

Project Title:

Effect of vineyard cover crop management on grape and wine quality II-grape composition and wine aroma

Principal Investigator(s):

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Cooperator(s):

Patty Skinkis, Department of Horticulture, Oregon State University: Vine physiology
Allen Holstein, Stoller Vineyards: collaborate with vineyard management
Leigh Bartholomew, Archery Summit Vineyards: collaborate with vineyard management

Objective(s) of Proposed Research or Outreach Project:

1. Investigate cover crop management in commercial vineyards on aroma and aroma precursor composition in grapes
2. Investigate cover crop management in commercial vineyards on flavor quality of wine
3. Investigate the feasibility to use aroma and aroma precursor analysis in grapes as an additional measurement for grape quality evaluation.

Progress:

Three cover crop management regimes, including clean cultivated (C), alternate row tillage (A), and solid cover (S) of an established grass mix cover crop, were evaluated at two commercial Pinot noir vineyard sites in the Willamette Valley of Oregon during 2007 (Table 1). Vine vegetative growth and grape quality was investigated by Patty Skinkis (See separate report). Basically, cover crop management did not show any significant impact on vine vegetative growth during 2007. Winegrape parameters such as berry weight, soluble solids, pH, and titratable acidity were evaluated during the ripening period up to harvest, and differences were not found for cover crop management treatments. Three important components of fruit quality for wine production were measured after harvest, including Yeast Assimilable Nitrogen Concentration (YANC), total berry skin phenolics and anthocyanins. Yeast available nitrogen concentration did not differ in fruit analyzed from different cover crop management or irrigation treatments. Berry skin anthocyanin concentration was lowest in the clean cultivated treatments. However, the clean cultivated and solid cover treatments at AS vineyard site yielded higher total berry skin polyphenols than alternately tilled. At the Stoller vineyard, solid cover and alternate tilled treatments had higher total berry polyphenols than clean cultivated which may be due to higher vine vigor at this site when compared to AS. Continuation of this study over several years will increase the understanding the impacts of cover crop management on vine growth and grape quality.

Table 1. Site Details for Cooperating Vineyards

Vineyard	Stoller	Archery Summit
Treatment 1	Alternate row tillage (A)	Alternate row tillage (A)
Treatment 2	Solid Cover Crop (S)	Solid cover Crop (S)
Treatment 3	Complete row removal (C)	Complete Row Removal (C)
Design	Complete randomized block	Complete randomized block
Replication	5 reps, 16 vines/rep	5 reps, 24 vines/rep
Irrigation	50% Split Rep irrigated vs. non-irrigated. (July-Sept)	Non-irrigated
Vegetative cover between rows	Mix of reemerging red fescue, 3 year old stand	Perennial blend 60% Elf perennial ryegrass, 20% creeping red fescue, 20% hard fescue, 2 year stand.
Tillage	May	May
Cultivar and Clone	Pinot noir 115/101-14 (planted 1998)	Pinot noir 667/101-14 (planted 1997)
Spacing	7' x 5'	6' x 3.5'

Wine Volatile Aroma Study

The fruit from Archery Summit was placed in 0.5 gal fermenting jars after being destemmed for whole berry fermentation. The must was sulfited with KMS to 50 ppm, and RC212 yeast was used. After primary fermentation, the wines were pressed and racked off the berry skins into 0.5 gal carboys. They remained there for 2 months and were bottled in January. Fermentations were replicated 3 times, except in treatment A, in which there was only enough berries and juice for 2 fermentation replicates.

Totally there were 8 bottles of Pinot Noir wine samples were analyzed. The samples labeled as “C” were from fruit taken from vines that received the “clean cultivation” treatment with triplication (C1, C2, and C3). Likewise, “A” received the “alternative row” treatment with duplication (A1 and A2); “S” received the “solid cover crop” treatment with triplication (S1, S2, and S3). Each bottle of wine was analyzed for one time using SBSE-GC/MS.

An internal standard solution was made by mixing 0.96 mg/mL of 3-heptanone, 1.03 mg/mL of hexyl formate, 1.08 mg/mL of 4-octanol, and 1.14 mg/mL of octyl propanoate in methanol, and stored at -15°C.

A 10 mL of wine sample was diluted with 10 mL of water in a 20 mL vial, in which a 20 uL of internal standard solution was added. A twister bar coated with PDMS was constantly stirred in the sample for 1 hour at a speed of 1000 rpm. After extraction, the twister was dried with tissue paper, and placed into a glass tube of the TDS tray. The analytes were thermally desorbed at the TDU in splitless mode and cryofocused in a CIS 4 at -80 ° C with liquid nitrogen.

