

USE OF TISSUE ANALYSIS IN VITICULTURE

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Tissue and soil analysis each have their application for monitoring vineyard mineral nutrition. Of these, tissue analysis is far more useful in determining the grapevines' nutritional status and guiding a fertilizer program. It has not been possible to correlate soil nutrient levels with the grapevines' status and requirements. Reasons include the wide variability of vineyard soil depth, physical conditions and chemistry; differing vineyard design, cultural requirements and irrigation practices; diverse macro- and micro-climates of viticulture regions and vineyard sites; and the inherent differences in nutrient uptake and demand by grape varieties and rootstocks. The value of soil analysis is mostly in the determination of problems related to certain chemical imbalances or excesses such as pH problems (alkalinity and acidity), salinity, cation imbalances (magnesium:calcium:potassium) and excess boron. Soil nutrient analysis can be used in vineyard establishment to determine if there are extremes in mineral nutrient levels. This can serve as a forewarning of fertilizer requirements or the need for immediate treatment prior to or during vineyard establishment. Further information on soil appraisal of chemical problems is available in "Salinity Appraisal of Soil and Water for Successful Production of Grapes", University of California ANR Leaflet 21056 and "Interpretation of Soil and Water Analysis", Chapter 15, *Raisin Production Manual*, University of California ANR Publication 3393.

Tissue analysis is a direct measure the vine's mineral nutrient status. Mineral concentrations in select tissues can account for a vine's root uptake, mobilization, accumulation and demand of most nutrients. However, there are limitations. Nutrient levels vary with tissue type, growth stage, shoot and canopy position, cultivar and growing season. Critical values or standards must be established with these variables in mind. Ongoing experience and data collection is still needed in our widely differing vineyard conditions and regions.

SAMPLING

The method of sampling is dependent on the objective: (1) survey of nutrient status, (2) follow-up nutrient sampling, and (3) diagnosing visual symptoms and disorders.

1. Survey of nutrient status.

This approach is used when surveying a vineyard for general nutrient status and evaluating fertilizer needs or practices. Most of the data and experience in California are based on leaf petioles sampled at bloom. The petioles are taken from opposite flower clusters near the base of the shoot. This provides a clean, easily sampled, repeatable tissue that tends to accumulate nutrients more than other plant parts. The samples should be taken during bloom, the nearer to full bloom the better ("full bloom" is when approximately two-thirds of the caps have loosened or fallen from the flowers). The full bloom sample assures that the tissue will be at the same physiological stage regardless of district and seasonal differences.

Samples should be taken from a single block or management unit and should represent a single variety and rootstock. Areas of distinctly different soil, vine appearance, or other condition should be sampled separately. Each sample should consist of 60-80 petioles collected from representative vines uniformly distributed over the area. Sample from minimally shaded, normal growing shoots on both sides of the vine canopy. Some laboratories may request 100 petioles per sample when petioles are small (e.g. Pinot noir and Gewurtztraminer) or multiple analyses are to be run.

Growers who routinely sample vineyard blocks from year to year may wish to designate specific rows or vines in a representative area. Re-sampling the same vines each time improves consistency in the results when tracking the vineyard's nutrient status and adjusting fertilizer practice over years.

Foliage contamination from a nutrient spray will give erroneous laboratory results. Do not sample after a nutrient spray unless you: (1) are not analyzing for any nutrient element contained in the spray, (2) thoroughly wash the samples (see HANDLING SAMPLES below) or have made arrangements with the laboratory for sample washing, or (3) are sampling uncontaminated tissue later in the season. Sampling uncontaminated tissue is preferable, as it is difficult to remove all the nutrient residue, especially when adjuvants are used with the spray.

2. Follow-up nutrient sampling

Certain nutrients that are in the questionable range at bloom can be re-checked later in the season to determine if deficiency has developed. This is particularly useful with K, which declines in the vegetative parts and can become deficient during fruit ripening. Sampling is best performed at veraison (berry softening at beginning of ripening) as it represents a physiological stage with supportive data and when leaf tissue is still healthy and functioning. Select petioles from recently matured leaves. This would be the second fully expanded leaf, usually the 6th to 7th leaf from the tip, on an actively growing shoot. The sample leaves should have the color and texture of the other mature leaves rather than the lighter and more shiny, tender appearance of young, expanding leaves.

