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Geospatial variation of grapevine water status, soil water availability, grape composition and sensory characteristics in a spatially heterogeneous premium wine grape vineyard

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Abstract

The geoscience component of terroir in wine grape production continues to be criticized for its quasi-mystical nature, and lack of testable hypotheses. Nonetheless, recent relational investigations are emerging and most involve water availability as captured by available water capacity (AWC, texture) or plant available water (PAW) in the root zone of soil as being a key factor. The second finding emerging may be that the degree of microscale variability in PAW and other soil factors at the vineyard scale renders larger regional characterizations questionable. Cimatic variables like temperature are well mixed, and its influence on wine characteristic is fairly well established. The influence of mesogeology on mesoclimate factors has also been characterized to some extent. To test the hypothesis that vine water status mirrors soil water availability, and controls fruit sensory and chemical properties at the vineyard scale we examined such variables in a iconic, selectively harvested premium winegrape vineyard in the Napa Valley of California during 2007 and 2008 growing seasons. Geo-referenced data vines remained as individual study units throughout data gathering and analysis. Cartographic exercises using geographic information systems (GIS) were used to vizualize geospatial variation in soil and vine properties. Highly significant correlations ($P < 0.01$) emerged for pre-dawn leaf water potential (Ψ_{PD}), mid-day leaf water potential (Ψ_L) and PAW, with berry size, berry weight, pruning weights (canopy size) and soluble solids content (°Brix). Areas yielding grapes with perceived higher quality had vines with (1) lower leaf water potential (LWP) both pre-dawn and mid-day, (2) smaller berry diameter and weight, (3) lower pruning weights, and (4) higher °Brix. A trained sensory panel found grapes from the more water-stressed vines had significantly sweeter and softer pulp, absence of vegetal character, and browner and crunchier seeds. Metabolomic analysis of the grape skins showed significant differences in accumulation of amino acids and organic acids. Data vines were categorized as non-stressed ($\Psi_{PD} \geq -7.9$ bars and $\Psi_L \geq -14.9$ bars) and stressed ($\Psi_{PD} \leq -8.0$ bars and $\Psi_L \leq -15.0$ bars) and subjected to analysis of variance. Significant separation emerged for vines categorized as

non-stressed versus stressed at véraison, which correlated to the areas described as producing higher and lower quality fruit. This report does not advocate the use of stress levels herein reported. The vineyard was planted to a vigorous, deep rooted rootstock (*V. rupestris* cv. St. George), and from years of management is known to be able to withstand stress levels of the magnitude we observed. Nonetheless, the results may suggest there is not a linear relationship between physiological water stress and grape sensory characteristics, but rather the presence of an inflection point controlling grape composition as well as physiological development.

1 Introduction

1.1 Geospatial scale and the concept of terroir

The concept of terroir as a space, time and anthropogenic continuum has received much criticism for its quasi-mystical basis (Hancock, 1999), relation to marketing hoaxes (Hugget, 2006, from Busby, 1825) and errors in geological and climatological interpretation. The quasi-mystical, non-quantifiable scientific hypothesis applied to terroir is not unique. In its modern conception, terroir is theoretically similar to the "*n* dimensional hyper-volume" concept of an ecological niche of Hutchinson (1957) that was widely accepted. Hutchinson's theory considered that an environmental continuum in *n* dimensions constituted an ecological niche. This niche concept is somewhat analogous to the *n* dimensions of geologic, climatologic, microbiologic (cf. Bokulich et al., 2014) and anthropogenic influences that are hypothesized to determine wine sensory characteristics. A problem with a geoscience component of terroir is that while climate and temperature characteristics are well mixed at the regional scale, soils are extremely and abruptly heterogeneous at the local scale, thus rendering questionable any broad generalizations.

The original concept of terroir seems to be 14th century Burgundy (Wilson, 2001), where it did refer to geospatial properties of vineyards based on soils and fruit quality.

Thus soils have always formed an important dimension in the terroir continuum in spite of our inability to define its scale. Terroir comes from the Latin root “terrae,” meaning Earth, which may help to explain, even in its modern conception, its strong connection to soils. Nonetheless, clear quantitative measures of geospatial “terroir” at the regional scale (macro- and mesogeology) are lacking, and for this reason in particular, geologic terroir at these larger scales remains speculative (White, 2003). Bonfante and colleagues (Bonfante et al., 2011) integrated several environmental variables including soil properties in describing and mapping terroir. The effect of this kind of descriptive analysis is that it evens out microscale variation, while the primary model drivers like crop water stress index (CWSI) and solar radiation interception are mostly influenced by the influence of geology on mesoclimate variation. Reynolds and co-workers found correspondence between flavor aromas, astringency, soluble solids and pH of Reisling with soil texture (sand versus clay content) but the correlations were highly inconsistent among vintages (1998–2002). The studied vineyard was only 4 hectares in size. Nonetheless these emerging studies allow us to establish a basis for the nature of geology and terroir.

Huggett (2006) reviewed the chemical nature of geological terroir and concluded there were only a few specific cases where soil chemistry is unique to an area. For example, she cites the calcareous soils of the Champagne AOC, but indicated it was unclear that it imparts a clear characteristic on wines. Hugget points out one exception may apply to saline areas where there may be a “slight saltiness of wines produced” (Huggett, 2006). Reynolds and colleagues did a comprehensive analysis of geospatial variation in soil sand, silt, clay content, pH and extractable P, K, Mg, Mn, Ca, Zn, Cu, Fe, and B, and tissue concentrations of N, K, Mg, Ca and B, versus yield components and must characteristics in a Riesling vineyard and found almost no consistent discernible relationships. Soils are geospatially extremely diverse and abrupt changes can occur even at the vineyard level. Thus, the definition of the notion of geospatial scale for terroir is an important subject and still lacking definition. Greater than 80 % of the grapevine root system generally resides in the upper 1.2 m of soil depth, or less, depending on

SOILD
1, 1013–1072, 2014

root limiting horizons (Smart et al., 2006). The major macronutrients absorbed by vines (N, P, K, Mg, Ca and S) can vary in soils as can the primary absorbed micronutrients of importance (B, Zn, Mn, Mo, Fe and Cu). But fertilization procedures to correct deficiencies for the above macro- and micronutrients and other chemical imbalances through ground based and foliar fertilizer applications are generally well recognized and the mitigation of deficiencies calls into question a relationship between soil minerology and terroir. This report focuses more specifically on soil water and vine water relations in response to geospatial variation of soil within a single vineyard. We posed the critical question of whether or not it can impart unique sensory and chemical characteristics upon the fruit produced. Thus, it is a primary hypothesis of this report that the most important factor conferring differences in fruit flavor and chemical profiles related to geology and soils is the soil water reservoir. As early as 1825, James Busby recognized factors such as good drainage and air porosity as critical in stating that "The conclusion may even be drawn, that the intrinsic nature of the soil is of less importance, than that it should be porous, free, and light." (Busby, 1825).

In contrast to geology, climatic influences on fruit development are fairly well known and described, and generally resolved using heat unit accumulation exercises (Amerine and Winkler, 1944; Huglin, 1978; Coombe, 1987; Gladstones, 1992). Historic development of regional appropriate varieties and growing systems is a clear result of climatic influence on terroir. Tonietto and Carboneau (2006) recognized the geological contribution of soil water and recently expanded upon the heat unit accumulation approach at the regional level by creating a model incorporating a dryness index (DI), which corresponded to the potential water balance of soil versus evapotranspiration demand and its contribution to presence or absence of water stress (after Riou et al., 1994). The DI was calculated as the balance between the regional average transpiration demand and soil evaporation, weighted for precipitation and a beginning soil water reservoir of 200 mm (Wo). Dry regions were those with water deficits based on the above model and thus, negative soil water balances. The model was used to define global wine growing regions in terms of variety, vintage quality and wine "typeness".

SOILD
1, 1013–1072, 2014

The model doesn't approach either meso- or microgeographic variation in soils where total available water can range from 50 to > 200 mm at the microgeologic (within vineyard) scale depending on factors that influence depth like slope, parent material and historic alluvial activity.

It is only from recent studies concerning mesoscale geologic (and climatic) influences on terroir (Jones et al., 2004; Bonfante et al., 2011) that some information is emerging on other environmental soil factors important to geologic terroir and that the soils parameters of focus concerns available water capacity. But much of this effort has really been directed towards the influence of mesogeology (10–100 km) on mesoclimate forcing by factors like precipitation, altitude (Mateus et al., 2002; Miguel-Tabares et al., 2002), slope and aspect (Failla et al., 2004; Jones et al., 2004; Shapland et al., 2012) and vine water relations (Reynolds et al., 2007 and 2010; Zufferey and Murisier, 2006; Zsofi et al., 2009). Soil minerology, on the other hand, has never really been brought to bear upon the question of why the same cultivars may produce different vineyard specific grape compositions as well as contributing to variation in wine styles of different regions (but see Huggett, 2006). The analyses approaching this have generally been conducted at small spatial scales (e.g. from 1 : 24 000 to 1 : 250 000). Several aspects of a growing area at large spatial resolution have indicated a high degree of spatial heterogeneity and may allow for a more targeted understanding at an extremely local level (Pierce and Nowak, 1999; Bramley, 2005; Reynolds et al., 2010; Scarlett et al., 2014). Morlat and co-workers found within-appellation differences to be greater in some cases than between-appellation differences (Morlat et al., 1984). These investigations call into question the validity of a macro- or mesoscale level of geologic terroir.

Recent work on *Vitis vinifera* cv. Cabernet Sauvignon vineyards in the Stellenbosch region of South Africa (Carey et al., 2008), an area of mixed soils and volcanic uplift much like the Napa Valley of California supports this contention. They employed the use of "natural terroir units" (NTUs) based on environmental and geological factors, linking above-ground and below-ground influences into a single unit of study. The

SOILD
1, 1013–1072, 2014

South African researchers determined that their delineation method produced far too many units for practical use and ultimately proposed a method of parameter simplification. Their internal debate illustrates the difficulty inherent in attempts to characterize viticulture areas in geological terms: when data is smoothed too much, important detail is lost, but when detail is too great, patterns cannot be discerned. As a consequence, their debate supports the hypothesis that geologic terroir may exist primarily at the microscale (vineyard specific) level of interpretation. This report is concerned with understanding the physiological basis of within vineyard heterogeneity. The primary hypothesis is that soil water availability is the main factor contributing to within vineyard variation in fruit quality in complex, hillside vineyards.

1.2 The influence of vine water status on fruit composition

A key component of management of premium quality winegrape vineyards in California is water status (or stress) and a relatively large body of evidence exists for water status, as indicated using measures of leaf water potential (LWP), influencing a number of grape chemical and sensory attributes. In as much as one of the key components of water provision and the time it takes for a vine to become stressed (growth limitation), is the size of the soil water reservoir, we adopted total plant available water in soil (PAW), pre-dawn LWP (Ψ_{PD}) and mid-day LWP (Ψ_L) as key factors to use in establishing a physiological pattern of spatial variation across the subject vineyard. Many previous investigations dealing with water stress have evaluated controlled irrigation treatments based on percentage deficit amount versus grape crop evapotranspiration (ET_c) or some arbitrarily chosen level of irrigation. We tested the hypothesis that using LWP as a "bio-indicator" in cartographic exercises would reveal geospatial variability of the site in terms of vine PAW and fruit characteristics.

Measurement of LWP at midday (Ψ_L) is a well-known method of assessing grapevine water status and serves as a relative metric of water stress condition (Smart and Coombe, 1983; Williams and Matthews, 1990). Midday LWP (Ψ_L) can be influenced by solar radiation, wind, vapor pressure deficit and temperature. Thus, it is not generally

SOILD
1, 1013–1072, 2014

a consistent measure of vine water status relative to the soil water status since the environmental parameters can quickly change. Measuring LWP during pre-dawn hours (Ψ_{PD}) provides an approximate estimate of soil water potential (Ψ_s) (van Zyl, 1987), but see Donovan and colleagues (Donovan et al., 2003) where in some extreme conditions a Ψ_{PD}/Ψ_s disequilibrium exists. While Ψ_L and Ψ_{PD} of grapevine have been shown to be highly correlated (Williams and Araujo, 2002), measuring LWP at pre-dawn is still important to this study because stomates are mostly closed and the influence of ambient factors on Ψ_L that might compromise the detection of micro-geospatial differences in the soil water reservoir, like wind and local vapor pressure deficit, are removed from the equation (Correia et al., 1995).

