2010 OWB final progress report

Project Title:

Effects 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
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:

Wine grape analysis:

Grape Samples

Two batches of Pinot Noir grape samples were analyzed. One cultivated in Stoller vineyard, and the other cultivated in Archery Summit vineyard. There were 30 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). Samples received the same treatment at two different vineyard spots ("I" and "D"). Analogously, "A" received the "alternative row cover crop" treatment and "S" the "solid cover crop" treatment. There were 15 samples in batch 2.

Sample treatment

Grape sample was blended using the liquid nitrogen, 50 grams grape powder mixed with 50 ml 0.2M citric buffer (pH=3.2) and 30 grams sodium chloride. Whole mixture was extracted at room temperature for 24 hours without light, and then centrifuged for 30 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

Twenty mL of grape juice extract were placed 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 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 extracted using BAKERBOND™ SPE C18 disposable extraction columns and then hydrolyzed by enzyme-acid. Each 20 mL of grape juice extract was loaded onto a C18 cartridge that was pre-conditioned with 10 mL of methanol followed by 10 mL of Milli-Q water. After sample loading, 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 45ºC, under vacuum. An aliquot of citrate buffer solution (20 mL) and 100 µL of Macer8™ FJ enzyme solution were added into the dried glycoside extracts. The mixtures were incubated at 45ºC 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 to extract the released aglycones for GC-MS analysis.

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GC-MS analysis

The analytes were thermally desorbed at the TDU unit in splitless mode and cryofocused in a CIS 4 (Gerstel) at -80 º C with liquid nitrogen. A solvent vent injection was employed and the temperature of the PTV was programmed from -80 ºC to 250 ºC at a rate of 10 ºC/sec. A HP-5 MS column (60m*0.32mm*0.25um) was used to separate the analytes. The oven temperature was programmed at 40 ºC for a 2 min holding, then to 220 ºC at 4 ºC/min, to 250 ºC at 8 ºC/min with 6 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 9.4 mg/L of 3-hexanone, 2.4 mg/L of 4-octanol, 15.1 mg/L of octyl propionate and 1.3 mg/L naphthalene in methanol, and stored at -15ºC.

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

The S-PLUS Version 7.0 software (Insightful Corp., Seattle, WA) was used to test the statistical variances of volatile constituents. The volatile compounds were grouped into three categories: C6 compounds, terpene alcohols, and norisoprenoids. The concentration of each compound among the different treatments was analyzed by the analysis of variance (ANOVA).

Results:

In this study, SBSE-GC-MS technique was used to analyze the concentration of C6 compounds, terpene alcohols, and norisoprenoids in 'Pinot noir' grapes. The free volatiles were directly extracted by SBSE and analyzed by GC-MS. The precursors were first extracted using C18 SPE chromatography. After the enzymatic hydrolysis, the released aglycones were analyzed by SBSE GC-MS. Volatiles and volatile precursors in grapes with different cover crop treatment from two vineyards were shown in **Table 1-3**.

Volatile analysis of 2008 grapes shown similar trends on irrigated grape and non-irrigated grape aroma composition, this is in agreement with Patty Skinkis's data that cover crop treatment didn't show any effect on the in-row vine water status and soil moisture in 2008.

However, some nitrogen (N) competition was observed in 2008 with clean treatment (C) vines having the highest N content compared to solid treatment (S) and alternative treatment (A). We see clear impact of cover crops managements on grape volatile composition. These changes could be related to N status.

C6 Compounds. C6 compounds are also called 'green' compounds. Generally, the origin of C6 compounds is related mainly to the lipoxygenase activity of the grape. C6 alcohols and aldehydes have the herbaceous and green odors, and are related to detrimental effects in the wine if present in concentrations above their sensorial perception limits. However, in most of cases, these compounds will disappear after wine fermentation process. The concentration of individual C6 compound including hexanal, *trans*-2-hexenal, and hexanol are present in **Table 1-3**. Grapes with solid treatment show higher concentration in free form C6 compound. Similar trends discovered in both Stoller vineyard and AS vineyard.

C6 aldehydes and alcohols contribute to green notes, although most of these compounds are oxidized during wine making process, the concentration of C6 compounds could be used as markers for grape maturity and overall grape quality. Although the difference was not statistically significant in many cases, a general trend was observed. Alternative cover had lowest free hexanal, t-2-hexenal, in irrigated treatment in Stoller (table 1), lowest free haxanal, t-2-hexenal, hexanol, and glycoside bound hexanol in non-irrigated treatment in Stoller (Table 2). This trend also true for the experiment in AS and lower content C6 was observed in alternate cover crop treatment. In addition, a general increasing trend of free C6 aldehyde was observed for solid cover crop treatment. A similar response was observed from a separate soil nutrient study that low soil nitrogen increases free C6 aldehyde formation in Pinot noir grapes. Since the contents of C6 compounds are typically negatively associated with grape maturity, these initial results suggest that alternative cover crop treatment could give better mature grape.

Terpene Alcohols. Terpene alcohols are a diverse class of natural products. Monoterpenes, particularly linalool, geraniol, nerol, and citronellol can contribute important floral and citrus characters to grapes and wines. Terpene alcohols also exist as aglycones, which will release the aroma molecule by acidic or enzymatic hydrolysis during winemaking. The concentrations of five terpene alcohols including *trans*-linalool oxide, *cis*-linalool oxide, linalool, α-terpineol, and geraniol in the grapes were determined in the present study. The concentrations of free, and glycoside of each individual terpene alcohol compound are presented in **Table 1-3**. Most of *trans*-linalool oxide and *cis*-linalool oxide exist in the glycoside form. The bound form of linalool, α-terpineol, and geraniol was over ten times of the amount of the free form. Solid treatment shows the highest concentration of free form terpene alcohols, however, alternative treatment has highest concentration of glycoside form terpene alcohols. Similar trends discovered in both Stoller vineyard and AS vineyard.

The impact of cover crops managements on terpene alcohols in grapes is complex because terpene alcohol is a large class of compounds and metabolism is unique for each compound. The free terpene alcohol contents are very low in Pinot noir grapes, and majority of them are present at glycoside precursor forms. These glycosides could be converted to free form during wine making and maturation process, and contribute to wine aroma. The individual terpene alcohol responded well with cover crop management with statistical differences observed for many compounds such as linalool (p-value<0.05), geraniol (p-value <0.005), *trans*-linalool oxide (p-value <0.05), and *cis*-linalool oxide (p-value<0.01).

