higher vigor) and moderate thinning (lower yield).

Table 5 Carotenoid content (mg/kg berry) in Pinot noir grape at different sampling time

	Treatment [a]	Neochrome *a*	Neoxanthin	Neochrome *b*	Flavoxanthin	Lutein	β-Carotene
12-Aug	GCON	17.6 (3.3) ab	40.0 (9.6)	3.42 (0.20) b	13.5 (2.7)	271 (56)	245 (1)
	GMT	16.3 (3.9) ab	42.2 (10.9)	2.27 b (1.78)	24.6 (16.6)	402 (235)	241 (38)
	TCON	24.0 (2.7) a	46.8 (7.5)	13.2 (2.8) a	19.0 (8.0)	289 (75)	292 (42)
	TMT	13.7 (2.27) b	40.2 (8.4)	4.54 (1.92) b	16.9 (2.0)	375 (54)	252 (12)
	P-value	*0.050*	*ns*	*0.002*	*ns*	*ns*	*ns*
19-Aug	GCON	11.4 (4.7)	23.9 (8.7) ab	nd b	12.1 (2.1) b	208 (17) b	186 (16)
	GMT	13.0 (2.0)	27.8 (3.3) ab	3.32 (0.5) a	25.6 (3.4) a	310 (9.9) a	176 (25)
	TCON	14.5 (3.0)	35.9 (3.2) a	nd b	15.4 (0.7) b	296 (40) ab	220 (11)
	TMT	8.58 (0.54)	17.1 (0.4) b	3.01 (0.51) a	23.4 (2.1) a	300 (32) a	184 (17)
	P-value	*ns*	*0.029*	*<0.001*	*0.001*	*0.021*	*ns*
26-Aug	GCON	4.66 (0.31)	15.7 (0.8)	nd	9.24 (0.70) b	227 (16) c	138 (4) bc
	GMT	4.84 (1.4)	20.8 (3.7)	0.60 (0.02)	10.4 (0.5) b	267 (24) bc	123 (3) c
	TCON	4.88 (1.16)	19.9 (5.5)	1.42 (0.57)	11.9 (0.4) ab	328 (24) ab	169 (7) a
	TMT	5.85 (1.58)	27.6 (7.7)	0.56 (0.79)	13.3 (1.4) a	346 (28) a	164 (14) ab
	P-value	*ns*	*ns*	*ns*	*0.007*	*0.003*	*0.002*
3-Sep	GCON	6.37 (0.97) a	34.1 (3.9)	0.81 (1.02)	20.8 (1.2) a	309 (24) ab	106 (1) c
	GMT	2.60 (0.58) b	27.1 (4.0)	nd	11.2 (1.7) b	287 (39) b	114 (19) bc
	TCON	7.01 (0.94) a	44.0 (5.2)	0.96 (0.36)	26.1 (2.6) a	429 (52) a	147 (10) ab
	TMT	5.72 (1.82) ab	31.8 (7.6)	1.83 (0.49)	11.3 (4.0) b	385 (35) ab	156 (5) a
	P-value	*0.022*	*ns*	*ns*	*0.001*	*0.022*	*0.004*
10-Sep	GCON	2.07 (0.13)	23.2 (1.9)	nd	11.3 (0.7)	282 (13) b	105 (4) b
	GMT	1.99 (0.18)	28.2 (5.9)	nd	12.2 (1.7)	300 (37) ab	105 (21) b
	TCON	2.68 (0.40)	30.8 (4.2)	nd	14.5 (0.3)	382 (28) a	145 (17) a
	TMT	2.45 (0.23)	30.9 (3.3)	nd	14.5 (1.9)	369 (35) ab	133 (9) ab
	P-value	*ns*	*ns*	-	*ns*	*0.023*	*0.005*
18-Sep	GCON	6.02 (0.62)	28.1 (3.3) b	nd	17.4 (1.2) ab	299 (32) b	106 (11) ab
	GMT	3.45 (1.51)	24.8 (2.7) b	nd	12.0 (3.6) b	300 (14) b	94.3 (4.1) b
	TCON	6.89 (1.45)	39.5 (2.6) a	nd	23.0 (0.8) a	397 (33) a	130 (9) a
	TMT	4.41 (1.92)	28.0 (3.2) b	nd	11.3 (0.7) b	368 (21) ab	117 (2) ab
	P-value	*ns*	*0.005*	-	*0.001*	*0.012*	*0.008*

