Report on the definition of PocketPlant3D thresholds for quantifying initial water stress

Sub-action B1.1

31.12.2022

Contents

Abstract

This deliverable focuses on the activities conducted during the first two years of the project to define relationships between canopy architecture and physiological variables of plant water conditions (e.g., stomatal conductance) for multiple grape cultivars, with the ultimate goal of identifying thresholds of synthetic indexes of canopy architecture corresponding to well-known thresholds of leaf stomatal conductance for moderate and severe water stress.

Dedicated field activities were conducted in 2021 and 2022, involving seven cultivars evaluated in open field or in pots with water treatments applied. Multiple samplings were conducted during the season, to explore a wide range of plant water status. Each time, measurements involved both synthetic indexes of canopy architecture (e.g., parameter χ of the Campbell' ellipsoidal distribution estimated with the app PocketPlant3D) and water stress physiological variables (e.g., stomatal conductance and net assimilation derived with a portable gas exchange analyser, leaf water potential measured with a pressure chamber). Overall, 671 leaves were sampled for physiological measurements and 5631 leaves for canopy architecture.

Results highlighted clear relationships between canopy architecture (parameter χ) and stomatal conductance, with R^2 ranging from 0.71 to 0.97 (0.85 on average) according to the grape variety analysed. Correlation of canopy architecture with leaf water potential and net assimilation was instead less clear (R^2 equal to 0.47 and 0.63 respectively, on average), highlighting the role of factors other than water stress in defining these specific physiological responses. The good results achieved for stomatal conductance allowed to use the relationships to identify the values of χ corresponding to a stomatal conductance of 200 mmol m^{-2} s^{-1} (well-known threshold for moderate stress in grapevine) and 100 mmol m^{-2} s^{-1} (well-known threshold for severe stress) for each variety. This would enable farmers to easily detect stressful conditions by simply using the MT, which implements the app PocketPlant3D (see deliverable B2.1 for details on MT functions), and the identified thresholds for χ.

Results also allowed to define a protocol for canopy architecture measurements. The analysis of the data collected with the app showed indeed how the angles of around ten leaves are enough to derive reliable estimates of canopy architecture, thus confirming the suitability of this approach for water stress evaluation under operational contexts (only few minutes are needed for conducting these measurements, few seconds per leaf).

Introduction

This deliverable focuses on the field activities conducted during 2021 and 2022 to define relationships between canopy architecture (evaluated with the app PocketPlant3D as implemented in the project's app PocketDRIVE) and water stress physiological variables for seven grape cultivars. The ultimate goal was to use such relationships to define threshold values of synthetic indexes describing canopy architecture corresponding to well-known thresholds of stomatal conductance for moderate and severe water stress. This would allow to use the MT to easily and promptly detect early water stress in vineyards (see deliverable B2.1 for details on the MT functions and on the app PocketDRIVE).

Field activities

To derive a first evaluation of the relationships between canopy architecture and plant water stress (*milestone B1.3*), dedicated field activities were conducted by UNIMI and UCSC **during 2021** in two demo-vineyards, one in Ziano Piacentino (Piacenza province) with the cultivar Croatina, and one in Castel San Giovanni (Piacenza province) with the cultivar Malvasia di Candia Aromatica (Malvasia hereafter). Three sampling events were conducted from mid-June to mid-August, with both measurements of plant water status and canopy architecture acquired at each event.
Measurements of plant water status and canopy architecture were conducted at five points for each vineyard during the field visits with no clear water stress (i.e., June 21 and July 30), and for each inter-row treatment within each vineyard during the last sampling (August 10, marked water stress), to better capture the within-field variability in plant water status. In the demo-vineyards indeed, different inter-row treatments affecting soil water storage capability were being applied according to the planned activities of action B2.
Physiological measurements of plant water status were conducted by UCSC using a portable gas exchange analyser (ADC Biosystems) – for evaluating stomatal conductance (gs, mmol m^{-2} s^{-1}), transpiration (E, mmol m^{-2} s^{-1}) and net assimilation (A, mmol m^{-2} s^{-1}) – and a pressure chamber, to measure leaf water potential (ΨL, MPa). Canopy architecture was evaluated by UNIMI using the app PocketPlant3D (as implemented in PocketDRIVE) to collect the angles (i) of the same leaves on which physiological measurements of water status were acquired and (ii) of additional leaves sampled in the same area (around 20 additional leaves, on average). These additional samplings were carried out to define a protocol of acquisition that provides the best compromise between a small number of leaves to be measured and a high accuracy of canopy architecture estimates. All measurements were conducted around midday. Overall, 124 leaves were sampled for physiological measurements, and more than 650 leaves for canopy architecture.

