**Water Status Indices of Young Apple Tree Respond to Water Stress under the Environment Sheltered from Rain**

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### Abstract

The main aim was to provide theoretical support for scientific regulation of the water and precision irrigation in orchards. This study focused on the water status indices of trees to soil water stress, and their relationship to water balance of the tree body. The results showed that stem maximum daily shrinkage (MDS) was the most sensitive to soil water stress, followed by midday stem water potential ($\psi_{stem}$). MDS also had obvious response to reference evapotranspiration (ET$_0$, R = 0.6759, P < 0.01, N = 62). The $\psi_{stem}$ was also sensitive to soil water stress and atmosphere water status, which was negatively correlated to ET$_0$ (R = -0.47071, P < 0.01, N = 30). Relative MDS, and relative soil water potential ($\psi_{soil}$) was highly correlated (R = 0.6582, P < 0.01, N = 30); and correlation between relative midday stem potential ($\psi_{stem}$) and $\psi_{soil}$ was very significant (R = 0.6143, P < 0.01, N = 30). Moreover, MDS was measured in succession and recorded automatically, but so far it has been difficult automatically to measure either leaf or stem potential. The other tree water status indices like predawn water potential ($\psi_{pd}$), daily growth of stem diameter (DG), stomatal conductance (g$_s$), had different response to water stress. But, in general, their response was not very sensitive to change in soil water potential. In conclusion, MDS is an optimal tool to aid for irrigation in orchards, followed by $\psi_{stem}$. © 2018 Friends Science Publishers

### Keywords: Apple trees; Water stress; Water status index; Maximum daily shrinkage; Midday stem water potential

### Introduction

The yield formation, quality development and economic benefit for fruit trees are closely related to the plant water status as it directly affects basic plant physiological processes like transpiration, photosynthesis, and matter metabolism and distributions (Shackel et al., 1997; Moriana et al., 2002). Tree water status depends not only on the water status of the soil (as represented by soil moisture content or soil water potential), but also has a close relationship with the state of atmospheric water (meteorological conditions related to soil evaporation and plant transpiration) and water metabolism in fruit trees (Liu, 1997; Yang et al. 2012; Girón et al., 2015). Currently, one of the most common approaches, used for irrigation scheduling, is the water balance (P-M method), which is based on reference evaportranspiration and requires the use of crop coefficients (Allen et al., 1998; Yang et al., 2013; Jia et al., 2014). When the P-M method is applied to fruit tree orchards, determination of the crop coefficients is complicated and variable, as it is related to the canopy size and the tree height. Both these are age dependent and influenced by the orchard management technology (e.g. tree canopy shaping and pruning techniques). In addition, uneven spatial and temporal distribution of the leaf area in orchards also influence the application (Chen and Liu, 2000; Doltra et al., 2007).

The soil water status indicators such as soil water content and water potential are generally used to indicate the crop water shortage and to guide irrigation (Wang et al., 2010; Moriana et al., 2012; Tao et al., 2014). In recent years, since advances in electronics and computers are used to agriculture, the soil moisture sensors have been applied widely (Hilhorst and Dirksen, 1994; He et al., 1999). Most sensors connected to a data logger can be used for instant fixed-point, timing or continuous soil water monitoring. However, soil moisture content has temporal-spatial differences, and fruit trees root system is uneven distribution and water absorption and utilization by root system exist variations in different soil layer. Thereby an adequate

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placement and the number of sensors installed must take into account soil spatial heterogeneity and fruit tree root distribution and water absorption characteristic (Doltra et al., 2007; Zhao et al., 2015).

The tree water status indicators are a comprehensive system which can reflect the ecological environment (air and soil) and the biological characteristics of plants. Predawn leaf water potential and midday stem water potential are considered to be two of the ideal indicators of tree water status (Moriana and Fereres, 2002; Doltra et al., 2007). At present, the application of pressure chamber to measure the water potential is not only time-consuming but also difficult to achieve continuous measurement of leaf or stem water potential (Liu et al., 2011). Trunk diameter changes (TDC) had a close relationship with plant water potential and water content of the tree body (Feng, 2012). The application of Linear Variable Differential Transformer (LVDT) can be very convenient to measure the change of trunk diameter, which allows the calculation of variable such as maximum daily shrinkage (MDS), daily growth (DG), and cumulative growth (CG), etc. Monitoring TDC has been widely used in plant water stress research and accurate and efficient water management of many kinds of fruit species (Goldhamer and Fereres, 2001; Fernández and Cuevas, 2010; Moriana et al., 2013). The purpose of this work was to study the response of tree water status to ET0 and soil water potential (ψstem) in the condition of strict water control in container grown plants. The feasibility of accurate water management and automatic irrigation in orchards, which based on tree water status indicators, has been discussed.

