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Maria P. Diago[1*], Andres Sanz-Garcia[1], Borja Millan[1], Jose Blasco[2], Javier Tardaguila[1]

[1]Instituto de Ciencias de la Vid y del Vino (University of La Rioja, CSIC, Gobierno de La Rioja), Madre de Dios 51, 26006 Logroño, La Rioja, Spain.

[2]Centro de Agroingeniería. Instituto Valenciano de Investigaciones Agrarias (IVIA). Cra. Moncada-Náquera km 5, 46113 Moncada (Valencia), Spain.

*Corresponding author: Maria P. Diago, telephone +34 941 299760, fax +34 941 299721. e-mail: mpaz.diago.santamaria@gmail.com

Running title: Grapevine flower number per inflorescence by image analysis

Maria P Diago[1], Andres Sanz-Garcia[1], Borja Millan[1], Jose Blasco[2], Javier Tardaguila[1]*
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Abstract

BACKGROUND: Flowers, flowering and fruit set are key determinants of grapevine yield. Currently, practical methods to assess the flower number per inflorescence, necessary for fruit-set estimation, are time and labour demanding. This work aims at developing a simple, cheap, fast, accurate and robust machine vision methodology to be applied to RGB images taken under field conditions, to estimate the number of flowers per inflorescence automatically.

RESULTS: 90 images of individual inflorescences of *Vitis vinifera* L. cultivars Tempranillo, Graciano and Carignan were acquired in the vineyard with a pocket RGB camera prior to flowering, and used to develop and test the "flower counting" algorithm. Strong and significant relationships, with R^2 above 80% for the three cultivars were observed between actual and automated estimation of inflorescence flower numbers, with a precision exceeding 90% for all cultivars.

CONCLUSION: The developed algorithm proved that the analysis of digital images captured by pocket cameras under uncontrolled outdoors conditions was able to automatically provide a useful estimation of the amount of flowers per inflorescence of grapevines at early stages of flowering.

Keywords: fruit set, flowering, berry number per cluster, vineyard, *Vitis vinifera* L., non-invasive.

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INTRODUCTION

The development and application of innovative techniques aimed at objectively monitoring the vineyard is a key issue in viticulture research in order to improve grape growing sustainability as well as grape and wine quality.

Flowering and fruit-set are the main determinants of grapevine yield[1]. These two physiological processes define the number of berries per cluster, which together with berry volume influence cluster architecture and compactness (looser or tighter clusters), considered indicators of grape and wine quality2. The reproductive performance, conducive to poor or good fruit-set has been reported to be variety and clone-dependent[1, 3, 4] and impacted by physiological, environmental and pathological factors [5].

Fruit-set rates have been estimated in various works evaluating the effects of viticultural practices since the 19th century[1, 6], including late pruning[7], shoot tipping[8], topping[9], early defoliation[10], girdling[11] and spray applications of growth regulators [9, 12] and nutrients[13, 14]. In most of these studies, berry number per cluster was used to estimate fruit set and a sample of clusters was measured assuming that initial flower number per inflorescence was invariable between treatments. However, flower number per inflorescence shows a strong variability among vines and within inflorescences of the same vine[1]. Therefore, a count of the flower number per inflorescence is essential for accurate assessment of fruit set.

Several practical methods have been used to determine the number of flowers per inflorescence. May[15] and Keller et al.[16] proposed to enclose each 'sample inflorescence' with a fine mesh bag secured with a plastic tie from the beginning of anthesis until complete fruit-set, and manually count the collected flower caps in order to estimate the number of flowers per cluster. Though effective, this method is time-consuming and labour-demanding. Poni et al.[10] photographed each 'sample inflorescence' against a dark background with a digital camera held perpendicular to the inflorescence. Then, they estimated initial flower number on tagged inflorescences using a linear regression between actual flower number and the flower number manually counted on photo prints established for 20 inflorescences taken from extra vines. Automating this operation

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would be advantageous in saving costs and labour effort, and may be technically feasible using a computer vision-based counting system.

