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

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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'IC.

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 'I C with liquid nitrogen. A solvent vent injection was employed and the temperature of the PTV was programmed from -80 'IC to 250 'IC at a rate of 10 'IC/sec. A RTX-1 column (60m*0.25mm*0.25um) was used to separate the analytes, and the oven temperature was programmed at 40 'IC for a 2 min holding, then to 210 'IC at 3 'IC/min, and to 270 °C at 5 'IC/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. The aroma compound comparison of different vineyard cover crop treatment.

Compounds	Treatment C			Treatment A			Treatment S			p-value
	Mean	SD	R.P.	Mean	SD	R.P.	Mean	SD	R.P.	
hexyl formate (IS)										
ethyl acetate	1.097	0.135	100	1.225	0.199	111	1.179	0.119	106	0.628
ethyl isobutyrate	0.207	0.061	100	0.268	0.035	136	0.255	0.047	128	0.419
isobutyl acetate	0.150	0.028	100	0.178	0.022	122	0.183	0.054	123	0.593
ethyl butyrate	0.238	0.034	100	0.246	0.036	106	0.263	0.051	111	0.779
ethyl 2-methylbutanoate	0.031	0.009	100	0.042	0.003	145	0.042	0.002	145	0.116
ethyl 3-methylbutanoate	0.057	0.016	100	0.086	0.006	158	0.082	0.011	148	0.087
3-methylbutyl acetate	3.225	0.828	100	3.710	0.350	120	3.350	0.133	108	0.647
2-methylbutyl acetate	0.745	0.203	100	0.816	0.068	115	0.842	0.066	118	0.699
octyl propionate (IS)										
ethyl hexanoate	0.701	0.073	100	0.894	0.121	128	0.821	0.089	117	0.143
hexyl acetate	0.023	0.006	100	0.026	0.000	114	0.025	0.002	112	0.708
ethyl octanoate	1.881	0.326	100	2.122	0.249	114	2.238	0.180	120	0.319
ethyl phenylacetate	0.004	0.001	100	0.005	0.002	147	0.005	0.000	138	0.198
phenethyl acetate	0.020	0.006	100	0.029	0.002	146	0.026	0.003	131	0.145
ethyl decanoate	0.842	0.196	100	0.973	0.076	117	0.906	0.082	108	0.615
ethyl cinnamate	0.008	0.001	100	0.010	0.004	129	0.009	0.002	113	0.595
4-octanol (IS)										
linalool	0.047	0.003	100	0.049	0.003	104	0.055	0.003	117	0.068
benzeneethanol	2.383	0.178	100	2.533	0.279	107	2.572	0.396	108	0.741
citronellol	0.088	0.021	100	0.100	0.015	114	0.071	0.002	80	0.191
geraniol	0.042	0.003	100	0.042	0.004	101	0.044	0.005	105	0.793
γ-nonalactone	0.022	0.002	100	0.031	0.004	138	0.039	0.003	179	0.003
β-damascenone	0.330	0.083	100	0.359	0.024	111	0.346	0.039	106	0.859
t-β-farnesene	0.052	0.021	100	0.058	0.025	115	0.069	0.010	133	0.581
β-ionone	0.030	0.002	100	0.039	0.015	132	0.040	0.003	133	0.279
farnesol	0.437	0.082	100	0.403	0.035	91	0.533	0.025	121	0.099
nerolidol	0.713	0.080	100	0.579	0.074	81	0.924	0.087	129	0.014

Treatment C: clean cultivation
Treatment A: alternative row treatment
Treatment S: solid cover crop
Mean: the average of the ratio of the peak area of target compounds to the peak area of internal standard
SD: standard deviation
R.P.: relative percentage