Leaf Blade versus Petiole Nutrient Tests as Predictors of Nitrogen, Phosphorus, and Potassium Status of ‘Pinot Noir’ Grapevines

R. Paul Schreiner1 and Carolyn F. Seagel
USDA-ARS-Horticultural Crops Research Unit, 3420 NW Orchard Avenue, Corvallis, OR 97330

Abstract. Grape growers rely on tissue tests of leaf blades or petioles for routine monitoring of vine nutritional health and for diagnosing potential nutrient deficiency or toxicity. There has been a long-standing debate as to which tissue better reflects the nutrient status of vines. A comparison of leaf blade and petiole nutrient concentrations was carried out to investigate which tissue better relates to vine growth, yield, and must nutrient responses of ‘Pinot noir’ grapevines to varying levels of nitrogen (N), phosphorus (P), and potassium (K) supply using data from a pot-in-pot vineyard over 4 years. Leaf blades and petioles were collected at 50% bloom and 50% veraison in each year and N, P, and K concentrations were assessed as predictors of leaf area at veraison, pruning mass at dormancy, yield, and must nutrient concentrations at fruit maturity. Data from commercial ‘Pinot noir’ vineyards were also used to investigate the relationship between leaf blade and petiole N concentrations with must N levels. Results indicated that leaf blades were superior to petioles in predicting vine growth, yield, and must yeast assimilable nitrogen (YAN) response. Leaf blade and petiole N concentrations at both sampling times were better predictors of vine productivity than petiole N concentrations. Relationships between leaf blade and petiole concentrations of P and K and vine response variables generally did not differ and both tissues appeared to equally effective in predicting P and K effects on growth, yield, and must P or K levels. Although petiole P was slightly better than leaf blade P at bloom in predicting must P levels, and models including both leaf and petiole K simultaneously as predictors relied only on leaf K. For all three nutrients, sampling at bloom and veraison had a similar predictive strength for response variables. Based on these findings, we recommend using leaf blades as opposed to petioles for diagnosing the N, P, and K status of ‘Pinot noir’.

The use of tissue nutrient tests to understand and diagnose the nutritional status of grapevines dates back to the 1920s work of Lagatu and Maume in France who developed the first recommendations for adequate N, P, and K levels in whole leaves (Cook, 1966). The early work focused on sampling at four times, and this was later reduced to bloom and veraison. The leaf blades (or lamina, being the primary site of photochemistry) subsequently became the standard tissue for diagnosing vine nutrients in European vineyards (Failla et al., 1995; Fregoni, 1980; Gärtel 1996; OIV, 1996). Work conducted in California vineyards focusing on N and K in the 1940s and 1950s indicated that petiole levels of K and NO3-N were better correlated to yield responses when these nutrients were limiting than was leaf K or leaf or petiole N values (Cook and Kishaba, 1956; Cook, 1966; Ulrich, 1942a, 1942b). These findings shifted subsequent research and development of tissue standards used in the United States and Australia to adopt petioles as the tissue of choice for routine diagnosis of vineyard nutrient status (Christensen 1969, 1984; Robinson, 1992; Robinson et al., 1978).

Part of the argument for using petioles came from observations that P, K, and NO3-N levels in petioles showed larger ranges than P, K, or total N levels in leaf blades. However, compared with leaves, the wider range of nutrient concentrations observed in petioles is also associated with greater variability (wider scatter) in petiole values temporally and across years and cultivars (Christensen, 1969, 1984; Cook and Kishaba, 1956). This greater variability in petiole values as compared with leaf values, however, can also be interpreted as a less reliable tissue than leaf blades as a diagnostic tool in vineyards (Benito et al., 2013; Bertoni and Morard, 1982; Romero et al., 2013). Petioles offer the advantage of being more convenient to collect, wash, and dry owing to their small size. Hence, the nutrient guidelines used by growers and testing laboratories in many regions of the United States still rely heavily on petiole values.

The guidelines to diagnose adequate nutrients in either leaf blades or petioles have been developed in large part by conducting surveys of numerous vineyards in a given region, with occasional plot trials where nutrient supply (fertilizer use) has actually been manipulated and altered the productivity of vines (Assimakopoulou and Tsougrianis, 2012; Christensen 1969, 1984; Conradie, 2001; Conradie and Saayman, 1989; Cook, 1966; Davenport et al., 2012; Robinson, 1992; Robinson et al., 1978). The reliance on surveys to provide ranges of nutrient status that are used as production guidelines can be misleading. For example, if a particular nutrient is generally high or low within the soils of a given region, then corresponding levels in leaf blades or petioles may also be high or low, biasing what are thought to be healthy levels. Grape growers and the testing laboratories that serve them will benefit by using guidelines that are not just based on regional averages, but rather where significant effects of nutrients on vine productivity or must quality are realized.

The goal of this study was to evaluate whether leaf blade or petiole nutrient concentrations better reflected vine growth, yield, and fruit quality responses to varying N, P, and K supply using an experimental system where each of these nutrients is carefully controlled. ‘Pinot noir’ was grown in a pot-in-pot vineyard with varying levels of either N, P, or K supply so that vine responses to each nutrient could be examined and to test if leaf and petiole nutrient tests are equally good predictors of vine productivity and must nutrient composition. Fruit was thinned on all vines by retaining a single cluster per shoot to achieve yields that were similar to current industry standards for premium ‘Pinot noir’ wines. A second goal was to compare whether bloom or veraison sampling times are better for predicting vine responses to N, P, or K supply.

