

LEAF BLADE VERSUS PETIOLE NUTRIENT SAMPLING AT BLOOM AND
VERAISON

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ABSTRACT

Grape growers collect and analyze petioles to assess plant nutrient concentrations, however it's unclear how accurately petioles reflect the nutrient concentrations of the leaves. Leaf blades and petioles of Cabernet Franc and Riesling were collected during bloom and veraison for analysis of 11 macro and micronutrients in grapevines grown on five sites in New York: Lansing Vineyard, Boundary Breaks Vineyard, Dr. Konstantin Frank Vineyard, Hazlitt 1852 Vineyard, and Hermann Weimer Vineyard. Results demonstrated that in most cases, phenological stage and tissue type impacted vine nutrient concentrations significantly. The three-way interactions (tissue type*phenological stage*cultivar) were significant for most nutrients in 2015 and 2017. The impacts of tissue, phenology stage, cultivar and their interactions on nutrients were different in 2015 and 2017 although the samples were collected from the same vineyard. This study contributes to the understanding of nutritional behaviors of eleven elements in blades and petioles at two phenological stages. It emphasizes the importance of creating specific guidelines for nutrient analysis that relies on different tissues at different phenological stages in different cultivars. These more specific guidelines can improve the accuracy and reliability of grapevine nutrient diagnosis.

BIOGRAPHICAL SKETCH

Yanan Lu was born in Shenyang, China on May 14th, 1997 and grew up in Shenyang. She graduated from Nanjing Agricultural University (NAU) with a Bachelors of Science in Horticulture in 2015. During her undergraduate study, she participated in research and projects in Dr. Jinggui Fang's lab identifying 106 Chinese grape cultivars with Microsatellites. Her experience in the Conference on One Health for Food Safety and Security at UC Davis inspired her enthusiasm for interdisciplinary communication and to pursue her master's degree in the US. She was the recipient of Merit-based NAU Scholarship from 2016 to 2019, the first prize of the Fifth National Forum on Practical Innovation of Plant Production for College Students (top 10%) in 2018, Outstanding Student Leader and Outstanding Graduates in NAU. Pursuing a love of grapes and a curiosity of wines, she then joined the Cornell University Department of Horticulture for her professional studies in Viticulture, where Russell Moss served as her advisor. She was fascinated by the conversion of grapes to wines. She will be applying what she learned at Cornell in her future career and travelling to embrace the diversity of vineyard management and winemaking practices around the world.

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LIST OF ABBREVIATIONS

CF, Cabernet Franc;

RS, Riesling;

Boundary Breaks Vineyard, BB;

Dr. Konstantin Frank Vineyard, FR;

Hazlitt 1852 Vineyard, HZ;

Hermann Weimer Vineyard, ME.

P, phosphorus;

K, potassium;

Mg, magnesium;

Ca, calcium;

Na, sodium;

B, boron;

Zn, Zinc;

Mn, manganese;

Fe, iron;

Cu, copper;

Al, aluminum;

1. Introduction

Grape growers are concerned about the concentration of macro and micro nutrients in the vine due to their influences on vine growth and fruit quality (Wolf 2008). Therefore, nutrient status of grapevines is monitored. It is important for grape growers to make adjustments in grapevines based on the nutrient status to achieve desired yield and quality of grapes. Grape growers believe that leaf analysis is reliable for monitoring the nutrition status of grapevines and for diagnosing potential nutrient deficiency and/or toxicity (Atalay 1978; Bertoni and Morard 1982). There have been various opinions among viticulturists regarding which phenological stage and tissue type can best reflect the nutrient status of grapevines.

The use of tissues to monitor and diagnose the nutritional status of grapevines could date back to Lagatu and Maume's work using whole leaves (Cook 1966). Original recommendations were to analyze the whole leaf at the beginning of flowering, the end of flowering or fruit set, veraison and harvest (Levy 1967). There are different phenological stages for sampling. Some researchers suggested one stage: bloom (Cook and Kishaba 1956), or fruit set (Ciesielska et al. 2002), or veraison (Stringari et al. 1997), while others recommended two phenological stages (Loué 1990; Failla et al. 1995).

