Rapid Screening of Apple Genotypes for Drought Tolerance by a Simplified Model of Canopy Conductance

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ABSTRACT

Expansion of apple growing to dry areas requires reliable approaches to select genotypes tolerant to water stress. We have simulated apple tree transpiration ($E_c$) from different commercial orchards and compared it with actual transpiration ($E_a$) derived from sap flow measurements. The modelling approach lies in rearranging a parameterized P–M Equation and incorporating in it a stomatal conductance model derived from meteorological variations. The model uses diurnal courses of variables instead of commonly used daily means. We then wrote a complex P–M Equation which already includes the canopy conductance as the function of radiation and vapour pressure deficit. For model calibration, an extensive experimental dataset of tree transpiration was collected from 2011 to 2013 in various sites of Albania and Kosovo, ranging from very warm to temperate. The daily pattern of canopy conductance for water vapour at canopy level provided by the model offers an unprecedented insight into rapid response to humidity of different cultivars as well as their light saturation levels. The satisfactory performance of this functional model should improve the precision of current empirical approaches followed to compute fruit tree water requirements and can be a promising tool for early and quick evaluation of water stress tolerance in apple genotypes.

Keywords: Irrigation, Modelling, Sap flow, Stomatal kinetics.

INTRODUCTION

Climatic changes are rapidly shifting the focus of fruit breeding programs to selection for tolerance to abiotic stress, in particular drought stress. Drought is a major factor limiting yields of fruit trees in many areas, but irrigation is often either not possible or uneconomic, so that much effort has been (and is being) devoted to breeding drought–tolerant cultivars (Jones, 2014). This is particularly relevant for irrigation in fruit growing in arid and semi–arid areas, like the Mediterranean. Here, water demand for irrigation is very high and is rising given the increasing world demand for fruits and the high water productivity of perennials as compared to that of many annual crops; moreover, large investments required and high production costs make the optimal use of water critical for the sustainability of commercial orchards (Villalobos et al., 2013). Improvement of water use efficiency and quality in agricultural practices remain a great challenge. This requires, among others, a better understanding of transpiration from plant canopies (Kumar et al., 2012). Drought events and heat waves that are supposed to increase in frequency and intensity have increased the importance of knowing what to do. Moreover, we need to study how fruit trees react to such climatic variability. Rapid transition to clonal rootstock with a less extensive root system has made the fruit trees that we grow more vulnerable to water stress. This is particularly important under
Mediterranean growing environments, which alter the ecophysiology of fruit trees, especially during summer as a consequence of the combined effects of high light, high air temperature, high vapour pressure deficit and low rainfall (Xiloyannis et al., 2012). In many cases, different cultivars are planted within the same orchard and beside different water requirements, similar irrigation rates are applied reducing the water use efficiency (WUE) (Kullaj et al., 2013).

Because of the central role that stomata have in the control of water loss, hence drought tolerance, much effort has been concentrated on the use of stomatal characters in physiological breeding for drought tolerance, especially stomatal closure (Jones, 1987). Stomata that close only in response to severe stress may be better for an agricultural crop than stomata that maintain a consistently low conductance (Jones, 1987). Similarly, stomatal closure in response to increasing humidity deficit (Jones, 1983) would also tend to enhance water-use efficiency and drought tolerance by restricting stomatal opening to the morning and evening hours of low evaporative demand (Jones, 1987). However, due to biological and environmental variability (particularly with tree fruits in the field), direct measurement of canopy conductance with porometers requires a large number of measurements to distinguish different genotypes (Jones, 1987). Modelling is an approach to overcome such a difficulty. There are a number of transpiration models varying in physiological detail (Dekker et al., 2000) but their application outside the academic environment has frequently failed, especially due to high variable requirements which introduce inaccuracy in measuring the actual transpiration as well as modelling it. Canopy conductance is another very useful plant-based stress indicator (Kullaj et al., 2014). Although a number of empirical models have been proposed to describe stomatal behaviour in the field, continuous and real-time calculation remains difficult and expensive. Thus, there are still gaps in terms of a model explicitly oriented to detect water stress using fewer meteorological variables broadly available to fruit growers and simple measurements in the field. Due to the complexity of fruit tree architecture, results from screening young potted plants differ significantly with mature genotypes in field collections. Furthermore, the ideotype for any environment involves a compromise between water conservation and productivity mechanisms with the optimum balance depending on the aridity of the environment. This would depend mainly on stomata behaviour. Thus, a fast and reliable screening method for stomata behaviour and transpiration would be highly appreciated by the fruit breeder.