For simplified comparison, the total terpenoids (both free and glycosidically bound) were lowest in solid cover crops under both irrigated and non-irrigated treatment in Stoller (Table 1 and 2) and also lower in AS vineyard (Table 3) although it is comparable with alternative cover treatment. Similarly, clean cover treatment had two out of three highest total terpene alcohols. Results suggested that solid cover could yield low terpene alcohols and clean cover could yield higher terpene alcohols, contribute positively to wine aroma.

Norisoprenoids. Norisoprenoids are important odorants in wines and are thought to originate from carotenoid degradation present in grapes. In present study, we analysis both free and bound form of beta-damascenone, beta-ionone. Alternative cover crop treatment showed the highest concentration of beta-damascenone precursors. Similar trends discovered in both Stoller vineyard and AS vineyard.

C13-isoprenoids including β-damascenone, β-ionone and α-ionone are very important to red wine aroma. β-ionone only exist as free form and its content was not affected by cover crop management. This is in agreement with our several other studies that β-ionone does not response with viticultural practices such as deficit irrigation and nutrient manipulation. β-Damascenone can be exist in free form or precursors. Cover crop treatment had clear impact for free β-damascenone, clear cover crop treatment had highest β-damascenone and solid cover crop treatment had the lowest. This trend was consistent for both irrigated and non-irrigated in Stoller vineyard (Table 1 and 2) and AS vineyard (Table 3). For the precursor form of β-damascenone, however, the alternative treatment seemed to have the highest content.

In summary, 2008 volatile study clearly demonstrated that cover crop management had impact on grape quality. Solid cover crop treatment yielded grape with the highest free C6 aldehydes, lowest terpene alcohols, lowest C13 isoprenoids. Alternative cover crop management yielded grapes with lowest C6 aldehyde, and clean cover crop management had the highest free β-damascenone.

Table 1. Concentrations of volatile Compounds in Irrigated Cover Crop management Pinot Noir grape harvested in Stoller vineyard in 2008 (μg/kg±SD).

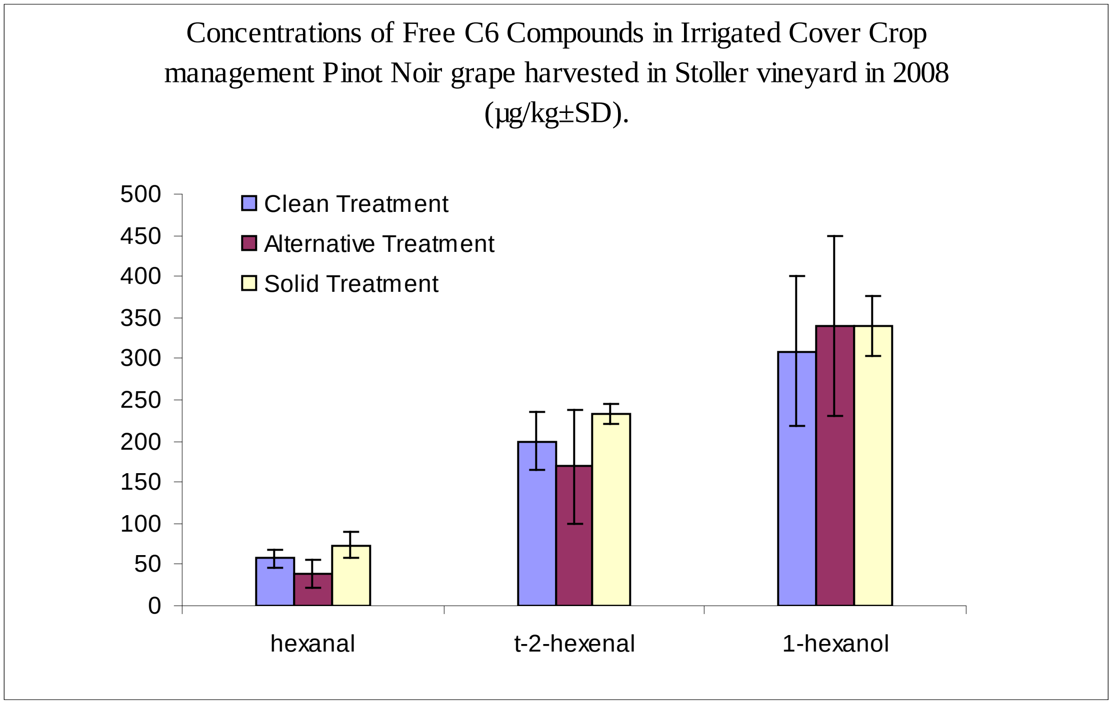
C6 compounds		CI	AI	SI	P-value
Free	Hexanal	57±11	39±17	73±16	0.01
	Trans-2-hexenal	200±34	168±69	234±12	0.12
	Hexanol	309±90	340±109	340±37	0.81
Glycoside	Hexanol	545±115	407±151	393±64	0.11
Terpenoids	Total	516	457	348	
Free	*Linalool*	0.8±0.1	1.5±0.2	1.8±0.3	0.00
	α-terpineol	2.3±0.5	2.4±0.8	2.9±0.2	0.22
	Geraniol	4.6±1.0	4.3±1.0	4.7±1.0	0.74
Glycoside	*Trans*-linalool oxide	108±31	116±37	81±13	0.17
	Cis-linalool oxide	94±28	100±34	67±10	0.14
	Linalool	12±2	13±2	11±1	0.27
	α-terpineol	280±88	204±35	166±36	0.03
	Geraniol	14±3	16±3	14±2	0.37
Norisoprenoids					
Free	β-damascenone	1.39±0.25	1.02±0.12	0.86±0.19	0.00
	β-ionone	0.05±0.01	0.07±0.01	0.06±0.01	0.11
	α-ionone	-	-	-	-
Precursors	β-damascenone	9.6±1.2	11.2±2.1	7.0±0.9	0.00