Computer vision-based systems are being used to automate inspection tasks in agriculture and food processing[17-19]. In addition to other characteristics such as colour or surface defects, shape, size and texture are features that make image analysis an objective and reliable tool for quality inspection, automatic recognition or crop forecasting[20-22]. However, the performance of a computer vision system only based on colour information is really dependent on many factors, particularly the illumination conditions, acquisition angle and object composition. In viticulture, some works on image analysis methods of red-green-blue (RGB) images have been conducted to estimate the number of berries per cluster at harvest time, based on simple image colour discrimination[23]. Grossetête et al.[24] presented an application for automatic counting of berries (at pea-size) on RGB images taken at night with smartphones with a simple image-processing algorithm by identification of a unique and bright spot in the centre of the berries created by the reflection of the light from the camera flash. However, this method is not technically useful to detect flowers because of the shapes of these, which do not create the bright spot mentioned.

The goal of the present work was to develop a simple, cheap, fast, accurate and robust image analysis methodology to be applied to RGB images taken under field conditions to estimate the number of flowers per inflorescence automatically.

MATERIALS AND METHODS

Image acquisition

Images of the inflorescences of *Vitis vinifera* L. cvs. Tempranillo, Graciano and Carignan were acquired at pre-flowering, when inflorescences are swelling, and flowers closely pressed together (phenological stage BBCH 55, following the scale of Lorenz et al.[25]) between 10 am and 4 pm, during season 2008 in three commercial vineyards located in Ollauri (La Rioja, Spain). For each cultivar 30 inflorescences were photographed. Images with a size of 2048 x 1536 pixels were manually acquired (no tripod was used) under field conditions with a Canon Digital Ixus 850 IS (Canon Inc.,

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Japan) compact colour camera. For each individual inflorescence a single image was taken. The camera was set up to "automatic mode" to let it choose all settings of exposure, white balance, and focusing. For this reason, the exposure time and shutter aperture varied between images. The distance between the inflorescence and the photographic lens (camera lens) was not initially pre-established but this was approximately considered around 30-40 cm (50-70 pixels per centimetre). The lighting system was intentionally neither controlled in order to mimic flexible outdoors working conditions, hence the brightness of some areas of the images changed due to the reflection of the sunlight at different hours. Finally, to assure a high colour contrast that eased the segmentation process of inflorescences, a uniform background of black colour was used.

Image Processing

Images were processed using Matlab (MatlabR2010b, MathWorks, Massachusetts, USA) and the processing method developed for flower counting was fully automatic and involved three stages: 1. Image pre-processing; 2. Flower counting, and 3. Image post-processing.

The first stage involved the conversion of the image from RGB to CIELAB colour space, and an initial segmentation by means of a threshold, separating the background from the flowers. This was accomplished based on the histogram from coordinate *b* of the CIELAB colour space, and a subsequent filtering process. The CIELAB colour space[26] is an international standard for colour measurement developed by the "Commission Internationale d´Eclairage" (CIE) in 1976 being widely used for colour analysis. It is represented by three coordinates so called *L**, *a**, and *b**. The term *L** is the luminance ranging from 0 to 100, that is combined with the other two chromatic components or coordinates: *a** represents the greenness-redness whereas *b** represents the blueness-yellowness[26]. The threshold selected for the segmentation process was automatically set for every image based on the clusters present on the histogram of the b* coordinate. Pixels representing background had similar colour and thus similar values in b* coordinate and the same occurs for pixels representing the inflorescence.