Materials and Methods
Orchard Characteristics
The experiments were carried out under rain-sheltered condition on July 2013 and July 2014 in Beihu Orchard (N35°36′, E116°16′), located at Jiaxiang county, Shandong province (China). The culture containers (50x50x50 cm cubes) were made of glass, filled with sandy loam with field moisture capacity 27.15%, soil bulk density 1.01g·cm⁻³, available N content 52.65 mg·kg⁻¹, available P content 45.51 mg·kg⁻¹, available K content 253.37 mg·kg⁻¹, organic matter 14.73 g·kg⁻¹, and full irrigation in November 2012. Two years old ‘Fuji’ (Malus pumila Mill cv. ‘Fuji’/Malus hupehensis) with comparatively uniform sizes were selected for the study. A total of 192 trees were planted in containers (one plant per container) on 10 March 2013 spaced at 1x2 m. Pest control and fertilization practices were commonly used by the growers in local orchard.

Water Treatments
All trees were irrigated with 100% of ETc in order to obtain non-limiting soil water conditions at the beginning of experiments. In the control treatment (CK), daily irrigation supplied was 100% of ETc (ETc values were calculated following the way of FAO; Allen et al., 1998). In the water stress treatment (WS), three was no irrigation from July 1st to July 31st in 2013 and 2014, and soil water potential decreased gradually. Sixty trees were selected for each treatment.

Meteorological Observations
An automatic weather station (TRM-ZS1, Jinzhou Meteorological Science & Technology Ltd in China) was installed in the orchard, which was 100 m away from the experimental plots. The weather station provided all kinds of meteorological data for calculating ETc and ETc.

Water Relations
In the test periods, ψp, ψx, and ψstem were measured by WP4C (Decagon company, USA) every two days with three replications. Soil samples, 15 cm far away from the plant, were taken with a small earth boring auger in 4 layers (0–10, 10–20, 20–30 and 20–40 cm) at 17:00–18:00, and the soil samples were mixed with each other for the measurement of ψx (Doltra et al., 2007; Tao et al., 2014). The gsc of functional leaves was measured before the sunrise, and ψstem was measured between 12:00–13:00 (local time). For midday ψx determination, leaves close to trunk were sealed with plastic bags made of reflective films 2 h before the measurement to achieve water balance between the leaves and the trunk (Doltra et al., 2007).

Stomatal Conductance (gsc)
The gsc of functional leaves were measured by LI-1600 (LICOR company, USA) every 2 days (Doltra et al., 2007; Liu and Shi, 2010). In selected days, the measurements were taken every 2 h with three replications, and the daily means of gsc were calculated.

Measurements of Trunk Diameters
Trunk diameters were measured with linear variable Differential transformers (DD-LVDT, Ecomatik company, Germany) mounted in the northern part of experimental tree trunks at a height of 30 cm above the ground. Measurements were taken every 30s and recorded on the datalogger (DL2e, Delta Device company, UK) programmed to calculate 20 min means (Liu and Shi, 2010; Feng, 2012). From these measurements the maximum daily shrinkage (MDS) and daily growth (DG) were obtained. MDS is defined as the difference between the maximum trunk diameter (reached normally before sunrise) and the minimum trunk diameter (found usually in the afternoon); DG is the difference between two consecutive daily maximum diameters.
Statistical Analysis

Figures were performed by using Origin 7.5 software. The data were analyzed by one-way ANOVA and the means were compared (p≤0.05) by Duncan’s Multiple Rang Test (DMRT). Relative values of all kinds of water status indices were calculated by using the equation below:

\[ X_i = (X_i - X_{ck})/X_{ck} \]

Where, \( X_i \) was relative value of certain water status index, \( X_i \) and \( X_{ck} \) were the corresponding water status indices under WS and CK, respectively.

Results

Change of Reference Evapotranspiration

Fig. 1 shows the dynamic changes of \( \text{ET}_0 \) during the test periods. In July 2013 and July 2014, the mean daily reference evapotranspiration was 4.9 and 4.6 mm, the maximum values were 6.6 and 6.4 mm, and the minimum values were 3.4 and 3.6 mm, respectively. Mean, maximum, or minimum values, were no significantly different in 2013 and 2014; however, the \( \text{ET}_0 \) had distinct daily variations during the test period, which mainly depended on weather conditions, such as temperature, air humidity, wind speed, air pressure and net radiation, etc.