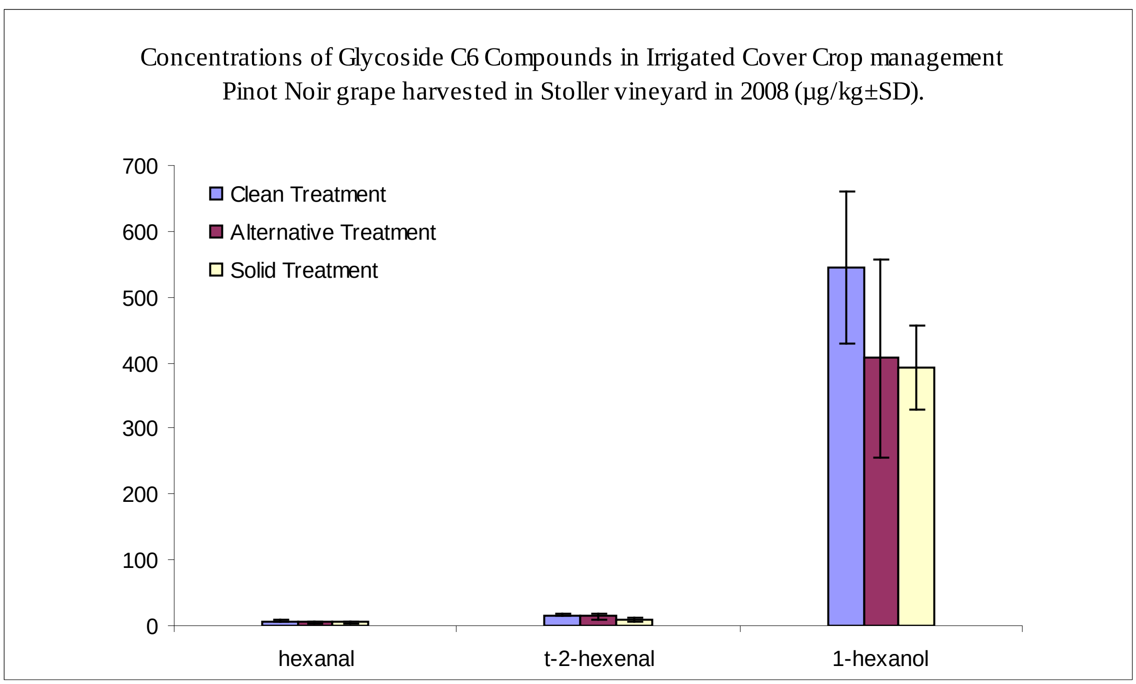
Table 2. Concentrations of volatile Compounds in Non-irrigated Cover Crop management Pinot Noir grape harvested in Stoller vineyard in 2008 (µg/kg±SD).

C6 compounds		CD	AD	SD	P-value
Free	Hexanal	58±8	37±11	70±11	0.00
	Trans-2-hexenal	181±39	181±29	258±55	0.02
	Hexanol	344±45	313±80	383±96	0.38
Glycoside	Hexanol	505±55	378±44	433±104	0.05
Terpenoids	Total	419	494	354	
Free	Linalool	1.0±0.2	1.8±0.4	2.0±0.3	0.00
	α-terpineol	2.3±0.4	3.3±0.4	3.2±0.5	0.01
	Geraniol	4.8±0.9	4.9±1.2	6.5±1.8	0.13
Glycoside	*Trans*-linalool oxide	97±23	120±13	79±14	0.01
	Cis-linalool oxide	84±18	102±15	67±10	0.01
	Linalool	10±1	13±2	12±0.8	0.01
	α-terpineol	208±47	232±68	167±26	0.16
	Geraniol	12±1	17±3	17±1	0.00
Norisoprenoids					
Free	β-damascenone	1.23±0.19	1.10±0.05	1.00±0.25	0.18
	β-ionone	0.05±0.01	0.06±0.01	0.07±0.01	0.20
	α-ionone	-	-	-	-
Glycoside	β-damascenone	8.6±0.4	10±0.5	7.0±0.4	0.00

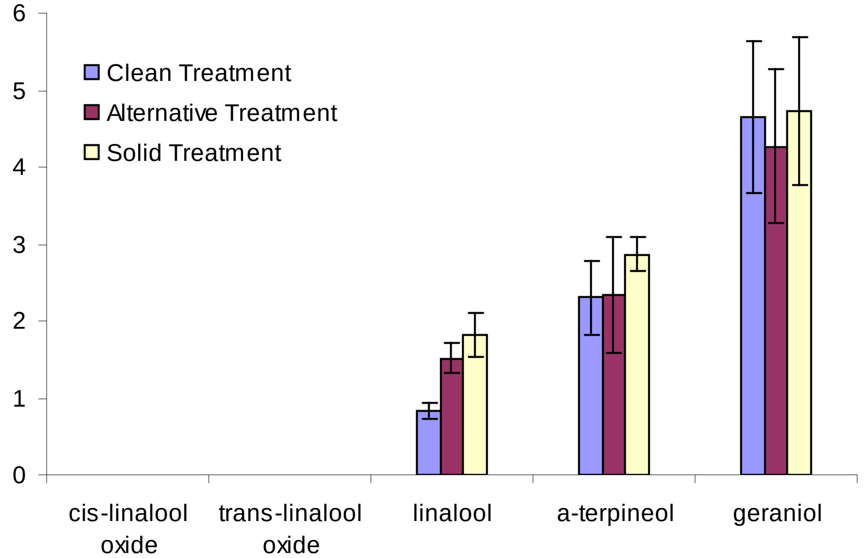
Table 3. Concentrations of volatile Compounds in Cover Crop management Pinot noir grape harvested in AS vineyard in 2008 (µg/kg±SD).

C6 compounds		C	A	S	P-value
Free	Hexanal	95±10	71±17	95±26	0.15
	T-2-hexenal	402±104	344±109	368±118	0.73
	Hexanol	662±186	505±39	484±94	0.10
Glycoside	Hexanol	439±111	301±52	374±23	0.05
Terpenoids	total	501	460	457	
	Linalool	1.79±0.30	1.75±0.25	1.78 ±0.29	0.97
	α-terpineol	3.54±0.42	3.54±0.79	2.75±0.67	0.13
	Geraniol	5.95±0.77	5.88±1.16	7.73±1.71	0.08
Glycoside	*Trans*-linalool oxide	118±35	93±4	106±8	0.27
	Cis-linalool oxide	94±28	71±11	78±9	0.19
	Linalool	12±2	10±1	12±1	0.38
	α-terpineol	250±39	260±67	232±24	0.54
	Geraniol	16±2	15±2	17±3	0.37
Norisoprenoids					
Free	β-damascenone	1.94±0.21	1.53±0.19	1.11±0.23	ND
	β-ionone	0.05±0.01	0.06±0.01	0.07±0.02	0.24
	α-ionone	-	-	-	-
Glycoside	β-damascenone	10.6±1.6	10±1.0	9.0±1.4	0.21