Mean (SD) presented. Different letters represent significantly (Tukey HSD, $P < 0.05$) different in means (n=5 dependent field sample). *ns*, not significant. nd, not detected. [a] GCON: grass between vine rows (lower vigor) and full crop (no crop thinning occurred, higher yield); TCON: tilled between vine rows (higher vigor) and full crop (no crop thinning occurred, higher yield); GMT: grass between vine rows (lower vigor) and moderate thinning (lower yield); TMT: tilled between vine rows (higher vigor) and moderate thinning (lower yield).

Table 6 Free and potential C13-norisoprenoids in Pinot noir grape under different treatment during the grape development

		Free C13-norisoprenoids		C13-norisoprenoid potential								
	Treatment	β-Damascenone	β-Ionone	β-Damascenone	α-Ionone	Vitispirane A	Vitispirane B	TDN	TPB	β-Ionone	3-Oxo-β-ionone	3,4-Dehydro-β-ionone
12-Aug	GCON	0.24 (0.04) c	0.58 (0.09) b	2.91 (0.27) b	0.36 (0.05)	3.34 (1.39)	1.45 (0.37)	5.96 (2.29)	nd	0.89 (0.10) b	1.01 (0.23)	0.31 (0.03) ab
	GMT	0.28 (0.05) bc	0.71 (0.34) ab	2.86 (0.65) b	0.38 (0.15)	3.28 (0.85)	1.29 (0.77)	5.28 (2.28)	nd	0.85 (0.15) b	0.88 (0.29)	0.26 (0.06) b
	TCON	0.39 (0.05) a	0.93 (0.15) a	4.71 (0.04) a	0.44 (0.09)	3.67 (0.66)	0.92 (0.19)	4.20 (1.67)	nd	1.48 (0.33) a	1.00 (0.32)	0.42 (0.12) a
	TMT	0.37 (0.07) ab	1.02 (0.07) a	4.59 (0.55) a	0.44 (0.11)	3.71 (0.73)	0.99 (0.23)	5.42 (2.00)	nd	1.42 (0.24) a	0.89 (0.17)	0.37 (0.06) ab
	P-value	*0.01*	*0.05*	*<0.001*	*ns*	*ns*	*ns*	*ns*	-	*<0.001*	*ns*	*0.021*
19-Aug	GCON	0.25 (0.04) ab	0.77 (0.13) b	2.97 (0.57)	0.29 (0.08)	2.75 (0.76)	1.07 (0.39)	2.57 (1.15)	nd	0.91 (0.15) b	1.13 (0.16)	0.26 (0.07)
	GMT	0.19 (0.03) b	0.73 (0.13) b	2.71 (0.19)	0.33 (0.06)	2.09 (0.39)	1.28 (0.23)	4.38 (1.83)	nd	0.95 (0.20) b	1.37 (0.60)	0.23 (0.09)
	TCON	0.27 (0.03) a	1.13 (0.23) ab	2.96 (0.61)	0.39 (0.17)	3.18 (0.35)	1.12 (0.32)	2.96 (1.39)	nd	1.37 (0.20) a	1.16 (0.56)	0.28 (0.08)
	TMT	0.26 (0.05) ab	1.42 (0.34) a	3.16 (0.44)	0.41 (0.11)	2.80 (0.68)	0.93 (0.22)	1.98 (0.62)	nd	1.49 (0.17) a	0.95 (0.10)	0.28 (0.03)
	P-value	*0.025*	*<0.001*	*ns*	*ns*	*ns*	*ns*	*ns*	-	*<0.001*	*ns*	*ns*
26-Aug	GCON	0.14 (0.01)	0.59 (0.07) b	3.38 (0.48) b	0.31 (0.06)	1.80 (0.36)	1.16 (0.39)	2.64 (1.54)	0.05 (0.03)	0.88 (0.12) b	2.11 (0.43) a	0.40 (0.08)
	GMT	0.14 (0.02)	0.60 (0.07) b	3.65 (0.59) ab	0.30 (0.07)	1.78 (0.30)	1.30 (0.48)	3.23 (0.07)	0.07 (0.02)	0.88 (0.14) b	1.63 (0.28) ab	0.39 (0.07)
