Assessment of Vineyard Canopy Porosity Using Machine Vision

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Abstract: Canopy porosity is an important viticultural factor because canopy gaps favor fruit exposure and air circulation, both of which benefit fruit quality and health. Point quadrat analysis (PQA) is standard for assessing canopy gaps but has limited utility because the method is laborious and time consuming. A new, objective, noninvasive, image-based method was developed and compared with PQA to assess the percent canopy gaps in vineyards with diverse viticultural conditions and grape varieties in New Zealand, Croatia, and Spain. The determination coefficient (R^2) of the regressions between the percent gaps using both methods exceeded 0.90 ($p < 0.05$) at each site, and R^2 of the global regression was 0.93 ($p < 0.05$). The time of day and side of the canopy photographed did not significantly affect the performance of the algorithm. With this new image-based assessment method, canopy management may be optimized to configure a desired amount of canopy gaps and thereby improve fruit quality and health.

Key words: canopy management, fruit exposure, image analysis, noninvasive sensing, point quadrat analysis, *Vitis vinifera* L

Canopy features such as leaf area, number of leaf layers, canopy porosity, and fruit exposure are important viticulture factors that can be regulated by canopy management. In terms of canopy porosity, the ideal grapevine canopy has 10 to 20% (Palliotti and Silvestroni 2004) or 20 to 40% gaps (Smart 1987) to ensure adequate sunlight capture and reduce shading. Canopy gaps are important for the fruit because airflow reduces the chance of crop loss to fungal disease (English et al. 1990, Austin et al. 2011), and exposure of the fruit to sun induces synthesis of aroma and flavor compounds (Reynolds and Wardle 1989, Diago et al. 2010) as well as anthocyanin pigments and other phenols (Bubola et al. 2012, Diago et al. 2012a, Tardaguila et al. 2012). However, excessive fruit exposure, especially in warm growing regions, can lead to sunburn and a reduction in grape color quality (Kliewer 1977, Mori et al. 2007). Optimizing both canopy porosity and fruit exposure is a challenge to viticulturists worldwide, and climates with diverse rainfall and temperature patterns require different canopy management strategies to maximize quality.

One of the most common ways to quantify canopy porosity and density is point quadrat analysis (PQA), which was adapted from the protocol described by Wilson (1959) for application in grapevines (Smart 1987). PQA involves inserting a probe through the grapevine canopy and counting the number and parts of the vine the probe comes in contact with, including the leaves, clusters, canes, and gaps. The proportion of gaps is quantified by dividing the number of gaps by the total number of insertions. A minimum of 50 passes through the canopy is recommended to accurately quantify the gaps (Smart 1987). In addition to its subjective nature, PQA is labor- and time-intensive and can potentially damage the fruit, which limits its usefulness in the wine industry. Carrying out 10 insertions per vine in 20 vines may take ~1.5 hr (Hill et al. 2011), which limits the number of vines that can be measured in a given time frame. Recently, new PQA metrics have been developed that integrate the spatial information relative to leaf and cluster position in the canopy, which is collected from PQA data sets and simplified whole-canopy photosynthetic photon flux (PPF) measurements (Meyers and Vanden Heuvel 2008). This new calibrated biomass and photon flux distribution model provides a reliable quantitative description of canopy biomass distribution and light environment and how the canopies respond to a thinning treatment.

Machine vision is a noninvasive technology based on image analysis. In viticulture, several works have focused on using image analysis to assess cluster and berry features under laboratory conditions (Diago et al. 2015, Cubero et al. 2014, 2015). The use of computer vision based on still photography with visible cameras outdoors to characterize the grapevine canopy, estimate factors affecting production, or assess health of the berries is rarely explored mainly due to the difficulties associated with uncontrolled illumination in the field. Some researchers have used methods based on the analysis of digital red, green, and blue (RGB) images for the quantification of various parameters such as the number of flowers per inflorescence (Diago et al. 2014); yield (Dunn and Martin 2004, Diago et al. 2012b, Nuske et al. 2014); the

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exposed leaf area, fruit, cane, and gaps (Tardaguila et al. 2010); and foliage density (Hill et al. 2011). Hill et al. (2011) showed a negative correlation between leaf layer number (determined by PQA) and percent gaps (determined by image analysis). However, a direct comparison between PQA and image analysis in terms of measuring the percent gaps of a vine has not been published. Therefore, a method that accurately assesses canopy porosity and correlates strongly with the standard PQA method is warranted. Development of such a method may help viticulturists refine canopy manipulations and configure the goal quantity of canopy gaps, with the overall aim of increasing fruit quality and health.

The goal of this study was to develop a new, objective, noninvasive automated system to assess the percent gaps of grapevine canopies using image analysis in the vineyard. We tested this image-based method in vineyards under diverse viticultural conditions (e.g., grape varieties, climates, and cultural practices) in New Zealand, Croatia, and Spain, and investigated the effects of the time of day and canopy side photographed.

Materials and Methods

Image acquisition and PQA. An adapted protocol for in-field image acquisition was developed based on the procedure described by Tardaguila et al. (2010) and used at all sites of the three countries. At each site, *Vitis vinifera* L. grapevines of several cultivars were photographed under similar natural light conditions (within ~1 hr) using a standard digital reflex camera with resolution greater than 7-megapixels and equipped with a flash light (to avoid or minimize the presence of shadows in the canopy images). The camera was mounted on a tripod set normal to the canopy, 2.0 m away from the row axis and 1.0 m above the ground, and placed on the vertex of a “simulated” triangle. To avoid photographing the canopies of adjacent vines in rows behind the vine of interest (and thus interfering with the desired image), a white cloth was placed behind the canopy of the vine to be photographed. The camera was configured in auto mode, which compensated for changes in luminosity and white balance. Delimitation of the canopy area to be measured by PQA (Smart and Robinson 1991) and image analysis, called the region of interest (ROI), was performed using two methods that were later compared. The first mode was used in New Zealand and involved use of a wooden frame that hung from the top of the foliage wire with the bottom of the frame even with the fruiting canes (Figure 1A, 1B). The second ROI delineation method (used at the Spain and Croatia sites) was simpler and involved placing two wooden or plastic sticks (Figure 1C, 1D) or segments of colored plastic tape (Figure 1E) at both left and right sides of the cordons of each grapevine canopy to delimitate the width of the ROI. The height was confined to the first 40 cm above the cordons.