The smaller petioles at this time may require a higher number in the sample as compared to bloom. Take 75 to 100 petioles per sample if more than one determination is needed. For blade samples, 25 to 35 are sufficient due to their greater mass as compared to petioles.

3. Diagnosing visual symptoms and disorders

Visual deficiency or toxicity symptoms more commonly appear during mid-summer to harvest time. Thus, sampling at this time is useful in diagnosing vine disorders or verifying a deficiency or toxicity. For such situations, sample the affected leaves regardless of location on the shoot and at any time when abnormal appearance is noted. When there are no reasonable clues, take both the petioles and blades but analyze them separately. If sodium (Na) and/or chloride (Cl) toxicity are suspected, analyze only the

petioles (these elements accumulate in the petioles, even though the blades may show the symptoms). For excess boron (B), use blades where much greater levels will be found.

It is also a good practice to take a sample of "non-symptom" tissue for comparison when the cause is in question. The "non-symptom" sample can be taken from an area adjoining the "symptom" area while making sure that the comparative samples are taken from the same position on the vine.

HANDLING SAMPLES

Place each sample in a well-labeled, clean paper bag and deliver to the laboratory as soon as possible. If there is a delay, keep the bags open in a dry, well-ventilated place. This will begin the drying process and prevent molding. Never use plastic bags.

Petioles need not be washed unless collected from a dusty vineyard or have residue remaining from a nutrient spray. Leaf blades have a greater surface area for collecting dust and should be washed if they are noticeably dirty. Foliar nutrient sprays can be difficult to wash off because of the adjuvants that are sometimes used. Wash in water containing a small amount of detergent followed by a tap water rinse and two distilled water rinses. Remember, however, that some elements (particularly potassium (K), Na, and Cl) are easily leached from necrotic or dead tissue. Washing should therefore be accomplished quickly, and excess water blotted from the leaves. Blades, in particular, should be dried quickly to avoid mold growth. Refrigeration is also a way to maintain sample freshness if there is a delay in getting samples to the laboratory for washing.

A forced-air or well-ventilated oven at 60° to 80°C (140° to 176°F) is ideal for sample drying. Growers have also successfully dried samples in a vehicle parked in the sun with the windows slightly open.

ANALYSES NEEDED

Only those nutrients known to be deficient or excessive in the district need to be analyzed. For example, nitrogen (N), K, magnesium (Mg), Na, Cl, zinc (Zn), and B are more commonly of concern. Nutritional problems of phosphorus (P), manganese (Mn), and calcium (Ca) are less common, but should be included in initial testing. Copper (Cu) and molybdenum (Mo) problems have not been confirmed in California. Iron (Fe) deficiencies cannot be accurately confirmed with tissue analysis due to lack of correlation of tissue levels with visual symptoms.

Growers may wish to include a broad analysis in their initial sampling, even if there is little chance for deficiency. This will, at least, verify that infrequently deficient nutrients such as P, Ca, and Mn are not necessary in most fertilizer programs. Also, some laboratories will run a "complete" analysis for the same cost as four or five elements.

Survey sampling establishes a base line for general nutritional status. This may be practiced for two or three years to determine yearly differences. Thereafter, only questionable nutrients or those involved in a fertilizer program require further testing. However, fluctuating yields or changes in trellising, pruning, or irrigation practices would warrant continued sampling.

INTERPRETATION OF LABORATORY ANALYSIS

The following interpretations in table 1 give critical values for important grapevine nutritional elements in opposite cluster petioles at bloom unless otherwise noted. The deficiency level is that at which deficiency symptoms may develop and/or a measurable response to fertilization with the nutrient in question can be expected.

Critical levels for nitrate-nitrogen (NO₃-N) are only established for Thompson Seedless. The levels for other nutrients are based on experience with several or more varieties and thus can be applied generally among varieties, as far as is known.

