Year-End Report
Virginia Wine Board, July 31, 2013

Title: Assessment of grapevine nitrogen status and optimized nitrogen fertilization practices

Principal Investigator:

Tony K. Wolf
AHS Jr. Agricultural Research and Extension Center
Virginia Tech
595 Laurel Grove Rd., Winchester VA 22602
(540) 869-2560 extn. 18 vitis@vt.edu

Type of Project: Research
Amount funded: $39,975

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) To reassess our tissue sampling protocol and diagnostic standards for evaluating vine nitrogen nutritional status with vigorous grapevines.

2) To determine optimal rates, materials, and timing of nitrogen fertilization in situations where companion cover crops are grown under the trellis to regulate vine growth and/or to minimize the potential for soil erosion. We aim to provide growers with recommendations to amend low vine nitrogen nutritional status for vines with adequate vigor and for small, weak vines.
3) To 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 thirteen-year old Sauvignon blanc vines at Glen Manor Vineyards near Front Royal VA during the 2011 season. 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 over the past 5 years. 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.

We have utilized many methods of data collection to date to evaluate the effects of different nitrogen fertilizers, rates and application methods on Sauvignon blanc vines. Nutrition analysis for nitrogen status has been measured by petiole and blade samples, chlorophyll content index, and berry YAN. Soil samples were collected to consider the confounding role of soil fertility on other measured parameters. Ceptometer readings were recorded to assess light exposure and an enhanced point-quadrat-analysis (EPQA) was conducted at véraison to describe general canopy architecture. Yield data and primary fruit chemistry (Brix, TA, YAN) data were collected at harvest. Berry samples were pressed at harvest and the juice was frozen for future analysis of amino acids—an area of interest due to their importance in fermentation and some aspects of wine aroma and flavor evolution—using Ultra Performance Liquid Chromatography (UPLC). Winter pruning were collected to assess the affect of treatments on vine growth.

Treatments at Glen Manor involve:
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)

**In 2013 we saw a greater degree of phytotoxicity in response to urea treatments made. In order to reduce the likeliness of leaf burn, we reduced the rate and are applying foliar N 9 times, for a total of 35 kg N/ha. Applications will end at the same phenological stage in both years.*

Treatment applications began in the 2011 growing season and will be repeated at least 3 years. Plant tissue analysis conducted at bloom in 2011, before the treatments were initiated, showed similar nutrient levels in the treatment vines (Table 1). Plant tissue analysis has been conducted at véraison and bloom in subsequent years, with observable differences only occurring at the véraison sampling time. At véraison 2011 both the high N treatment and foliar N treatment had a statistically significant increase in petiole N compared to the control; at véraison 2012 only the high N rate had a statistically significant increase in petiole N compared to the control. Chlorophyll is the green pigment in plants that is responsible for conversion of sunlight energy into chemical energy that the plant can use. Given that the nitrogen concentration of leaves has a direct impact on chlorophyll concentration and physiologic function, we are interested in monitoring its concentration. These measurements were taken with Minolta SPAD 502DL chlorophyll meter, and the resulting data will be calibrated against adequately fertilized set of vines in each vineyard. Leaf chlorophyll concentration was measured optically at véraison in 2011 and 2012, but minor differences were observed across treatments (Table 2). We decided to collect CCI readings at four time points in 2013 (pre-véraison, véraison, pre-harvest, harvest). The data from our first collection indicate the foliar N treatment has the greatest CCI compared to all other treatments, and the low and high soil N treatment are similar to one another but have a higher CCI than the control. These values are likely to shift over the season, after the second application has been made to the high soil N treatment.

Yield data were collected in late-August at the time of commercial harvest (Table 3). In 2011, we did not anticipate substantial treatment effects on components of yield and would not expect to see treatment differences in the first year of treatment, in that flower buds and cluster number were determined in the previous year. Differences across treatments were also minor in 2012.

Primary fruit chemistry and yeast-assimilable N levels in fruit at harvest are shown in Table 4. In 2011 the only statistically significant difference in YAN content was between the high N treatment and the control. In 2012 the foliar N treatment had a statistically higher YAN content compared to all other treatments.

Table 1 – Glen Manor: Tissue concentration of Nitrogen in leaf blades and petioles at two growth stages.

	Nitrogen (%)						
	Bloom 2011		Véraison 2011		Bloom 2012		Véraison 2012
Treatment	Blades	Petioles	Blades	Petioles	Blades	Petioles	Petioles
Control	2.87	0.88	2.50 a	0.43 b	2.56 a	0.85 a	0.81 b
30 kg/ha N soil (bloom)	.	.	2.53 a	0.47 ab	2.62 a	0.85 a	0.91 ab
30 + 30 kg/ha N soil (bloom + véraison)	.	.	2.59 a	0.48 a	2.51 a	0.86 a	0.94 a
35 kg/ha N foliar (7-9 applications)	.	.	2.53 a	0.48 a	2.63 a	0.89 a	0.84 ab

***Results from the bloom 2013 petiole samples are not yet available.**

Table 2 – Glen Manor: Leaf chlorophyll concentration index by treatment.