Our research has sought to develop a simplified modelling approach to screen tree fruit genotypes for their canopy conductance behaviour using minimum variables available by growers.

**MATERIALS AND METHODS**

**Modelling**

The followed modelling approach takes into consideration the tighter coupling of fruit tree canopies to the atmosphere due to the large roughness (Villalobos et al., 2009), implying that transpiration will be mainly modulated by canopy conductance and leaf to air vapour pressure deficit \( D \) (Dragoni and Lakso, 2008) as opposed to short crops (including the reference grass surface), where transpiration is mostly dependent on solar radiation (Jarvis, 1985). The modelling procedure is based on the calculation of the potential evapotranspiration based on physical analysis at three levels: (a) Penman Equation; (b) Monteith–modified Penman Equation, (c) Parameterization of P–M Equation with respect to dependence of canopy conductance on meteorological variables (Kučera et al. 2016). Penman Equation (Penman, 1948) describes water evaporation from a homogeneous short trimmed lawn well saturated with water.
according to the formula, here in terms of energy \[\text{Equation (1)}\].

\[\lambda E_p = \frac{\Delta(R_n - G) + \gamma Da(1 - b \cdot w)}{\Delta + \gamma} \]

Where, \(R_n\): Net radiation (W m\(^{-2}\)); \(G\): Soil heat flux (W m\(^{-2}\)); \(D\): Vapour pressure deficit of the air (Pa); \(w\): Wind speed (m s\(^{-1}\)); \(\gamma\): Psychrometric constant = 66 Pa K\(^{-1}\); \(\Delta\): Slope of saturation water vapour pressure deficit (Pa K\(^{-1}\)); \(\lambda\): Water heat capacity = 2.45e6 J kg\(^{-1}\); \(a, b\): Empirical parameters.

To reduce the variables required, as the dataset seldom contains direct measurement of net radiation and soil heat flux, we calculated net Radiation \((R_n)\) from global Radiation \((R_g)\) by estimated albedo and soil heat flux is neglected. As in many cases wind data are not accurate or available to fruit growers, we replaced the uncertain second “wind” part of Penman Equation with a turbulent diffusion theory based expression \[\text{Equation (2)}\].

\[\lambda E_p = \frac{\Delta(R_n - G) + \rho c_p D g_o}{\Delta + \gamma (1 + g_o \gamma_{\text{canopy}})} \]

Where, \(c_p\): Specific heat of air = 1004 J kg\(^{-1}\) K\(^{-1}\); \(\rho\): Density of dry air = 1.22 kg m\(^{-3}\); \(g_o\): Aerodynamic conductance (ms\(^{-1}\)).

The (wind speed dependent) aerodynamic conductance was calculated from canopy parameters \[\text{Equation (3)}\].

\[g_o = \frac{k^2 u}{\ln^2 \left(\frac{z-d}{z_0}\right)} \]

Where, \(g_o\): Aerodynamic resistance as the function of wind speed (ms\(^{-1}\)); \(k\): von Karman constant (dimensionless); \(u\): Wind speed (ms\(^{-1}\)); \(d\): Zero plane displacement (3 m); \(z\): Wind speed measurement height (3 m).

Parameterization of Penman–Monteith Equation was based on the assumption that the stomatal conductance depends on intercepted solar radiation (Monteith, 1977) and \(D\) values according a suitable formula (e.g., Lohammar et al., 1980). This takes into consideration the fact that in tall, discontinuous canopies (like orchards), transpiration processes are strongly coupled, in particular, with atmospheric \(D\) (Dragoni and Lakso, 2008). Thus, we account for the main difference with other transpiration models that use grass as a transpiration reference.