The leaf blade was the standard tissue for monitoring and diagnosing nutrient status of grapevines in Europe (Bennett 1993; Failla et al. 1995). The advantage of using petiole is that petioles are smaller in size than blades, hence they are easier to collect and prepare for the test. Some arguments for using petioles resulted from observations that P and K levels in petioles showed wider ranges than P and K levels in leaf blades, which can be explained with wide variability in petiole values across years and cultivars (Cook and Kishaba 1956; Christensen 1969; Christensen 1984).

However, the relatively large variability in petiole nutrition values compared to blade nutrition level was considered less reliable than blades as a tissue to test in nutrition monitor and diagnosis (Bertoni and Morard 1982; Benito et al. 2013; Romero et al. 2013). Currently, some researchers analyze leaf blades or petioles only, others analyze both to compare the two (Benito et al. 2013).

In this study, samples were collected in two tissues (blades and petioles) at two phenological stages (bloom and veraison). The goal of this study was to evaluate whether phenological stages, tissue types, cultivars, and interactions among them have impacts on nutrients level in grapevines grown in New York vineyards. Another goal is to compare samples from different years and different sites to detect the environmental factors that can potentially influence the results of nutrients measurement.

2. Materials and Methods

2.1 Nutrients data collection

Samples for nutrient data were collected in 2015, 2016 and 2017. P, K, Mg, Ca, Na, B, Zn, Mn, Fe, Cu and Al concentrations were measured. In 2015 and 2017, nutrient data were from Cabernet Franc and Riesling in Cornell University Vineyard, Lansing, NY. Blades and petioles were collected during bloom and veraison. In 2016, the nutrients were measured in four different sites: Boundary Breaks Vineyard, Lodi, NY; Dr. Konstantin Frank Vineyard, Hector, NY; Hazlitt 1852 Vineyard, Hector, NY and Hermann Weimer Vineyard, Dundee, NY. Only Riesling was chosen. Blades and petioles were collected during veraison.

2.2 Statistics analysis

A statistical analysis on the nutrient concentration in leaves and petioles was conducted in order to evaluate whether there was significant differences in the results between the two tissue types, two phenology stages and two cultivars in 2015 and 2017, and between the two tissue types and four sites in 2016. Data that fell out of three times of standard deviation were removed as outliers prior to analysis. 13 samples in 2015 were removed as outliers. Data were analyzed using a mixed model for each year separately. In 2015 and 2017, tissue, phenology stage, cultivar and all their interactions were entered as fixed effects and replication nested within cultivar was entered as random effect. In 2016, tissue, site and their interaction were entered as fixed effects and replicates within sites was entered in the model as a random effect. Non-significant terms were removed from the model in a stepwise backwards method. Posthoc multiple comparisons with a Tukey correction were performed to further assess the significant terms. The significance was determined at 0.05. Data were analyzed using R version 3.6.1.

3. Results

The linear mixed effect models for 2015 data and 2017 data are similar, but the results are different. In 2015, the impact of three-way interaction among tissue, phenology stage and cultivar on all nutrients were highly significant (Table 1), which means all these three factors could influence nutrient level together, and the results could not be interpreted based on only one or two factors. Tissue had a significant impact on P, K, Fe, Cu and Al. Tissue had significant effects on Mg and Mn only at bloom. For Ca, Na, B and Zn, most samples were significantly influenced by different tissues with exceptions. Tissue didn't show significant impacts on Ca in Riesling at veraison, Na in Cabernet Franc at bloom, B in Cabernet Franc at veraison and Zn in Riesling at bloom. Phenological stage had a significant impact on Mg, Ca, B, Zn, Mn and Fe. For P, K, Cu and Al,

most samples were significantly influenced by different phenology stages with exceptions. Phenology stage didn't show significant impacts on P, Cu and Al in petiole of Cabernet Franc and K in blades of Riesling. Phenological stage only had a significant effect on Na levels in petioles. The impacts of different cultivars on nutrient levels were less significant than that of phenology stage and tissue. It's hard to get a general conclusion from cultivar impacts on nutrients because they were dependent on phenology stage and tissue.