Additional field activities were carried out **in 2022** (*milestone B1.6*), involving three grape varieties (Malvasia, Pinot Noir, Sangiovese), which were grown in demo-vineyards (Malvasia, Pinot Noir) or in pots with irrigation treatments applied (Sangiovese, Pinot Noir). The choice of conducting pot experiments was due to the need of ensuring the exploration of a wide range of plant water conditions within a single season. Pot experiments were set up by UCSC in Piacenza. Nine sampling events were conducted from June to August to explore a wide range of water stress conditions, using exactly the same protocol of measurements adopted in 2021 (UNIMI conducted PocketPlant3D measurements, UCSC those with ADC and pressure chamber).

To extend the analysis to more cultivars and to collect more data for improving the robustness of results, **complementary activities outside LIFE** were carried out by UNIMI during 2021 and 2022.
In 2021, the same protocol of data acquisition described above was used to collect measurements of canopy architecture and physiological variables of plant water status for the cultivar Chardonnay grown in two vineyards, one in Adro (Brescia province) and one in Monzambano (Mantova province). Both vineyards had irrigation treatments applied (including one not irrigated and one fully irrigated), which allowed to explore a wide range of plant water status during a single season.
In 2022, additional data were collected on Chardonnay in the Monzabano vineyard and on Pinot Blanc, Sangiovese, and Montepulciano grown in pots at UNIMI with water treatments applied. The same protocol of data acquisition previously described was used, with measurements of canopy architecture collected with the app PocketPlant3D, data of stomatal conductance (gs, mmol m^{-2} s^{-1}), transpiration (E, mmol m^{-2} s^{-1}) and net assimilation (A, mmol m^{-2} s^{-1}) collected with a portable gas exchange analyser, and data on leaf water potential (ΨL, MPa) derived with a pressure chamber.

Overall, the dataset collected in the two-year campaign refer to seven cultivars (Croatina, Chardonnay, Malvasia, Montepulciano, Pinot Blanc, Pinot Noir, Sangiovese), and it includes data from 671 leaves sampled for physiological measurements and 5631 leaves for canopy architecture.

Data analysis

Leaf angles collected with PocketPlant3D (θL) were used to estimate two synthetic indices of canopy architecture: the parameter χ (unitless) of the Campbell's ellipsoidal leaf angle distribution (Campbell, 1990; Eq. 1) and the light extinction coefficient of solar radiation (k; Eq. 2) (Campbell, 1986).

The parameter χ represents the ratio between the horizontal and the vertical semi-axis of an ellipsoid, thus providing a synthetic representation of the degree of erectness of the photosynthetic tissues (Campbell, 1986, 1990). The lower the value of χ, the higher the tendency of the distribution to approximate a prolate spheroid (erectophile canopy).

The parameter χ is estimated as follows:

$$\chi = -3 + \left(\frac{MTA}{9.65}\right)^{-0.6061} \tag{1}$$

Where MTA is the mean tilt angle (rad), estimated as the complementary of θL because it represents the angle between the normal to the screen and the zenith (Campbell, 1990).

