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Relevance of sink-size estimation for within-field zone delineation in vineyards

I. Urretavizcaya[1] · J. B. Royo[1] · C. Miranda[1] · B. Tisseyre[2] · S. Guillaume[2] · L. G. Santesteban[1]

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Abstract Source to sink size ratio, i.e.: the relative abundance of photosynthetically active organs (leaves) with regards to photosynthate demanding organs (mainly bunches), is widely known to be one of the main drivers of grape oenological quality. However, due to the difficulty of remote sink size estimation, Precision Viticulture (PV) has been mainly based on within-field zone delineation using vegetation indices. This approach has given only moderately satisfactory results for discriminating zones with differential quality. The aim of this work was to investigate an approach to delineate within-vineyard quality zones that includes an estimator of sink size in the data-set. The study was carried out during two consecutive seasons on a 4.2 ha gobelet-trained cv. 'Tempranillo' vineyard. Zone delineation was performed using Normalized Difference Vegetation Index (NDVI), soil apparent electrical conductivity (EC_a) and bunch number (BN) data. These variables were considered separately, in pairs, or the three altogether, using fuzzy *k*-means cluster analysis for combinations. The zones delineated based on single variables did not allow a sufficient discrimination for grape composition at harvest, NDVI being the only variable that by itself resulted in zones that to some extent differed in grape composition. On the contrary, when two variables were combined, discrimination in terms of grape composition improved remarkably, provided the sink size estimation variable (BN) was included in the combination. Lastly, the combination of the three variables yielded the best discriminating zoning, improving slightly on those provided by NDVI + BN and EC_a + BN combinations. Thus, the relevance of including a variable related to sink size (in this case the number of bunches per plant) has been confirmed, which makes its consideration highly advisable for any PV work aiming at zone delineation for grape quality purposes.

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✉ L. G. Santesteban
gonzaga.santesteban@unavarra.es

[1] Departamento de Producción Agraria, Universidad Pública de Navarra, Pamplona, Navarre, Spain

[2] UMR ITAP, IRSTEA/Montpellier SupAgro, Montpellier, France

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Introduction

The delineation of within-vineyard zones that would potentially produce grapes of differential quality has been one of the main aspects that Precision Viticulture (PV) has dealt with in the last decade. Zone delineation has been based mainly on the information given by remotely obtained vegetative indices, and has been proved to be suitable to distinguish zones within the vineyard with different vegetative development (Santesteban et al. 2010; Acevedo-Opazo et al. 2008a, 2008b, 2010; Hall et al. 2003; Bramley et al. 2011c). However, the performance of this approach with regards to grape composition is much more erratic. For instance, Bramley et al. (2011b) used PCD index to delineate zones, observing oenological differences for just two of the five wine composition parameters considered. Similarly, Acevedo-Opazo et al. (2008a) found that the zones defined with Normalized Difference Vegetation Index (NDVI) were not coherent with most grape composition parameters, and Santesteban et al. (2010) only found differences between NDVI-defined zones for one of the six grape composition parameters considered. On the contrary, Hall et al. (2011), observed that grape composition was generally correlated with vegetation indices, although the correlation coefficients found were inferior to 0.4. King et al. (2004) reported a very good correspondence between the zones defined with NDVI obtained with a proximal sensor mounted to an all-terrain vehicle and grape composition.

The zone delineation process has been completed by some authors combining the information given by vegetation indices with other variables such as soil apparent electric conductivity (EC_a) or elevation (Bramley et al. 2011a; Bramley and Lamb 2003; Corwin and Lesch 2005; Santesteban et al. 2013; Urretavizcaya et al. 2011; Tagarakis et al. 2013), but the degree of agreement between the zones defined and grape composition was still just moderate. Santesteban et al. (2013) hypothesised that introducing any variable that estimated sink size (i.e.: fruit load) in multivariate based methods for zone delineation would provide an improvement in their performance, since an adequate balance between photosynthate sources (leaves) and sinks (majorly the bunches) is known to determine, to a great extent, grape quality (Howell 2001).

