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(SSD AGR03) XXII ciclo

Water status, growth, yield and fruit quality of 'Valencia' orange trees under partial root-zone drying

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International PhD course in Mediterranean Fruit Crops

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ABA	Abscisic Acid
B.D.	Bulk density
CI	Conventional irrigation
CO_2	Carbon dioxide
c_p	The specific heat of the air;
DI	Continuous Deficit Irrigation
DW	Dry weight
E	Water flux
IRR	irrigation
e_a	actual vapor pressure (kPa)
e_s	saturation vapor pressure for a given time period (kPa)
ET	Evapotranspiration
ET_c	Crop evapotranspiration
ET_o	Reference evapotranspiration
F.C.	Field capacity
FW	Fresh weight
G	Soil heat flux (MJ m^{-2} d^{-1});
g_s	Stomatal Conductance
Kc	Crop coefficient
PRD	Partial rootzone drying
PVPP	Polyvinylpolypyrrolidone
r_a	Aerodynamic resistance (s m^{-1})
RDI	Regulated Deficit Irrigation
RER	Relative elongation rate
RGR	Fruit relative growth rate
R_{leaf}	Resistance to leaf
R_n	The net radiation (MJ m^{-2} d^{-1})
R_{root}	Resistance to root
r_s	Surface or canopy resistance (s m^{-1})

R_{soil}	Resistance to soil
R_{total}	Total resistance
RWC	Relative water content
R_{xylem}	Resistance to xylem
SPAC	Soil-Plant-Atmosphere Continuum
SWP	Soil water potential
T	Air temperature at 2 m height [°C],
TA	Titratable acidity
TCSA	Trunk cross-sectional area, cm^2
TSS	Total soluble solids
u_2	Wind speed at 2 m height ($m\ s^{-1}$)
VPD	Vapor pressure deficit
W.P.	Wilting point
WUE	Water use efficiency
γ	Psychrometric constant ($kPa\ C^{-1}$)
Δ	Slope of saturation vapor pressure ($kPa°C^{-1}$)
λET	Latent heat of flux ($MJkg^{-1}$)
ρ_a	Air density ($kg\ m^{-3}$)
Ψ	water potential
Ψ_g	The gravitational potential
Ψ_l	Leaf Water Potential
Ψ_m	The matric potential
Ψ_p	Pressure or hydrostatic potential
Ψ_s	The osmotic potential
Ψ_{stem}	Stem Water Potential

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ABSTRACT

The Citrus genus includes tropical and subtropical species, which, as a group, represent an important share of the world economy. The sustainable production of citrus depends on the availability of adequate water throughout the year. Insufficient and non uniform water supply for irrigation in many parts of the world are leading to an increased effort toward the improvement of water use efficiency (WUE), including more efficient irrigation strategies. This study was conducted to evaluate the effect of partial rootzone drying (PRD) on water status, growth, yield and fruit quality of adult 'Valencia' orange trees. Three irrigation strategies were used during three crop seasons starting in spring 2007 and ending with the harvest of the third crop, in spring 2010. Treatments were conventional irrigation (CI) where water at 100% of crop evapotranspiration was applied to entire root-zone, partial root-zone drying (PRD) where 50% of CI water was applied to one alternated side of the root-zone, and continuous deficit irrigation (DI) where 50% of CI water was applied to both sides of the root-zone. Climate parameters and soil water potential (SWP) were monitored continuously; relative water content (RWC), stomatal conductance (g_s), fruit and shoot growth were monitored at regular intervals during the irrigation periods; yield and fruit quality was determined each year at harvest. RWC and g_s reductions under reduced irrigation were sporadic and varied depending on the year. No shoot growth reduction was observed in response to deficit irrigation treatments, whereas fruit size was consistently reduced by DI particularly in 2008. Yield parameters were generally not affected by irrigation, although DI trees tended to have lower yield, number of fruits, and yield efficiency than CI and PRD trees. Fruits of DI trees were smaller than those of CI trees. Fruits of DI trees also yielded less juice than fruits of CI and PRD trees, whereas juice of DI and PRD fruits showed higher soluble solids, acidity, vitamin C and carotenoids than juice of CI fruits. No significant difference was found between PRD and DI in terms of WUE, although PRD values were always higher on the average. Overall, our results suggest that PRD irrigation may represent a valid strategy for cultivation of oranges in the Mediterranean, while continuous deficit irrigation eventually causes a stress response, resulting in fruit size and juice yield reductions.

Keyword:

Citrus sinensis; crop WUE; deficit irrigation; PRD; peel color; relative water content; stomatal conductance; vitamin C

1

GENERAL PART

Literature Review

1. LITERATURE REVIEW

1.1 Climate of Sicily and citrus growing conditions

Exhaustive information is provided on citrus species grown in different countries of the world with different climates, soils, and harvesting seasons. Tree growth and fruit development require adequate light, water and appropriate temperature. Citrus is produced best under a mild subtropical climate. The best-quality fruit is produced in the Mediterranean climate, which is characterized by relatively dry (low rainfall), hot summers, and cool, wet periods (winter rains) during fruit maturity, in winter. When grown in this climate, fruits express relatively high sugar content and acidity, as well as uniform and intense peel color, as insect-pest problems on young fruit are generally few or of minor intensity (Milind, 2008).

Sicily can be defined as an area of typical Mediterranean climate (mild and rainy winters and hot and dry summers). In most part of the territory, the average annual rainfall is between 500-700 mm. The average temperature is around 26 $^{\circ}$C in summer (July – August), whereas the minimum temperature occurs between December and February with an average of 10-14 $^{\circ}$C (Cartabellotta et al., 1998).

Sicily possesses a favorable climate for growing a large number of cultivars of the major citrus species (53.9 % of total citrus production in Italy, ISTAT). The most commonly grown citrus in Sicily are sweet orange (*Citrus sinenis* L. Osbeck), lemon and mandarin. The major cultivars of sweet orange are 'Valencia' as processed blond, Navelina, Washington Navel (Ribera), Navelate, and New Hall as fresh-market blond, Moro, Tarocco, and Sanguinello as fresh and processed pigmented. A small amount of acidless oranges are also produced for the domestic market. Low temperatures are required for the development of the highly pigmented rind of oranges and mandarins (Spiegel-Roy and Goldschmidt, 1996). Blood orange varieties do well when grown in the Catania area (Barbagallo et al., 2007) as cool winter enhance the anthocyanin content and the red flesh color.

Rainfall in Sicily generally satisfies citrus evapotranspirative demand only in fall, winter and in part of spring, whereas in summer precipitations are scarce and generally not adequate for citrus growing (Cartabellotta et al., 1998). Hence, careful irrigation management during summer is essential for a constant citrus production and viable industry.

1.2. General concepts of irrigation

Irrigation is the application of water to living plants. Uphoff, (1986) defines irrigation as a practice of applying water to the soil to supplement the natural rainfall and provide moisture for plant growth. According to FAO (1994), irrigation is defined as the artificial application of water to the crop for the purpose of food and fiber production overcoming deficiencies in rainfall and help in creating stabilized agriculture.

1.2.1. Irrigation scheduling

The irrigation scheduling indicates how much irrigation water has to be given to the crop, and how often or when water is given. This ensures that water is applied to the crop when needed with the required amount (Brouwer et al., 1989; Evans et al., 1991). There are many factors to consider when determining a successful irrigation schedule according to (Doorenbos and Kassam 1979; Vedula and Nagesh Kumar 1996; Fereres and Evans, 2006) such as:

- Soil water holding capacity;
- Crop water use and crop sensitivity to moisture stress at different growth stages;
- Effective rainfall and availability of irrigation water.
- Prevailing climatic norms.

Water availability strongly influences flowering and fruit set and can affect fruit drop, fruit size, yield, internal fruit quality characteristics and canopy development (Falivene et al., 2006). Therefore, the planning of seasonal supply of the available amount of water must be directed towards meeting the water requirements of the plant during the most sensitive growth stages instead of spreading the available water to the plant equally over the all growing periods. For the non-sensitive growth stages, the amount of water allocated must be sufficient enough to prevent any effect on the yield and quality.

Reducing water supply during the initial and final stages of fruit development will delay size increases in lemons (*C.limon*), however, the final yield will be unaffected (Torrecills et al., 1993).

Koo (1969), Tucker (1986), and Parsons (1989) reported that optimal citrus production requires the maintenance of available soil water during the growing stages as follows:

1. **Flowering, fruit setting** and new flush development should have good soil moisture by applying water if rain does not fall at this time;
2. **Fruit development:** Fruit development: remarked by a high transpiration therefore, water deficiency would influence photosynthesis and consequently water requirements will be very high;
3. **Fruit maturation:** a high moisture content may have a harmful effect on fruit quality and flower initiation so that, it is recommended to reduce the soil water content at this stage and the soil should be kept fairly dry;
4. **Post-harvest:** after the crop has been harvested, trees require less amounts of water to restore growth.

On the other hand, irrigation scheduling saves water, energy, labor and maximizes yield response to other management practices. A careful use of irrigation water may also help reduce pollution and environmental risks (Ali, 2010). Irrigation scheduling can also help prevent several problems (Trimmer and Hansen, 1994), such as:

1- Water loss by deep percolation

2- Soil salinity

3- Water availability for irrigation

4- Low crop yield

Wample and Smithyman (2002) define the irrigation management as a tool for improving the production that can have many goals such as, control of tree vigor, prevention of occasional periods of water stress, improvement of fruit quality through its influence on the content of soluble solids and pH.

1.2.2. Water budgeting for irrigation scheduling

Irrigation scheduling involves determining both the timing of irrigation and the quantity of water to apply. It is an essential daily management practice for a farm manager growing irrigated crops. Proper timing of irrigation can be done by monitoring the soil water content or monitoring the crop in the field. Plant stress responses provide the most direct measure of identifying the plant demand for water. However, it should be noted that while plant stress indicators provide a direct measure of when water is required, they do not provide a direct volumetric measure of the volume of water required to be applied.

The crop water requirement is defined as the amount of water required to compensate the water loss from the cropped field (Allen et al., 1998). Many researchers describe it as the total water needed for evapotranspiration (ET). Therefore, the water requirement can be decided by determining the actual ET.

The crop water requirement can be related to the amount of water used by a reference crop. The reference crop typically is grass or alfalfa that is well irrigated and cov-ers 100 % of the ground. The reference evapotranspiration (ET_o) includes the water evaporated from the soil surface and the water transpired by the plants.

The daily ET_o can also be calculated from daily climate data like temperature, wind speed, sunshine and relative humidity. There are several methods used to calculate or measure ET_o. The most common methods are Penman method, Pan Evaporation and Blaney-Criddle method. The climate data can be obtained from a weather station.

The successful irrigation scheduling requires good understanding to the knowledge of soil water holding capacity, crop water use, and crop sensitivity to moisture stress at different growth stages. This requires consideration about the effective rainfall and availability of irrigation water (Waskom, 1994).

A good irrigation schedule requires an accurate quantification of ET. The most common approach to calculate ET is given by multiplying ET_o by the crop coefficient (k_c) which depends on ground cover and crop characteristics (Doorenbos and Pruitt, 1977; Allen et al., 1998; Villalobos et al., 2000). Figure 1.1 shows the components of the water budget for a micro-irrigated citrus tree.

1.2.3. Evapotranspiration (ET)

Evapotranspiration is a term used to describe the combined process between evaporation from soil and plant surfaces transpiration through canopies to atmosphere. Numerous factors must be considered when estimating ET (Fig. 1.1and 1.3), the major ones are:

1- Solar radiation;

2- Air temperature;

3- Wind speed;

4- Humidity.

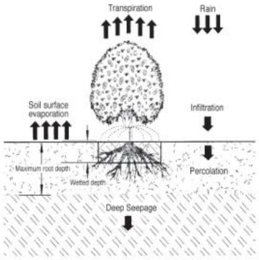

Figure 1.1. *Components of water budget for a micro-irrigated citrus tree (adapted from FAO series 2, 1997).*

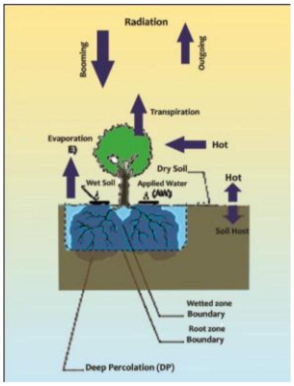

Figure 1.2. *Radiation and water balance on a plant under localized irrigation (adapted from FAO series 2, 1997).*

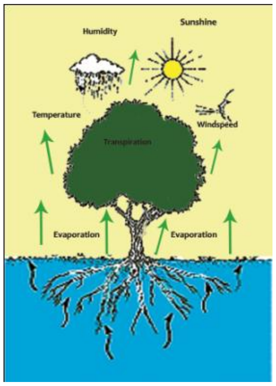

Figure 1.3. *Weather variables affecting evaporation, transpiration and soil moisture uptake by roots (adapted from FAO series 2, 1997).*

1.2.4. Reference crop evapotranspiration

The international FAO definition of ‘reference crop’ evapotranspiration is the evapotranspiration rate from a hypothetical reference crop with an assumed crop height of 0.12 m, a fixed surface resistance of 70 sec m^{-1} and an albedo of 0.23, closely resembling the evapotranspiration from an extensive surface of green grass of uniform height, actively growing, well-watered, and completely shading the ground (Doorenbos & Pruitt, 1977).

The Penman-Monteith equation is valid for different agro-climatic regions, with a 10% margin of error (Allen et al., 1998).

$$\lambda ET = \frac{\Delta(R_n - G) + \rho_a c_p \frac{(e_s - e_a)}{r_a}}{\Delta + \gamma\left(1 + \frac{r_s}{r_a}\right)}$$

Where:

λET: latent heat of flux “MJkg^{-1};

R_n: the net radiation “MJ m^{-2} d^{-1}”;

G: soil heat flux “MJ m-2 d-1”;

ρa: air density kg m-3;

cp: the specific heat of the air;

es: saturation vapor pressure for a given time period “kPa”;

ea: actual vapor pressure “kPa”;

$(e_s - e_a)$: saturation vapor pressure deficit “kPa”;

Δ: slope of saturation vapor pressure “kPa°C^{-1}”;

γ: psychrometric constant “kPa°C^{-1}”;

r_s : surface or canopy resistance “s m^{-1}”;

r_a : aerodynamic resistance “s m^{-1}”

From the original Penman-Monteith equation and the equations for aerodynamic and canopy resistance, the FAO Penman-Monteith equation has been derived as the following:

$$ET_o = \frac{0.408\Delta(R_n - G) + \gamma \frac{900}{T + 273} u_2 (e_s - e_a)}{\Delta + \gamma(1 + 0.34u_2)}$$

Where:

ET_o: reference evapotranspiration [mm day^{-1}],

T: air temperature at 2 m height [°C],

u_2 : wind speed at 2 m height [m s^{-1}],

1.2.5. Crop coefficient (K_c)

Allen et al. (1998) mentioned that in the arid and semi-arid regions of the Mediterranean, the recommended K_c values for citrus range from 0.70 in winter to 0.65 in summer with no ground cover. With growing ground cover or weed, the values range from 0.75 to 0.70, respectively for winter and summer.

