

Year-end Report
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Assessment of grapevine nitrogen status and optimized nitrogen fertilization practices

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Background on research problem: The aggressive use of cover crops, including under-trellis sward, has been shown to help regulate vine size and vine vigor with overly-vigorous vines in Virginia vineyards (Hatch et al., 2011). Under-trellis cover crops favorably reduce vine size therefore improving vine balance and lowering vineyard management costs. Competition between the under the trellis cover crop and vine for the same soil water and nutrients appears to be the principal mechanism behind the reduction in vine size. Under-trellis cover crops are also important in those situations (e.g., Figure 1) where vineyards are being located on steep slopes in order to minimize the potential for soil erosion. The under-trellis (also called intra-row) cover crops are becoming more widely used in the Virginia industry and are either intentionally planted, or adopted as native vegetation (weeds). These companion crops, however, do have some undesirable effects. They can become over-competitive with vines for water, leading to drought stress. This can be avoided by judicious use of irrigation during dry weather to avoid water stress. Another problem encountered with the cover crops is that under-trellis cover crops can compete with the vines for essential nutrients, chiefly nitrogen (N). This research addresses growers' questions about how best to manage the competing goals of suppressing vine size with under-trellis cover crops, while minimizing the negative effects of those cover crops on vine and berry nitrogen status. Our goal is to develop a set of vineyard fertilization recommendations that are consistent with our Sustainable Vineyard Practices recommendations, and that promote optimal fruit composition, including acceptable levels of Yeast-Assimilable Nitrogen at harvest.



Figure 1. Glen Manor vineyard illustrating steep, hillside plantings (erosion potential).

Objectives:

- 1) Determine the most efficient method and optimal time for annual nitrogen applications, such that cover crop growth is maintained, while adequate vine nitrogen and final berry YAN are achieved.
- 2) Reassess our tissue sampling protocol and diagnostic standards for evaluating vine nitrogen nutritional status with vigorous grapevines.
- 3) Explore differences in nitrogen contributions from foliar nitrogen applications and sustainable alternatives, such as composting and utilizing leguminous cover crops.
- 4) Evaluate the influence of various nitrogen fertilization strategies on basic berry chemistry, must fermentable nitrogen levels, berry amino acid composition, and other potential wine quality attributes.

Experiment 1, Glen Manor: Four treatments were applied to eighteen-year old Sauvignon blanc vines at Glen Manor Vineyards near Front Royal VA beginning during the season and continued each year hence, including 2014. The vineyard block was identified to have a perennial problem with low N status in the vines and in the must after being managed with an under-trellis cover crop, predominately *Festuca arundinacea*. The treatments were applied to 3-vine panels on an open lyre trellising system, each replicated 6 times in a randomized, complete block experimental design.

Glen Manor treatments:

- 1) Control: no additional nitrogen added to system
- 2) 30 kg N/ha applied to soil at bloom (as calcium nitrate)
- 3) 30 kg N/ha applied to soil at boom and 30 kg N/ha applied 6 weeks post bloom (as calcium nitrate) total application of 60 kg N/ha per season
- 4) Foliar N (5kg N/ha) applied starting at bloom, 7-9 total applications equivalent to a total of 35 kg N/ha applied during the season (as urea at rate of 60 gal. water per acre application rate)

Experiment 2, Chateau O'Brien: A second experiment was added in January 2012 at Chateau O'Brien vineyard near Markham, VA. Vineyard block of interest is a ten-year-old planting of Merlot planted on a relatively steep slope where intra-row cover cropping is used to suppress soil erosion and vine vigor. The block has chronically inferior vine capacity and severely low nitrogen levels. Treatments at Chateau O'Brien were applied to 6-vine panels, replicated 5 times in a randomized, complete block experimental design. Floor management was standardized with intra-row zones (50-85-cm wide) planted to a mixed stand of *Festuca rubra* L. and native (weed) vegetation, maintained with a hand-held line trimmer.