New Zealand sites. The Merlot site was located in the Bridge Pa Triangle region (lat. 39°38′20.6″S; long. 176°42′44.7″E). Merlot vines were clone 181 on 101-14 rootstock. Vines were planted in 2001, cane-pruned to two canes with 10 to 12 buds per cane, and trained on a vertical shoot positioning (VSP) trellis with 2.5 m row spacing and 2.0 m vine spacing. The Syrah site was located in the Te Awanga region of Hawke’s Bay (lat. 39°37′27.9″S; long. 176°57′10.3″E). The vines were mass select (MS) clone on Riparia Gloire rootstocks. Vines were planted in 2008, cane pruned to two canes with 10 to 12 buds per cane, and trained on a VSP trellis with 2.5 m row spacing and 1.8 m vine spacing. Every two weeks, digital photographs were taken with a Sony Cyber-shot 7.2-megapixel camera with a Carl Zeiss Vario-tessar lens. Twelve vines per treatment were photographed at each site. Image acquisition started before bloom and continued until veraison when the bird nets went up. A wooden frame (1.2 m × 0.7 m) was used to delimitate the ROI (Figure 1A). PQA was performed at all of the sites on the same day the photographs were taken. A stainless steel probe 5 mm in diameter was used for point quadrat (PQ) insertions. The PQA for percent canopy gaps consisted of 11 passes through the canopy at 10-cm horizontal intervals at heights of 10, 30, 50, and 70 cm above the cane (44 passes per vine) for each vine. All measurements were made during the 2012-2013 growing season.

Croatian sites. The experiment in Croatia was carried out in two Merlot vineyards, one Cabernet Sauvignon vineyard, and one Sauvignon blanc vineyard. The first Merlot site was located in Poreč (lat. 45°13′20.51″N; long. 13°36′00.52″E; 11 m asl, Istra, Croatia). Merlot vines (clone ISV-F-V6) were grafted onto SO4 rootstock, dry-farmed, spur-pruned on a bilateral cordon to retain eight spurs and two buds per spur, and trained onto a VSP trellis system with 2.5 m row spacing and 0.8 m vine spacing. Grapevines were planted in NNE-SSW orientation in 2006. The second Merlot site was located in Dajla (lat. 45°20′47.00″N; long. 13°33′13.61″E; 23 m asl, Istra, Croatia). Merlot vines (clone R12) were grafted onto 1103P rootstock, dry-farmed, Guyot-pruned to single cane with 10 buds and one replacement two-bud spur, and trained onto a VSP trellis system with 2.4 m row spacing and 0.9 m vine spacing. Grapevines were planted in NW-SE orientation in 2004. The Cabernet Sauvignon site was located in Dajla (lat. 45°20′48.48″N; long. 13°33′12.09″E; 20 m asl, Istra, Croatia). Cabernet Sauvignon vines (clone R5) were grafted onto 1103P rootstock, dry-farmed, Guyot-pruned to a single cane with 10 buds and one replacement two-bud spur, and trained onto a VSP trellis system with 2.4 m row spacing and 0.9 m vine spacing. Grapevines were planted in NW-SE orientation in 2004. The Sauvignon blanc site was located in Funtana (lat. 45°10′01.55″N; long. 13°38′06.98″E; 32 m asl, Istra, Croatia). Sauvignon blanc vines (clone R3) were grafted onto 110R rootstock, dry-farmed, Guyot-pruned to a single cane with 10 buds and one replacement two-bud spur, and trained onto a VSP trellis system with 2.6 m row spacing and 0.9 m vine spacing. Grapevines were planted in N-S orientation in 2005. The first Merlot site located in Poreč was measured in both the 2012 and 2013 seasons, whereas the rest of the sites were measured in the 2013 season only. Photos of the canopy were taken with an Olympus FE-47 digital compact camera one week before harvest. Two red plastic rods of dimensions 370 × 32 mm were positioned on both sides of the vine 80 cm apart at the first Merlot site located in Poreč and 90 cm apart at the other sites to measure the width of ROI (Figure 1C). The area between these plastic rods and from the basal wire to 40 cm above was taken for image analysis and PQA. PQA was performed on the same day and on the same vines as those used for image analysis for accurate comparison between the methods. A sample for image acquisition and PQA consisted of six vines at the first Merlot site and five vines at all other sites and cultivars. Nine samples were taken per year for each site and cultivar, resulting in a total of 45 samples for image analysis and PQA in Croatia. A stainless steel probe 3 mm in diameter and 1 m in length was used for PQ insertions. PQA consisted of 100 rod insertions per sample of five vines in all plots carried out randomly to cover the entire fruiting zone (from the basal wire where cordons or canes were positioned to 40 cm above the cane or cordon), which was the same zone used for image analysis.

Spanish site. The experiments were conducted in 2010 in a commercial dry-farmed cv. Tempranillo vineyard located in Cidamón (lat. 42°29′8.83″N; long. 2°50′22.57″W; 181 m asl, La Rioja, Spain). Tempranillo vines (Clone 43) were grafted onto 41B rootstock, spur-pruned to 10 to 12 buds on a bilateral cordon, and trained onto a VSP trellis system with 2.7 m row spacing and 1.15 m vine spacing. Grapevines were planted NW–SE orientation in 2004.

To assess the correlation between digital image analysis and PQA, a two-step defoliation and thinning experiment was performed. Photos of the canopy were taken with a Canon EOS-1D digital still camera one week before harvest. Two 50-cm pieces of plastic labeling tape were positioned on both sides of each vine 115 cm apart (from the end of one cordon to the end of the other cordon) to delimitate the width of the ROI (Figure 1E). The area between these pieces of plastic tape and 40 cm above the basal wire was used for image analysis and PQA. Each vine was photographed prior to any defoliation or thinning intervention. In the first step, the first six main basal leaves and every second cluster of each shoot were removed. In the second step, another six leaves and the basal cluster of each shoot were removed (total of 12 leaves removed). PQA was conducted after each step of image acquisition. A wood probe 5 mm in diameter was used for PQ insertions. The PQA for percent canopy gaps consisted of