	TCON	0.17 (0.02)	0.92 (0.01) a	4.47 (0.55) a	0.32 (0.08)	2.18 (0.61)	0.98 (0.42)	2.40 (1.40)	0.05 (0.01)	1.19 (0.17) a	1.44 (0.21) ab	0.38 (0.04)
	TMT	0.17 (0.04)	1.00 (0.12) a	4.21 (0.64) ab	0.38 (0.09)	1.93 (0.30)	0.97 (0.17)	2.56 (0.91)	0.08 (0.03)	1.16 (0.17) ab	1.38 (0.47) b	0.36 (0.09)
	P-value	*ns*	*<0.001*	*0.041*	*ns*	*ns*	*ns*	*ns*	*ns*	*0.011*	*0.031*	*ns*
3-Sep	GCON	0.15 (0.02)	0.50 (0.06) bc	4.58 (0.38) b	0.13 (0.06)	1.48 (0.33)	2.57 (0.67)	6.22 (1.38)	0.32 (0.09)	0.51 (0.21) c	3.01 (0.50)	0.64 (0.12)
	GMT	0.14 (0.01)	0.47 (0.09) c	4.28 (0.72) b	0.14 (0.08)	1.59 (0.42)	1.97 (0.33)	5.50 (1.88)	0.27 (0.06)	0.57 (0.20) bc	2.83 (0.36)	0.60 (0.12)
	TCON	0.17 (0.03)	0.68 (0.08) a	5.87 (0.72) a	0.23 (0.09)	1.53 (0.13)	1.76 (0.12)	4.87 (0.54)	0.27 (0.10)	0.95 (0.18) a	2.42 (0.31)	0.66 (0.09)
	TMT	0.17 (0.03)	0.63 (0.07) ab	5.98 (0.39) a	0.15 (0.06)	1.56 (0.21)	2.12 (0.80)	4.29 (1.65)	0.27 (0.08)	0.82 (0.03) ab	2.56 (0.87)	0.71 (0.12)
	P-value	*ns*	*0.001*	*<0.001*	*ns*	*ns*	*ns*	*ns*	*ns*	*0.002*	*ns*	*ns*
10-Sep	GCON	0.08 (0.05)	0.41 (0.07) b	5.04 (0.59)	0.32 (0.24)	2.61 (0.39)	2.22 (0.94)	4.41 (2.02)	0.29 (0.18)	0.80 (0.23)	2.64 (0.60) ab	0.68 (0.21)
	GMT	0.06 (0.04)	0.40 (0.10) b	4.60 (0.59)	0.18 (0.06)	2.45 (0.53)	2.29 (0.81)	5.40 (2.02)	0.26 (0.09)	0.70 (0.13)	2.69 (0.50) a	0.59 (0.07)
	TCON	0.12 (0.02)	0.65 (0.09) a	5.20 (0.86)	0.25 (0.03)	2.13 (0.44)	1.45 (0.49)	3.38 (1.53)	0.12 (0.05)	0.90 (0.18)	1.79 (0.46) c	0.48 (0.13)
	TMT	0.09 (0.06)	0.61 (0.08) a	5.74 (0.41)	0.13 (0.07)	2.07 (0.79)	1.89 (0.48)	5.23 (1.61)	0.30 (0.09)	0.69 (0.07)	1.74 (0.32) bc	0.50 (0.07)
	P-value	*ns*	*<0.001*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*0.007*	*ns*
18-Sep	GCON	0.07 (0.02) b	0.32 (0.05) b	4.93 (0.63) b	0.21 (0.05)	1.99 (0.68)	2.28 (0.49)	4.85 (1.59)	0.35 (0.05)	0.71 (0.03)	2.87 (0.54)	0.59 (0.11)
	GMT	0.10 (0.03) ab	0.38 (0.07) b	5.25 (0.23) ab	0.21 (0.04)	1.59 (0.66)	2.47 (1.14)	5.44 (1.02)	0.40 (0.17)	0.57 (0.11)	3.09 (0.54)	0.68 (0.12)
	TCON	0.12 (0.02) a	0.49 (0.06) a	5.45 (0.49) ab	0.26 (0.04)	1.95 (0.56)	2.20 (0.83)	4.45 (1.50)	0.30 (0.16)	0.72 (0.09)	2.28 (0.47)	0.51 (0.08)
	TMT	0.13 (0.03) a	0.51 (0.02) a	6.02 (0.47) a	0.25 (0.09)	1.99 (0.69)	1.90 (0.58)	5.20 (1.63)	0.36 (0.09)	0.74 (0.13)	2.47 (0.41)	0.61 (0.10)
	P-value	*0.003*	*<0.001*	*0.018*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*