A solvent vent injection was employed and the temperature of the PTV was programmed from -80 °C to 250 °C at a rate of 10 °C/sec. A RTX-1 column (60m*0.25mm*0.25um) was used to separate the analytes, and the oven temperature was programmed at 40 °C for a 2 min holding, then to 210 °C at 3 °C/min, and to 270 °C at 5 °C/min with 5 min holding. The selected target aroma compounds were quantified by comparison the peak area of each compound to the peak area of internal standard. Comparison of treatments was achieved by assigning the amount of each aroma compound in “C” as 100% to get a relative percentage of each aroma compound in “A” and “S”. Meanwhile, one-way ANOVA and Bonferroni significant difference were used to test the difference among treatments with the statistical software of S-Plus.

Results:

Totally, 25 important aroma compounds in experimental wines were analyzed in this study, which included 15 esters, 6 terpenoids, 2 norisoprenoids, 1 alcohol and 1 lactone. All those target compounds were previously reported as key aroma compounds in wines. Based on their biochemical formation, they could be either varietal aroma or yeast fermented aroma. Generally, most of esters are fermentation derived, and their concentrations are more controlled by the yeast and fermentation condition. Terpenoids and norisoprenoids are varietal aroma, which is the expression of the environment-genotype interaction.

To investigate the vineyard treatment on wine flavor, the “C” treatment (clean cultivation) was used as control, and the amount of each aroma compound was assumed as 100. Compared to “C”, “A” (alternative row treatment) and “S” (solid cover crop) appeared to have higher amount (20% or more) of branch-chained esters such as ethyl isobutyrate, isobutyl acetate, ethyl 2-methylbutyrate, ethyl 3-methylbutyrate, ethyl phenylacetate, phenylethyl acetate, r-nonalactone and β -ionone (raspberry aroma). However, due to large variation of fermentation treatment, these differences were not found to be statistically significant by one-way ANOVA. Other esters, terpenoids and β -damascenone did not show any difference among the treatments. These preliminary results are surprising and need to be confirmed in multiple years at different sites.

Table 1 Concentrations of Aroma Compounds in Pinor Nior wine Made from Grape undergoing Different Cover Crop Treatments and without irrigation in Vineyard(ug/L).

Compounds	Clean	SD	Alternate	SD	Solid	SD
ethyl isobutyrate	102.34	7.18	112.90	3.99	85.15	16.90
isobutyl acetate	253.54 ^a	4.75	218.51 ^b	15.43	175.89 ^c	25.39
ethyl butyrate	527.56 ^a	1.98	443.33 ^b	16.81	437.96 ^b	50.49
ethyl 2-methyl butyrate	7.12	0.03	8.31	0.61	7.38	0.82
ethyl 3-methylbutyrate	8.04	0.08	10.37	0.81	9.52	0.91
Methylbutylacetate(mg/L)	1.35	0.01	1.40	0.15	1.41	0.19
ethyl hexanoate	645.92 ^a	6.28	638.60 ^a	50.74	524.17 ^b	28.33
hexyl acetate	11.27	0.95	11.07	0.83	8.51	0.52
ethyl octanoate	536.13 ^a	9.63	464.58 ^b	102.42	418.09 ^c	3.66
ethyl nonanoate	0.24	0.03	0.24	0.00	0.33	0.04
ethyl decanoate	173.53	1.94	165.45	5.33	161.93	3.55
ethylphenyl acetate	0.82	0.00	1.11	0.13	0.94	0.00
phenylethyl acetate	19.17	0.66	26.23	2.70	28.56	0.62
ethyl cinnamate	3.19	0.04	2.71	0.18	3.30	0.00
ethyl vanillate	858.86 ^a	66.30	551.46 ^c	33.71	668.78 ^b	7.24
hexanol(mg/L)	2.48	0.02	2.82	0.15	2.07	0.02
linalool	10.04	0.11	10.11	0.10	11.74	0.23
1-octanol	42.94	0.17	34.03	0.81	37.52	0.14
nonanol	11.93	0.30	11.24	1.22	11.77	0.35
citronellol	16.95	0.21	19.11	2.70	22.03	0.52
nerol	1.74	0.17	2.11	0.09	2.21	0.21
t-beta damascenone	7.53	0.33	6.82	0.59	7.03	0.18
hexanoic acid(mg/L)	4.91	0.36	4.91	0.72	3.96	0.20
geraniol	10.94	0.48	10.68	0.34	11.81	0.55
benzenemethanol	1083.25	119.60	912.90	111.16	835.90	21.50
benzeneethanol(mg/L)	23.96 ^b	1.36	32.08 ^a	2.84	31.69 ^a	1.21
beta ionone	0.17	0.01	0.15	0.01	0.15	0.01
gamma nonalactone	6.06 ^b	0.40	4.96 ^c	0.28	8.18 ^a	0.17
nerolidol	4.59 ^b	0.35	10.40 ^a	1.44	10.13 ^a	0.92
octanoic acid(mg/L)	4.02	0.07	3.67	0.46	3.58	0.19
eugenol	3.44 ^a	0.17	2.43 ^b	0.01	3.62 ^a	0.23
nonanoic acid	6.04 ^b	0.62	6.84 ^b	0.03	9.05 ^a	0.95
delta dodecalactone	2.72 ^b	0.11	2.57 ^b	0.10	3.33 ^a	0.47