Regarding flower counting, the flowers present a higher degree of light reflection than other areas which is used to detect them. This stage was focused on counting the

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number of brighter areas which usually corresponded to the presence of flowers. The specific steps were the following:

a. Computation of the extended-maxima transform, which was the regional maxima - connected components of pixels with a constant intensity value, and whose external boundary pixels all have a lower value- of the H-maxima transform[27] (suppression of all local maxima lower than a threshold), to find and identify the brighter points of the lightness of the image (L* coordinate). This converted the initial image into a binary one. The threshold was manually selected based on a set of images.
b. Finding the connected components in the binary image computed to label them as flowers for each image using the "bwconncomp" function from the Matlab Image Processing Toolbox. This function groups as the same component pixels with a neighbourhood.
c. Measurement of the size and centroids of all the candidate regions.

Finally, the third stage was intended to remove other material than flowers from the brighter areas selected. The filtered data was obtained with the Matlab function "regionprops". This filtering was subsequently conducted as a sequential process on the basis of three criteria; one per step:

a. Region size filtering: This filtering step refers to the size of the brightness areas representing the flowers, and involves deleting the regions which were bigger than 2.5 times the median of the region zones.
b. Distance between brighter areas: This filtering process required the calculation (and sorting) of the distances between the centroids and their corresponding histograms, and subsequent filtering of these histograms using the highest distance values between centroids. The flowers in the inflorescence were grouped so the histogram of distances shows a cluster than can be used to filter false positives without the constraint of a fixed distance when taking the image.
c. Shape of the brighter areas: This involved the computation of the ratio between the maximum and minimum length values of the axis for each region. The shape of the bright areas was similar between them and different to bright areas from

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stems so results can be grouped and filtered automatically using the ratio length/width and the histogram.

Actual flower counting and estimation from photo prints

The main goal was to determine the linear relationship between the experimental measurements, the manual counting procedure, and the automatic flowers detection by the implemented method. Therefore, in order to validate the image analysis method, the actual flower number per inflorescence was determined manually after image acquisition by individually detaching the flowers from the rachis. Moreover, for all cultivars, estimation of the flower number on imaged inflorescences was also done by manually counting the flowers on printed images.

Statistical performance analysis

The performance of the developed method was mainly evaluated by the Pearson's correlation coefficient (r) and coefficient of determination (R^2).

Additionally, five randomly selected images from each of the three cultivars were manually and automatically processed using the developed algorithm to estimate the number of flowers per inflorescence. *Total count* (TC), which refers to the number of flowers automatically detected by the developed method, *true positive* (TP), which is the number of flowers identified that were actual flowers, *false positive* (FP), that represents the number of false detections, and *false negative* (FN), which corresponds to the number of flowers that were not automatically detected but were manually counted, were computed for each cultivar. In addition, precision and recall were used for measuring the performance of this binary classification. Recall was defined as the percentage of actual flowers detected[28]:

$$\text{Recall} = \frac{\text{TP}}{\text{TP} + \text{FN}} \quad \textbf{(1)}$$

and precision was the percentage of flowers detected correctly, defined as:

$$\text{Precision} = \frac{\text{TP}}{\text{TP} + \text{FP}} \quad \textbf{(2)}$$

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Statistical analysis was conducted using R-project v.3.0.1. (RCore Team: 'R: a language and environment for statistical computing'; 2012, Vienna, R Foundation for Statistical Computing).

RESULTS AND DISCUSSION

Image processing

Figure 1 shows the colour histograms, used for segmentation in the first stage (image pre-processing), for the colour distribution (RGB and CIELAB spaces) in an inflorescence image of cultivar Tempranillo. Two different sets are observed only for coordinates a^* (Figure 1E, 1H) and b^* (Figure 1F, 1I) of the CIELAB colour space, indicating that their histograms were able to separate correctly the background from the interesting information in the image. In the histogram of coordinate b*, the local minimum after the first local maxima was the threshold used for the image segmentation process (Figure 1I). A larger distribution was found for the background cluster, because of the higher number of pixels corresponding to the background as compared to the flowers. On the other hand, the histograms in Figure 1A, 1B and 1C evidenced that no differentiation between the target and background in the image could be achieved in the RGB space as no clustering was detected. Although the algorithm worked with component b^* demonstrated that either component $^*a$ or b^* could be successfully used (Figure 1H and 1I). These results were consistent in all the images analysed but the threshold to separate the inflorescence from background varied slightly from one image to another due to fluctuations in the lighting source during image acquisition.