The average yearly K_c value of 0.69 is in good agreement with reports from Arizona (Hoffman et al., 1982), Valencia (Castel and Buj, 1993), Cyprus (Eliades, 1994), Iran (Sepaskhah and Kashefipour, 1995), Crete (Chartzoulakis et al., 1999), California (Grismer, 2000) and Uruguay (Garcia Petillo and Castel, 2007).

Garcia Petillo and Castel, (2007) indicated that K_c has a clear seasonal trend. The minimum K_c is 0.60 in summer, intermediate values in autumn and spring (0.77 and 0.80, respectively) and a maximum of (0.87) in winter.

1.2.6. Crop evapotranspiration (ET_c)

By adopting a reference crop (grass), it has become easier and more practical to select consistent crop coefficients and to make reliable actual crop evapotranspiration (ET_c) estimates in new areas. The calculation of ET_c (mm day^{-1}) requires the selection of appropriate K_c for use with standardized reference evapotranspiration (Allen et al., 1998).

$$ET_c = K_c \times ET_o$$

1.3. Basics of water relations in plants

Water relations are important to the functioning of trees, as water is the greatest component of the active tree (by mass), and all biological processes and growth may be limited by an inappropriate water status (Lakso, 2003). Literature shows that changes in plant water relation parameters, like leaf water status and stomatal response, can be explained in terms of changes in the hydraulic architecture of trees (Tyree and Cochard, 1996; Salleo et al., 2000).

Plant water status is usually described by two basic parameters: the content of water in the plant or the energy status of the water in the plant, expressed as the (total) water potential, ψ (Kirkham, 2005). It can be regarded as a very sensitive indicator for the degree of water stress experienced by the plant (McCutchan and Shackel, 1992).

Different irrigation regimes and their effect on water relations have been studied in many tree crops and other perennial and annual plants. The emphasis of most studies has mainly been on yield, vegetative growth and water relations in response to reducing water supply. Citrus fruits are frequently grown in areas where water supply limits optimum growth and production. Many experiments considering water relation of citrus trees have been carried out under different conditions (Levy, 1980; Habermann et al., 2003; Pérez-Pérez et al., 2007). Although these results may be of scientific value, they are of limited applicability to orchard conditions due to different environmental conditions within orchards.

1.3.1. The soil-plant-water-atmosphere continuum (SPAC)

The Soil-Plant-Atmosphere Continuum (SPAC) is the pathway for water moving from soil through the plant to the atmosphere. The transport of water along this pathway occurs in components only as separate defined and differently between a scientific discipline in the environment (John, 1966):

- Soil physics characterizes soil water in terms of tension,
- Plant physiology characterizes plant water in terms of *diffusion pressure deficit,*
- Meteorology uses vapor pressure or relative humidity to characterize atmospheric water.

An analog of ohm’s law was proposed to describe and analyze the path of water flow from the soil through plants, and the atmosphere (Van den Honert, 1948). The ability of plants to maintain a favorable water status depends on the resistance to water flow in the SPAC (Jones et al., 1982; Ruggiero et al., 2003).

Total resistance (R_{total}) in the water transport pathway in the SPAC controls water f low. The analogy equates water flux to an electrical current, the water phase to the electromotive force, the resistance to either liquid or gaseous diffusion, and water flux to an electrical resistance analog (Bravdo, 2000), e.g.,

$$E = \frac{\Delta\Psi_{soil-root\,surface}}{R_{soil}} = \frac{\Delta\Psi_{root\,surface-xylem}}{R_{root}} = \frac{\Delta\Psi_{xylem}}{R_{xylem}} = \frac{\Delta\Psi_{leaf-atmosphere}}{R_{leaf}}$$

Where E = water flux, Ψ = water potential, R_{soil} = resistance to soil, root, xylem and leaf.

The water absorbed by the root hairs is translocated upwards through the xylem. The ascent of sap or movement of water from root to leaf is explained by the cohesion-tension theory. A number of theories have been put forward at various times to explain the mechanism of ascent of sap. These are (i) Vital theories, (ii) Root Pressure theory and (iii) Transpiration pull (Rajan, 2003).

1.3.2. Water potential concepts

Water potential denotes the state of water in the plant or soil as compared to that of pure water (0 MPa). It depends on three factors: concentration, pressure and gravity. The following equation expresses the components of water potential (Tromp et al., 2005):

$$\Psi_t = \Psi_p + \Psi_s + \Psi_g + \Psi_m$$

Where Ψ_p is the pressure or hydrostatic potential, Ψ_s the osmotic potential, Ψ_g the gravitational potential and Ψ_m the matric potential.

Leaf water potential (Ψ_l) and stomatal conductance (g_s) are responding to the interacting environmental factors such as radiation, temperature, availability of moisture and vapor pressure deficit (VPD) (Elfving et al., 1972; Hall et al., 1976; Pereira and Kozlowski, 1978). Such stomatal responses to VPD generally act to minimize the effect of changing environment on ET, and hence Ψ_l. The response of leaf g_s to these environmental and edaphic parameters has been reviewed (Camacho, 1977). Fereres et al., (1979) showed that low Ψ soil had an overriding influence on leaf g_s. This relationship has been observed to various degrees by others (Kriedemann and Barrs, 1981). A humidity response is probably also involved in the gradual closure of stomata during the day as this has been observed in apple (Landsberg et al., 1975; Lakso, 1983; 1986) and in citrus (Sinclair and Allen, 1982; Cohen and Cohen, 1983).

Elfving et al., (1972) have shown a close positive correlation between Ψ_l and transpiration rate of citrus. Subsequently, it has been shown that stomata opening results in immediate onset of transpiration which peaks late in the morning (Davies and Kozlowski, 1974 and Hall et al., 1975). However, water relations of citrus are also influenced to a large extent by high resistances to water transport within the plant. As the soil dries, and water uptake rate from the soil falls below the potential transpiration rate, so the actual transpiration rate falls. This situation arises when the soil-plant conducting system cannot sustain the rate of water loss driven by atmospheric demand, and so g_s

must fall. Therefore, the effects of falling gravimetric water content θ_g in the root zone are reflected in changing leaf g_s values of canopy/stomata conductance values.

In citrus, changes in plant water potential and atmospheric demand (humidity and temperature) influence primarily stomatal behavior (Hall et al., 1975; Levy, 1980). Transpiration of citrus trees is much more sensitive to changes in total leaf g_s than in many other agricultural crops due to the generally high boundary layer conductance of orchard canopies (Jarvis et al., 1981). This high boundary layer conductance implies a close coupling with the environment.

Measurement of plant water status by compensation, sap flow, porometry and pressure chamber methods are useful in establishing the degree of water stress in plants (Slavik, 1974; Kramer, 1983).

1.4. Photosynthesis and transpiration

1.4.1. Water use efficiency (WUE)

Efficiency is generally defined as the ratio of output over input. Historically, the term “water use efficiency” has been used in two very different senses, hydrological and physiological (Stanhill, 1986). WUE includes any measure that reduces the amount of water used per unit of any given activity, consistent with the maintenance or enhancement of water quality (Richards et al., 2002).WUE is often considered an important determinant of yield under stress and even as a component of crop drought resistance. It has been used to imply that rainfed plant production can be increased per unit water used, resulting in ‘‘more crop per drop’’ (Blum, 2009). Increasing WUE in irrigated agriculture and promoting dry land farming will both play a significant role in maintaining food security (Deng et al., 2006).

Chaves et al. (2004) defined WUE as the ratio of carbon gained in dry matter over a given period, to water loss over the same period. In C_3 plants, the actual rate of CO_2 assimilation, which is dictated by CO_2 availability (g_s), corresponds to the internal CO_2 partial pressure. If g_s increases above the operational point, leaf photosynthetic rate would only marginally increase and WUE would decrease (Schulze, 1986, Schulze, et al., 1994, Chaves, et al., 2004). Schulze et al. (1994) summarized WUE as follows: stomata are able to balance CO_2 entry into the cellular space for photosynthesis to occur, and also control cell dehydration by minimizing water loss; thus stomata will open to the extent required to provide sufficient CO_2 to meet the requirements for photosynthesis. During water stress periods, when the midday stomatal conductance is high, the daily net CO_2 assimilation decreases, leading to low CO_2 availability, which further leads to decline in carboxylation efficiency and lower intrinsic WUE (WUEi) (Valladares and Pearcy, 2002; Chaves, et al., 2004).

1.4.2. Stomata hydraulics

Stomata act as valves, opening and closing in response to changing conditions and so, control water loss from plants. An important plant function that is influenced by internal water status is the regulation of stomata aperture. The greatest loss of water by plants is through leaf stomata during transpiration (Mullins et al., 1992).

In response to soil drying, many plant species close their stomata without any change in plant water status (Bates and Hall, 1981). The suggestion of another controlling mechanism was supported by an experimental split-root system that demonstrated that plants respond to a signal from the roots (Blackman and Davies, 1985; Coutts, 1982). The plant hormone abscisic acid (ABA) has been identified as a compound that plays a role in stomatal closure as soil dries.

Stomata are connected hydraulically to each other throughout leaf (Raschke, 1970) and to the rest of the transpiration stream through the xylem vessels in the leaf petiole (Fig 1.1). This connectivity has been demonstrated by many researchers in experiments where a perturbation introduced on one part of the leaf, e.g. by imposing irradiance (Buckley and Mott, 2000) or a step change in the vapor pressure deficit (VPD) of the air (Nonami et al., 1990), caused the stomata on the same leaf but distal from the perturbation to respond despite no local change in either the irradiance or VPD. Thus the behavior of individual stomata is strongly influenced by developments elsewhere in the transpiration stream. This is substantiated by the fact that plants behave in such a way that their water status is maintained above a certain threshold value by ensuring that their stomatal conductance somewhat matches the hydraulic conductance of the transpiration stream (Meinzer et al., 2003). For instance, trees with high stomatal conductance generally tend to have high hydraulic conductance as well. Therefore, Limiting water supply to the leaves e.g. by transverse cuts to the xylem vessels or by inducing air embolisms causes the stomata conductance to decrease (Sperry et al., 1993).

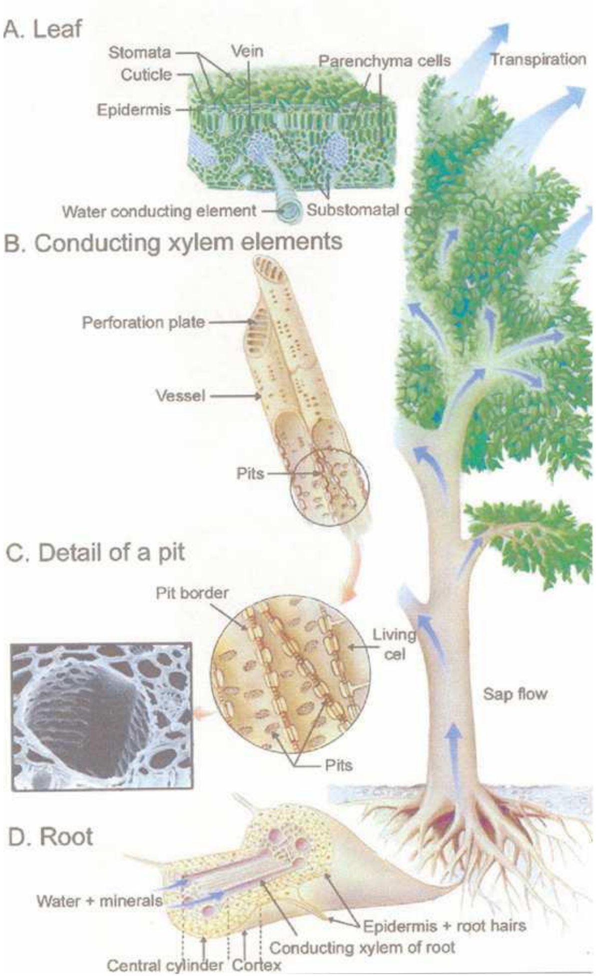

Fig. 1.4.*Components of the transpiration pathway for a typical tree from the roots to the leaves (from Dzikiti, 2007). The detailed characteristics of each component are shown on the left hand side of the diagram.*

1.5. Deficit irrigation

Deficit irrigation practices differ from traditional irrigation practices. The manager needs to know the level of transpiration deficiency allowable without significant reduction in crop yield. Deficit irrigation is a promising water management strategy to achieve increased crop water productivity (English, 1990). It is an optimization strategy in which irrigation water is applied during sensitive growth stages of a crop and whereby, outside these periods, irrigation is limited or can even be absent if rainfall guarantees a minimum supply of water.

1.5.1. Regulated deficit irrigation (RDI)

Behboudian and Mils (1997) define Regulated Deficit Irrigation (RDI) as a system of managing soil water supply to impose periods of predetermined plant or soil water deficit that can result in some economic benefit. Over the last two decades, extensive research work conducted on fruit trees have demonstrated a positive yield response to mild water deficits (Marsal et al., 2002; Chalmers et al., 1981).

Originally, RDI was developed primarily to control vegetative growth in high density orchards, and secondarily to maximize fruit size, fruitfulness and fruit quality (Goodwin and Jerie, 1992). Thus, it consists on deliberately applying an amount of water at a level below water requirement during the irrigation season in order to save water and control vigor (Goodwin and Boland, 2000). A certain reduction in yield is observed, but fruit quality (e.g. sugar content) tends to be equal to or even greater than that of fully irrigated trees (Marouelli and Silva, 2007; Spreer et al., 2007; Cui et al., 2008; Hueso and Cuevas, 2008).

A certain level of drought stress is maintained in the root zone and the primary objective of its use in tree crops is to control the canopy size and save water. Usually a fraction of ET_c is replaced during each irrigation rather than applying the full ET_c rate. Successful implementation of the RDI strategy depends on a precise knowledge of the vegetative and reproductive development of the fruit tree under investigation. For example, periods when vegetative growth is normally high and fruit growth is low are ideal for reducing irrigation rates (RDI) since a significant portion of resources can be allocated in favor of fruit development rather than toward vegetative growth. On the other hand, application of RDI during the sensitive periods, e.g. flowering and fruit set, is not advisable. For those reasons, much research has focused on the appropriate timing of the application of this strategy, also in citrus (Goldhamer and Salinas, 2000).