Parameterization process is usually based on daily mean values of main environmental factors \((R_n, D)\) and the most common approach is to rearrange the P–M Equation for canopy conductance and then to find the best fit to \(R_n\) and \(D\). The common Lohammar Equation describing the \(R_n\) and \(D\) influence on canopy conductance to water vapour is written in the form \[\text{Equation (4)}\] supposing stomata opening due to solar activity and closure due to high evaporating demands.

\[g_o = \frac{R_g}{R + R_0} \left[1 + aD\right] \]

Where, \(R_g\): Global solar radiation; \(R_0\): Minimum values of solar radiation for stomata opening; \(a\): Empirical parameter; \(D\): Vapour pressure deficit of the air (Pa).

Contrary to this approach, we have used diurnal courses of variables instead of commonly used daily means (Kullaj et al., 2014). For the parameterization and experimental validation of transpiration of this model we used sap flow measurement (see below).

We have used a different formula \[\text{Equation (5)}\]. Describing the influence of \(D\) on stomata closure and the parameterization process is performed as a direct non-linear multi-regression analysis of P–M Equation.

\[g_o = \frac{R_g}{R + R_0} \left[0.5 \cdot \frac{1}{\pi} \arctg\left(\frac{D}{a}\right) + g_m\right] \]

Instead of standard rearrangement of P–M Equation for \(g_o\) calculation and following discrete regression analyses (Kullaj et al., 2016a, Kullaj et al., 2016b) for \(R_n\) and \(D\) we wrote a complex P–M Equation which already includes the \(g_o\) as the function of \(R_n\).
and $D$ [Equation (6)]. Transpiration ($E$) is calculated using a scaling procedure from sap flow data.

$$E = \frac{(\Delta + \gamma)(1 + \frac{R_o}{R_s} \cdot \frac{g_{\text{lim}}(0.5 - \frac{1}{\pi} \arctan(D/b) + g_{\text{min}})}{\lambda})}{\lambda}$$

(6)

Using a nonlinear multiregression analysis provided by Mini32 software, PrgmClc module, parameters $a$, $b$, $R_o$, $g_{\text{lim}}$ and $g_{\text{min}}$ best fitting to (usually sap flow based) measured canopy transpiration and the one calculated by P–M Equation are obtained. The P–M Equation for the fit analysis was written but for faster analysis, the numerator is calculated separately. The fit module estimates the time lag between radiation and sap flow using a cross-correlation analysis. The programming code is created automatically although an editing is advisable. During the calculation, a new file with calculated canopy transpiration variable is created. The model was calibrated by regressing the observed values for each experimental dataset.

**Experimental Setup**

In all the field experiments conducted from 2011 to 2014 for the purposes of calibrating the transpiration model, tree transpiration was measured with sap flow sensors EMS 62 (EMS Brno, CZ www.emsbrno.cz), based on SHB (Stem hHeat Balance) method (Lindroth et al., 1995) (Čermak, 2004). Sensors were installed on the main leader on trees for each cultivar, using a special cordless drill for the holes. The measuring interval was every minute with 1 s warm-up and storing interval every 15 minutes. A portable meteorological station Minikin RTHi (EMS, Brno). Gypsum block’s sensors were used to measure Soil Water Potential (SWP) at two different depths, 20 and 40 cm respectively. For calibration of the model, no water deficit was applied during most of the period. For the purposes of evaluating changes in stomatal behaviour between cultivars and eventually closure during water deficit, irrigation was withheld at the end of the measuring period, to produce a peak of water deficit of $-3.0$ MPa. Plant materials used in each experiment were 5 trees for each commercial cultivars of apple, which are frequent in cultivation but with some distinctive behaviour, namely ‘Golden Delicious’ (GD), ‘Idared’ (ID), ‘Gala’ (GL), and ‘Pink Lady’ (PL). Trees were trained as central leader and were grafted either on the M9 or Pajam 2 (P2) rootstocks. Replicates were selected based on their similar values of trunk diameter and other biometric measurements, i.e. scion/rootstock affinity index (Kullaj et al., 2014), vigour, number of branches and shoots, etc.