Water deficits that result in Ψ_L of less than approximately −1.0 MPa generally slow or arrest growth of grapevine and diminish fruit set. Fruit yield declines through decreased berry number and decreased berry size (Matthews et al., 1987; Medrano et al., 2003). A decrease in berry size can sometimes lead to higher specific phenolic concentration (Esteban et al., 2001), but what has often been cited as the reason for increase in phenolic concentration was an increase in surface area (skin) to volume (pulp) ratio. Thus, lowered water potential does not appear to be the sole mechanism (Roby et al., 2004); nonetheless, there are more polyphenols in smaller, water-restricted grapes. Other factors seem to be related to the fact that smaller grapes tend to have higher polyphenolic concentrations in and of themselves (Roby et al., 2004; Chapman et al., 2004). Nonetheless, a positive relationship between more negative LWP and an increase of both gross concentration of polyphenols and the smaller population of non-water extractable polyphenols has been demonstrated (Sivilotti et al., 2005). This was accomplished by extracting in ethanol (EtOH). Although extracting with EtOH is not a complete analog to fermentation and maceration, the results give insight into the links between water stress and the development of berry compounds that may translate into wine constituents.

Low vegetative growth due to restricted photosynthetic activity has been extensively linked to water stress (Escalona et al., 2002; Flexas et al., 1998; Liu et al., 1978; Schultz

SOILD
1, 1013–1072, 2014

and Matthews, 1988; Winkel and Rambal, 1993). The direct effect of water stress on expansive vegetative growth varies somewhat among cultivars (Flexas et al., 2002; Gomez-del-Campo et al., 2002; Kaiser et al., 2004a; Kaiser et al., 2004b; Medrano et al., 2003; Mullins et al., 1998; Silvestroni et al., 2004). Water status is therefore a consistent predictor of decreased or arrested expansive vine growth, or "vigor". Deficit irrigation, for example, can cause a difference of up to 61 percent in a grape yield (Alleweldt and Ruhl, 1982). Expansive growth may be a good indicator of vintage quality, as excessive vine foliage production (vigor) has been shown to be correlated with lower polyphenol concentrations (Cortell et al., 2005). However, the mechanism by which a decrease in polyphenols occurs is unknown. Sun exposure has been positively correlated with phenolic concentration (Crippen and Morrison, 1986). Production of canopy foliage (i.e., vigor) is often reduced under stress conditions, allowing more sunlight into the fruiting zone. Thus, increased light exposure as a contributing factor in the role of water stress in phenolic development cannot be ruled out, even though it is unlikely that sunlight is the only driving factor in phenolic development across all of the preceding studies.

A primary objective of the investigation described here was to approach the hypothesis that sensory attributes of fruit would have patterns similar to those detected in terms of the soil water reservoir and vine water status (Ψ_L and Ψ_{PD}) at the microgeologic scale. Grape aroma compounds beyond those that have been shown to contribute to vegetal vs. fruity character of wines are important factors in describing varietal characteristic and overall wine quality (Ebeler and Noble, 2000). Elevation of organic acid concentrations in fruit of well-irrigated vines has been demonstrated (Bravdo et al., 1985; Esteban et al., 1999; Hepner et al., 1985) and has been considered a mark of low quality. Some reports have found soluble solids (sugars) to be unaffected by water application (Ballatore et al., 1970; Esteban et al., 1999; Sivilotti et al., 2005). In cases of severe drought (De La Hera Orts et al., 2004), sugar ripening has been reported to be restricted and in this case it is likely caused by limited photosynthetic activity, which is less sensitive to low leaf water potentials than expansive growth. However, a greater

SOILD
1, 1013–1072, 2014

number of reports show evidence of an increase in sugar and decrease in acidity under water stress conditions (Bravdo et al., 1985; Jackson and Lombard, 1993; Koundouras et al., 2006; Seguin, 1983; Tregoat et al., 2002).

This study sought to understand relationships between physiological responses of vines based on vine available soil water within the vineyard (PAW), and sensory and metabolomic analyses. Given the large body of evidence (above) for water availability and mild stress conditions influencing flavor and mouthfeel constituents, regardless of mechanism, it was expected that chemical and sensory differences would correspond to physiological phenomena such as: (1) Ψ_{PD} and Ψ_L, (2) berry size, (3) pruning weights as a proxy for canopy leaf area, and (4) soluble solids content. A primary hypothesis we tested was that vines exhibiting physiological signs of water stress (lower LWP, smaller berries, lower pruning weights) should yield berries with different sensory and chemical profiles.

2 Materials and methods

2.1 Vineyard site location

Stags Leap Vineyard 4 (SLV 4, 38°24′4.65″ N by 122°18′55.62″ W) was planted in 1973. It's a 2.28 ha (5.36 acre) vineyard of *Vitis vinifera* cv. Cabernet Sauvignon (var. Concannon) on St. George rootstock (*V. rupestris*) with a 12 by 7 foot row by vine spacing and trained to bilateral cordons on a U trellis. Vine rows were laid out in a general northeast-southwest orientation. SLV 4 was an older planting (35 years, 2008) and many vine cordons have been infected with *Eutypa* spp. wood disease, so replanting and cordon re-establishment has resulted in a somewhat age diverse vine environment, both in terms of overall vine and cordon age. Every vine in SLV 4 was evaluated for probable age, state of cordons, and a detailed map of the vineyard created to aid data vine selection. From that map, vines from the original planting with two mostly original cordons were chosen from the top, middle, and bottom of the slope, at regular

SOILD
1, 1013–1072, 2014

intervals (rows 2, 6, 10, 14 etc.). Where possible, vines surrounded by similar aged individuals were preferentially selected. Data vines were chosen from random locations using a vineyard map rather than while in the field to avoid visual bias. The vineyard was divided into three irrigation blocks, so an equal number of vines were chosen for irrigation-blocks 4N, 4C, and 4S (for North, Center and South, Fig. 1).

2.2 Physiological measurements

Physiological data were collected starting at bloom in 2007 on an original group of 36 geo-referenced data vines. Thirty five additional data vines, taken from the intervening rows, were added at véraison in 2007 to give greater spatial resolution to the cartographic exercises described below. For some more labor-intensive or costly tests, a smaller subset of 12 to 36 representative vines from the original 36 were used. All irregularities were accounted for in the statistical analysis. Data vines were geo-referenced using a hand-held GPS unit (Trimble Ag GPS 132 using TDS Recon and running HGIS ARM), and imported into ArcGIS for spatial statistical analysis.

Vine physiological data (Ψ_L, Ψ_{PD}, berry size and weight, Brix, cane production) were taken from geo-referenced data vines at the major developmental stages of bloom, pea-size berries, véraison, harvest and dormancy. Timing of phenological stages is variable depending on climate, cultivar, and geographic location. Taking measurements at defined stages allowed for seasonal continuity for comparison of the data across the 2007 and 2008 vintages (Jones and Davis, 2000). Calendar dates for each phenological stage above during 2007 and 2008 were remarkably similar.

Leaf water potential (LWP) was measured at bloom, pea-size, and véraison using a pressure chamber with a 0.5 L chamber (Soil Moisture Equipment Corp. model 3008 Santa Barbara, CA). Fully expanded sunlit leaves (Ψ_L) were sampled in duplicate (triplicate if there was a leaf to leaf discrepancy of ±0.5 bars or greater) at midday (Ψ_L, 1–3 p.m. (PST)) and pre-dawn (Ψ_{PD}, 3–6 a.m.). Leaves for pre-dawn sampling were taken from the same geo-referenced data vines and position in the canopy as those taken at midday.

SOILD
1, 1013–1072, 2014

Berry diameter was measured shortly after véraison using digital calipers (Mitutoyo model 500-682 Aurora, IL). From each of the vines sampled, 36 berries were measured by selecting one each of a perceived large and small berry from 18 randomly selected clusters in the canopy. Berry samples were taken at harvest by randomly picking 100 berries (blind) from each of the 71 data vines, from different locations along the vine cordon and within the cluster. Berries were re-counted upon returning to the laboratory from the field, weighed as a group and means were taken. Berry samples were kept on ice in a cooler in the field and through weighing. They were transferred to a freezer (−20 °C) immediately after weighing.

Dormant grapevine canes were pruned from the cordons to one-bud spurs according to conventional management practice at SLV 4 in February 2007 and February 2008. Bundled canes were weighed with a field balance. The resulting "pruning weight" represents an approximate measurement of shoot dry matter accumulation during the previous season, and thus a relative measure of canopy size (vigor). All vines were under similar evaporative conditions throughout the winter months, so field-measured pruning weights were considered to be adequate to meet this goal.

Soluble solids (°Brix) were measured using an Atago pocket refractometer (Atago, model PAL-1 #3810, Bellevue, WA). Three randomly selected grapes from each data vine sampled in 2007 and 2008 were thawed for 2 h and crushed. Approximately 1 mL of liquid exudate from each crushing was placed on the refractometer and read for °Brix.

2.3 Soil assessment

SLV 4 sits on a southwest-facing slope with highly developed volcanic soils of mixed Boomer-Forward-Felta complex, consisting of a Fine-loamy, mixed, superactive, mesic Ultic Haploxeralf; a Medial, mixed, mesic Typic Vitrixerand; and, a Loamy-skeletal, mixed, superactive, thermic Pachic Argixeroll, respectively. Boreholes were taken at 36 of the original geo-referenced data vines in May of 2008 using a high-pressure hydraulic tool (Geoprobe Systems model 66DT, Salina, Kansas). At many locations, the

“Geoprobe” was unable to penetrate to 1.0 m because of bedrock layers, but where possible, cores were taken to a depth of 1.0 to 1.2 m. Accurate estimates of rooting depth (to bedrock) were facilitated for most boreholes by estimating depth to either bedrock or a root limiting argillic horizon. For the deeper soils, rooting depth was estimated at 1.2 m (Smart et al., 2006). The Geoprobe often crushed layers, causing backsliding from the soil tubes but care was taken to make as accurate an estimation of effective rooting depth (ERD) as possible (to horizons where roots were absent or scarce). Soil cores were separated into horizons using color and texture by feel. Each horizon was dried, sieved at 2 mm and tested for soil pH, particle size distribution (sand, silt, clay content), and moisture retention at 0.033 and 1.5 MPa applied pressure. A soil based plant available water (PAW) was calculated as:

$$\mathrm{PAW} = (\theta_\mathrm{v}0.033\ \mathrm{MPa} - \theta_\mathrm{v}1.5\ \mathrm{MPa})/100) \times \mathrm{BD} \times (1 - \text{rock fraction}) \times \text{depth (mm)}, \quad (1)$$

where θ_v is volumetric water content (%) of the < 2 mm particle size fraction and BD is bulk density ($\mathrm{g\,cm^{-3}}$).

Mineral soil samples (< 2 mm fraction) were analyzed by the UC Davis Agriculture and Natural Resources (DANR) Analytical Laboratory according to their standard procedures. Soil pH was determined in a saturated paste using a pH electrode, according to USDA Agricultural Handbook 60 (Staff, 1954). The method has reproducibility within 0.2 pH units. Soil texture was analyzed by hydrometer suspension, using sodium hexametaphosphate solution to disperse soil aggregates (Sheldrick and Wang, 1993). Analysis of the retention of moisture from field capacity (0.033 MPa pressure), was conducted on the < 2 mm particle size fraction using a pressure plate (Klute, 1986). Pressure was applied at 0.033 and 1.5 MPa, to approximate the soil moisture retention at field capacity (FC) and the wilting point (PWP), respectively.