1.5.2. Citrus and water deficit

Water stress is the physiological condition to which a tree is subjected whenever the rate of water loss from the leaves by transpiration exceeds the rate of water absorbed by the root system; this reduces plant growth and biomass production. McCarthy et al. (2002) reported that vegetative growth (shoot growth) is more sensitive to water stress than fruit growth.

A readily available soil water supply appears essential for the development of optimum fruit size and yield in citrus (Levy et al., 1978; Moreshet et al., 1983). Plant water deficits, resulting from a reduction in the flow of water through the soil-plant system, affect a wide range of physiological and developmental processes involved in fruit production, including reduced growth due to inhibited cell division and cell expansion, and reduced photosynthetic capacity due to stomatal closure (Slatyer, 1967; Kozlowski, 1974; Hsaio, 1973).

Pérez-Pérez et al., (2007) showed that the effect of water stress on growth and yield depends on the amount and time of occurrence of the water stress. It also varies with crop species and variety. The yield response to water shortage can vary depending on the particular growth period. The initial period of fruit growth is the most sensitive phase to drought stress. Total soluble sugars and titratable acidity increased when a severe drought stress occurred only in the final period of fruit growth, near ripening and harvest. On the contrary, it only increased the peel/pulp ratio if it occurred in the initial period of fruit growth.

Citrus trees have the tendency to conserve water through relatively low transpiration rates and relatively high leaf and root resistances (Kriedemann and Barrs, 1981). But a large part of the resistance to water stress in citrus also appears to rest in its ability to adapt to water deficits under a range of environmental conditions. There is, however, little unequivocal information available concerning the effect of decreasing soil water potential on total root hydraulic resistance within citrus trees. These characteristics can potentially be manipulated to improve WUE, but to achieve this goal there is a continuing need for more knowledge about the physiological processes that control water use.

Moss and Muirhead (1971a, b) in their study on 'Valencia' orange showed that temperature and evaporation rates during November were the most consistent predictors of yield in New South Wales. After allowing for the effects of tree age and yield in the previous season, 44.5% of the remaining variability in Valencia was attributed to temperature, with 32.1% due to evaporation conditions. Similar responses for yield variability (ranging from 26 to 150 kg per tree) from one season to the next, have been reported by Jones and Cree (1964) who showed that yield appeared negatively correlated with the previous year's crop and with daily maximum temperature following bloom.

On the other hand, (Hilgeman, 1977) showed that fruit quality (total soluble solids and total acidity) was improved when late summer water stress was induced. Also other studies indicate that reduced water supply, particularly late in fruit development, is associated with higher total soluble solids and titratable acidity (Castel and Buj, 1990; Peng and Rabe, 1998; Hutton et al., 2007). Mantell et al. (1976) reported similar results in 'Shamouti' orange where fewer summer irrigations resulted in improved juice quality (higher levels of vitamin C, sugar, citric acid, and higher total soluble solids to acid ratio), despite increasing the number of small fruit per tree.

Manner et al., (2006) indicated that citrus growth in dry Mediterranean climates, with average rainfall less than 250 mm/yr and dry summers, is only possible with irrigation. Citrus can generally tolerate 3–4 months of minimal rainfall. Drought tolerance depends on temperatures, soils, wind, and the desired level of fruit production. Citrus loses productivity in drought and requires irrigation during the summer months to sustain production also in Florida and Central and Southern California.

Syvertsen (1982) reported that citrus leaf water potential is related to age, and minimum leaf water potential for stomatal closure of young leaves were as high as -1.6 MPa which coincides with previously measured leaf potentials at zero turgidity. As for mature leaves, water potential was as low as −3.5 MPa in 3- to 6-month-old leaves. The rate at which new leaves are produced and become mature may be an important mechanism by which the tree can adjust to water stress.

González-Altozano and Castel (1999) reported that Clementine yields were reduced when water stress was applied at an early stage of fruit ontogeny through increased fruit drop. However, stress applied at a later stage can affect fruit yield by decreasing fruit weight.

Syvertsen (1985) showed that vegetative growth in citrus is very sensitive to water stress. Long water stress period reduces yield of orange about 25% (Pires et al., 2008).

Shalhevet and levy (1990) reported that size, rind appearance and internal maturity are the major quality parameters for citrus fruit, and these variables are strongly influenced by irrigation management. Water application may enhance some performance variables while minimizing effects on others.

Research on citrus irrigation has been reviewed by several authors (Doorenbos and Kassam, 1979; Kriedemann and Barrs, 1981; Shalhevet et al., 1981). Functional relationships between yield and water deficits in citrus have been given by Shalhevet and Bielorai (1978) and Doorenbos and Kassam (1979). In some of these irrigation studies, the sensitivity of yield to water stress has been found to depend on the phenological growth stage at which the deficit occurred.

1.5.3. RDI studies on other crops

Many studies have been performed on deficit irrigation and other water related concepts of different fruit crops:

- Dry et al., (2000) reported that RDI is widely practiced in the red wine orchard in Australia.

- Levy et al. (1978) indicated that in the long term, vegetative growth can be reduced by deficit irrigation more than fruit growth. Water relations and vegetative development are a function of water available to plants (Tognetti et al., 2006).

- The impact of deficit irrigation on fruit quality has been investigated for several fruit species “apple, olive and mango”. In many cases, a positive influence on fruit quality was reported (Bussakorn et al., 2000; Motilva et al., 2000; Pickering et al., 2002).

- Leib et al., (2004) found that fruit weight per tree was reduced by 10% in the deficit irrigation treatment as compared to the conventional irrigation and partial root-zone drying treatments. Also, the deficit irrigated treatments produced higher soluble solids and greater firmness than the control treatment. Gómez-Rico et al. (2007) noted that trees under RDI produce an olive oil similar in composition and quality to that of trees irrigated with 100% Et_c.

1.5.4. Partial root-zone drying (PRD)

PRD is a deficit irrigation strategy designed to maintain half of the root system in a dry or drying state, while the other half is irrigated. The theory behind PRD is based on root-to-shoot chemical signaling in drying soil, which is supposed to reduce stomatal conductance (Loveys et al., 2000; Gowing el al., 1990; Düring et al., 1996). This strategy involves exposure of roots to alternate drying and wetting cycles and enables plants to grow with reduced stomatal conductance but without signs of water stress, (Zhang et al., 1987; Davies et al., 1994; Davies et al., 2002; Santos et al., 2003; Kang and Zhang 2004). Figure (1.2) shows a schematic diagram indicating how roots might interact with drying soil to generate chemical signals in the xylem in a typical PRD setting.

A practical inconvenience of PRD is that it requires the use of twice the amount of tubing than RDI or conventional irrigation, thus increasing installation costs. Nevertheless, the underlying mechanisms of PRD functioning are still a matter of discussion. Bravdo (2005) stated that it is not possible to have absolute control of root drying under field conditions and that hydraulic redistribution from deeper to shallower roots may prevent the clear results that can be obtained in potted plants.

The PRD seems to give better results in soils that ensure high rates of infiltration and deep roots. However, the depth of the root-zone is not necessarily directly correlated with the infiltration rate. Low infiltration rates may be associated with both superficial and deep root systems.

The partial root-zone drying irrigation technique has been shown to reduce the need for pruning (Dry et al., 1996) due to a reduced vegetative vigor. In addition, opening up of the canopy increases light penetration to the fruit which increases the color e.g. of grape fruit and increases the content of compounds associated with flavor and aroma. Most important also, half the amount of water applied to control plants can be added to apple trees under PRD without significant yield reductions (Talluto et al., 2008). Interesting results of the application of PRD on cotton showed that the crops were ready for harvest three weeks earlier than the control treatment (Mingo and Davies, 2001).

PRD improve WUE in various tree crop species (Kang et al., 2002; Grant et al., 2004; Romero et al., 2004; van Hooijdonk et al., 2004; Cifre et al., 2005; Tognetti et al., 2005). PRD clearly improved yield per unit of applied water with respect to conventional irrigation when high irrigation volumes are applied (Davies et al., 2002; Kirda et al., 2007; Morison et al., 2008).

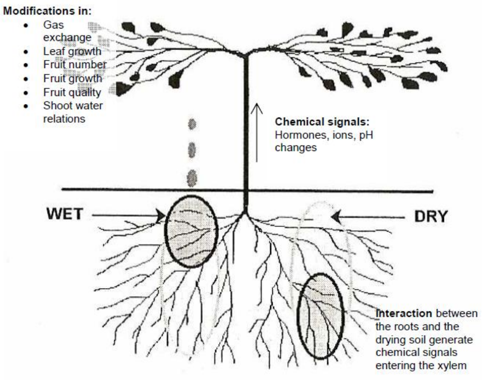

Fig. 1.5. *Schematic diagram shows how roots might interact with drying soil to generate chemical signals in the xylem in a typical partial root-zone drying setting (from XXX).*

1.5.5. The role of abscisic acid (ABA)

Many studies demonstrate that ABA can play a central role in the long distance drought signaling process in many plants (Wensuo Jia and Zhang, 2008). The PRD technique is based on plant root-to-shoot chemical signaling that influences shoot physiology. The production of ABA by roots in a drying soil restricts gas exchange and photosynthesis through its action on stomata. Theoretically, roots of the watered side of the soil will keep a favorable plant water status, while dehydration on the other side will promote the synthesis of hormonal signals, which will reach leaves via the transpiration stream and further reduce stomatal conductance. This will decrease water loss and vegetative growth and increase WUE (Dry et al., 1996; Davies et al., 2000). The PRD strategy may also increase root growth at deeper layers of the soil as it has been described for grapevine (Dry et al.,2000b; Santos et al., 2003) or in the entire root system, as shown for tomato (Mingo et al., 2003). PRD strategies have also resulted in higher xylem pH (Davies and Zhang 1991; Dry et al. 1996; Dry and Loveys 1999; Stoll et al. 2000) and lower cytokinins levels (Davies et al. 2005) which restricts stomatal opening. The PRD may also bring about other benefits to the crop besides higher WUE.

It can influence carbohydrate partitioning between the different plant organs and affect the quantity and quality of the harvest (Kang and Zhang, 2004).

1.5.6. Previous studies on PRD

Different experiments were conducted on PRD to understand the impact of this strategy on different crops:

- Dry et al., (1996) explained that the effects of PRD in grapevine are:
    1- Increase in water use efficiency
    2- Reduction of vegetative vigor, which can affect light penetration and consequently improve grape quality
    3- There are no significant reductions in yield and berry size.
- Spreer et al. (2007) Reported that in a mango orchard under PRD, fruit size was increased and had a higher fraction of edible parts compared to RDI.
- The PRD technique also allows for reduction of irrigation water, good fruit size and reduction of shoot growth in field-grown 'Braeburn', 'Fuji', 'Gala' 'Pacific Rose™' and 'Pink Lady' apple (Caspari et al. 2004; Einhorn and Caspari 2004; Lombardini et al. 2004; Talluto et al., 2007 e 2008; Zegbe and Behboudian, 2008).
- Leib et al., (2004) demonstrated that a 35 to 45% reduction of water with PRD strategies caused minimal apple yield and size reductions.
- O'connell and Goodwin (2007a) showed that PRD is not recommended for micro-irrigated pear orchards on fine-textured soils in the Goulburn Valley, Australia.
- Kang et al. (2003) showed that in a pear orchard, PRD results in higher WUE compared to conventional flood irrigation due to water loss by percolation.

- Wahbi et al. (2005) showed that in an olive orchard, PRD has not improved the crop response over an RDI regime that applied the same amount of water under arid condition in southern Morocco.

- Kang and Zhang (2004) reported water savings under PRD of 52% in peach and 23% in pear, respectively.

- Water reduction in grapevine may reduce vegetative growth without any effect on the grape quality (Stoll et al., 2000; dos Santos et al., 2003; Souza et al., 2004), and wine quality was consistently improved under PRD (Dry et al., 2000).

- In maize, PRD irrigation reduced water consumption by 35% with a total biomass reduction of 6–11% as compared to fully watered plants (Kang and Zhang, 2004).

- Another experiment with hot peppers and drip irrigation showed that PRD reduced water used for irrigation by about 40% and maintained similar yield as in fully watered plants (Kang et al., 2001).
- The limited data available for PRD effects on **citrus** have pointed to a water volume rather than a PRD effect on yield and fruit size (Eliades et al., 2004).
- Kidra et al., (2007) showed that WUE under PRD was increased in mandarin (*Citrus reticulata* cv. Marisol) in Turkey.
- Dzikiti et al. (2008) showed that in navel oranges PRD decreased g_s, while maintained leaf water potential to control levels.
- Treeby et al. (2007) showed that size of navel oranges was reduced by both PRD and simple reductions in irrigation volumes.

2

EXPERIMENTAL PART

2.1. INTRODUCTION

Cultivation of fruit trees in semi-arid regions is often characterized by increased evapotranspiration, high soil salinity and limited water availability. Those conditions are expected to spread and intensify, especially in the Mediterranean, due to climatic changes, demographic pressure and greater allocation of water resources to municipalities and touristic areas. For those reasons increasing crop water use efficiency through the rationalization of irrigation becomes critical also for citrus fruit production.

Regulated deficit irrigation (RDI) has been long recognized as a potent tool to manage fruit trees, and specifically to reduce shoot growth, improve fruit quality, and save some irrigation water by imposing plant water stress in a controlled manner (Chalmers et al. 1981; Mitchell and Chalmers 1982). Results of RDI experiments have been promising in certain regions and for some fruit crops, such as peach (Chalmers et al. 1981), pear (*Pyrus communis* L.) (Caspari et al. 1994; Mitchell et al. 1989), French prune (*Prunus domestica* L.) (Lampinen et al. 1995), almond (*Prunus dulcis* L.) (Goldhamer et al. 2006), pistachio (*Pistacia vera* L.) (Goldhamer and Beede 2004), apricot (*Prunus armeniaca* L.) (Ruiz-Sàncez et al. 2000), and olive (Goldhamer 1997; Moriana et al. 2003). In citrus, where yield seems to proportional to shoot growth, the control and regulation of water deficit is quite complex resulting often in significant fruit drop or reduced final fruit size and yields, depending on the period of deficit imposition (González-Altozano and Castel, 1999; Hutton, 2004; Pérez-Pérez et al. 2008).