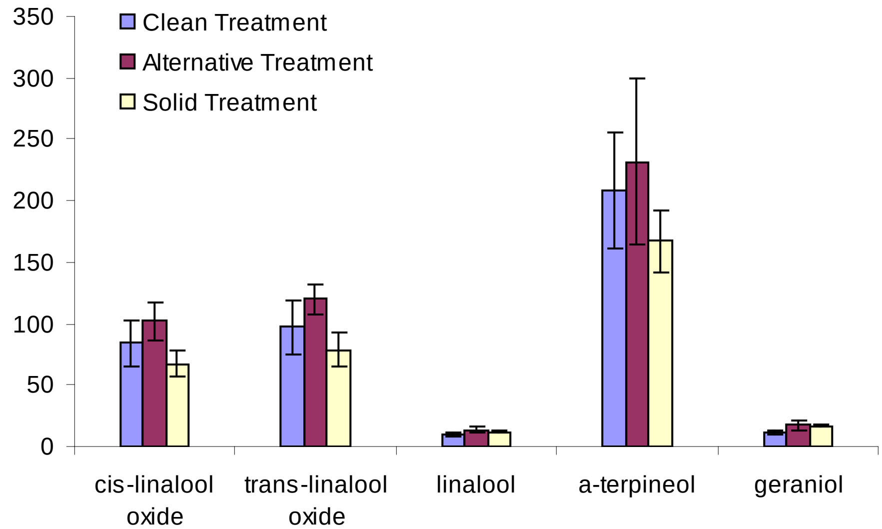

Concentrations of Free C6 Compounds in Irrigated Cover Crop management Pinot Noir grape harvested in Stoller vineyard in 2008 (µg/kg±SD).
500
450
400
350
300
250
200
150
100
50
0
Clean Treatment
Alternative Treatment
Solid Treatment
hexanal
t-2-hexenal
1-hexanol

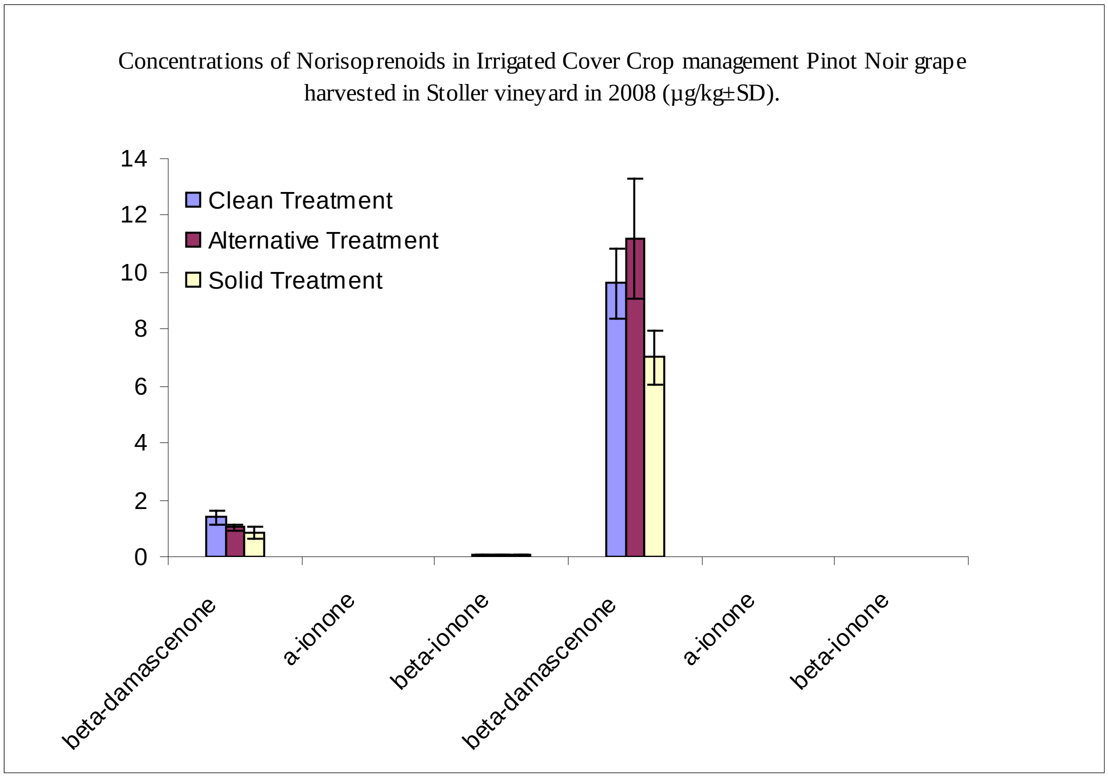

Concentrations of Glycoside C6 Compounds in Irrigated Cover Crop management Pinot Noir grape harvested in Stoller vineyard in 2008 (µg/kg±SD).
700
600
500
400
300
200
100
0
Clean Treatment
Alternative Treatment
Solid Treatment
hexanal
t-2-hexenal
1-hexanol

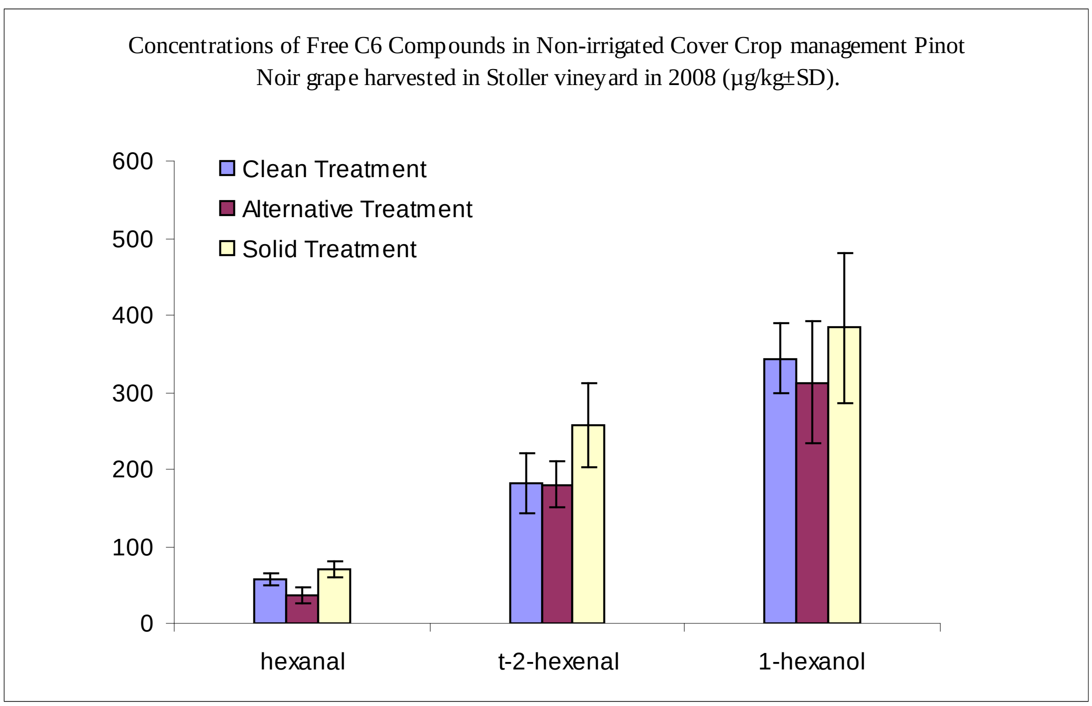
Concentrations of Free Terpene Alcohols in Irrigated Cover Crop management Pinot Noir grape harvested in Stoller vineyard in 2008 (µg/kg±SD).