Materials and Methods

Pot-in-pot vineyard system. The majority of the data used here were obtained from a pot-in-pot ‘Pinot noir’ vineyard over 4 consecutive years (2012–15) where N, P, or K were each independently manipulated. The pot-in-pot vineyard used a similar design as described earlier (Schreiner et al., 2013, 2014), with different levels of either N, P, or K supplied to vines at the beginning of their 4th year after transplanting. Grafted ‘Pinot noir’ grapevines (Vitis vinifera, L.
Pommard clone, FPS 91 on 101-14 Rootstock; Duarte Nursery Inc., Hughson, CA) were grown in (60 L) pot-in-pot, microplots (Grip Lip 6900T; Nursery Supplies Inc., McMinnville, OR), installed at the Oregon State University, Lewis Brown Research Farm, Corvallis, OR (44.553°N, 123.216°W). Pots were filled with 50 L of a 3:1 coarse sand (Pre-stress sand mix; Knife River Inc., Corvallis, OR; Jory soil series (fine, mixed, active, mesic Xeric Paleudult collected from the Oregon State University, Woodhall Research Vineyard) mix and 1-year-old dormant vines were planted in 2009. Vines were spaced at 1.0 m x 3.2 m, and trained on a single Guyott system using vertical shoot positioning. Head height of vines was set at 0.5 m from ground level and main shoots were trimmed (hedged) ≈2 weeks after fruit set at a height of 2.2 m from ground level. From 2011 to 2015, vines were pruned to 12 buds plus one renewal spur, and later thinned to 10 shoots and a single renewal spur after threat of frost had passed each spring. All vines received complete nutrient solution (half-strength Hoagland’s solution; Hoagland and Arnon, 1950) for the first 3 years after planting (2009 to 2011), delivered through the drip irrigation system three times per week from budbreak to veraison, and about two times per week from veraison to harvest. Vines received water on other days during the growing season as needed. Vines were drip irrigated using four pressure compensating emitters (2.0 L h⁻¹) per microplot attached to a ring of spaghetti tubing to disperse water evenly throughout the pots. Irrigation inputs were managed based on volumetric soil water content (θv) and vine water status, using the same approach as described in Schreiner et al. (2010). The θv was measured by time domain reflectometry (TDR; Soil Moisture Equipment Corp., Santa Barbara, CA) using 45 cm steel waveguides (rods) installed vertically in the pots halfway between the vine trunk and the pot edge. One set of waveguides was installed in each plot replicate in the center vine in each plot.

Treatments with varying N, P, or K levels were applied to vines from 2012 to 2015. The concentration of N was supplied at four lower rates (75%, 50%, 30%, and 15% of Control rate) along with the Control (100%), where the total concentration of N during fertigation was 7.5 mm (equivalent to half-strength Hoagland’s solution). Phosphorus was supplied at three reduced rates (50%, 20%, and 0% of Control) along with the Control (100%) where the total P concentration delivered during fertigation was 0.5 mm in the Control. Potassium was supplied at three lower rates (50%, 20%, and 0% of Control) along with the Control (100%), where total K concentration during fertigation was 4.5 mm. A total of 11 treatments were applied to 20 vines each in a randomized complete block design with four replicates in each treatment consisting of five continuous vines per plot (experimental unit). Treatments are designated as Control (100% N–P–K), 75%N, 50%N, 30%N, 15%N, 50%P, 20%P, 0%P, 50%K, 20%K, and 0%K. Vines in the 50% N treatment received 50% of the concentration of N supplied to vines in the Control treatment, but all other nutrients (including P and K) were supplied at the same rate as the Control. In this way, only N supply was altered in the low N treatments, only P supply was altered in the low P treatments, and only K was altered in the low K treatments. Vines were fertigated with varying nutrient supply three times per week from budbreak to veraison and about two times per week from veraison to harvest by supplying 4 L of the respective nutrient solution per microplot. The application of 4 L of fertilizer solution increases the θv by 8% and was sufficient to drain out of the inner pots (confirmed by visual inspection) early in the growing season when soil moisture was maintained at a higher level. Later in the summer when vines were exposed to moderate water stress, this was not always sufficient to drain out of the inner pots. To ensure that fertilizer salts did not accumulate during the late summer, vines were irrigated for a 2-h period (16 L) at the beginning of July, August, and September in each year. The potential accumulation of salts was monitored by measuring the soil electrical conductivity (EC) using a soil probe at multiple depths (Model no. 2265FSTP; Spectrum Technologies Inc., Plainfield, IL). The EC values were always below 1.2 mS cm⁻¹.

In 2015, after 3 years of lowest N supply (15% N), vines in this treatment were boosted back to the Control rate of N supply (7.5 mm total N per fertigation event) to examine recovery from N deficiency. Therefore, this treatment was excluded from our data set in 2015 so the N treatments that year only had data from 16 plots (4 treatments x 4 replicates) instead of 20 plots, thus giving 76 observations (n = 76) for N treatment plots when all years were used in models.

Fruit clusters were thinned ≈2 weeks after fruit set in each year to 1.0 cluster per shoot including the renewal shoot (spur) by retaining either the basal cluster, or the second cluster. Although the specific clusters that were removed from each shoot were not recorded, basal clusters were usually retained on count shoots (10 per vine) and the second cluster was more often retained in the case of spur shoots (one per vine). In no case was a third cluster (rare) retained. Fungicides were used to manage powdery mildew [Erysiphe necator (Schw. Burr.)] and bunch rot (Botrytis cinerea L.) as per standard practices in the region (Skinkis et al., 2012). Differences in fruit cluster solar exposure and vine water status resulting from differences in canopy size among the different treatments (i.e., indirect effects of nutrient supply) were minimized by applying variable leaves of leaf removal and irrigation in different treatments as previously described (Schreiner et al., 2013). However, new targets for soil water content were determined for this sand:soil mixture based on relationships between θv and midday leaf water potential (Ψ_leaf) (pressure chamber; PMS Instrument Company, Albany, OR) and stomatal conductance (gs) (LI-COR 6400 photosynthesis system or LI-COR 1600 steady state leaf porometer; LI-COR Inc., Lincoln, NE) using the same approach as previously described (Schreiner et al., 2013).