The balance between source and sink size has been classically estimated by the Ravaz Index, defined as the ratio between grape and pruning wood mass (Ravaz 1903) and constitutes one of the most widely used indices among viticulturists. More recently, the ratio between leaf area and the amount of fruit has been introduced as an index to estimate vineyard balance (Howell 2001) and is frequently referred as crop load. In opposition to the relevance that has been given to vine balance in classic viticulture, PV has only taken into account "source size", since vegetation indices are good surrogates for leaf area development (Baluja et al. 2012; Hall et al. 2011; Johnson et al. 1996; Tagarakis et al. 2013) and can, to some extent, represent canopy microclimate as canopy size conditions it (Smart and Robinson 1991), but are not good surrogates of the amount of fruit (Hall et al. 2011; Bonilla et al. 2015). The main reason for not considering "sink size" relative variables for zone delineation is probably that, to date, there is not a readily usable method that would allow the estimation of its spatial variability. Nevertheless, there is a growing interest in developing methods for accurate and cost effective estimation of fruit load early in the season, based on RGB (red, green and blue) and multispectral imagery (Diago et al. 2012; Nuske et al. 2011; Fernandez et al. 2013). In that sense, the work performed by

Grocholsky et al. (2011), where both sink and source sizes were simultaneously estimated using a camera and laser system, is particularly relevant. The inclusion of sink size estimators for within zone delineation will have a great impact for vineyard management (for example, adjusting cultural practices to get more balanced vines by practices such as cluster or leaf thinning).

The aim of this work was to investigate an approach to delineate within-vineyard grape quality zones from information obtained earlier in the season. The originality of this approach is the inclusion of an estimator of sink size in the data-set.

Materials and methods

Experimental layout

The study was carried out during two consecutive seasons (2010 and 2011) on a 4.2 ha gobelet-trained cv. 'Tempranillo' vineyard, located in Leza (42°33′22″N, 2°38′07″W, 572 m asl, Basque Country, Spain). The vineyard was 17-years old at the beginning of the experiment, with a 2.4 m × 1.2 m planting distance and no irrigation system, as this is a traditionally rain-fed vineyard area. This climate of the area belongs to Classes II and III in Huglin's classification (Huglin and Schneider 1998). The climatic conditions in 2010 were drier and warmer than average, whereas in 2011 the weather was similar to average in terms of spring rainfall, and slightly cooler in temperature, but the lack of rainfall from mid-August to late-September led to moderate water deficit and basal defoliation after NDVI image acquisition. More detailed data on the climatic conditions during the study period and some vineyard characteristics are detailed in Urretavizcaya et al. (2014).

During the season previous to the start of the experiment (2009), Normalized Differential Vegetative Index (NDVI) information was obtained from QuickBird satellite images, taken shortly after veraison (first half of August). Pixel size is 0.61 m for panchromatic, and 2.4 m for multispectral. NDVI was used to define a field sampling grid. The distance between each sampling point (SP) of the grid was chosen as the range of the NDVI semi-variogram model corresponding to 25 % of the total semi variance (C0 + C1), which implies that 75 % of spatial variability was encompassed. The resulting sampling grid consisted of 60 SP, with a 30 m × 30 m distance between them. At each SP, 15 adjacent plants located in three consecutive rows were marked, and all of them were used for the experimental measurements and sampling.

In order to delineate within-vineyard zones, information related to (i) vegetative development, (ii) sink size and (iii) soil characteristics were considered.

– Vegetative development was estimated using the NDVI calculated from QuickBird multiband satellite images acquired shortly after veraison each year, once vegetative growth had stopped. Once a map for the whole vineyard was obtained, the average value observed at the pixels in the zone that included the 15 vines at each SP was calculated and assigned to it. In order to test the ability of NDVI to estimate vegetative development in *goblelet* trained vines, for which no references are available, mean total shoot cross sectional area (SCSA) was calculated for each SP by measuring the basal diameter of all the shoots in the 15 vines at the end of July, once apical growth was stopped.

- Sink size was estimated by counting at veraison the total number of bunches (BN) of all the 15 vines at each SP.
- Soil variability was characterized by measuring soil electrical apparent conductivity (EC_a) at each SP using a handheld EM38 (Geonics Ltd., Ontario, Canada). At each sampling point, EC_a measurements were performed every second for 3–5 min, and the obtained data averaged after removing values shown to be outliers in box-and-whisker diagram. The measurements were performed in 2010 winter, after a heavy rainfall, in order to minimize the differences between measurements caused by soil water content.