Table 1. *P*-values from three-way ANOVA: Effect of tissue (leaf blade vs. petiole), phenological stage (bloom vs. veraison), cultivar (Cabernet Franc vs. Riesling) and their interactions on eleven nutrient concentration of Riesling and Cabernet Franc in 2015. CF, Cabernet Franc; RS, Riesling. Tis, Tissue; Phe, Phenology stage; Cul, Cultivar.

	Tissue impact		Phenology impact		Cultivar impact		Triple interaction
	Phe + Cul	P - value	Tis + Cul	P - value	Phe + Tis	P - value	P- value
P	Bloom + CF	0.014	Blade + CF	<0.0001	Bloom + Blade	0.5328	<0.001
	Veraison + CF	0.0255	Petiole + CF	0.1608	Veraison + Blade	0.6123	
	Bloom + RS	<0.0001	Blade + RS	<0.0001	Bloom + Petiole	<0.0001	
	Veraison + RS	0.0134	Petiole + RS	<0.0001	Veraison + Petiole	0.3095	
K	Bloom + CF	<0.0001	Blade + CF	<0.0001	Bloom + Blade	0.0566	<0.001
	Veraison + CF	<0.0001	Petiole + CF	<0.0001	Veraison + Blade	0.2333	
	Bloom + RS	<0.0001	Blade + RS	0.6246	Bloom + Petiole	<0.0001	
	Veraison + RS	<0.0001	Petiole + RS	0.0203	Veraison + Petiole	0.004	
Mg	Bloom + CF	<0.0001	Blade + CF	<0.0001	Bloom + Blade	0.0001	<0.001
	Veraison + CF	0.0681	Petiole + CF	<0.0001	Veraison + Blade	0.0369	
	Bloom + RS	<0.0001	Blade + RS	<0.0001	Bloom + Petiole	<0.0001	
	Veraison + RS	0.1634	Petiole + RS	<0.0001	Veraison + Petiole	0.4563	
Ca	Bloom + CF	<0.0001	Blade + CF	<0.0001	Bloom + Blade	<0.0001	<0.001
	Veraison + CF	<0.0001	Petiole + CF	<0.0001	Veraison + Blade	0.792	
	Bloom + RS	<0.0001	Blade + RS	<0.0001	Bloom + Petiole	<0.0001	
	Veraison + RS	0.0001	Petiole + RS	<0.0001	Veraison + Petiole	0.2922	
Na	Bloom + CF	0.6695	Blade + CF	0.2703	Bloom + Blade	0.8053	<0.001
	Veraison + CF	0.0134	Petiole + CF	0.0003	Veraison + Blade	0.5814	
	Bloom + RS	<0.0001	Blade + RS	0.8314	Bloom + Petiole	<0.0001	
	Veraison + RS	0.012	Petiole + RS	<0.0001	Veraison + Petiole	0.9271	
B	Bloom + CF	<0.0001	Blade + CF	<0.0001	Bloom + Blade	<0.0001	<0.001
	Veraison + CF	0.8614	Petiole + CF	<0.0001	Veraison + Blade	<0.0001	
	Bloom + RS	<0.0001	Blade + RS	<0.0001	Bloom + Petiole	<0.0001	
	Veraison + RS	0.0038	Petiole + RS	<0.0001	Veraison + Petiole	0.1413	
Zn	Bloom + CF	<0.0001	Blade + CF	<0.0001	Bloom + Blade	<0.0001	<0.001
	Veraison + CF	<0.0001	Petiole + CF	<0.0001	Veraison + Blade	0.0787	
	Bloom + RS	0.1148	Blade + RS	<0.0001	Bloom + Petiole	<0.0001	
	Veraison + RS	<0.0001	Petiole + RS	<0.0001	Veraison + Petiole	0.9703	
Mn	Bloom + CF	<0.0001	Blade + CF	<0.0001	Bloom + Blade	<0.0001	<0.001
	Veraison + CF	0.7045	Petiole + CF	<0.0001	Veraison + Blade	0.2921	
	Bloom + RS	<0.0001	Blade + RS	<0.0001	Bloom + Petiole	0.2927	
	Veraison + RS	0.2596	Petiole + RS	<0.0001	Veraison + Petiole	0.0768	
Fe	Bloom + CF	<0.0001	Blade + CF	<0.0001	Bloom + Blade	<0.0001	<0.001
	Veraison + CF	<0.0001	Petiole + CF	0.033	Veraison + Blade	0.5174	
	Bloom + RS	<0.0001	Blade + RS	<0.0001	Bloom + Petiole	0.0275	
	Veraison + RS	<0.0001	Petiole + RS	<0.0001	Veraison + Petiole	0.6996	
Cu	Bloom + CF	<0.0001	Blade + CF	<0.0001	Bloom + Blade	0.6074	<0.01
	Veraison + CF	<0.0001	Petiole + CF	0.4238	Veraison + Blade	0.0021	
	Bloom + RS	0.0342	Blade + RS	<0.0001	Bloom + Petiole	<0.0001	
	Veraison + RS	<0.0001	Petiole + RS	<0.0001	Veraison + Petiole	0.4819	
Al	Bloom + CF	<0.0001	Blade + CF	<0.0001	Bloom + Blade	<0.0001	<0.001
	Veraison + CF	<0.0001	Petiole + CF	0.495	Veraison + Blade	<0.0001	
	Bloom + RS	<0.0001	Blade + RS	<0.0001	Bloom + Petiole	<0.0001	
	Veraison + RS	<0.0001	Petiole + RS	<0.0001	Veraison + Petiole	0.1332	