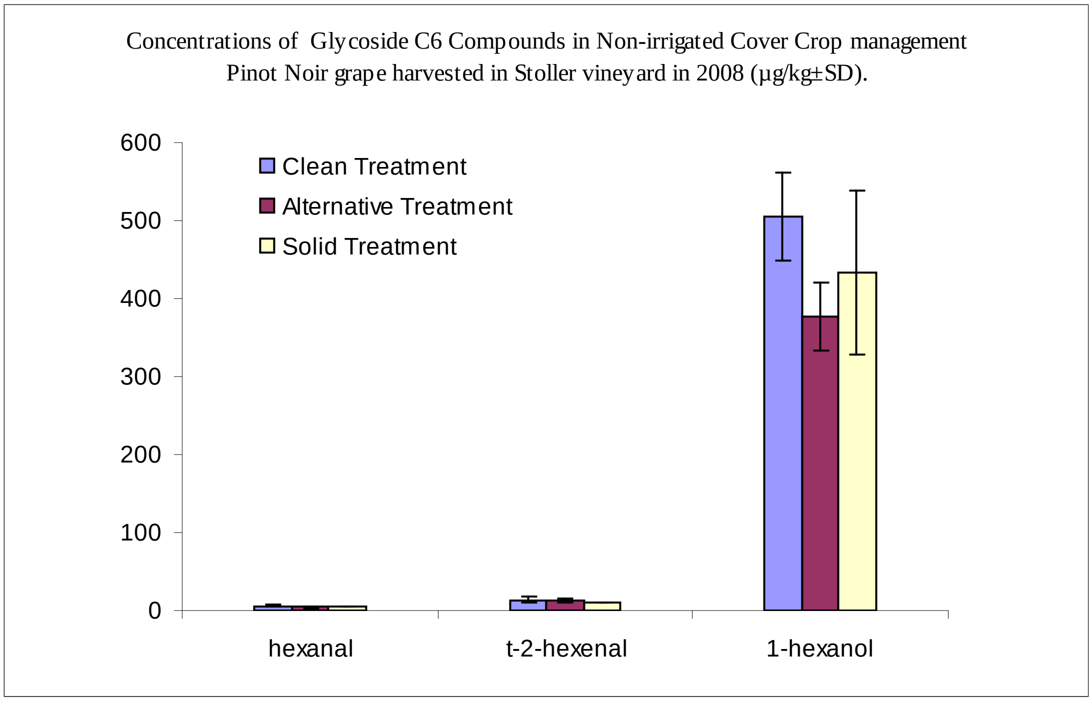


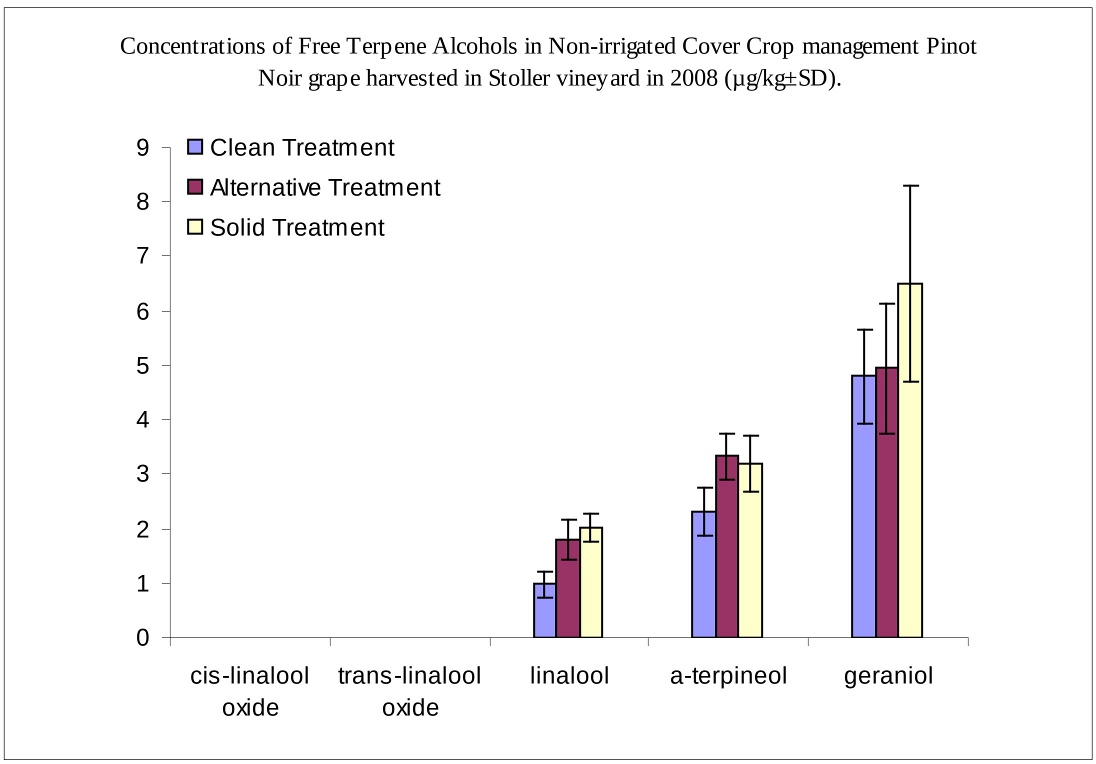
Concentrations of Glycoside Terpene Alcohols in Irrigated Cover Crop management Pinot Noir grape harvested in Stoller vineyard in 2008 (µg/kg±SD).



