Image analysis techniques applied to canopies, berries, plant tissues and leaves.

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1. Introduction

Image analysis has become a powerful tool that offers the advantage, over other monitoring methods in agriculture, of having the data (images or video) available to be assessed using different methodologies and algorithms after data acquisition. This type of analysis has gained great importance in research under a climate change scenario, in which novel monitoring methods are required to assess non-discrete spatial and temporal physiological responses to changes in the environment. Our group has developed a variety of novel image analysis tools applied to automatic infrared thermography, image and video analysis of plants and plant organs in the field and laboratory. This research is part of The Vineyard of the Future (VoF) initiative from The University of Adelaide, that aims to establish a fully instrumented vineyard, including high definition stereoscopic cameras and infrared cameras to assess the soil-plant-atmosphere continuum and as a test bed for new technologies.

2. Materials and Methods

The automated and semi-automated image analysis techniques described are: (i) canopy structure assessment to extract critical parameters using gap analysis from cover photography and video, (ii) infrared thermography of canopies to automatically extract water stress indices, iii) analysis of fluorescent microscopy images to assess berry morphometrics, mesocarp living tissue and shrivel and (iii) morpho-colorimetric analysis of organs (leaves and petioles) and discrimination using principal component analysis (PCA) techniques. The customised analysis codes were developed in MATLAB® 2011b and the Image Analysis toolbox ® (Mathworks Inc., Natick, MA, USA).

2.1. Canopy structure assessment using cover photography

A digital camera can be mounted on a tripod with a bubble level and used to acquire digital photos (as .JPEG files) at the zenith angle from canopies. Digital images are collected at 0.3 m from the ground for trees and at ground level for grapevine. Digital images usually are collected in parallel with measurements using the allometry techniques or other established instrumentation, such as Ceptometers or LiCOR 2000 as ground truth. The analysis script has been fully described in Fuentes et al. (2008). The analysis methodology performs a gap analysis of upward-looking digital images by automatically dividing each binary image into a number of sub-images defined by the user (Figure 1). From each sub-image, the program automatically counts the total number of pixels corresponding to sky (S) and leaves (L). A big gap is considered when the ratio S/L in each sub-image is larger that a user-specified value. When this threshold is met per sub-image, the pixel count for S is added to the big gap count for that particular full image. If the ratio observed is smaller than the user-specified value for a define sub-image, the pixel count contribution to the total
big gap count is equal to zero. The fractions of foliage cover \((f_f)\) and crown cover \((f_c)\) are calculated from Mcfarlane et al. (2007) by the following equations:

\[
f_c = 1 - \frac{g_{LT}}{T_P} \quad (1) \quad f_f = 1 - \frac{g_T}{T_P}\]

where \(g_{LT}\) is the total number of large gap pixels; \(g_T\) is the total number of gap pixels and \(T_P\) is the total pixels. Using the \(f_f\) and \(f_c\) calculated values, the crown porosity \((\Phi)\) can be calculated as follows:

\[
\Phi = 1 - \frac{f_f}{f_c} \quad (3) \quad LAI_M = -f_c \frac{\ln \Phi}{k} \Omega(0)
\]

Finally, the effective leaf area index \((LAI_M)\) is calculated from Beer’s Law and clumping index at the zenith \((\Omega(0))\) (Eq. 5). A reference light extinction coefficient \((K_M)\) was calculated by inverting Equation 4 (Eq. 6) (Macfarlane et al., 2007):

\[
\Omega(0) = \frac{(1 - \Phi) \ln (1 - f_f)}{\ln (\Phi)} \quad (5) \quad K_M = -f_c \frac{\ln \Phi}{LAI_A} \Omega(0)
\]

Having available a variable \(K_M\), by either using formula 6 or by measuring directly \(K\) with a light sensor over and under the canopy, helps to increase accuracy of the LAI estimation (Poblete-Echeverria et al. unpublished).

2.2. Computational analysis of infrared thermography from canopies

Infrared image analysis of canopies is based on the computation of three reference temperatures, described as \(T_{dry}\), \(T_{wet}\), and \(T_{canopy}\). The first two thresholds can be obtained using physical reference leaves (Gowing et al., 1993; Idso, 1982; Jones, 1999a; Jones et al., 2002) and mathematically using the leaf energy balance method (Jones, 1999b; Jones et al., 2002).