In 2017, the pattern of how nutrients were affected by tissue, phenology stage and cultivar are different from that in 2015 although they were both from Lansing vineyard. The three-way interaction among three factors are less significant than that in 2015 (Table 2). In terms of Mn, Ca, B, Zn, Mn and Fe, the three-way interactions among tissue, phenology stage and cultivar were significant. Tissue had a significant impact on Mg, Fe, and Zn, on Mn only at bloom, and on Ca only in Cabernet Franc at veraison. It didn't significantly affect B in Cabernet Franc at veraison in Cabernet Franc at bloom. Phenological stage showed significant impacts on Ca, B and Mn. Most data showed the significant impacts of phenology stage on Fe with some exceptions. Phenological stage didn't have a significant impact on Fe in petioles of Riesling. Phenological stage only had significant effects on Mg levels in Riesling, and Zn levels in blades. The impacts of different cultivars were less significant than that of phenology stage and tissue.

Table 2. *P*-values from three-way ANOVA: Effect of tissue (leaf blade vs. petiole), phenological stage (bloom vs. veraison), cultivar (Cabernet Franc vs. Riesling) and their interactions on Mg, Ca, B, Zn, Mn and Fe concentration of Riesling and Cabernet Franc in 2017. CF, Cabernet Franc; RS, Riesling. Tis, Tissue; Phe, Phenology stage; Cul, Cultivar.

