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## Comparison of water status indicators for young peach trees

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**Abstract** We measured a series of physiological and physical indicators and compared them to xylem sap flow, to identify the most sensitive and reliable plant water status indicator. In the growing season of 1998, 4-year-old peach trees (*Prunus persica* Batsch cv. ‘Suncrest’, grafted on ‘GF 677’ rootstock) were studied under two irrigation treatments, 25 l day<sup>-1</sup> and no irrigation, and during recovery. Trials were conducted near Pisa (Italy) in a peach orchard situated on a medium clay loam soil and equipped with a drip-irrigation system (four 4 l h<sup>-1</sup> drippers per tree). Measurements of leaf water potential ( $\Psi_w$ ), stem water potential ( $\Psi_s$ ), and leaf temperature ( $T_l$ ) were taken over 5 days (from dawn to sunset) and analyzed in conjunction with climatic data, sap flow (SF), trunk diameter fluctuation (TDF) and soil water content (SWC). Physiological indicators showed substantial differences in sensitivity. The first indication of changes in water status was the decrease of stem radial growth. TDF and SF revealed significant differences between the two irrigation treatments even in the absence of differences in pre-dawn leaf water potential ( $pd\Psi_w$ ), up until now widely accepted as the benchmark of water status indicators. Irrigated trees showed a typical trend in SF rate during the day, while in non-irrigated plants the maximum peak of transpiration was anticipated. Measurements of water potential showed  $\Psi_s$  to be a better indicator of tree water status than  $\Psi_w$ .  $T_l$  was found to have poor sensitivity. In conclusion, we found the sensitivity of the indicators from the most to the least was: TDF > SF rate > SF cumulated =  $pd\Psi_w = \Psi_s > md\Psi_w > T_l$ .

### Introduction

Plant water status can be determined using both physiological and physical indicators. Physiological indicators are direct and indirect measurements of plant water status. Physical indicators refer to environmental factors that influence plant water balance (Katerji et al. 1988).

Direct physiological indicators express relative water content (Bennett 1990) or leaf water potential (Scholander et al. 1965; Meyer and Green 1980). Indirect physiological indicators describe processes induced by changes in plant water status, including variations in stomatal conductance, leaf temperature, and plant organ diameter as well as qualitative morphological alterations. Physical indicators include soil water content, soil water potential in the root area, and vapor pressure deficit (VPD). They are currently used, although somewhat controversially, to measure environmental evaporative demands (Katerji et al. 1988).

Several studies have shown that information on crop water status, required when planning irrigation programs, is provided more reliably by physiological indicators. However, there is no general agreement on the most suitable indicator (Katerji et al. 1988).

Pre-dawn leaf water potential (Ferreira et al. 1997; Valancogne et al. 1997) is frequently used, while other indicators have also been employed, such as stem water potential (McCutchan and Shackel 1992; Naor et al. 1999), variations in plant organ diameter (Garnier and Berger 1985; Hugué et al. 1992; Simonneau et al. 1993; Goldhamer et al. 1999), variations in stomatal conductance (Harrison et al. 1989; Sellés and Berger 1990; Tan and Layne 1991), leaf temperature (Glenn et al. 1989; Girona et al. 1993; Cohen et al. 1997; Jones et al. 1997; Massai et al. 2000a), and soil water content (Goldhamer et al. 1999). Xylem sap flow measurement has been proposed to monitor plant water consumption (Améglio et al. 1993; Massai and Remorini 2000) and, together with physiological and physical indicators, adequately estimates crop water status (Nadezhdina 1999; Massai et al. 2000b).

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The purpose of the present study is to assess the most sensitive and reliable indicator of plant water status. We compared xylem sap flow with a wide range of water stress indicators in peach trees during a growing season.

## Materials and methods

Trials were conducted at one of the experimental farms of the University of Pisa located at Colignola (Pisa, Italy, 43°43' N 10°23' E), on a peach orchard (*Prunus persica* Batsch), cv. 'Suncrest', planted in February 1995, grafted onto GF 677 and trained to an open-center canopy with 5×5 m spacing with inter-row grass cover and a herbicide strip along the row. Horticultural care was that typically adopted in the area of production. Trees received standard commercial dormant pruning and fertilization (120 kg ha<sup>-1</sup> N, 80 kg ha<sup>-1</sup> P and 100 kg ha<sup>-1</sup> K) in the spring before the experiment.

The peach orchard is situated on flat land, on a medium clay loam soil (42% sand, 38% lime, and 20% clay), and equipped with a drip-irrigation system (four 4 l h<sup>-1</sup> drippers per tree placed at distances of 1 m and 2 m from the trunk).