Treatment	Chlorophyll Concentration Index 2011(véraison)	Chlorophyll Concentration Index 2012 (véraison)	Chlorophyll Concentration Index 2013 (pre-véraison)
Control	19.5 a	14.9 a	12.5 c
30 kg/ha N soil (bloom)	20.0 a	14.7 a	14.0 b
30 + 30 kg/ha N soil (bloom + véraison)	20.6 a	16.0 a	13.7 b
35 kg/ha N foliar (7 applications)	20.4 a	16.3 a	15.5 a

Table 3 – Glen Manor: Yield components by treatment.

	Number of clusters		Yield per vine (kg)		Cluster weight (g)		Berry weight (g)		Berries per cluster	
Treatment	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
Control	21.4	40.6	6.1	5.2	291.0	128.5	2.0	.	144.3	.
30 kg/ha N soil (bloom)	21.6	42.1	6.5	5.4	296.7	127.0	2.1	.	142.8	.
30 + 30 kg/ha N soil (bloom + véraison)	22.3	41.4	6.5	4.9	284.8	119.8	2.0	.	142.4	.
35 kg/ha N foliar (7 applications)	22.1	37.6	6.0	4.5	277.2	122.3	2.0	.	138.2	.

Table 4 – Glen Manor: Primary fruit chemistry and fruit yeast assimilable nitrogen (YAN) by treatment.

	pH		TA (g/L)		°Brix		YAN (mg/L)	
Treatment	2011	2012	2011	2012	2011	2012	2011	2012
Control	3.42	3.28	6.42	4.56	21.9	21.4	119.1b	157 b
30 kg/ha N soil (bloom)	3.43	3.28	6.05	4.80	21.5	21.5	138.2ab	175 b
30 + 30 kg/ha N soil (bloom + véraison)	3.43	3.27	6.13	5.57	21.8	21.4	153.9 a	174 b
35 kg/ha N foliar (7 applications)	3.44	3.31	6.14	4.75	22.2	21.4	154.6ab	228 a

Experiment 2, Chateau O'Brien: A second experiment was added in January 2012 at Chateau O'Brien vineyard near Markham, VA (approximately 20 miles from Winchester and within 15 miles of the Glen Manor Vineyard). 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 exhibited low nitrogen levels; severely in some cases. Treatments at Chateau O'Brien were applied to 6-vine panels, replicated 5 times in a randomized, complete block experimental design. Pruning weights were gathered by panel in February 2012, before the start of the experiment. Floor management will be standardized as permanent row middle fescue, with intra-row zones (50-85-cm wide) planted to mixed stand of red fescue and native (weed) vegetation, maintained with a hand-held line trimmer.

Treatments at Chateau O'Brien involve:

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)

Treatments began at bloom-time in 2012 and will be repeated each year for a minimum of three consecutive years. Plant tissue analysis conducted at bloom in 2012, before the treatments were initiated, showed similar nutrient levels in the treatment vines (Table 1). A follow-up plant tissue analysis was conducted at véraison (start of final stage of fruit ripening) in late-summer 2012, but the differences in petiole N between treatments were not statistically different.

Minor differences were observed across treatments for leaf chlorophyll concentration, measured optically at véraison in 2012 (Table 2), yield components (Table 3), primary fruit chemistry, and YAN levels (Table 4). Given the nature of fertilization studies, we did not expect to observe differences in these parameters during the first year of this study. The 2013 pre-véraison SPAD readings indicate that the high N treatment had significantly greater CCI measurements compared to the control, low compost rate, and low soil N treatments. The clover+low compost rate, clover+high compost rate, and post-harvest N treatments had significantly greater CCI measurements than the control and low compost rate treatments.

Research note: the red clover in treatments 4 and 5 exhibited poor establishment in 2012. We suspect this was due to dry conditions after seeding and competition with other weeds. To ensure better establishment next in 2013, the panels were treated with a nonselective herbicide prior to seeding, the rate of seed applied was increased, and we opted to sow a mix of both red clover and crimson clover. In 2013 we obtained a minimum of 60% establishment in each panel and a maximum of 95% establishment (relative to the abundance of native vegetation).

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

Table 1 – Chateau O'Brien: Tissue concentration of Nitrogen in petioles by treatment.

