PHYSIOLOGICAL MEASUREMENTS OF DAILY DAYLIGHT FERTIGATED CITRUS TREES.

BY

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously, in its entirety or in part, been submitted at any university for a degree.

SUMMARY

Daily daylight fertigation regimes (involving drip fertigation, on a daily basis, during the daylight hours) are becoming widely used in the South African citrus industry in the endeavor to enhance tree productivity. Such regimes could provide sensitive nutrient and moisture management, reducing plant stress in general as well as the response time to root-directed cultural activities.

There is a need to evaluate the efficacy of daily daylight fertigation systems relative to conventional irrigation systems. Standard horticultural evaluation of orchard management practices is very time consuming. We opted for physiological studies comparing plant stress levels, in an attempt to quantify plant performance under each system. Citrus trees under daily daylight fertigation and conventional micro-jet and drip-irrigated regimes were monitored to establish plant stress levels as indicated by sap flow, xylem water potential, stomatal conductance and chlorophyll a fluorescence. Plants under a daily daylight fertigation regime are believed to have good soil water conditions in their rooting volume, and therefore experience negligible baseline levels of stress. The trees do, however, experience midday depression in stomatal conductance, to a lesser degree, but not unlike trees under conventional regimes. It appears as if a larger rooting volume of micro-jet irrigation regimes enhances recovery from the midday depression. It is recommended that producers optimise productivity during the morning hours, by early irrigation, so that plants can function optimally, whilst environmental conditions are most favourable for high physiological activity.

We also assessed the effect of withholding water from trees adapted to a daily daylight fertigation regime to evaluate the risk involved with short-term water deficits in trees adapted to this regime, as well as the usefulness of physiological techniques for identifying water stress. Stomatal conductance and xylem water potential indicated water stress sooner than the other physiological parameters. Citrus trees seem to be relatively insensitive to water deficit stress as measured by sap flow and chlorophyll a fluorescence.

Sap flow is buffered by tree capacitance, and although mediated via stomatal conductance, atmospheric conditions and not the soil water content primarily determine it. As daily fertigation is applied to trees under DDF regimes, they exhibit more optimal levels of xylem water potential and stomatal conductance, compared to trees from which water is withheld. Although alleviating it to a degree, daily irrigation did not mitigate the midday depression in these values. Seen over a season, even small enhancements of stomatal conductance (and with it photosynthesis and possibly, growth) and xylem water potential, could incrementally produce higher yields.

OPSOMMING

In die strewe na verhoogde boomproduktiwiteit, word daaglikse sproeibemesting (deur 'n drupbesproeiingsstelsel toegedien tydens die dagligure) al meer algemeen in die Suid Afrikaanse sitrusbedryf gebruik. Hierdie praktyk verminder algemene plantstres deur baie spesifieke voedings- en vogbeheer, en verkort ook die plant se reaksietyd op wortelgerigte bewerkingsaktiwiteite.

Dit is nodig om die relatiewe voordeel van daaglikse sproeibemesting teenoor konvensionele besproeiingssisteme te evalueer. Huidige tuinboukundige evaluering van boord-bestuurspraktyke is baie tydrowend. In 'n poging om plantreaksie onder verskillende praktyke te beskryf, het ons besluit om die plantstresvlakke met fisiologiese metodes te vergelyk. Sitrusbome onder daaglikse sproeibemesting, en konvensionele mikro- en drupbesproeiing, is onderskeidelik gemonitor om die plant se stresvlakke vas te stel, soos aangedui deur sapvloei, xileem-waterpotensiaal, stomatale geleiding en chlorofil a fluoresensie. Die plante onder daaglikse sproeibemesting ondervind lae vlakkke van waterstremming, waarskynlik weens hoë grondvogtigheid in die wortelsone. Die bome ondervind wel, soos dié onder konvensionele besproeiing, middagdepressie in stomatale geleiding, hoewel tot 'n mindere mate. Dit blyk asof die groter wortelvolume van mikrospuit besproeide bome die herstel na middagdepressie bespoedig. Produsente word aangeraai om die oggendure optimaal te gebruik deur vroeg te besproei sodat plantproduktiwiteit hoog is terwyl die omgewingsfaktore op hul gunstigste is en wanneer die hoogste fisiologiese aktiwiteit voorkom.