Table 1. Interpretive Guide for Grape Tissue Analysis at Bloom and Veraison

| Nutrient | Deficient (below) | Adequate (above) | Excessive ² (above) | Toxic ³ (above) |
|-------------------------|-----------------------------|-----------------------------|-----------------------------------|--|
| NO ₃ -N, ppm | 350 ¹ | 500 | 2,000 | 8,000 |
| P (total), % | 0.10 (0.08) ⁴ | 0.15 (0.12) ⁴ | | |
| K (total), % | 1.0 (0.5) ⁴ | 1.5 (0.8) ⁴ | | |
| Mg (total), % | 0.2 | 0.3 | | |
| Zn (total), ppm | 15 | 26 | | |
| Mn (total), ppm | 20 | 25 | 300 | 2,000 |
| B (total), ppm | 25 | 30 | | 80 (100) ⁴ 120 (300) ⁴ in blades |
| Na (total), % | | | | 0.5 0.3 in blades |
| Cl (total), % | | | 0.5-1.0 | 1.5 0.5 in blades |

¹Critical NO₃-N levels are based on Thompson Seedless data only. Some laboratories report as % NO₃. Multiply % NO₃ by 2258 for ppm NO₃-N (i.e. 1.0% NO₃ = 2258 ppm NO₃-N).

²Excessive levels may be cautionary rather than indicating known effects on vine performance.

³Critical toxicity values are not well defined due to variety, growing condition, and seasonal differences.

⁴Veraison (berry softening) petiole values are in parenthesis.

Nitrate-nitrogen (NO₃-N). It is well known that grape varieties have inherently different NO₃-N levels. This is, at least, partly attributed to differences in nitrogen metabolism (reduction of NO₃-N in the foliage). For example, Malbec, a high nitrate (NO₃) variety, shows lower nitrate reductase activity than Zinfandel, a moderate NO₃ variety. Nitrate reductase is an important enzyme involved in NO₃ reduction, the first step toward conversion to other nitrogen compounds; its lower activity explains why NO₃ may accumulate more in varieties such as Malbec. Petiole NO₃-N is also influenced by climatic conditions prior to and during bloom.

Thus, critical levels will need to be developed for each variety in various regions. This has only been accomplished with own-rooted Thompson Seedless in the San Joaquin Valley.

Meanwhile, varieties have been grouped according to their typical differences in NO₃-N levels as shown in Table 2. This information is based on data from where varieties are grown on own roots in uniform trial sites. It shows Thompson Seedless to be of medium NO₃-N status while some of the other important varieties are in comparatively high or low categories. Obviously, NO₃-N interpretation with varieties other than Thompson Seedless could be misleading.

Table 2. Ranking of Grape Varieties by Their Comparative Bloomtime Petiole NO₃-N Levels When Grown on Own Roots.

| High | High-medium | Medium | Low-medium | Low |
|-----------------|--------------------|--------------------------|-------------------|------------|
| Malbec | Petite Sirah | Pinot noir | Barbera | Sylvaner |
| Merlot | Chenin blanc | Semillon | Colombard | Salvador |
| Grenache | Emerald Riesling | Cabernet Sauvignon | Gerwurztraminer | Ribier |
| Tinta Madeira | Muscat of | Rubired | Tokay | Flame |
| White Reisling | Alexandria | Ruby Cabernet | | Seedless |
| Sauvignon blanc | Emperor | Chardonnay | | Perlette |
| Black Corinth | Christmas Rose | Zinfandel | | |
| Redglobe | | Carignane | | |
| | | Thompson Seedless | | |
| | | Ruby Seedless | | |
| | | Calmeria | | |
| | | Exotic | | |
| | | Italia | | |

¹Based on data of L. P. Christensen, W. M. Kliewer, and J. A. Cook, UC Davis.

For example, Flame Seedless and French Colombard can be highly vigorous with relatively low NO₃-N levels of 100-200 ppm. This points to the inappropriateness of using the Thompson Seedless data to diagnose deficiency in other varieties. Petiole NO₃-N with other varieties should only be used in a general way to follow fertilizer practices over years or to look at extremely low or high levels along with vigor assessment.

Nitrate-N status can also be strongly influenced by rootstock. Higher petiole values are commonly experienced with Freedom, Ramsey (Salt Creek), St. George, and 3309C. Lower values are more common with 5C, 420A, Harmony and 110R. The interaction of rootstock and fruiting variety is not well understood. However, the NO₃-N status of some varieties such as Flame Seedless and Merlot can be strongly influenced by rootstock. The use of any rootstock will typically increase and decrease the NO₃-N status of Flame Seedless and Merlot, respectively, as compared to own roots.