Changes in Water Relations Parameters

Fig. 2 shows changes in \( \psi_{soil} \), \( \psi_{pd} \) and \( \psi_{stem} \) during 2a tests. \( \psi_{soil} \) under CK treatment ranged from -0.01 to -0.04 MPa, as a result of daily relatively high and stable water supplement, which was sufficient to meet normal growth and development of plant for water requirements. \( \psi_{soil} \) under WS treatment showed a gradually declining trend; the difference compared with \( \psi_{soil} \) under CK gradually widened in due course of time. By the end of tests, a minimum \( \psi_{soil} \) under WS in 2013 and 2014 reached to -0.19 and -0.18 MPa, respectively, and the plants experienced no stress, mild stress, moderate stress, and severe stress in the whole process.

Difference in \( \psi_{pd} \) between treatments was not significant from the test beginning day 20, but from 20 day till the end, the difference increased gradually, which reflected that \( \psi_{pd} \) was less sensitive to mild and moderate water stress, much sensitive to severe stress, therefore, \( \psi_{pd} \) could not reflect the mild and moderate water stress as the tree water status indices. At night stomata closed and transpiration ceased, while root water uptake and water conduction were carrying on, leaves or trunk can return to normal water status after root water uptake and water conduction in mild or moderate conditions. Under severe water stress root water uptake was not sufficient to make the tree body return to normal water status, so the difference of \( \psi_{pd} \) between WS and CK treatments was significant (Doltra et al., 2007).

Fig. 1: Change of reference evapotranspiration during test periods in 2013 and 2014

In the condition of no water stress or mild stress, difference in \( \psi_{stem} \) was not significant between CK and WS. With an increase of stress level, the difference between them was becoming more and more significant. At later stage of the tests in 2013 and 2014, the difference was about -1.3 and -1.2 MPa, respectively. The variability of \( \psi_{stem} \) was relatively large between days whether in CK or WS treatments. Correlation data showed that \( \psi_{stem} \) and \( \text{ET}_0 \) had a significant linear correlation (Table 1). \( \psi_{stem} \) not only responded to the changes of \( \psi_{soil} \) but also responded to atmospheric evapotranspiration (\( \text{ET}_0 \)) as well.

Changes of DG and MDS

Fig. 3 shows the changing trends of DG and MDS under CK and WS treatments. During the tests, DG was relatively small, but its variability was relatively large. The average values under CK treatment in 2013 and 2014 were 0.07 and 0.06 mm, their minimum values 0.01 and 0.00 mm, and their maximum values 0.13 and 0.11 mm, respectively. The mean DGs under WS treatment were 0.05 and 0.04 mm, respectively, their minimum values both 0.00 mm, and their maximum values 0.10 and 0.09 mm, respectively. During the whole tests in 2013 and 2014, the growth of the tree trunk was slow, and no significant differences in DG between treatments were found. This was due to the fact that DG was not sensitive to water deficit under our experimental conditions.

Compared to DG, MDS changed obviously between CK and WS treatments in 2a tests. The mean values under CK treatment in 2013 and 2014 were 0.22 and 0.16 mm, the minimum values were 0.06 and 0.03 mm, and the maximum were 0.42 and 0.38 mm, respectively; and the mean values MDS under WS treatment were 0.33 and 0.22 mm, the minimum were 0.11 and 0.06 mm, and the maximum were 0.52 and 0.45 mm, respectively.
Table 1: Correlation between tree water status indices and reference evapotranspiration (ET₀)

<table>
<thead>
<tr>
<th>Water status indices</th>
<th>Linear models</th>
<th>R</th>
<th>P</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predawn leaf water potential (MPa)</td>
<td>$ψ_{pd} = 0.5995 + 0.0604 \times ET₀$</td>
<td>-0.3256</td>
<td>0.0791</td>
<td>30</td>
</tr>
<tr>
<td>Midday stem water potential (MPa)</td>
<td>$ψ_{pd} = -1.2610 - 0.1277 \times ET₀$</td>
<td>-0.4707</td>
<td>0.0226</td>
<td>30</td>
</tr>
<tr>
<td>Daily stem growth (mm·d⁻¹)</td>
<td>$DG = 0.0576 - 0.0305 \times ET₀$</td>
<td>-0.1028</td>
<td>0.4267</td>
<td>62</td>
</tr>
<tr>
<td>Maximum stem shrinkage (mm·d⁻¹)</td>
<td>$MDS = -0.2240 + 0.1018 \times ET₀$</td>
<td>0.6759</td>
<td>0.0001</td>
<td>62</td>
</tr>
<tr>
<td>Daily mean stomatal conductance(mm·m⁻²·s⁻¹)</td>
<td>$g_s = 298.9991 - 24.3344 \times ET₀$</td>
<td>-0.2002</td>
<td>0.2888</td>
<td>30</td>
</tr>
</tbody>
</table>