Partial rootzone drying (PRD) is an irrigation technique that was recently developed for grapes (*Vitis vinifera* L.) in Australia, with the primary aim of saving irrigation water (Dry et al. 1995; Dry and Loveys 1998). With PRD only one half of the rootzone is irrigated, whereas the other half is not, with 50-60% savings over conventional irrigation. The physiological basis for PRD is that roots in drying soil produce abscisic acid (ABA), The physiological basis for PRD is that roots in drying soil produce abscisic acid (ABA); if there is sufficient sap flow through those roots, ABA is translocated to the shoots (Dodd et al. 2008a) where it signals a developing soil-water deficit (Dry et al. 1995). Accounting for the relative contributions of different parts of the root system to sap flow can also explain shoot xylem ABA concentrations (Dodd et al. 2008b). In the leaves, ABA induces partial stomatal closure, which reduces transpiration and may increase water use efficiency. However, as the other half of the rootzone is kept well watered, the effect on plant water potential is minimal (Gowing et al. 1990) and other metabolic and physiological processes associated to water stress are not affected (Dry et al. 1995; Dry et al. 2000). The technique relies on cyclical wetting and drying of parts of the rootzone in order to maintain root derived ABA signals (Zhang and Davies 1987). Increased xylem ABA and pH (Davies et al. 2002), both probably responsible for partial stomatal closure, and reductions of cytokinin levels in roots, shoot apexes, and buds (Stoll et al. 2000) have been reported in plants under PRD regime. The resulting changes in hormonal balance may contribute to reduce vegetative growth under those conditions.

The impact of PRD has been extensively investigated in grapevine (Dry and Loveys, 1998 and 1999; Stoll and Loveys, 2000; Santos et al., 2003) and, to a lesser extent, in other woody perennials (e.g. olive, Wahbi et al., 2005; apple, Talluto et al., 2008). Many studies support a PRD effect (e.g. grapevines, Antolín et al., 2006; apple, Leib et al., 2006; mango, Spreer et al., 2007), but some studies support a water volume effect (e.g. grapevines, Gu et al., 2004). The limited data available for PRD effects on citrus have pointed to a water volume rather than a PRD effect on yield and fruit size (Eliades et al., 2004).

In sub-tropical evergreen citrus trees, vegetative growth is sensitive to water stress (Syvertsen, 1985). Growth is often positively related to yield, and drought stress almost always results in reductions in growth and yield. Thus, large amounts of irrigation water are generally supplied to citrus trees to prevent drought stress. In addition, water savings by DI and PRD in citrus have not been entirely successful in Mediterranean-like climates, where tree growth, fruit size and yield decreased in response to insufficient amounts of water (Hutton, 2004).

Valencia oranges are grown in various subtropical and Mediterranean regions and primarily intended for production of juice and concentrates. For this purpose, major quality parameters are juice yield, TSS, vitamin C and other dietary supplements. In Mediterranean regions, navel oranges represent the major source of oranges for fresh consumption, but their production covers market demand only until March. Valencia oranges may therefore represent a good option to extend orange fruit production into late spring. Size, rind appearance and internal maturity are the major quality parameters for fresh citrus fruit, and these variables are known to be influenced by irrigation management (Shalhevet and Levy, 1990). Reduced water supply, particularly late in fruit development, is associated with higher total soluble solids (TSS) and titratable acidity (TA) (Castel and Buj, 1990; Peng and Rabe, 1998; González-Altozano and Castel, 1999; Hutton et al., 2007). Reducing water supply during the initial and final stage of fruit development will delay size increases in lemons (*C. limon*), despite the final yield being unaffected (Torrecillas et al., 1993). Clementine mandarin yields may be reduced by water deficit at an early stage of fruit growth through increased fruit drop, but at a later stage by decreasing fruit weight (González-Altozano and Castel, 1999).

RDI strategies during the intermediate stage of citrus fruit growth have been reported to save water under semi-arid conditions (Pérez-Pérez et al. 2008). However, the use of PRD in citrus trees has not been extensively investigated as there is a lack of understanding of growth response to seasonal drought stress. The objectives of the present study was to determine physiological, growth, yield and fruit quality responses of 'Valencia' orange trees to PRD and continuous deficit irrigation under field conditions of a typical Mediterranean climate.

2.2. MATERIAL AND METHODS

2.2.1. Experimental site

The experiment was performed on an area of about 800 m^2, at the experimental farm of the School of Agriculture, University of Palermo, Italy (30.06N, 13.21E, and 31 m a.s.l.). Type, physical and hydraulic properties of the soil are indicated in table 2.1.

Tab. 2.1. Soil properties at the experimental site. F.C. = field capacity, W.P. = wilting point, B.D. = bulk density.

Soil sample depth (cm)	Particle Size Distribution (%)			F.C.	W.P.	B.D.	Texture Class
	Sand	Silt	Clay				
15-30	52	29.1	18.9	0.239	0.123	1.44	Loam

The study was carried out on 48 adult orange trees (*Citrus sinensis*, cv Valencia) grafted onto sour orange (*Citrus aurantium*, L.) rootstock, spaced at 4×4 m, trained at full globe canopy branched at about 0.8 m from the ground and reaching 3-3.5 m in height. The study considered three crop seasons starting in spring 2007 and ending with the harvest of third crop in spring 2010. Typically, the 'Valencia' orange trees at the experimental site bloom in the month of April and fruits reach full maturity after about 400 days.

With the exception of irrigation all trees received same conventional cultural cares: Complex solid fertilizers (20N:10P:10K at the rate of 1.5 kg/tree) were applied to the soil once a year in winter and weeds were controlled by regular tilling of inter-rows and rows. Irrigation was provided by a micro-sprinkler system with a single line per tree row and two emitters per tree located at about 1 m from the trunk and 0.5 cm from the ground. Specifically, micro-sprinklers with 360° circular spray pattern were used and nominal delivery rates are reported in Table 2.2. Micro-sprinklers were connected to PE lines with 5-mm diameter polyethylene tubing of various length and wood sticks were used for exact positioning of emitters with respect to tree trunk, soil humidity sensors, and portion of rootzone to be irrigated.

Before the irrigation season started each year, the system was flushed to clean various deposits out of the pipes and the plugged micro-sprinklers and filters were replaced with new ones. Uniformity of water distribution was checked by measuring delivery rates of all emitters each year before the irrigation season started.

2.2.2. Irrigation treatments and experimental design

In spring 2007, 48 trees uniform in size were selected and labeled according to a randomized block design with four replicates (blocks), each including four trees per irrigation treatment (total 12 trees per replicate). In June, three irrigation treatments were imposed, 1) conventional irrigation (CI), where two 3-mm-diameter emitters per tree were used so that trees received 100% of water consumed by crop evapotranspiration on both sides of the rootzone; 2) partial rootzone drying (PRD), where one 3-mm-diameter emitter per tree was used so that trees received about 50% of CI water only on one alternated side of the rootzone; 3) continuous deficit irrigation (DI), where two 2-mm-diameter emitters per tree were used so that trees received about 50% of CI water on both sides of the rootzone. The interval between irrigation events and the duration of each event (minimum 40 min maximum 2 h) were adjusted to maintain soil moisture of wetted volume above 80% of field capacity (-50 kPa) in the rootzone of CI trees but avoid spreading of wet areas into the dry sides of PRD trees. Wet and dry sides of PRD trees were alternated every 2-3 weeks when soil water potential in the dry side reached values of approximately -100 to -150 kPa.

Table 2.2 Characteristics of the micro-sprinklers used in the experiment.

***Φ* mm**	**Flow rate (l/h)**	
	1 bar	**1.5 bar**
2	183	210
3	384	445

2.2.3. Climate, water requirements, and water status.

Climate parameters were recorded hourly with a μMetos weather station (Pessl, Austria) positioned within the experimental plot. These data were compared to regional agro-meteorological data (Servizio Informativo Agrometeorologico Siciliano) and used to calculate daily reference evapotranspiration ($ET_{\circ}$) according to the FAO Penman-Monteith equation. Irrigation volumes to CI trees were calculated to meet crop evapotranspiration (ET_c), which was determined as

$$ET_c = ET_{\circ} \times K_c$$

where K_c was derived from FAO indications and set to 0.7 from October through May, and to 0.65 from June through September, due to the specific soil management, climate conditions and to the fact that 'Valencia' trees bear fruits all year around (Allen et al. 1998).

Vapor pressure deficit (VPD) was calculated from air temperature (in °C) and relative humidity (in %) measured hourly by the weather station located nearby the trees, and plotted against time to give an indication of daily variation in atmospheric vapor demand at sampling dates. Also instantaneous VPD was caculated from canopy air temperature and relative humidity measured on the same dates and at the same time of stomatal conductance measurements and used to establish associations with other parameters. Soil water potential (SWP) was monitored continuously at a fixed depth of 45 cm with six Watermark sensors (Irrometer Co., Riverside, CA, USA) directly connected to the weather station. Sensors were positioned at about 80 cm from emitters and 1 m from the tree trunk in opposite sides of the rootzone.

Stomatal conductance (g_s) was monitored bi-weekly throughout the irrigation period and at longer intervals during rainy periods using an AP4 Delta-T porometer (Delta-T Devices, Cambridge, UK). On each date, the device was calibrated and g_s was measured between 10:00HR and 12:00HR on one mature, sun-exposed leaf per tree.

In the same sampling dates, two leaves similar to those used for g_s measurements were wrapped in parafilm and aluminum foil, detached, enclosed in zip-lock bags, and transported in ice to the laboratory for determination of fresh weight (FW). Leaf samples were rehydrated for 24 h in the dark for determination of turgid weight (TW) and subsequently oven dried at 60 °C to constant weight for determination of dry weight (DW). Relative water content (RWC) was calculated as:

$$RWC = [(FW - DW)/(TW - DW)] \times 100.$$

2.2.4. Growth measurement

In all three years, fruit growth was estimated by measuring on the same dates as previous measurements height and width of one fruit per tree with a digital caliper and calculating changes in average diameters and relative growth rate (RGR). Concurrently, also a growing shoot per tree was selected, labeled and measured. Shoot relative elongation rate (RER) was calculated as the increase in shoot length per meter of shoot per day. Trunk circumference was also measured above the graft union each year before harvest and trunk growth was estimated as the increase in trunk cross-sectional area (TCSA) from harvest 2008 to harvest 2010 normalized by the initial TCSA. TCSA was also used to calculate yield efficiency (kg of fruit per cm^2 TCSA) and crop load (number of fruits per cm^2 TCSA).

2.2.5. Yield and fruit quality

Each year, harvest time was determined by monitoring sugar and acid contents and fruits were harvested in 2-3 picks within the first three weeks of May (Tab.2. 3). Fruits of each tree were weighed and counted directly in the field.

Table 2.3. Harvest dates and picks of the three crop seasons.

Crop season	Fruit picks	Date
2007-2008	1	05 May
	2	20 May
2008-2009	1	04 May
	2	12 May
	3	21 May
2009-2010	1	03 May
	2	10 May

In every pick date, a sub-sample of 5-10 fruits were collected randomly from each tree and brought to the laboratory. In the laboratory, each fruit was weighed, measured in height and width, and photographed with a Nikon Coolpix L10 digital camera. Pictures were taken under controlled light conditions and sample fruits from each tree and pick were included in a single picture. Digital images were edited, cropped to one fruit per image, labeled and used to determine intensity of peel color in each fruit. Digital images were analyzed using an algorithm developed with MATLAB® software (The MathWorks Inc., Natick, MA, USA) that converts images from RGB to CIE 1976 L*a*b format, extracts the fruit from the image (removing the image background) and quantifies color characteristics as the weighted distance of each pixel in the image from a reference sample (best colored area interactively chosen from a well-colored fruit). The output is an index ranging from 0 (no orange) to 1 (full orange).

The juice was extracted from each fruit with a squeezer and weighed to determine juice yield as a percentage of total fruit weight. Total soluble solids (TSS) were measured in the juice of each fruit using a digital refractometer (Atago Co., Ltd, Tokyo, Japan). Juice pH and titratable acidity were determined using a compact titrator (Crison Instruments, SA, Barcelona, Spain) and acidity was expressed as grams of citric acid per 100 ml of juice. All measurements were performed at room temperature. Juice water content was also measured by evaporating pre-weighed amounts of juice at 60 °C.

Total carotenoids content was analyzed by UV-visible spectrophotometry using a Beckman DU-640 spectrophotometer (Beckman Coulter, Inc., CA, USA) and according to Benk's method (Benk, 1961). Briefly, 0.5-1.0 g of orange juice was mixed with 50 ml of a 1:1 (v: v) methanol and petroleum ether solution in presence of sand. The mix was filtered on cotton wool and residues were repeatedly extracted with the methanol-petroleum ether solvent until the elute was colorless. Five ml of water were added, the solution was mixed well and the aqueous layer was removed. The remaining petroleum-ether phase was washed with small quantities of methanol (90%) until colorless. After washing with 25 ml of water and filtering, the extract containing all the carotenoids was re-diluted with petroleum ether to 50 ml final volume. The optical density of the solution was read on the spectrophotometer at 450 nm. Potassium bichromate was used to construct standard curves and quantify total carotenoids.

Sample solutions of juice were prepared and filtered to remove any particles, and dark juices were clarified with 1% polyvinylpolypyrrolidone (PVPP). Ascorbic acid content was measured according to procedure no. 409677 of Boehringer (Mannheim, Germany). This colorimetric method is based on the reduction of the tetrazolium salt MTT [3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazoliumbromide] by L-ascorbic acid in the presence of the electron carrier PMS (5-methylphenazinium methyl sulphate) at pH 3.5 to a formazan. The MTT-formazan is determined by measuring absorbance at 578 nm. Vitamin C content was expressed as milligrams of L-ascorbic acid per liter of juice.

2.2.6. Data analysis

Yield and fruit quality data were compared by two-way analysis of variance with year and irrigation treatment as main factors, fruit pick and block as replicate factors, and year x irrigation as the sole interaction. Vitamin C and carotenoids were compared by one-way analysis of variance with irrigation treatment as the main factor, and fruit pick and block as replicate factors. Fruit quality data were also analyzed using crop load as a covariate to correct for differences in fruit number among treatments. However, since differences in fruit number were mainly due to irrigation treatments, unadjusted means and statistics with no covariate in the model are reported in tables. Trunk growth rates over the three years were compared by one-way analysis of variance with irrigation treatment as the main factor, and block as replicate factor. When appropriate means were separated using Tukey's multiple range test ($P \leq 0.05$). Repeated measures analysis of variance followed by orthogonal polynomial contrasts was used to evaluate differences in g_s, RWC, shoot and fruit growth across sampling dates and between treatments for each year separately. Linear regression analysis was performed to establish relationships among g_s, RWC and VPD. All described statistical tests were performed using SYSTAT procedures (Systat Software Inc., Richmond, Ca., USA).