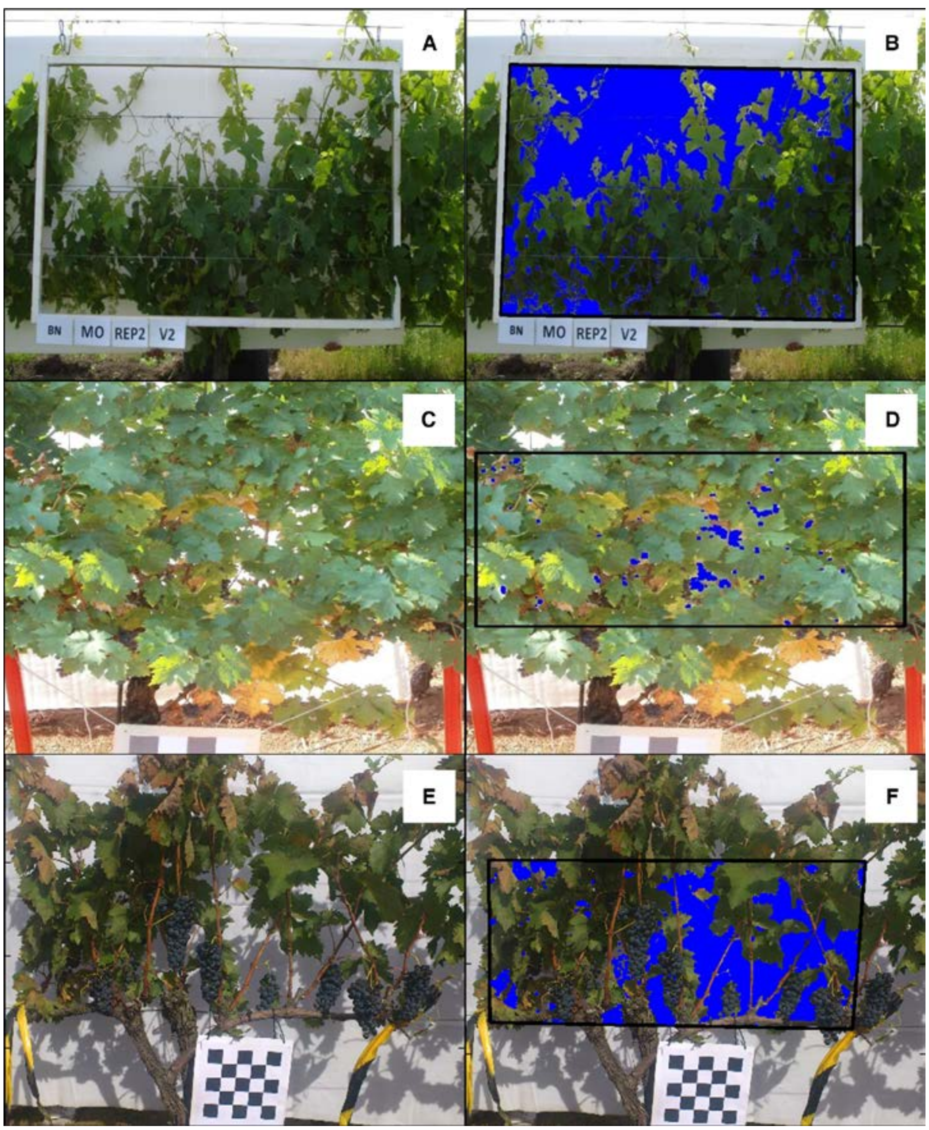

Figure 1 Examples of red, green, and blue images and their detected gaps (in blue shading) using the developed algorithms within selected regions of interest, respectively, in (**A**, **B**) Merlot in New Zealand; (**C**, **D**) Merlot in Croatia; and (**E**, **F**) Tempranillo in Spain.

three rod insertions through the canopy at heights of 20, 30, and 40 cm above the cane (nine passes per vine) in a total of eight grapevines.

Image processing. The image analysis application was developed in MATLAB (MATLAB R2010b, The Mathworks) with the goal of automating the process (main steps are described in Figure 2). A clustering algorithm based on the Mahalanobis distance was used to identify the pixels corresponding to the canopy gaps or porosity as described in Diago et al. (2012b). The Mahalanobis distance measures the similarity between an unknown sample group and a known sample group, and it accounts for correlations of the data set with different variances for each direction and is scale-invariant. These properties make this clusterization algorithm ideal for image segmentation under uncontrolled conditions, especially when segment illumination varies among images. The modified algorithm used a known sample of values to classify an unknown batch of pixels into groups or classes based on a characteristic vector (the RGB color values of each pixel). The first step involved a supervised selection of a representative number of points to be used as reference (also called seed) for each group of classification. Seeds of 20 pixels corresponding to each group or class (green leaf, background, trunk, grape cluster, and canopy gap) were manually extracted from images for each trial.

Prior to image analysis, ROI delineation was required to analyze the canopy gaps in the same area that the PQA was carried out. At the New Zealand site, the ROI was delimited by the wooden frame whereas at the Croatia and Spain sites, the ROI was a rectangle whose width was demarcated by the plastic rods or tapes and height was 40 cm above the basal wire. The percentage of gaps was calculated as the number of pixels tagged as gaps by the algorithm divided by the total number of pixels in the ROI.

Influence of the side of the canopy and time of day on image acquisition and processing. To assess the influence of the canopy side that was photographed and the time of day at which images were taken (both of which contribute to different shadowing effects), a separate trial was conducted on 20 Aug 2015 in Croatia. The experiment was performed on *V. vinifera* L. cv. Cabernet Sauvignon vines, clone ISV-FV5, grafted on SO4 rootstock (*V. berlandieri* × *V. riparia*), in the experimental vineyard of the Institute of Agriculture and Tourism in Poreč, Croatia (lat. 45°13′19.70″N; long. 13°36′01.39″E). Vines were planted in a NNE-SSW orientation with a declination of 26° from N-S, in 2006 and spur-pruned on a bilateral cordon to retain eight spurs and two buds per spur, and trained onto a VSP trellis system with 2.5 m row spacing and 0.8 m vine spacing. Images were taken on 30 vines positioned in one vineyard row at three different