2.4 Cartographic exercises

Geographic information system software (ArcGIS, ESRI, Redland, CA) was used to geospatially characterize the vineyard. Maps were created using the Universal

SOILD
1, 1013–1072, 2014

Transverse Mercator (UTM) graticule and the North American Data system of 1984 (NAD 1984). ArcGIS was used to correlate the geo-referenced vineyard data vine locations with Ψ_L, Ψ_{PD}, berry weight, berry diameter, and pruning weight. Using ordinary kriging analysis paired with vector analysis, it was possible to map and measure the areas of those differences. Ordinary kriging enables statistical interpolation of areas surrounding spatially explicit data points to generate predictions about the spatial extent of the variable of interest, so interpolating the physiological data from the 71 data vines with ordinary kriging was used to characterize the larger set of 2373 vines in SLV 4.

Predictive maps generated with ordinary kriging employed a spherical model that included 5 "neighbor" data points for the data sets of 71 geo-referenced vines and 3 neighbor data points for 2007 and soil sets containing 36 geo-referenced individuals. This model was used after testing other available models, as well as higher and lower numbers of neighbors. The 5-member Spherical model produced the lowest root mean square (RMS), standard errors closest to zero, and standardized RMS closest to 1.0. For some data sets, the K-Bessel and J-Bessel models produced smaller differences between the average standard error and the RMS, which is suggested by ESRI, (ArcGIS, Redlands California USA), as the deciding factor when examining the many possible statistical outcomes of kriging. However, when performing a manual check of the prediction vs. actual measured values, the Spherical model showed much greater accuracy across all data sets. As discussed above, during kriging the weighting factor of neighbor data points decreases with increasing distance, so as both soil and physiological changes were abrupt, and tight resolution of within-vineyard variability was the goal, increasing the number of neighbor data points included in the model created greater smoothness and therefore undesirable for the objectives of this investigation. The same model was used for all predictive maps. The Kriging exercises were then converted to vector format for measurement of the areas classified by interpolation.

SOILD
1, 1013–1072, 2014

2.5 Sensory evaluation

We are not aware of rigorous sensory evaluation of grape fruit in the study of terroir. Most studies have concentrated on must or wines in an attempt to describe both variety characteristic and heightened quality for specific cultivars (Abbott et al., 1991; Falque et al., 2004; Francis et al., 1992; Heymann and Noble, 1987; Ohkubo et al., 1987; Preston et al., 2008; Vilanova et al., 2009; Bonfante et al., 2011). Grapes rather than wine were analyzed in this report for several reasons: First, the commercial value of the fruit was way too high to allow for microvinification studies. Secondly, the spatial resolution needed for this investigation was at a very large scale (an approximate 1 : 2500 map scale) and thus it would be nearly impossible to carry out sufficient microvinifications. Thirdly, if grapes from many vines were to be combined to create wine, spatial resolution needed for geostatistical analysis would be lost. In addition, micro-fermentation continues to confound researchers (Graves, 2008), and consistency of results has not yet been achieved. Finally, as this study was concerned with differences in berry constituents that could be present in trace amounts, it was determined that berries would be a preferable testing medium, both for sensory and chemical trials.

A sensory panel of 6 individuals trained with Cabernet Sauvignon grapes blind-tasted 6 previously frozen grapes per data vine using descriptive analysis procedures (Lawless and Heymann, 1987). Beyond the superior convenience of working with frozen grapes, preliminary work has shown that previously frozen berries show better separation in sensory trials than fresh. Grapes were tested individually, and each panelist dissected the grape for evaluation of skin, pulp, and seeds individually for 18 parameters: squishy pulp, dissolvable pulp, sweet pulp, sour pulp, thick skin, bitter skin, sour skin, astringent skin, vegetal skin, fruity skin, raisined skin, green seed, brown seed, hard seed, crunchy seed, bitter seed, astringent seed, nutty seed. Sensory parameters were selected by the panel during a preliminary consensus training session but largely followed established methods (Rousseau, 2001) and conformed to previously mea-

SOILD
1, 1013–1072, 2014

Geospatial variation of grapevine water status

D. R. Smart et al.

sured characteristics both of grapes in general and Cabernet Sauvignon in particular (Heymann and Noble, 1987).

Following analysis by sensory evaluation, data vines were separated into two groups – those with midday véraison $\Psi_L > -14.9$ bar (non-severely stressed) and those with $\Psi_L \leq -15.0$ bar (severely stressed). The groups are heretofore referred to as the categories of non-stressed (Group I) and stressed (Group II). Vines were divided in the same manner using pre-dawn LWP measurements: non- severely stressed individuals had $\Psi_{PD} > -7.9$ bars while stressed individuals had $\Psi_{PD} \leq -8.0$ bars. Analysis of variance using least squares means (LS means) were conducted using other levels of LWP divisions (−13.0, −14.0, and −16.0 bar for Ψ_L, and −6.0, −7.0 and −9.0 bar for Ψ_{PD}). LWP divisions of −8.0 and −15.0 bars (Ψ_{PD} and Ψ_L respectively) had the most significant results across all categories, both for sensory and physiological data.

2.6 Metabolomics analysis

Sixty samples from each year were used for metabolomic analysis by GC-TOFMS (Kind et al., 2009). For each year, 30 data vines were randomly selected from Group 1 and Group 2. Ten grapes from each of the selected data vines were peeled and skins rinsed twice with deionized (DI) water. The skins were then freeze-dried in an FTS Systems Dura-Dry freeze dryer (FTS Systems, Stone Ridge, NY) to afford easier handling. Fresh berry tissue was kept frozen throughout the grinding and extraction steps that followed to prevent enzyme activity and subsequent changes in berry composition. Each skin sample was ground in a ball-bearing grinder for 60 s. Extractions of 5, 2.5, and 1 mg of grape skin were tested. Five-mg samples contained too much sugar, obscuring many metabolite peaks, and 1 mg samples insufficient for detection of a range of metabolite peaks. All samples were thus prepared using 2.5 mg of freeze-dried grape skin.

The skins were extracted with 1.5 mL cold (−20 °C) 5 : 2 : 2 vol/vol methanol : chloroform : water (MeOH : CCl_4 : H_2O) solvent, vortexed for 10 s, placed on an agitator for 20 min at room temperature (approx. 22 °C), and centrifuged for 3 min at 14 000 relative centrifugal force (RCF). A subsample of 35 µL of supernatant

SOILD
1, 1013–1072, 2014

was then transferred to sterile Eppendorf tubes, evaporated in a vacuum chamber for 1 h, and transferred to a freezer (−20 °C) until just prior to injection, at which point the samples were derivatized using 10 µL of 40 mg mL^{-1} methoxylation (MeOX) and agitated 90 min at 30 °C at maximum speed. 2 µL of fatty acid methyl esters (FAMEs) and 90 µL of 2,2,2-trifluoro-N-methyl-N-trimethylsilyl-acetamide (MSTFA) were then added to increase the volatility of metabolites, and the samples agitated again for 30 min at 37 °C at maximum speed. Each sample was prepared in triplicate and injected twice, for a total of 6 injections per sample. Results of the current investigation were compared against libraries based on a fatty-acid methyl ester retention index system and were established by GC/MS based on time-of-flight mass spectrometry (GC-TOF) and quadrupole mass spectrometry (GC-Quad) (Kind et al., 2009).

2.7 Statistical approach

Leaf water potential, pruning weight, °Brix, berry weight, and berry diameter were evaluated by linear regression using SYSTAT Systems (2008). All results – physiological, sensory, and chemical – were evaluated by ANOVA using LS means with SAS statistical software (SAS, 2008). Vines were divided into unstressed and stressed groups for ANOVA and LS means testing by the above-mentioned LWP-based stress groups. Significance was designated at 95 % certainty ($p \leq 0.05$).

3 Results

3.1 Physiological data

Leaf water potential (ψ_{PD} and ψ_L) was extremely variable across the vineyard (see Table 1). In both years, some vines had LWPs more negative at bloom (late May) than others had achieved by véraison (mid-August, Table 1). There was a difference in LWP between lowest and highest observations for ψ_{PD} of −10.1 bars in 2007 at véraison, while it was nearly identical at −10.8 bars in 2008. For ψ_L the difference at véraison

SOILD
1, 1013–1072, 2014

was −7.5 bars in 2007 and −8.5 bars in 2008 with the extreme observations being very similar (Table 1). The geospatial pattern for ψ_{PD} and ψ_L was highly consistent between vintages (Figs. 2 and 3). This difference did not converge as the season progressed in either year; rather, the range of differences across the vineyard continued to increase from bloom up to the pea-size phenological stage and véraison (Table 1).

The number of vines for both years that fell into either the more stressed ($\psi_{PD} \leq -8.0$ bars and $\psi_L \leq -15.0$ bars at véraison) or the "less stressed" ($\psi_{PD} \geq -7.9$ bars and $\psi_L \geq -14.9$ bars) categories of vines was approximately equal. While the number of vines in each category remained largely the same at véraison for ψ_{PD} and ψ_L observations in 2007, the 2008 categories showed a slightly greater number of vines (7) that did not fit the above category of "water stressed" at pre-dawn but then did fit into the stressed category of vines at mid-day.

Physiological parameters measured (e.g. ψ_{PD}, ψ_L berry diameter and weight, °Brix and pruning weight) showed similar geospatial patterns of variation across the vineyard (Figs. 2, 3, 4, 5, 6, and 7), and were also very consistent across the 2007 and 2008 vintages (Figs. 8 and 9). The correlations with LWP were consistent with perhaps the exception of °Brix. The correlations with pre-dawn LWP at véraison ($P \leq 0.01$) in 2007 were $r^2 = 0.616$, 0.626, 0.144 and 0.541 for berry diameter, berry weight, °Brix and pruning weight, respectively, and the correlations with mid-day LWP were $r^2 = 0.607$, 0.675, 0.120 and 0.341 for the same respective parameters. In a like manner, the correlations of pre-dawn LWP at véraison in 2008 were $r^2 = 0.191$, 0.593, 0.163 and 0.466 for berry diameter, berry weight, °Brix and pruning weight and were also highly statistically significant ($P < 0.01$). The correlations with mid-day LWP at véraison in 2008 were also significant ($P < 0.05$) with $r^2 = 0.307$, 0.473, 0.100 and 0.513, respectively for berry diameter, berry weight, °Brix and pruning weight. This indicated a surprising degree of consistency for the two seasons.

Maps of both water stress indicators (ψ_{PD} and ψ_L) at the pea-size phenologic stage showed consistent patterns across both years (see Figs. 2 and 3). But the correlations of ψ_{PD} and ψ_L at the pea-size phenologic stage (berry expansion) was not as good in

SOILD
1, 1013–1072, 2014

2007, but we note the full complement of data vines had not been established at the pea-size stage in 2007. The correlations with pre-dawn LWP at pea-size ($P < 0.05$) in 2007, 60.5 mm precipitation March–May, were $r^2 = 0.085$, 0.081, 0.012 and 0.095 for berry diameter, berry weight, °Brix and pruning weight, respectively, and the correlations with mid-day LWP were $r^2 = 0.185$, 0.185, 0.006 and 0.048 for the same respective parameters. However, the correlations of pre-dawn LWP at the pea-size stage in 2008, 7.9 mm precipitation March–May, were $r^2 = 0.413$, 0.554, 0.215 and 0.394 for berry diameter, berry weight, °Brix and pruning weight and were statistically significant ($P < 0.01$). The correlations with mid-day LWP at the pea-size phenological stage in 2008 were also statistically significant ($P < 0.01$) with $r^2 = 0.378$, 0.466, 0.176 and 0.296, respectively for berry diameter, berry weight, °Brix and pruning weight.

Berry size (diameter and weight) showed the least overall variation across the vineyard with only a less than 3 mm difference in diameter in 2007 and 2008 and less than a half gram difference in weight (Table 2). Pruning weights, on the other hand had nearly a 4 fold difference (kg) among data vines and °Brix, surprisingly, had a range of difference greater than 7 °Brix units (Table 2) in both 2007 (7.2) and 2008 (7.7), but similar geospatial patterns emerged (Fig. 6). While pruning weight showed the greatest change between 2007 and 2008 at −20.8 % of the mean (Table 2), it nonetheless showed good correlations with water status, both ψ_{PD} and ψ_L in both seasons (see Figs. 8 and 9). Pruning weight was also the most highly variable among data vines. The range of difference observed between the highest and lowest pruning weights were 3.83 kg in 2007 and 3.24 kg in 2008 (Table 3) or more than a 4-fold difference.