Chateau O'Brien treatments:

- 1) Control (no additional N)
- 2) Compost, low rate (roughly 33.5 kg/ha of actual N total analysis)
- 3) Compost, high rate (roughly 67 kg/ha of actual N total analysis)
- 4) Clover and compost, low rate (roughly 33.5 kg/ha of actual N total analysis)
- 5) Clover and compost, high rate (roughly 67 kg/ha of actual N total analysis)
- 6) Calcium nitrate, low rate (15 + 15 + 0) [numbers reflect kg/hectare N at one of 3 points in time: early-season + mid-season + post-harvest]
- 7) Calcium nitrate, high rate (30 + 30 + 0)
- 8) Calcium nitrate, low rate, applied post-harvest (0 + 0 + 30)

Experiment 3, Winchester: A third experiment was implemented in June 2012, and repeated in 2013 at the Agricultural Research and Extension Center in Winchester, VA. Vineyard block of interest is a seven-year-old planting of Petit Manseng vines with under trellis cover crops, which consist primarily of *Festuca rubra* L. infested with *Trifolium arvense* L. and *Medicago lupulina* L. Treatments are in 5-vine panels, replicated 5 times in a completely randomized design. The trial was added to further explore the potential use of late-season foliar N applications to increase vine and berry N in cover cropped vines.

Winchester treatments:

- 1) Cover crop control – no nitrogen additions*
- 2) Herbicide control– no nitrogen additions*
- 3) Foliar urea application to cover cropped vines – 5 kg/ha applied 2 weeks prior to véraison, and 5kg/ha applied 1 week prior to véraison (10kg/ha total)
- 4) Foliar urea application to cover cropped vines– 5 kg/ha applied 1 week post véraison and 5kg/ha applied 2 weeks post véraison (10kg/ha total)

* All panels received 10kg/ha calcium nitrate via soil application on June 1st, 2012 and on June 13th, 2013. Given that all treatments received the same application, we plan to attribute differences in berry chemistry and other data collection to experimental treatments.

Methods

1. To determine treatments effects on vine nitrogen status, petiole tissue analyses were conducted at bloom and véraison (blades were collected at Glen Manor only) and season-long chlorophyll content index readings were recorded. Given that the nitrogen concentration of leaves has a direct impact on chlorophyll concentration and physiologic function, we were interested in monitoring its concentration with a handheld chlorophyll meter.
2. To determine treatment effects on berry nitrogen status, YAN values were measured at harvest. Our goal was to increase YAN levels to the recommended minimum of 140 mg N/L to prevent the need for N supplementation in the winery.
3. To determine treatments effects on amino acid chemistry, berry samples were collected at harvest and analyzed using ultra performance liquid chromatography (UPLC). Our interest in analyzing amino acid chemistry was due to the integral role amino acids play in the development of wine aroma compounds.
4. To determine if treatments interfered with yield or vine growth, components of yield and pruning weight data were recorded.
5. To determine treatments effects on general canopy architecture, an enhanced point-quadrat-analysis (EPQA) was performed at véraison.
6. To determine if treatments interfered with berry maturation, primary fruit chemistry (Brix, TA, pH) were determined at harvest.
7. To monitor potentially confounding variables, annual soil samples and season-long weather data of rainfall and temperature were recorded.

Results & Discussion

Sauvignon blanc (Glen Manor Vineyards)

Nitrogen content in petioles was increased by the high calcium nitrate rate at véraison 2011 and 2012 and by foliar fertilization at véraison 2011 (Table 1). Nitrogen content in blades was increased by the high calcium nitrate rate and foliar fertilization at véraison 2013 (Table 1). No treatment significantly affected CCI values at véraison in 2012, but all treatments increased average season-long CCI values in 2013, with the high calcium nitrate doing so most dramatically. Treatments had no effect on primary fruit chemistry, pruning weights, canopy density, or components of yield in any year.