2.2.1 Physical and estimated temperature thresholds to filter non-leaf material

Physical threshold temperatures can be obtained using “painted” leaves with petroleum jelly and water to obtain \(T_{dry}\) and \(T_{wet}\) respectively (Figure 2) (Jones et al. 2002; Fuentes et al. 2005). Thresholds obtained using the leaf energy balance method require micrometeorological data obtained in parallel with the infrared thermal images. The following algorithms describe the computation of these indices and the data required:

\[
T_{dry} - T_a = \frac{r_{HR} R_{ni}}{\rho c_p} \quad (7)
\]

where \(T_a\) is the air temperature measured at the same position and time as infrared thermography acquisition, \(r_{HR}\) is the parallel resistance to heat and radiative transfer, \(R_{ni}\) is the net isothermal radiation (the net radiation that would be received by an equivalent surface at air temperature), \(\rho\) is the density of air and \(c_p\) is the specific heat capacity of air. This formula uses the concept of isothermal radiation and assumes a dry surface with the same aerodynamic and radiative properties, in which the sensible heat loss will equal the net radiation absorbed (Jones, 1992).

\[
T_{wet} - T_a = \frac{r_{HR} r_{aw}}{\rho c_p [\gamma (r_{aw} + s)]} - \frac{r_{HR} \delta e}{\gamma (r_{aw} + s) + r_{HR}} \quad (8)
\]

were \(r_{aw}\) is the boundary layer resistance to water vapour transfer (assumed to be largely determined by the stomatal resistance), \(\gamma\) is the psychrometric constant, \(s\) is
the slope of the curve relating saturation vapour pressure to temperature, \( \delta e \) is the water vapour pressure deficit in the air.

Once obtained \( T_{\text{dry}} \) and \( T_{\text{wet}} \), \( T_{\text{canopy}} \) can be determined automatically by filtering infrared thermal images using matrix analysis as follows:

\[
A(m, n) = \begin{pmatrix}
T_{1,1} & \cdots & T_{1,n} \\
\vdots & \ddots & \vdots \\
T_{m,1} & \cdots & T_{m,n}
\end{pmatrix}
\]

(9)

where \( A(m,n) \) is the infrared thermal image with \( (m,n) \) pixels and temperature readings per pixel \( (T) \) in \(^\circ\text{C}\).

\[
A_{\text{if}} = T_{\text{wet}} \geq A_{(t,m,b)} \geq T_{\text{dry}} ; (A_{(t,m,b)} \neq A_{\text{if}}) \notin A_{\text{if}}
\]

(10)

Equation 10 describes the criteria used to filter images using \( T_{\text{dry}} \) and \( T_{\text{wet}} \) as thresholds. Physical thresholds allow the estimation of water stress indices from infrared thermal images in a semi-automatic manner.

2.2.3 Algorithms to compute water stress indices using physical thresholds
Crop water stress index (CWSI) can be calculated using equation 11. An index \((I_g)\), proportional to leaf conductance to water vapour transfer \((g_L)\) can be obtained (Eq. 12) using the relationship proposed by Jones et al. (2002).

\[
\text{CWSI} = \frac{T_{\text{canopy}} - T_{\text{wet}}}{T_{\text{dry}} - T_{\text{wet}}} \quad I_g = \frac{T_{\text{dry}} - T_{\text{canopy}}}{T_{\text{canopy}} - T_{\text{wet}}} = g_L\left( r_{\text{aw}} + \left( \frac{S}{Y} \right) r_{HR} \right)
\]

(11)  

(12)

2.3. Fluorescence microscopy analysis of berries
A MATLAB® R2008a code was created to analyse fluorescent digital images (Figure 3). This analytical tool allows the semi-automated measure of the following variables: i) berry perimeter \((P, \text{cm})\), ii) diameter \((D, \text{cm})\), iii) area \((A, \text{cm}^2)\), and iv) tissue vitality as percentage of living tissue \((\text{LT} \%)\).

Berry shrivel (ShI) was calculated as a normalised index (Fuentes et al., 2010):

\[
\text{ShI} = \frac{R - R_{\text{min}}}{R_{\text{max}} - R_{\text{min}}}
\]

(13)

where \( R \) is the ratio between the diameter and the perimeter for a single berry, \( R_{\text{max}} \) is the maximum ratio from all data sampled per variety, \( R_{\text{min}} \) is the minimum ratio from samples per variety. The ShI index ranges from 1 (maximum turgor) to 0 (maximum shrinkage).