All measurements were conducted during the 1998 growing season. Six trees were selected for uniformity (mean cross-sectional trunk area 72.8±4.1 cm<sup>2</sup>): three irrigated with roughly 25 l water per day (20 m<sup>3</sup> ha<sup>-1</sup> day<sup>-1</sup>) from 1 June to 30 September, and three that received no irrigation from the beginning of June until 4 September (in coincidence with the first abundant rains). Volume of water for daily irrigation maintained the soil water content in the root zone around 80% of field capacity. At harvest time (last week of July), yields per tree were 35.4 kg (irrigated) and 30.5 kg (non-irrigated).

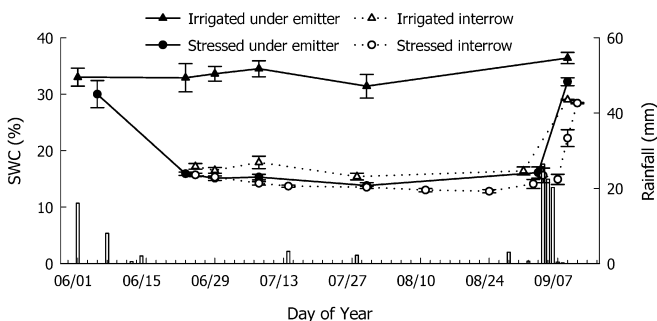
The following water status indicators were measured: soil water content (SWC), sap flow (SF), trunk diameter fluctuation (TDF), diurnal trend of leaf water potential ( $\Psi_w$ ), stem water potential ( $\Psi_s$ ) and leaf temperature ( $T_l$ ).

### Climatic data

Air temperature, relative humidity, solar radiation, rainfall, and potential evapotranspiration were measured by an automated meteorological station (ETG, Florence), placed in the proximity of the orchard. Rainfall is showed in Fig. 1.

### Soil water content

Soil water content (SWC) was monitored by time domain reflectometry (TDR). A Moisture Point TDR system model MP-917 (Environmental Sensors, Canada), equipped with four PRB-K



**Fig. 1** Seasonal trend of rainfall (mm) and volumetric soil water content (SWC, %) monitored in a peach orchard in Colignola, Pisa, during 1998. Data points represent the average of at least 16 measurements; bars show standard errors. Vertical dotted lines indicate beginning and end of treatment

multilevel probes (Environmental Sensors), was used to measure soil moisture content (in percent volume, m<sup>3</sup>/m<sup>3</sup>) at 15, 30, 45, and 60 cm depths. Measurements were taken at two different positions; along tree rows at a distance of 0.75 m from the tree (under the dripline) and inter-row at 2.5 m from the dripline.

### Plant water potential

$\Psi_w$  was measured before dawn (predawn, pd) and at the hottest time of the day (midday, md).  $\Psi_s$  was determined throughout the day (hourly, from predawn to sunset) at 20-day intervals.

Leaf and stem water potential were measured using a Scholander type (Scholander et al. 1965) pressure chamber (Technogas, Pisa) equipped with a three-hole lid allowing three measurements to be made simultaneously. Pressure was increased in increments of 0.2 MPa every 30 s (Turner 1981).  $\Psi_w$  was measured on three fully expanded leaves of similar age per tree. Leaves, situated on the median portion of shoots and well exposed to sunlight, were cut off halfway along the stalk and immediately processed (Turner 1981).  $\Psi_s$  was measured on three leaves per tree, wrapped in aluminum foil and encased in polyethylene bags at least 3 h before measurement. Leaves for predawn readings (pd $\Psi_s$ ) were bagged the previous evening immediately after sunset to avoid night-time dew formation. In all cases, leaves were placed in the chamber within a few seconds after excision.

### Leaf temperature

$T_l$  was determined for a whole day (from dawn to sunset) at 20-day intervals, using a Cyclops Compac 3 infrared portable thermometer (Land Infrared, Sheffield, UK). The sensor was placed at a distance (~10 cm) from the leaf blade to obtain a target area of 35 mm diameter. The entire area detected by the sensor was totally occupied by a single leaf in full sunlight.  $T_l$  measurements were performed on five leaves per tree at 1-h intervals throughout the day.