Foliar fertilization consistently increased berry YAN at harvest in all three years by 29.4%, 45.2%, and 88.8% respectively (Table 2). The high calcium nitrate rate increased berry YAN in 2011 and 2013, while the low calcium nitrate rate only increased berry YAN in 2013. The high rate of calcium nitrate increased the concentrations of two amino acids (Ser and Gln) in 2012. In 2013, the low rate of calcium nitrate increased the concentration of one amino acid (Arg); the high rate of calcium nitrate increased the concentration of five amino acids (Gln, Arg, Thr, Ala, and Ile); and season long foliar nitrogen applications increased the concentration of eleven amino acids (Gln, Arg, Thr, Ala, Lys, Tyr, Met, Val, Ile, Leu, and Phe). Berries treated with season-long foliar nitrogen (Foliar-GMV) exhibited a 65.6% increase in total free amino acids (1164.77 mg/L) in 2013 (Table 3).

Merlot (Chateau O'Brien)

Nitrogen concentration in petioles was increased by both clover treatments at bloom 2013 (Table 1). No treatment significantly affected CCI values at véraison in 2012; however, the clover treatments, the high calcium nitrate rate, and the post-harvest calcium nitrate rate increased average season-long CCI values relative to the control in 2013 (Table 1). The clover combined with the high compost rate increased pruning weights in 2012, but all other treatments had no effect. Treatments had no effect on primary fruit chemistry, canopy density, or components of yield in any year.

The high calcium nitrate rate increased berry YAN at harvest by 66.2% in 2013; however, treatments had no effect on total free amino acids or individual amino acid concentrations in 2012 or 2013 (Table 2).



Figure 2. Mowing established red+crimson clover at Chateau O'Brien.

Petit Manseng (AREC)

Vines grown in the presence of an herbicide strip consistently had the greatest nitrogen concentration in petioles and the highest CCI values (Table 1). The herbicide vines exhibited a 50.0% increase in total vine yield in 2012, and a 66.6% increase in 2012 pruning weights. These findings reaffirm our research concern, in that under-trellis cover crops may decrease vine nitrogen status. Brix at 2013 harvest was decreased in herbicide vines. Treatments had no effect on canopy density, pH, or TA in any year.

The post-véraison foliar application increased berry YAN at harvest by 69.8% in 2013 (Table 2). Treatments had no effect on total free amino acids or individual amino acid concentrations in 2012. In 2013, the post-véraison foliar treatment increased the concentration of total free amino acids by 30.3%, which corresponded to an increase in 9 individual amino acids (Ser, Gln, Arg, Gly, Glu, Thr, Ala, Lys, and Met) (Table 4).

Wines made from two treatments from the Sauvignon blanc at Glen Manor Vineyards in 2013 were subjected to a trained consumer preference panel at the University of Arkansas in June 2014. The two treatments were the control (no N) and the 30 kg N/ha rate of N applied to the foliage of the vines. The panel detected subtle differences between the wines but the results were equivocal as to whether one wine was preferred over the other; thus, the sensory analyses were inconclusive.

Outcomes and Benefits Expected:

The primary objectives of this work aim to develop a set of recommendations for accurately assessing vine nitrogen status and providing guidance on the optimal means of augmenting the vine's nitrogen needs in low nitrogen environments. Results from this study varied, possibly due to the different varieties being tested, different sites under consideration, varying degrees of vineyard floor establishment, and other disparities in routine vineyard management practices (e.g., spray schedule, leaf pulling, pruning, fungicide applications, fertilization of other nutrients). Nonetheless, this study helped identify nitrogen treatments that effectively increase berry YAN and the concentration of specific amino acids, without interfering with berry ripening or inhibiting the primary purpose of utilizing cover crops to curtail vine vigor. Although no treatment in this study wholly increased all parameters of vine nitrogen status (as determined by nutrient analyses of petioles and blades, and chlorophyll fluorescence), it did provide a baseline of how growers might amend traditional fertilization approaches. Given the combined success of foliar nitrogen applications to Petit Manseng and Sauvignon blanc increasing berry YAN and individual amino acids, with the positive effects of high rates of soil-applied calcium nitrate on improving chlorophyll content and petiole nitrogen in Merlot and Sauvignon blanc, we believe a combined approach may be an effective fertilization approach that could overcome and circumvent the competition for nitrogen uptake between established cover crops and grapevines. Coupling a high rate of soil-applied calcium nitrate with a post-véraison foliar application of urea may facilitate adequate vine nitrogen status while significantly increasing berry YAN and individual amino acids, thereby boosting the potential to improve the overall aroma profile of resultant wines. The results from this study suggest establishing clover as the under-trellis cover crop may aid in improving vine nitrogen content; as such, future work could focus on improving establishment of clover in vineyards and potentially combining leguminous cover crops with post-véraison foliar nitrogen applications.