Treatment C: Complete tillage”(no cover crop);A: alternative row cover crop;

S: solid cover crop. The result was the mean of two analysis; SD: standard deviation

Table 2 Concentrations of Aroma Compounds in Pinor Nior wine Made from Grape undergoing Different Cover Crop Treatments and with regular irrigation in Vineyard(ug/L)

	Clean	Sd	Alternate	Sd	Solid	Sd
ethyl isobutyrate	91.39	5.30	94.57	3.30	102.03	3.04
isobutyl acetate	270.54 ^a	6.96	221.70 ^b	11.12	183.36 ^c	10.66
ethyl butyrate	575.87 ^b	36.27	634.78 ^a	126.40	378.83 ^c	29.74
ethyl 2-methyl butyrate	7.06	0.14	8.25	0.47	9.01	0.01
ethyl 3-methylbutyrate	7.58	0.08	9.94	0.17	10.93	0.07
Methyl butylacetate(mg/L)	1.64	0.04	1.52	0.09	1.33	0.01
ethyl hexanoate	533.20 ^a	32.22	471.10 ^b	5.91	513.07 ^a	76.47
hexyl acetate	12.49	0.47	7.46	0.17	8.32	1.47
ethyl octanoate	579.31 ^a	40.04	456.54 ^b	26.98	377.00 ^c	37.28
ethyl nonanoate	0.26	0.03	0.33	0.04	0.26	0.03
ethyl decanoate	190.28 ^a	6.56	166.55 ^b	2.19	147.78 ^b	3.85
ethylphenyl acetate	0.69	0.10	0.85	0.01	1.15	0.10
phenylethyl acetate	21.89	4.68	30.46	3.03	27.44	3.62
ethyl cinnamate	3.17	0.66	2.94	0.11	3.82	0.51
ethyl vanillate	735.89 ^a	129.98	616.47 ^b	17.35	629.09 ^b	123.64
hexanol(mg/L)	2.40	0.14	1.91	0.09	2.00	0.02
linalool	8.92	0.68	9.43	1.03	9.94	0.12
1-octanol	46.56	0.62	47.11	2.81	37.62	1.30
nonanol	10.94	0.54	13.96	1.44	12.39	0.07
citronellol	11.33	0.13	10.57	2.64	22.61	1.43
nerol	1.88	0.08	1.63	0.31	2.23	0.58
t-beta-damascenone	8.49	0.52	6.96	0.23	7.02	0.34
hexanoic acid(mg/L)	5.66	0.43	6.54	0.84	3.20	0.25
geraniol	11.50	0.93	11.79	0.83	11.58	0.23
benzenemethanol	933.75	49.06	820.47	10.03	739.94	49.87
benzeneethanol(mg/L)	22.66 ^b	1.81	32.61 ^a	4.08	32.73 ^a	3.14
beta ionone	0.19 ^a	0.02	0.14 ^b	0.03	0.15 ^b	0.01
gamma nonalactone	4.55 ^b	0.04	7.10 ^a	1.19	7.01 ^a	0.24
nerolidol	5.24 ^c	0.20	7.39 ^b	0.49	10.09 ^a	1.34
octanoic acid(mg/L)	4.44	0.18	4.73	0.56	3.18	0.06
eugenol	3.40	0.14	3.04	0.03	3.00	0.03
nonanoic acid	5.84 ^b	0.31	6.73 ^a	1.65	9.32 ^a	2.52

delta dodecalactone	3.17	0.21	2.81	0.33	2.98	0.11
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Wine grape analysis (2007 samples, 2008 samples are still in analysis):

Grape Samples

Two batches of Pinot Noir grape samples were analyzed. One harvested on 2007.10.04 (Batch 1), and the other on 2007.09.21 (Batch 2). There were 15 samples in batch 1, which were cataloged to 3 different treatments. Sample C was grape received the “clean cultivation” treatment in vineyard with five replications (C1, C2, C3, C4 and C5). Analogously, “A” received the “alternative row cover crop” treatment and “S” the “solid cover crop” treatment. There were 30 samples in batch 2; samples received the same treatment at two different vineyard spots (“I” and “D”).

Sample treatment

Grape sample was placed in a plastic bag, crushed by hand after thawed in cold water. The puree was settled for about 5 min to extract the aroma compounds into the juice, and then was centrifuged for 20 min at 7000 rpm. The supernatant was filtered through filter papers (Whatman No. 1 filter paper with particle retention 11 μm , followed by VWR 413 filter paper with particle retention 5 μm). The clear juice was used for free and bound aroma analysis.