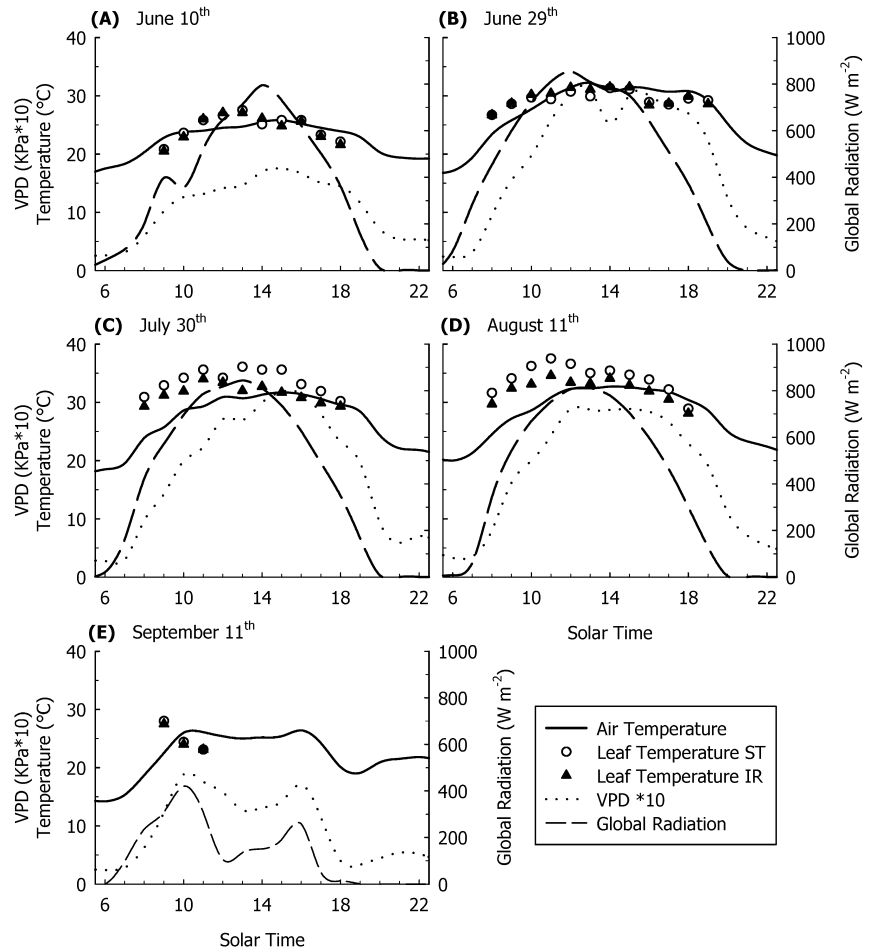
### Trunk diameter fluctuations

TDF was measured by a micrometric system (INRA-patented "Pepista 2000" INRA system), based on TDF permanent measurement, with 10  $\mu$ m accuracy. Three LVDT, model DF 2.5 linear transducer sensors (Solartron Metrology, Bognor Regis, UK), mounted on Pecapto 05 trunk sensor carriers and connected to a Campbell CR10X datalogger (Campbell Scientific, Logan, Utah, USA), were applied to the trunks of two selected trees. Readings were taken every 15 s and processed every 30 min to provide mean values. The resulting values also allowed calculation of the two most significant parameters of TDF: daily evolution (DE) and maximum daily shrinkage (MDS). DE (diameter measurements) is the overall change in diameter measured each day at dawn. MDS is the difference between the maximum diameter, usually observed in the early morning, and the minimum diameter, generally reached in mid-afternoon (Huguet et al. 1992).

### Sap flow

SF measurements were carried out using a system based on heat balance in a portion of the branch (Sakuratani 1981; Steinberg et al. 1989). Gauges were installed on straight sections of the stem with no swellings or lumps that could weaken the contact between stem surface and the heater or thermocouples. Any loose bark and small branches sprouting from the section were carefully removed (Smith and Allen 1996). Sensors were moved to different branches at 20-day intervals to allow normal growth during the experimental period. The system consisted of ten SGA10 and SGA13 sensors (Dynamax, Houston, Tex., USA) placed on branches (8–15 mm diameter) well

**Fig. 2** Diurnal trend of leaf and air temperature ( $^{\circ}\text{C}$ ), vapor pressure deficit ( $VPD$ ,  $\text{kPa}$ ) and global radiation ( $\text{W m}^{-2}$ ). On 10 June (A), 29 June (B), 30 July (C), 11 August (D), and 11 September (E), leaf temperatures were measured (in a peach orchard in Colignola, Pisa) in irrigated (IR, filled triangles) and non-irrigated (ST, empty circles) trees. Data points represent the average of at least 15 measurements. Leaf temperature standard error  $P < 0.5$



exposed to sunlight. To ensure good contact, sensors were installed at the hottest time of the day (when the diameter shrinks to its smallest size), and the bark was slightly thinned, taking care not to affect the epidermis, in order to increase gauge sensitivity. In addition, type G4 silicone paste was applied to achieve good thermal contact (Smith and Allen 1996). Sensors and adjacent branch portions were protected from solar radiation by aluminum foil to avoid the development of external temperature gradients.