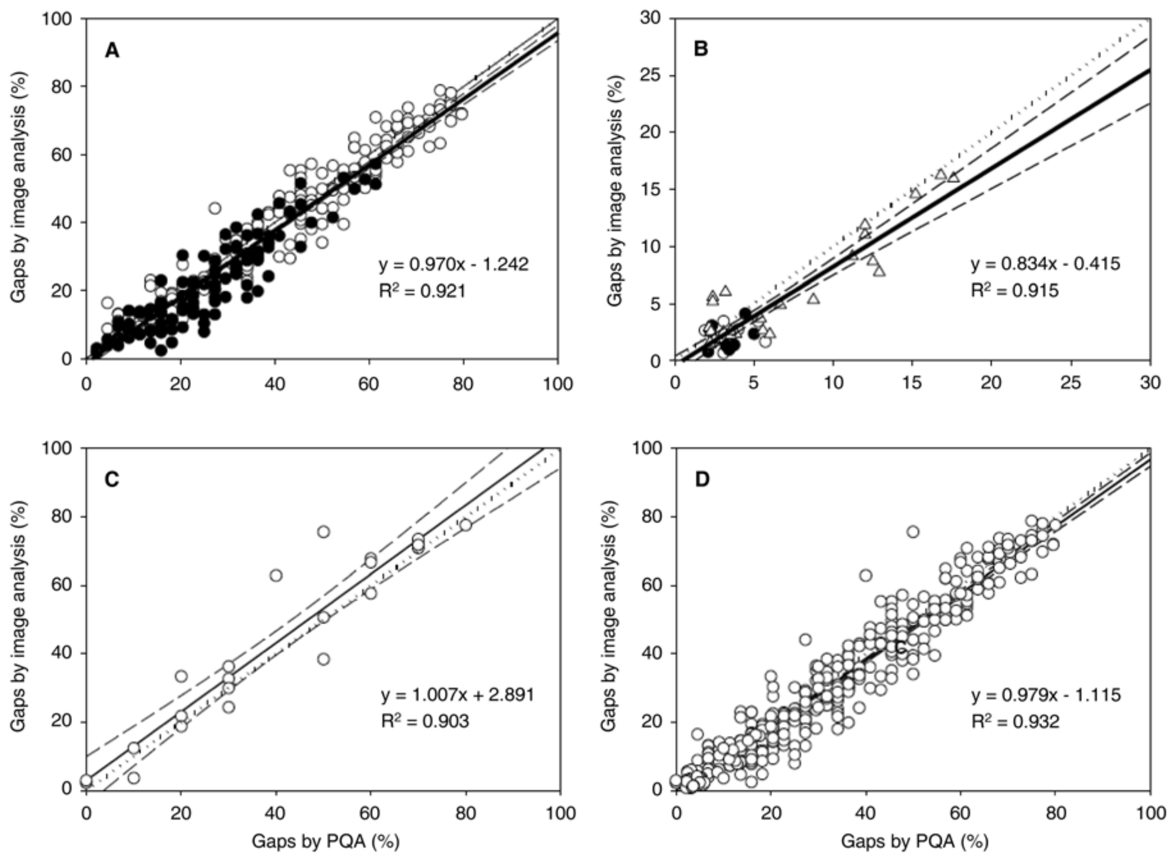

Figure 2 Regressions of percent canopy gaps measured by image analysis compared to canopy gaps measured by point quadrat analysis (PQA; solid line) for (**A**) Merlot (open circles) and Syrah (black circles) in New Zealand in 2012 and 2013 (n = 288); (**B**) Sauvignon blanc (open circles), Cabernet Sauvignon (black circles), and Merlot (open triangles) in 2012 and 2013 in Croatia (n = 45); and (**C**) Tempranillo in Spain (n = 24) in 2010; (**D**) all cultivars and sites are pooled (n = 357), with a 1:1 reference line (dotted line), and a 95% confidence interval (dashed line). Slope coefficients were significant for $\alpha = 0.05$.

times during the day from both sides of the vineyard row. Prior to image acquisition, vines were manually defoliated to create three different canopy porosity levels around the fruiting zone. Likewise, 10 vines were not defoliated to yield a small proportion of gaps (4% gaps), 10 vines were defoliated to create a medium number of gaps (17% gaps), and 10 vines were defoliated more extensively to increase the canopy porosity around the clusters by generating a larger number of gaps (33% gaps). The first set of images was taken in the morning (1100 hr), when the sun was positioned to the east side of the vineyard rows. The second set of images was taken at midday (1400 hr), when the sun was positioned over the vines, and the third set of images was taken in the afternoon (1700 hr), when the sun was positioned to the west side of the vineyard rows. At each time, images were acquired from both sides of the canopy, east and west of vine rows. Vines were photographed with the digital single-lens reflex camera Nikon D5100 using the lens Nikon AF-S DX NIKKOR 18-55mm f/3.5-5.6G VR with the flashlight. A white screen was placed behind the vines before taking the images. Two red plastic rods (370 × 32 mm) were positioned on both sides of the vine and 80 cm apart to delimit the width of the ROI. A checkered reference with black and white squares 50 × 50 mm in size was used as a dimension reference and positioned on the vine trunk under the fruit zone. The area comprised between these plastic rods and 40 cm above the cordon was the ROI used for image analysis and PQA. PQA was performed on the same day as the vines were photographed. A stainless steel probe 3 mm in diameter was used for PQ insertions, and a plasticized steel net with holes of 10 × 7 cm was used to guide the insertions. The PQA for percent canopy gaps consisted of 40 passes per vine divided in four horizontal intervals at heights of 5, 15, 25, and 35 cm above the cordon. Ten insertions were made 7 cm apart at each height. Images were analyzed using the developed algorithm, and the percentage of canopy gaps were computed.

Statistical analysis. Correlations between the number of gaps in the canopy assessed by PQA and by image analysis were calculated using the determination coefficient (R^2). Statistical *t*-tests were used to assess whether the value of the slope coefficient for each regression was equal to 1, and the 95% confidence intervals of the slope coefficients were calculated. All calculations and plots were performed using Sigma Plot 12.0 (Systat Software Inc.). To test the influence of the time of day and the side of the canopy on the percent gaps (canopy porosity) measured by image analysis, a two-way ANOVA using daytime and canopy side as factors with interactions was performed using Infostat/L software.

Results

Figure 1 shows sample RGB images of the canopy gaps detected using the image-based algorithm in the selected ROIs in New Zealand (Figure 1A, 1B), Croatia (Figure 1C, 1D), and Spain (Figure 1E, 1F). Differences in canopy density were evident among sites, from very dense (Figure 1C, 1D) to very porous (Figure 1E, 1F). Despite the general recommendation of using flash and diffuse ambient light to avoid the appearance of shadows in the images, some shadows did occur (Figure 1E, 1F), but the developed algorithm was able to overcome their effect.

Canopy porosity by image analysis versus PQA: Regression models. The differences in percent gaps in the different imaged canopies of the three sites were confirmed by the plots in Figure 2. A vast range of canopy porosity was sampled in New Zealand (Figure 2A), as quantified percent gaps spanned from almost 0% (extremely dense canopy) to 80% gaps (extremely porous canopy). In Croatia (Figure 2B), the most porous canopy barely reached the 15% gaps. In Spain (Figure 2C), percent gaps ranged from 0 to 80% gaps, although the wide range was due to the assessment of the same grapevine canopies after two consecutive defoliation/thinning steps, which led to an increase in canopy porosity. At this site, the increased canopy porosity was quantified after each defoliation step. Image analysis and PQA showed that percent gaps increased to an average of 17% and 46% after the first and second defoliation/thinning steps, respectively (n = 8).

A strong correlation between percent canopy gaps measured by image analysis and those measured by PQA was observed regardless of vineyard location, grapevine variety, or type of ROI delimitation (Figure 2). An even higher determination coefficient (R^2) was obtained when all sites and varieties were pooled together (Figure 2D). In addition to the global regressions computed for all three locations, individual regressions between the percent canopy gaps measured by image analysis versus PQA were also calculated for each individual cultivar in New Zealand (Figure 3) and in Croatia (Figure 4). In New Zealand, significant relationships (intercept and slope coefficients statistically significant at $p < 0.001$) were observed with Merlot ($R^2 = 0.93$, Figure 3A), and Syrah ($R^2 = 0.83$, Figure 3B), whereas in Croatia, only the regression for Merlot was statistically significant (Figure 4).

Table 1 shows the 95% confidence intervals for the slope and intercept coefficients for all statistically significant regressions, as well as the *t*-test and *p*-values of the statistical tests to verify whether the slope coefficients were different from 1 and intercept coefficients were different from 0. The slope coefficient was not statistically different from 1 ($p > 0.05$) for the Tempranillo in Spain, the global regressions within each country, and the global regression for all cultivars and sites. These results were confirmed by the confidence intervals, as the range delimited by the lower and upper confidence intervals only included the value 1 for those regressions with slope coefficient statistically equal to 1. The intercept values equaled 0 for all except the global regression, for which the 95% confidence interval of the intercept did not include 0 and the corresponding *t*-test had a $p < 0.05$ (Table 1).