The maps generated for each of the fruit size characteristics visually corresponded very well to those of ψ_{PD} and ψ_L, (e.g. compare Figs. 2 and 3 with 4 and 5) showing a discernable pattern with a "kidney-shaped" center of lowered water status. The interpolated kriging exercises for ψ_{PD} and ψ_L at véraison (Figs. 10 and 11, right panel) were also similar in spatial pattern with that of berry diameter and weight (Figs. 4 and 5) and correlations were generally statistically significant in 2007 and 2008 at véraison ($p < 0.01$, Figs. 8 and 9). The cartographic exercises for pruning weight (Fig. 7) also

SOILD
1, 1013–1072, 2014

fit well with the ψ_{PD} and ψ_L geospatial patterns with pruning weights being from 2 to 4 fold lower in the areas with vines categorized as more stressed. Again the correlations were highly statistically significant in 2007 and 2008 ($p < 0.001$, Figs. 8 and 9). The cartographic exercises for °Brix also displayed the same kidney shaped pattern but were surprisingly less well correlated with leaf water status at véraison, with $r^2 = 0.176$ in 2007 and $r^2 = 0.100$ in 2008 (Figs. 8 and 9). Nonetheless it should be pointed out the area with advanced sugar ripening (°Brix > 24.0, Fig. 6) corresponded well with the area found to have lower water status (Figs. 2, 3, 10 and 11). The area found on the eastern edge of the northern end of Block 4C was particularly striking in that it displayed higher °Brix, lower berry weight, lower pruning weight, and smaller berry diameter very consistently across both years. This area corresponded to the area of greatest water stress found in SLV 4.

As discussed above, the fruit and vine parameters we measured were generally statistically significantly correlated ($P \leq 0.05$) with the physiological measurements of vine water status at véraison (ψ_{PD} and ψ_L, Figs. 8 and 9). Further, when vines were grouped according to vine water status categories of mid-day LWP more positive than −15.0 bars (non-stressed) and more negative than −15.0 bars (stressed), the results were also generally highly statistically significant (Table 3) with few exceptions. The two parameters that showed best linear correlations with ψ_{PD} and ψ_L, pruning weight and berry weight at véraison also showed the highest level of being statistically significantly different when evaluated using analysis of variance ($p \leq 0.0001$ for all seasonal water status measurements, Table 3). These corresponded to areas of lower berry weights (Fig. 5) and pruning weights (Fig. 7) as well as lower LWPs (see Figs. 10 and 11, left panel for generalized areas with more stressed vines).

Analysis of the soil wetness index using geographic information systems (ArcGIS) ruled out slope-related geomorphic controls on water availability. Maps showing measured vine water status (Figs. 2, 3, 10 and 11) indicated bands of lowered vine LWP radiating out from a central area rather than exhibiting a slope-related pattern of bands (down the slope). This suggested there was a soil-related difference influencing

SOILD
1, 1013–1072, 2014

water-holding capacity independent of position on the hillslope. Patterns in soil texture (Fig. 13) corresponded well to moisture-release since the fraction analyzed was the < 2 mm particle size fraction, particularly the soil clay content, but were not well representative of the patterns observed for ψ_{PD} and ψ_L. Rather, they appeared in areas more related to transitional soils, representing a confluence of the areas of rocky, well-developed volcanic soils where water status was categorized as stressed, and areas of lesser vine stress where soils were deep, rich and composed primarily of alluvial deposits from historic events and changes in stream channels.

The changes we observed in soil plant available water (PAW) were surprisingly abrupt for an area of approximately two and one quarter hectares (Fig. 12, right) and also did not reflect a downslope pattern. Total plant available water varied throughout a range of from 68.5 to 177.5 mm of water. Soil texture (sand and clay) were not statistically significantly correlated with soil PAW with $r^2 = 0.017$ ($p = 0.445$) for PAW versus sand content, and $r^2 = 0.060$ ($p = 0.147$) for PAW versus clay content. The mapping exercises (Fig. 13) represent weighted mean averages (depth) for sand and clay content, but extensive horizonation renders these interpolations questionable and points to effective rooting depth as a primary factor controlling vine water status. PAW showed statistically significant correlations ($P < 0.01$, Fig. 14) with the physiological parameters of ψ_{PD}, ($r^2 = 0.146$) and ψ_L ($r^2 = 0.283$), berry diameter ($r^2 = 0.301$), berry weight ($r^2 = 0.436$) and pruning weights ($r^2 = 0.256$).

3.2 Sensory

Findings of the sensory panel were evaluated by ANOVA using the −15.0 bars standard for mid-day LWP (see Sect. 2.5) and −8.0 bars for pre-dawn LWP as a division between water stressed and non-water stressed groups respectively. The panel found significant differences for 8 of 18 parameters between the two mid-day groups and for 9 of 18 parameters for the two pre-dawn groups. Grapes from the less water stressed vine category of non-stressed were described as having sour pulp; thick, bitter, sour, and vegetal skin; green, hard, bitter, and astringent seeds (Fig. 15). Grapes from vines in the more water stressed category were described as having squishy, sweet, dissolvable pulp; raisined skin; and brown, crunchy, and nutty seeds (Fig. 15). Measured variables account for 87 % of the variance.

3.3 Chemical profiling

Metabolomic analysis yielded information on 67 known compounds and 128 unknown compounds (Fig. 16). When subjected to ANOVA, water-status categories were statistically significantly different for 15 compounds (11 known and 4 unknown). These differences followed a distinct pattern among the known compounds: 5 were amino acids (leucine, valine, isoleucine, phenylalanine and tryptophan) found in greater abundance in the more stressed vines, and 5 were organic acids (idonic, threonic, shikimic, malic and citric), found in greater abundance in the category of non-water stressed vines. The eleventh was conduritol-beta-epoxide, a compound that inhibits alpha-glucosidase activity in both animals and plants and is sometimes considered a natural antibiotic, and found in greater abundance in the stressed vines. There were more significant differences between the 2007 and 2008 seasons (20 known and 28 unknown compounds differed), than between water stress category, though the between year results do not form as consistent a pattern as did water stress category (see Fig. 16). Measured variables account for 100 % of the variance.

4 Discussion

We examined the hypothesis that plant available water in soil (PAW), and geospatial heterogeneity thereof, has a major influence on wine grape leaf water status and, in turn, fruit chemical and sensory properties. Our observations for a complex slopes vineyard in the Stags Leap American Viticulture Area of the Napa Valley supported this contention, but will require further verification in other “less complex” vineyard settings.

SOILD
1, 1013–1072, 2014

4.1 Geospatial variation in vine physiology

Our results were more consistent over vintage as compared with other emerging investigations examining a relationship between the soil water reservoir with physiological variables of importance to wine grape quality. For example, Bodin and Morlat (Bodin and Morlat, 2006) found broad based differences in number of days to reach specific phenologic stages that corresponded to an estimated soil available water based on the "terroir" categories of "weak", "medium" and "strongly" weathered rock. The relationship between stomatal conductance (integrated using δ^{13}C in leaves) and pre-dawn LWP (ψ_{PD}) in their investigation was strong. On the other hand, the relationship between ψ_{PD} and terroir category was not clear and complicated by precipitation. Water status was more dependent on seasonal precipitation in a high rainfall region that varied inter-annually, as compared with their geologic terroir categories (Bodin and Morlat, 2006). Our investigation differed in a critical way from those of Bodin and Morlat (2006), Reynolds et al. (2007) and Bramley (2005) who all noted a high degree of inconsistency between vintages in wine characteristics and vine physiological attributes within single vineyards. Our results, in contrast, were highly consistent for water status (ψ_{PD} and ψ_L) between 2007 and 2008 with a few exceptions. Springtime precipitation (March–May) was 60.5 mm in 2007, when correlations between ψ_{PD} and ψ_L broke down at the pea-size phenological stage, while in 2008, when Mar-May precipitation was only 7.9 mm correlations between physiological and growth variables with ψ_{PD} and ψ_L were statistically significant.

Bonfante and colleagues (Bonfante et al., 2011) identified crop water stress index (CWSI) and soil available water capacity (AWC, measured for the < 2 mm particle size fraction) as imparting an influence on wine characteristic. CWSI is closely linked to stomatal behavior, and thus somewhat indirectly linked to the supply function of soil water (see Sperry et al., 2002). The information for AWC was gathered from soil survey data taken at a small scale (1 : 50 000). Our investigation differed in that we identified a direct correlation with soil plant available water (PAW), where

SOILD
1, 1013–1072, 2014

PAW = [AWC × ERD × (1-fraction rock + gravel volume)] mapped at a large scale of approximately 1 : 2500 and with an observed PAW range (68–177 mm) that greatly exceeded the smoothing exercises of both Bonfante et al. (2011) and Jones et al. (2004). Our results indicated that PAW was well correlated with physiological and growth variables measured in this investigation (Fig. 14). Thus, the soil water reservoir, when integrated for rock and gravel content and effective rooting depth and not precipitation or other environmental variables emerged as being more explanatory. This brings up two important aspects of the concept of geologic terroir: (1) when data is smoothed too much, critical detail is lost, but when detail is too great (Carey et al., 2008), large scale patterns cannot be discerned, and, (2) our data and that of others highlights the *n* dimensionality of terroir and how an environmental variable like precipitation can negate, e.g., a geologic factor. This highlights the extreme degree of spatial heterogeneity of soils, along with frequent and abrupt transitions in depth, horizonation and chemical composition.

The areas of greatest water stress in the vineyard block studied (Figs. 2, 3, 10 and 11), showed consistent vine physiological responses for both the 2007 and 2008 vintages. The geospatial patterns detected corresponded well with other interpolated maps of physiological variables assessed like berry size, berry weight, °Brix and pruning weight (Figs. 4, 5, 6 and 7). The consistency we observed is an important finding with respect to the observations of Reynolds et al. (2007), who found no consistency over 4 vintages (1998–2002) for yield parameters, key aroma compounds, volatile terpenes, titratable acidity, pH and soluble solids (°Brix), and Bramley (2005) who found no inter-vintage consistency for quality parameters like anthocyanin and polyphenolic content. In this investigation berry size (mm diameter), berry weight (g) were both strongly positively correlated with both ψ_{PD} and ψ_L at véraison in both 2007 and 2008 (Figs. 8 and 9). Pruning weight (vine size, Fig. 7) was similarly statistically significantly correlated with ψ_{PD} and ψ_L ($P < 0.001$) indicating that more negative LWP and therefore higher stress levels resulted in smaller canopies with respect to fruit load (Cortell et al., 2005; Tisseyre et al., 2008; Winkel et al., 1995) . This may have indicated that the canopies of the water stressed categorized vines ($\psi_L \leq -15.0$ bars) were more open, perhaps allowing for greater light interception by fruit, but this was not directly evaluated. It is likely a smaller canopy of the stressed vines was achieved over long time periods (years), considering the advanced age of the vineyard. Finally, °Brix was consistently statistically significantly negatively correlated with ψ_{PD} and ψ_L, albeit weakly, in both 2007 and 2008 (Figs. 8 and 9) which again was surprising considering its geospatial pattern (Fig. 6) was strongly similar to water status (Figs. 2, 3, 10 and 11) and PAW (Fig. 12).

The crop evapotranspiration demand during the 2007 growing season was 274 mm and during 2008 it was 280 mm of water (CIMIS, 2014), as corrected using a grape crop coefficient of 0.8 and a maximum canopy estimated at approximately 0.6 $m^2\,m^{-2}$ leaf area index using shadow casting estimates (Williams and Ayars, 2005). It is acknowledged there are competing factors that could influence fruit chemical composition other than a limited supply function (A_R, cf. Sperry et al., 2002) versus evapotranspiration demand (A_L). Related factors such as drainage (air filled porosity) and the volume of soil roots occupy (root : shoot ratio) may also play a role. For example, the balance of root : shoot hormonal relationships under water stress conditions could influence root to shoot hormone transport and ripening (Munns and Sharp, 1993; Okamoto et al., 2004). One of the most dramatic examples of shallowness was found at the northeastern section of the vineyard block, where the vine rows shorten, on the border between irrigation-blocks 4N and 4C (Fig. 1). This corresponded well with areas of vines categorized as water stressed in the LWP interpolations (Figs. 10 and 11, left panel). The geospatial pattern of LWP was observed starting as early as the pea-size phenological stage (2008) for both ψ_{PD} and ψ_L. As noted above, both physiological measures of water status strongly resemble patterns that emerged for berry diameter (Fig. 4) berry weight (Fig. 5), °Brix (Fig. 6) and pruning weight (Fig. 7). The soils found in the consistently non-stressed categorized irrigation block 4S, at the southern end of the vineyard, are deeper with consistently higher PAW. These soils were derived from historicalluvial

SOILD
1, 1013–1072, 2014

deposits and alluvial (landslide) events as compared with the volcanic soils found elsewhere in the more north and central extent of SLV 4.