2.3. RESULTS

2.3.1. Climate, irrigation volumes and soil moisture

In all three years, evapotranspirative demand was highest during the dry season, in June, July and August (Fig. 2.1). Rainfall of 2009 was by far the greatest, although 30% of annual rainfall was concentrated in January and February, a lot before the irrigation season started, and 43% between mid September and the end of October, after the irrigation season was over (Fig. 2.1). Due to scarce winter rainfall and climate trends in 2008 (Fig.2. 1) irrigation events started a few days earlier compared to 2007 and 2009 (Tab. 2.4). Relevant and frequent precipitations resumed earlier in 2009 (Fig. 2.4A) compared to 2007 (Fig. 2.2A) and 2008 (Fig. 2.3A). As a consequence, the 2009 irrigation season was a few days shorter than in 2007 or 2009 (Tab. 2.4). Despite large differences in annual rainfall and small differences in the duration of irrigation seasons, CI trees received basically the same number of irrigation events and volumes in the three years (Tab.2. 4).

Table 2.4 Period and total volumes of the three irrigation seasons.

Irrigation season	First event	Last event	N. events	Volumes (mm)			Annual rainfall
				CI	PRD	DI	
2007	29 Jun	18 Sep	21	345	160	154	664
2008	18 Jun	11 Sep	20	340	140	132	456
2009	23 Jun	7 Sep	21	345	149	140	1238

In all three years, SWP of CI treatment showed fluctuations due to wetting and drying cycles between consecutive irrigation events, but staying generally above -50 kPa (about 80% of field capacity) during the irrigation period. Only in a few cases, at the end of August in 2007 (Fig.2.2B) and in mid July in 2008 (Fig. 2.3B), SWP reached minimum values of -70 to -80 kPa. In DI trees, SWP followed sharper fluctuations than in CI trees reaching minimum values of -160 to -180 kPa only in late August in 2007 (Fig. 2.2B), of -160 kPa often throughout July and August in 2008 (Fig. 2.3B), and of -190 to -200 kPa at the end of June, mid July and mid August in 2009 (Fig.2.4B). In all three seasons, average SWP of wet and dry sides of PRD was lower than SWP of CI, but generally a bit higher than SWP of DI. In 2007, wet and dry sides of PRD trees were alternated on 27 July, 14 August, and 4 September; in 2008, on 11 and 29 July, 17 August, and 11 September; in 2009, on 10 and 20 July, 2 and 13 August, and 7 September. In the non-irrigated side of PRD, the relatively long period of progressive drying determined strong SWP fluctuations reaching at the end of each dry cycle minimum values of -160 kPa in late July of 2007 (Fig. 2.2C), of -190 to -200 kPa in mid July and early September of 2008 (Fig.2. 3C), and in early and mid August and early September of 2009 (Fig. 2.4C). Irrigation by the micro-sprinkler system and volumes applied in each event allowed for a good separation of wet and dry sides of the rootzone in PRD and no significant transfer of moisture to the dry side occurred on the days of irrigation.

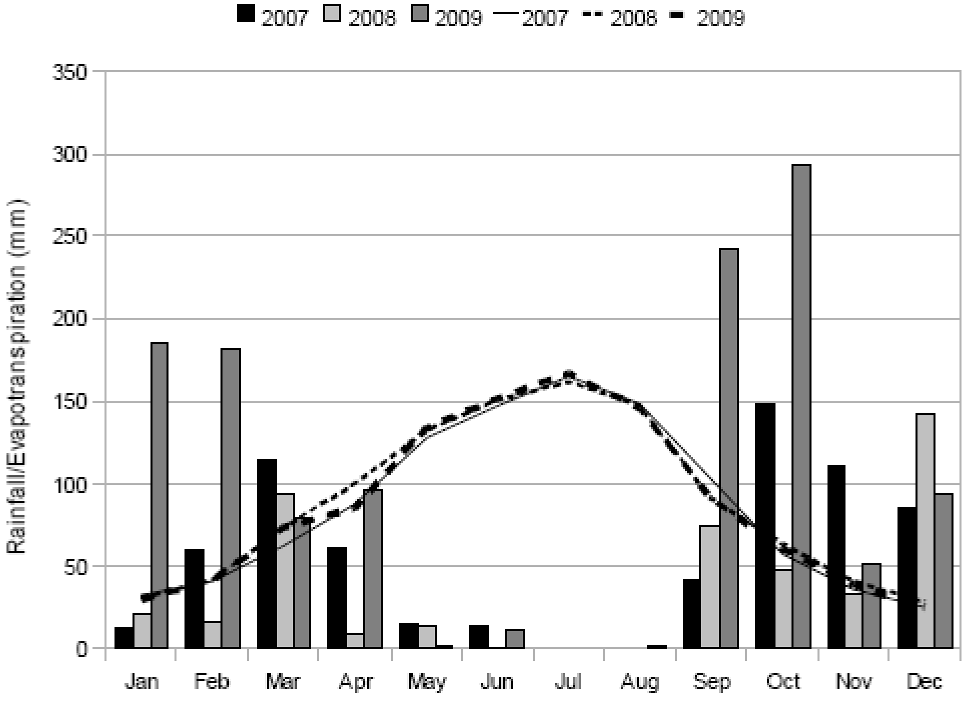

Fig. 2.1. *Distribution of total monthly rainfall (bars) and reference evapotranspiration (lines) during the three years of observations at the experimental site in Palermo (30°06' N, 13°21' E, and 31 m a.s.l.), Sicily.*

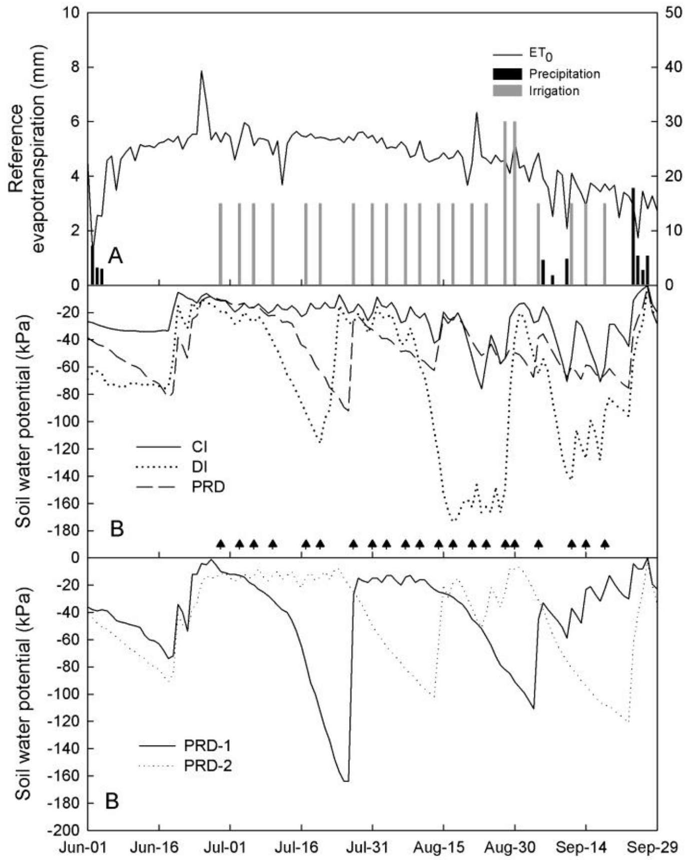

Fig. 2.2. *Water losses and inputs (A) recorded in summer 2007 at the experimental site in Palermo (30°06' N, 13°21' E, and 31 m a.s.l.), Sicily. Soil water potential in the rootzone of 'Valencia' orange trees under conventional irrigation (CI), continuous deficit irrigation (DI), and partial rootzone drying (PRD, average of wet and dry side; panel B) and in the two sides of the rootzone of trees under PRD (C) during the 2007 irrigation season.*

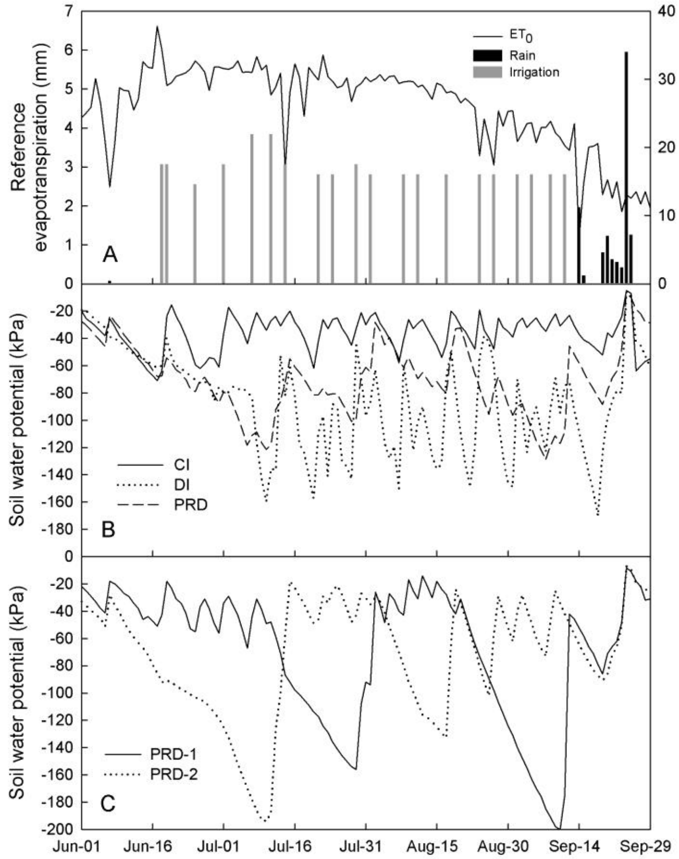

Fig. 2.3. *Water losses and inputs (A) recorded in summer 2008 at the experimental site in Palermo (30°06' N, 13°21' E, and 31 m a.s.l.), Sicily. Soil water potential in the rootzone of 'Valencia' orange trees under conventional irrigation (CI), continuous deficit irrigation (DI), and partial rootzone drying (PRD, average of wet and dry side; panel B) and in the two sides of the rootzone of trees under PRD (C) during the 2008 irrigation season.*

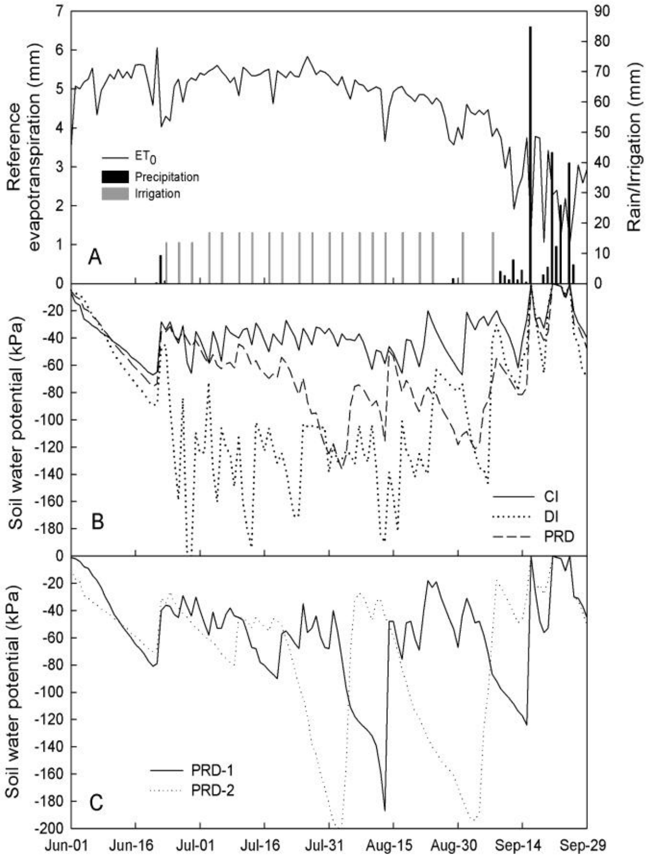

Fig. 2.4. *Water losses and inputs (A) recorded in summer 2009 at the experimental site in Palermo (30°06' N, 13°21' E, and 31 m a.s.l.), Sicily. Soil water potential in the rootzone of 'Valencia' orange trees under conventional irrigation (CI), continuous deficit irrigation (DI), and partial rootzone drying (PRD, average of wet and dry side; panel B) and in the two sides of the rootzone of trees under PRD (C) during the 2009 irrigation season.*

2.3.2. Tree water status and stomatal conductance

In 2007, RWC was measured only on 17 July and 31 August and, although DI leaves tended to be somewhat more dehydrated than CI and PRD leaves in both occasions, the irrigation treatment did not affect significantly leaf hydration level (Tab.2. 5). On the contrary, RWC decreased significantly from mid July to the end of August.

Tab. 2.5. Leaf relative water content (%) of adult 'Valencia' orange trees under conventional irrigation (CI), partial rootzone drying (PRD), and continuous deficit irrigation (DI). Means ± standard errors.

Irrigation	17-Jul	31-Aug
CI	87.2 ± 1.01	84.2 ± 1.70
PRD	88.2 ± 0.84	85.8 ± 1.07
DI	86.4 ± 1.15	82.0 ± 1.45
ANOVA	IRR	0.095
	TIME	0.002
	IRR × TIME	0.672

In both 2008 and 2009, leaf RWC followed different trends in the three treatments (significant interaction between irrigation treatment and sampling date). Specifically in 2008, RWC of DI leaves was lower than RWC of CI leaves on 29 July and 12 August, whereas RWC of PRD leaves was highest on 12 September (Fig.2.5A). By the end of the first week of October, RWC reached a minimum value of about 79% equal for all treatments. In 2009, RWC was generally stable throughout the season with PRD lower than CI only in a couple of occasions (12 August and 8 September), and increased significantly on 14 October in CI and PRD leaves, whereas remained significantly lower in DI leaves (Fig.2.5B). It is worth to notice that RWC followed generally opposite trends toward the end of the two years, decreasing in 2008 and increasing in 2009.