Influence of the time of the day and side of canopy imaged. The inconsistent presence of shadows in the images caused by the time of day and the side of the canopy may influence the accuracy of the model at each site. Figure 5 shows the images of a given Cabernet Sauvignon grapevine from the east side of the canopy in the morning (Figure 5A), at midday (Figure 5B), and in the afternoon (Figure 5C), and from the west side of the canopy in the morning (Figure 5D), at midday

(Figure 5E), and in the afternoon (Figure 5F). Larger presence of shadows were observed when images were taken on the east side in the morning (Figure 5A) and on the west side in the afternoon (Figure 5F), whereas almost no shadows in the fruiting zone can be detected for images taken under all other conditions. To test the effect and interaction of the time of day and side of the canopy, a two-way ANOVA with time × side interaction was calculated for the percent gaps measured by image analysis of the same set of 30 Cabernet Sauvignon vines photographed from the east and west sides of the canopy in the morning, midday, and afternoon. The time of day (p = 0.120), the side of the canopy photographed (p = 0.618), and their interaction were not significant (p = 0.778).

For the experiment involving image acquisition of the same grapevines at different times of day and on each sides of the canopy, statistically significant ($p < 0.001$) regressions of the percent gaps computed by image analysis versus PQA were calculated and are shown in Figure 6. The best fit was achieved for images taken at midday (Figure 6C, 6D) and afternoon (Figure 6E, 6F), with R^2 higher than 0.90, regardless of whether the east or west side of the canopy was photographed. *T*-tests were performed to assess whether the slope coefficients of the regression models was affected by image acquisition conditions and to determine whether their values were different from 1. Table 2 shows the 95% confidence intervals for the slope and intercept coefficients of all statistically

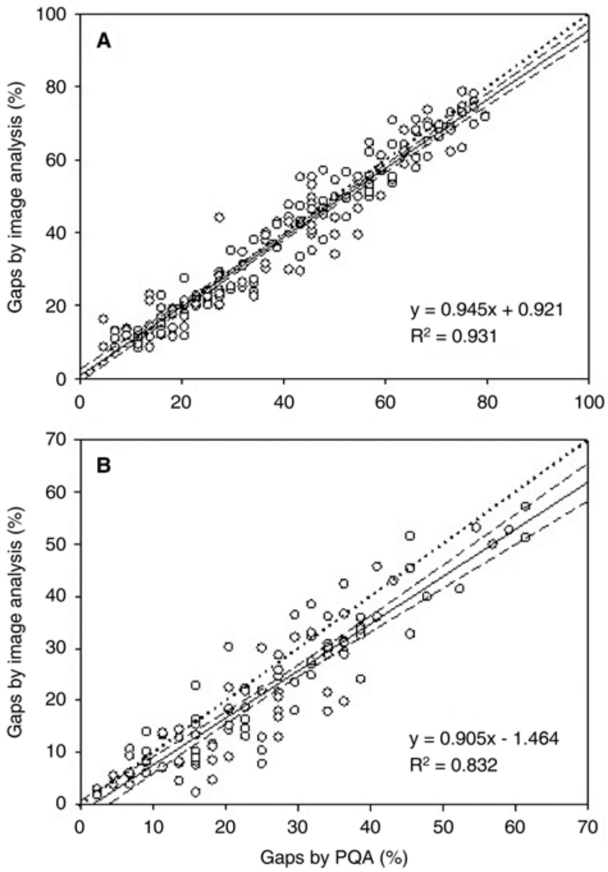

Figure 3 Regressions of percent canopy gaps measured by image analysis compared to canopy gaps measured by point quadrat analysis (PQA; solid line) for (**A**) Merlot (n = 180) and (**B**) Syrah (n = 108) in New Zealand in 2012 and 2013, with a 1:1 reference line (dotted line), and a 95% confidence interval (dashed line). Slope coefficients were significant for $\alpha = 0.001$.

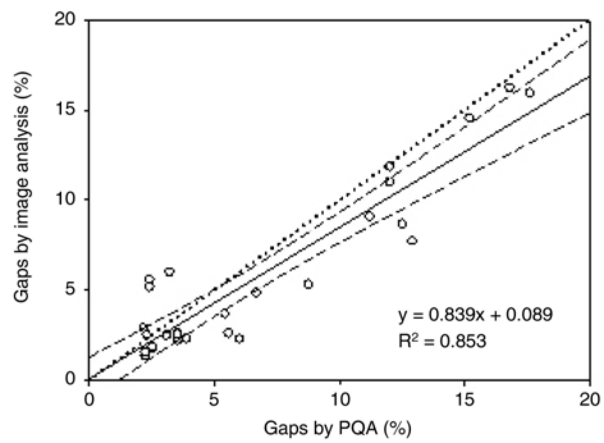

Figure 4 Regressions of percent canopy gaps measured by image analysis compared to canopy gaps measured by point quadrat analysis (PQA; solid line) for Merlot (n = 27) in Croatia in 2012 and 2013, with a 1:1 reference line (dotted line) and a 95% confidence interval (dashed line). Slope coefficient was significant for $\alpha = 0.001$.

Table 1 Slope coefficients of the regression models of the canopy percent gaps estimated by image analysis versus point quadrat analysis (PQA) for different cultivars and sites. The 95% confidence intervals for the slope coefficients are indicated in brackets. The *t*-test values and their corresponding *p* values for the null hypothesis of H_0: slope = 1 and H_0: intercept = 0 are presented.

Site/cultivar	N	Slope	*t*-test value[a]	*p* value	Intercept	*t*-test value[a]	*p* value
New Zealand							
Merlot	180	0.945 (0.907; 0.984)	2.85	0.004	0.921 (-0.826; 2.669)	1.04	0.299
Syrah	108	0.905 (0.826; 0.983)	2.44	0.016	-1.464 (-3.731; 0.803)	-1.28	0.203
All	288	0.970 (0.937; 1.003)	1.76	0.079	-1.243 (-2.581; 0.095)	-1.83	0.068
Croatia							
Merlot	27	0.839 (0.939; 0.983)	2.30	0.030	0.089 (-1.111; 1.289)	0.15	0.880
All	45	0.864 (0.746; 0.981)	0.18	0.856	-0.415 (-1.220; 0.390)	-1.04	0.304
Spain							
Tempranillo	24	1.007 (0.861; 1.153)	0.14	0.888	2.890 (-4.143; 9.926)	0.85	0.403
All sites							
All	357	0.979 (0.952; 1.007)	1.50	0.134	-1.115 (-2.176; -0.054)	-2.07	0.039

[a] *t*-tests for the null hypothesis H_0: slope =1 and H_0: intercept = 0 were only tested for those regressions with statistically significant slope coefficients ($p < 0.05$). All cultivars in Croatia also included measurements on Cabernet Sauvignon and Sauvignon blanc.

significant regressions, and the *t*-test and *p*-values to determine if the slope coefficients were statistically different from 1 and the intercept coefficients were statistically different from 0. The slope coefficient of the regression for the images taken on the west side of the canopy in the afternoon was significantly different (p = 0.0024) from the other five slope coefficients and was the only condition in which the slope was not significantly different from 1 (p = 0.293) (Table 2). The intercept coefficients were statistically different from 0 in all conditions.