The extinction coefficient for solar radiation (k, unitless) was then estimated by using the parameter χ according to Eq. 2 (Campbell, 1986):

$$k = \frac{\sqrt{\chi^2 + tan^2\theta_L}}{A} \tag{2}$$

Where A was calculated as proposed by (Campbell, 1990):

$$A \approx \chi + 1.774\,(\chi + 1.182)^{-0.733} \tag{3}$$

Relationships between the values of χ and k and physiological variables describing crop water status (stomatal conductance, net assimilation, and leaf water potential) were evaluated through linear regression for each cultivar separately. In case of cultivars evaluated during both 2021 and 2022 (Malvasia, Chardonnay), data from the two years were pooled together. Relationships between canopy architecture and stomatal conductance were defined by considering values averaged for each combination sampling date × water treatment for pot experiments and sampling date × inter-row treatment for field experiments.

Such relationships were then used to derive the threshold values of χ and k corresponding to a stomatal conductance (gs) of 200 mmol m^{-2} s^{-1} (threshold for moderate stress) and 100 mmol m^{-2} s^{-1} (threshold for severe stress). There is indeed a general consensus in the literature that well-watered vines have midday gs values higher than 300 mmol·m^{-2} ·s $^{-1}$, moderately stressed vines have midday gs values close to 200 mmol·m^{-2} s $^{-1}$, and severely-stressed vines have gs values lower than 100 mmol·m^{-2} s $^{-1}$ (e.g., Williams and Araujo 2002; Bellvert et al., 2014). Information about similar thresholds regarding leaf water potential were also available, but the best results achieved for stomatal conductance (as described in the following chapter) led to use this physiological variable for defining χ and k thresholds.

Results and discussion

Results showed that the experimental activities allowed to explore a wide range of crop water status, with conditions close to optimum during the early field visits and for well-watered treatments, and water stress increasingly higher while moving through the summer, especially for not irrigated treatments. Overall, observed values of stomatal conductance ranged indeed between values lower than 5 mmol·m^{-2} s $^{-1}$ to values higher than 600 mmol·m^{-2} s $^{-1}$.

In general, a good agreement was found between plant water status and canopy architecture, without clear differences due to the index of canopy architecture used (parameter χ or light extinction coefficient). For this reason, results are hereafter discussed only for the first index (χ), being the light extinction coefficient derived from it (Eq. 2).

Figure 1 shows the relationships found between canopy architecture and stomatal conductance for the cultivars Montepulciano, Sangiovese and Pinot Blanc, which were grown in pots with water treatment applied. Figure 2 refers instead to relationships found for cultivars Malvasia, Chardonnay, and Pinot Noir evaluated in open field in 2022 and for cultivar Croatina evaluated in open field in 2021. For Malvasia and Chardonnay data from the first year of the project were also available, so all the data (2021 and 2022) were pooled together to derive more robust relationships between canopy architecture and stomatal conductance (Figure 3).

Results highlighted clear relationships between canopy architecture and stomatal conductance, regardless of the cultivar analysed. The R^2 ranged indeed between 0.71 and 0.97 (0.85 on average) according to the grape variety analysed. Best results were achieved for Pinot Blanc and Moltepulciano (R^2 equal 0.97 and 0.96, respectively), whereas Malvasia and Sangiovese showed the lowest agreement (R^2 eqal to 0.72 and 0.71, respectively). Nevertheless, all the relationships were statistically significant (*p*-value <0.001).