This work was presented by DeAnna DeAttilio, with appropriate attribution of the funding sources, at the Virginia Vineyards Association's winter technical meeting (January 2014), and as a poster at the American Society of Enology and Viticulture annual meeting in Austin, TX

(June 2014). In addition, Tony Wolf presented aspects of the work at the Eastern Wine Expo in Lancaster, PA (March 2014).

Future Work:

A renewal proposal was submitted but declined in 2014. We feel, however, that certain aspects of the work should be continued for several reasons. The first is that we still have questions about the value of legumes as contributors of nitrogen to grapevines. We also believe that naturally assimilated nitrogen may be much less expensive to apply as urea to vines, than as yeast supplements in the winery. Finally, there remain industry questions about the YAN concentrations deemed adequate or desirable to optimize wine stylistic goals. We (Drs. Amanda Stewart and Tony Wolf) are therefore supporting and advising an additional student (James Russell Moss) with further field and lab studies with nitrogen adjustments to the vines.

Table 1 –Treatment effect on tissue concentration of Nitrogen in petioles and chlorophyll content index.

Sampling Period	% Nitrogen concentration in petioles						Chlorophyll Content Index	
	Bloom 2011	Véraison 2011	Bloom 2012	Véraison 2012	Bloom 2013	Véraison 2013	Véraison 2012	Season-long 2013
Sauvignon blanc (Glen Manor)								
Control	0.88	0.43b	0.89	0.81b	0.82	0.52	14.92	15.24 c
Low Soil	0.88	0.47ab	0.85	0.91ab	0.80	0.53	14.68	17.15 b
High Soil	0.88	0.48a	0.86	0.94a	0.86	0.55	15.99	19.20 a
Foliar	0.88	0.48a	0.89	0.84ab	0.81	0.52	16.32	17.29 b
Significance	n/a	0.0112	ns	0.0138	ns	ns	ns	<.0001
Merlot (Chateau O'Brien)								
Control			1.06	0.74	0.86c	0.63	19.14	12.94 cd
Low Compost			1.06	0.75	0.96bc	0.63	18.98	12.25 d
High Compost			1.06	0.75	0.88c	0.62	19.34	15.69bc
Clover+Low Compost			1.06	0.74	1.16ab	0.60	20.58	17.44 ab
Clover+High Compost			1.06	0.72	1.23a	0.62	21.48	17.21 ab
Low Soil			1.06	0.77	0.97bc	0.63	19.75	14.92 bcd
High Soil			1.06	0.82	0.92bc	0.67	21.41	20.39 a
Post Harvest			1.06	0.77	0.93bc	0.61	20.02	17.81ab
Significance			n/a	ns	<.0001	ns	ns	<.0001
Petit Manseng (AREC)								
CC Control				0.40	0.80	0.61 b	14.72 b	15.33b
Herbicide Control				0.48	0.92	0.81 a	18.39 a	19.68a
Pre-Véraison Foliar				0.40	0.84	0.68 ab	17.13 ab	17.74ab
Post-Véraison Foliar				0.40	0.79	0.63 b	16.82 ab	17.01b
Significance				n/a	ns	0.0072	0.0012	0.0003

Table 2. Treatment effect on yeast assimilable nitrogen levels at harvest.