The evaluation of the agronomic significance of the zones delineated was tested using yield and grape composition data obtained at harvest. Yield was determined by weighting all the bunches produced by the 15 vines at each SP. Then, a 300-berry sample was gathered per SP, and berry weight (BW) as well as the main composition parameters were determined using standard procedures. Shortly, after crushing, total soluble solids concentration (TSS) was measured with a temperature compensating refractometer RFM840 (Bellingham-Stanley Ltd., Kent, UK), pH and titratable acidity (TA) using a pH-Burette 24 auto-titrator (Crison, Barcelona, Spain), malic (MalA) and tartaric acid (TarA) concentration was measured enzymatically using an autoanalyzer (Easychem, Systea s.p.a., Italy), whereas yeast assimilable nitrogen (YAN) was estimated following the procedure described by Aerny (1996) with the modifications detailed in Garcia et al. (2011). Phenolic maturity was evaluated after 4 h extraction in two different media (pH = 1.0, pH = 3.2) following the methodology described in Glories and Augustin (1993) that allows estimating the concentration of total (TAnt) and extractable (EAnt) anthocyanins and of total phenolics (TP).

Data analysis

For both years of the experiment, the 60 SP were classified considering three clustering variables (NDVI, EC_a and BN) and their possible combinations. Classes (i.e.: clusters) were defined through fuzzy *k*-means analysis. The potential number of classes was considered to be 2, 3 and 4, and the best-performing number was chosen taking into account the values obtained for fuzziness performance index (FPI) and modified partition entropy (MPE) (Boydell and McBratney 2002) for fuzzy exponents ranging from 1.0 to 1.9. Clustering was performed applying fuzzy *k*-means analysis to pixel values in NDVI, EC_a and BN maps, obtained using ordinary kriging as detailed in Urretavizcaya et al. (2014).

The oenological significance of the classes defined was evaluated with two different procedures, using yield, berry weight and the nine grape composition variables measured at harvest (TSS, pH, TA, MalA, TarA, YAN, TAnt, EAnt and TP). Firstly, a one-way ANOVA considering the classes as the factor was used, allowing a separated evaluation for each variable. When the ANOVA showed statistically significant differences ($P < 0.05$), a Tukey–Kramer's test to determine post hoc separation between means was used, as it is a test suitable for unbalanced data sets (Sahai and Ojeda 2004). Secondly, MANOVA was done with the nine grape composition variables, considering as factors the classes defined by the seven input data (NDVI, EC_a and BN, and their four possible combinations). ANOVAs were performed using IBM SPSS Statistics v. 21 (IBM SPSS Statistics Inc., Chicago, IL, USA), and MANOVAs with R (v. 3.1.0, the R Foundation for Statistical Computing).

Results and discussion

Significance of NDVI measurements in gobelet trained vines

NDVI was shown to be a good estimator of vegetative development in *goblelet* trained vines, since it was related to total shoot cross sectional area (SCSA) measured at the end of July in 2010, when apical growth was already stopped (SCSA = 122.9 + 1722.6 × NDVI, $R^2 = 0.58$, Fig. 1). Although there were no earlier references on the performance of NDVI as a vegetative development surrogate in *gobelet* trained vines, it was reasonable to consider its usefulness since, some of NDVI limitations in vertical shoot positioned (VSP) canopies such as narrow canopies that create problems with aerial and satellite sensing (Taylor et al. 2005) or saturation effect of red NDVI when the canopies are big (Stamatiadis et al. 2010) apply to a much lower extent in a free-trained system.

Within-vineyard variability for grape composition

The average values observed for quality parameters were relatively similar in the two seasons considered (Table 1), the main differences being observed for TSS (25.2 vs. 24.0 °Brix) and MalA (2.80 vs. 1.87 g L^{-1}), as similarly reported by Baluja et al. (2013) for the same seasons for cv. Tempranillo, in a nearby area. The reason behind these differences may be that during the last part of the season in 2011, moderate to severe water deficit occurred across the vineyard, which resulted in a considerable basal shoot defoliation that probably implied a reduction coupled to a decrease in photosynthetic activity (Tomás et al. 2012) and an increase in malic acid degradation (De Souza et al. 2005).