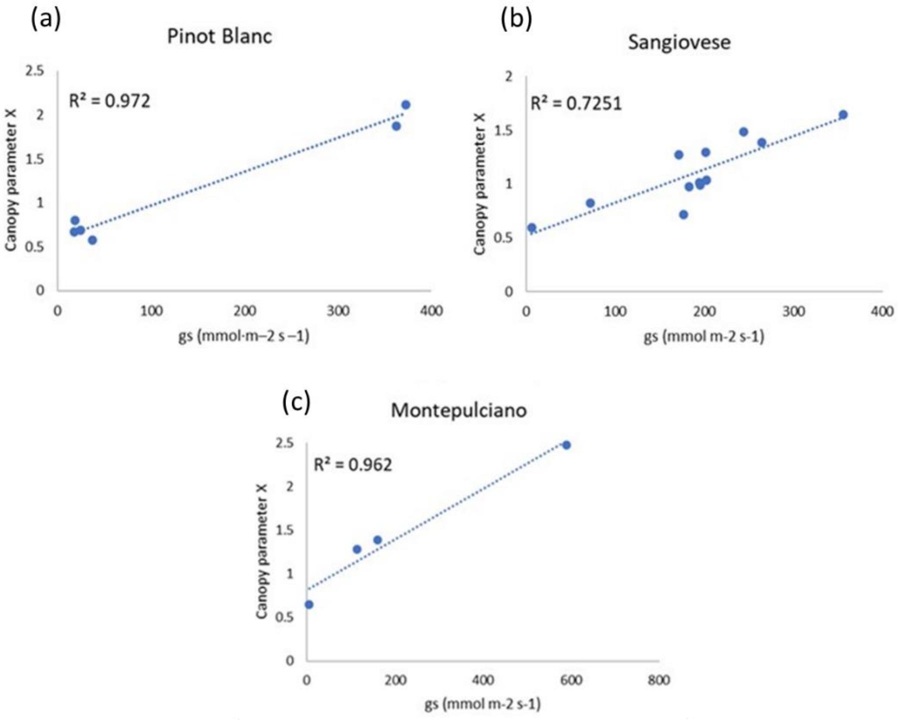

Figure 1. Relationships between canopy architecture (χ parameter of the Campbell's elipsoidal distribution) and stomatal conductance for three cultivars evaluated in pot experiments. All the measurements were collected at midday. Each point represents the average values for each combination sampling event × water treatment. Cultivar Sangiovese has more data because it was involved in both UNIMI and UCSC experiments.