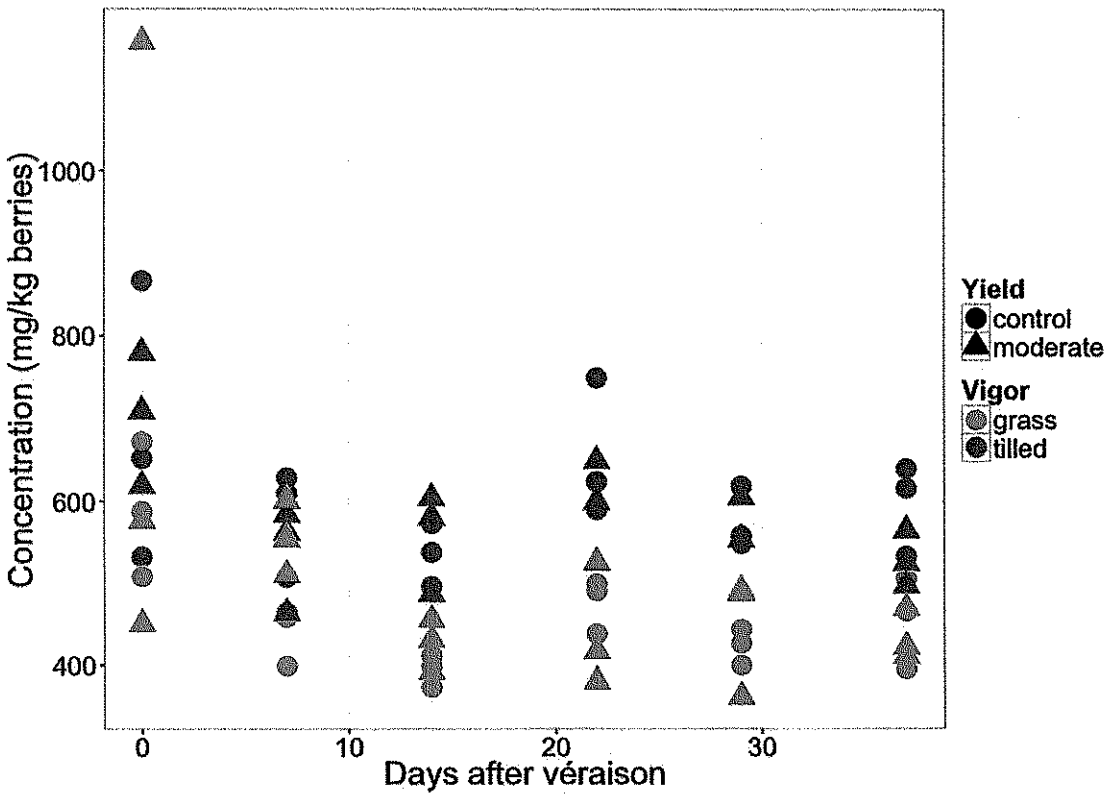

Figure 2 Total carotenoid content in Pinot noir grape under different treatment.