The locations of each experiment and measurement periods were deliberately selected in order to represent a graduation in terms of heat represented by the Heliothermic Index (HI) (Huglin, 1978), varying from temperate to very warm. Thus, in 2011, the experiment (Exp. 1) was conducted in a 5–year old commercial orchard of 2.5 ha. The orchard was in the area of Lushnja (40° 58’ 33 N 19° 40’ 16 E) categorised as very warm following the HI index. Measurement took place during July. In 2012, we repeated the measurements in the same plot in Lushnja from May to July (Exp. 2). In the same year, another experiment (Exp. 3) was conducted in a commercial orchard of 2 ha, in the 5th year in Tirana (41° 25’ 30 N 19° 42’ 02 E) categorised as temperate warm. Measurements were conducted from May to July. In 2013, the experiment (Exp. 4) was conducted in a small experimental orchard in Mitrovica, Kosovo (42° 50’ 18” N 20° 50’ 29” E), categorised as temperate under
this scale. Measurement took place from July to September. Some details of all experiments are given in Table 1.

**RESULTS**

**Environmental Conditions**

Differences in terms of temperatures from one location to the other as well as measuring period is shown by the intra- and inter-annual variation in meteorological variables driving transpiration of apples trees. Data on net radiation and air temperature for each experiment are given in Table 2. Meteorological variables for Experiments 2 and 4, representing two contrasting climates, a very warm one and temperate as well as two different periods are given as supplemental files.

**Vapour Pressure Deficit and Potential**

Vapour pressure Deficit (D) and Potential Evapotranspiration (PET) rates which are highly correlated varied among sites and through the measuring periods usually increasing from spring to summer and then decreasing again towards autumn [Figure 1, (A and B)]. Net radiation values averaged 454.8 W m\(^{-2}\) with maximum values above 1,000 W m\(^{-2}\). There were very few cloudy days which means there was no limitation to stomata opening. Average temperature did not differ too much between sites (±25.7°C) and was constantly high, especially during June – August, leading to high D and PET values with maxima averaging 4.6 MPa and 0.86 mm h\(^{-1}\) respectively (see Table 2).

**Tree Transpiration**

Transpiration rate depends on a balance among water supply to the plant, energy...
supply to evaporate water within the leaf and the ease with which water vapour can escape from the leaves (Forbes and Watson, 1992). These processes are dominated by two environmental factors, namely solar radiation and the water potential of the atmosphere. Tree transpiration (E) was variable among experiments and through the year but more variable in spring and in autumn, as weather conditions were also more variable (summer is often steadily hot and dry in the Mediterranean climates where the experiments took place). Table 3 shows both observed (Ea: Actual transpiration from trees) and simulated (Ec: Calculated transpiration) transpiration rates for the entire dataset of experiments. Calculation of modelled E values in PrgmClc module was approached directly from the fit module. Maximum transpiration rates in all our

![Figure 1](image-url)
Table 3. Comparison of actual transpiration and calculated transpiration values for the entire dataset of all experiments and cultivars. Data did not include rainy days or conditions of water deficit.  

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>$E_a$ (mm d$^{-1}$)</th>
<th>$E_c$ (mm d$^{-1}$)</th>
<th>$r$</th>
<th>RMSE (mm d$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Max</td>
<td>Average</td>
<td></td>
<td>Max</td>
</tr>
<tr>
<td>Golden Delicious</td>
<td>4.9</td>
<td>1.2</td>
<td>4.2</td>
<td>1.4</td>
</tr>
<tr>
<td>Pink Lady</td>
<td>7.4</td>
<td>1.8</td>
<td>7.3</td>
<td>2.2</td>
</tr>
<tr>
<td>Gala</td>
<td>5.7</td>
<td>1.4</td>
<td>5.2</td>
<td>1.7</td>
</tr>
<tr>
<td>Idared</td>
<td>6.1</td>
<td>1.5</td>
<td>6.0</td>
<td>1.8</td>
</tr>
<tr>
<td>Average</td>
<td>6.0</td>
<td>1.5</td>
<td>5.7</td>
<td>1.8</td>
</tr>
</tbody>
</table>