4.2 Sensory characteristics

A key hypothesis was that berries from vines under greater water stress within the SLV 4 vineyard would have higher °Brix earlier in the season and sensory characteristics more typical of riper fruit – sweeter, softer, less acidic berries (Bravdo et al., 1985; Jackson and Lombard, 1993; Koundouras et al., 2006; Peterlunger et al., 2007; Seguin, 1983; Tregoat et al., 2002). A less explored area of ripeness concerns the relationship between fruit maturity at harvest and the appearance or disappearance of volatile compounds (Canuti et al., 2009). While this raises more questions concerning the role of fruit "maturity" in the sensory experience, this report was limited to detection of non-volatile compounds. Nonetheless, the characteristics in the sensory analysis associated with advanced ripeness and heightened quality were more heavily weighted by LWP category (Fig. 15).

Many of the significant sensory characteristics found in this study were indicators of ripeness with respect to the factors of texture, color and flavor. As discussed above, early ripening and particularly earlier onset of véraison has been positively correlated with water stress. The significant results in the sensory panel indicated that, in accordance with the original hypothesis, fruit of the more stressed categorized vines within the SLV 4 vineyard was ripening sooner as indicated by detection of advanced ripening characteristics (Fig. 15). Earlier ripening in this case was significantly correlated, albeit weakly, with more negative water potentials – leading to higher soluble solids content (Figs. 6, 10 and 11). Unexpectedly, astringency, which is a sensory term often linked with higher polymeric phenol composition, was judged by the sensory panel to be higher in the non-stressed vine category; whereas, heightened polyphenolic levels of grapes from vines experiencing water stress is well established, as discussed in the Introduction. Nonetheless, sugar is known to mask astringency, and phenolic development is strongly tied to sugar development (Pirie and Mullins, 1977), so it is hard

SOILD
1, 1013–1072, 2014

to uncouple these two compositional factors without further chemical analysis of the berries. As the soluble solids content was found to be higher in the vines categorized as water stressed, perhaps this masking effect acted to elude astringency detection by the sensory panel. Nonetheless, phenolic composition of the skin increases at the greatest rate during the latter stages of véraison (Pirie and Mullins, 1980) so this remains an open area for further investigation. As the bulk of phenolic components are found in the skin (Ribereau-Gayon and Stonestreet, 1964), water stress may be driving a berry compositional difference perhaps unrelated to berry size and therefore likely unrelated to phenolic content.

The sensory results were significant not just for taste and mouthfeel characteristics associated with ripening, but for other characteristics as well, suggesting that the sensory differences associated with water stress were not merely a result of the sort of early ripening that water stress promoted. At least one investigation has focused on naturally occurring water deficits (non-irrigated, dryland conditions), and found early water stress ($\psi_{PD} < -3.0$ bars at about pea-size) increased the concentration of anthocyanins and total phenolics in berry skins (Kondouros et al., 2006). In comparison to this investigation, the stress conditions were lesser. In addition, like this investigation, some other metabolic phenomenon like hormonal relationships not directly related to stress but root:shoot ratio might have been driving the development of these characteristics. Thicker berry skin is associated with higher water stress levels (Esteban et al., 2001) but that did not seem to be the case in this investigation. The significant sensory and physiological results observed here were simply contexts for developing further hypotheses into how the soil environment is involved mechanistically in separation of chemical constituents of the berry.

While the positive effects of water stress on quality have previously been thought to have a limit, particularly on the accumulation of sugar and the decrease in acidity (Chalmers et al., 2008; Girona et al., 2006; Ojeda et al., 2005), our findings indicated that positive effects extend well beyond what is considered severe stress. The theoretical “wilting point” as described for less hardy plants than grapevines, is at −15.0 bars.

SOILD
1, 1013–1072, 2014

The most stressed vines, those with LWP at or more negative than the wilting point, were those found to have the most positive characteristics by the sensory panel. In this study, almost half of the data vines in both 2007 (49.3 %) and 2008 (43.7 %) had mid-day water potentials at or below the wilting point. Interestingly, an additional 19.7 % (14 of 71 vines both years but not the same 14 vines) in SLV 4 were under LWP levels considered stressful at véraison, in example for $\psi_L \leq -14.0$ bars accounted for 69 % (2007) and 63 % (2008) of the data vines. This difference between our results and others cited, however, may be due to cultivar, rootstock and/or age of the vines at the time of the investigation (35 years). Cabernet Sauvignon has been shown to be relatively stress-tolerant (Gaudillere et al., 2002) as compared with other varieties like Syrah and Pinot Noir, the varieties studied in the above-cited paper. This may be related to its elevated activation of abscisic acid ABA synthetic pathways under deficit irrigation conditions (Deluc et al. 2009), a putative signal molecule for stomatal closure (Okamoto et al. 2004; Soar et al., 2004).

The natural range of water status seen in SLV 4 was in most cases more extreme than prescribed by controlled irrigation trials (Bravdo et al. 1985; Chalmers et al., 2008,; Esteban et al., 1999; Girona et al., 2006; Hepner et al., 1985; Ojeda et al., 2005), but consistent across both years (see Table 1). Again, this observation indicated that permanent site-specific characteristics like soil texture, stoniness and rootimg depth, the primary factors in estimating PAW were causative. Soil boreholes taken at the vineyard showed no strong pattern in any single physical characteristic that would contribute to water stress. While it is generally accepted that water-holding capacity of the soil is a limiting factor in both plant reproductive and vegetative productivity, vine-soil relations are complicated by complex geomorphology and the deep rooting nature of grape (Smart et al., 2006; Winkel et al., 1995). Temporal stability of within-vineyard variation as tied to soil composition has been previously reported (Gaudillere et al., 2002) as well as refuted (Reynolds et al., 2007; Winkel et al., 1995), which only reinforces the possibility that conditions beyond soil composition per se are in play. At the site used

SOILD
1, 1013–1072, 2014

for this study, a non- atypical diverse hillside vineyard with heterogeneous soil depths and types, it seems that a suite of factors contributing to PAW were causative.

4.3 Metabolomic profiles

The strength of metabolomics as a tool in viticulture has not been fully explored. It has been used to characterize wine styles (Schmidke et al., 2013) and to show differences in wines from fruit grown on "different soils" and in different vintages (Pereira et al., 2007). Another recent study used genomic pathway analysis to explore the role of water stress in grape (Deluc et al., 2009), but that study focused on hormone regulation, particularly ABA. A difference in metabolic profile between plants experiencing extreme water stress and those that were less stressed might be expected, and so a key question is whether that correlates to flavor compound development.

Wine grapes are harvested at relatively high soluble solids content, and the high sugar concentrations in the samples have tended to obscure detection of metabolites found in lower-concentrations so compounds of interest in a metabolomics study are generally found in low concentrations, it was determined that using skins only could confer greater resolution to chromatograms. Molecular groups important to this study can be found in the skins while only a small proportion of that population set is found in the pulp and seeds (Harbertson et al., 2002).

The results of the metabolomic analysis were both expected and unexpected. The elevation of organic acids has been demonstrated in well-irrigated vines (Bravdo et al., 1985; Esteban et al., 1999; Hepner et al., 1985) and has been considered a mark of low quality. The vines in SLV 4 were irrigated with quantities of water during both the 2007 and 2008 seasons that did not meet ET_c demand, and only after the vines had reached apparently extremely stressful LWP levels. Less stressed vines in this investigation were planted in areas with higher soil available water (Figs. 10, 11 and 12), although the irrigation quantities applied were less than ET_c demand even when added to the soil water reservoir. The variable effect of irrigation on sugar accumulation observed in the numerous reports discussed in the Introduction section may account for the greater number of differences across years and the simultaneous lack of a clear pattern among compounds in that group.

A large suite of polyphenolic compounds increase in concentration as a consequence of water stress and/or light interception by fruit clusters in grape canopies. The linkage between water stress and restriction of vegetative growth (shoot and leaf expansion) and thus light penetration into the canopy, makes it challenging to separate the effects of light interception and temperature versus water stress per se. Polyphenolic compounds are also different for different cultivars (Adams, 2006; Wenzel et al., 1987) and this may help to explain a large degree of a sense of regional terroir. A positive relationship between more negative LWP and elevation of both gross concentration of polyphenols and the smaller population of inextractable polyphenols by extracting with EtOH has been demonstrated (Sivilotti et al., 2005; Cassasas et al., 2013).

Amino acids are typically found in greater concentration in stressed vines (Vasconcelosi et al., 2005). Not expected, however, was the clear way in which five amino acids – valine, tryptophan, phenylalanine, leucine, and isoleucine – were highly elevated in the vines experiencing severe water stress, especially as these amino acids are not those typically elevated in grape. A study of Muscat of Alexandria showed elevated total amino acid levels in berries from deficit-irrigated vines (El-Ansary and Okamoto, 2007), with arginine being the predominant amino acid identified. Proline has been shown to be significantly elevated in the water stressed treatments in controlled irrigation trials (Deluc et al., 2009; Freeman and Kliewer, 1985; Ginestar et al., 1998; Matthews and Anderson, 1988). However, neither proline nor arginine was one of the significantly different amino acids found in this investigation. The effect found in the Deluc study (Deluc et al., 2009) was pronounced in Cabernet Sauvignon and not significant in Chardonnay. Given the variable effect by cultivar, perhaps there is also a rootstock-scion influence (Stockert et al., 2013) contributing to the differences in amino acid profiles found in this investigation. It is also possible that as this study concentrated on the skins of the grape berry rather than the juice, the proline and arginine concentrations found here would not show the same elevated levels found elsewhere.

This presents a good example of why it can be so challenging to characterize a single general variable (geology) in an *n* dimensional response plane.

5 Summary

While many observations supported our original hypotheses, that the soil water reservoir and the establishment of water stressed conditions is a major driving variable in geologic studies of terroir, we cannot entirely rule out other soil properties in conditioning physiological responses in this vineyard. Another aim of this study was to examine grapevines in the field on a vine-by-vine basis to achieve greater understanding of the selective harvesting process as it relates to within-vineyard variability. Our mapping exercises and sensory quality assessments of fruit (which were conducted by a blind panel) agreed very well with the geospatial selective harvest area. The use of grapes rather than wine in the sensory and chemical trials was unique, and contributed to understanding, or perhaps verifying that in-field evaluation of fruit makes sense when evaluated in a more objective manner. We found that basic monitoring techniques already used in many vineyards to make selective harvesting decisions, for example monitoring for water stress and preferentially picking smaller berries, was significant when evaluated by unbiased sensory trials. Further, spatially relating the data using geostatistical analyses other more conventional relational analyses proved invaluable in assessing site variation

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SOILD
1, 1013–1072, 2014

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SOILD

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Geospatial variation of grapevine water status

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Table 1. Predawn (ψ_{PD}) and midday (ψ_L) leaf water potential during 2007 and 2008 in bars.

	ψ_{PD}, range	ψ_{PD}, mean	ψ_L, range	ψ_L, mean
Bloom 2007	ND	ND	−10.70 to −6.50	−8.44
Pea−Size 2007	−4.33 to −0.55	−2.00	−12.80 to −7.65	−10.78
Véraison 2007	−12.00 to −1.90	−6.82	−18.20 to −10.70	−14.74
Bloom 2008	−4.80 to −0.90	−1.93	−10.80 to −5.80	−7.93
Pea Size 2008	−6.67 to −1.20	−3.07	−14.10 to −6.25	−9.73
Véraison 2008	−13.00 to −2.25	−7.00	−18.73 to −10.20	−14.75

SOILD

1, 1013–1072, 2014

Geospatial variation of grapevine water status

D. R. Smart et al.

Table 2. Mean, range and % change in mean of physiological parameters of grape berries, pruning weight and °Brix for the two vintages, 2007 and 2008 measured in the investigation.