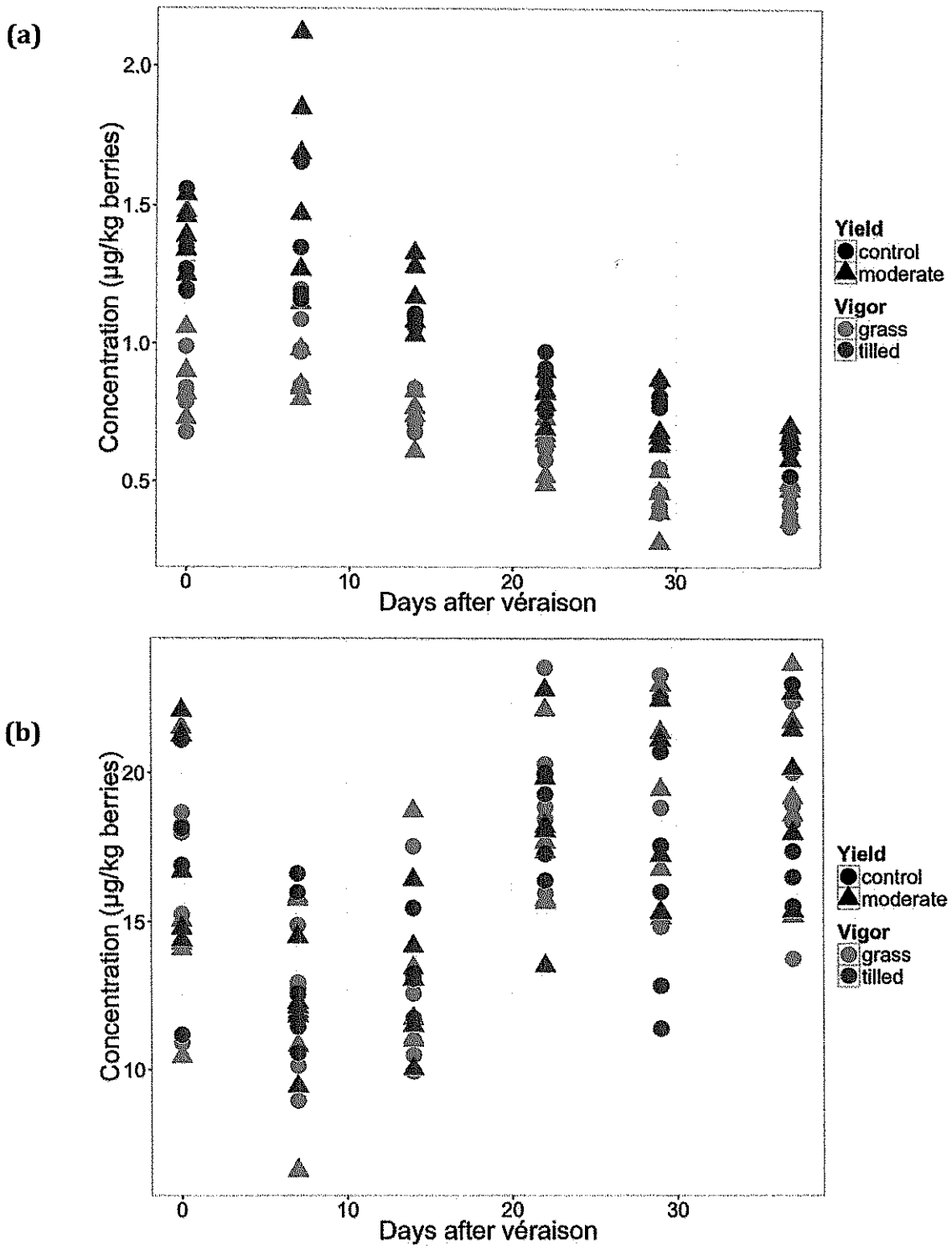

Figure 3 Total C_{13}-norisoprenoid content in Pinot noir grape under different treatment. (a) Free C_{13}-norisoprenoids. (b) hydrolytically released C_{13}-norisoprenoids.

Table 7 MANOVA and post hoc ANOVAs for carotenoids

	Tests of Between-Subjects Effects (ANOVA)							Multivariate tests (MANOVA)
		P-value						*P-value*
Effect	*Df*	Neochrome *a*	Neoxanthin	Neochrome *b*	Flavoxanthin	Lutein	β-Carotene	Wilks' Lambda
Intercept	1	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Time	5	**<0.001**	**0.005**	**<0.001**	**0.004**	0.099	**<0.001**	**<0.001**
Vigor	1	0.138	0.127	**<0.001**	0.192	**<0.001**	**<0.001**	**<0.001**
Yield	1	**0.001**	0.334	0.25	0.632	0.162	**0.026**	**<0.001**
Time * Vigor	5	0.795	0.985	**<0.001**	0.925	0.435	0.809	**<0.001**
Time * Yield	5	**0.038**	0.602	**<0.001**	**<0.001**	0.208	0.437	**<0.001**
Vigor * Yield	1	0.057	0.202	**0.02**	**0.049**	0.238	0.254	0.255
Time * Vigor * Yield	5	**0.021**	0.904	**<0.001**	0.705	0.988	0.719	**0.01**
Error	48							

Design: Intercept + Time + Vigor + Yield + Time * Vigor + Time * Yield + Vigor * Yield + Time * Vigor * Yield