$E_a$ = Actual or observed transpiration; $E_c$: Calculated or simulated transpiration from Equation (6), RMSE = Root Mean Square Error.

experiments (Table 3) and for each cultivar are similar to published values of maximum transpiration rates in the summer (in the range 2.96–7.45 mm d$^{-1}$) with the same age (Dragoni et al., 2005, Gong et al., 2006, Gong et al., 2007, Villalobos et al., 2013) although similar rates have been found in studies using older trees (Cohen and Naor, 2002, Auzmendi et al., 2011, Green and Clothier, 1999). A rational comparison of these values is not straightforward for several reasons. Most studies provide only a few days of measurement (Auzmendi et al., 2011). In some cases transpiration was limited by water deficit (Dragoni et al., 2005, Gong et al., 2006, Gong et al., 2007) and there are many differences between these studies in terms of cultivars and rootstocks, plant vigour, training system, Leaf Area Index (LAI), intercepted radiation, etc. The presence of intercepted water on the foliage after rainfall events adds further variability (Villalobos et al., 2013). Average rates are relatively low as they include many zero values or very low values during hours with no or very low light. Table III presents also the Pearson correlation coefficients (R) and the Root Mean Square Error (RMSE) (in mm d$^{-1}$) of the results from comparing Equation (6) with the given parameters to the observed or actual transpiration ($E_a$).

As the datasets of various experiments follow the same behaviour and to reduce the number of figures and tables, Figure 2 shows a representative sample of actual transpiration rates for each cultivar from Experiment 4 with a temperate climate, more typical for apple growing compared to the climate of other experimental sites.

The $E_c$ output of Equation (6) has also been plotted over the measured data in Figure 3 only for one of the cultivars, Golden Delicious, as a visual test of the goodness–of–fit.

The visual appraisal of the calibrated model output in Figure 3 suggests the following observations that in all the cultivars (figures for other cultivars have been omitted due to limitation in the size of paper) and experiments, the output of Equation (6) matches the seasonal pattern without any clear period of over– or underestimation, suggesting that in the climates where these experiments took place – this model accounts for the seasonal variability of transpiration. As a corollary, the RMSE of Table III does not seem to be biased in any part of the irrigation season, which implies that the capacity of Equation (6) of predicting transpiration will increase over periods longer than a day (irrigation amounts are often calculated at a weekly or a monthly basis). The goodness–of–fit of Equation (6) is very similar for all the cultivars (Table III), suggesting that the assumptions made in this model (for example those associated with the high coupling with the atmosphere) are of a general nature, and the physiological differences between cultivars can be handled by calibration parameters.
Figure 2. Actual evaporation ($E_a$) rates of four apple cultivars under study (upper window) and daily patterns (lower window) in a temperate location (Exp. 4).

Figure 3. Actual ($E_a$) and calculated ($E_c$) rates of four apple cultivars under study (upper window) and daily patterns (lower window) in a temperate location (Exp. 4).

Last, it seems that short periods of low transpiration (associated to occasional cloudy days or in general transitory low evaporative demand) are sometimes cause of worse fit over the measured data (example of high RSME for ‘Pink Lady’ or ‘Idared’).

Canopy Conductance

With our model, we can provide canopy conductance as a diurnal dynamic pattern offering an unprecedented tool for monitoring water stress and stomatal behaviour. For canopy conductance, as with
transpiration, we have shown only a period of Experiment 4 (Figure 4) in order to show how stomata close in response to increased $D$.