Discussion

Canopy porosity by image analysis versus PQA: Regression models. A new, objective, noninvasive, automated method was developed using RGB images of grapevine canopies in the vineyard to analyze canopy porosity. The method was remarkably effective in vineyards of three different wine producing countries where grapevines of various cultivars were grown under distinct viticultural conditions (e.g., soil, climate, plant density, row orientation).

Regression analyses showed that assessment of canopy porosity by image analysis with the developed algorithm and by PQA yielded very similar results. For both New Zealand and Croatian trials in which models were built individually for each cultivar, PQA tended to overestimate the percent gaps as the slope coefficients of the regressions were significantly lower than 1. However, the slope coefficients were not statistically different from 1 for the Spanish site with Tempranillo, the global models for each country, and the global model for all sites.

With PQA, the minimum gap size is limited by the probe diameter and the working precision and experience of the operator, whereas with image analysis, the minimum gap size is fixed by the image resolution or pixel size. Moreover, using PQA to assess canopy porosity, which involves a limited number of insertions, means that each insertion (of size corresponding to the diameter of the probe) represents the porosity of a canopy surface that may be several-fold greater than the probe's diameter. This effect, which gets diminished as the number of insertions increases, may lead to enhanced uncertainty in the percent gap values measured by PQA. On the other hand, in canopies with high density (percent of gaps less than 6%) and small range in percent gaps (which occurred with the Cabernet Sauvignon and Sauvignon blanc in Croatia in the 2012-2013 season), the regression model of the percent gaps determined by image analysis versus PQA was inaccurate and no correlation could be established between the methods.

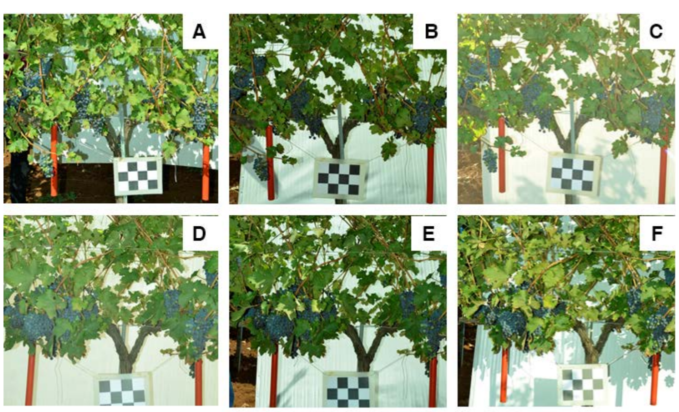

Figure 5 Red, green, and blue (RGB) images of a given Cabernet Sauvignon grapevine taken in Croatia (September 2015) from the east side of the canopy (**A**) in the morning, (**B**) at midday, and (**C**) in the afternoon, and from the west side of the canopy (**D**) in the morning, (**E**) at midday, and (**F**) in the afternoon. Morning time: 1100 hr; midday: 1400 hr; afternoon: 1700 hr.

Regardless of whether the slope coefficient differed from 1 or the intercept values differed from 0, the significant regression models developed for different cultivars, sites, and image acquisition conditions confirmed that the developed image analysis algorithm could accurately assess the canopy porosity in VSP-trained grapevines.

In addition to the canopy porosity, PQA provides additional information about the density of the vegetation in the canopy, including leaf layer number, cluster exposure, percent interior clusters, leaf exposure, and percent interior leaves. Some of this canopy information can also be assessed using image analysis, as Tardaguila et al. (2010) measured cluster exposure and yield in a defoliation trial. The algorithm developed for this study was adapted from the procedure described by Tardaguila et al. (2010) and distinguishes between leaves and clusters. However, the present work focused on the assessment of the canopy gaps in VSP-trained grapevines, as this training system is used worldwide. For this type of trellising, the vertical training of the shoots between the catch wires leads to a tightly packed configuration of vegetation (width of VSP canopies usually ranges from 30 to 40 cm). Under these conditions, the canopy gaps are allocated so that they are assumed to be in a 2D distribution. This 2D approach underlying the image analysis method mimics the human visual assessment but provides an objective figure that corresponds to the number of pixels matching the canopy gaps. Although visual estimations are rapid, they are subjective and prone to observer bias (Wilson et al. 2007). Additionally, visual estimates may yield confounding results from different observers and cannot provide robust, reliable, and consistent information. Appropriate and regular training is required to mitigate the subjectivity of visual estimation (Balehegn and Berhe 2015). The developed image analysis method is more conclusive when applied to VSP systems and less responsive to non-vertical trellising. Applying the image analysis method to open canopies requires consideration of additional factors, such as the internal distribution of leaves and clusters as well as additional images taken from above or at the bottom of the canopy. The integration of information on PPF was used by Meyers and Vanden Heuvel (2008) to enhance the precision and spatial acuity of PQA in open canopies. Other alternatives involve the use of LiDAR to better characterize the depth of the canopy gaps in open trellis systems, as this type of terrestrial laser scanner operates based on the time-of-flight principle to estimate the distance to the canopy (Arnó et al. 2015). Conversely, several authors (Fuentes et al. 2012, De Bei et al. 2015) have estimated the leaf area index of grapevine canopies by imaging from the bottom of the plant. Hill