Fig. 2: change of soil water potential, predawn leaf water potential and midday stem water potential during test period in 2013 and 2014
○: (CK) Control; ●: water stress treatment

It was clear that the mean values, the minimum or maximum values of MDS was higher in WS treatment than in CK treatment, and the difference between the two treatments was more significant with the increase of ET₀ and the aggravation of water stress. Although MDS volatility under two treatments was great, the changing trends were consistent.

Changing Trends of Stomatal Conductance

Fig. 4 shows the dynamic changing of $g_s$ in the 2a test periods. Mean values of $g_s$ under CK were 180 and 230 mmol·m⁻²·s⁻¹, the minimum values were 60 and 70 mmol·m⁻²·s⁻¹, and the maximum were 378 and 485 mmol·m⁻²·s⁻¹, respectively. However, the mean values under WS were 150 and 209 mmol·m⁻²·s⁻¹, the minimum values were 54 and 78 mmol·m⁻²·s⁻¹, and the maximum values were 289 and 475 mmol·m⁻²·s⁻¹, respectively. During the 2a tests, the daily average, minimum and maximum of $g_s$ were less in WS treatment than in CK treatment, and no significant differences between CK and WS treatments were found under the conditions of no stress, mild and moderate stress, but at the later stage of the experiment (in the condition of severe stress), obvious differences occurred between two treatments. The daily fluctuations of $g_s$ were large during 2a test periods, but their changing modes under CK and WS were consistent, which indicated that the soil water potential was not the main factor to affect the $g_s$ under...
mild or moderate stress for apple trees. So $g_s$ cannot be used as an ideal index to indicate the water status of the apple tree. First, the apple tree root system, trunk and branches had abundant storage water, which could guarantee the water supply to meet the needs of transpiration in mild or moderate water stress; secondly, the atmospheric environment, such as the solar radiation flux density, wind speed and atmospheric humidity had importantly impact on $g_s$ (Girona et al., 2012).

Response of Different Water Status Indices to Water Stress

In order to study effects of soil water stress on tree water status indices, the relative value of the water status indices (the ratio of tree water status indices under WS treatment and the corresponding indices under CK treatment) were calculated. As shown in Fig. 5 and Table 2, $\Psi_{rs}$ reflected the degree of soil water stress, that is, $\Psi_{rs}$ increased as degree of soil water stress aggravated. During the tests, $\Psi_{rs}$ was close to linear increase with the test processing, which performed consistent results in 2013 and 2014.

At early stage of the experiments, changes $g_{rs}$ was small with an increase of water stress, which showed a significant downward trend only at the late stage of experiments under severe water stress. Correlation analysis showed that there was no significant relation between $g_{rs}$ and $\Psi_{rs}$ ($R = -0.38$, $P > 0.05$). $\Psi_{pd}$ increased with an increase in water stress (ranging from 0.0 to 3.6), and the change was relatively smaller during the early stage, but at the later stage, especially in 2014 after 20 days from beginning treatment, $\Psi_{pd}$ showed a sharp rise. Correlation analysis showed that $\Psi_{pd}$ was significantly correlated with $\Psi_{rs}$ ($R = 0.51$, $P < 0.05$, $N = 30$). Two years’ data showed that $\Psi_{stem}$ variation range was between -0.01 to -2.0, and it had significant correlation with $\Psi_{rs}$. $\Psi_{stem}$ indicated a slow downward trend with the increase of water stress, and had the characteristic of obvious fluctuation, no significant correlation with $\Psi_{rs}$. $\Psi_{soil}$, which changed between -0.2 and 3.7, had a relatively stable increasing trend with the enhanced WS, and showed a significant correlation with $\Psi_{soil}$.
Based on the analysis, MDS\textsubscript{r} was not only significantly correlated with atmospheric evapotranspiration (ET\textsubscript{0}), but also indicated significant correlation with the \( \psi\text{soil} \), which comprehensively reflected the changes of atmospheric water and soil water status. With an increase in water stress, the change of MDS\textsubscript{r} was relatively large and quite stable, which reflected that it was more sensitive to water stress than other water status indices. MDS\textsubscript{r} was convenient to be automatically continuously observed. Therefore, it was the relatively ideal water status indices and powerful tools for precise irrigation for applying to trees. \( \psi\text{stem} \) was also quite sensitive to atmospheric water and soil water status, but it was difficult to measure automatically and continuously. \( G_p \), DG, and \( \psi_{pd} \) could respond to water stress in some content, but they were not stable, so they should not be used as ideal water status indices for irrigation.

