Research Project Title: Foliar Nitrogen Application in Wine Grapes to Enhance Wine Quality

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Objectives:
Evaluate late season foliar nitrogen (N) application on plant N concentration and yeast assimilable N (YAN) in the fruit.

Justification:
Although adequate N availability is required to support optimum grape yield and fruit quality, elevated N increases canopy leaf area, which increases disease pressure (Poling, 2007). In addition, extensive shoot growth requires additional thinning to optimize canopy microclimate for fruit and wood maturation (Christensen, 2005). In the southeast, where excessive plant available water accelerates vine growth, little or no N is applied to avoid the potential negative effects of excessive N (Jackson and Lombard, 1993; Keller, 2005). Consequently, YAN in grape musts is frequently below the minimum threshold (140 mg N/L) required to avoid stuck fermentation and atypical aging (Monteiro and Bisson, 1991; Spayd et al., 1995; Hannam et al., 2014).

One potential solution to increase YAN, while minimizing vine growth and disease potential, is through late-season foliar N. Grape leaves readily absorb urea-N, which is translocated to the clusters (Conradie, 1986; Dong et al., 2002). In contrast, late-season soil applied N is not effective due to low surface soil moisture (Howard, 2014). Lacroux et al. (2008) demonstrated soil applied N increased vigor and *Botrytis* incidence, whereas foliar N improved vine N status and enhanced aroma characteristics of Sauvignon Blanc without increasing vigor or *Botrytis* susceptibility. Other studies confirm the positive effects of foliar N on increased YAN and wine aromatics (Garde-Cerdan et al., 2014; Ancín-Azpilicueta et al., 2013; Lasa et al., 2012; Dufourcq et al., 2009).

Methodologies
Two research sites were located on a Fairview sandy clay loam (fine, kaolinitic, mesic Typic Kanhapludults) located on Shelton Vineyards in Surry Co., NC (Fig. 1). Soil properties (check plots) were typical of vineyard soils (relatively high P, K, micronutrients) in the Yadkin Valley appellation with a previous history of manure applications (old dairy farm; Table 1). Soil pH (0-20 cm) was optimum (Site A) for vinifera wine grape production; however, at Site B pH was below optimum. At Site A, soil samples were also collected from the "soil applied N" plots, although results were not significantly different from the soil test data obtained from the "check plots" (data not shown).

Figure 1. Aerial view of the two field research sits at Shelton Vineyards (Dobson, NC).

Table 1. Selected soil properties from the research plot area.

Plot	Depth	OM	CEC	BS	pH	Ca	Mg	P	K	S	Mn	Zn	Cu
	cm	*%*	*meq 100g^{-1}*	*%*		*------- % -------*		*---------- ppm ----------*					
A	**0-10**	0.43	8.9	76.5	5.7	51.9	21.7	86	115	17	24	21	11
	10-20	0.36	6.9	83.8	6.2	56.4	21.4	118	67	15	13	7	3
B	**0-10**	0.60	5.0	65.3	5.4	42.8	17.9	76	119	15	17	10	9
	10-20	0.47	4.7	66.3	5.4	42.2	18.6	34	107	25	10	5	6

Soil samples were collected prior to bud-break from "check plots" at 0-10 and 10-20 cm depths. Soils were air dried and sent to the NC Department of Agriculture & Consumer Services Laboratory for analysis (Hardy et al., 2003).

Plant tissue (petiole and leaf) samples were collected at full bloom (pre-treatment). At full bloom, 40-50 petiole/leaf samples were collected from opposite the first or second cluster from the bottom of the shoot in each treatment. Petioles and leaves were analyzed separately and total N (and other macro- and micronutrients) was determined in each sample (Hardy et al., 2003).

A multispectral camera mounted on an Unmanned Aerial Vehicle (UAV) was used to test the potential for measuring N status remotely using a common vegetative index known as NDVI (normalized difference vegetative index). NDVI is based on the measurement of 2 distinct wavelengths, red and near-infrared, within the electromagnetic spectrum. When used in the mathematical ratio these two wavelengths are known to correlate well to plant vigor and N content. A modified cannon EOS camera was used as the sensor to measure the red and near-infrared energy reflected from the grape canopy. The sensor was mounted on a custom fabricated rotary UAV and programmed to collect pictures at 1Hz. A pre-programmed flight path was developed before each flight and used to fly a serpentine pattern over the experimental areas. The images were collected with 70% overlap and later used to develop a single, complete aerial orthomosaic of each site. These orthomosaics were georeferenced using the locations of known ground control points.