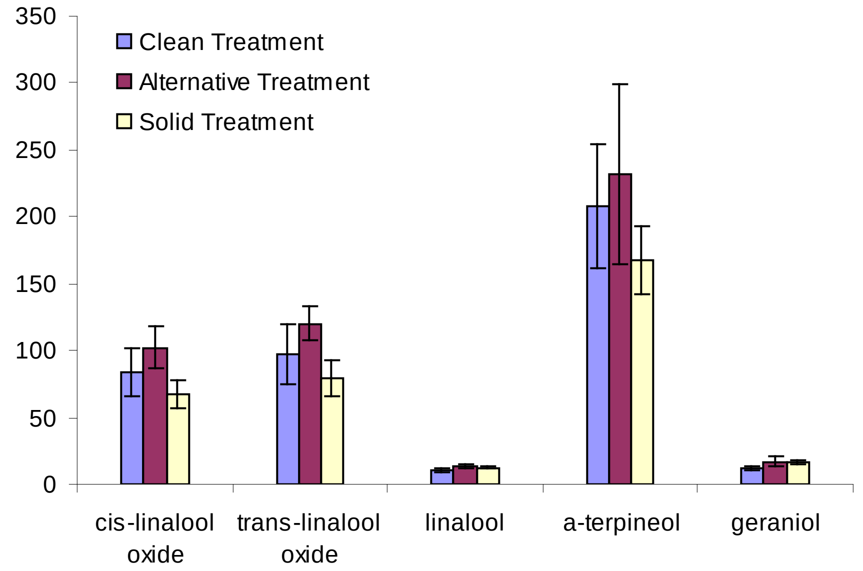

Concentrations of Norisoprenoids in Irrigated Cover Crop management Pinot Noir grape harvested in Stoller vineyard in 2008 (µg/kg±SD).
14
12
10
8
6
4
2
0
Clean Treatment
Alternative Treatment
Solid Treatment
beta-damascenone
a-ionone
beta-ionone
beta-damascenone
a-ionone
beta-ionone


Concentrations of Free C6 Compounds in Non-irrigated Cover Crop management Pinot Noir grape harvested in Stoller vineyard in 2008 (µg/kg±SD).
600
500
400
300
200
100
0
Clean Treatment
Alternative Treatment
Solid Treatment
hexanal
t-2-hexenal
1-hexanol

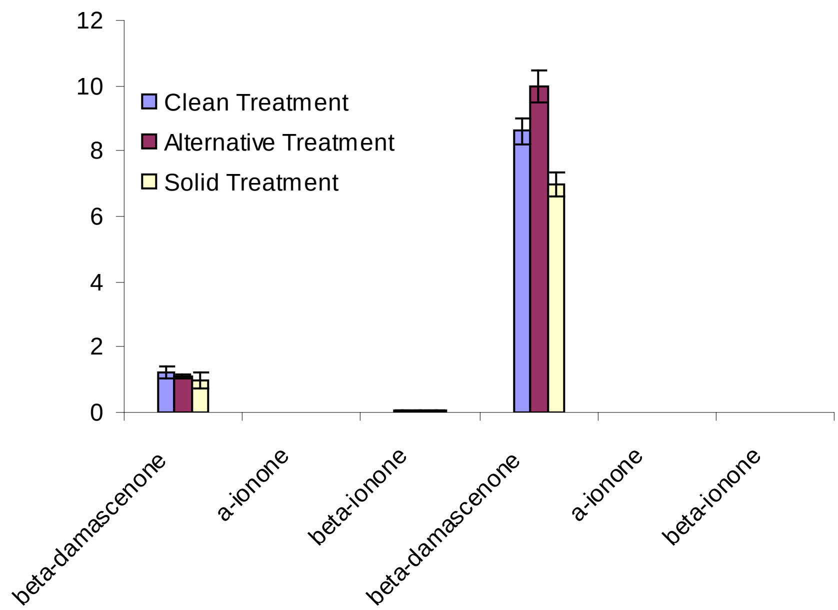

Concentrations of Glycoside C6 Compounds in Non-irrigated Cover Crop management Pinot Noir grape harvested in Stoller vineyard in 2008 (µg/kg±SD).
600
500
400
300
200
100
0
Clean Treatment
Alternative Treatment
Solid Treatment
hexanal
t-2-hexenal
1-hexanol