Free aroma compound analysis in grape juice

The 10 mL of grape juice, 10 mL of citrate buffer, and 6 g of sodium chloride were mixed in a 20 mL vial. 20 μL of internal standard solution was also added to the vial. A pre-cleaned Twister stir bar was placed into the vial and stirred for 3 hours at a speed of 1000rpm. After extraction, the stir bar was rinsed with Milli-Q water, dried with Kimwipe tissue paper, and placed into the glass sample holder of the thermal desorption system autosampler tray for GC-MS analysis.

Bound glycoside precursor analysis in grape juice

Bound glycoside precursor analysis was also achieved by SBSE-GC-MS after they were separated using BAKERBONDTM SPE C18 disposable extraction columns and then enzyme-acid hydrolyzed. Each 10 mL of grape juice was loaded onto a C18 cartridge which was pre-conditioned with 10mL of methanol followed with 10 mL of Milli-Q water. Then the cartridge was washed with 10 mL of Milli-Q water and then 20 mL of dichloromethane. The glycoside extracts were finally eluted from the cartridge with 6 mL of methanol into a 40 mL vial, and concentrated to dryness at 45°C, 100rpm under vacuum. 20 mL of citrate buffer solution and 100 μL of Macer8TM FJ enzyme solution were added into the dried glycoside extracts. The mixtures were incubated at 45°C for 20 hours. After enzymatic hydrolysis, the mixture was cooled to room temperature, and 6 g of sodium chloride as well as 20 μL of internal standard solution were added to the vial. A pre-cleaned Twister stir bar was placed into the vial and stirred for 3 hours at a speed of 1000rpm. After extraction, the stir bar was rinsed with Milli-Q water, dried with Kimwipe tissue paper, and placed into the glass sample holder of the thermal desorption system autosampler tray.

GC-MS analysis

The analytes were thermally desorbed at the TDU in splitless mode and cryofocused in a CIS 4 (Gerstel) at -80 ° C with liquid nitrogen prior to injection. A solvent vent injection was employed and the temperature of the PTV was programmed from -80 °C to 250 °C at a rate of 10 °C/sec. A RTX-1 column (60m*0.25mm*0.25um) was used to separate the analytes. The oven temperature was programmed at 40 °C for a 2 min holding, then to 80 °C at 6 °C/min, to 210 °C at 3 °C/min and to 270 °C at 6 °C/min with 2 min holding. The mass selective detector in the scan mode was used for acquiring the data. The concentrations were calculated based on the standard curves of the corresponding compounds.

Internal Standards:

An internal standard solution contained 7.01ppm of 4-octanol, 9.99ppm of isobutyl heptanone and 10.36ppm of octyl propionate in methanol, and stored at -15°C.

Quantification Method:

Each standard stock solution was mixed and diluted with citrate buffer, combined with a 20 µL of internal standard solution and 6g a sodium chloride. The stir bar extraction and GC-MS procedure was same to the sample analysis. The calibration curve built up by plotting y-axis as peak area ration of target compounds to internal standard and x-axis as concentration ratio of target compounds to internal standard.

Statistical analysis

Comparison of treatments was achieved by one-way ANOVA and Bonferroni significant difference with the statistical software of Orgin6.1.

Results:

Totally, 25 important aroma compounds in experimental wines were analyzed in this study, which included 15 esters, 6 terpenoids, 2 norisoprenoids, 1 alcohol and 1 lactone. All those target compounds were previously reported as key aroma compounds in wines. Based on their biochemical formation, they could be either varietal aroma or yeast fermented aroma. Generally, most of esters are fermentation derived, and their concentrations are more controlled by the yeast and fermentation condition. Therefore, it probably is difficult to correlate esters to cover crop treatment. Terpenoids and norisoprenoids are varietal aroma, which is the expression of the environment-genotype interaction. Their concentration is assumed to be correlated to vineyard cover crop treatment. However, since all these compounds are important to wine flavor, they were compared individually from each treatment.

“C” treatment (clean cultivation) was completely bare soil, which was considered as control, and the amount of each aroma compound was assumed as 100%. Although compared to “C”, “A” (alternative row treatment) and “S” (solid cover crop) had higher percentage of some aroma compound (Table 1), one-way ANOVA indicated that there was no significant difference among

the treatments. There is no significant difference for bound aroma compounds among the treatments.