Flights were conducted at 200 feet altitude on May 24, 2016 during full bloom. Color images were flown and used to develop corresponding aerial surveys (Fig. 2).

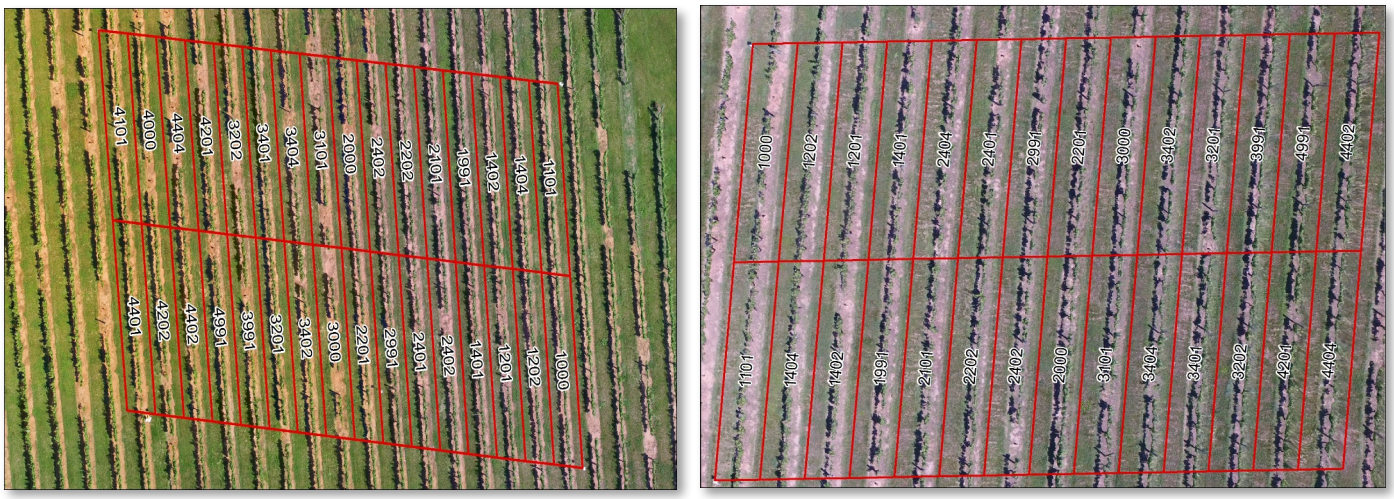

Figure 2. Georeferenced color image of site A (left) and B (right) captured on May 24, 2016 by Unmanned Aircraft Vehicle (UAV). Plots are outlined in red with and labeled with treatment numbers.

Georeferenced aerial photographs from the multispectral sensor were used to calculate average NDVI for each plot. The calculation excludes all non-grape vegetation and soil so as to assure the values are representative of the grape canopy (Fig. 3).