**Discussion**

Under the conditions of global climate change and water resource shortage, accurate and automatic irrigation has become the development tendency of water management in modern orchards, and the identification and diagnosis of tree water status have also become theoretical principles to realize accurate and automatic irrigation (Wang et al., 2010). In this study, the sensitivity of each index to water stress and their relationships has been discussed under the rain shelter conditions in order to schedule precise irrigation in orchard.
The study showed that $\psi_{pd}$ had less sensitivity to mild and moderate water stresses, but more sensitive to severe water stress, therefore $\psi_{pd}$ could not be regarded as an index to reflect mild and moderate water stress. Under mild and moderate water stress, leaves or trunks can restore the normal water status, because stomata close and transpiration reduces at night, while the root water uptake and water conduction still proceeded at the same time. Under severe water stress, the difference of $\psi_{pd}$ between CK and WS became evident, while water uptake by roots was not sufficient to make the trees return to normal water status. Angelocci and Valancogne (1993) found that $\psi_{pd}$ was not significantly different between non-irrigation treatment (from the end of July to the end of August) and normal irrigation treatment under moderate water stress ($\psi_{soil} \geq -0.15$ MPa), which was about -0.20 and -0.18 MPa, respectively.

The $\psi_{stem}$ was considered to be sensitive water index to indicate tree water status, being often looked as an effective tool to guide irrigation. The study showed that difference of $\psi_{stem}$ was significant between CK and WS under moderate or severe water stress, which became clearer with an increase of stress level. These results showed that $\psi_{stem}$ was very sensitive to moderate and severe water stress. In addition, irrespective of CK or WS treatment, the daily fluctuations of $\psi_{stem}$ were large, which had a significant linear correlation with $ET_0$ (Table 1). This reflected the relative sensitivity of $\psi_{stem}$ for $ET_0$ Naor and Cohen (2003) showed that when $\psi_{stem}$ was less than -0.13 MPa at noon, it had a great decline in the growth and development of Golden Delicious apple tree. $\psi_{stem}$ at noon can also reach -0.15 MPa compared with daily irrigation under large day fluctuation, which was similar to results of this study.

During the whole experimental period, the trunk growth was slow, and DG was not significantly different between the two treatments. This indicated that DG was not sensitive to water stress in our study. Different result was obtained in research of peach tree, which showed that DG was obvious smaller under severe water stress than under fully irrigated condition, as the water uptake by root at night had not been enough to make tree recover to normal water conditions. This impacted the growth of peach tree under severe water stress in their study (Marsal et al., 2002). Tree species and tree-ages might be related to the difference between the researches. In addition, the correlation between DG and $ET_0$ was poor. DG was not suitable to be used as threshold values for the control and management of irrigation based on our research.

The results of this study showed that the changing trends of $\psi_{stem}$ and MDSr changed similarly with $\psi_{soil}$, which was also significantly correlated to $\psi_{root}$. These results were similar to the data reported for plum (Samperio et al., 2015), apricot (Fereres and Goldhamer, 2003), and apple trees (Doltra et al., 2007). The authors found that the MDSr was relatively sensitive and stable to the response of water stress. Compared with their results, MDSr in this study was relatively smaller, which may be related to the differences in the test environment and test materials.

### Conclusion

MDS and midday $\psi_{stem}$ were appropriate indicators for tree water status, which were the two most sensitive water status indicators under water stress among the tree water status, indices, followed by $\psi_{pd}$ and DG, and $e_s$ was the most insensitive to water stress. When guiding apple orchard irrigation and water management with the application of tree body water status, the comprehensive effects of ages, growing period should be also considered. In the whole development period of apple tree, the sensitivity and practicability of the water status index need to be further studied, especially the relationship of the tree water status index and yield production and qualities of the apples.

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