Significant results are printed in bold ($P < 0.05$).

Table 8 MANOVA and post hoc ANOVAs for total C13-norisoprenoids

	Tests of Between-Subjects Effects (ANOVA) *P-value*									Multivariate Tests (MANOVA) *P-value*
Effect	Df	β-Damascenone	α-Ionone	Vitispirane A	Vitispirane B	TDN	TPB	β-Ionone	3-Oxo-β-ionone	3,4-Dehydro-β-ionone	Wilks' Lambda
Intercept	1	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Time	5	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Vigor	1	**<0.001**	**0.003**	0.223	**0.002**	**0.013**	0.223	**<0.001**	**<0.001**	0.434	**<0.001**
Yield	1	0.911	0.441	0.366	0.935	0.268	0.170	0.326	0.995	0.795	0.384
Time * Vigor	5	**<0.001**	0.791	0.316	0.743	0.995	0.814	**<0.001**	0.029	**<0.001**	**<0.001**
Time * Yield	5	0.780	0.637	0.837	0.981	0.619	0.323	0.824	0.964	0.382	0.666
Vigor * Yield	1	0.382	0.456	0.570	0.375	0.533	0.129	0.491	0.433	0.175	0.944
Time * Vigor * Yield	5	0.262	0.958	0.996	0.294	0.274	0.193	0.317	0.372	0.816	0.305
Error	82										

Design: Intercept + Time + Vigor + Yield + Time * Vigor + Time * Yield + Vigor * Yield + Time * Vigor * Yield
Significant results are printed in bold ($P < 0.05$).