The sensors were connected to a Campbell CR7 datalogger (Campbell Scientific). Readings were taken every 15 s and processed with Dynamax DGSF 5.0 software to supply accumulated values every 30 min (Steinberg et al. 1989). For each branch, measured for SF, leaf area was measured (see section below) to express SF in milligrams  $\text{H}_2\text{O m}^{-2} \text{s}^{-1}$ . Plant water consumption was calculated from daily accumulated SF values.

#### Leaf area

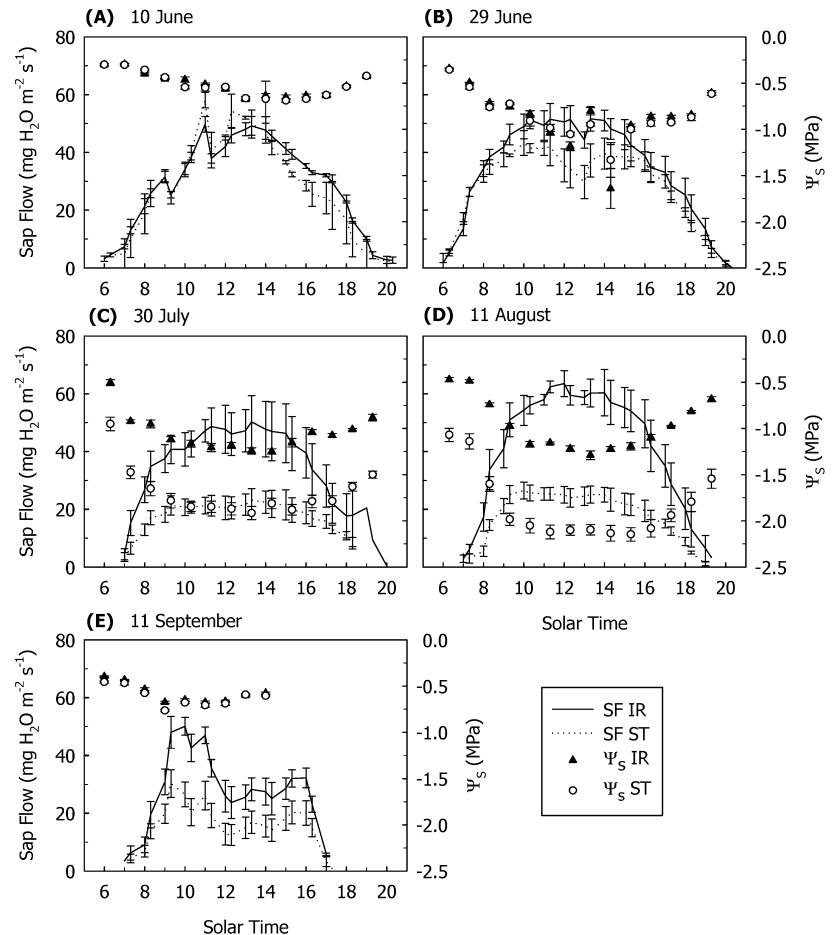
We derived regression equations in order to estimate LA on the branches used for SF measurements. Shoot length (cm) and leaf area ( $\text{cm}^2$ ) were measured (Licor 3000 area meter; Licor, Lincoln, Neb., USA) in 200 shoot samples randomly collected in the experimental orchard, including 80 shoots, 70 lateral shoots, and 50 spurs (less than 3 cm length). The estimated mean LA was constant in the spurs ( $= 49.59 \text{ cm}^2$ ), while two different regression equations were derived for 1 cm main shoot ( $y = 13.87x - 2.86$ , where  $y$  is the leaf area and  $x$  is the shoot length,  $R^2 = 0.92$ ) and for 1 cm lateral shoot ( $y = 13.17x - 89.09$ ,  $R^2 = 0.87$ ), respectively. In all experimental branches spurs were counted and shoots were measured, to calculate LA values.