	2007		2008		%change
	Range	Mean	Range	Mean	in mean
Berry Diameter (mm)	9.23–12.20	10.51	8.58–10.76	9.99	−4.9 %
Berry Weight (g)	0.56–1.08	0.80	0.49–0.90	0.74	−7.5 %
Pruning Weight (kg)	0.75–4.58	1.83	0.45–3.69	1.45	−20.8 %
Soluble Solids (°Brix)	21.0–28.2	25.3	20.4–28.1	25.1	−0.5 %

SOILD
1, 1013–1072, 2014

Geospatial variation of grapevine water status

D. R. Smart et al.

Table 3. Statistical probability of committing a Type I error when accepting the hypothesis that physiological characteristics of °Brix, berry diameter, berry weight and pruning weight differed between the leaf water potential groupings at véraison of stressed ($\psi_{PD} \leq -8.0$ bars and $\psi_L \leq -15.0$ bars) versus non-stressed individuals ($\psi_{PD} \geq -7.9$ bars or and $\psi_L \geq -14.9$ bars).

	ψ_{PD}, 2007	ψ_L, 2007	ψ_{PD}, 2008	ψ_L, 2008
°Brix	$p = 0.0540$	$p = 0.0450$	$p = 0.0008$	$P = 0.0230$
Berry Diameter	$p \leq 0.0004$	$p \leq 0.0001$	$p = 0.0643$	$P = 0.0013$
Berry Weight	$p \leq 0.0001$	$p \leq 0.0001$	$p \leq 0.0001$	$P \leq 0.0001$
Pruning Weight	$p \leq 0.0001$	$p \leq 0.0001$	$p \leq 0.0001$	$P \leq 0.0001$

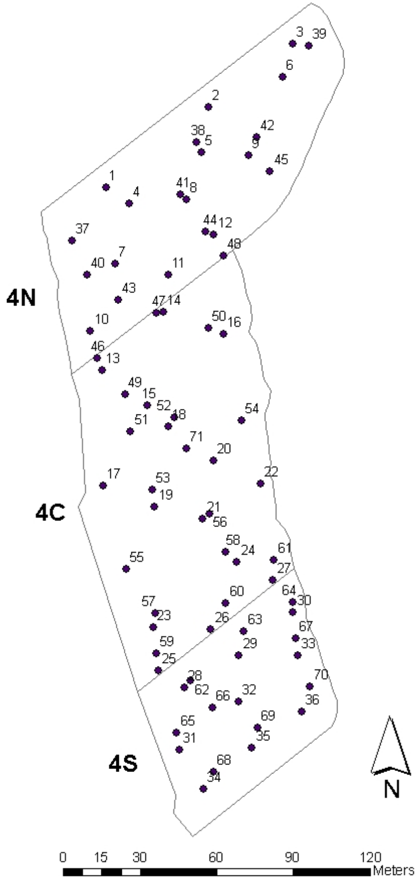

Figure 1. Numbers correspond to data vines; numbers start over at 37 in 4N because of the addition of data vines at véraison 2007. Rows run up the slope in a southwest-northeast direction, so, e.g., data vines 1, 2, and 3, are in row 2. Areas 4N, 4C, and 4S denote irrigation blocks 4-North, 4-Center, and 4-South, respectively.

SOILD
1, 1013–1072, 2014

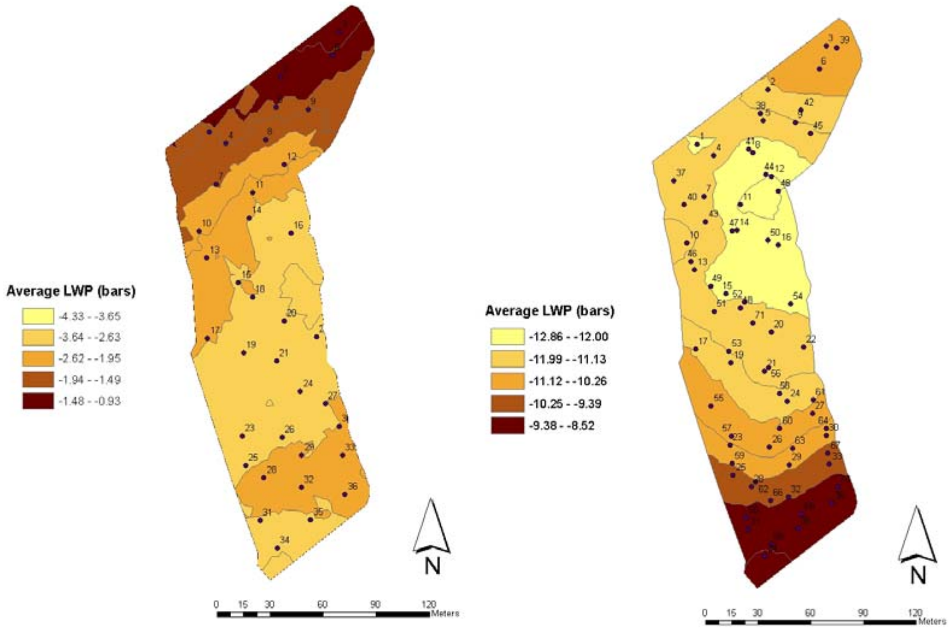

Figure 2. Interpolated map using ordinary kriging analysis of 2007 pre-dawn leaf water potential (LWP, left) at the pea-size phenologic stage and measured on 19 June 2007, and 2007 pea-size mid-day LWP (right), measured on the same day.

SOILD
1, 1013–1072, 2014

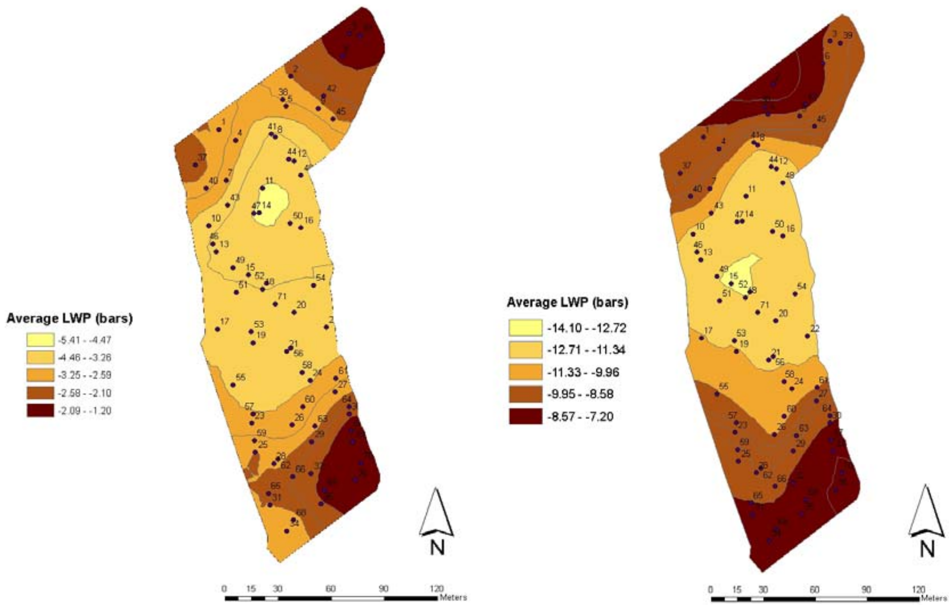

Figure 3. Interpolated map using ordinary kriging analysis of 2008 pea-size phenologic stage pre-dawn leaf water potential (LWP, left), measured on 26 June 2008 and 2008 midday LWP (right), measured on 25 June 2008.

SOILD
1, 1013–1072, 2014

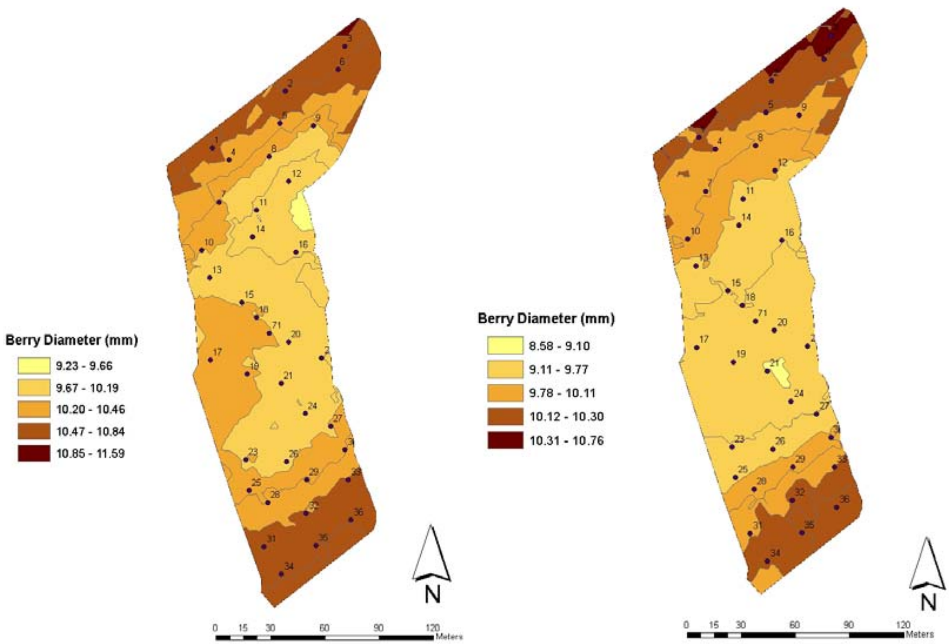

Figure 4. Interpolated map using ordinary kriging analysis of 2007 berry diameter (left) measured on 23 August 2007, and 2008 berry diameter (right) measured on 28 August 2008.

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1, 1013–1072, 2014

Geospatial variation of grapevine water status

D. R. Smart et al.

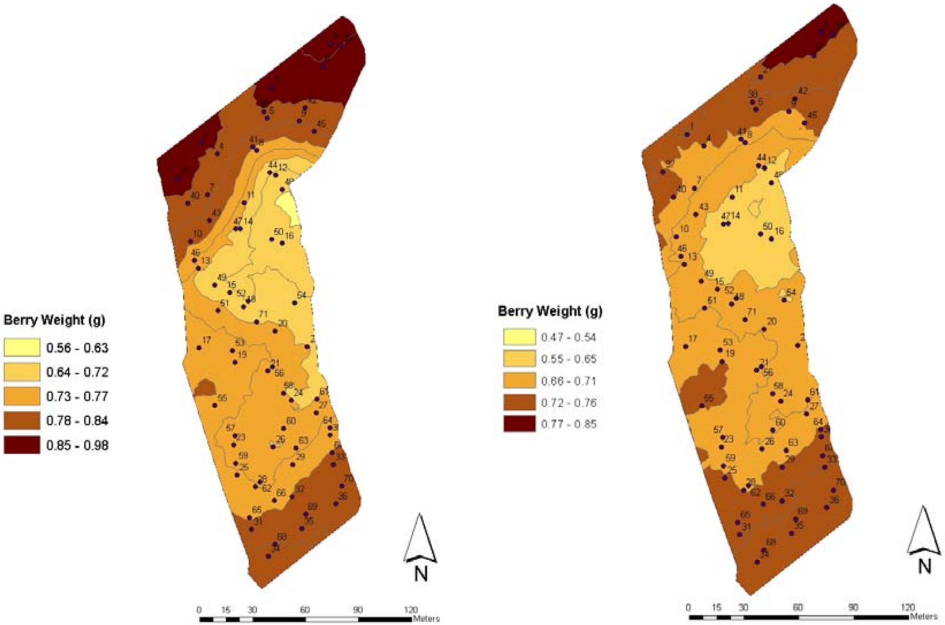

Figure 5. Interpolated map using ordinary kriging analysis of 2007 berry weight (left) measured on 5 September 2007, and 2008 berry weight (right) measured on 4 September 2008.