Table 1 Content of free aroma compounds in Pinot Nior grape with different covercrop treatment (ppb) (10.04.2007)

COMPOUND	C	A	S	P-value
4-octanol (IS)	7.01	7.01	7.01	
pentanal	nd	nd	nd	
hexanal	505±113	516.2±86.9	566.1±157.0	p = 0.7111
t-2-hexenal	586.1±161.7	776.1±548.0	621.0±172.6	p = 0.6596
c-3-hexenol	13.8±9.0	13.2±8.1	15.6±5.0	p = 0.8729
1-hexanol	225.2±22.5	268.9±69.3	238.2±82.0	p = 0.5482
heptanal	1.6±0.2	1.7±0.3	1.6±0.3	p = 0.6352
2-heptanol	4.2±2.1	4.6±2.5	4.8±1.8	p = 0.8802
heptanol	1.0±0.6	1.2±0.6	1.3±1.0	p = 0.7050
1-octen-3-ol	4.5±0.6	5.9±0.9	5.3±1.5	p = 0.1517
octanal	1.4±0.1	1.5±0.2	1.4±0.03	p = 0.1229
t,t-2,4-heptadienal	nd	nd	nd	
2-ethyl-hexanol	0.8±0.5	0.6±0.3	1.1±0.4	p = 0.1797
2-octenal	3.5±0.4	3.4±0.3	3.6±0.3	p = 0.4712
octanol	16±1.0	15.1±1.9	15.5±1.0	p = 0.8116
nonanal	2.2±0.1	2.3±0.1	2.2±0.1	p = 0.1294
t-2-nonenal	2.3±0.04	2.3±0.1	2.3±0.1	p = 0.9084
t,t-2,4-nonadienal	nd	nd	nd	
isobutyl heptanone(IS)	9.99	9.99	9.99	
limonene	nd	nd	nd	
t-beta-ocimene	nd	nd	nd	
t-linalool oxide	nd	nd	nd	
c-linalool oxide	nd	nd	nd	
a-terpimolene	nd	nd	nd	
linalool	nd	nd	nd	
a-isophorone	nd	nd	nd	
4-ketoisophorone	nd	nd	nd	
citronellal	nd	nd	nd	
menthol	nd	nd	nd	
4-terpineol	nd	nd	nd	
myrtenal	nd	nd	nd	
a-terpineol	nd	nd	nd	
citronellol	nd	nd	nd	
nerol	1.3±0.9	1.0±0.6	2.2±0.9	p = 0.0771
citral	nd	nd	nd	
geraniol	7.3±4.3	4.8±2.8	11.5±4.3	p = 0.0501
octyl propionate (IS)	10.36	10.36	10.36	
t-beta-damascenone	nd	nd	nd	
alpha-ionone	nd	nd	nd	
geranyl acetone	nd	nd	nd	
beta-ionone	nd	nd	nd	

Treatment C: clean cultivation

Treatment A: alternative row treatment

Treatment S: solid cover crop

I and D: two different vineyard spots

Value presented as Mean \pm standard deviation

Table 2 Content of bound aroma compounds in Pinot Nior grape with different covercrop treatment(ppb) (10.04.2007)

COMPOUND	CI	AI	SI	P-value
4-octanol (IS)	7.01	7.01	7.01	
pentanal	nd	nd	nd	
hexanal	7.5±2.3	13.0±7.0	9.9±5.5	p = 0.2867
t-2-hexenal	78.2±35.5	128.8±74.5	101.0±63.9	p = 0.4377
c-3-hexenol	3.6±2.0	2.0±1.5	2.1±2.7	p = 0.8869
1-hexanol	64.7±1.0	93.8±29.7	85.6±40.0	p = 0.3176
heptanal	1.2±0.03	1.3±0.1	1.3±0.04	p = 0.4331
2-heptanol	1.9±0.8	2.5±0.9	2.1±1.4	p = 0.6313
heptanol	3.6±1.8	3.1±2.5	3.4±1.4	p = 0.6313
1-octen-3-ol	3.6±0.2	4.1±0.7	4.2±1.1	p = 0.6313
octanal	1.5±0.2	1.2±0.7	1.6±0.2	p = 0.6313
t,t-2,4-heptadienal	nd	nd	nd	
2-ethyl-hexanol	0.56±0.48	1.2±1.0	0.7±0.5	p = 0.2615
2-octenal	3.9±0.4	3.9±0.4	3.9±0.2	p = 0.9088
octanol	4.1±1.2	4.8±2.2	4.4±0.7	p = 0.7769
nonanal	2.1±0.1	2.2±0.1	2.2±0.1	p = 0.4038
t-2-nonenal	2.3±0.1	2.4±0.2	2.4±0.2	p = 0.7045
t,t-2,4-nonadienal	0.7±0.9	1.0±0.9	1.0±0.9	p = 0.7993
isobutyl heptanone(IS)	9.99	9.99	9.99	
limonene	nd	nd	nd	
t-beta-ocimene	3.3±2.6	3.7±2.9	4.4±0.9	p = 0.7478
t-linalool oxide	39.5±23.3	46.1±31.2	44.6±36.7	p = 0.9402
c-linalool oxide	62.6±35.8	71.0±40.8	70.8±51.0	p = 0.9394
a-terpimolene	4.8±1.6	5.3±1.8	5.4±2.0	p = 0.8575
linalool	0.8±1.8	2.0±2.3	2.2±2.3	p = 0.5827
a-isophorone	0.40±0.8	0.4±0.6	1.4±2.0	p = 0.3754
4-ketoisophorone	49.9±11.6	44.6±13.0	68.3±21.3	p = 0.0840
citronellal	0.6±0.6	1.9±1.6	1.1±0.8	p = 0.2013
menthol	nd	nd	nd	
4-terpineol	nd	nd	nd	
myrtenal	3.6±3.2	3.2±2.5	4.1±3.7	p = 0.9070
a-terpineol	139.3±46.3	99.8±31.2	148.4±76.1	p = 0.3568
citronellol	2.17±2.27	3.0±2.5	3.4±3.4	p = 0.7719
nerol	3.495±1.4	4.3±1.1	4.3±1.6	p = 0.6234
citral	nd	nd	nd	
geraniol	15.6±8.0	20.6±8.5	21.5±8.9	p = 0.5087
octyl propionate (IS)	10.36	10.36	10.36	
t-beta-damascenone	11.0±4.4	16.6±7.9	15.6±6.3	p = 0.3540
alpha-ionone	nd	nd	nd	
geranyl acetone	1.7±1.1	1.6±1.1	1.6±1.2	p = 0.9922
beta-ionone	nd	nd	nd	