Every 1 to 3 during the growing season (θv) was monitored, and irrigation was applied whenever θv approached the target soil water content desired. The θv, at field capacity and the permanent wilt point was previously determined at a bulk density of 1.40 g cm⁻³ with a psychrometer (SC-10; Decagon Devices, Pullman, WA). The θv at field capacity was 25.2% and the θv at the permanent wilt point [soil water potential (Ψ_soil) = –1.5 MPa] was 7.0%. In situ wilt tests were conducted on 20 nonexperimental (extra) vines in the vineyard by withholding irrigation on two separate occasions in summer of 2011 and another in 2012. On all three occasions, vines showed visual signs of leaf wilt and minor berry shrivel, and gs was below 50 mmol m⁻² s⁻¹ whenever θv averaged 8.9%, and Ψ_leaf values were ≈–1.5 MPa. Moderate water stress, defined as Ψ_leaf between –1.0 and –1.3 MPa and gs values between 50 and 150 mmol m⁻² s⁻¹ (Lovisolo et al., 2010), occurred at θv between 10% and 13%. Vines were thus irrigated accordingly: before fruit set, irrigation was applied to maintain θv above 17%; from fruit set to veraison, vines were irrigated when θv was between 10% and 13% to help control canopy growth; and after veraison, vines were irrigated to maintain θv of 14% to 15%. Irrigation was applied after 21:00 PST and rates were adjusted daily as needed based on mean θv, values per treatment.

Commercial vineyard systems. Data from four commercial vineyards were used to compare leaf and petiole N relationships to must YAN levels. Data from the commercial sites used here were all ‘Pinot noir’ vineyards in the Willamette Valley region in western Oregon. Data from two of the sites came from a vineyard floor cover crop trial in 2004 and 2005, where seven different cover crop treatments were imposed at each site (Sweet et al., 2010). Both sites were planted to Pommard clone on 3309C rootstock, and one was located on a Jory soil series, and the other on Yamhill soil series (fine, mixed, superactive, mesic Ultic Haploxeroll). Data from the third site was obtained from a foliar N fertilizer trial conducted in 2013 using low rates of urea-N to boost fruit YAN levels on a Woodburn soil series (fine-silty, mixed, superactive, mesic AquUltic Argixeroll) with clone 115 on 3309C rootstock. The fourth site was from a vineyard floor trial on Jory soil series using clone 115 on 101–14 rootstock and included data from 2010 to 2013. All leaf and petiole nutrient data used here were derived from individual treatment plots at each site and analyzed in our facility using the same equipment and methods as per the pot-in-pot data.

Leaf and petiole nutrient status (predictors). Vine leaf blades and petioles were collected to determine nutrient status at 50% bloom.
and at 50% veraison (color change). Ten leaves per plot were sampled from count shoots at both bloom and veraison between the hours of 9 and 11 AM PST. Opposite cluster leaves were collected at bloom and paired leaf samples comprising a leaf opposite a cluster and a recently expanded leaf were collected at veraison. Leaf blades and petioles were separated, rinsed in distilled water, dried at 65 °C for 48 h (Shel Laboratory FX 28-2; Sheldon Manufacturing Inc., Cornelius, OR), and ground to pass through a 425-μm sieve. Nitrogen was determined via combustion analysis (Leco Inc., St. Louis, MO), and P and K concentrations were measured by inductively coupled plasma-optical emission spectrometry (ICP-OES) (Perkin Elmer Optima 3000DV; Wellesley, MA) after microwave digestion in HNO₃ (Jones and Case, 1990). Reference standard apple (Malus domestica L.) leaves (no. 151, National Institute of Standards and Technology) were included in each set of samples to ensure instrument and digestion procedures were accurate. Leaf blade and petiole concentrations are expressed based on tissue dry weight (DW).

Vine growth, yield, and juice nutrients (response variables). Leaf area per vine was measured at veraison in each year by first obtaining the primary shoot length and the length of all lateral shoots for every shoot on the middle three vines per plot. The area of leaves on main shoots and lateral shoots was then determined on 20 random shoots per treatment (ensuring that both larger and smaller shoots were included from each treatment) by comparison with series of concentric circles of known area as in Schreiner et al. (2012). Leaf area per vine was calculated from the relationships between leaf area and shoot length for each main shoots and lateral shoots and summing the calculated areas for all shoots per vine. Dormant season cane (pruning) weights (fresh) from all vines were determined in the winter by weighing the count shoots from the previous season. Fruit was harvested in each year based on a random sampling of berries from all plots (3 berries per plot) when berries reached ≈22–23 °Brix. However, in 2013, high rainfall just before fruit maturity decreased fruit sugars below 20 °Brix and fruit was eventually harvested at ≈21 °Brix. All plots were harvested on the same day each year. Fruit clusters were removed from each vine, counted, and weighed. A subsample of five randomly selected clusters from each plot were juiced using a stainless steel hand-crank press to obtain 625 mL/kg⁻¹ fresh weight of clusters using at least two pressings that were combined. Fruit maturity indices (soluble solids, pH, and titratable acidity) were determined as previously described (Swete and Schreiner, 2010). Yeast assimilable nitrogen concentration in must was determined by summing primary amino acid-N obtained by o-phthalaldehyde (OPA) assay (Dukes and Butzke, 1998) and ammonia-N by an enzymatic assay (Sigma ammonia assay kit; Sigma Chemical Co., St. Louis, MO). Must P and K concentrations were measured by ICP-OES after microwave digestion in HNO₃ as per leaf blade and petiole samples. Must concentrations are expressed based on juice volume.