Identification and evaluation of the number of flowers per inflorescence

The effectiveness of the implemented algorithm was tested against the "destructive" manual counting of the actual flower number per inflorescence, and also compared to the manual estimation from photo prints. Figure 2A shows the original image, the resulting image after manually counting the flowers in the printed image (Figure 2B), the detected flowers on the binary image (Figure 2C), and the detected flowers superimposed on the original image (Figure 2D) after the automatic processing of one inflorescence image from 'Graciano'. The automatic counting using the developed

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method allowed to distinguishing the flowers from other parts of the inflorescence and also from the background.

The relationship between the number of flowers detected both manually and automatically from the images, and the actual flower number per inflorescence, determined by destructive removal and counting, are reported for Graciano (Figure 3A), Carignan (Figure 3B), Tempranillo (Figure 3C) and all three cultivars together (Figure 3D). For these three cultivars, considering each cultivar individually, the determination coefficient (R^2) of the relationships between the number of flowers estimated either manual or automatically with the developed method, to actual flower number per inflorescence was always higher than 0.80 ($p<0.001$). No strong differences in R^2 values were found between manual and automated flower estimation. However, R^2 diminished to 0.76 when the three varieties were considered together in the automated mode.

In comparison to our results, Grossetête et al.[24] reported a strong nonlinear relationship ($R^2=0.92$ using a polynomial model) for the estimation of berry number per cluster from images taken between berry set (BBCH 71, fruits begin to swell and there are some remains of flower lost) and veraison (BBCH 79, or berry touch complete). Differently from our work, Grossetête et al.[24] took the pictures at night, using the camera-integrated flash light. The rationale behind their algorithm relied in using the reflection of light on the berry surface, which behaved as a specular reflection whose maximum was found on the center of the berry as a unique and distinct peak, and decreased dramatically from the center to the boundary. This methodology cannot be applied to inflorescences prior to flowering because of the geometry of the flower buttons which are not spherical. Fruit set rates in grapevines do not usually exceed 50%[1], meaning that only a proportion of the flowers in the inflorescence will become berries in the cluster, and the potential occlusion of berries in an image can be different from that of flowers. This would explain the better fitting of a non-linear model [24] instead of a linear one as presented in this work and the differences in the R^2 values.

Underestimation of the flower number per inflorescence occurred for all cultivars, regardless the method employed, manual or automated counting from images, but it occurred more intensely in the automated mode (Figure 3). Since the number of flowers per inflorescence was estimated from a single 2D image per inflorescence,

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underestimation was expected, as those flowers on the opposite side of the inflorescence were "not visible" either for the algorithm, nor for the human eye, hence they were not computed. However, the larger underestimation observed for the automated process is explained by its conservative nature, as one of the priorities of the developed algorithm was to minimize the false positives, in other words, not computing material other than flowers. Following this criterion, the consecutive application of the filters (third stage) based on the size of the brightness areas (which represented the flowers), the distance between the brightness zones, and their shape may have led to a very severe filtering, eliminating false negatives but increasing the number of true positives.

Performance of the application proposed

It was important to demonstrate the performance of each filtering step in the third stage of the algorithm. Table 1 shows the statistical results after the application of each filtering step: region size, distance and shape of the brightness. Considering the evolution of the number of false negatives (FN) and false positives (FP), it can be said that there was not a drastic increase or reduction in any of these steps. Nevertheless, the algorithm shows a slightly increase in FN after application of shape filtering which led to a reduction of the recall ratio, from 75.4% (after size filtering) to 74.3% (Table 1). This was caused by the fact that the shape filtering process "removed" more true positives (TP) flowers than avoided FP ones. Even though, the algorithm subsequently improved its precision, from 91.0% (after size filtering) to 92.2% (after distance filtering) and finally 92.9% (after shape filtering). Comparing these results with other approaches for visual grape detection[29], similar performance indicators of recall and precision were achieved. Further additional work should look at modifying the calculation and applications of the filter by distance in order to improve the detection of more true positives and achieve better recall values. Another approach to solve the problem of the low recall performance could be the combination of our algorithm with the detection of the intensity edges of the flowers or even their shadows in order to deploy a more robust method.