## Results

Physical indicators were analyzed first. SWC was monitored throughout the experimental period (Fig. 1). Under the dripline of irrigated trees, SWC was around 33% before (0700 hours) and 40% after (1300 hours) irrigation; values comparable to field capacity in the trial plot (42%). SWC was found to be 16–18% under the driplines of non-irrigated trees and in the inter-rows of both irrigated and non-irrigated trees, as early as 20 days after the beginning of treatment. Such values are close to the permanent wilting point determined for this soil type. During the rest of the season, under conditions of persistent high temperatures and absence of rainfall, SWC decreased from 17% to 12% in the inter-rows of non-irrigated trees. When irrigation was restored (4 September), inter-row SWC remained close to 15% even after 5 days of irrigation. Only after abundant rainfall, from 4 to 6 September, did inter-row SWC recover to values around 30% in both treatments.

Other physical indicators, namely VPD ( $\text{kPa}$ ), global radiation ( $\text{RG}$ ,  $\text{W m}^{-2}$ ), and air temperature ( $T_a$ ,  $^{\circ}\text{C}$ ) were measured on five selected days (Figs. 2, 3 and 4) and compared to physiological indicators. In particular, SF

**Fig. 3** Diurnal trend of sap flow rate ( $SF$ ,  $\text{mg H}_2\text{O m}^{-2} \text{s}^{-1}$ ) and stem water potential ( $\Psi_S$ , MPa) measured in a peach orchard in Colignola, Pisa, on 10 June (A), 29 June (B), 30 July (C), 11 August (D), and 11 September (E), in irrigated ( $IR$ , filled triangles) and non-irrigated ( $ST$ , empty circles) trees.  $\Psi_S$  values represent the average of at least nine measurements; bars show standard errors



rate diurnal trend ( $\text{mg H}_2\text{O m}^{-2} \text{s}^{-1}$ ) correlated well both with VPD and RG (see for example Figs. 2E and 3E).

Physiological indicators were measured on five selected days and compared, using  $\Psi_S$  as a reference. Trees with no symptoms of water stress showed an increase in SF rate during the day from dawn to the hottest time (Fig. 3). Daytime trends of SF were closely correlated to  $\Psi_S$  trends (Fig. 3), except for 29 Jun (Fig. 3B) when SF showed differences of about 20% in the hottest part of the day, whereas no significant  $\Psi_S$  variation was found.

TDF indicated a night-time increase in diameter, followed by a progressive decrease through the day (Fig. 4). In the afternoon trunk diameter started to increase, with restoration of the midnight value some hours later and continuous increase until dawn. Differences in  $\Psi_S$  were associated to a 2–3 h delay in trunk diameter recovery (Fig. 4C, D). On 29 June such delay was evident even in the absence of  $\Psi_S$  variation (Fig. 4B).

Non-irrigated trees were slow-growing, compared to irrigated trees. This phenomenon was detected as early as 4–5 days after the beginning of treatment, even in the absence of significant  $\text{pd}\Psi_W$  differences. Furthermore, a reduced increase in trunk diameter was observed, due to limiting xylem deposition (data not shown). The range of MDS was more pronounced in non-irrigated than irrigated trees (Fig. 4B–D).

$T_1$  was higher than  $T_a$  throughout the morning and up to early afternoon, when it became lower than  $T_a$ . In non-irrigated trees, 3–4°C higher  $T_1$  values were recorded (Fig. 2C, D), in association with significant  $\text{pd}\Psi_W$  differences. In addition,  $T_1$  of non-irrigated trees remained higher than  $T_a$  for longer than  $T_1$  of irrigated trees (Fig. 2C, D).