VI. OUTSIDE PRESENTATIONS OF RESEARCH

A poster presentation was given at the 2014 "OWRI Grape Days" (April 1st). Results to date from the study have also been shared and discussed with the Willamette Valley Enology Technical group during two meetings in 2014. A manuscript is under preparation for publication.

VII. RESEARCH SUCCESS STATEMENTS:

The result has showed that wine volatile profile has changed when the corresponding vine vigor was different, while it was barely affected by the different level of crop load. Moreover, lower vine vigor contributed to higher branched-chain esters, alcohols and C_{13}-norisoprenoids in wine in year 2011 and 2012. The changes in profiles of ester and alcohols are possibly due to the nitrogen competition between grape vine and cover crop, which leads to a lower nitrogen content in the fruit. C_{13}-norisoprenoids, the degradation product of carotenoids, are important grape-derived compound and their concentrations are reported to be associated with many factors, i.e. sunlight exposure and water status. Our results indicated that besides climate factors, different vine balance could also alter the grape composition and thus affect the wine aroma.

VIII. FUND STATUS:

This was a two and half year proposal, a PhD graduate student was hired and assigned to this project at the first year. However, the funding was severely reduced to $10, 000 for the second year's research. We have to allocate other resources to finish this project.

LITERATURE CITED:

Baumes, R., J. Wirth, S. Bureau, Y. Gunata and A. Razungles (2002). "Biogeneration of C13-norisoprenoid compounds: experiments supportive for an apo-carotenoid pathway in grapevines." Analytica Chimica Acta **458**(1): 3-14.

Bravdo, B., Y. Hepner, C. Loinger, S. Cohen and H. Tabacman (1985). "Effect of irrigation and crop level on growth, yield and wine quality of Cabernet Sauvignon." American Journal of Enology and Viticulture **36**(2): 132-139.

Celette, F., A. Findeling and C. Gary (2009). "Competition for nitrogen in an unfertilized intercropping system: The case of an association of grapevine and grass cover in a Mediterranean climate." European Journal of Agronomy **30**(1): 41-51.

Chapman, D. M., M. A. Matthews and J.-X. Guinard (2004). "Sensory attributes of Cabernet Sauvignon wines made from vines with different crop yields." American Journal of Enology and Viticulture **55**(4): 325-334.

Coelho, E., S. M. Rocha, I. Delgadillo and M. A. Coimbra (2006). "Headspace-SPME applied to varietal volatile components evolution during Vitis vinifera L. cv. 'Baga' ripening." Analytica Chimica Acta **563**(1–2): 204-214.

Crupi, P., A. Coletta and D. Antonacci (2010). "Analysis of carotenoids in grapes to predict norisoprenoid varietal aroma of wines from Apulia." Journal of Agricultural and Food Chemistry **58**(17): 9647-9656.

Diago, M. P., M. Vilanova, J. Blanco and J. Tardaguila (2010). "Effects of mechanical thinning on fruit and wine composition and sensory attributes of Grenache and Tempranillo varieties (Vitis vinifera L.)." Australian Journal of Grape and Wine Research **16**(2): 314-326.

Fang, Y. and M. C. Qian (2006). "Quantification of selected aroma-active compounds in Pinot noir wines from different grape maturities." Journal of agricultural and food chemistry **54**(22): 8567-8573.

Guidoni, L., E. Beaurepaire and J. Y. Bigot (2002). "Magneto-optics in the ultrafast regime: thermalization of spin populations in ferromagnetic films." Physical review letters **89**(1): 17401.

Humpf, H. U., P. Winterhalter and P. Schreier (1991). "3, 4-Dihydroxy-7, 8-dihydro-. beta.-ionone. beta.-D-glucopyranoside: natural precursor of 2, 2, 6, 8-tetramethyl-7, 11-dioxatricyclo [6.2. 1.01, 6] undec-4-ene (Riesling acetal) and 1, 1, 6-trimethyl-1, 2-dihydronaphthalene in red currant (Ribes rubrum L.) leaves." Journal of Agricultural and Food Chemistry **39**(10): 1833-1835.