Statistical analysis. Relationships between predictor (leaf blade and petiole nutrient concentrations) and response variables (leaf area at veraison, pruning mass at dormancy, yield, and juice/must nutrient concentrations, and pH) were assessed by regression for each nutrient separately. All data used were derived from the individual replicate plots for various treatments (i.e., raw data, not the means). The treatment impacts of varying N, P, and K supply on vine productivity and fruit and wine composition from this trial will be presented in future publications. For end-user decision-making, we wanted to determine the most simplistic models for predicting vine productivity and juice composition. Therefore, we focused on assessing linear models, even though some data may be better reflected by more complex models (e.g., logistic). In addition, we wanted to determine models with broad inference capability over time. Therefore, we focused on selecting models that were least influenced by plant age and phenology. Data for any given year used for regressions between nutrient status and response variables were only included in the overall model if the relationship for that year was also significant. For example, there were some years where the relationship between P and K status and some response variables was not significant. When this occurred, the data from that year were not used in the final model, and is indicated in text.

Linearity and homoscedasticity were assessed by plotting residuals vs. predicted values. Normality was assessed using normal probability plots and the Kolmogorov–Smirnov and Shapiro–Wilk tests. To assess whether leaf blade or petiole nutrients were better predictors of selected response variables, both the adjusted coefficient of determination (r²_adj) and root mean square error (RMSE) were used to evaluate model fit. The r²_adj is a unit-less relative measure of the fit and assesses the proportion of total variance that is explained by the model. The RMSE is an absolute measure of the fit in response variable units (lower RMSE indicate better fit) and assesses how accurately the model predicts the response. The RSME and r²_adj between models were compared only if their units were the same and they were fitted to the same sample of the same dependent variable. The correlations between leaf and petiole nutrients and response variables were compared using Fisher’s r to z transformation. The RMSE was used to validate that r²_adj was a reliable parameter for comparing models (e.g., larger r²_adj associated with smaller RMSE). The proportion of the variation accounted for by predictor variables in mixed models that included year (plant age), and phenology (bloom vs. veraison), or when both leaf and petiole concentrations were used simultaneously was assessed using eta squared (η²). When applicable, differences in regression coefficients were assessed at P < 0.05 and Iₛₚ > 2. After conducting regressions separately for leaf and petiole, we also explored modeling both tissues together by either forcing both in models, or doing stepwise, or best subsets with Mallows’ C_p test models to confirm which tissue performed better for each response variable.

Results

Data used for modeling from pot-in-pot system. In the pot-in-pot trial, leaf blade N concentrations had a wider range than petiole N concentrations at bloom and at veraison (Table 1). In contrast, petiole P and K concentrations had a wider range than leaf blade concentrations of these nutrients at bloom and at veraison. The range of leaf blade N concentration values was about two times greater than the range for petiole N at bloom and about four times greater at veraison. Compared with the range of concentrations in leaves, the range of petiole P concentrations was about 0.5 times greater and the range of petiole K concentrations was 3.5 times greater. Leaf blade concentration data were less variable than petiole concentration data (Table 1). In general, the coefficients of variation (CV) for leaf blade N, P, and K concentrations were smaller than for petioles.

There were linear relationships between N, P, and K supply and corresponding leaf and petiole concentrations for each of these nutrients (data not shown). There were strong linear relationships between N status (leaf blade and petiole N concentrations) and all response variables, including yield, in all four years (Table 1). Phosphorus and K supply did not alter response variables in all years, therefore not all years were included in models for P and K (Table 1). Varying P and K supply only altered growth in the last 2 years of this study (2014 or 2015). Yield was only altered by low P or K supply in the final year of this study (2015). However, P supply altered must P concentrations in all years, and K supply altered must K concentrations and must pH in the last 3 years (2013–15).

Relationships between N and response variables in pot-in-pot system. Linear regressions between leaf blade and petiole N concentrations and vine growth parameters (leaf area and pruning mass), yield, or must YAN showed that leaf N was superior compared with petiole N for predicting all response variables (Table 2). Leaf blade N models had better fit (r²_adj) and lower error (RMSE) than petiole N models at both bloom and veraison. In addition, correlation coefficients (r) for leaf blade N models were significantly greater than for petiole N for each response variable examined at both bloom and veraison (based on Fisher’s r to z comparison). The strength of the models did not differ for either leaf blade N or petiole N when comparing bloom to veraison. The slope (β₁) of the regression models for leaf
blade N did not differ between bloom and veraison for any response variable. In contrast, the slope of the petiole N models was larger at veraison than at bloom for each of the response variables. Therefore, leaf blade N is better than petiole N for predicting vine responses as sampling time does not affect the strength of the leaf blade N relation to vine responses only the y-intercept at different sampling times (β0 not shown).

Further confirmation that leaf blade N was a better predictor than petiole N was evaluated by best subsets and stepwise linear regression using both tissues for inclusion in models. Results indicated that at bloom, only leaf blade N was included in models for leaf area and yield, whereas for pruning mass and YAN, both leaf blade and petiole N were included in models. However, when both leaf blade and petiole N were included in models, the β1 for petiole N was negative and petiole N accounted for less of the variation than leaf blade N (lower r²). Similar results occurred at veraison, where only leaf blade N was included in models for leaf area, pruning weight, and yield, whereas both leaf blade and petiole N was included in models for YAN, but again the β1 for petiole N was negative and the r² for petiole N was lower than leaf blade N.

Leaf blade N was a better predictor for all vine responses than petiole N when comparing all years because petiole N varied greatly by year, whereas leaf blade N concentrations had less year-to-year variation (Figs. 1 and 2). Differences in petiole N concentrations among years were especially evident in 2012, for leaf area, pruning mass, and YAN and in 2014 for yield. The lowest concentrations for both leaf blade and petiole N had occurred in 2012, the first year vines were exposed to varying N supply. There were slight differences among years with leaf blade and petiole N models; however, removing specific years from leaf blade N models did not alter r² adj. In contrast, removing specific years from petiole N models improved r² adj. This was mainly because the slopes (β1) for petiole N regressions differed greatly among years, whereas year-to-year variation in slopes for leaf blade N regressions had less impact on model fit. For example, even though β1 for the relationship between leaf blade N and leaf area at veraison was greater in 2015 than in 2013, and β1 for the relationship between leaf blade N and pruning mass was greater in the last 3 years as compared with 2012; removing the year with different β1 (2013 for leaf area, 2012 for pruning mass) did not significantly improve model fit. Whereas for petiole N models, removing the 2012 data for leaf area, pruning mass and YAN improved model fit.