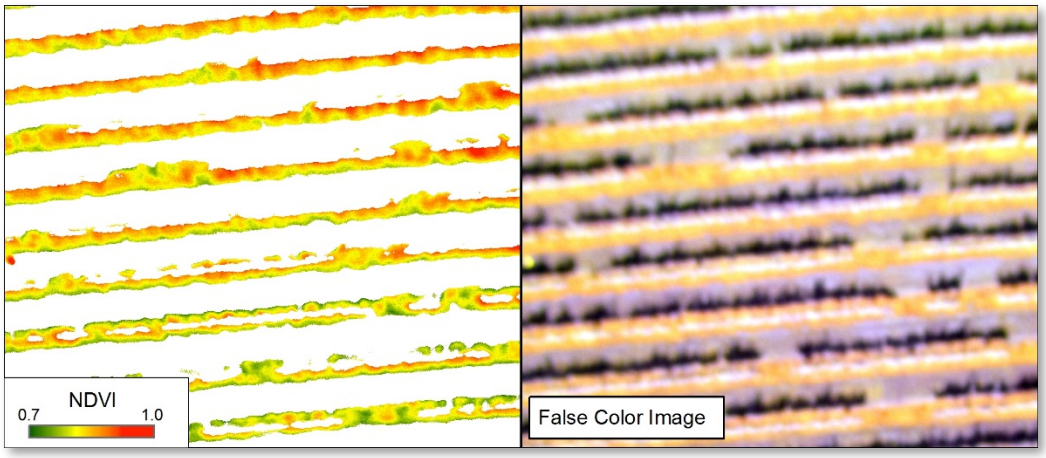

Figure 3. Example of a Normalized Difference Vegetative Index (NDVI – left) raster and the original false-color image collected by UAV (right). Non-grape vegetation and soil were removed from the false-color image before calculating NDVI. NDVI ranges from 0 to 1 with higher values corresponding to greater plant vigor. (darked areas in false color image represent shadow from the ~ 6 ft high grape vines).

Foliar and soil N treatments included liquid urea (21-0-0) and granular urea (45-0-0) applied to four replications (Table 2). The liquid urea was diluted with distilled water in variable proportions in 2L bottles to facilitate application of the N rates with the sprayer. A backpack CO_2 sprayer (R&D Sprayers, Inc.) equipped with 4-80° flat spray nozzles on 0.30 m spacing was used to apply treatments. The 1.2 m spray boom was held vertically along each side of the treatment row to facilitate optimum canopy coverage. Each treatment was applied to 10 m of row on ~3 m row spacing.

Table 2. Treatments used in the 2016 study at both sites.

N treatment designation	**N Treatment description**	
	Total N applied (lb N/a)	***N application times***[1]
0	check	
10	10	14 d pre-veraison
20	20	14 d pre-veraison
10 x 2	20 (2 - 10 lb/a)	14 d pre-veraison; veraison
10 x 4	40 (4 - 10 lb/a)	14 d pre-veraison; veraison; 5 & 10 d post veraison
Soil N	180	pre- bud break

[1]full bloom-May 24; veraison-July 20

Grape clusters were collected at harvest from each treatment, sent to the Enology Services Laboratory (Appalachian State Univ.), and analyzed for pH, total acidity (TA), Brix, Malic acid, YAN (yeast assimilable N), and FAN (free amino acid N). Grape juice samples were also sent to the Enology Analytical Services Lab. (Virginia Tech), where additional aromatic compound analyses are being conducted.

Plant N measurements and harvest fruit quality data were analyzed using Analysis of Variance (ANOVA) as the plot design is a randomized complete block design.

Results

Leaf and petiole N analysis at full-bloom (prior to foliar N application) was necessary to establish background plant N levels that could be used to assess foliar N need (Table 3). At both sites, petiole N content at full bloom was below the established critical level of 1.2-1.6% N (Poling, 2007). No significant differences in plant N or NDVI were detected between foliar treatment areas since foliar N applications did not begin until July 9. With 180 lb N a^{-1} soil applied at bud break treatment, leaf N at both sites and petiole N at site A were significantly increased, although NDVI and petiole N (site B) were not affected (Table 3). Compared to the 2015 data where a lower N rate was soil applied (only 90 lb N a^{-1}), these data illustrate that soil N applied at relatively high rates will slightly increase plant N content at full bloom.

Table 3. Full bloom (May 24) plant N content and NDVI using a UAV sensor platform.

	Site A				Site B		
N treatment	Plant N		NDVI[1]		Plant N		NDVI[1]
	Petiole	Leaf			Petiole	Leaf	
	--------- % ----------				--------- % ----------		
0	0.98 a	3.55 a	0.86		1.00	3.19 a	0.88
10	1.06 a	3.66 a	0.92		0.90	3.10 a	0.87
20	0.94 a	3.51 a	0.85		0.93	3.28 ab	0.89
10x2	0.95 a	3.68 a	0.87		1.05	3.24 a	0.87
10x4	1.03 a	3.64 a	0.90		1.05	3.03 a	0.85
Soil N	1.16 b	3.75 ab	0.91		1.02	3.35 b	0.89
P > f	0.018	0.031	ns		ns	0.048	ns

[1]NDVI = normalized difference vegetative index

In order to develop foliar N recommendations based on UAV-acquired imagery, NDVI measurements must relate to plant leaf or petiole N content. Site A showed a significant relationship (p = 0.05) between NDVI and leaf N, but not between NDVI and petiole N (Fig. 4).