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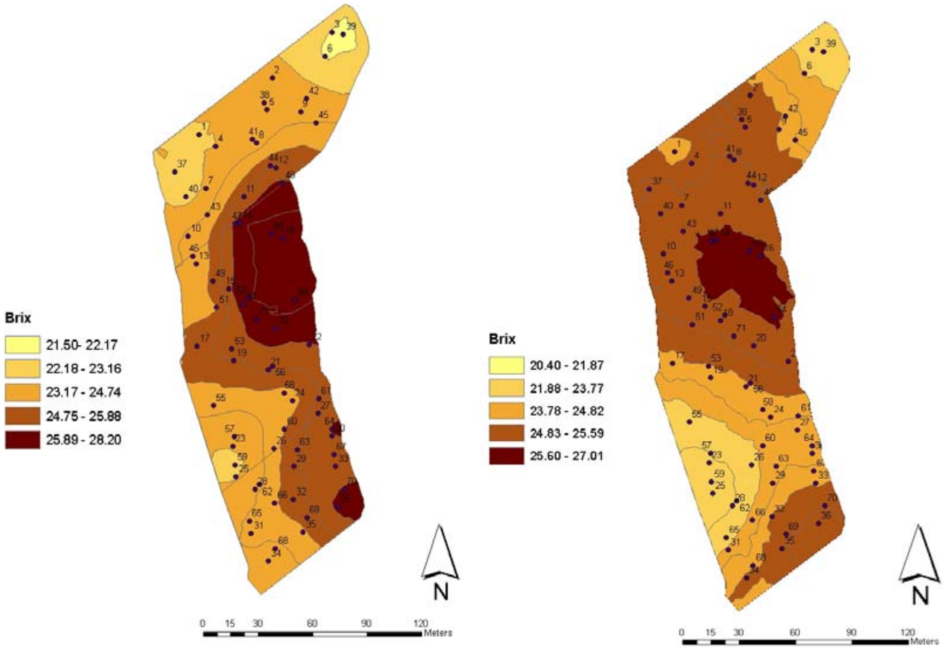

Figure 6. Interpolated map using ordinary kriging analysis of 2007 soluble solids (Brix) measured on 5 September 2007 (left), and 2008 soluble solids measured on 4 September 2008.

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1, 1013–1072, 2014

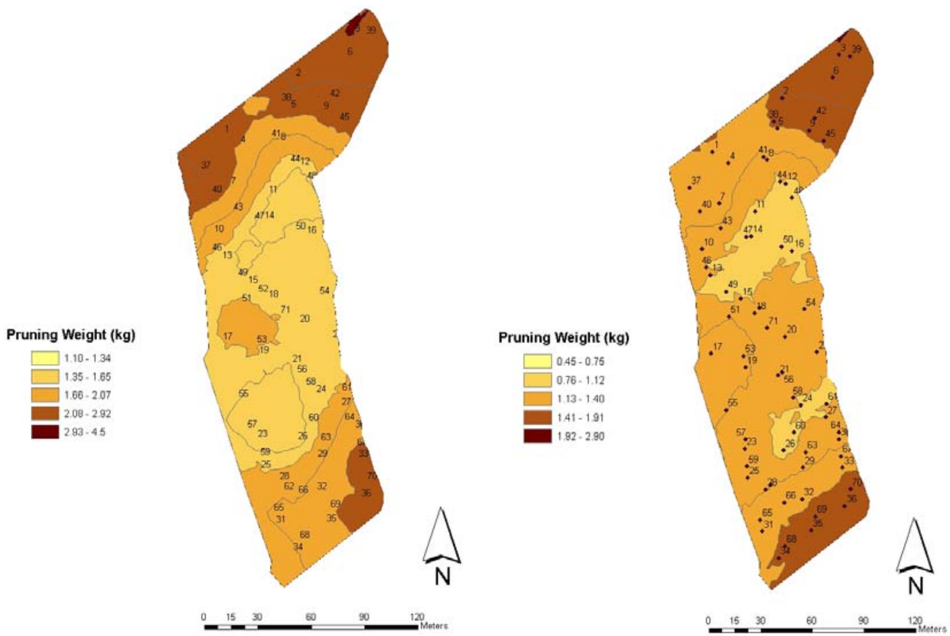

Figure 7. Interpolated map using ordinary kriging analysis of 2007 pruning weight (left), measured 4 February 2008, and of 2008 pruning weight (right), measured 12 February 2009.

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1, 1013–1072, 2014

Geospatial variation of grapevine water status

D. R. Smart et al.

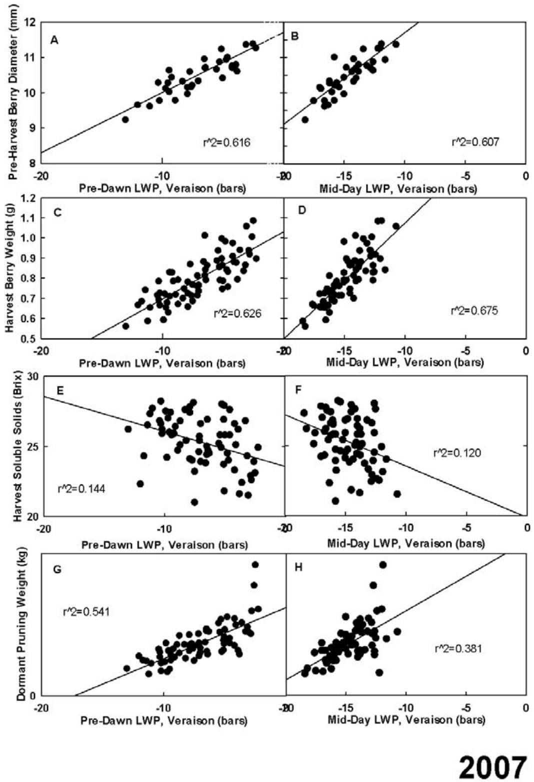

Figure 8. Correlations of physiological responses, berry size and weight, soluble solids (°Brix) and canopy size (pruning weight) versus pre-dawn and mid-day leaf water potential (LWP) at véraison in 2007.

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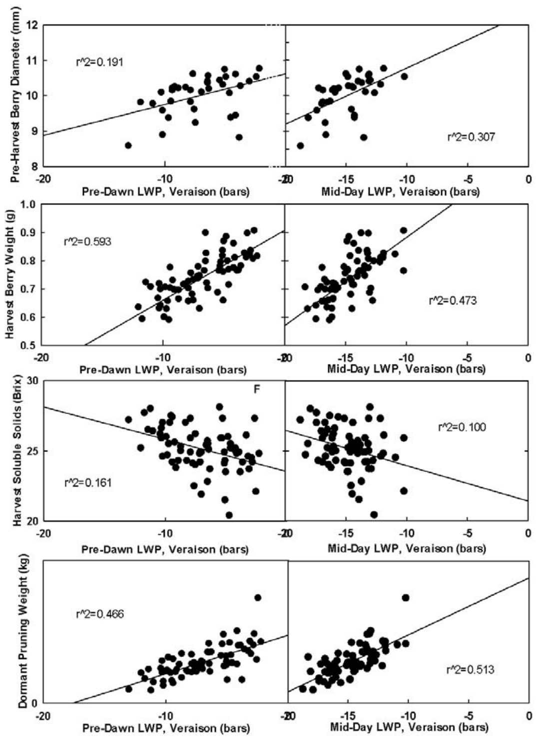
1, 1013–1072, 2014

Geospatial variation of grapevine water status

D. R. Smart et al.



2008

Figure 9. Correlations of physiological responses, berry size and weight, soluble solids (°Brix) and canopy size (pruning weight) versus pre-dawn and mid-day leaf water potential (LWP) at véraison in 2008.

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1, 1013–1072, 2014

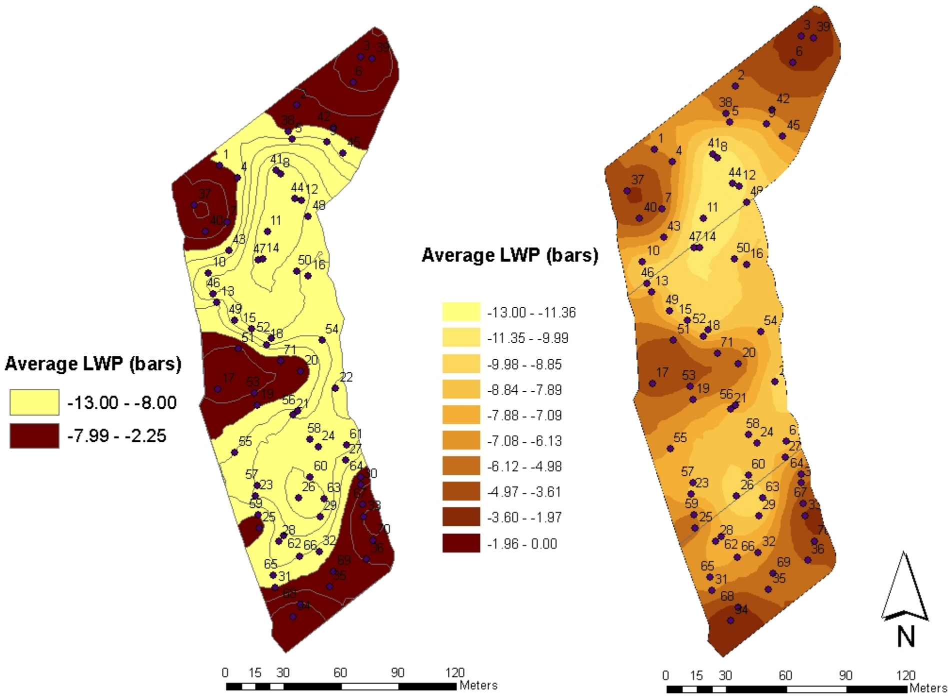

Figure 10. Interpolated map using ordinary kriging analysis showing 2007 vineyard area showing pre-dawn LWP at vériason (right) and locations (left) as divided into analysis groups of vines categorized as water stressed ($\psi_L \leq -15.0$ bars) and vines categorized an non-stressed ($\psi_L \geq -14.99$ bars).

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1, 1013–1072, 2014

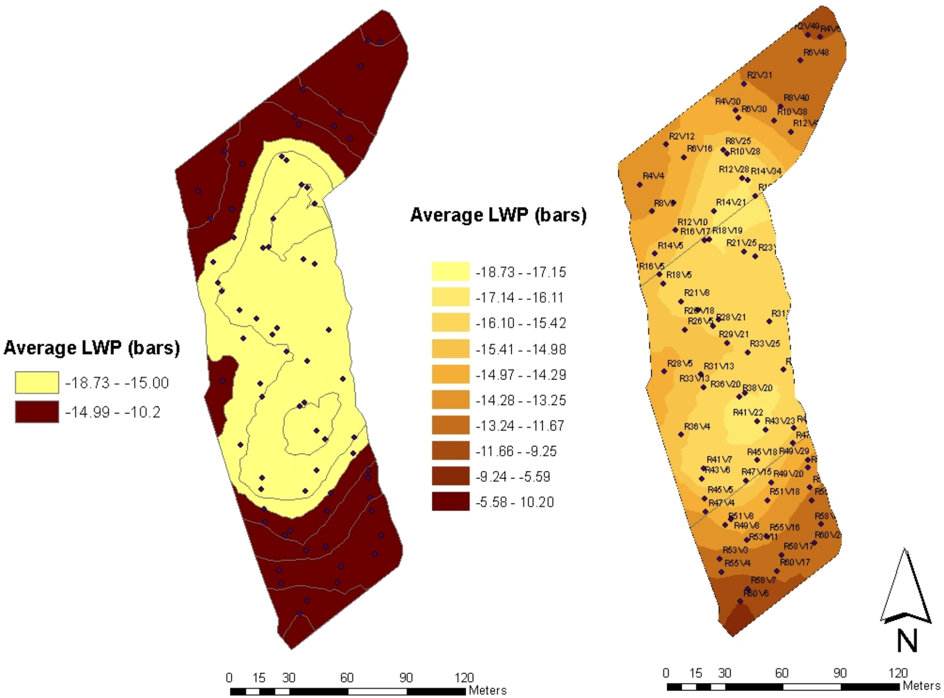

Figure 11. Interpolated map using ordinary kriging analysis showing 2008 vineyard area showing mid-day LWP at vériason (right) and locations (left) as divided into analysis groups of vines categorized as water stressed ($\psi_L \leq -15.0$ bars) and vines categorized an non-stressed ($\psi_L \geq -14.99$ bars).