Table 3 Content of free aroma compounds in Pinot Nior grape with different covercrop treatment(ppb) (09.21.2007)

COMPOUND	CI	AI	SI	P-value
4-octanol (IS)	7.01	7.01	7.01	
pentanal	nd	nd	nd	
hexanal	91.7±19.6	133.9±43.2	122.9±95.4	p = 0.7350
t-2-hexenal	328.3±41.3	505.8±84.3	449.2±178.4	p = 0.2766
c-3-hexenol	0.00±0	0.00±0	3.7±4.5	p = 0.1117
1-hexanol	197.7±24.9	208.1±31.2	199.4±41.2	p = 0.9393
heptanal	1.4±0.04	1.4±0.1	1.4±0.1	p = 0.9720
2-heptanol	2.1±0.3	2.6±0.9	2.0±0.7	p = 0.5563
heptanol	0.7±0.1	0.5±0.2	0.7±0.2	p = 0.4859
1-octen-3-ol	3.7±0.2	3.9±0.3	3.9±0.3	p = 0.7578
octanal	1.2±0.4	0.8±0.5	1.1±0.4	p = 0.6516
t,t-2,4-heptadienal	nd	nd	nd	p = 0.3966
2-ethyl-hexanol	nd	nd	nd	
2-octenal	3.2±0.1	3.3±0.1	2.6±1.0	p = 0.3054
octanol	29.1±9.2	20.3±11.5	25.5±12.7	p = 0.6782
nonanal	2.2±0.1	2.4±0.1	2.3±0.1	p = 0.3100
t-2-nonenal	2.2±0.02	2.30±0.098	2.3±0.1	p = 0.4765
t,t-2,4-nonadienal	1.7±0.0	1.7±0.0	1.4±0.5	p = 0.3858
isobutyl heptanone(IS)	9.99	9.99	9.99	
limonene	nd	nd	nd	
t-beta-ocimene	nd	nd	nd	
t-linalool oxide	nd	nd	nd	
c-linalool oxide	nd	nd	nd	
a-terpimolene	nd	nd	nd	
linalool	nd	nd	nd	
a-isophorone	nd	nd	nd	
4-ketoisophorone	nd	nd	nd	
citronellal	nd	nd	nd	
menthol	1.01±1.352	1.40±1.396	2.3±2.9	p = 0.7751
4-terpineol	nd	nd	nd	
myrtenal	nd	nd	nd	
a-terpineol	nd	nd	nd	
citronellol	nd	nd	nd	
nerol	0.7±0.4	1.4±0.7	1.2±0.4	p = 0.3821
citral	nd	nd	nd	
geraniol	5.4±4.4	8.1±3.9	9.6±2.8	p = 0.4782
octyl propionate (IS)	10.36	10.36	10.36	
t-beta-damascenone	nd	nd	nd	
alpha-ionone	nd	nd	nd	
geranyl acetone	nd	nd	nd	
beta-ionone	nd	nd	nd	

Table 4 Content of bound aroma compounds in Pinot Nior grape with different covercrop treatment(ppb) (09.21.2007)