Keller, M., L. J. Mills, R. L. Wample and S. E. Spayd (2005). "Cluster thinning effects on three deficit-irrigated Vitis vinifera cultivars." American Journal of Enology and Viticulture **56**(2): 91-103.

Kliewer, W. M. and N. K. Dokoozlian (2005). "Leaf area/crop weight ratios of grapevines: Influence on fruit composition and wine quality." American Journal of Enology and Viticulture **56**(2): 170-181.

Kurtural, S. K., I. E. Dami and B. H. Taylor (2006). "Effects of Pruning and Cluster Thinning on Yield and Fruit Composition ofChambourcin'Grapevines." HortTechnology **16**(2): 233-240.

Loscos, N., P. Hernandez-Orte, J. Cacho and V. Ferreira (2007). "Release and formation of varietal aroma compounds during alcoholic fermentation from nonfloral grape odorless flavor precursors fractions." Journal of Agricultural and Food Chemistry **55**(16): 6674-6684.

Naor, A., Y. Gal and B. Bravdo (2002). "Shoot and Cluster Thinning Influence Vegetative Growth, Fruit Yield, and Wine Quality ofSauvignon blanc'Grapevines." Journal of the American Society for Horticultural Science **127**(4): 628-634.

Ough, C. and R. Nagaoka (1984). "Effect of cluster thinning and vineyard yields on grape and wine composition and wine quality of Cabernet Sauvignon." American Journal of Enology and Viticulture **35**(1): 30-34.

Prajitna, A., I. E. Dami, T. E. Steiner, D. C. Ferree, J. C. Scheerens and S. J. Schwartz (2007). "Influence of cluster thinning on phenolic composition, resveratrol, and antioxidant capacity in Chambourcin wine." American Journal of Enology and Viticulture **58**(3): 346-350.

Reynolds, A., C. Edwards, D. Wardle, D. Webster and M. Dever (1994). "Shoot Density AffectsRiesling'Grapevines II. Wine Composition and Sensory Response." Journal of the American Society for Horticultural Science **119**(5): 881-892.

Reynolds, A. G., J. Schlosser, D. Sorokowsky, R. Roberts, J. Willwerth and C. de Savigny (2007). "Magnitude of viticultural and enological effects. II. Relative impacts of cluster thinning and yeast strain on composition and sensory attributes of Chardonnay Musqué." American journal of enology and viticulture **58**(1): 25-41.

Reynolds, A. G., S. Yerle, B. Watson, S. F. Price and D. A. Wardle (1996). "Fruit environment and crop level effects on Pinot noir. III. Composition and descriptive analysis of Oregon and British Columbia wines." American Journal of Enology and Viticulture **47**(3): 329-339.

Salinas, M. R., A. Zalacain, F. Pardo and G. L. Alonso (2004). "Stir bar sorptive extraction applied to volatile constituents evolution during *Vitis vinifera* ripening." Journal of Agricultural and Food Chemistry **52**(15): 4821-4827.

Strauss, C. R., E. Dimitriadis, B. Wilson and P. J. Williams (1986). "Studies on the hydrolysis of two megastigma-3, 6, 9-triols rationalizing the origins of some volatile C13 norisoprenoids of Vitis vinifera grapes." Journal of Agricultural and Food Chemistry **34**(1): 145-149.

Tesic, D., M. Keller and R. J. Hutton (2007). "Influence of vineyard floor management practices on grapevine vegetative growth, yield, and fruit composition." American Journal of Enology and Viticulture **58**(1): 1-11.

Winterhalter, P., M. Sefton and P. Williams (1990). "Volatile C13-norisoprenoid compounds in Riesling wine are generated from multiple precursors." American Journal of Enology and Viticulture **41**(4): 277-283.