To verify our conclusion that leaf blade N was more robust predictor across years, we also evaluated whether including year as a categorical factor in our regressions between N concentration and response variables improved overall model fit for both leaf blade and petiole. The resulting proportion of variance accounted for by year in leaf blade N models was lower (r²), than for petiole N models. For example, at bloom in the leaf blade N model, year accounted for ≈4% of the variation in YAN, whereas year accounted for ≈13% of the variation in YAN in the petiole N model.

Relationships between N and response variables in commercial vineyards. Given the clear differences in predictive strength between leaf and petiole N from the pot-in-pot system, we compared these results to data from commercial vineyards. The largest data set from commercial vineyards available to us included nutrient status in paired samples from leaf blades and petioles along with must YAN concentrations from replicated trials conducted in western Oregon. The field data support our results from the pot-in-pot trial, where leaf blade N was a better predictor than petiole N across a number of sites at both bloom and veraison (Fig. 3). The overall fit of the regressions was not as good for the field data compared with data from the pot-in-pot system, given the greater variability that occurred across commercial blocks. The β1 between pot-in-pot and field data sets did not differ for bloom leaf blade N or petiole N models nor for veraison leaf blade N models in estimating must YAN levels. In contrast, the β1 from the petiole N model for the pot-in-pot vineyard at veraison was greater than the β1 from field data set model.

Vines with low N status had less green leaves (more yellow) and smaller canopies than vines with high N status (data not shown). This was obvious in the pot-in-pot trial, where different N treatments were in close proximity. However, in the commercial sites, differences in leaf color among various treatments that impacted N status were not obvious, even though differences in vigor could be seen visually among different treatment plots.

Relationships between P and response variables in pot-in-pot system. Linear regressions between leaf blade and petiole P concentrations and vine growth, yield or must P concentrations from the pot-in-pot trial indicated that P concentrations from both tissues generally have similar predictive strength (Table 3). However, petiole P at bloom had a stronger correlation to must P concentrations than leaf blade P at bloom and the model RMSE was lower for petiole P. On the other hand, the r² adj from leaf blade P models for the other response variables (growth and yield) were slightly but not significantly higher than petiole P models and RMSE values were generally lower for leaf blade P than petiole P models. The strength of the relationship for either leaf blade P or petiole P did not differ between bloom and veraison sampling times. The β1 for both leaf blade P and petiole P models and each response variable were greater at veraison than at bloom, indicating that small changes in P concentrations at veraison can cause a greater magnitude of change in the measured response variable than at bloom. However, the range in P concentrations was smaller at veraison than it was at bloom (Fig. 4) and the r² adj for bloom and veraison models were similar, indicating that both tissues have similar reliability at each sampling time.

Of all response variables measured, must P concentrations had the strongest relationship to plant P status at both bloom and veraison (Table 3; Fig. 4). The relationship between must P and petiole P is linear at bloom but appear to be curvilinear at veraison (Fig. 4). Indeed, a quadratic model of the relationship between veraison petiole P and must P was
Leaf area (m²) Bloom 0.176 a 0.689 a 0.33 <0.001 0.274 b 0.440 a 0.45 <0.001 0.012

Veraison and must P did not have a better quadratic relationship between petiole P at veraison and must P. The relationship between leaf blade P and petiole P was assessed as curvilinear, but a quadratic fit was not significantly better than the linear model with the same coefficient. However, this quadratic relationship between petiole P at veraison and must P did not have a better quadratic relationship than the linear model between leaf blade P at veraison and must P. The relationship between bloom leaf blade P and must P also appears to be curvilinear, but a quadratic fit was not better than the linear fit (based on $r^2_{adj}$).

Further evaluation of the predictive ability of leaf blade P and petiole P was assessed by best subsets and stepwise linear regression using both tissues for inclusion in models. Results indicated that at bloom, both leaf blade P and petiole P were included in the models for must P, and petiole P had a greater $\eta^2$ than leaf blade P. In contrast, at veraison, although both leaf blade P and petiole P were included in models for must P levels, petiole P had a lower $\eta^2$ than leaf blade P.

Leaf symptoms of P deficiency (anthocyanin accumulation, red color) were observed in years 2013 to 2015, first appearing in 2013 after fruit harvest and in subsequent years just before the time of fruit harvest. The symptoms were most obvious in the 0%P treatment, but were also evident in the 20%P treatment when veraison leaf blade P was ≤1.0 g·kg⁻¹ DW and veraison petiole P was ≤0.50 g·kg⁻¹ DW. Not surprising the presence of leaf symptoms was more closely correlated to leaf P than to petiole P values (data not shown).

**Relationships between K and response variables**

- **Linear regression**: Linear regressions of K status and vine growth, yield, and must K or must pH indicated that both leaf blade K and petiole K were similar in predicting vine responses (Table 4; Fig. 5). There was no case where either leaf blade or petiole models were better predictors to any of the response variables. Similar to leaf P models, the $r^2_{adj}$ and RMSE associated with leaf blade K models were slightly higher and lower, respectively, than for petiole K models; however, these differences were not significant. In addition, sampling time had little influence on model $r^2_{adj}$ and RMSE, although leaf blade K and petiole K models for leaf area, pruning mass, and yield had slightly higher $r^2_{adj}$ at bloom than at veraison. In contrast, petiole K models had slightly higher $r^2_{adj}$ for must K levels and must pH at veraison than at bloom. Further evaluation of the predictive ability of leaf blade K and petiole K was assessed at postharvest by best subsets and stepwise linear regression using both tissues for inclusion in model. Results indicated that only leaf blade K was included in models for pruning mass, leaf area, must K, and must pH, and this occurred at both bloom and veraison. In contrast, only leaf blade K was included in must K models for yield at bloom, but both blade K and petiole K were included in models for yield at veraison. Similar to the relationship between N and yield, when both leaf blade K and petiole K were included in models for pruning mass, leaf area, must K, and must pH, this occurred at both bloom and veraison. In contrast, only leaf blade K was included in models for yield at bloom, but both blade K and petiole K were included in models for yield at veraison. Similar to the relationship between N and yield, when both leaf blade K and petiole K were included in models for yield at veraison; however, this quadratic relationship between petiole K at veraison and must K did not have a better quadratic relationship than the linear model between leaf blade K at veraison and must K. The relationship between bloom leaf blade K and must K also appears to be curvilinear, but a quadratic fit was not better than the linear fit (based on $r^2_{adj}$).