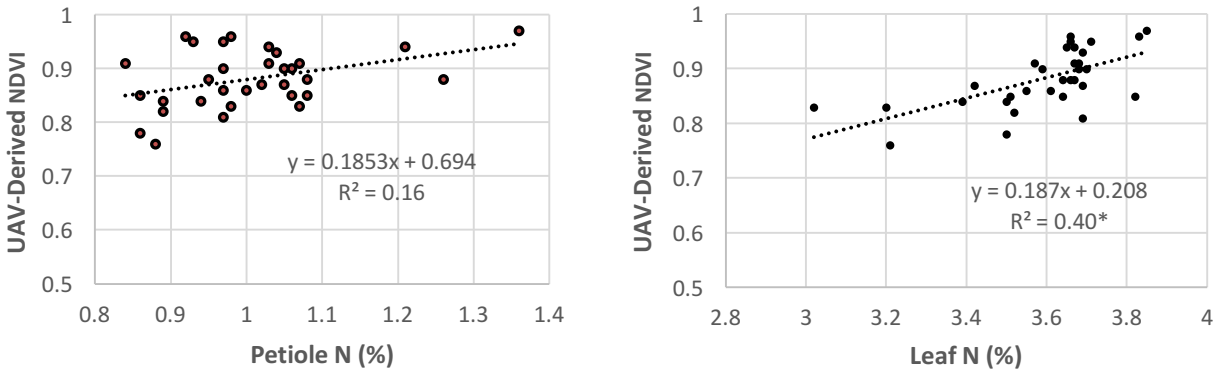

*Figure 4. Relationship between petiole (left) and leaf (right) N and UAV-based NDVI at experimental site A. *significant at p=0.05*

Similarly, at site B, no significant relationship was observed between petiole N and NDVI; however, the relationship between leaf N and NDVI was significant (Fig. 5). When using single detector sensors, as is this study, the ability to resolve differences at higher N contents decreases. Typically, when NDVI values approach 0.9, the ability to resolve differences in tissue N are limited. UAV-based sensors designed with separate detectors for each wavelength are now available for UAV-based data acquisition. Future use of these sensors will allow for finer control of measurement

in high-biomass crops like *Vitis vinifera* and will improve foliar N recommendations when substantial ranges in tissue N are observed. When only small differences in tissue N are present, UAV-based measurements are of less value and singe rate applications are more appropriate.

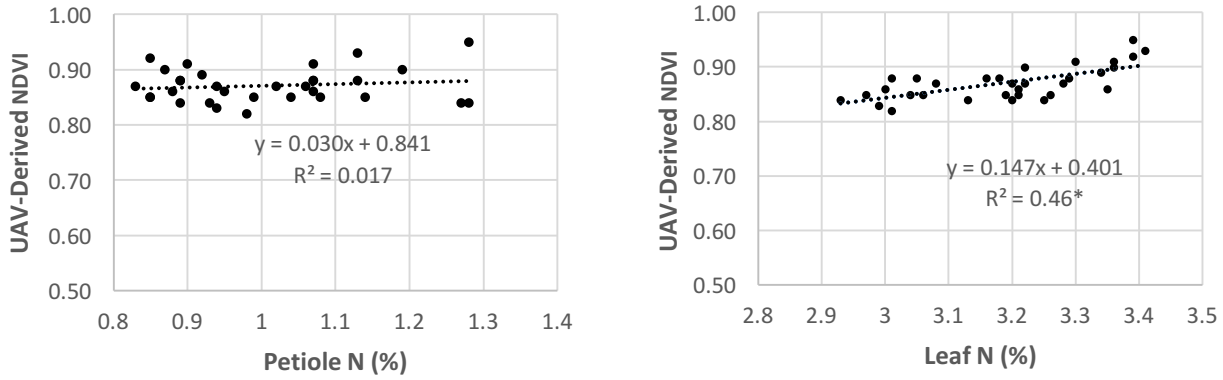

*Figure 5. Relationship between petiole (left) and leaf (right) N and UAV-based NDVI at experimental site B. *significant at p=0.05*

Soil or foliar applied N had no effect on brix and pH of grape juice at either site (Table 4, 5). In contrast to 2015, the higher soil applied N rate in 2016 resulted in increased TA, malic acid YAN, and FAN at site A compared to the check treatment (Table 4). Although not as pronounced, similar increases were observed at site B (Table 5).