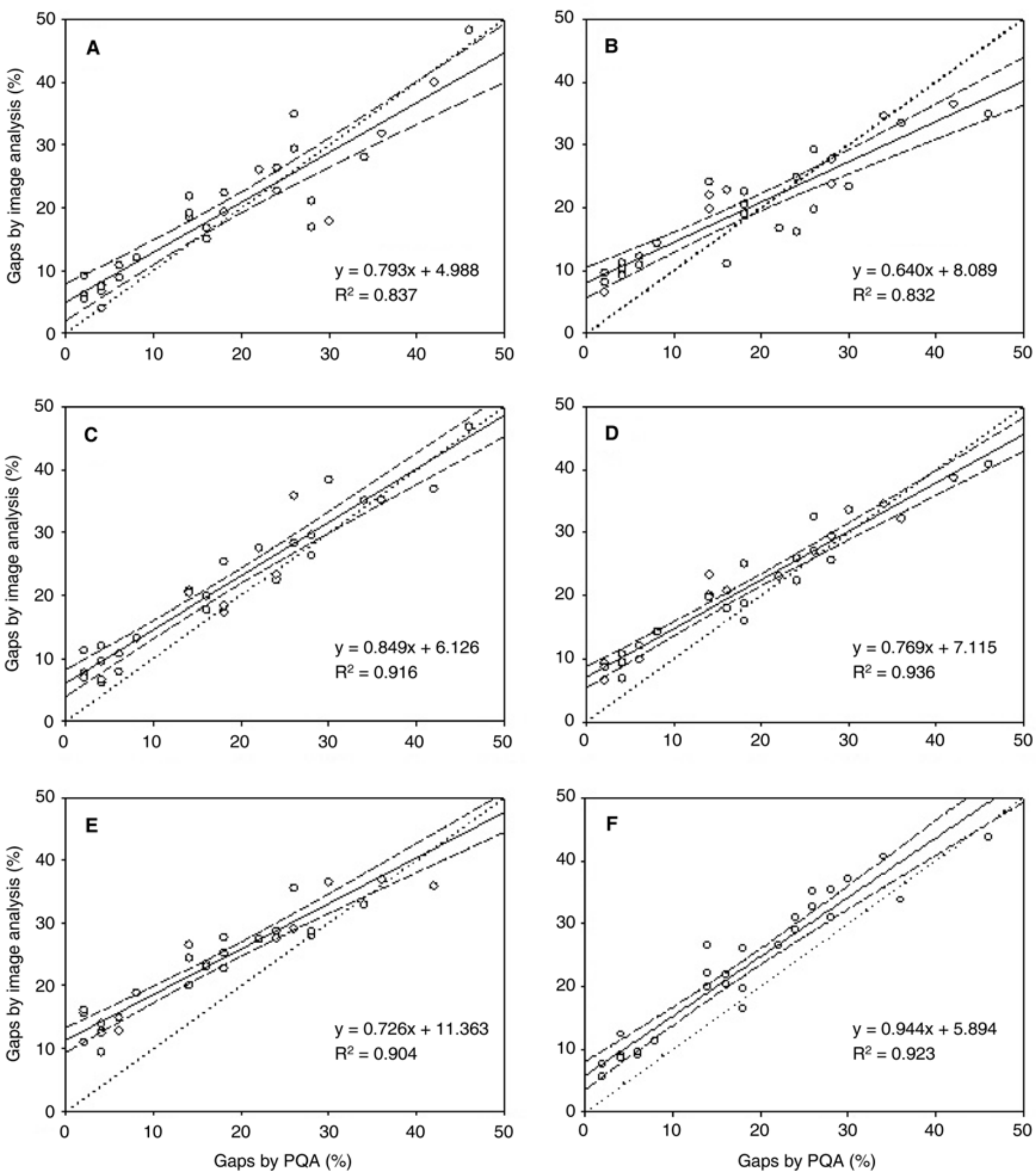

Figure 6 Regressions of percent canopy gaps measured by image analysis compared to canopy gaps measured by point quadrat analysis (PQA; solid line) for Cabernet Sauvignon in Croatia in 2015 for images taken from the east side of the canopy (**A**) in the morning, (**C**) at midday, and (**E**) in the afternoon, and from the west side of the canopy (**B**) in the morning, (**D**) at midday, and (**F**) in the afternoon, with a 1:1 reference line (dotted line), and a 95% confidence interval (dashed line). Slope coefficients were significant for $\alpha = 0.001$. Morning time: 1100 hr; midday: 1400 hr; afternoon: 1700 hr; n = 30.

Table 2 Slope coefficients of the regression models of the canopy percent gaps estimated by image analysis (under different image acquisition conditions) versus point quadrat analysis for a given set of Cabernet Sauvignon grapevines. The 95% confidence intervals for the slope coefficients are indicated in brackets. The *t*-test values and their corresponding *p* values for the null hypothesis of H_0: slope = 1 and H_0: intercept = 0 are presented.

Time of day/side of canopy	N	Slope	*t*-test value[a]	*p* value	Intercept	*t*-test value[a]	*p* value
Morning							
East	30	0.793 (0.657; 0.928)	3.14	0.004	4.988 (2.059; 7.917)	3.49	0.002
West	30	0.640 (0.529; 0.751)	6.66	<0.001	8.089 (5.681; 10.496)	6.88	<0.001
Midday							
East	30	0.849 (0.750; 0.949)	3.14	0.004	6.126 (3.978; 8.275)	5.84	<0.001
West	30	0.769 (0.691; 0.847)	6.06	<0.001	7.115 (5.427; 8.804)	8.63	<0.001
Afternoon							
East	30	0.726 (0.634; 0.817)	6.12	<0.001	11.363 (9.376; 13.351)	11.71	<0.001
West	30	0.944 (0.836; 1.051)	1.07	0.293	5.849 (3.638; 8.061)	5.43	<0.001

[a] *t*-tests for the null hypothesis H_0: slope = 1 and H_0: intercept = 0 were only tested for those regressions with statistically significant slope coefficients ($p < 0.05$).

et al. (2011) compared the leaf layer number of the canopy assessed by PQA with the gap area percentage estimated by image analysis and observed a correlation between the log-transformed variables ($R^2 = 0.87$, $p < 0.001$). However, no studies have directly compared the two methods for measuring gap percent as Hill et al. (2011) focuses on assessing the density of the foliage.

Factors influencing image analysis. Several factors may influence the performance of the developed image-analysis method, including the method of ROI delimitation and shadowing induced by natural illumination, which depends on the time of day and canopy side photographed. Unlike the two other sites, a wooden frame was used both for image acquisition and PQA at the New Zealand site. The frame simplified delineation of the edges of the ROI for image analysis and ensured a higher correspondence between the canopy area assessed by the two methods. However, from an operational point of view, the use of a frame at the New Zealand site did not seem to yield a substantial increase of R^2 and accuracy. Therefore, to simplify the application under field conditions, use of a frame is not needed.

The results obtained from the imaging trial performed at different times of day and on both sides of the canopy showed that the algorithm was able to overcome differences in shadowing, as the percent gap was not significantly different among the six environmental conditions tested. Therefore, the algorithm was robust enough (provided the proper seeds for class establishment are given at the beginning of the process) to assess percent gaps in the grapevine canopy using images with variable shadow distribution and taken at different times of day. To improve the practicality of the image analysis method, further research should investigate eliminating its dependence on the background color to improve its functionality for applications in the vineyard and promote the adaptation of this image-based methodology to an on-the-go approach.

Practical implications of the developed image-analysis method. According to Hill et al. 2011, image-based estimation of canopy porosity is an objective method whereas PQA is subjective. These authors report some subjectivity to the choice of where to frame the photographs to most accurately represent the whole grapevine when using the image-assisted method to estimate leaf layer number. To evaluate the canopy porosity in our study, we targeted the fruiting zone, which is the ROI from the viticultural point of view because the fruit zone microclimate is a substantial determinant of grape quality and health. Our developed image-based method is simple enough for production vineyards to measure canopy characteristics, which may allow vineyard managers and grapegrowers to refine their manipulations of the canopy, thereby saving money and improving wine quality. Information about percent canopy gaps may guide the producer's choice of management practices in the following or current season, provided the assessment of the canopy gap is performed early enough in the season, (i.e., after flowering) to support decisions about leaf removal during the early stages (such as at berry set, pea size, or veraison). From a practical perspective, assessing canopy gaps using PQA may not be difficult for an experienced and skilled grapegrower. However, considering the importance of canopy management for the health and quality of fruit, a method that allows a grapegrower or vineyard manager to quickly measure, control, and manage canopy porosity is valuable for producing high-quality grapes in both experimental and commercial operations. Future work will focus on simplifying the image acquisition process by avoiding the use of a background color, adapting the algorithm to be implemented in low-cost, easy-to-use devices (such as smartphones), and enabling image acquisition on-the-go.