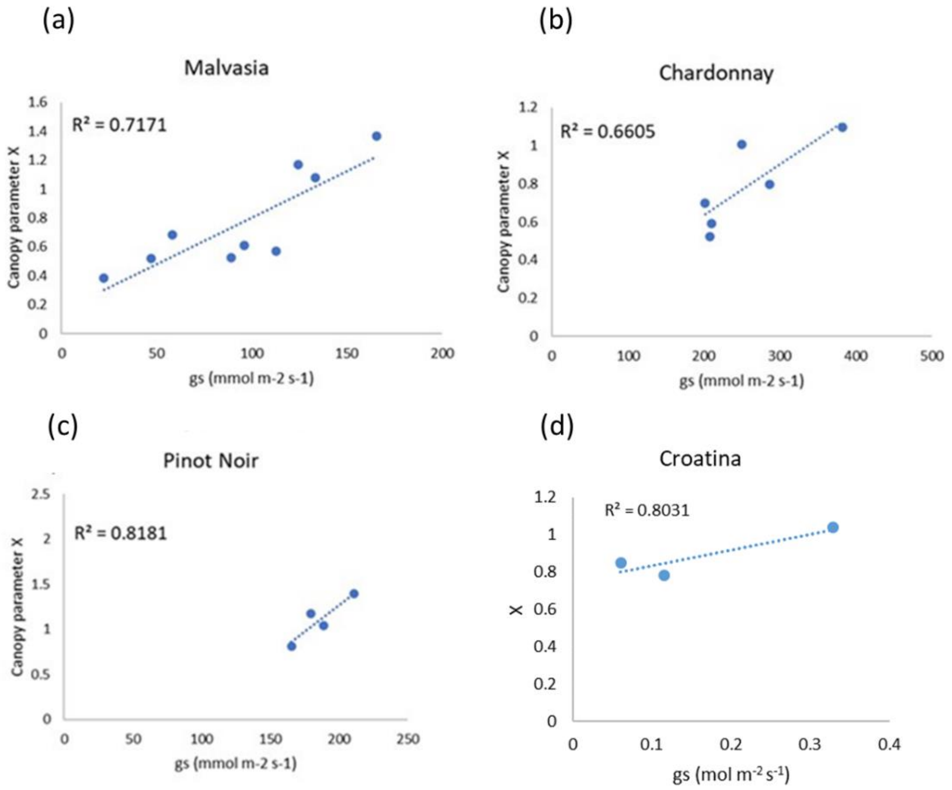

Figure 2. Relationships between canopy architecture (χ parameter of the Campbell's elipsoidal distribution) and stomatal conductance for cultivars Chardonnay, Malvasia, Pinot Noir and Croatina grown in the field. All the measurements were collected at midday. Each point represents the average values for each combination sampling event × inter-row treatment.

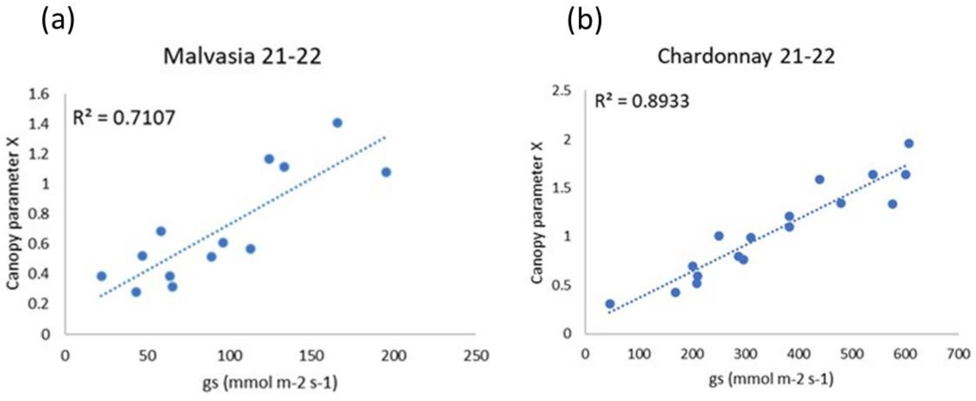

Figure 3. Relationships between canopy architecture (χ parameter of the Campbell's elipsoidal distribution) and stomatal conductance for cultivars Chardonnay and Malvasia obtained by pooling together data from 2021 and 2022. All the measurements were collected at midday. Each point represents the average values for each combination sampling event × inter-row treatment. Being based on more data, these relationships were those used to define the threshold values of χ for moderate and severe water stress for these two cultivars (see Table 1).

Correlation of canopy architecture with leaf water potential and net assimilation was instead less clear (R^2 equal to 0.47 and 0.63 respectively, on average), highlighting the role of factors other than water stress in defining these specific physiological responses. Examples of the relationships found between canopy architecture and net assimilation are reported in Figure 4, which highlights how the correlation was satisfactory in same cases (Pinot Blanc, Montepulciano, Malvasia) and decidedly less in others (Sangiovese). Poor agreement between canopy architecture and net assimilation was found also for the cultivar Croatina (R^2 of 0.11) and for the cultivar Chardonnay (R^2 equal to 0.37).