Yeast Assimilable Nitrogen (mg N/L)			
Year	2011	2012	2013
Sauvignon blanc (Glen Manor)			
Control	119 b	157 b	125 b
Low Soil	138 ab	175 b	189 a
High Soil	154 a	174 b	194 a
Foliar	154 a	228 a	236 a
Significance	0.0097	0.0061	0.0005
Merlot (Chateau O'Brien)			
Control		73	65 b
Low Compost		58	66 b
High Compost		63	73 ab
Clover+Low Compost		75	94 ab
Clover+High Compost		69	94 ab
Low Soil		78	75 ab
High Soil		88	108 a
Post Harvest		67	100 ab
Significance		ns	0.0090
Petit Manseng (AREC)			
CC Control		147	212 b
Herbicide Control		147	263 b
Pre-Véraison Foliar		138	249 b
Post-Véraison Foliar		222	360 a
Significance		ns	0.0003

Table 3. Treatment effects on amino acid concentration (mg/L) in Sauvignon blanc juice at harvest 2013.

Free amino acid concentration (mg/L)					
Treatment					
Amino Acids	Significance	Control	Low Soil	High Soil	Foliar
His	ns	46.45	46.05	48.78	56.03
Ser	0.0046	32.63b	41.89ab	44.29ab	53.33a
Gln	0.0064	46.30b	69.94ab	77.78a	87.06a
Arg	0.0006	103.04b	194.70a	200.19a	269.44a
Gly	ns	3.39	4.02	4.33	4.82
Asp	0.0318	25.03ab	22.97b	26.83ab	29.95a
Glu	ns	108.55	119.71	134.40	139.75
Thr	0.0010	39.11b	54.85ab	60.26a	73.03a
Ala	0.0043	86.65b	117.95ab	134.94a	147.35a
Pro	ns	158.14	195.53	202.66	222.31
Lys	0.0227	2.75b	3.29ab	3.43ab	4.12a
Tyr	0.0369	4.27b	5.90ab	5.30ab	6.12a
Met	0.0169	2.43b	2.911ab	2.64b	4.29a
Val	0.0230	18.98b	23.01ab	24.26ab	26.82a
Ile	0.0056	9.81b	12.44ab	13.12a	14.11a
Leu	0.0147	9.47b	12.34ab	13.24ab	14.31a
Phe	0.0237	8.45b	9.81ab	10.10ab	11.93a
Total:	0.0063	703.47b	937.33ab	1006.64ab	1164.77a
Number AA significantly increased by treatment			1	5	11

Table 4. Treatment effects on amino acid concentration (mg/L) in Petit Manseng juice at harvest 2013.

Amino Acids	Significance	Free amino acid concentration (mg/L)			
		Treatment			
		CC Control	Herb Control	Pre-Véraison	Post-Véraison
His	ns	65.12	68.52	63.91	78.45
Ser	0.0101	80.38b	105.93ab	100.07ab	145.72a
Gln	0.0042	67.72b	90.48ab	80.06b	135.76a
Arg	0.0131	284.74b	371.32ab	357.62ab	612.26a
Gly	0.0076	6.17b	7.34ab	7.82ab	10.14a
Asp	0.0265	28.63	43.05	30.70	40.68
Glu	0.0069	36.35b	50.37ab	40.78b	58.74a
Thr	0.0268	90.00b	115.40ab	110.65ab	158.66a
Ala	0.0003	37.34b	57.44b	48.67b	86.35a
Pro	ns	2309.71	2520.20	2284.17	2591.74
Lys	0.0096	3.44b	4.43ab	4.83b	3.64a
Tyr	ns	24.57	26.25	26.99	36.55
Met	0.0061	5.11b	6.17b	5.57b	9.36a
Val	ns	43.99	51.41	48.56	58.94
Ile	ns	26.46	27.67	28.77	25.99
Leu	ns	40.87	43.40	41.00	48.40
Phe	ns	21.35	24.97	23.58	30.11
Total:	ns	3171.94	3616.37	3299.80	4135.46
Number AA significantly increased by treatment	--	--	0	0	9