As a whole, the range and variability observed for grape composition parameters was high (Table 1), particularly for TSS, YAN, and for all parameters related to phenolic maturity (TAnt, EAnt and TP), for which nearly two-fold differences were observed

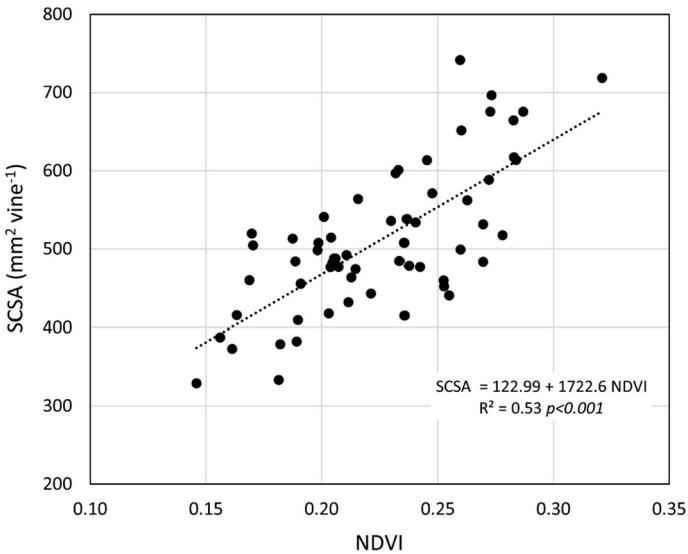

Fig. 1 Relationship between NDVI and the sum of shoot cross-sectional area (SCSA)

Table 1 Descriptive statistics for grape composition parameters in samples gathered at harvest in the whole field

	Mean		Range		CV (%)		Spread (%)	
	2010	2011	2010	2011	2010	2011	2010	2011
TSS (°Brix)	25.16	24.00	23.34–26.52	21.8–25.4	2.80	2.87	12.48	14.96
pH	3.50	3.55	3.38–3.61	3.41–3.69	1.49	1.67	6.58	7.88
TA (g AT L^{-1})	3.86	3.52	3.11–4.58	3.04–4.58	9.19	9.08	38.28	44.80
MalA (g L^{-1})	2.80	1.87	2.20–3.40	1.32–2.33	10.47	14.49	42.93	53.43
TarA (g L^{-1})	6.22	5.92	5.82–6.93	5.53–6.51	3.54	3.68	17.68	16.89
YAN (mg L^{-1})	118.26	104.18	80.02–195.15	73.36–153.85	15.74	17.42	97.64	76.74
TAnt (mg L^{-1})	896.48	930.20	646.25–1238.84	731.41–1137.48	12.99	9.79	66.09	43.68
EAnt (mg L^{-1})	347.77	343.84	251.31–437.26	261.52–444.33	12.33	12.71	53.54	53.39
TP (mg L^{-1})	1149.11	1144.31	949.20–1474.36	870.25–1400.45	9.01	9.08	45.73	46.31

CV coefficient of variation, *Spread* the range divided by the median, expressed as a percentage, *TSS* total soluble solids, *TA* titratable acidity, *MalA* malic acid concentration, *TarA* tartaric acid concentration, *YAN* yeast assimilable nitrogen, *TAnt* total anthocyanins, *EAnt* extractable anthocyanins, *TP* total phenolics

between the lowest and the highest values each season. On the contrary, a much lower range was observed for must acidity (TA, MalA, TarA), for which the difference between the highest and lowest values within the same year was ≈ 1 g L^{-1}. The range of variability of grape composition within this vineyard can be therefore considered large enough to justify grape segregation into batches with different quality and, as a consequence, a good target vineyard for PV implementation. The SP with higher anthocyanin and phenolics concentrations would be suitable for aged wines, those within the lower range would have been suitable only for generic wine. Similarly, grapes within the lower range of soluble solids content would have benefited from a later harvest to reach TSS >23.5 °Brix, the threshold for harvesting in the region. CV values were between 1/2 and 1/3 of the CV values observed during the same year in samples taken from a network of 74 cv.

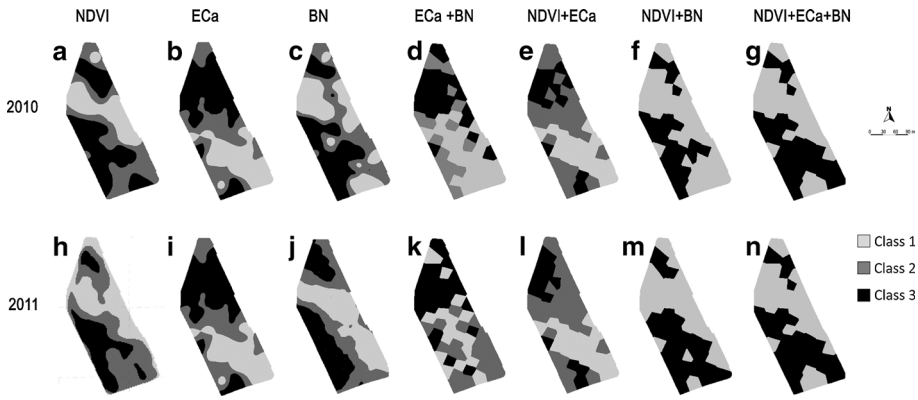

Fig. 2 Maps of zones delineated using NDVI, EC_a, BN and their in 2010 and 2011

Table 2 Evaluation of the agronomical significance the classes obtained through ANOVA from NDVI, EC_a, BN and their combinations