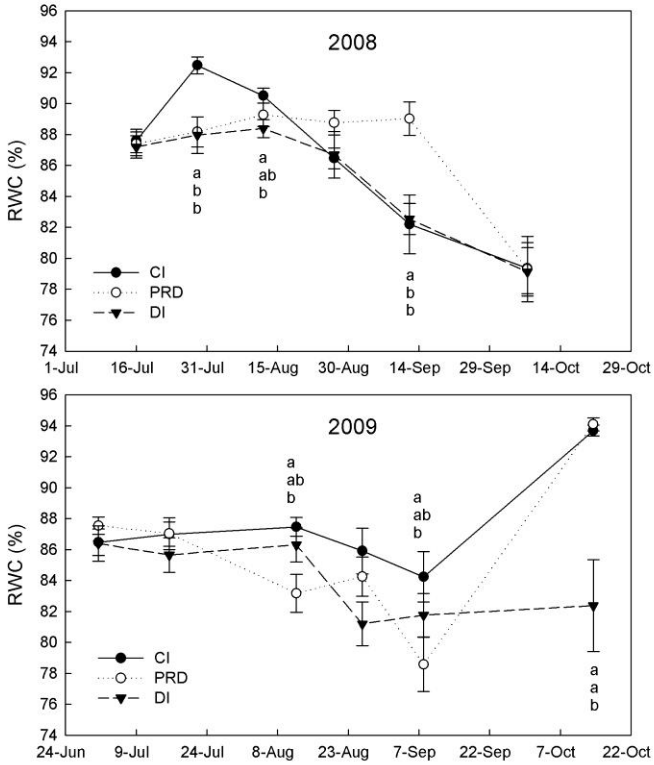

Fig.2.5. *Relative water content (RWC) in leaves of 'Valencia' orange trees under conventional irrigation (CI), partial rootzone drying (PRD), and continuous deficit irrigation (DI) during the 2008 and 2009 irrigation seasons. Error bars represent standard errors of the means. When present, different letters indicate significant differences among irrigation treatments for a specific date (P < 0.05).*

Reductions of g_s in deficit irrigation treatments were relatively minor and more consistent in 2007 and 2009 than in 2008, where no difference between treatments was observed at any sampling date (Fig.2.6). In 2007, g_s of DI trees was lowest, and g_s of PRD intermediate, on 21 August, 4 and 18 September (Fig.2.6A). In 2009, g_s of CI trees was significantly higher than g_s of PRD and DI on 29 July and 12 August (Fig.2.6C). When significant, reductions of g_s levels were similar in PRD and DI trees. No difference in g_s was observed on dates away from the irrigation periods (i.e., late fall, winter, or early spring).

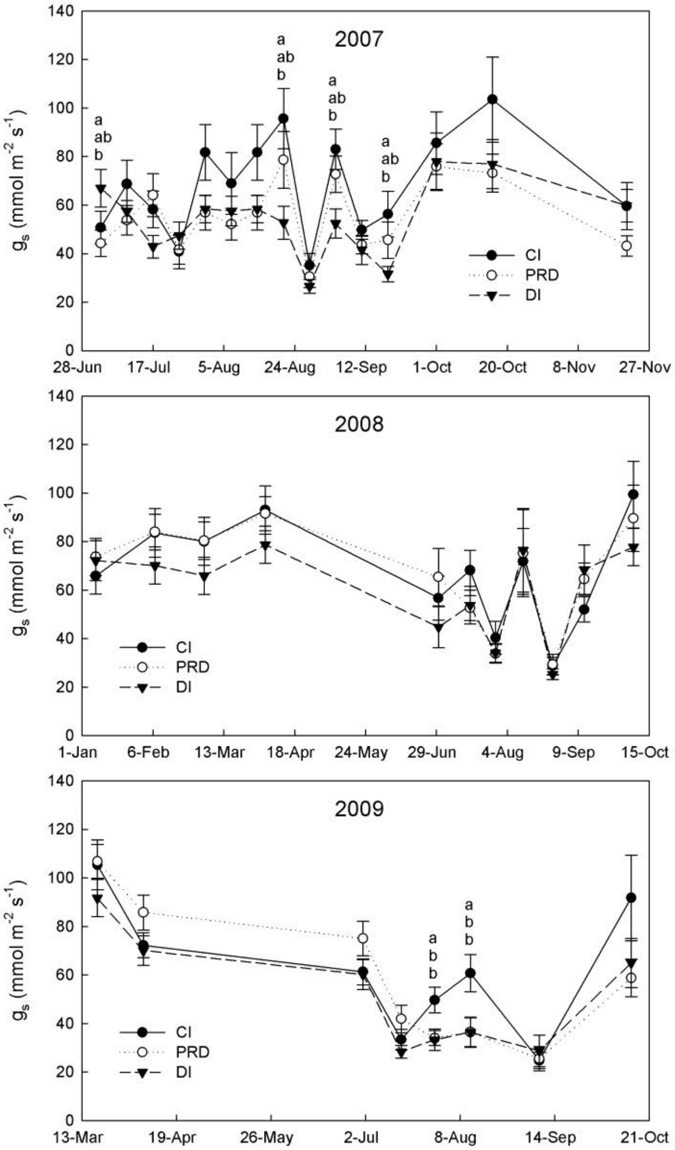

Fig.2.6. *Stomatal conductance (g_S) in leaves of 'Valencia' orange trees under conventional irrigation (CI), partial rootzone drying (PRD), and continuous deficit irrigation (DI) during the three years of observations. Error bars represent standard errors of the means. When present, different letters indicate significant differences among irrigation treatments for a specific date ($P < 0.05$).*

When data of sampling dates were pooled together, g_s showed a linear inverse relationships with VPD in all treatments and for both 2008 and 2009 (Figs.2.7 and 2.8). Regression slopes were generally steeper for PRD compared to CI and DI and in 2009 compared to 2008, but analysis of covariance did not show any significant interaction with treatment suggesting that a general inverse relationship exists between VPD and g_s in the 'Valencia' orange trees under the experimental conditions.

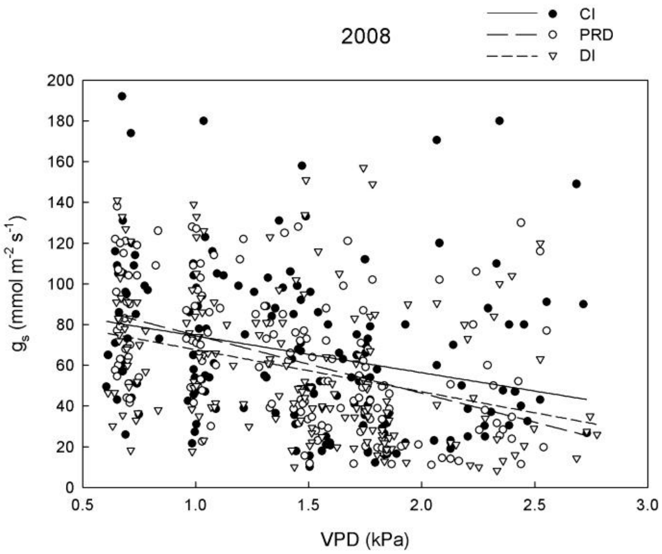

Fig.2.7. *Relationship between vapor pressure deficit (VPD) and stomatal conductance (g_s) in leaves of 'Valencia' orange trees under conventional irrigation (CI), partial rootzone drying (PRD), and continuous deficit irrigation (DI) during the 2008 sampling period. In CI, $g_s = 92.5 - 18.0*VPD$ ($P < 0.001$); in PRD, $g_s = 103.2 - 28.5*VPD$ ($P < 0.001$); in DI, $g_s = 88.3 - 20.6*VPD$ ($P < 0.001$).*

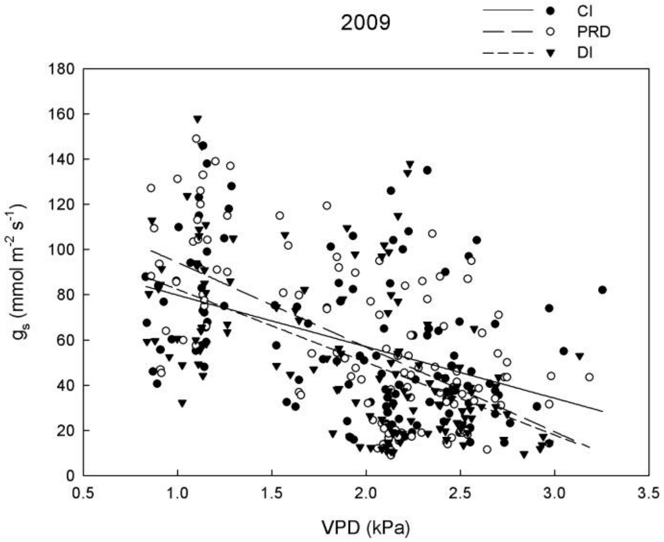

Fig.2.8. *Relationship between vapor pressure deficit (VPD) and stomatal conductance (g_s) in leaves of 'Valencia' orange trees under conventional irrigation (CI), partial rootzone drying (PRD), and continuous deficit irrigation (DI) during the 2009 sampling period. In CI, $g_s = 102.6 – 22.8 * VPD$ ($P < 0.001$); in PRD, $g_s = 131.6 – 37.4 * VPD$ ($P < 0.001$); in DI, $g_s = 114.5 – 32.2 * VPD$ ($P < 0.001$).*

On the other hand, RWC of PRD and DI leaves (not of CI) exhibited an inverse linear relationship with g_s in 2008 (Fig.2.9), and in this case the interaction with the irrigation treatment was significant confirming the different response of CI leaves. In 2009, only RWC of PRD leaves exhibited a non-linear (logarithmic) positive association with g_s (Fig.2.10).

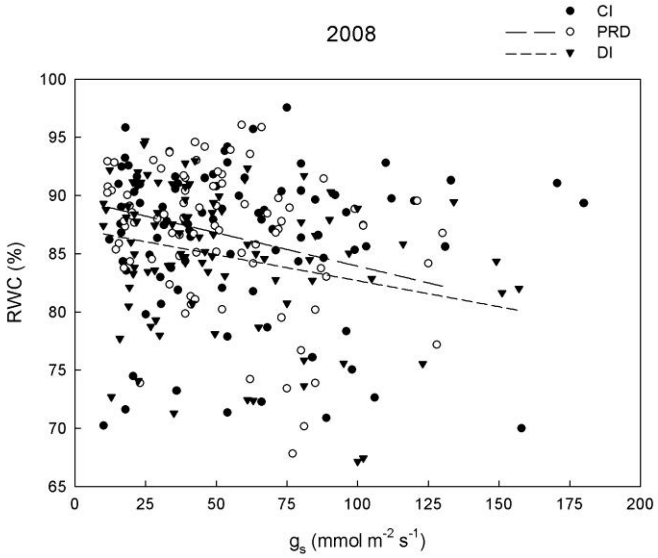

Fig.2.9. *Relationship between stomatal conductance (g_s) and relative water content (RWC) in leaves of 'Valencia' orange trees under conventional irrigation (CI), partial rootzone drying (PRD), and continuous deficit irrigation (DI) during the 2008 sampling period. In PRD, RWC = 89.7 – 0.057 * g_s (P = 0.008); in DI, RWC = 87.1 – 0.045 * g_s (P = 0.015).*

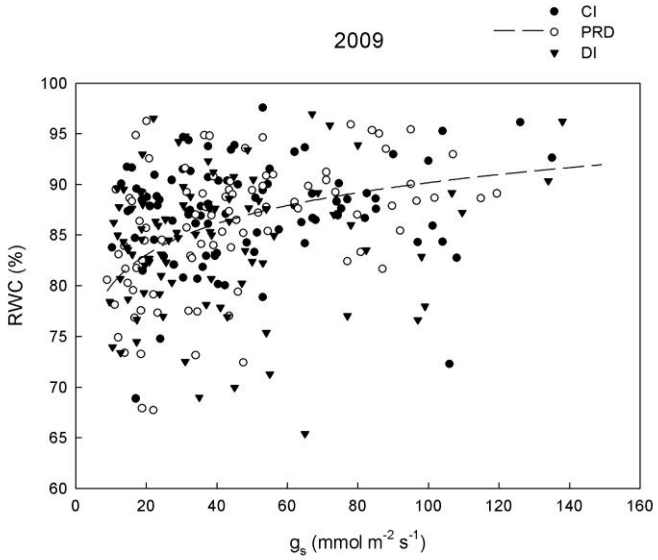

Fig.2.10. *Relationship between stomatal conductance (g_s) and relative water content (RWC) in leaves of 'Valencia' orange trees under conventional irrigation (CI), partial rootzone drying (PRD), and continuous deficit irrigation (DI) during the 2009 sampling period. In PRD, RWC = 69.8 + 4.42 * ln[g_s] (P < 0.001).*

Only in 2008, RWC was positively associated to VPD according to a saturating type of exponential trend (Fig.2.11). Also in this case the absence of interaction with the irrigation treatment suggests a similar response of CI, PRD and DI leaves to the specific environmental conditions. No significant relationship between VPD and RWC was observed in 2009.

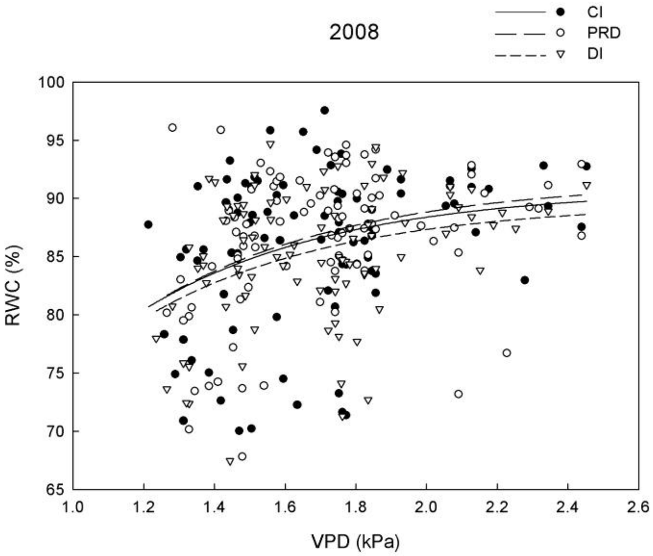

Fig.2.11. *Relationship between vapor pressure deficit (VPD) and relative water content (RWC) in leaves of 'Valencia' orange trees under conventional irrigation (CI), partial rootzone drying (PRD), and continuous deficit irrigation (DI) during the 2009 sampling period. In CI,* $RWC = 90.8 * (1-0.164^{VPD})$ *(*$P = 0.002$*); in PRD,* $RWC = 91.5 * (1-0.172^{VPD})$ *(*$P < 0.001$*); in DI,* $RWC = 89.6 * (1-0.160^{VPD})$ *(*$P < 0.001$*).*

In 2008, when measurements on all trees of each irrigation treatment were averaged per date, g_s was directly related to SWP according to a linear trend (Fig.2.12). In 2009, the same relationship followed an exponential trend, indicating a SWP threshold value (between -80 and -100 kPa) below which g_s did not respond anymore to SWP (Fig.2.13).