	Tissue impact		Phenology impact		Cultivar impact		Triple interaction
	Phen + Cul	P - value	Tis + Cul	P - value	Phe + Tis	P - value	P - value
Mg	Bloom + CF	<0.0001	Blade + CF	0.0759	Bloom + Blade	0.005	
	Veraison + CF	<0.0001	Petiole + CF	0.1725	Veraison + Blade	0.0406	<0.001
	Bloom + RS	<0.0001	Blade + RS	0.024	Bloom + Petiole	0.0001	
	Veraison + RS	0.0157	Petiole + RS	<0.0001	Veraison + Petiole	0.1256	
Bloom + CF	0.1819	Blade + CF	<0.0001	Bloom + Blade	0.7638		
Ca	Veraison + CF	0.0154	Petiole + CF	0.0011	Veraison + Blade	0.026	<0.05
	Bloom + RS	0.2383	Blade + RS	<0.0001	Bloom + Petiole	0.0367	
	Veraison + RS	0.4456	Petiole + RS	<0.0001	Veraison + Petiole	0.431	
	Bloom + CF	<0.0001	Blade + CF	<0.0001	Bloom + Blade	<0.0001	
B	Veraison + CF	0.3124	Petiole + CF	<0.0001	Veraison + Blade	0.0022	<0.001
	Bloom + RS	<0.0001	Blade + RS	<0.0001	Bloom + Petiole	0.1074	
	Veraison + RS	<0.0001	Petiole + RS	<0.0001	Veraison + Petiole	0.5129	
	Bloom + CF	<0.0001	Blade + CF	<0.0001	Bloom + Blade	<0.0001	
Zn	Veraison + CF	<0.0001	Petiole + CF	0.1053	Veraison + Blade	0.7062	<0.05
	Bloom + RS	<0.0001	Blade + RS	<0.0001	Bloom + Petiole	<0.0001	
	Veraison + RS	<0.0001	Petiole + RS	0.0884	Veraison + Petiole	<0.0001	
	Bloom + CF	<0.0001	Blade + CF	<0.0001	Bloom + Blade	<0.0001	
Mn	Veraison + CF	0.5397	Petiole + CF	<0.0001	Veraison + Blade	0.8183	<0.001
	Bloom + RS	<0.0001	Blade + RS	<0.0001	Bloom + Petiole	0.007	
	Veraison + RS	0.0899	Petiole + RS	<0.0001	Veraison + Petiole	0.0569	
	Bloom + CF	<0.0001	Blade + CF	<0.0001	Bloom + Blade	<0.0001	
Fe	Veraison + CF	<0.0001	Petiole + CF	0.0252	Veraison + Blade	0.7543	<0.001
	Bloom + RS	<0.0001	Blade + RS	<0.0001	Bloom + Petiole	0.0763	
	Veraison + RS	<0.0001	Petiole + RS	0.2	Veraison + Petiole	0.4506	

For some nutrients, there was no significant interaction among tissue, phenological stage and cultivar, such as P, K, Na, Cu and Al (Table 3), so the three-way interaction terms were removed from their models. There was no significant interaction between tissue and cultivar in P, K and Al, no significant interaction between phenology stage and tissue on P, K and Na, and no significant interaction between phenology stage and cultivar in P, Na and Al. These non-significant two-way interactions were removed from their models. Tissue had significant impacts on P while phenological stage and cultivar didn't did not significantly impact P. Tissue impacts on Na were dependent on phenological stage, and its impacts on Al were dependent upon cultivar. Phenological stage impacts on K were dependent on tissue, indicating significance only in petioles.

Phenological stage had a significant effect upon Al only in Cabernet Franc. Cultivar impacts on K were dependent on tissue, while it's impacts on Na depended on phenological stage. Compared to data in 2015, some similar trends were observed in 2017; such as phenological stage impacts on Ca, B and Mn but most impacts were different, some even showed opposite results from the other year. The three parameters that were measured can be influenced by other environmental factors, such as rainfall, temperature and evaporation (Longbottom 2009).

Table 3. *P*-values from three-way ANOVA: Effect of tissue (leaf blade vs. petiole), phenology stage (bloom vs. veraison), cultivar (Cabernet Franc vs. Riesling) and their interactions on P, K, Na, Cu and Al concentration of Riesling and Cabernet Franc in 2017.

	Tissue impact		Phenology impact		Cultivar impact		Triple interaction
	two-way interaction	P - value	two-way interaction	P - value	two-way interaction	P - value	P - value
P	No significant interaction	<0.0001	No significant interaction	0.9352	No significant interaction	0.0054	0.933
K	Bloom	<0.0001	Blade	0.0038	Blade	0.4892	0.278
	Veraison	<0.0001					
	CF	<0.0001	Petiole	<0.0001	Petiole	0.0001	
	RS	<0.0001					
Na	Bloom	<0.0001	Blade	0.2443	Bloom	0.0558	0.533
			Petiole	<0.0001			
	Veraison	0.0172	CF	<0.0001	Veraison	0.1606	
			RS	0.0775			
Cu	Bloom	0.0174	Blade	<0.0001	Bloom	0.0001	0.773
	Veraison	<0.0001	Petiole	0.5836	Veraison	0.4173	
	CF	0.0018	CF	0.0866	Blade	0.4009	
	RS	<0.0001	RS	<0.0001	Petiole	0.0007	
Al	CF	<0.001	CF	0.0023	Bloom	0.8299	0.568
					Veraison	0.0034	
	RS	<0.0001	RS	0.5934	Blade	0.0028	
				Petiole	0.7684		