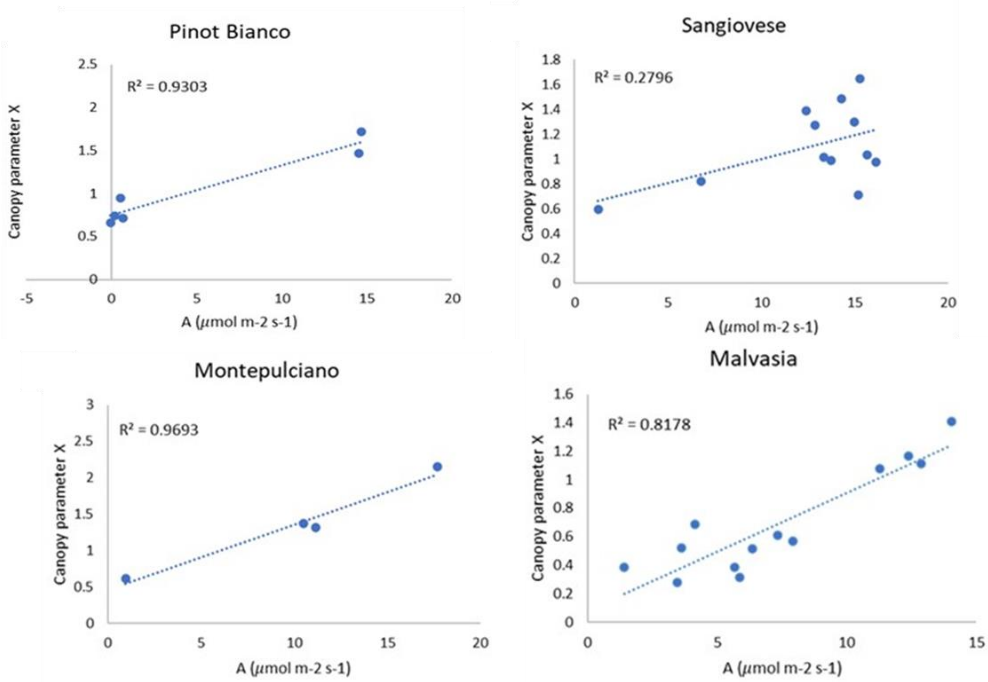

Figure 4. Relationships between the χ parameter of the Campbell's elipsoidal distribution and net assimilation for four sample varieties.

This led to use the relationships between χ and stomatal conductance as the most reliable to derive the thresholds values of χ indicating the onset of water stress. The calibration curves obtained are reported in Table 1, together with the χ values representing thresholds for moderate and severe water stress. These thresholds are those currently implemented in the beta version of PocketDRIVE.

Table 1. Calibration curves derived from field data collected in 2021 and 2022 to identify the threshold values of χ corresponding to a stomatal conductance of 200 mmol m^{-2} s^{-1} (threshold for moderate water stress) and of 100 mmol m^{-2} s^{-1} (threshold for severe water stress). These relationships are also shown in Fig.1 (Sangiovese, Montepulciano, and Pinot Blanc), Fig. 2 (Croatina and Pinot Noir) and Fig. 3 (Malvasia and Chardonnay).

			Threshold value of χ	
Cultivar	**R^2**	**Calibration curve**	**Moderate stress**	**Severe stress**
Montepulciano	0.96	χ = 0.0029 · gs + 0.8173	1.4	1.1
Pinot Blanc	0.97	χ = 0.0038 · gs + 0.5894	1.35	0.96
Malvasia	0.71	χ = 0.0061 · gs + 0.121	1.34	0.73
Pinot Noir	0.82	χ = 0.0117 · gs - 1.0642	1.28	0.11
Sangiovese	0.73	χ = 0.0031 · gs + 0.5184	1.14	0.83
Chardonnay	0.89	χ = 0.0027 · gs + 0.099	0.64	0.37
Croatina	0.8	χ = 0.0085 · gs + 0.074	0.92	0.83

The variability observed between the calibration curves obtained for the different cultivars led to retain cultivar-specific calibration curves at this stage. Merging the data from multiple cultivars led indeed to a marked reduction of R^2. Neverthessl, further analysis would be directed towards understanding the possible role of plant stomatal behaviour (isohydric and anisohydric) on the relationships between canopy architecture and stomatal conductance. Preliminary analysis highlighted how some varieties show a marked reduction in χ (thus more vertical leaves) even at low water stress levels (e.g., Chardonnay, Figure 5), whereas others like, e.g., Pinot Noir, show a steep decrease in χ only when severe stress levels are reached. However, more data are need to support these preliminary results.