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1, 1013–1072, 2014

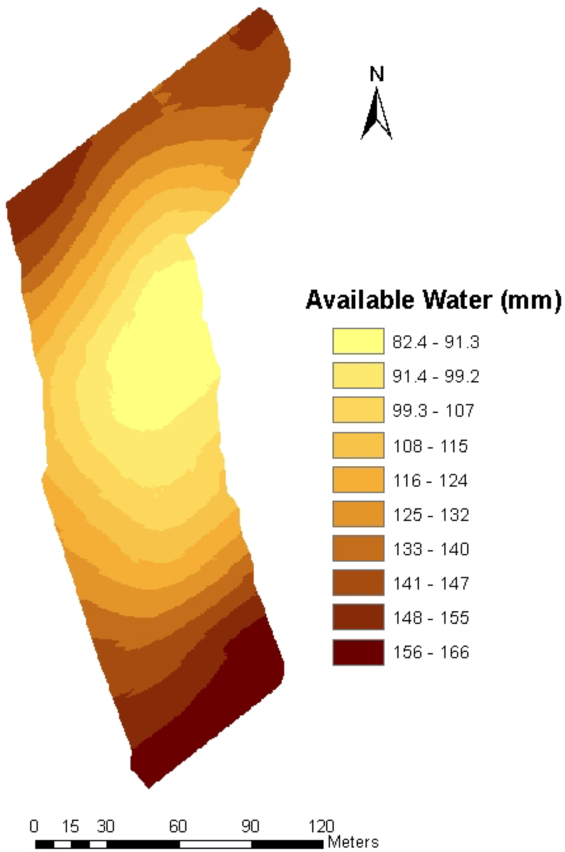

Figure 12. Interpolated map using ordinary kriging analysis showing predictive map of calculated plant available water content (PAW, mm).

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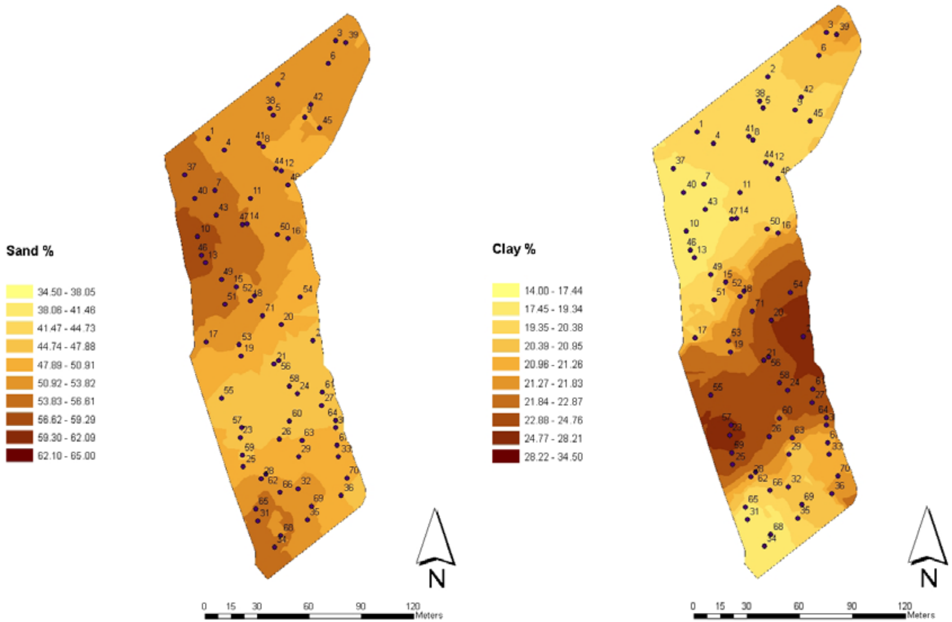

Figure 13. Interpolated map using ordinary kriging analysis of SLV 4 percent sand content (left) and percent clay content (right).

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1, 1013–1072, 2014

Geospatial variation of grapevine water status

D. R. Smart et al.

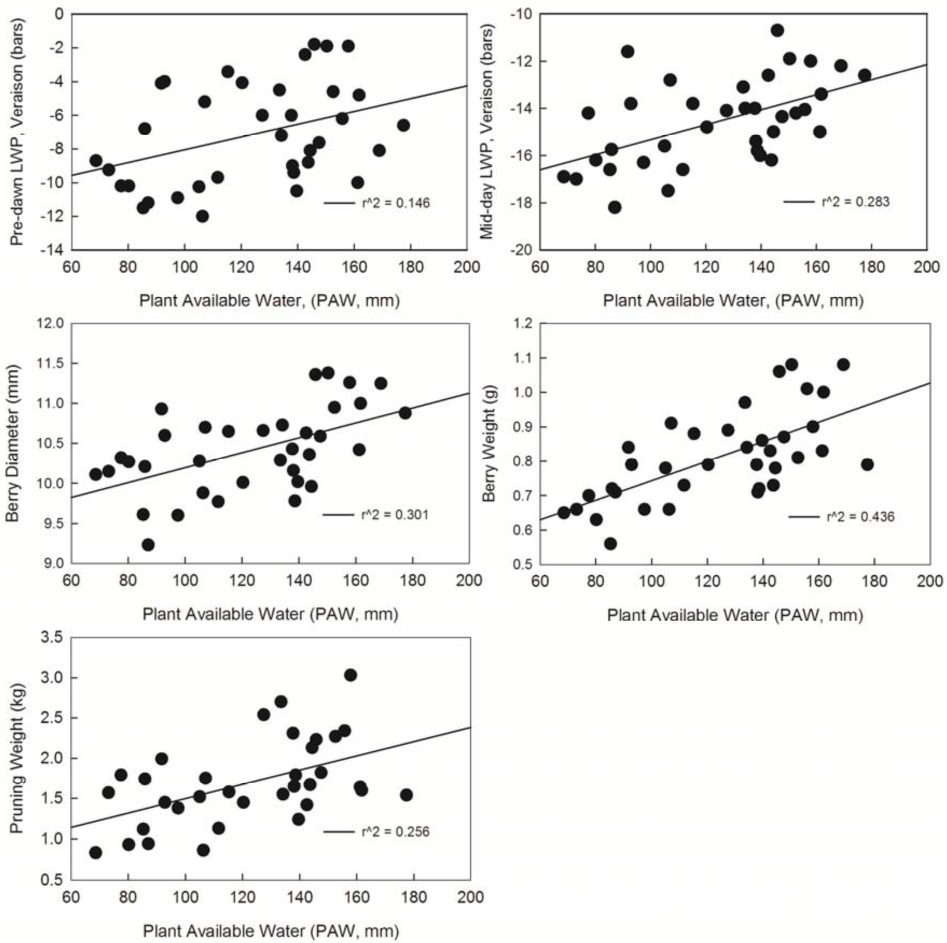

Figure 14. Correlations of physiological data versus plant available water in soil (PAW). Data are shown for 2008 and similar results were encountered for 2007.

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1, 1013–1072, 2014

Geospatial variation of grapevine water status

D. R. Smart et al.

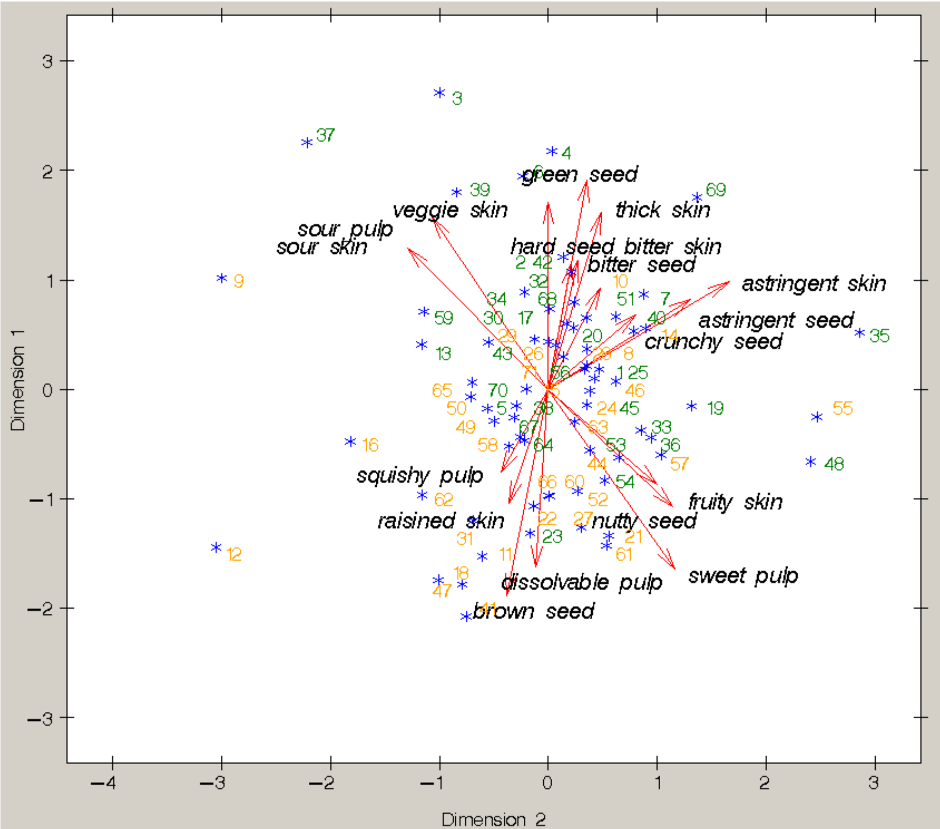

Figure 15. Principle components analysis (PCA) of identified sensory characteristics. Significant differences emerged between the group of vines categorized as non-water stressed (elements seen in upper quadrants), and the group of vines categorized as water stressed (elements seen in lower quadrants). Numbers are data vine numbers; data vines categorized as non-water stressed are labeled in green type, and data vines categorized as stressed are indicated by orange labels.

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1, 1013–1072, 2014

Geospatial variation of grapevine water status

D. R. Smart et al.

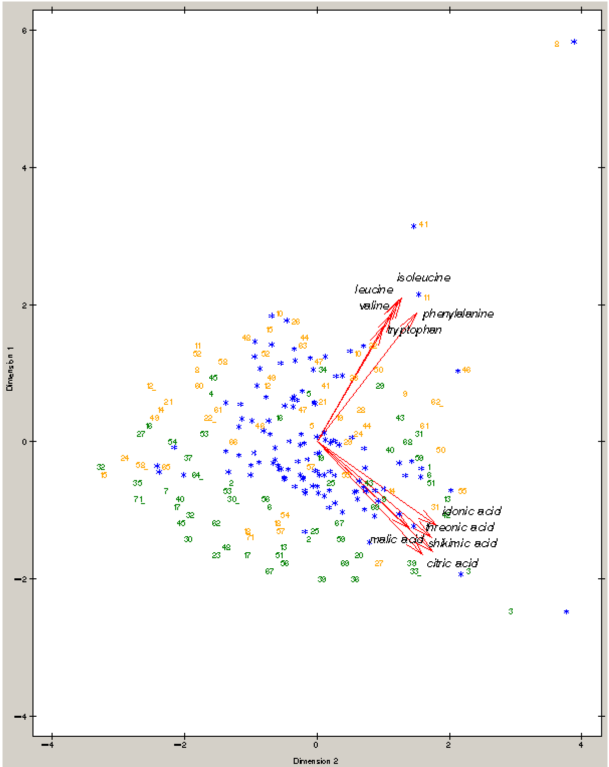

Figure 16. Principle components analysis (PCA) showing strong separation of amino acids from organic acids across both years, and indicating a significant water stress effect. Number labels correspond to data vine numbers; green labels represent non-water stressed categorized vines and orange labels represent stressed vine category.

SOILD
1, 1013–1072, 2014

Geospatial variation of grapevine water status

D. R. Smart et al.