The resulting statistics of the performance analysis of the developed algorithm for each group of five images per cultivar are presented in Table 2. Concerning the precision

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percentage, which is the fraction of detections being flowers, a very high value, exceeding 90.0% in all cases, was achieved for the three cultivars, meaning that the algorithm did not essentially detect areas that were not flowers. On the contrary, there appears to be a divergence between manual and automated flower counting as shown by the false negative value (number of flowers not detected automatically but manually reported), which led to a lower overall recall (74.3%). In this way, the aim of robustness and flexibility (uncontrolled outdoors conditions for the easiness of future applications) of the developed algorithm at risk of lower accuracy is also affecting the recall values. In contrast to earlier implementations [24], no artificial lighting source was utilized in the experiments. This caused a high variation in the position of the brightness on the black background due to the natural light. Another important advantage of the developed algorithm is that flower dimensions were not taken into consideration, and the distance between the camera and the inflorescence did not need to be pre-established in any case. Should image acquisition settings be more restrictive and constant, an increase in the accuracy of the method is expected.

The processing speed was less than 1 image per second. A trade off between the algorithm speed, robustness and accuracy was intended by avoiding iterative methods. In fact, the system did not need any prior calibration and its setting parameters were valid to the complete range of distances between the inflorescence and the camera. This was a clear advantage to other systems in which red, green and blue threshold values have to be manually adjusted to select the right pixels for all the digital images [23].

The low cost, simplicity and flexibility of the in-field image acquisition and processing of the developed method for the estimation of the number of flowers per inflorescence in grapevines (ie. by implementation of the presented methodology in tablet or smartphone-like devices) may be of great help to the wine industry aimed at assessing the initial flower number per inflorescence for fruit set and yield calculations.

CONCLUSIONS

Our results show that the developed algorithm to analyze digital images captured by pocket cameras under uncontrolled outdoors conditions was able to automatically provide useful estimations of the amount of flowers per inflorescence of grapevines at early stages of flowering. This could help vineyard managers with automated prediction

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of fruit set rates and potential yield. In a short time, the developed algorithm may be implemented on a mobile device such as a smartphone or even using a distributed management system (cloud computing), to provide flower count information at each georeferenced position of a given vineyard for mapping.

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FIGURES' CAPTIONS:

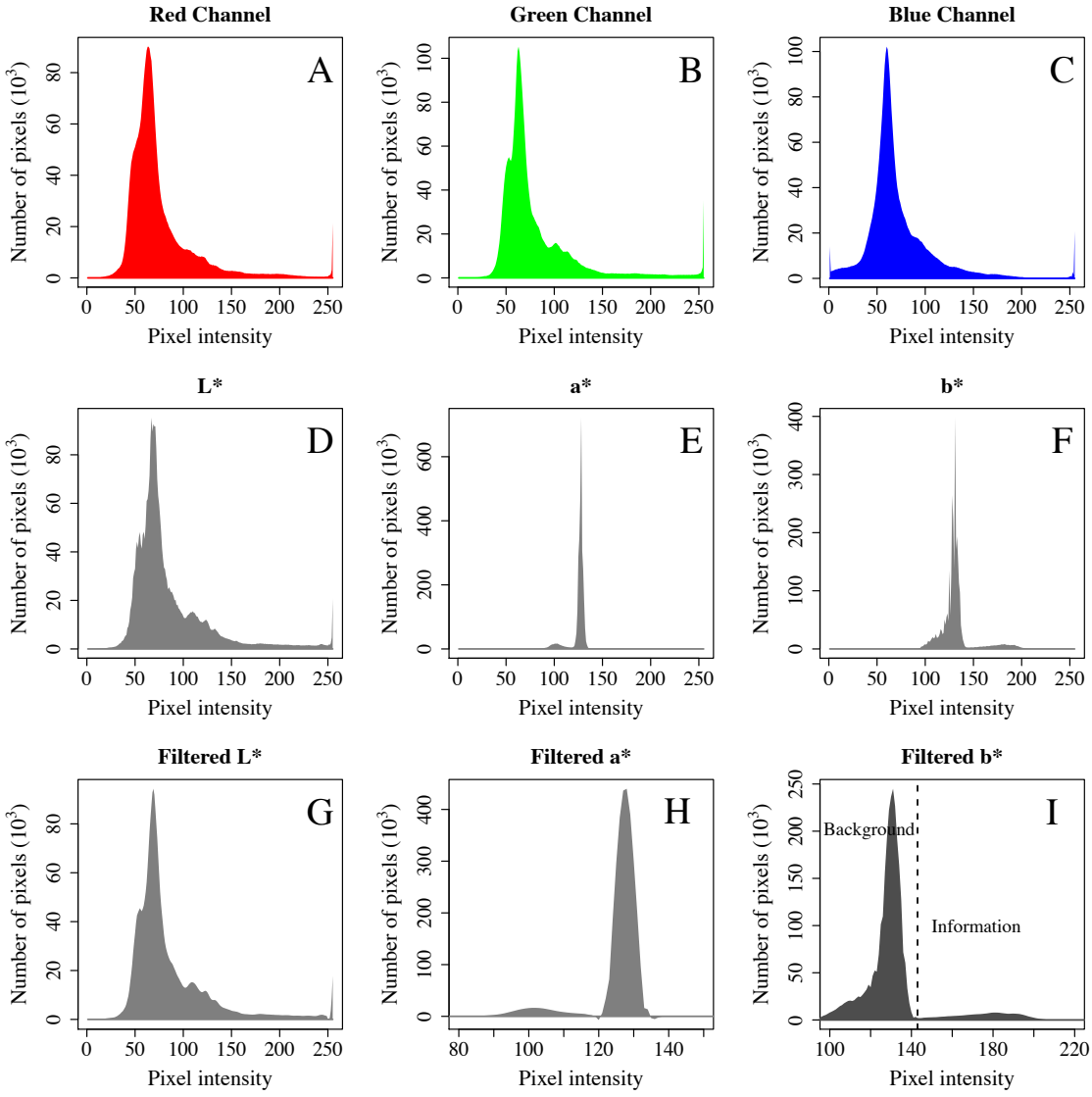

Figure 1: Histograms showing the number of pixels for an inflorescence image of the cultivar Tempranillo. A) Red channel, B) Green channel and C) Blue channel of the RGB colour space; D) *L** component, E) *a** component and F) *b** component of the CIELAB colour space; G) Filtered *L** component, H) Filtered *a** component and I) Filtered *b** component of the CIELAB colour space.