Ons het ook die effek van wateronthouding gemeet op die bome wat aangepas is vir daaglikse sproeibemesting. Sodoende is die risiko verbonde aan 'n korttermyn watertekort op hierdie bome ge-evalueer, asook die bruikbaarheid van fisiologiese tegnieke om waterstremming in sitrus te identifiseer. Stomatale geleiding en xileem-waterpotensiaal het waterstremming vroëer aangedui as die ander fisiologiese parameters. Sitrusbome blyk redelik onsensitief te wees teenoor droogtestremming soos

gemeet deur sapvloei en chlorofil a fluoresensie. Sapvloei word gebuffer deur boom-kapasitansie, en alhoewel sapvloei gereguleer word deur stomatale geleiding, is dit die atmosferiese toestande (hoofsaaklik dampdruk verskil) wat dit primêr beïnvloed, en nie die grond-water inhoud nie. Omdat bome daagliks sproeibemes word, het hulle meer optimale vlakke van xileem-waterpotensiaal en stomatale geleiding in vergelyking met bome waarvan water weerhou is. Alhoewel daaglikse sproeibemesting die middagdepressie verlaag het, is dit nie daardeur opgelos nie. Oor die typerk van 'n seisoen kan selfs minimale verhogings in stomatale geleiding (en daarmeesaam fotosintese en moontlik groei) en xileem-waterpotensiaal, hoër opbrengste tot gevolg hê.

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EFFECTS OF WATER DEFICIT STRESS ON WATER RELATIONS AND PHOTOSYNTHESIS OF EVERGREEN SUBTROPICAL TREE CROPS: PHYSIOLOGY AND METHODOLOGY.

1   INTRODUCTION

Syvertsen and Lloyd (1994) state that most agricultural practices involve modifications of carbon assimilation and dry matter allocation patterns. These physiological and developmental manipulations are attempts to minimise environmental stress and maximise productivity, fruit yield and quality (Grierson et al., 1982; Syvertsen and Lloyd, 1994). The objective of this review is to quantify the effects of mild water stress on water potential (Ψ), sap flow, stomatal conductance and gas exchange in fruit trees, with specific reference to citrus and other evergreen subtropical crops. The midday depression of photosynthesis, transpiration and the effects of water deficit on the photosynthetic apparatus are included in the discussion, but osmotic adjustment in response to water deficit is not covered in detail.

Grierson et al. (1982) describes stress as any external factor that results in a restriction, acceleration or interruption of normal metabolic processes of plants or plant parts. Environmental stress may also be beneficial and mild stress levels can help plants to acclimatise and raise the threshold at which lethal effects become evident. Grierson et al. (1982) cite many examples, e.g. reduced water supply can improve fruit quality and induce frost hardiness; and by subjecting plants to a drying cycle, stomatal conductance is lowered and sensitised, so that stomata respond by rapid closing following recurring water deficits. Not only the extent (Fereres et al., 1979) but also the rate of induction of the stress factors influences the effects of water deficit and other stresses. The speed at which drought is induced may significantly alter gas exchange in many plants (Escalona et al., 1999), including vines (Flexas et al., 1999) and also reported in papaya (Marler and Mickelbart, 1998). In open hydroponic and daily drip fertigation regimes, the daily nutritional requirements of plants are given via drip irrigation, as a nutrient and pH balanced solution. Contrary to conventional irrigation regimes, where trees have extensive root systems and stress develops much more slowly, such systems limit tree rooting volume very effectively (Woods, 1999) and water stress can develop rapidly

when trees experience high temperatures and vapour pressure deficits. Soil texture influences the soil water holding capacity, and thus, relative to trees growing in heavier soils, trees in sandy soils usually experience water deficit stress much more quickly. As water deficit develops, a higher electrical conductivity is needed to continue water uptake and retain turgor (Hsiao, 1973; Fereres et al., 1979; Morgan, 1984), and osmotic substances increase in the roots, shoots and leaves (Grierson et al., 1982; Levy, 1983). Downton (1983) noted that the photosynthetic apparatus was protected from photoinhibition under water deficit conditions when the stress developed slowly enough to allow osmotic adjustment. In the short term, osmotic adjustment to mild water stress entails an increase of the sucrose/starch ratio of the recently fixed carbon (Quick et al., 1989; Vassey and Sharkey, 1989) and an increased pool of low molecular weight compounds (e.g., proline, other amino-acids and inert sugars) in the longer term (Chaves, 1991).