At both sites, foliar N significantly increased YAN, FAN, and malic acid compared to the check treatment, and were also significantly greater than with soil applied N (Table 4, 5). In addition, the split N treatments exhibited a larger response than N applied at one time. For example, the 20 lb a^{-1} split N rate increased YAN 24 and 12% over the single 20 lb N a^{-1} rate at site A and B, respectively. Compared to the check, the 40 lb a^{-1} split N rate treatment (10x4) increased YAN 58 and 49% at site A and B, respectively, while increasing YAN 25 and 37% at site A and B over the soil applied N treatment, respectively. Foliar N treatment effects on malic acid were similar to those on YAN/FAN, although the increases were not as pronounced (Table 4, 5).

Table 4. Foliar and soil applied N effects on selected wine grape quality parameters in site A.

TRT	Brix	pH	Titratable Acidity	Malic Acid	YAN	FAN
			---------- *g L^{-1}* ----------		---------- *ppm* ----------	
0	19.5 a	3.55 a	3.41 a	2.28 a	183 a	124 a
10	19.2 a	3.59 a	3.34 a	2.48 a	218 b	148 b
20	18.9 a	3.56 a	3.52 a	2.40 a	209 b	137 b
10x2	20.8 a	3.69 a	3.68 b	2.95 b	260 d	176 d
10x4	19.3 a	3.67 a	3.69 b	2.77 b	290 e	205 e
Soil N	19.9	3.61 a	3.67 b	2.65 b	233 c	157 bc
P > f	ns	ns	0.031	0.023	<0.001	<0.001

[1]means followed by the same letter are not significantly different (p=0.01)

Table 5. Foliar and soil applied N effects on selected wine grape quality parameters in site B.

TRT	Brix	pH	Titratable Acidity	Malic Acid	YAN	FAN
			---------- *g L^{-1}* ----------		---------- *ppm* ----------	
0	19.6 a	3.66 a	2.83 a	2.19 a	185 a	137 a
10	19.5 a	3.72 a	2.83 a	2.42 b	181 a	138 a
20	19.2 a	3.66 a	2.95 a	2.24 a	194 ab	143 a
10x2	20.1 a	3.72 a	2.93 a	2.48 b	218 b	162 b
10x4	19.3 a	3.80 a	3.03 a	2.77 c	275 c	210 c
Soil N	19.6 a	3.70 a	2.93 a	2.39 b	201 b	152 ab
P > f	ns	ns	ns	0.021	<0.001	<0.001

[1]means followed by the same letter are not significantly different (p=0.01)

Conclusions

These preliminary results suggest that foliar N applied pre- and post-veraison can significantly improve grape N content and other parameters critical to enhancing flavor compound concentrations, without increasing vine vigor. Split N applications generally increased wine grape quality parameters to a greater extent than single foliar applied N rates or pre-bud break soil applied N. These preliminary data also demonstrate the potential use of remote sensing (UAV) in assessing N status in the vineyard. *Therefore, identifying N deficient grape plants at full bloom by either plant sampling/analysis or through remote sensing can direct the vineyard manager to initiate late-season foliar N management to improve wine grape quality*.

Impact Statement

To reduce vine vigor and leaf disease pressure, wine grape growers in the southeastern U.S. minimize or avoid use of soil applied N. As a result, wine makers frequently add N (DAP) to the must to complete the fermentation process. Low N plants result in low YAN in the must, potentially reducing flavor in the final wine product. This research will establish the value of foliar N applied through grape maturation (pre → post veraison) to enhance the flavor profile of *vinifera* grapes.