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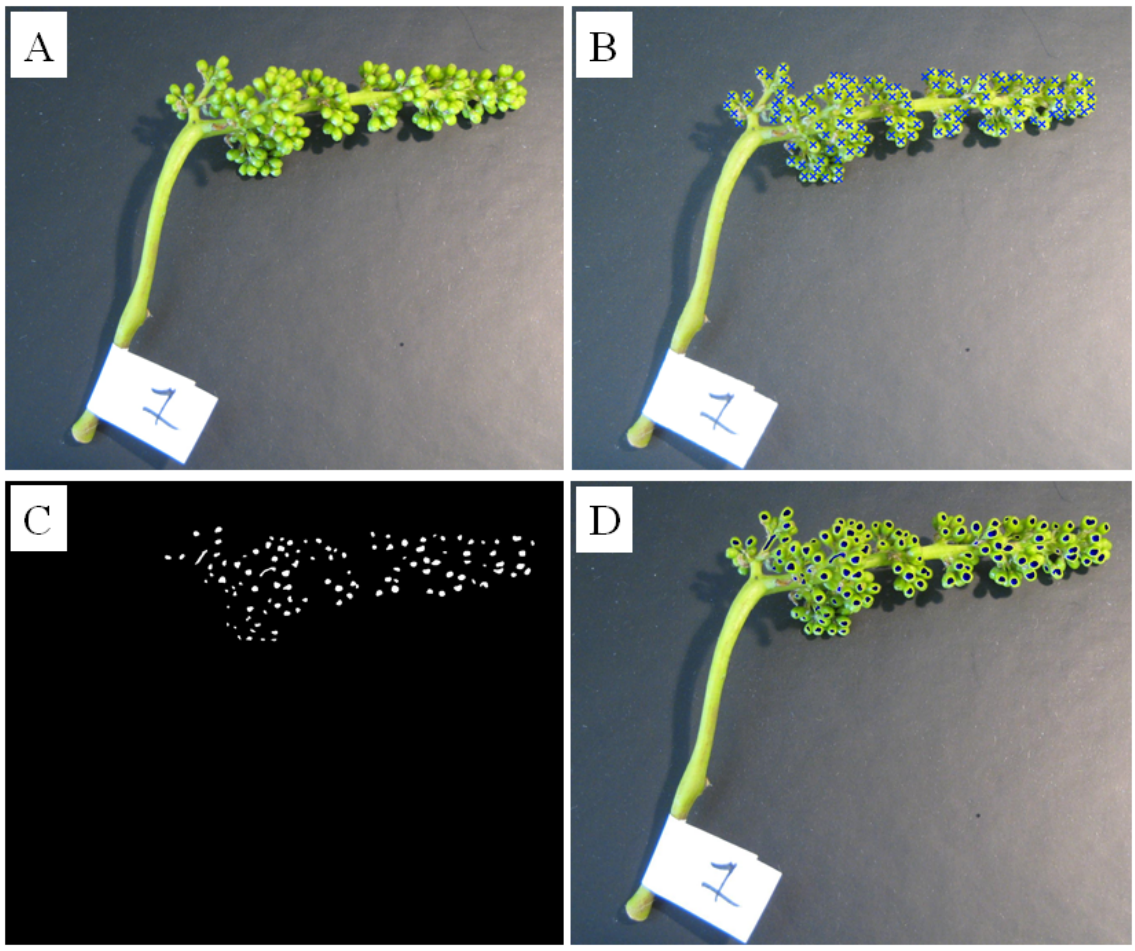

Figure 2: A) Raw RGB image, B) same image with flowers manually marked with an "X", C) same image after the segmentation process and D) raw RGB image combined with the regions detected (blue colour) after the automatic counting of one representative inflorescence of the cultivar Graciano at phenological stage BBCH 55.