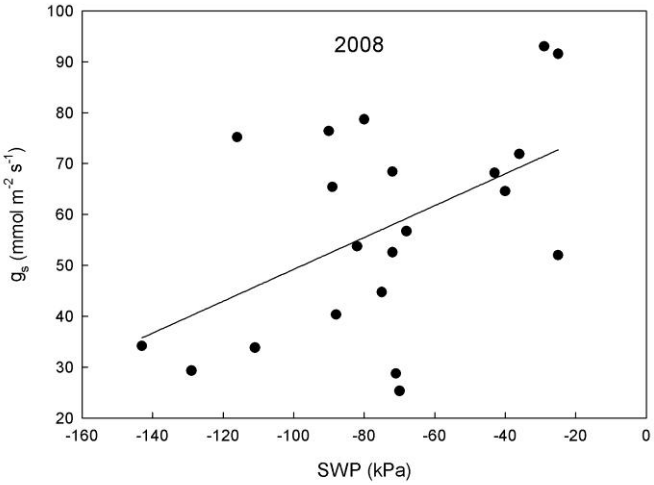

Fig.2.12. *Relationship between soil water potential (SWP) and leaf stomatal conductance (g_s) of 'Valencia' orange trees under conventional irrigation (CI), partial rootzone drying (PRD), and continuous deficit irrigation (DI) during the 2008 sampling period.* $G_s = 80.5 + 0.31*SWP$ *(P = 0.018).*

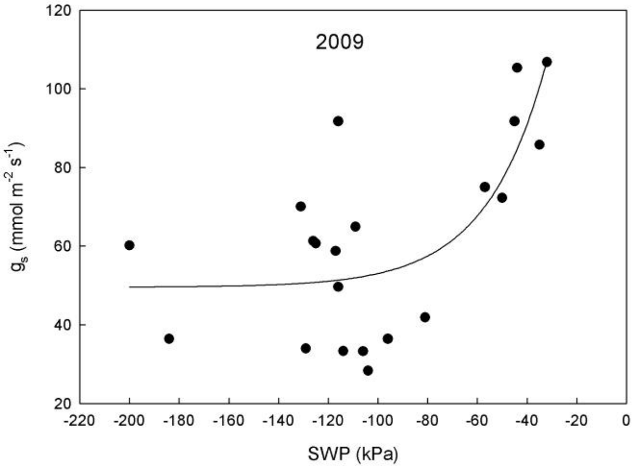

Fig.2.13. *Relationship between soil water potential (SWP) and leaf stomatal conductance (g_s) of 'Valencia' orange trees under conventional irrigation (CI), partial rootzone drying (PRD), and continuous deficit irrigation (DI) during the 2009 sampling period.* $G_s = 49.6 + 217*1.04^{SWP}$ *(P = 0.001).*

2.3.3. Fruit and shoot growth

Changes in fruit size, as shown by the 2007 data with a broad time-range of measurements (from 75 to 300 days after full bloom), followed a multi-modal curve, with lag periods at the end of August, in mid September, and mid October, to reach a plateau (and nearly final size) by mid November (Fig.2.14).

Fruit diameter was significantly and consistently affected by irrigation only in 2008, although a similar tendency to gradually show differences with time was also present in the other two years (Fig.2.14). In particular, most of the difference between treatments occurred during the month of August in 2007 and 2009, and during the month of July in 2008. In addition, both PRD and DI irrigation tended to reduce fruit size in 2007 and 2009, whereas only DI irrigation reduced fruit size over CI (PRD intermediate) at all dates but the first of 2008.

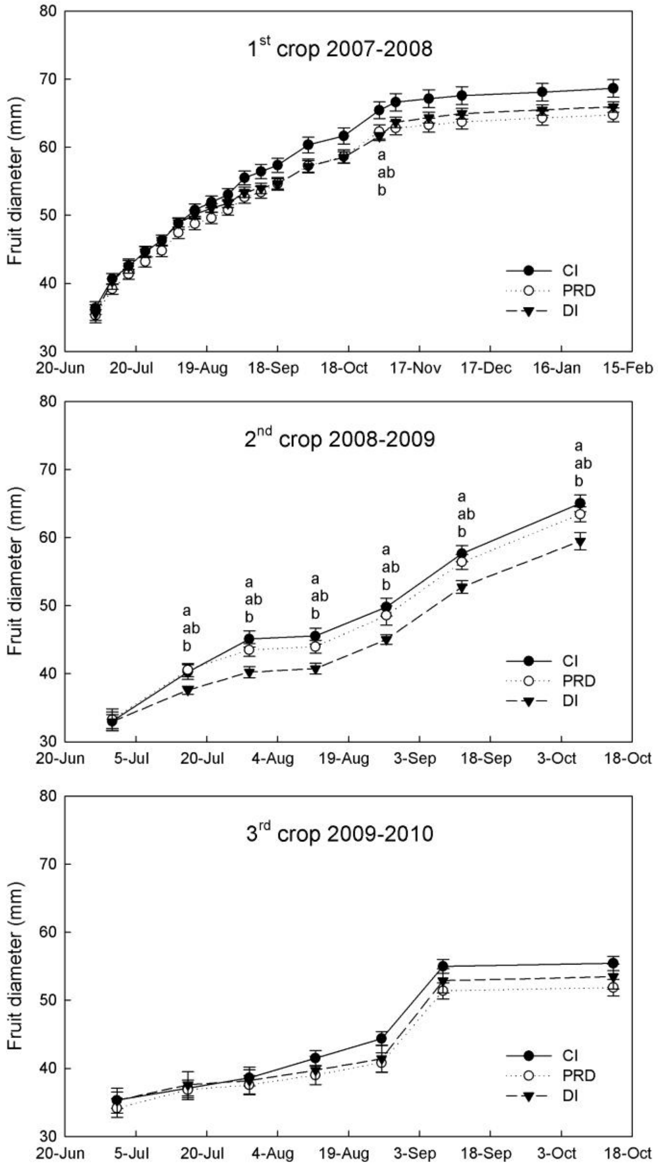

Fig.2.14. *Fruit diameter of 'Valencia' orange trees under conventional irrigation (CI), partial rootzone drying (PRD), and continuous deficit irrigation (DI) during the three years of observations. Error bars represent standard errors of the means. When present, different letters indicate significant differences among irrigation treatments for a specific date ($P < 0.05$).*

Peaks of fruit growth were registered in early July of 2007 and 2008, early August of 2007, and early September of all three years (Fig.2.15).

Differences in fruit RGR among irrigation treatments were significant only sporadically, especially in 2009. In particular, RGR of DI fruits was highest on 7 November in 2007, whereas RGR of CI fruits was highest on 29 July in 2008 (Fig.2.15). In 2009, RGR of CI fruits was highest on 29 July and 12 August, whereas RGR of DI fruits was higher than RGR of CI fruits on 8 September. In general there was a common tendency for fruits of DI trees to resume and compensate for growth differences after resumption of precipitations in autumn.

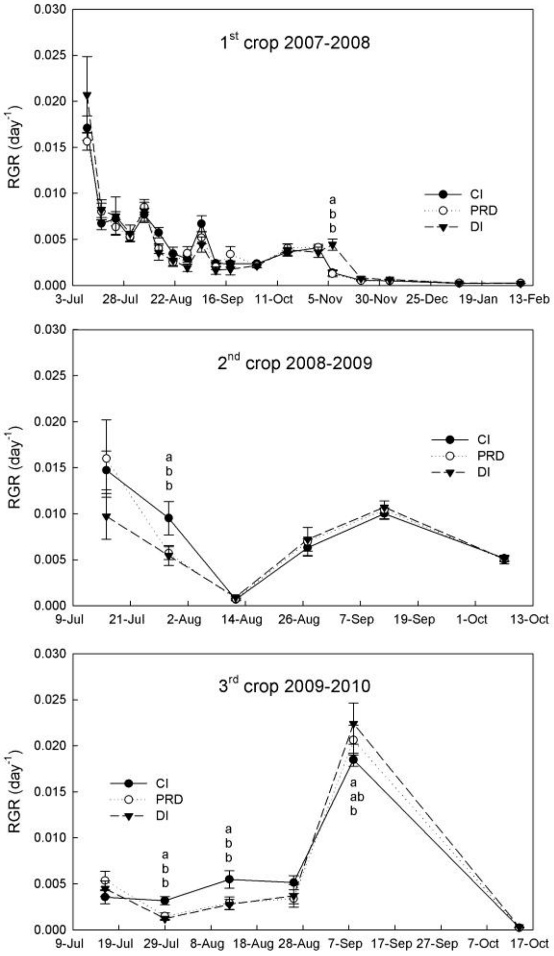

Fig.2.15. *Fruit relative growth rate (RGR) of 'Valencia' orange trees under conventional irrigation (CI), partial rootzone drying (PRD), and continuous deficit irrigation (DI) during the three years of observations. Error bars represent standard errors of the means. When present, different letters indicate significant differences among irrigation treatments for a specific date ($P < 0.05$).*

Shoot growth was higher in 2009 compared to 2007 or 2008 (Fig.2.16). The summer flush of growth occurred from mid to the end of July in all three years. A second peak at the beginning of September was evident only in 2009. The autumn flush was evident from early to late November only in 2007, when measurements were taken through the winter. Irrigation never affected shoot RER, although PRD shoots tended to have the highest RER in July of all three years (Fig.2.16). Also trunk growth over the three years was unaffected ($P = 0.579$) by irrigation with the average trunk growth being 0.15, 0.17, and 0.16 cm cm^{-2} for CI, PRD, and DI, respectively.

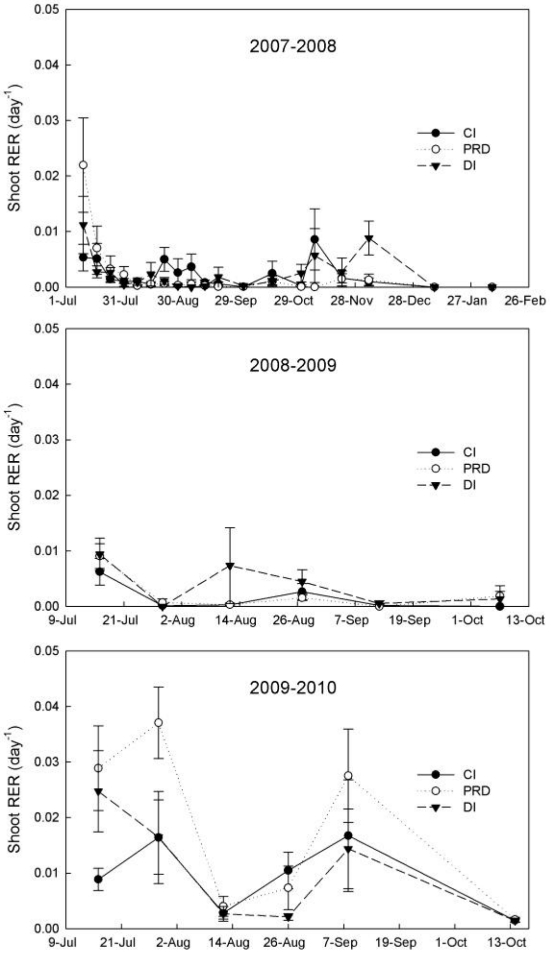

Fig.2.16. *Shoot relative elongation rate (RER) of 'Valencia' orange trees under conventional irrigation (CI), partial rootzone drying (PRD), and continuous deficit irrigation (DI) during the three years of observations. Error bars represent standard errors of the means. When present, different letters indicate significant differences among irrigation treatments for a specific date ($P < 0.05$).*

2.3.4. *Yield and fruit quality*

Yield responses of 'Valencia' trees to irrigation followed similar trends during the years of observations, as indicated by the lack of interaction between year and irrigation (Tab. 2.6). Yield, number of fruits, and crop load of 2009 were lower than those of 2008 and 2010, whereas yield efficiency of 2009 and 2010 was similar and lower than yield efficiency of 2008. Yield parameters were generally not affected by irrigation, although DI trees tended to have lower yield, number of fruits, and yield efficiency than CI and PRD trees (Tab. 2.6).

Tab. 2.6 Production of adult 'Valencia' orange trees under conventional irrigation (CI), partial rootzone drying (PRD), and continuous deficit irrigation (DI). Means ± standard errors. The bottom section reports P-values from analysis of variance (ANOVA). When present, lower-case letters indicate significant differences among levels of a given factor (Tukey's multiple range test, P < 0.05).

Factor	Level	Yield (kg)	N. fruits (count)	Yield efficiency (kg cm^{-2})	Crop Load (Number cm^{-2})
Year	2008	32.5 ± 1.90a	213 ± 13.0a	0.076 ± 0.0052a	0.498 ± 0.036a
	2009	24.5 ± 1.44b	170 ± 12.2b	0.050 ± 0.0032b	0.349 ± 0.027b
	2010	32.6 ± 1.96a	246 ± 14.1a	0.061 ± 0.0035b	0.467 ± 0.026a
Irrigation	CI	31.6 ± 1.98	210 ± 14.4	0.065 ± 0.0046	0.425± 0.030
	PRD	30.5± 1.77	215 ± 13.1	0.063 ± 0.0045	0.445 ± 0.034
	DI	27.6 ± 1.81	205 ± 14.1	0.060 ± 0.0040	0.443± 0.31
ANOVA	Year	0.001	< 0.001	< 0.001	< 0.001
	Irrigation	0.276	0.873	0.657	0.937
	Y×IRR	0.437	0.330	0.499	0.342

Again, the lack of interaction between year and irrigation indicated consistent responses in terms of fruit quality of 'Valencia' trees to irrigation in the three years (Tab. 2.7). Fruit weight was similar in 2008 and 2009 but lower in 2010; specific weight and juice yield were higher in 2008 compared to the following two years; juice TSS was higher in 2009 than in 2008 and 2010; juice TA was highest in 2009, followed by 2008 and lowest in 2010; fruit peel was better colored in 2008 and 2010 than in 2009 (Tab.2. 7).

In this case, irrigation significantly affected fruit quality. Fruits of DI trees were smaller than those of CI trees, whereas PRD fruits exhibited intermediate weight. Fruit size and volume showed the same response to irrigation (data not shown), as also indicated by similar fruit specific weight in the three treatments (Tab. 2.7). Fruits of DI trees yielded also less juice than fruits of CI and PRD trees, whereas juice of DI and PRD fruits showed higher TSS and TA than juice of CI fruits. Peel color was unaffected by irrigation, although CI and PRD fruits tended to be better colored than DI fruits (Tab.2.7). Sugar yield expressed in tons per hectare was higher in CI (1.21) and PRD (1.22) compared to DI (1.06).

Tab. 2.7. Fruit quality of adult 'Valencia' orange trees under conventional irrigation (CI), partial rootzone drying (PRD), and continuous deficit irrigation (DI). Means ± standard errors. The bottom section reports P-values from analysis of variance (ANOVA). When present, lower-case letters indicate significant differences among levels of a given factor (Tukey's multiple range test, P < 0.05).