**Fig. 1. Relationships between leaf blade and petiole nitrogen (N) concentrations at veraison and vegetative growth (leaf area and pruning mass) of 'Pinot noir' grapevines grown in a pot-in-pot vineyard.** Plants were transplanted in 2009 and data collection began in 2012. Leaf blades and petioles were collected at 50% veraison. Leaf area was measured at veraison and pruning mass at dormancy. Data points are N concentrations [g·kg⁻¹ dry weight (DW)] from five vine plot replicates. Line for linear regression over 4 years (n = 76). Root mean square error (RMSE) from linear regression.
occurred in both years in plots where veraison leaf blade K was $4.2 \text{ g} \cdot \text{kg}^{-1} \text{ DW}$ and veraison petiole K was $3.6 \text{ g} \cdot \text{kg}^{-1} \text{ DW}$. Some fruit clusters in the 0%K and 20%K treatments also developed late bunch stem necrosis (usually affecting the tip of clusters or shoulders) in 2014 and 2015. Bunch stem necrosis symptoms occurred when veraison leaf blade K was $5.5 \text{ g} \cdot \text{kg}^{-1} \text{ DW}$ and when veraison petiole K was $5.0 \text{ g} \cdot \text{kg}^{-1} \text{ DW}$ (data not shown).

**Discussion**

Nutrient concentrations in leaf blades were clearly better than in petioles for predicting the effects of N on growth, yield, and must YAN levels in our trial with ‘Pinot noir’. In every case for each response variable tested here, leaf blade N models were significantly better than petiole N models. This was true at bloom and at veraison. In addition, when both leaf blade and petiole N were simultaneously assessed in regression models, in most models, only leaf blade N was included, and whenever petiole N was included, it had a negative slope and accounted for only a small proportion of variance of response variables ($r^2$). Field data from commercial sites relating leaf blade or petiole N to must YAN levels also revealed that leaf blades were better than petioles.

One of the primary reasons that leaf blade N concentrations were better than petiole N in our study is due to the greater range of N concentrations that occur in leaves as opposed to petioles and that leaf blade N varies less petiole N among and within years. A greater year-to-year variability in petiole N concentrations compared with leaf blade N in grapevines has been observed by others (Benito et al., 2013; Romero et al., 2013; Schreiner, 2010; Schreiner et al., 2012). Petiole NO$_3$-N concentrations have shown large fluctuations both within and across growing seasons (Christensen, 1969; Cook and Kishaba, 1957; Cook and Lider, 1964; Robinson et al., 1978). Why petiole N varies more among years is not clear, although petiole NO$_3$-N levels were negatively related to spring rainfall amounts in California, and petiole NO$_3$-N was not related to yield (Cook and Lider, 1964).

The N concentrations in leaf blades have been recommended over petioles in diagnosing the N status of grapevines by others. Results from a survey of Washington vineyards combined with a N fertilizer trial in cultivars Riesling and Merlot showed that leaf blade N was superior to petiole N in reflecting where N fertilizer applications had increased yield, and that veraison was a better sampling time for nutritional diagnosis than was bloom in that region (Davenport et al., 2012). Leaf blades were also preferred over petioles in diagnosing N status based on the greater stability and lower CV of leaf blade N concentrations over that in petioles in ‘Garnacha tinta’ and ‘Tempranillo’ vineyards (Benito et al., 2013; Romero et al., 2013). Bertoni and Morard (1982) working in table grape vineyards came to the conclusion that both blades and petioles have a similar diagnostic potential, although petioles could be preferred for sensitivity (for example when conducting N trials in vineyards) while leaf blades are better for reliability across seasons and cultivars.

Numerous authors working on wine grape cultivars have shown that that leaf blade N was better related to vine N status than was petiole N; however, most have also concluded that petiole NO$_3$-N is the more sensitive indicator for N status (Christensen, 1984; Conradie, 2001; Conradie and Saayman, 2011).
In most cases, the yearly variation of petiole NO₃-N levels has not been reported. The well-known variation of petiole NO₃-N concentrations across years (Christensen, 1969; Cook and Kishaba, 1957; Cook and Lider, 1964) combined with highly variable petiole NO₃-N data collected from Pacific Northwest vineyards (Davenport et al., 2012) indicates that petiole NO₃-N is not a robust indicator of vine N status.

The importance of vine N status as a driver for growth, yield, and must YAN levels was reflected in our data as the slopes ($\beta_1$) for relationships between leaf blade N concentrations and all response variables were similar between bloom and veraison sampling times (only the intercept had shifted). Petiole N concentrations, however, had different slopes at bloom vs. veraison. The similar slopes for leaf blade N at both phenological stages indicate further that leaf N has greater utility for predicting vine responses than petiole N. Our data show that the year-to-year variation for leaf N relationships to four...
The comparison of leaf blades to petioles as a diagnostic tissue for understanding and predicting vine responses to P and K was less clear than our findings with N. This was due in part to having less overall impact of P and K supply on vine growth and yield, as opposed to the rapid and strong effect of varying N supply. Low levels of either P or K supply here reduced vine growth and yield only after 3 or 4 years in those treatments with zero added P or K to the soils via fertigation.