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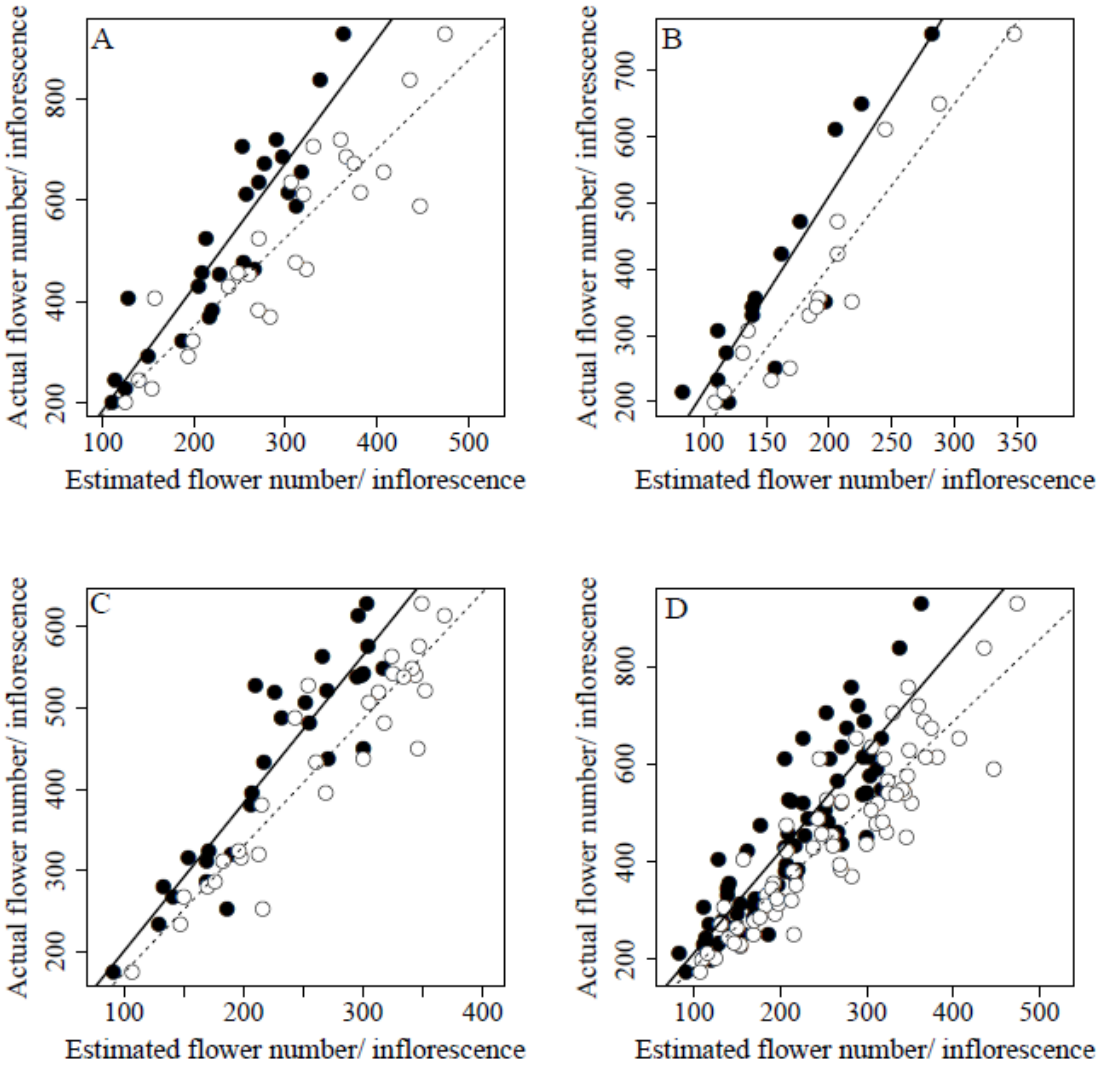

Figure 3: Relationship between the actual number of flowers per inflorescence and those detected both manually (○, dashed line) and automatically (●, solid line) from digital images in A) Graciano (manual: y=1.75x+0.93, R^2=0.81 at p<0.001; automate: y=2.44x-59.5, R^2=0.83 at p<0.001); B) Carignan (manual: y=2.46x-89.1, R^2=0.89 at p<0.001; automate: y=2.96x-81.2, R^2=0.84 at p<0.001); C) Tempranillo (manual: y=1.56x+17.1, R^2=0.87 at p<0.001; automate: y=1.82x+18.1, R^2=0.83 at p<0.001); D) All cultivars (manual: y=1.68x+16.2, R^2=0.81 at p<0.001; automate: y=2.08x+5.4, R^2=0.76 at p<0.001).