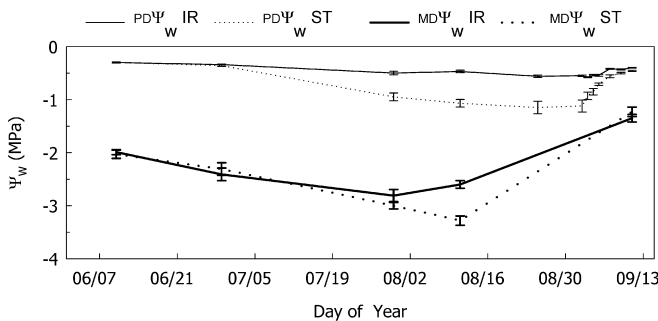
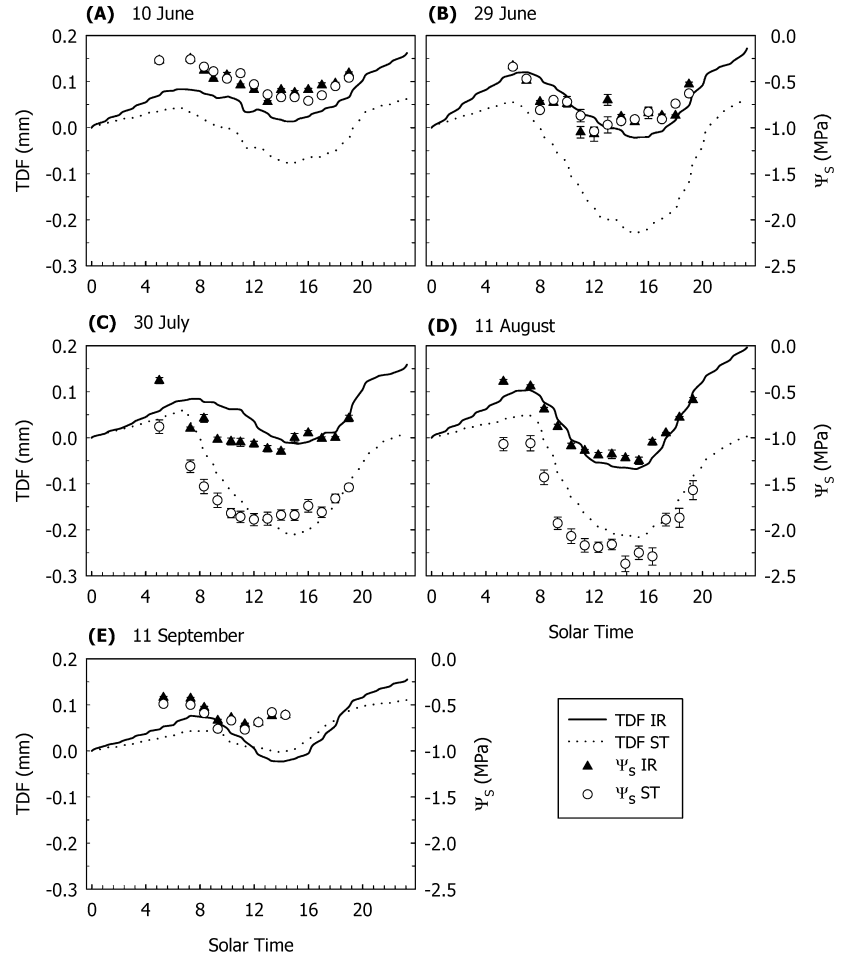
$\text{pd}\Psi_W$  and  $\text{md}\Psi_W$  were measured throughout the experimental period (Fig. 5). Differences in  $\text{pd}\Psi_W$  between the two treatments were in the order of 0.3 MPa (starting from 30 July), while substantial differences in  $\text{md}\Psi_W$  measures were observed only on 11 August when both the measures shown evident variations between the two treatments at 0.6 MPa.

$\Psi_W$  was compared to  $\Psi_S$  on three selected days throughout the daytime at 1-h intervals (Fig. 6). The difference in  $\Psi_S$  between irrigated and non-irrigated trees remained constant throughout the whole day (best seen on 7 July), while differences in  $\Psi_W$  varied, particularly around midday.

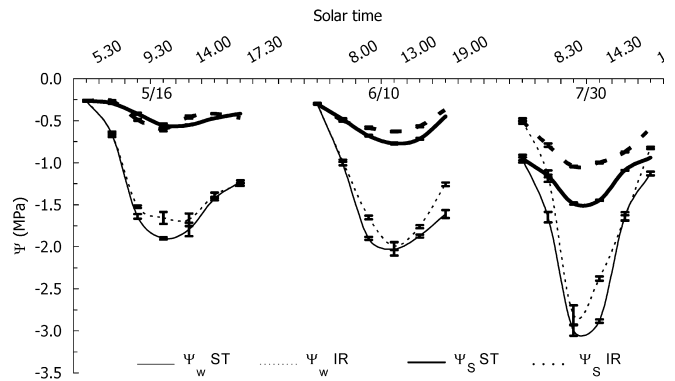
## Discussion

Up until now,  $\Psi_W$  has been widely accepted as the benchmark of water status indicators. Unlike other tree water status indicators,  $\Psi_W$  measured at dawn is a true

**Fig. 4** Diurnal trend of trunk diameter fluctuation (*TDF*, mm) and stem water potential ( $\Psi_s$ , MPa) measured in a peach orchard in Colignola, Pisa, on 10 June (A), 29 June (B), 30 July (C), 11 August (D), and 11 September (E), in irrigated (*IR*, filled triangles) and non-irrigated (*ST*, empty circles) trees. Data points represent the average of at least three measurements; bars show standard errors