2.4. Automated morphometric and colorimetric analysis of scanned organs
This code is capable of analysing automatically scanned images from leaves, berries and other organs to obtain and analyse morphometric parameters, such as: Area \( (\text{cm}^2)\), Perimeter \( (\text{cm})\), maximum and minimum length \( (\text{cm})\), eccentricity \( (\text{adimensional})\). From each image, the code is also capable of obtaining color using the RGB (red, blue and green) and the CieLab colour codes.

3. Results and Conclusions
Results have shown that the image analysis techniques developed have helped to gather and analyse significant amount of physiological and morphometric data from plants. From field measurements, the canopy architecture technique has offered a robust ground truthing method for established airborne or satellite remote sensing
techniques for eucalyptus trees (Fuentes et al. 2008) grapevines (Fuentes et al. unpublished) and apple trees (Poblete-Echeverria et al. unpublished). Image analysis techniques have also helped to characterise physiological relationships between berry cell death and living tissue in grapes (Fuentes et al. 2010) and to obtain key physiological data for management purposes from infrared thermography (Fuentes et al. 2012). All image analysis tools offer the possibility not only to automate the acquisition of images or videos but also automatically analyse the data in real time. Furthermore, image analysis tools can be associated to statistical analysis techniques, such as PCA, allowing automatic validation of data obtained (Figure 5).

References:
Feature of the image analysis protocol. (a) Image luminosity module can be activated to avoid inclusion of seeds in the image of the berry (Figure 2b). The GUI components are: (i) an intensity image for the original image (a). A binary or segmented image is calculated (b) by selecting an adequate threshold (white pixels) and the intensity image is produced from this masked image. A seed extraction percentage calculated from the comparison between the binary or segmented image and the original image Enhances the selection (line d in Figure 2c). The intensity component of the original or segmented image presents the dead tissue (Figure 2). The GUI components are: (i) an intensity histogram produced to detect the edge of berries. The first upward peak from the left corresponds to the background, the first valley to the edge of the berry and the second peak to the berry. An interactive cursor selector is used to obtain a suitable threshold. Inset (top right) presents an example of edge and diameter detection for a Chenin Blanc berry and the transect method to obtain the perimeter (dashed line a). Long axis (line c) to the minimum diameter (medial). The later is considered for the case of oblate spheroid shapes (e.g. Flame Seedless). In the case of ellipsoidal shaped berries (e.g. Red Globe), or the long axis in the case of prolate spheroid shapes (e.g. Seedless Ruby), the long axis can be used as the berry diameter automatically for ellipse fitting tool applied to a berry (Figure 1b). (b) Ellipse fitting tool applied to a berry and the transect method to obtain the perimeter (dashed line a). Long axis (line c) to the minimum diameter (medial). The later is considered for the case of oblate spheroid shapes (e.g. Flame Seedless). In the case of ellipsoidal shaped berries (e.g. Red Globe), or the long axis in the case of prolate spheroid shapes (e.g. Seedless Ruby), the long axis can be used as the berry diameter automatically for ellipse fitting tool applied to a berry (Figure 1b).

Figure 1: Digital RGB upward-looking digital image of almond trees (left) and filtered image (right) using the intensity component to isolate clouds. After filtering, the binary image is sub-divided for gap analysis and architectural parameter extraction.

Figure 2: Infrared thermal image (left) with physical thresholds from painted leaves. Filtered image is obtained using T\textsubscript{dry}, T\textsubscript{wet} and isolating non-leaf material through a matrix analysis (right). Filtered image is obtained using T\textsubscript{dry}, T\textsubscript{wet} and isolating non-leaf material through a matrix analysis (right).

Figure 3: Automatic analysis of fluorescent microscopy images of berries (left). Once recognised the contour of berries, morphometric analysis is made to obtain the berry shrivel index (ShI) using formula 13 (right).
Figure 4: Output of scanned image analysis to extract morphometric and colorimetric parameters from objects (grapevine leaves, cv. Chardonnay) identified automatically as blobs. Fractal dimension (Fd) was obtained using the "Boxcount" function from File Exchange - Matlab central (Frederic Moisy, 2006). Fractal dimension separates petioles from leaves for box sizes higher than $10^2$.

Figure 5: Principal component analysis (PCA) from data obtained by the morpho-colorimetric analysis from grapevine leaves.