References:

Ancín-Azpilicueta, C., Nieto-Rojo, R., Gómez-Cordón, J. 2013. Effect of foliar urea fertilization on volatile compounds in Tempranillo wines. *J. Sci. Food Agri.* 93:1481-1485.

Christensen, L.P. 2005. Foliar fertilization in vine mineral nutrient management programs. *In* Proc. Soil Envir. Vine Mineral Nutr. Symp. L.P. Christensen and D.R. Smart (eds.), p. 83-90. Am. Soci. Enol. Vitic., Davis, CA.

Conradie W.J., 1986. Utilisation of nitrogen by grape-vine as affected by time of application and soil type. S. Afr. J. Enol. Vitic. 7:76-83.

Dong, S., L. Cheng, C. Scagel, L. Fuchigami. 2002. Nitrogen absorption, translocation and distribution from urea applied in autumn to leaves of young potted apple (Malus domestica) trees. Tree Physiol. 22:1305–1310

Dufourcq, T., Charrier, F., Poupault, P., Schneider, R., Gontier, L., Serrano, E. 2009. Foliar spraying of nitrogen and sulfur at veraison: a viticultural technique to improve aromatic composition of white and rosés wines. *In* Proc.16th Intern. GiESCO Symposium (pp. 379–383). UC Davis, Dept. Viticulture and Enology.

Garde-Cerdan, T., R. Lopez, J. Portu, L. Gonzalez-Arenzana, I. Lopez-Alfaro, P. Santamaria. 2014. Study of the effects of proline, phenylalanine, and urea foliar application to Tempranillo vineyards on grape amino acid content. Comparison with commercial nitrogen fertilisers. Food Chemistry 163:136-141.

Hannam, K., G. Neilsen, D. Neilsen, W. Rabie, A. Midwood, P. Millard. 2014. Late-Season Foliar Urea Applications Can Increase Berry Yeast-Assimilable Nitrogen in Winegrapes (*Vitis vinifera* L.). Am. J. Enol. Vitic. 65:89-95.

Hardy, D.H., M. R. Tucker, and C. E. Stokes. 2003. Crop Fertilization Based on North Carolina Soil Tests. http://www.ncagr.com/agronomi/obook.htm.

Havlin, J.L., D.H. Hardy, R.J. Gehl, S.E. Spayd. 2012. Survey of Nutrient Status in Vitis vinifera Grapes in North Carolina. Comm. Soil Sci. Plant Anal. 43:299-314.

Howard, A. 2014. Water Dynamics in the Yadkin Valley American Viticultural Area – Observations and Modeling. M.S. Thesis. NC State University. Raleigh, NC.

Jackson, D.I., and P.B. Lombard. 1993. Environmental and management practices affecting grape composition and wine quality-A review. Am. J. Enol. Vitic. 44:409-430.

Keller, M. 2005. Deficit irrigation and vine mineral nutrition. Am. J. Enol. Vitic. 56:267-283.

Lacroux, F., O. Tregoat, C. Van Leeuwen, A. Pons, T. Tominaga, V. Lavigne-Cruege, D. Dubordieu. 2008. Effect of foliar nitrogen and sulphur application on aromatic expression of *Vitis vinifera* L. cv. Sauvignon blanc. International J. Vine and Wine Sci. 42(3):125-132.

Lasa, B., S. Menendez, K. Sagastizabal, M. Cervantes, I. Irigoyen, J. Muro, P. Aparicio-Tejo, I. Ariz. 2012. Foliar application of urea to ‘‘Sauvignon Blanc’’ and ‘‘Merlot’’ vines: doses and time of application. Plant Growth Regul. 67:73–81.

Monteiro, F., and L. Bisson. 1991. Amino acid utilization and urea formation during vinification fermentations. Amer. J. Enol. Vitic. 42:199–208.

Poling, E.B. (ed.). 2007. NC Winegrape Grower's Guide. NC Coop. Ext. Ser. 196p.

Spayd, S.W., C.W. Nagel, and C.G. Edwards. 1995. Yeast growth in Riesling juice as affected by vineyard nitrogen fertilization. Am. J. Enol. Vitic. 46:49-55.