**Fig. 5** Seasonal trend of predawn (*pd*) and midday (*md*) leaf water potential ( $\Psi_w$ , MPa) measured in a peach orchard in Colignola, Pisa, during 1998, in three non-irrigated (*ST*) and three irrigated (*IR*) trees. Data points represent the average of at least nine measurements; bars show standard errors. Vertical dotted line shows the end of treatment

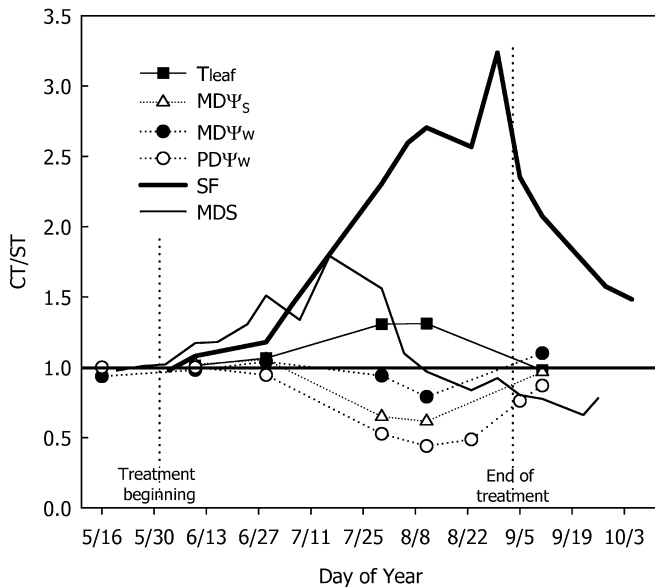


**Fig. 6** Diurnal trend of leaf water potential ( $\Psi_w$ , MPa) and stem water potential ( $\Psi_s$ , MPa) measured in a peach orchard in Colignola, Pisa, on 16 May, 10 June, and 30 July, in irrigated (*IR*) and non-irrigated (*ST*) trees. Data represent the average of at least nine measurements; bars show standard error

soil–plant–atmosphere balance indicator, independent of micrometeorological conditions (Meyer and Green 1980; Katerji et al. 1988). However,  $\Psi_w$  is not ideal for detecting early phases of tree water stress.

We made extensive measurements and compared a series of water status indicators throughout the peach-tree growing period. We found that the first indication

of water stress is a decrease in stem radial growth, in accordance with previously reported data (Huguet et al. 1992; Simonneau et al. 1993; Goldhamer et al. 1999). MDS values in water-stressed trees were higher than in irrigated trees, but became lower under severe water stress conditions, confirming Huguet et al.’s (1992) results. We found significant differences in daily shrink-



**Fig. 7** Seasonal trends of physiological indicator ratios measured in irrigated and non-irrigated trees.  $pd\Psi_w$  (predawn leaf water potential),  $md\Psi_w$  (midday leaf water potential) and  $md\Psi_s$  (midday stem water potential) data represent the average of at least nine measurements;  $T$  (midday leaf temperature) data represent the average of at least 15 measurements;  $SF$  (sap flow cumulated) data represent the average of at least three measurements;  $MDS$  (maximum daily shrinkage) data points represent one measurement. All data were measured in a peach orchard in Colignola, Pisa, in 1998

age, suggesting that increasing amounts of water reserves were recruited to sustain leaf transpiration with the progression of water stress. It is widely accepted that oscillations in trunk diameter are related to changes in water content of external tissues (phloem, cambium, and bark) with little contribution from the xylem. However, more work needs to be done to determine the contribution of sapwood to trunk water content oscillations (Simonneau et al. 1993; Goldhamer et al. 1999).

The heat balance method is a valid and sensitive tool to measure sap flow, and it has been successfully used not only to estimate water consumption but also as an indicator of tree water status. In the present study, significant differences in  $SF$  were observed, even when

$pd\Psi_w$  failed. Whenever differences were shown by  $pd\Psi_w$  these were consistent to  $SF$  rate data. According to previous reports (Nadezhdina 1999; Massai et al. 2000b)  $SF$  rate turned out to be a reliable indicator of tree water status. Distinctive trends in  $SF$  rate during the day were associated to the irrigation regime. In fact,  $SF$  rate in stressed trees reached a peak in the early morning (1000 hours) and decreased thereafter, while irrigated trees maintained elevated water consumption even in the middle of the day. In non-irrigated trees, the maximum peak of transpiration was anticipated, indicating stomata closure and therefore limitation of water consumption.