Conclusion

A new, objective, noninvasive, image-analysis based method to assess grapevine canopy porosity was successfully developed and tested in vineyards with diverse viticultural conditions and cultivars in three wine regions around the world and may be a viable alternative to the more laborious PQA. Considering the easy-to-use implementation of the image-based method, which is accurate with images acquired from any side of the canopy at any time of day, and its potential to refine canopy management, image analysis techniques will likely gain acceptance in viticulture. The developed method may optimize canopy management by enabling the user to configure a desired amount of canopy gaps, which could be maintained or further manipulated as the season progresses, with the aim of improving fruit quality and health. New technologies such as machine vision could be adapted to develop decision support tools and provide fast and noninvasive monitoring, which would be helpful for precision viticulture.

Literature Cited

Arnó J, Escolà A, Masip J and Rosell-Polo JR R. 2015. Influence of the scanned side of the row in terrestrial laser sensor applications in vineyards: Practical consequences. Precis Agric 16:119-128.

Austin CN, Gove GG, Meyers JM and Wilcox WF. 2011. Powdery mildew severity as a function of canopy density: Associated impacts on sunlight penetration and spray coverage. Am J Enol Vitic 62:23-31.

Balehegn M and Berhe K. 2015. Training reduced subjectivity of comparative yield method of estimation of grassland biomass. Grass Forage Sci. doi: 10.1111/gfs.12194.

Bubola M, Peršurić Đ, Kovačević Ganić K, Karoglan M and Kozina B. 2012. Effects of fruit zone leaf removal on the concentrations of phenolic and organic acids in Istrian Malvasia grape juice and wine. Food Technol Biotech 50:159-166

Cubero S, Diago MP, Blasco J, Tardáguila J, Millán B and Aleixos N. 2014. A new method for pedicel/peduncle detection and size assessment of grapevine berries and other fruits by image analysis. Biosyst Eng 117:62-72.

Cubero S, Diago MP, Blasco J, Tardáguila J, Prats-Montalbán JM, Ibáñez J, Tello J and Aleixos N. 2015. A new method for assessment of bunch compactness using automated image analysis. Aust J Grape Wine Res 21:101-109.

De Bei R, Hook J, Fuentes S, Gilliham M, Tyerman S and Collins C. 2015. Linking canopy architecture to grape quality using the LAI canopy app. *In* Proceedings of the 19th International Meeting of Viticulture GIESCO, pp.585-588. Montpellier, France.

Diago MP, Vilanova M and Tardaguila J. 2010. Effects of timing of manual and mechanical early defoliation on the aroma of *Vitis vinifera* (L.) Tempranillo wine. Am J Enol Vitic 61:382-391.

Diago MP, Ayestarán B, Guadalupe Z, Poni S and Tardaguila J. 2012a. Impact of prebloom and fruit set basal leaf removal on the flavonol and anthocyanin composition of Tempranillo grapes. Am J Enol Vitic 63:367-376.

Diago MP, Correa C, Millán B, Barreiro P, Valero C and Tardaguila J. 2012b. Grapevine's yield and leaf area estimation using supervised classification methodology on RGB images taken under field conditions. Sensors 12:16988-17006.

Diago MP, Sanz-Garcia A, Millan B, Blasco J and Tardaguila J. 2014. Assessment of flower number per inflorescence in grapevine by image analysis under field conditions. J Sci Food Agric 94:1981-1987.

Diago MP, Tardaguila J, Aleixos N, Millan B, Prats-Montalbán JM, Cubero S and Blasco J. 2015. Assessment of cluster yield components by image analysis. J Sci Food Agric 95:1274-1282.

Dunn GM and Martin SR. 2004. Yield prediction from digital image analysis: A technique with potential for vineyard assessments prior to harvest. Aust J Grape Wine Res 10:196-198.

English JT, Bledsoe AM and Marois JJ. 1990. Influence of leaf removal from the fruit cluster zone on the components of evaporative potential within grapevine canopies. Agr Ecosyst Environ 31:49-61.

Fuentes S, De Bei R, Pozo C and Tyerman S. 2012. Development of a smartphone application to characterise temporal and spatial canopy architecture and leaf area index for grapevines. Wine Vitic J 27:56-60.

Hill GN, Beresford RM, Wood PN, Kim KS and Wright PJ. 2011. Image-assisted gap estimation, a simple method for measuring grapevine leaf canopy density. Crop Sci 51:2801-2808.

Kliewer WM. 1977. Effect of high temperatures during the bloom-set period on fruit-set, ovule fertility, and berry growth of several grape cultivars. Am J Enol Vitic 28:215-222.

Meyers JM and Vanden Heuvel JE. 2008. Enhancing the precision and spatial acuity of point quadrat analyses via calibrated exposure mapping. Am J Enol Vitic 59:425-431.

Mori K, Goto-Yamamoto N, Kitayama M and Hashizume K. 2007. Effect of high temperature on anthocyanin composition and transcription of flavonoid hydroxylase genes in 'Pinot noir' grapes (*Vitis vinifera*). J Hortic Sci Biotec 82:199-206.

Nuske S, Wilshusen K, Achar S, Yoder L, Narasimhan S and Singh S. 2014. Automated visual yield estimation in vineyards. J Field Robot 31:837-860.

Palliotti A and Silvestroni O. 2004. Ecofisiologia applicate alla vite. *In* Viticoltura ed Enologia Biologica. E Cozzolino (ed.), pp. 41-88. Edagricole, Bologna, Italy.

Reynolds AG and Wardle DA. 1989. Influence of fruit microclimate on monoterpene levels of Gewürztraminer. Am J Enol Vitic 40:149-154.

Smart RE. 1987. Influence of light on composition and quality of grapes. Acta Hortic 206:37-48.

Smart RE and Robinson M. 1991. Sunlight Into Wine: A Handbook for Winegrape Canopy Management. Winetitles, Adelaide, Australia.

Tardaguila J, Martinez de Toda F, Poni S and Diago MP. 2010. Impact of early leaf removal on yield and fruit and wine composition of *Vitis vinifera* L. Graciano and Carignan. Am J Enol Vitic 61:372-381.

Tardáguila J, Blanco JA, Poni S and Diago MP. 2012. Mechanical yield regulation in winegrapes: Comparison of early defoliation and crop thinning. Aust J Grape Wine Res 18:344-352.

Wilson JW. 1959. Analysis of the spatial distribution of foliage by two-dimensional point quadrats. New Phytol 58:92-99.

Wilson SK, Graham NAJ and Polunin NVC. 2007. Appraisal of visual assessments of habitat complexity and benthic composition on coral reefs. Mar Biol 151:1069-1076.