COMPOUND	CI	AI	SI	P-value
4-octanol (IS)	7.01	7.01	7.01	
pentanal	nd	nd	nd	
hexanal	5.1±0.8	6.9±2.79	7.0±2.5	p = 0.5082
t-2-hexenal	35.0±6.2	41.3±9.4	38.2±6.2	p = 0.5273
c-3-hexenol	2.1±1.2	2.5±2.0	1.7±1.3	p = 0.7730
1-hexanol	78.2±11.4	86.3±17.6	86.9±7.5	p = 0.6924
heptanal	1.2±0.1	1.3±0.04	1.3±0.03	p = 0.3376
2-heptanol	1.8±0.3	1.8±0.3	1.6±0.4	p = 0.5460
heptanol	1.5±0.9	1.7±1.0	1.8±0.8	p = 0.5460
1-octen-3-ol	3.1±0.1	3.2±0.1	3.1±0.1	p = 0.4521
octanal	2.0±0.5	2.0±0.5	1.9±0.4	p = 0.9621
t,t-2,4-heptadienal	nd	nd	nd	
2-ethyl-hexanol	nd	nd	nd	
2-octenal	3.5±0.1	3.5±0.2	3.6±0.1	p = 0.4967
octanol	53.9±39.1	57.1±39.8	57.3±40.8	p = 0.9914
nonanal	2.2±0.1	2.2±0.07	2.2±0.1	p = 0.7028
t-2-nonenal	2.4±0.04	2.5±0.08	2.5±0.1	p = 0.0647
t,t-2,4-nonadienal	nd	nd	nd	
isobutyl heptanone(IS)	9.99	9.99	9.99	
limonene	nd	nd	nd	
t-beta-ocimene	2.0±1.1	1.7±0.9	2.1±1.1	p = 0.8540
t-linalool oxide	23.2±15.1	39.1±9.5	39.0±11.9	p = 0.2505
c-linalool oxide	35.7±21.3	53.3±12.9	59.2±19.5	p = 0.2886
a-terpimolene	3.7±0.8	4.1±0.6	4.9±1.0	p = 0.1714
linalool	0.56±1.028366	1.5±0.6	2.1±1.0	p = 0.1366
a-isophorone	0.00±0	0.00±0	0.36±0.5696	p = 0.3966
4-ketoisophorone	26.4±17.6	32.9±8.2	39.2±19.0	p = 0.5873
citronellal	nd	nd	nd	
menthol	nd	nd	nd	
4-terpineol	nd	nd	nd	
myrtenal	4.2±4.8	8.8±3.5	8.5±2.3	p = 0.2692
a-terpineol	165.7±72.1	308.6±83.5	275.2±44.6	p = 0.0745
citronellol	3.0±2.8	5.4±2.1	7.6±2.6	p = 0.1357
nerol	4.0±1.8	5.3±1.1	6.2±1.3	p = 0.1997
citral	nd	nd	nd	
geraniol	10.2±7.5	15.4±4.7	19.8±6.1	p = 0.1820
octyl propionate (IS)	10.36	10.36	10.36	
t-beta-damascenone	6.1±2.1	11.3±2.7	10.3±2.1	p = 0.0490
alpha-ionone	0.00±0	0.00±0	0.00±0	
geranyl acetone	1.4±1.2	2.3±0.9	2.7±1.0	p = 0.3640
beta-ionone	nd	nd	nd	

Table 5 Content of free aroma compounds in Pinot Nior grape with different covercrop treatment(ppb) (09.21.2007)

COMPOUND	CD	AD	SD	P-value
4-octanol (IS)	7.01	7.01	7.01	
pentanal	nd	nd	nd	
hexanal	116.1±75.2	100.8±30.5	110.1±95.4	p = 0.9595
t-2-hexenal	414.2±174.9	531.8±196.8	407.6±203.0	p = 0.6133
c-3-hexenol	0.00±0	0.00±0	0.00±0	
1-hexanol	233.5±92.0	191.2±59.1	214.7±84.7	p = 0.7668
heptanal	1.4±0.1	1.5±0.02	1.3±0.1	p = 0.0223
2-heptanol	2.3±0.8	1.9±0.5	2.4±1.6	p = 0.7975
heptanol	0.3±0.1	0.7±0.5	0.5±0.2	p = 0.1942
1-octen-3-ol	4.3±0.5	4.0±0.4	4.1±1.2	p = 0.8135
octanal	1.1±0.4	1.1±0.6	0.5±0.7	p = 0.3121
t,t-2,4-heptadienal	nd	nd	nd	
2-ethyl-hexanol	nd	nd	nd	
2-octenal	2.7±1.1	1.9±1.7	2.7±1.5	p = 0.6719
octanol	23.5±6.6	22.7±18.1	28.6±9.4	p = 0.7373
nonanal	2.5±0.1	2.4±0.1	2.3±0.1	p = 0.2418
t-2-nonenal	2.3±0.1	2.3±0.1	2.3±0.18	p = 0.8987
t,t-2,4-nonadienal	1.7±0.01	1.7±0.0	1.4±0.89	p = 0.3918
isobutyl heptanone(IS)	9.99	9.99	9.99	
limonene	nd	nd	nd	
t-beta-ocimene	nd	nd	nd	
t-linalool oxide	nd	nd	nd	
c-linalool oxide	nd	nd	nd	
a-terpimolene	nd	nd	nd	
linalool	nd	nd	nd	
a-isophorone	nd	nd	nd	
4-ketoisophorone	nd	nd	nd	
citronellal	nd	nd	nd	
menthol	5.9±4.7	2.0±2.8	1.8±3.0	p = 0.2524
4-terpineol	nd	nd	nd	
myrtenal	nd	nd	nd	
a-terpineol	0.26±0.36	nd	nd	p = 0.3042
citronellol	nd	nd	nd	
nerol	1.6±0.6	0.7±0.63	1.2±0.7	p = 0.1846
citral	nd	nd	nd	
geraniol	11.1±5.1	5.5±3.4	8.4±5.2	p = 0.3064
octyl propionate (IS)	10.36	10.36	10.36	
t-beta-damascenone	nd	nd	nd	
alpha-ionone	nd	nd	nd	
geranyl acetone	nd	nd	nd	
beta-ionone	nd	nd	nd	