In addition to being able to acclimatise, plants are also well adapted to resist and recover from stress (Fereres et al., 1979), and this is especially true for the commonly-experienced water deficit-induced stress. A wide range of adaptations to water deficits exist for survival and for improved production under water stress conditions (Turner, 1986). Nevertheless, water deficit stress is the single most important factor limiting crop yields (Hsiao, 1973; Begg and Turner, 1976), directly and indirectly affecting numerous metabolic and developmental processes.

Leaf growth and canopy development are responsible for the production of photosynthesising tissues and for allowing light interception. Photosynthetic CO_2 assimilation is essential for plant growth and is the primary source of carbon for organic molecules and reducing power for carbohydrate production (Fernandez et al., 1997). Drought affects both factors of this interrelationship (Flore and Lakso, 1989; Fernandez et al., 1997). Reductions in growth affect photosynthesis on a whole plant level and alterations in physiological factors, directly or indirectly, the photosynthetic tissues (i.e., through stomatal conductance, leaf water potential, leaf abscisic acid levels, photosynthetic apparatus functioning and carbohydrate partitioning and distribution; Quick et al., 1989; Chaves, 1991; Fernandez et al., 1997).

Chaves (1991) described the impact of a given type of stress, as the result of the interaction between the plant (a controlled system functioning according to its genetic information) and the stress, which imposes limitations of variable intensity depending on its severity and duration. Davies and Zhang (1991) contend that the term "water stress" should not be restricted to situations where water relations variables are modified, but should also include shoot and leaf responses to drying soil (e.g. reductions in stomatal conductance and photosynthesis), without detectable changes in leaf water status. Thus, many plant responses under moderate drought stress levels are of a regulatory nature, rather than stress-induced damage (Chaves, 1991). The normal daily trend in water potential due to transpirational water loss, and the resulting or concurrent reductions in stomatal conductance and photosynthesis are therefore not strictly "stress" reactions. For the purposes of this paper, the term "water deficit" or "water stress" will be used liberally to include such reactions. From a possible role of ABA as a drought signal in plants, Davies and Zhang (1991) concluded that such root signals may not only exert relatively dynamic control over stomatal conductance, but may also be important mechanisms regulating development, over an extended period, as a function of soil water status.

2   WATER DEFICIT STRESS AND TREE PHYSIOLOGY

Stress interactions

Water deficit stress generally occurs simultaneously with high light and/or high temperatures, which may predispose plants to the occurrence of photoinhibition and leads to a decrease in mesophyll photosynthesis (Demmig-Adams et al., 1989; Chaves, 1991). Chaves (1991) indicates that it is not clear whether photoinhibition causes the decrease in photosynthesis, or whether the alterations of the primary photochemistry process are the consequence of protective or repair mechanisms of carbon metabolism, caused by the water deficit. In many cases the photosynthetic apparatus seems to be rather resistant to water deficit (Chaves, 1991; Syvertsen and Lloyd, 1994; Blaikie and Chacko, 1998). In citrus, CO_2 deprivation is induced by low stomatal conductances and enhanced by a very low internal conductance (g_i) to CO_2, resulting in low internal partial

pressures of CO_2 at the chloroplast (Lloyd *et al.*, 1992; Syvertsen and Lloyd, 1994). Under elevated temperatures and high irradiance levels, such CO_2 deprivation could enhance the sensitivity of the photosynthetic apparatus to high light stress, induce photoinhibition and promote damage to photosystem II (Powles, 1984; Marler and Mickelbart, 1998). Chaves (1991) mentioned that the photon flux density (PFD) at which photoinhibition occurs, decreases proportionally to increases in the intensity of superimposed stresses. Photoprotective mechanisms (e.g., the xanthophyll cycle) act under water deficit (Demmig *et al.*, 1988) as well as in well-watered trees and under mild atmospheric conditions (Epron *et al.*, 1992), and can be rapidly adjusted to changes in incident light. Mechanisms of avoiding permanent photoinhibition are important components of a strategy of tolerance to water deficits (Demmig *et al.*, 1988). This allows photochemical activity to restart rapidly as soon as conditions more favourable to photosynthesis arise (Epron *et al.*, 1992).