Factor	Level	Weight (g)	Specific weight (g ml^{-1})	Juice yield (%)	TSS (°Brix)	TA (g 100ml^{-1})	Color index
Year	2008	154.9± 3.69a	1.042± 0.0032a	52.9± 0.31a	11.8± 0.095b	1.05± 0.020b	0.939± 0.0009a
	2009	153.7± 6.10a	1.006± 0.0044b	49.8± 0.46b	12.8± 0.091a	1.20± 0.018a	0.851± 0.0019b
	2010	133.3± 3.10b	1.003± 0.0035b	50.8± 0.44b	11.9± 0.109b	0.99± 0.014c	0.944± 0.0015a
Irrigation	CI	156.7± 5.07a	1.020± 0.0042	51.5± 0.40a	11.9± 0.110b	1.01± 0.015b	0.904± 0.0045
	PRD	145.8± 4.22ab	1.013± 0.0046	52.0± 0.37a	12.3± 0.89a	1.14± 0.017a	0.904± 0.0047
	DI	139.3± 4.31b	1.012± 0.0042	49.3± 0.53b	12.5± 0.121a	1.15± 0.025a	0.900± 0.0049
ANOVA	Year	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Irrigation	0.016	0.365	< 0.001	< 0.001	< 0.001	0.254
	Y×IRR	0.420	0.934	0.150	0.362	0.069	0.491

In 2010, concentration of total carotenoids and vitamin C was higher in the juice of DI fruits than in the juice of CI fruits, and intermediate in the juice of PRD fruits (Tab. 2.8). Those differences were canceled if total carotenoids and vitamin C are measured on a fruit weight basis.

Tab. 2.8. Total carotenoids and vitamin C in juice and fruits of adult 'Valencia' orange trees under conventional irrigation (CI), partial rootzone drying (PRD), and continuous deficit irrigation (DI). Means ± standard errors. When present, lower-case letters indicate significant differences among irrigation treatments and within each column (Tukey's multiple range test, P < 0.05).

Irrigation	**Carotenoids (mg L^{-1})**	**Vitamin C (mg L^{-1})**	**Carotenoids (mg kg^{-1})**	**Vitamin C (mg kg^{-1})**
CI	29.1± 1.26b	362± 5.22b	15.1± 0.63	188± 3.48
PRD	32.3± 1.22ab	371± 6.55ab	16.5± 0.67	189± 4.43
DI	35.0± 2.08a	382± 4.76a	17.4± 1.13	188± 3.46

When testing for differences in WUE, year and irrigation treatment never interacted, and fruit and juice WUE were higher in 2010 compared to 2008 or 2009 (Tab. 2.9). Sugar WUE was similar in the three years. Across years, fruit, juice, and sugar WUE were higher in PRD and DI trees than in CI trees (Tab.2.9). No significant difference was found between PRD and DI in terms of WUE, although PRD values were always higher on the average.

Tab.2. 9. Fruit, juice, and sugar water use efficiency (WUE) of adult 'Valencia' orange trees under conventional irrigation (CI), partial rootzone drying (PRD), and continuous deficit irrigation (DI) in the three years of observations. Means ± standard errors. The bottom section reports P-values from analysis of variance (ANOVA). When present, lower-case letters indicate significant differences among levels of a given factor (Tukey's multiple range test, P < 0.05).

Factor	**Level**	**Fruit WUE (g l^{-1})**	**Juice WUE (g l^{-1})**	**Sugar WUE (g l^{-1})**
Year	2008	9.7± 0.65b	5.14± 0.36b	0.61± 0.044
	2009	9.3± 0.81b	4.61± .041b	0.60± 0.054
	2010	12.0± 1.01a	6.19±0.51a	0.73± 0.54
Irrigation	CI	5.8± 0.38b	3.07±0.20b	0.36± 0.24b
	PRD	12.8± 0.83a	6.62± 0.41a	0.81± 0.048a
	DI	12.3± 0.82a	6.16± 0.45a	0.76± 0.54a
ANOVA	Year	0.012	0.012	0.084
	Irrigation	< 0.001	< 0.001	< 0.001
	Y×IRR	0.189	0.378	0.530

2.4. DISCUSSION

In terms of reference evapotranspiration the three years of investigation were relatively homogeneous, whereas precipitations were particularly abundant in 2009 (Fig.2.1). However, over 70% of the 2009 annual rainfall was concentrated away from the irrigation period and did not contribute in any particular way to the water balance of the dry season.

Trends of SWP generally reflected changes in soil moisture following the imposition of irrigation treatments and indicated a good separation in time of wet and dry zones for the PRD treatment in all three years (Figs.2.2, 2.3, and 2.4). This is particularly important for the efficacy of the PRD technique, and the use of micro-sprinklers, along with duration and interval between irrigation events, proved to be effective. Particular care should be posed on this aspect, especially in field trials, as PRD functioning is based on the separation and alternation of wed and dry sides of the rootzone, and some negative outcomes of PRD studies may be also due to lack of this conditions. In this regard, continuous recording of SWP proved to be a useful and safe method to monitor time and spatial changes in soil moisture. Differences between SWP of DI rootzones and average SWP of wet and dry PRD rootzones are in part due to a small difference in irrigation volumes applied to the two treatments (Tab.2.4) and in part possibly due to differences in wetted soil surface and consequent soil evaporation (Marsal et al., 2008).

Comparative levels of RWC indicate that DI irrigation induced somewhat a generalized dehydration in all years, whereas PRD showed mild leaf dehydration only in 2009 (Fig.2.5), in correspondence of marked decreases in SWP of the dry side (Fig.2.4). These results are consistent with those obtained in apple (Einhorn and Caspari, 2004; Talluto et al., 2008) and with leaf water potentials of Swingle citrumelo seedlings (Melgar et al., 2010).

Reductions of g_s in PRD trees were relatively minor (on average for the three years 9% less than CI) and only sporadically significant (Fig.2.6). DI irrigation generated greater (on average for the three years 15% less than CI) and, in 2007, more consistent g_s reductions than PRD, whereas in 2009 reductions were similar for both deficit treatments. This degree of stomatal control in DI trees, however, was not sufficient to maintain levels of hydration similar to CI trees. Previous work with greenhouse-grown citrumelo seedlings did not show significant reductions of g_s in response to PRD in spite of increases in leaf ABA (Melgar et al., 2010). However, in our study the lack of g_s response in PRD trees cannot be explained by a decrease of stomatal sensitivity to ABA due to severe stress hydraulic signals (Tardieu et al., 1993), as DI leaves (lower RWC) did show consistent g_s reductions when PRD leaves did not (i.e., 2007).

In both 2008 and 2009, there was a general response of g_s to VPD common to all irrigation treatments (Figs.2.7 and 2.8). In particular, 'Valencia' trees tended to close stomates in response to VPD increases and the response was more marked in 2009 than in 2008. This would be consistent with a feed-forward type of stomatal response (Streck 2003) perhaps involving some root-generated chemical signaling, and it generally agrees with previous observations in 'Valencia' orange trees (Medina et al., 1999). In 2008, leaf hydration level was maintained at a constant level in CI trees by a simple stomatal control and no soil water limitation. In PRD and DI, leaf hydration decreased as g_s increased (Fig. 2.9), while it did not respond to SWP (data not shown). This shows a stomatal response to soil water deficit (Fig.2.12) and not to RWC, which is typical of long-term stomatal response to soil water deficit, possibly mediated by root-generated ABA. In 2009, stomates seemed to respond more directly to leaf hydration level, at least in the low range of leaf RWC (Fig.2.10), suggesting the presence of a hydraulic component and a sort of feedback mechanism. In this year, g_s also responded to SWP, but in a non-linear fashion. The two types of associations (g_s vs RWC, and SWP vs g_s) together seem to indicate that SWP may control g_s when soil moisture and leaf RWC are relatively high (above -80 kPa for SWP and above 85% for RWC), whereas g_s seems to respond to leaf hydration when RWC falls below 85% and guard cells may start sensing low water potentials in the surrounding cells (Streck, 2003). As a result, differences, rather than discrepancies, between the two years of observations are mainly due to a broader range of SWP in 2009 compared to 2008, which is also shown by lower SWP values reached in the rootzone of DI trees in 2009 (Fig. 2.4) than in 2008 (Fig. 2.3).

No shoot or trunk growth reduction was observed in response to deficit irrigation in this study (Fig. 2.16). Similarly, total plant growth was not reduced by PRD in Swingle citrumelo seedlings (Melgar et al., 2010). On the contrary, mandarin trunk growth was reduced by PRD (Kirda et al., 2007). Contrasting results are also reported in other woody crops, and studies with field-grown apples suggest that the vegetative growth response to PRD may depend on seasonal irrigation volumes (O'Connell and Goodwin 2007; Talluto et al., 2008).

Maintenance of relatively high leaf hydration levels under reduced soil moisture of PRD and DI treatments cannot be simply explained by reductions of tree water consumption through stomatal control, especially in PRD. Other mechanisms must have played a major role. Adjustments of leaf area should not have played any significant role as shoot and trunk growth were not diminished by PRD or DI irrigation. On the other hand, changes in root functionality and sap flow (not measured in this study) following partial drying of the rootzone, and specifically greatly increased root sap flows after re-wetting of the dry portion, have been observed in other fruit crops (Green and Clothier 1999; Green et al., 2006). Also, experiments on Swingle citrumelo seedlings (Melgar et al., 2010) suggest that root growth (especially length of the dry side) may contribute to sustain water absorption, root functionality, and ultimately maintain relatively high g_s levels under PRD conditions. This may help explain differences in leaf hydration level between PRD and DI even when g_s of PRD trees was not reduced.

Fruit growth was affected by irrigation only in sporadic occasions between late July and early August, when atmospheric water demand is relatively high and irrigation has generated significant water deficit in the soil, and fruit RGR did not show any specific difference between DI and PRD fruits (Fig.2.15). Our fruit size data (Fig.2.14), however, agree with the general concept that PRD irrigation does not affect fruit growth and final size in different crops (Leib et al. 2006; Spreer et al., 2007; Talluto et al. 2008; Van Hooijdonk et al. 2004). On the contrary, simple reductions of irrigation volumes may result in a typical water deficit response leading to fruit size reductions as documented in apple (Talluto et al. 2008), but also in citrus (González-Altozano and Castel, 1999; Hutton, 2004; Pérez-Pérez et al. 2008).

The lack of a major irrigation effect on vegetative growth, but also fruit growth, resulted in similar yields for all treatments. Interestingly, also number of fruits per tree and especially crop load were similar in all treatments, including DI. This agrees with previous observations on drip-irrigated navel oranges under PRD (Dzikiti et al., 2008), but is apparently inconsistent with other studies in citrus where yield were reduced by deficit irrigation (Eliades et al., 2004; González-Altozano and Castel, 1999; Hutton, 2004). However, citrus yield reductions under deficit irrigation have been associated to either increased fruit drop in case of early deficit, or to reduced fruit size in case of late deficit (González-Altozano and Castel, 1999). In our study, soil moisture conditions and the long and slow growth cycle of 'Valencia' fruit did not cause any significant fruit drop, and only a size reduction effect of DI fruit was significant at harvest. Evidently the fruit size effect alone was not sufficient to cause significant yield reductions. PRD trees in our case did not even suffer fruit size reductions, whereas size of navel oranges has been reduced by both PRD and reductions in irrigation volumes (Treeby et al., 2007). Juice TSS and TA were both increased to a similar degree by PRD and DI (Tab. 2.7), and this is a very well established response in citrus particularly when water deficit is imposed during late fruit growth (Castel and Buj, 1990; Peng and Rabe, 1998; González-Altozano and Castel, 1999; Hutton et al., 2007). Although our 'Valencia' fruits had completed only 1/3 of their growth period toward the end of treatment imposition (about 120 days after bloom in mid August, they had already reached 80 to 90% of final size, so water deficit occurred certainly during late growth stages.

DI irrigation also increased the concentration of total carotenoids and vitamin C in the juice and content in the fruit, whereas PRD irrigation induced intermediate levels of the two dietary supplements (Tab.2.8). This was not due to fruit dehydration as using percent juice water content as a covariate in the analysis did not cancel differences in carotenoids or vitamin C. On the contrary, differences were canceled when carotenoids and vitamin C were expressed on per kilogram of fruit due to differences in juice yield.

Yet, the simple reduction of irrigation volumes (DI) over conventional irrigation caused a 2.2% reduction of juice yield, corresponding to a loss of over 1.6 tons of juice per hectare and about 150 kg of sugars per hectare. On the contrary, water savings of over 1.9 megaliters per hectare and per year with PRD did not cause major or significant losses.

Juice and sugar losses of DI fruits were in part compensated by water savings associated to a 59% reduction of irrigation volumes. Important reductions of irrigation volumes (59% in DI and 57% in PRD), in fact, determined significant increases in fruit, juice and sugar WUE of both deficit treatments over CI. This translated into more grams of fruit, juice, or sugars per liter of irrigation water in DI and PRD indicating that withholding water, especially by the PRD technique, may be beneficial to the economy of Mediterranean citrus farmers.

2.5. CONCLUSIONS

Overall, our observations show that seasonal growth and final yields of 'Valencia' orange trees under the Mediterranean climate of Northern Sicily were not affected by PRD or continuous deficit irrigation. Tree responses to the two deficit irrigation treatments differed with respect to water status, but changes in stomatal control (either VPD- or ABA-mediated) alone were not sufficient to explain differences. It is likely that a combination of the amount and the way of applying irrigation water (PRD), rather than just the irrigation volume (DI), is responsible for the triggering of more efficient mechanisms to maintain sufficient leaf hydration levels. This translated into maintenance of shoot and fruit growth throughout the season, and ultimately to yields comparable to conventionally irrigated trees. Continuous deficit irrigation eventually resulted in a stress response, which penalized fruit size and juice yield, emphasizing the importance of alternated rootzone wetting over a simple reduction of irrigation volumes.

In areas like the Mediterranean regions, where water resources are a limiting factor for agricultural productions due to low and non-uniform precipitations, inefficient distribution or strong competition from municipalities and touristic activities, costs associated to water acquisition and in-farm distribution may be high and affect significantly the farmers' income. In those situations, saving water through appropriate irrigation strategies has a direct impact on farming economy, and reducing costs associated to irrigation without yield losses is by itself an outstanding achievement. If we add to this the environmental benefits associated to reducing irrigation volumes, i.e., saving energy (less pollution) but also maintaining a good quality of water and soil resources, deficit irrigation strategies should be taken into serious consideration even when certain yield losses occur. Future studies will have to explore into more detail the nutritional status of trees under PRD and deficit irrigation in general, as well as conduct site-specific economical analysis to evaluate where and when applying PRD irrigation strategies at a commercial scale may be viable to growers.

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