Nitrogen (%)		
Treatment	Petioles (Bloom 2012)	Petioles (Véraison 2012)
Control– no nitrogen	1.06	0.74 a
Compost– low rate of 30kg/ha of actual N	.	0.75a
Compost– high rate of 60 kg/ha of actual N	.	0.75a
Clover and low rate of compost	.	0.74a
Clover and high rate of compost	.	0.72a
30 kg/ha N (15 kg/ha at bloom + 15kg/ha 6 weeks post bloom)	.	0.77a
60 kg/ha N (30kg/ha N at bloom + 30 kg/ha 6 weeks post bloom)	.	0.81a
30kg/ha N (post-harvest)	.	0.77a

***Results from the bloom 2013 petiole samples are not yet available.**

Table 2 – Chateau O'Brien: Leaf chlorophyll concentration index by treatment.

Treatment	Chlorophyll Concentration Index 2012 (véraison)	Chlorophyll Concentration Index 2013 (pre-véraison)
Control– no nitrogen	19.2	13.6 c
Low rate compost–33.5 kg/ha of actual N	18.8	14.0 c
High rate compost– 67 kg/ha of actual N	19.3	16.9 abc
Clover and low rate of compost	20.6	17.8 ab
Clover and high rate of compost	21.5	18.3 ab
30 kg/ha N soil (15 kg/ha at bloom + 15kg/ha 6 weeks post bloom)	19.9	15.8 bc
60 kg/ha N soil (30kg/ha N at bloom + 30 kg/ha 6 weeks post bloom)	21.6	19.5 a
30 kg/ha N soil (post-harvest)	20.2	19.0 ab

Table 3 – Chateau O'Brien: Yield components by treatment.

Treatment	No. clusters	Yield per vine (kg)	Cluster weight (g)	Berry weight (g)	Berries per cluster
Control– no nitrogen	63.8	2.69	41.86	0.99	42.28
Low rate compost–33.5 kg/ha of actual N	60.0	3.12	48.82	0.99	49.43
High rate compost– 67 kg/ha of actual N	63.2	3.35	51.40	1.00	51.13
Clover and low rate of compost	70.2	3.36	48.09	0.95	50.34
Clover and high rate of compost	75.2	3.38	45.49	0.99	46.06
30 kg/ha N soil (15 kg/ha at bloom + 15kg/ha 6 weeks post bloom)	62.4	2.74	41.43	0.93	44.43
60 kg/ha N soil (30kg/ha N at bloom + 30 kg/ha 6 weeks post bloom)	67.8	2.60	38.06	0.94	38.41
30 kg/ha N soil (post-harvest)	72.0	3.66	51.85	1.06	48.72

Table 4 – Chateau O'Brien: Primary fruit chemistry and fruit yeast assimilable nitrogen (YAN) by treatment.

Treatment	**pH 2012**	**TA (g/L) 2012**	**°Brix 2012**	**YAN 2012**
Control– no nitrogen	4.02	1.29	18.74	72.6a
Low rate compost–33.5 kg/ha of actual N	4.11	1.42	20.04	57.6a
High rate compost– 67 kg/ha of actual N	4.16	1.40	29.96	63a
Clover and low rate of compost	4.15	1.60	20.18	75.2a
Clover and high rate of compost	4.07	1.72	20.4	69a
30 kg/ha N soil (15 kg/ha at bloom + 15kg/ha 6 weeks post bloom)	4.01	1.53	19.86	78a
60 kg/ha N soil (30kg/ha N at bloom + 30 kg/ha 6 weeks post bloom)	4.14	1.58	20.02	88a
30 kg/ha N soil (post-harvest)	4.16	1.60	20.16	67a

Experiment 3, Winchester: A third experiment was implemented in June 2012 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 *Trifolium arvense* L. and *Medicago lupulina* L. Treatments are in 5-vine panels, replicated 5 times in a randomized, complete block experimental 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. Data collection will mimic the work done at Chateau O'Brien and Glen Manor.

Treatments at Winchester involve:

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.

Similar to the results obtained from the other two sites in the first year of trial, minor differences were observed across treatments for leaf chlorophyll concentration (Table 2), yield components (Table 3), and primary fruit chemistry (Table 4) at the AREC site. Our 2012 data indicates there were significant differences in berry YAN levels for the post-véraison foliar application compared to the pre-véraison treatment. Both 2012 véraison and 2013 pre-véraison SPAD readings indicate there is a statistically significant difference between the herbicide control and the under trellis cover crop control, which further demonstrates the competition for nitrogen in cover cropped sites. These preliminary results support the implementation of the treatments at the AREC site and the further experimentation with foliar nitrogen applications.

Table 1 – AREC: Tissue concentration of Nitrogen in petioles by treatment.