	NDVI		EC_a		BN		EC_a + BN		NDVI + EC_a		NDVI + BN		NDVI + EC_a + BN	
	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011
Yield (kg)	*	ns	ns	ns	*	ns	*	*	*	*	*	*	*	*
BW (g)	*	ns	ns	ns	*	ns	*	*	*	ns	*	*	*	*
TSS (°Brix)	ns	*	ns	ns	ns	ns	*	ns	ns	*	ns	*	*	*
pH	*	*	ns	ns	*	ns	*	ns	*	*	*	*	*	*
TA (g AT L^{-1})	*	ns	ns	ns	ns	ns	*	*	ns	*	*	ns	ns	*
MalA (g L^{-1})	ns	*	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns
TarA (g L^{-1})	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns
YAN (mg L^{-1})	ns	*	ns	ns	ns	*	ns	*	ns	ns	ns	*	ns	*
TAnt (mg L^{-1})	*	ns	ns	ns	ns	*	*	*	ns	ns	*	*	*	*
EAnt (mg L^{-1})	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	*	*	*
TP (mg L^{-1})	*	ns	ns	ns	ns	ns	ns	*	ns	ns	*	*	*	ns
Total Q	4	4	0	0	1	2	4	6	1	3	4	6	5	6
Total	6	4	0	0	3	2	6	8	3	4	6	8	7	8

The number of classes was always 3, except for NDVI + BN in 2011 and for NDVI + EC_a + BN in 2010 and 2011, for which it was 2

NDVI Normalized Difference Vegetation Index, *BN* bunch number, EC_a soil apparent electric conductivity, *BW* berry weight, *TSS* total soluble solids, *TA* titratable acidity, *MalA* malic acid concentration, *TarA* tartaric acid concentration, *YAN* yeast assimilable nitrogen, *TAnt* total anthocyanins, *EAnt* extractable anthocyanins, *TP* total phenolics, *Total Q* number of berry composition variables for which significant differences appeared, *Total* number of variables for which significant differences appeared

* Significant differences between classes ($P < 0.05$), *ns* non-significant ($P > 0.05$)

Tempranillo vineyards in the same area (data not shown), which gives an idea of the magnitude of the variability in grape composition that can be found within a single vineyard.

Significance of the zones defined

The zones delineated with the seven different input data, i.e. NDVI, EC_a, BN and their combinations (Fig. 2), showed a very different performance when the agronomic significance of the classes was evaluated through ANOVA analyses of yield and the grape composition parameters obtained at harvest. Results are summarized in Table 2, and given in detail as Supplementary Material in Table S1. The zones delineated when NDVI, EC_a and BN were considered individually performed poorly: NDVI was the single variable that allowed the differentiation between classes for more grape composition variables (4 in 2010, 4 in 2011), whereas the classes defined solely with EC_a discriminated for none of the grape composition parameters, and BN discriminated for only 1 parameter in 2010 and for 2 in 2012. Quite surprisingly, BN classification performed poorly even for yield and BW discrimination, only being able to discriminate classes with different yield and BW in 2010. BN plays only a moderate role in yield definition, which is more closely related to berry number (Dunn and Martin 1998).

However, the classifications obtained by fuzzy *k*-means analysis when combining two out of the three variable clustering variables considered (NDVI + BN, EC_a + BN and NDVI + EC_a) performed much better (Table 2). The classification that included the three variables (NDVI + EC_a + BN) was the best-performing one (discriminated for 5 and 6 grape composition parameters in 2010 and 2011 respectively), followed by NDVI + BN and EC_a + BN (4 and 6 in 2010 and 2011 respectively). These three combinations also allowed discriminating for the two quantitative crop parameters considered (yield and BW) both seasons. The poorest-performing of the three combinations was the one that did not consider BN (NDVI + EC_a), which only discriminated for 1 and 3 grape composition parameters in 2010 and 2011 respectively. Therefore, the inclusion of an easily measurable