Table 4. Slope ($\hat{b}_1$), adjusted coefficient of determination ($r^2_{adj}$), and root mean square error (RMSE) for linear relationships between potassium (K) concentrations in leaf blades or petioles and vine productivity measures, K concentrations and pH in must from ‘Pinot noir’ grapevines in 2012–15.

| Response variable and phenology$^a$ | Leaf blade K$^b$ | Petiole K$^b$ | Leaf blade vs. petiole P value$^a$ |
|-----------------------------------|-----------------|---------------|----------------------------------|
|                                  | $\hat{b}_1$ | $r^2_{adj}$ | RMSE | $P$ | $\hat{b}_1$ | $r^2_{adj}$ | RMSE | $P$ | Leaf blade vs. petiole P value$^a$ |
| Leaf area ($m^2$)                 |                |               |      |     |                |               |      |     |                                    |
| Bloom                            | 0.102 a        | 0.534 a       | 0.212 | <0.001 | 0.029 a        | 0.524 a       | 0.214 | <0.001 | >0.050 |
| Veraison                         | 0.093 a        | 0.480 a       | 0.223 | <0.001 | 0.025 a        | 0.418 a       | 0.236 | <0.001 | >0.050 |
| Pruning mass (g)                 | 28.5 a         | 0.426 a       | 0.732 | <0.001 | 8.1 a          | 0.398 a       | 0.750 | <0.001 | >0.050 |
| Bloom                            | 26.9 a         | 0.408 a       | 0.744 | <0.001 | 7.1 a          | 0.345 a       | 0.782 | <0.001 | >0.050 |
| Veraison                         | 0.125 a        | 0.591 a       | 0.284 | <0.001 | 0.053 a        | 0.565 a       | 0.293 | <0.001 | >0.050 |
| Yield                            | 0.153 a        | 0.504 a       | 0.313 | <0.001 | 0.036 a        | 0.295 a       | 0.373 | 0.017 | >0.050 |
| Bloom                            | 0.656 a        | 0.319 a       | 0.195 | <0.001 | 27 a           | 0.436 a       | 0.250 | <0.001 | >0.050 |
| Veraison                         | 110 a          | 0.611 a       | 0.208 | <0.001 | 27 a           | 0.528 a       | 0.229 | <0.001 | >0.050 |
| Must K (mg L$^{-1}$)             | 0.067 a        | 0.414 a       | 0.163 | <0.001 | 0.015 a        | 0.333 a       | 0.173 | <0.001 | >0.050 |
| Bloom                            | 0.065 a        | 0.532 a       | 0.144 | <0.001 | 0.016 a        | 0.438 a       | 0.159 | <0.001 | >0.050 |
| Veraison                         |                |               |      |     |                |               |      |     |                                    |

$^a$Leaf area per vine at veraison (2014–15, n = 32), pruning mass per vine at dormancy (2014–15, n = 32), yield per vine (2015, n = 16), and must K and pH (2013–15, n = 48) at commercial maturity. Leaf blades and petioles collected at 50% bloom and 50% veraison.

$^b$Potassium concentrations in g kg$^{-1}$ dry weight. $\hat{b}_1$ followed by the same letter for each response variable does not differ between bloom and veraison ($P > 0.05$). $r^2_{adj}$ followed by the same letter for each response variable do not differ between bloom and veraison based on Fisher’s $r$ to $z$ comparison ($P > 0.05$).

$^c$From Fisher’s $r$ to $z$ comparison comparing leaf vs. petiole relationships.

Fig. 4. Relationships between leaf blade and petiole phosphorus (P) concentrations and must P concentrations of ‘Pinot noir’ grapevines grown in a pot-in-pot vineyard. Plants were transplanted in 2009 and data collection began in 2012. Leaf blades and petioles collected at 50% bloom and 50% veraison. Must P measured at commercial maturity. Data points are P concentrations [g kg$^{-1}$ dry weight (DW)] from five vine plot replicates. Line for linear regression over 4 years (n = 64). Root mean square error (RMSE) from linear regression.

Key vine responses within the same vineyard are minimal, and supports previous findings where leaf blade N was better correlated to must YAN levels than was petiole N in a previous pot-in-pot trial using own-rooted ‘Pinot noir’ (Schreiner et al., 2013). Petiole N status is not as reliable across years even in a highly controlled system like a pot-in-pot vineyard, indicating poor utility as a diagnostic tool for N status.

The comparison of leaf blades to petioles as a diagnostic tissue for understanding and predicting vine responses to P and K was less clear than our findings with N. This was due in part to having less overall impact of P and K supply on vine growth and yield, as opposed to the rapid and strong effect of varying N supply. Low levels of either P or K supply here reduced vine growth and yield only after 3 or 4 years in those treatments with zero added P or K to the soils via fertigation.
Both leaf blades and petioles collected at veraison were equally good at predicting vine responses to varying P supply. At bloom, petiole P was better than leaf blade P in predicting must P levels in the fruit at harvest, whereas the other vine responses (growth and yield) had similar relationships with petiole or leaf blade P. An argument can be made on this basis to choose petioles for P diagnosis at bloom, as this was the only case where leaf blade and petiole P relationships differed significantly. However, one could also argue that leaf blades might be preferred over petioles in diagnosing P status because leaf blade P values had better model fit ($r^2_{adj}$) and lower error than petiole P values for all the other vine responses (leaf area, pruning mass, and yield) at bloom and at veraison, even though these were not statistically significant.