In 2016, there were only two factors: tissue (blade vs. petiole) and site (BB, FR, HZ and ME). They interacted significantly with each other in most cases, but for Mn, Fe and Al, there was no significant interaction between tissue and site (Table 4). Tissue had significant effects on Fe, but the effects on Mn and Al were not significant. Site had significant effects on Mn and Fe, but no significant effects on Al. P, K, Mg, Ca, Na, B, Zn and Cu were significantly affected by interaction of tissue and site. Tissue had a significant impact on K and Zn level no matter where they were sampled but didn't influence Mn and Al significantly. Tissue had significant effects on Ca and Cu in only ME and FR site, Na only in ME site and P only in FR site. It didn't show significant effects on Boron when sampled in HZ site. Different sites had an impact on nutrient levels, but the trend is not clear.

Table 4. *P*-values from two-way ANOVA: Effect of tissue (blade vs. petiole) and the interactions between tissue and site (BB, FR, HZ and ME) on eleven nutrient concentrations of Riesling in 2016.

	Tissue impact		Interaction		Tissue impact		Interaction
	Site	P - value	P - value		Site	P - value	P - value
P	BB	<0.0001	<0.001	Zn	BB	<0.0001	<0.05
	FR	0.9208			FR	<0.0001	
	HZ	<0.0001			HZ	<0.0001	
	ME	<0.0001			ME	<0.0001	
K	BB	<0.0001	<0.01	Mn	No significant interaction	0.3819	0.087
	FR	<0.0001					
	HZ	<0.0001					
	ME	<0.0001					
Mg	BB	<0.0001	<0.001	Fe	No significant interaction	<0.0001	0.165
	FR	<0.0001					
	HZ	<0.0001					
	ME	<0.0001					
Ca	BB	0.5152	<0.001	Cu	BB	0.2519	<0.001
	FR	0.0184			FR	<0.0001	
	HZ	0.8363			HZ	0.4894	
	ME	<0.0001			ME	<0.0001	
Na	BB	0.0798	<0.001	Al	No significant interaction	0.3134	0.448
	FR	0.1849					
	HZ	0.0798					
	ME	<0.0001					
B	BB	0.0166	<0.05				
	FR	<0.0001					
	HZ	0.4472					
	ME	0.0113					

4. Discussion

The reliability of some guidelines that provide ranges of desired nutrient concentration can be suspicious and even misleading. It was observed that the nutrient concentrations could be effected by different environmental conditions (sampled years and sites in this study) and genetic background of grapevines (cultivars). Considering this and the significant interactions among phenological stage, tissue and cultivar in 2015, grape growers and the grapevine nutrition analytical laboratories may benefit from guidelines and reference values that are not just based on regional or previous means, but rather based on the factors that have significant effects on nutrient concentrations in grapevine. It is essential to set specific reference concentrations for the different

tissues at different phenological stages in different cultivars in order to improve the accuracy of nutritional diagnosis. Due to the difficulties of setting such specific reference nutrient concentrations in a large-scale production, we need to figure out how to reach desirable accuracy of measured nutrient levels without a heavy work. In the future studies, it will be interesting to determine whether leaf petiole nutrient concentration predicted leaf blade nutrient concentration and whether phenological stages affected how well petiole nutrients predicted leaf blade nutrients. It's possible that grape growers could obtain satisfying leaf nutrient data without excessive references if there is an obvious relationship between leaf petiole nutrients and leaf blade nutrients.

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