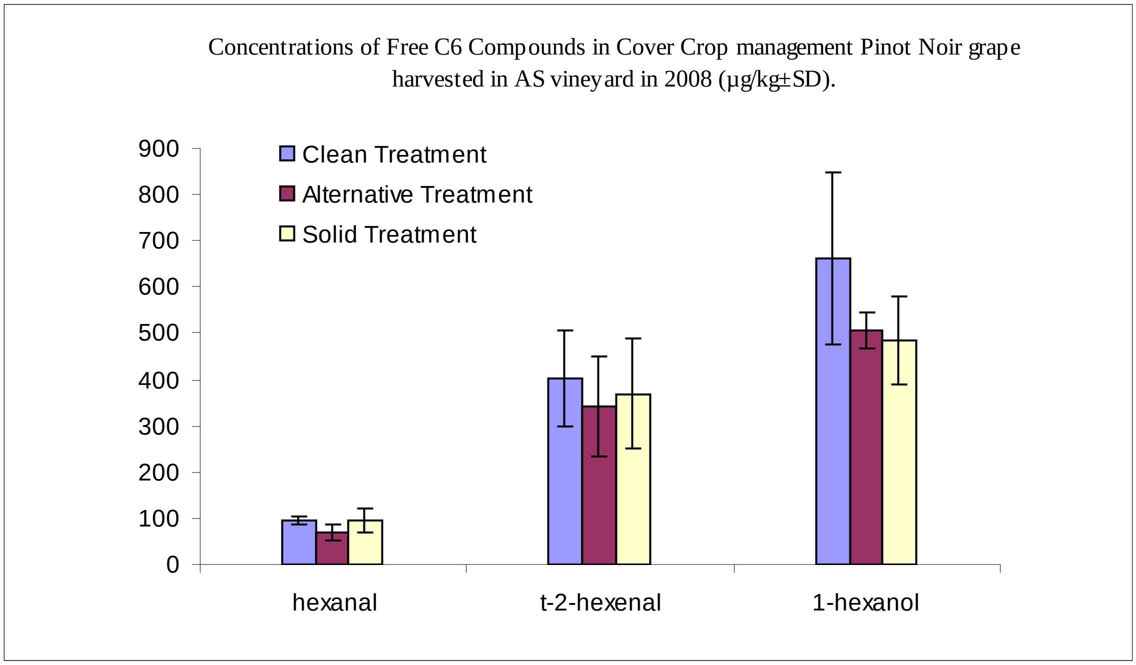

Concentrations of Free Terpene Alcohols in Non-irrigated Cover Crop management Pinot Noir grape harvested in Stoller vineyard in 2008 (µg/kg±SD).
9
8
7
6
5
4
3
2
1
0
Clean Treatment
Alternative Treatment
Solid Treatment
cis-linalool oxide
trans-linalool oxide
linalool
a-terpineol
geraniol

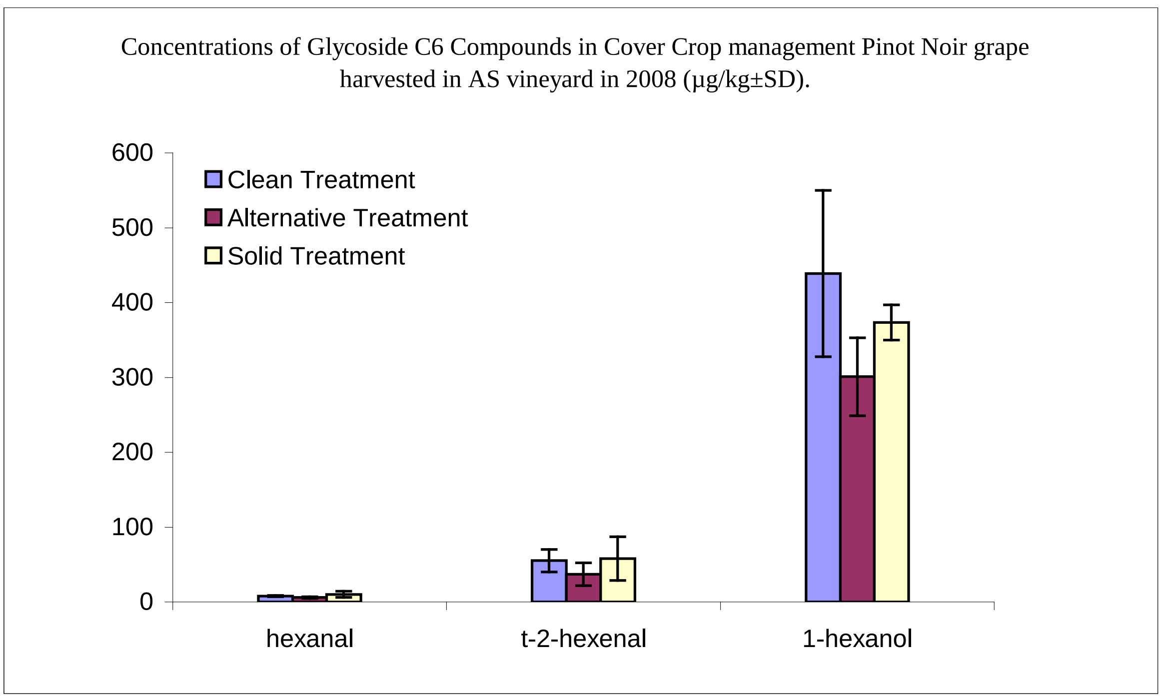
Concentrations of Glycoside Terpene Alcohols in Non-irrigated Cover Crop management Pinot Noir grape harvested in Stoller vineyard in 2008 (µg/kg±SD).



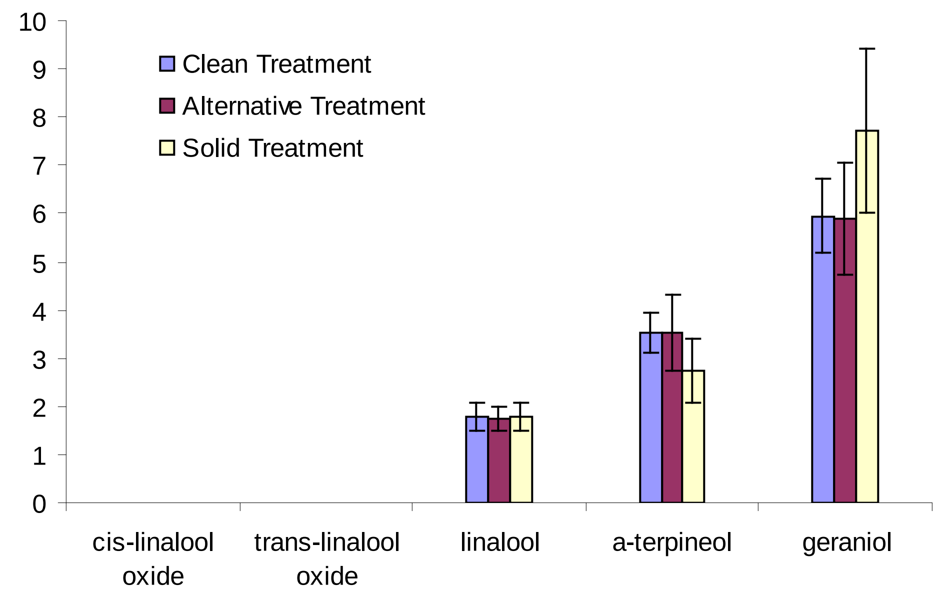
Concentrations of Norisoprenoids in Non-irrigated Cover Crop management Pinot Noir grape harvested in Stoller vineyard in 2008 (µg/kg±SD).