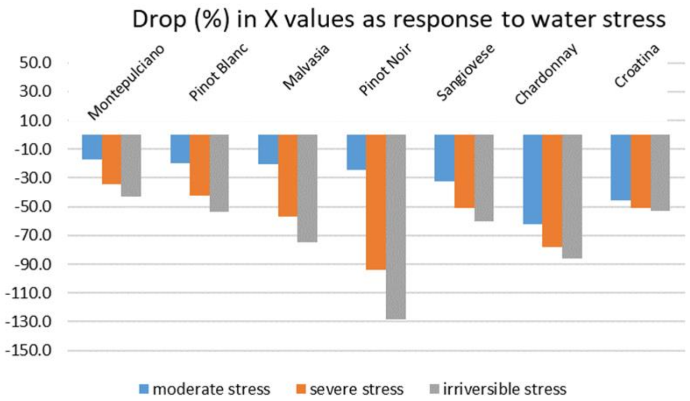

Figure 5. Percentage variation of χ values for moderate (blue bars), severe (orange bars) and irreversible (grey bars) water stress as compared to well-watered conditions for the seven cultivars analysed. These χ values were derived by using the relationships sreported in Table 1 and the values of stomatal conductance of 300, 200, 100, and 50 mmol m^{-2} s^{-1} for, respectively, well-watered, moderate stress, severe stress and irreversible stress.

The activities conducted in 2021 and 2022 also allowed to define a protocol for canopy architecture measurements. The analysis of of the data collected with the app showed indeed how the angles of around ten leaves collected in the middle of the canopy (Figure 6) are enough to derive reliable estimates of canopy architecture, thus confirming the suitability of this approach for water stress evaluation for operational contexts (only few minutes are needed for conducting these measurements, few seconds per leaf). This protocol has been implemented in the app PocketDRIVE, which requires the angles from at least ten leaves to estimate χ and provide indications about the actual occurrence of water stress.

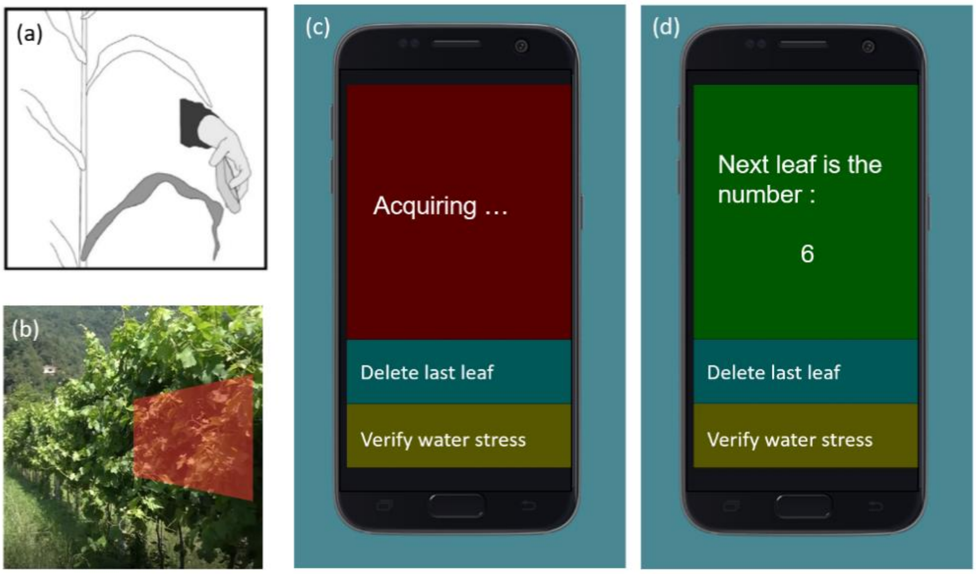

Figure 6. Collection of leaf angles with the app PocketDRIVE to evaluate canopy architecture and verify water stress occurrence. The device has to be kept parallel to the leaf main axis (a). The angle collection should involve leaves in the middle part of the canopy (b) and can be started by clicking on the red button (c). The app keeps count of the leaves measured (d) because at least 10 leaves are needed to derive reliable estimates of canopy architecture.

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