Nitrogen (%)	
Treatment	Petioles (Pre-treatment, July 2012)
Under Trellis Cover Crop Control	0.40
Herbicide Control	0.48
10 kg/ha N foliar (5 kg/ha 1 week pre-véraison + 5 kg/ha 2 weeks pre-véraison)	0.40
10 kg/ha N foliar (5 kg/ha 1 week post-véraison + 5 kg/ha 2 weeks post-véraison)	0.40

***Results from the bloom 2013 petiole samples are not yet available.**

Table 2 – AREC: Leaf chlorophyll concentration index by treatment.

Treatment	Chlorophyll Concentration Index 2012 (véraison)	Chlorophyll Concentration Index 2013 (pre-véraison)
Under Trellis Cover Crop Control	14.7b	14.9 b
Herbicide Control	18.4a	16.8 a
10 kg/ha N foliar (5 kg/ha 1 week pre-véraison + 5 kg/ha 2 weeks pre-véraison)	16.8ab	16.5 ab
10 kg/ha N foliar (5 kg/ha 1 week post-véraison + 5 kg/ha 2 weeks post-véraison)	17.2ab	15.5 ab

Table 3 – AREC: Yield components by treatment.

Treatment	Number of clusters	Yield per vine (kg)	Cluster weight (g)	Berry weight (g)	Berries per cluster
Under Trellis Cover Crop Control	40.44	4.19	104.77	1.13	92.90
Herbicide Control	36.52	2.82	74.00	1.18	62.56
10 kg/ha N foliar (5 kg/ha 1 week pre-véraison + 5 kg/ha 2 weeks pre-véraison)	38.78	3.06	79.56	1.16	68.40
10 kg/ha N foliar (5 kg/ha 1 week post-véraison + 5 kg/ha 2 weeks post-véraison)	39	3.32	84.05	1.15	73.24

Table 4 – AREC: Primary fruit chemistry and fruit yeast assimilable nitrogen (YAN) by treatment.

Treatment	pH 2012	TA (g/L) 2012	°Brix 2012	YAN (mg/L) 2012
Under Trellis Cover Crop Control	3.38	5.24	26.94	160 ab
Herbicide Control	3.35	5.43	26.96	147 ab
10 kg/ha N foliar (pre-véraison) (5 kg/ha 1 week pre-véraison + 5 kg/ha 2 weeks pre-véraison)	3.33	5.16	26.52	138 b
10 kg/ha N foliar (post-véraison) (5 kg/ha 1 week post-véraison + 5 kg/ha 2 weeks post-véraison)	3.37	5.02	26.64	222 a

Data not presented:

- Enhanced point quadrant analysis (EPQA) conducted at véraison in 2012 to describe canopy architecture and fruit exposure.
- 2011 and 2012 pruning weights.
- 2012 and 2013 block soil samples.
- Berry amino acid composition was analyzed using UPLC technology for all harvest samples during the week of January 28th, 2013. These analyses will continue to be conducted on harvest samples in future years. The purpose of employing this technology is to determine if different fertilization rates, methods, and materials have an effect on berry amino acid composition—a fruit quality component that has tremendous influence on the aromas formed in resultant wines.

Future modifications and additions:

- A season-long weather station was installed at Glen Manor to record daily rainfall, temperature and relative humidity at the site.
- Early and mid-season determination of cover-crop establishment was determined prior to mowing in July.
- The SPAD protocol was modified, such that individual leaves in experimental plots were tagged with the intent of returning to each of the tagged leaves at multiple sampling dates throughout the season (pre-véraison, véraison, pre-harvest, and harvest).

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 N environments. While we have historically relied upon bloom-time sampling of leaf petioles to determine N status, there is increased interest in including must analysis of YAN as a diagnostic criterion. Our experiments will allow a direct comparison of must and foliar N levels, addressing both viticultural needs of crop yield and vine size, but also recognizing the importance of N to fermentation and flavor and aroma chemistry.

Registering increased nitrogen reserves in the grapevine, as assessed by tissue analysis, may take two or more years. Three or more years of data collection would be necessary before conclusions can be made about the most efficient timing and rate of applied nitrogen. Total rates of N may be adjusted up or down depending on measured responses; however, we would tentatively aim to maintain leaf petiole total N at or above 0.90% N through véraison, and must (juice) levels of yeast-assimilable nitrogen at or above 150 mg/L, but avoid having soil nitrate-N levels in excess of 20 kg/ha 30 days after harvest. This last point relates to our desire to avoid a pool of unused, potentially leachable, nitrates in the soil profile during the dormant period. Depending on our access to wine-making equipment and analytical instrumentation, we would like to pursue more detailed analyses of musts and wines with respect to treatment impact on flavor and aroma compounds. We expect to make small lots of wine in the 2013 season and review the finished wines for consumer preference, at minimum, but perhaps for specific metabolites associated with flavor and aroma (e.g., certain thiol compounds in Sauvignon blanc).