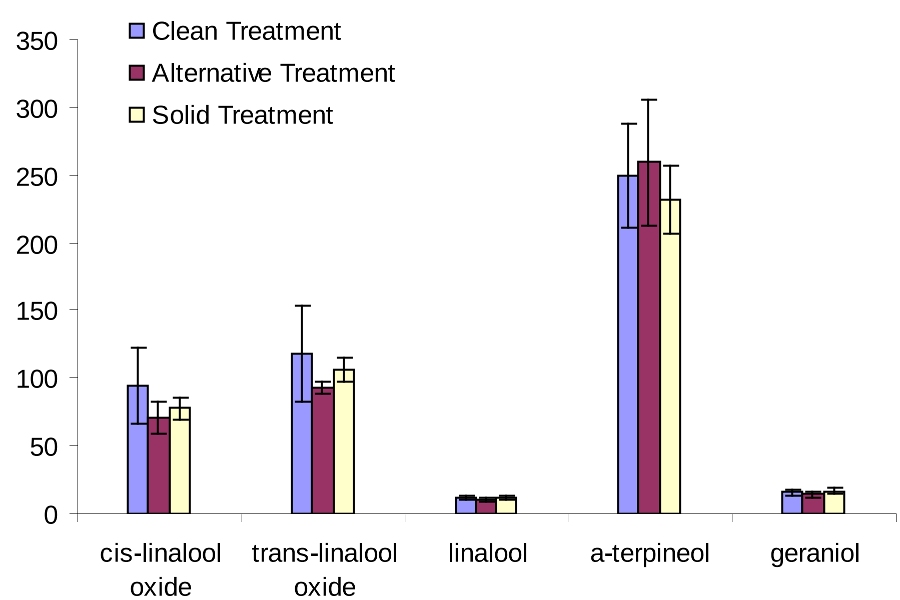

Concentrations of Free C6 Compounds in Cover Crop management Pinot Noir grape harvested in AS vineyard in 2008 (µg/kg±SD).
900
800
700
600
500
400
300
200
100
0
Clean Treatment
Alternative Treatment
Solid Treatment
hexanal
t-2-hexenal
1-hexanol

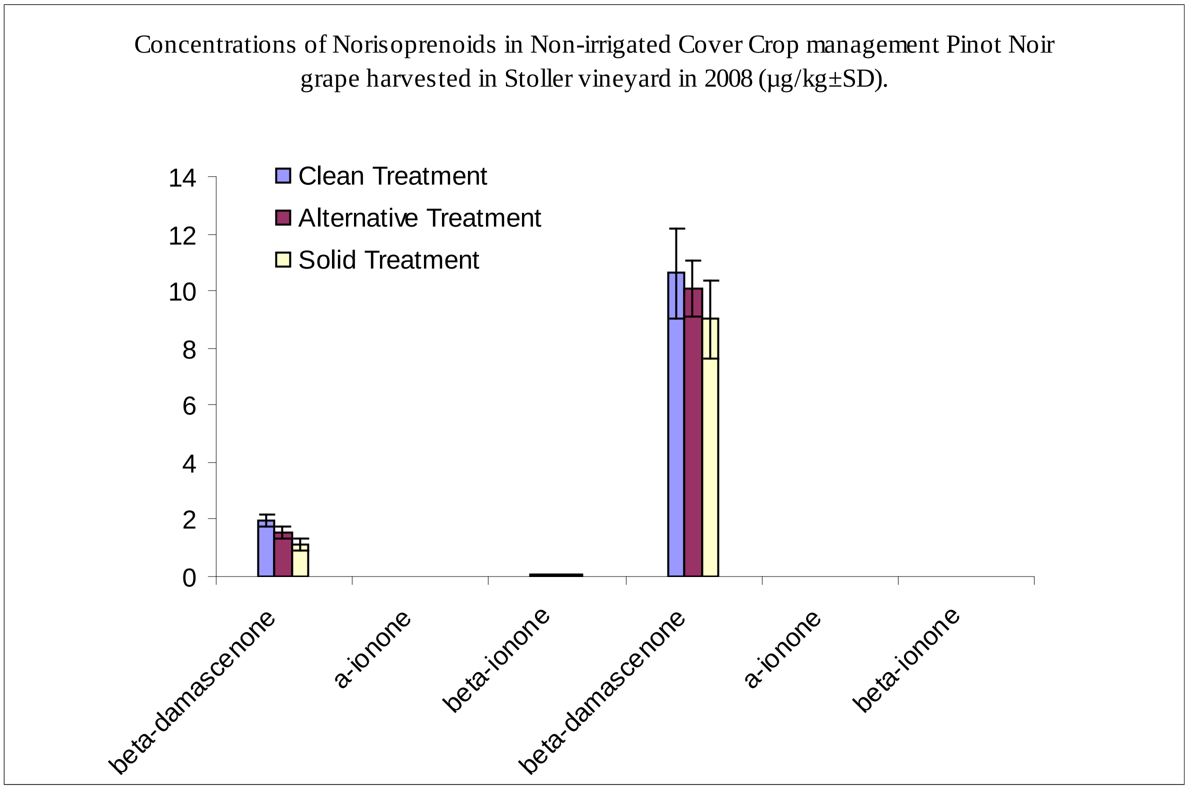

Concentrations of Glycoside C6 Compounds in Cover Crop management Pinot Noir grape harvested in AS vineyard in 2008 (µg/kg±SD).
600
500
400
300
200
100
0
Clean Treatment
Alternative Treatment
Solid Treatment
hexanal
t-2-hexenal
1-hexanol

Concentrations of Free Terpene Alcohols in Cover Crop management Pinot Noir grape harvested in AS vineyard in 2008 (μg/kg±SD).



Concentrations of GlycosideTerpene Alcohols in Cover Crop management Pinot Noir grape harvested in AS vineyard in 2008 (μg/kg±SD).




Concentrations of Norisoprenoids in Non-irrigated Cover Crop management Pinot Noir grape harvested in Stoller vineyard in 2008 (μg/kg±SD).
Clean Treatment
Alternative Treatment
Solid Treatment
0
2
4
6
8
10
12
14
beta-damascenone
a-ionone
beta-ionone
beta-damascenone
a-ionone
beta-ionone

Supplemental study: Wine Volatile Aroma Analysis (not funded by OWB)

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 -15ºC.

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 º C with liquid nitrogen. A solvent vent injection was employed and the temperature of the PTV was programmed from -80 ºC to 250 ºC at a rate of 10 ºC/sec. A RTX-1 column (60m*0.25mm*0.25um) was used to separate the analytes, and the oven temperature was programmed at 40 ºC for a 2 min holding, then to 210 ºC at 3 ºC/min, and to 270 °C at 5 ºC/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 4 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 acetatte	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
ethly 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
benzenementhanol	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 5 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 acetatte	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
ethly 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
benzenementhanol	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