Seasonal changes in NO₃-N levels can be rapid, especially with extreme weather changes before bloom. Increases are associated with cool temperatures and low light intensity while sunny,

warm weather favors decreased levels. Nitrate-N is typically highest 5 to 10 days before bloom, declines through the bloom period, and reaches a relatively stable, lower level 2 to 3 weeks after bloom.

Nitrate-N can also vary widely from year to year without changes in fertilization practices. This is most often associated with differences in springtime weather conditions as mentioned above. Non-irrigated vineyards subjected to wide differences in rainfall can be particularly affected.

Critical levels of **total N** analysis are not well defined. One problem is that there are relatively small differences in tissue levels between vines responding to N fertilization. This may be due to the masking effects of the protein N that form the leaf tissues as compared to the assimilable N forms, including NO₃-N. However, there is interest in using total N values due to the problems of seasonal, regional, and varietal variability of NO₃-N. Data from recent trials indicate that bloom petiole critical values for deficiency can range between 0.65 and 0.9% N, depending on cultivar. The effects of excess vine N were associated with bloom petiole total N of 0.9 to 1.2%, depending on cultivar. Thus, a tentative threshold level of 0.9% N in bloom petioles is suggested. Vineyards at 0.9% N or below have potential for deficiency while those above may be prone to excess. This threshold level declined gradually through the season to 0.85% N at veraison and 0.8% N at harvest.

Leaf blade total N was found to be inferior to petiole total N as an indicator of vine N status, especially at bloom. There appears to be no value in using bloom blade N levels due to their relative insensitivity to N fertilizer practice and vine response. Veraison blade total N levels demonstrated separation of low and high N status vines at a threshold level of 3.1% N. Overall, leaf blade total N analysis is of questionable value as compared to petiole total N, regardless of growth stage.

Finding a widely effective and useful laboratory method to guide N fertilization continues to be a research challenge. Irrespective of methodology, the final criteria of N fertilization should be an assessment of the vine canopy and rate of vine growth. Highly vigorous vines do not need N, regardless of tissue levels.

Phosphorus (P). Confirmed deficiencies are uncommon in California, although they are increasingly recognized in some coastal and foothill sites, especially in acidic and high Fe soils. Tissue analysis has been useful in separating them from similar-appearing problems such as Leafroll Virus and Willamette Mite injury. Petiole P levels tend to decline through the bloom period and level off through midsummer; hence, critical levels change somewhat during the season. Differences among mature leaf petioles along the shoot are minor. Levels in the same vineyard can fluctuate as much as 100% from year to year.

There are wide differences among grape varieties and rootstocks. Rootstocks which tend to increase P levels include Ramsey, 110R, 1103P, and St. George; rootstocks associated with lower P levels include 039-16, 3309C, 420A, 101-14Mgt, and Harmony.

Potassium (K). Tissue analysis will confirm deficiency symptoms and is particularly useful in identifying isolated areas that warrant treatment. Vines in the questionable range at bloom

should be rechecked 6-10 weeks later at veraison by sampling recently mature leaf petioles. This will determine if deficiency is developing at fruit ripening, a more common occurrence.

Petiole levels usually decline most rapidly from before bloom until 2 to 4 weeks afterwards. Thereafter they decline gradually or level off through midsummer. Heavy crop loads can dramatically lower petiole K levels during fruit ripening. Harvest-time levels associated with deficiency symptoms are 0.3% K and below. A critical level of 0.5% K at harvest is recommended.

Potassium levels are highest in the youngest mature leaf petioles where they peak at bloom and then decline with time and leaf age. Potassium levels can vary widely (30-50%) from year to year in the same vineyard and are strongly affected by variety, rootstock, and irrigation practice.

Rootstocks that tend to contribute in higher petiole K levels include Freedom, Harmony, St. George, and 039-16; lower-K rootstocks include 420A, 110R, 5BB, 3309C, and 140Ru. These differences may contribute to fruit pH effects as well as vine susceptibility to K deficiency.

Magnesium (Mg). Petiole levels increase as the growing season progresses and tend to be higher in older petioles. Critical levels are probably higher later in the season, but they have not been established. For example, mild deficiencies may occur in late season with levels of 0.5% Mg or more.