Table 3 Evaluation of the significance of the classes obtained from NDVI, ECa, BN and their combinations to delineate zones with different grape composition using MANOVA

	df	*df* error	2010		2011	
			F	*P*	*F*	*P*
NDVI	18	98	2.343	0.004	3.410	<0.001
EC_a	18	98	1.305	0.202	0.832	0.659
BN	18	98	1.164	0.306	0.907	0.572
ECa + BN	18	98	2.988	0.006	2.440	0.003
NDVI + EC_a	18	98	2.032	0.014	1.020	0.445
NDVI + BN	9	50	2.622	0.014	4.989	<0.001
NDVI + EC_a + BN	18	98	2.200	0.038	2.784	0.010

The number of classes was always 3, except for NDVI + BN in 2011 and for NDVI + EC_a + BN in 2010 and 2011, for which it was 2

NDVI Normalized Difference Vegetation Index, *BN* bunch number, EC_a soil apparent electric conductivity, *df* degrees of Freedom, *F* Fisher's *F*-ratio, *P P* value

variable that estimates sink size improved significantly the zone delineation procedure. For all input data combinations the optimum number of classes was 2 or 3 according to FPI and MPE indices (results not shown).

When the discrimination ability for grape composition of the zones delineated was tested according to MANOVA (Table 3), the trend observed with ANOVA was confirmed. NDVI was the only variable that, on its own, discriminated them significantly. The combination of variables resulted in general in an improvement, and particularly the inclusion of BN in the fuzzy *k*-means analysis increased the significance of the differences observed.

As a whole, the results presented clearly demonstrate the importance of using within-field zone delineation procedures that combine variables that are representative for the main factors that condition grape composition This probably explains why zoning performed using a vegetation index as the only information source in previous studies (Santesteban et al. 2010; Acevedo-Opazo et al. 2008a, 2008b, 2010; Hall et al. 2003; Bramley 2005) resulted in only a moderate correspondence with grape composition. According to wine managers in the area, the differences in grape composition between the zones delineated with the best performing classifications would justify up to 2.5-fold differences in grape price (from 0.8 to 2 €/kg), which clearly could lead to segregated harvest.

Grape ripening is a very complex process, driven by a wide set of factors that directly or indirectly affect it (Champagnol 1984; Jackson and Lombard 1993). Not taking into account any of the most relevant ones (such as the amount of fruit) hinders a good evaluation of grape quality potential. Vegetative development is certainly a relevant parameter for grape quality, since it is related to light interception and also to competition between vegetative and reproductive sinks during ripening (Smart and Robinson 1991) but, according to the results presented, it has been proved not to be—on its own—efficient enough to discriminate zones of different berry composition. Water availability is also one of the factors conditioning differences in grape quality; particularly in areas where summer conditions are dry (Santesteban and Royo 2006). The inclusion of EC_a is probably integrating several soil characteristics directly related to soil water holding capacity due to differences in soil texture and depth (Brevik et al. 2006; Friedman 2005), and has been proved to be useful for classification under our study conditions.

Lastly, the relevance of considering a parameter that estimated sink size has been particularly highlighted by the results obtained, since the performance of the classification method using BN performed better than those not considering it. The balance between fruit level and vegetative development is known to be very decisive for grape quality (Howell 2001), and therefore needs to be included for successful zoning. Although pruning, when skilfully performed, does somehow balance source to sink ratios (more fruiting buds are maintained in the more vigorous parts of the vineyard), differences in this balance still occur from year to year and within-vineyard for the same year, which justifies the inclusion of BN for zone delineation. All this supports the hypothesis set up in Santesteban et al. (2013), who considered sink size to be an essential aspect to be considered for classifications. Our results suggest that, although nowadays it is not possible to obtain a fruit-load related parameter remotely, it is worthwhile including it in any PV work aimed at grape quality estimation, since it is not a very time consuming measurement when it has to be done in field. These results support the necessity of developing procedures to estimate fruit load automatically, and highlights the importance of relevant works conducted in order to determine the number and weight of the clusters through artificial vision (Diago et al. 2012; Nuske et al. 2011; Fernandez et al. 2013; Grocholsky et al. 2011).

Conclusion

The early definition of within vineyard zones combining NDVI, EC_a and BN data was successful, since the zones delineated allowed a differentiation of grape batches with different characteristics at harvest. Interestingly, the inclusion of a variable related to sink size (in this case the number of bunches per plant) provided the most efficient classification, which makes its consideration highly advisable for any PV work aimed at zone delineation for grape quality estimation.

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