**DISCUSSIONS**

Breeders of physiological traits have been concerned with stomatal conductance but they have had difficulties to compare many genotypes, because of the large number of measurements of canopy conductance. In addition, it is necessary to measure plant water status in order to separate real differences in stomatal response at equal water potentials from differences arising from other factors (such as leaf area) that affect the rate of development of stress and hence affect stomatal closure (Jones, 1987). Our model increases the precision of the estimate and reduces data requirements (air temperature, vapour pressure deficit and net radiation) relative to other models. Use of diurnal courses brings more details to the analysis of tree transpiration and canopy conductance and it satisfactorily shows the agreement between observed and simulated transpiration patterns.

The conductance model seems to capture the environmental differences. As one can appraise visually, canopy (stomatal) conductance ($g_s$) is lower in summer than in autumn. This is due to a stomata closure because of very high $D$ levels. Other authors have also found a high correlation between $g_s$ and $D$ (Fanjul and Jones, 1982, Thorpe et al., 1980, Warrat et al., 1980, Watson et al., 1978). Comparison of the entire dataset of experimental data under non-water stress conditions show that canopy conductance of different apple cultivars seem to follow the same behaviour, especially for overlapping measurement periods in each experiment. However, when we compare the values during short water stress of several days at the end of the measuring periods, we notice significant changes between cultivars in their response to avoid excessive water loss through stomata (Figure 5). The results show differences in canopy conductance behaviour, in particular stomata closure, due to increases of vapour pressure deficit. The most contrasting behaviours are those of GL.

![Figure 4](image_url)

**Figure 4.** Daily patterns of vapour pressure Deficit ($D$) and calculated canopy conductance ($g_s$) during a selected measuring period for cv. GD. It is easily noticeable the reduction in stomata conductance when $D$ values increases.
Figure 5. Differences in stomata behaviour of various cultivars in response to vapour pressure Deficit (D).


and PL; the first react to increasing $D$ by closing stomata immediately while PL shows a gradual closure maintaining transpiration and photosynthetic activity. However, genotypes that maintain a high canopy conductance have a high temperature tolerance and therefore, benefit from increased evaporative cooling compared to other genotypes more prone to close the stomata.

Changes in stomata opening in relation to net radiation did not differ significantly (omitted due to space) but the model is highly sensitive and can also be used for evaluating the light saturation levels for different genotypes or stomatal kinetics in general. The model can be used to define the optimal pattern of water use in an unpredictable environment, which is important for the breeders to optimise plant behaviour.

Analysis suggests a missing degree of freedom in D part of Lohammar Equation (Lohammar et al., 1980). The formula applied is more complex but it gives more flexibility to stomata behaviour which does not mimic stomata closure so sharply under low $D$ conditions. The model could be used for assessing the genotype–to–environment interactions especially in relation to the stomatal response to abiotic factors as well as a proxy physiological marker in selection for resistance. Besides offering an insight on the different mechanism of stress avoidance/tolerance, this has a great relevance for irrigation scheduling as it tells us if a cultivar tolerates deficit irrigation and how important it is to avoid water stress.

This approach helps to understand canopy water status from the point of view of daily dynamics. The sensitivity of canopy conductance to vapour pressure deficit in our model offers an approach to study stomata behaviour toward environmental variables and agricultural practices. It is a promising tool for early and quick evaluation of water stress tolerance in apple genotypes.

REFERENCES


عنوان تابعی از تابیش و کمیابی فشار بخار، نوشته شد. برای کالیبراسیون مدل، مجموعه داده های تجربی در سال های 2011 تا 2013 که گسترده ای از تعریق درختان مناطق مختلف Albania و Kosovo شامل مناطق خیلی گرم تا معتدل می شود جمع آوری شد. الگوی روزانه هدايت تاج برای بخار آب در سطح تاج بخشی به شده توسط مدل، یک بخش پی سابقه واکنش سریع به رطوبت ارقام مختلف و همچنین اشاع نور خود را ارائه می کند. این عملکرد رضا نیز بررسی بخشی این مدل کاربردی باید دقت دروش تجربی مورد را توسط محاسبه آب مورد نیاز درخت بخار بیشتر و درمانات یک ایزد امیدوار کند. برای ارزیابی اولیه و سریع تحمیل تنش آب در زنویع های سیب باشد.