Table 6 Content of bound aroma compounds in Pinot Nior grape with different covercrop treatment(ppb) (09.21.2007)

COMPOUND	CD	AD	SD	P-value
4-octanol (IS)	7.01	7.01	7.01	
pentanal	nd	nd	nd	
hexanal	5.9±3.9	4.2±0.9	5.8±3.4	p = 0.6847
t-2-hexenal	40.1±15.3	45.6±16.7	29.5±12.1	p = 0.3381
c-3-hexenol	2.7±1.2	1.6±1.5	1.8±1.3	p = 0.5442
1-hexanol	90.5±7.4	82.0±18.1	92.8±13.9	p = 0.4744
heptanal	1.2±0.04	1.0±0.6	1.3±0.15	p = 0.4520
2-heptanol	2.0±0.3	1.9±0.2	1.9±0.3	p = 0.9211
heptanol	1.1±0.1	1.4±1.0	1.7±0.7	p = 0.4552
1-octen-3-ol	3.4±0.1	3.3±0.2	3.3±0.3	p = 0.4572
octanal	1.6±0.3	1.4±0.1	1.9±0.6	p = 0.3204
t,t-2,4-heptadienal	nd	nd	nd	
2-ethyl-hexanol	nd	nd	nd	
2-octenal	3.6±0.1	3.5±0.1	3.4±0.34	p = 0.3356
octanol	24.1±29.6	6.0±0.6	42.5±50.1	p = 0.34160
nonanal	2.3±0.1	2.3±0.2	2.5±0.4	p = 0.3189
t-2-nonenal	2.4±0.1	2.5±0.03	2.5±0.1	p = 0.3890
t,t-2,4-nonadienal	nd	nd	nd	p = 0.6186
isobutyl heptanone(IS)	9.99	9.99	9.99	
limonene	nd	nd	nd	
t-beta-ocimene	2.8±1.5	2.0±1.7	2.5±2.1	p = 0.82180
t-linalool oxide	40.9±5.9	27.9±12.0	37.4±23.1	p = 0.4290
c-linalool oxide	59.1±10.7	46.7±18.2	55.5±31.9	p = 0.6802
a-terpimolene	4.5±0.6	4.2±0.8	3.9±2.59	p = 0.8459
linalool	1.6±0.5	1.1±1.3	1.7±1.9	p = 0.7997
a-isophorone	0.1±0.1	0.00±0	1.0±1.7	p = 0.2498
4-ketoisophorone	49.8±10.8	35.4±9.9	36.7±31.0	p = 0.4969
citronellal	nd	nd	nd	
menthol	nd	nd	nd	
4-terpineol	nd	nd	nd	
myrtenal	9.8±3.5	5.2±3.8	7.0±4.0	p = 0.2829
a-terpineol	306.8±91.1	214.6±124.2	237.3±70.8	p = 0.4040
citronellol	7.8±2.1	5.9±3.2	7.4±3.8	p = 0.6206
nerol	6.4±1.0	5.6±1.2	5.8±1.6	p = 0.6464
citral	nd	nd	nd	
geraniol	18.6±3.0	18.0±5.5	18.9±6.9	p = 0.9685
octyl propionate (IS)	10.36	10.36±0	10.36	
t-beta-damascenone	11.4±1.6	7.9±3.4	9.2±3.5	p = 0.2399
alpha-ionone	0.00±0	0.00±0	0.00±0	
geranyl acetone	2.3±0.9	1.7±0.6	2.3±1.3	p = 0.6131
beta-ionone	nd	nd	nd	