$pd\Psi_w$  measurements showed differences in the order of 0.3 MPa in relation to irrigation treatments, while substantial differences in  $md\Psi_w$  values were only found after a longer period of severe stress (Fig. 5). In addition, daily variations of  $\Psi_s$  (0.3–0.4 MPa on 30 July 1998), were constant throughout the whole day, unlike  $\Psi_w$  (0.1–0.5 MPa on 30 July 1998) (Fig. 6). In fact, differences between irrigated and non-irrigated trees appeared statistically significant at predawn when stomata are closed. However, in the middle part of the day stomata regulation leads to similar  $\Psi_w$ , whereas large differences are still evident for  $\Psi_s$ . Such marked differences between  $\Psi_s$  and  $\Psi_w$  (1.0–1.5 MPa) could be explained by the stomatal resistance component of total  $\Psi_w$  (Garnier and Berger 1985; McCutchan and Shackel 1992). Our overall results confirm  $pd\Psi_w$  and  $\Psi_s$  to be better tree water status indicators than  $md\Psi_w$ , and  $\Psi_w$ , respectively.

The  $T_l$  indicator only detected differences when water stress was very pronounced, and these differences were limited to early afternoon. However, an interesting trend was observed: in irrigated trees,  $T_l$  remained higher than  $T_a$  until early afternoon (1500 hours) whereas in non-irrigated trees, it was persistently higher until late afternoon (1800 hours). When compared to the other indicators,  $T_l$  revealed the slowest response. Furthermore, late-summer water stress measured by  $T_l$  may remain undetected because of leaf senescence masking the effects.

To visualize the overall indicator profiling, we designed the panel shown in Fig. 7. Data sets referring to non-irrigated trees were normalized to irrigated controls. Sensitivity of the indicators is revealed by the “distance”

**Table 1** One-way analysis of variance based on the LSD method. The significance level of the differences between treatments in different days are marked with an asterisk (95% interval), two asterisks (99% interval) or ns (not significant).  $pd\Psi_w$  (predawn leaf water potential),  $md\Psi_w$  (midday leaf water potential) and  $md\Psi_s$

(midday stem water potential) data represent the average of at least nine measurements;  $T_l$  (midday leaf temperature) data represent the average of at least 15 measurements;  $SF$  (sap flow cumulated) data represent the average of at least three measurements. All data were measured in a peach orchard in Colignola, Pisa, in 1998

	10 June	29 June	30 July	11 August	23 August	5 September	11 September9
$pd\Psi_w$	ns	*	**	**	**	**	**
$md\Psi_w$	ns	ns	**	**			ns
$md\Psi_s$	ns	*	**	**			ns
$T_l$	ns	ns	**	**			ns
$SF$ cumulated	ns	*	**	**	**	**	**

of the normalized data from the value-1 line. Moreover, Table 1 shows the one-way analysis (ANOVA) for a selection of the most meaningful experimental days. Figure 7 and Table 1 demonstrate the sensitivity of all physiological indicators in determining the beginning of the trees' water deficit. TDF is confirmed to be the first physiological indicator of variations in tree water functioning. However, we were unable to perform a statistical analysis of MDS values because of the low number of available sensors.

Statistical analysis on selected days showed significant differences for  $pd\Psi_w$  and SF cumulated starting from 29 June. Between 30 July and 5 September all indicators showed significant differences, while a week after water restoration we observed differential responses. Restoration of irrigation was accompanied by abundant rainfall, leading to  $\Psi_s$  recovery. Nevertheless, differences in  $pd\Psi_w$ , SF cumulated and MDS were still evident. One week after water restoration, SF rate and cumulated values still showed about a 50% difference between treatments, indicating that the SF function was not yet fully restored in non-irrigated trees. When water stress develops over a prolonged period of time, water flow in stressed trees may remain permanently impaired, so that recovery is incomplete. One possible mechanism could be the high sensitivity of peach to cavitation phenomena, which drastically reduce hydraulic conductivity of the xylem and reduce plant recovery even after many days of irrigation (Massai et al. 2000b).

## Conclusions

The physiological indicators we analyzed showed different sensitivity in estimating tree water status.  $\Psi_s$ , measured both at dawn and throughout the day, SF and TDF revealed to be the best indicators. In contrast,  $T_1$  was found to have poor sensitivity, with results not easily interpretable. The physical indicator SWC gave clear results but not always easy to correlate with plant water status. Finally, the results presented here confirm and complement data previously reported by Goldhamer et al. (1999) allowing us to design the following scale of sensitivity: TDF > SF rate > SF cumulated =  $pd\Psi_w = \Psi_s > md\Psi_w > T_1$ .

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