Petiole P concentrations were greater than leaf blade concentrations at bloom when P status was high, but this was not apparent at veraison in the pot-in-pot vineyard. This confirms numerous studies where P has been shown to accumulate in petioles but not in leaves when vine P status is high (Christensen, 1984; Conradie and Saayman, 1989; Cook, 1966; Klein et al., 2000). Indeed, the higher level of P in petioles under conditions of high P status is the primary reason that petioles have been recommended over leaf blades as the diagnostic tissue for diagnosing P status. However, this requires collecting and examining P in both tissues which defeats the purpose of choosing one tissue to minimize costs. Conradie and Saayman (1989) concluded that petiole P was preferable to leaf blade P in diagnosing the P status of ‘Chenin blanc’ vines based on larger shifts in petiole P concentrations in response to P fertilizer. However, if one focuses on the relationships between leaf blade and petiole P concentrations and must P levels, leaf blade P also correlated better with must P than petiole P in Conradie and Saayman (1989). The greater variability associated with petiole P concentrations as compared with leaf blades has led others to conclude that leaf blades are better predictors of certain vine qualities depending on the time of year. When both leaf blade P and petiole P concentrations were simultaneously assessed in regression models for must P, petiole P at bloom accounted for more of the variability in must P (greater $r^2$) than leaf P at bloom. However, leaf blade P at veraison accounted for more of the variability in must P than petiole P at veraison. In models assessing both leaf blade P and petiole P for predicting pruning weights and yield, only leaf blade P was included in models at both sampling times. Romero et al. (2013) found that the CV for leaf blade and petiole P concentrations was similar at bloom, whereas the CV for leaf blade P concentrations was lower than petioles at veraison in ‘Tempranillo’ grapevines. Our data from ‘Pinot noir’ indicate petiole P has a greater CV than leaf blade P at both bloom and veraison. This suggests that the lower variability in leaf P may account for its better predictive ability at veraison but not at bloom. The results here and the results from other studies examining P suggest that both tissues are about equally good in diagnosing vine P status, although petioles may be preferred at bloom and leaf blades may be preferred at veraison.

For K, even though the concentration range was much greater in petioles compared with leaf blades, our leaf blade data were...
equally good in predicting vine responses. Model fit did not differ between leaf blades and petioles in predicting vine responses to K status. Others have concluded that petioles are more useful than blades in diagnosing K status of grapevines because of the higher concentrations of K in petioles and the larger changes in petiole K vs. leaf K in response to changing K supply (Christensen, 1984; Conradi and Saayman, 1989; Cook, 1966; Hepner and Bravdo, 1985; Ulrich, 1942a). However, in most of these studies, the variability associated with petiole and leaf blade K values was not presented.

As noted above for P, a greater concentration range of K in petioles than in blades does not necessarily lead to better diagnostic value if there is also wider scatter of the data. The data here with ‘Pinot noir’ support the notion that petioles and blades have similar diagnostic value to understand vine K status, because the larger concentration range and larger response in petioles are associated with greater variability or scatter (Bertoni and Morard, 1982). More recent findings have also shown petiole K values to have greater variability than leaf blade K values (based on cv) as observed here (Benito et al., 2013; Romero et al., 2013). Since leaf blade K concentrations are more stable near bloom and veraison, leaf blades were recommended over petioles as a more reliable indicator of K status (Benito et al., 2013; Romero et al., 2013). The wide temporal variation in petiole K in the weeks before and after bloom was demonstrated long ago (Christensen, 1969). Petiole K concentrations were, however, better correlated to yield, must acid levels, and K concentrations than were leaf blade K concentrations in a survey of Agiorgitiko (red wine grape cultivar) vineyards in Greece (Assimakopoulou and Tsougrianis, 2012). Unfortunately, only the correlation coefficients were given in that study, making comparisons to this and other trials difficult.

When both leaf blade K and petiole K concentrations were simultaneously assessed in regression models, leaf blade K was clearly a superior predictor to petiole K, which was only included in the model assessing yield using veraison K status. As with N, the slope (β1) for petiole K was negative when both leaf blade and petiole K at veraison were in the model and leaf blades accounted for a greater proportion of the variability in yield (greater r2). The argument that petioles are a better tissue than leaf blades to diagnose the K status of grapevines based on their greater concentration range is not supported by our findings. In our study, the concentration range of K was about 3.5 times greater in petioles than in blades, yet leaf blade models, while not statistically different, had a numerically better fit (lower r2adj) and lower errors (RMSE) than petiole K models. If one had to choose a single tissue, the overall findings from this study indicate that leaf blades are the better alternative to diagnosing the K status of ‘Pinot noir’.

Altering N, P, and K supply to vines provided us with leaf blades and petioles encompassing a range of tissue nutrient concentrations similar to values found in commercial vineyards and allowed us to determine which tissue type is a better choice for diagnosing the nutritional status of ‘Pinot noir’. Our results indicate leaf blades should be recommended over petioles because leaf blade K was clearly superior to petiole K in diagnosing vine responses to varying N status, and because both leaf blades and petioles were similar in predicting responses to P and K status.

Our data comparing predictive ability of tissues at bloom and veraison support recent findings where neither temporal sampling in wine grape vineyards over the season was used to understand variability and stability of leaf blade vs. petiole nutrients (Benito et al., 2013; Romero et al., 2013). In ‘Pinot noir’, leaf blade nutrient concentrations within the ranges reported here have a stronger relationship to productivity and must nutrient levels than petioles, and are less variable and more stable than petiole concentrations. This concept is logical as leaf blades are the primary metabolic tissue of the canopy (Cook, 1966).

The use of leaf blades as opposed to petioles for routine monitoring of nutrient status of grapevines is also supported by practical experience diagnosing magnesium (Mg) deficiency in vineyards in the Willamette Valley of Oregon. Leaf blade Mg concentrations were related to the presence or absence of Mg deficiency symptoms in three commercial vineyards, while petiole Mg concentrations did not differ between symptomatic and nonsymptomatic samples in two of the sites (R.P. Schreiner, unpublished data). The inconvenience of collecting leaf blades owing to their larger size and volume as compared with petioles could be overcome by using a leaf punch to sample only a portion of the blade.

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