Mg tends to be reciprocal to K in tissue concentration, a relationship that is strongly influenced by variety and rootstock, as well as soil levels. Ratios of K:Mg under deficiency situations vary too widely to be diagnostic. However, they can be indicative of extremes where antagonism may contribute to either Mg or K deficiency. For example, Mg deficiency is more commonly associated with K:Mg ratios of >10:1 with petiole Mg levels at <0.2%. General K deficiencies are more commonly noted in high Mg serpentine soils where bloom petiole levels may be above 3.0% Mg and with corresponding low levels of K. Even so, the absolute levels of Mg and K, rather than their ratios, are the most definitive indication of deficiency.

Calcium (Ca). Critical tissue levels are not given, as Ca deficiency has not been documented in California. The benefits of Ca in amending sodic (high Na) or acid soils are well known, but this practice is based on soil analysis. Direct vine response to Ca has only been demonstrated under extremely high Mg conditions, presumably due to Mg antagonism to Ca and K. "Normal" petiole levels begin at ~0.5% Ca, most commonly range from 1.0 to 2.0%, and can occur above 3.5% in petioles where Mg and K levels are low.

Zinc (Zn). Grape varieties and rootstocks vary widely in susceptibility to Zn deficiency. Most varieties fit within the critical levels given, although sensitive varieties such as Ribier, Merlot, and Muscat of Alexandria should always be maintained above the adequate threshold level. Low-susceptibility varieties such as Perlette and Chenin blanc will tolerate levels at or slightly below the deficient level of 15 ppm Zn.

Differences in petiole levels along the shoot and changes during the growing season are minor. Bloom time levels are most critical because of possible fruit set and "shot berry" effects.

Manganese (Mn). California experience with deficiencies is limited due to the small number of occurrences. Levels above 200 to 300 ppm usually indicate a low pH or acid soil. Soil pH's of 5.5 or less are very common at this range. This due to increased Mn availability in acid soils. High Mn is probably not a problem until 1,200 ppm or higher is reached. Toxic levels are based on European literature where toxic levels range up to 5,000 ppm.

Vines with iron chlorosis problems will sometimes show deficient levels of Mn. These problems have been corrected with iron treatments. The relationship to Mn nutrition has not been determined.

Boron (B). Both deficiency and toxicity of B are found in California vineyards. Deficiencies are found in North Coast soils of basaltic (volcanic) and granitic origin, Sierra Foothill soils and the more sandy alluvial soils along the east side of the Central Valley. Toxicities are mostly associated with soils derived from the sedimentary (marine) coastal mountain ranges, especially where well waters exceed 1 ppm B.

Petiole levels normally do not vary markedly along the shoot or during the growing season. However, in soils with high B, leaf tissue levels increase gradually throughout the season.

Boron accumulates more in the blades. Thus, in high-B areas, the levels increase markedly during the season and are higher in the older leaves. Samples of affected blades will readily confirm toxicity. Blade values of 120 ppm B and above at bloom indicate a potential toxicity problem; 300 ppm B and above in mid to late summer will confirm toxicity.

Iron (Fe). Critical levels have not been established, because there is no correlation between Fe deficiency and tissue levels. Normal, non-deficient leaves may have lower or similar petiole and blade Fe levels than those with mild to severe chlorosis symptoms. Petiole levels range widely from 20 to 300 ppm but most typically from 70 to 200 ppm. Leaf blade Fe levels are usually higher than those of the petioles. Iron contamination of the sample from dust, metal equipment, etc. is a common analytical problem.

Iron deficiency problems in high lime soils should utilize lime- tolerant rootstocks. Otherwise, correction with soil and foliar Fe treatment is often temporary and partial, as well as expensive.

Chlorine (Cl). Varieties and rootstocks differ widely in tolerance. Chlorine continues to accumulate during the growing season and does so predominately in the petiole, although the symptoms of excess appear in the blades. Leaf injury from Cl sometimes occurs at petiole levels down to 0.8% in sensitive varieties such as Barbera and when Na is high. Blade analysis may help to confirm toxicity.

Sodium (Na). Effects of excess Na have not been clearly defined, because they are usually associated with high Cl. Sodium may aggravate a Cl problem. Petiole levels of over 0.5% Na at bloom suggest problems, especially if K is relatively low. Visual symptoms of leaf margin discoloration (black or dark purple staining) and necrosis have been associated with blade levels above 0.5% Na.

Proceedings of Varietal Winegrape Production Short Course
University of California Davis Extension
March, 2005