Epron *et al.* (1992), working on *Quercus petraea*, reported that water depletion produced a reduction in the maximal rate of photosynthesis (A_{max}) and an increased sensitivity of gas exchange to summer atmospheric conditions (high light and temperature and high vapour pressure deficit). The reduction of photosynthesis was accompanied by enhanced thermal dissipation of excess excitation energy, via the xanthophyll cycle, resulting in a reduced photosystem II photochemical efficiency (F_v/F_m) (see 3.4). The increase in thermal de-excitation of photosystem II prevented permanent damage to the photosynthetic apparatus, so that a complete recovery of F_v/F_m occurred during the night.

Under conditions of water deficit, repair of heat-induced damage is decreased (Ludlow, 1987), leading to an increase in photoinhibition and even photodamage. Mild heat stress inhibits net CO_2 uptake by reversible conformational changes in the thylakoids (Weis, 1983), leading to a shift in light distribution in favour of photosystem I, and by deactivation of some Calvin cycle enzymes (Weis, 1981).

No interaction of light stress, superimposed on water deficit, was observed in cashew (Blaikie and Chacko, 1998), papaya (Marler and Mickelbart, 1998) or apple (Fernandez *et al.*, 1997), but contrasting reports on the significance of the interaction are abundant

(i.e., Björkman and Powles, 1984; Demmig et al., 1988). These substantial differences indicate the existence of diverse genotypic sensitivities to photoinhibition, either at the photoinhibitory sites, in the protective systems or in the repair mechanisms (Chaves, 1991). The variation in mechanisms regulating excitation energy dissipation in the photochemistry of photosystem II provides further support (Foyer et al., 1994). Some of these energy dissipation mechanisms will be described later (see 3.4).

Water deficit stress

Even short periods of drought stress lowered stomatal conductance and transpiration and increased the proline concentration (Levy, 1983) in potted citrus seedlings. Active osmotic adjustment by carbohydrate accumulation does not occur in citrus (Lloyd et al., 1987b). As already noted, the rate of the drought stress induction may significantly alter the plant response. Marler and Mickelbart (1998) found that A_{max} in papaya plants were reduced 85% in potted plants at a soil water potential (Ψ_s) of −70 kPa (reached after 1 week of stress), whilst plants in the field had a 50% A_{max} reduction at the same soil water potential (reached after 5 weeks).

Fereres et al. (1979) hypothesised that citrus plants are well-adapted to drought, because measured rates of evapotranspiration from well-irrigated orchards are significantly lower than the potential rates, and because leaf conductance decreases with increasing vapour pressure deficit, thus limiting transpiration in arid climates. Syvertsen and Lloyd (1994) also noted that citrus stomata close to conserve water at high temperatures and with increases in vapour pressure deficit, as do the stomata of numerous other plants (El-Sharkawy et al., 1985). Kriedemann and Barrs (1981) concluded that citrus has a high photosynthetic water use efficiency (WUE) because of the stomatal sensitivity to vapour pressure deficit. Increasing WUE under increasing soil water deficit was postulated (Levy, 1983). Kriedemann and Barrs (1981) reported a significant midday depression in photosynthesis, whilst transpiration remained relatively constant (confirmed by Sinclair and Allen, 1982), with the stomatal conductance decreasing only enough to stabilise transpiration. Although an increase in vapour pressure deficit reduces stomatal conductance, transpiration may even be enhanced because of the increased evaporative demand (Syvertsen and Lloyd, 1994; Prior et al.,

1997). It is generally accepted, though, that transpiration rate could also be depressed during midday (Mott and Parkhurst, 1991; Syvertsen and Lloyd, 1994), and there is ample evidence for reductions in transpiration under mild water deficit conditions (Hsiao, 1973; Levy, 1983; Kaiser, 1987).

Reductions in photosynthesis under water deficit conditions have generally been attributed to stomatal closure (Escalona et al., 1999), but non-stomatal factors could also be present, i.e. increased mesophyll resistance to CO_2 diffusion and the down-regulation of photochemical capacity (Correia et al., 1990). Water potential (Fereres et al., 1979) and photosynthesis (Thompson et al., 1965) recover more quickly than transpiration after a period of water deficit. Zhang and Davies (1990) postulated the role of ABA as a water stress signal. The possible role of ABA in the control of photosynthesis, directly and indirectly (via the control of stomatal conductance) has been a subject of great controversy for the past two decades. Recently, integrated control by both chemical and hydraulic root signals has been postulated (Tardieu and Davies, 1993; Tardieu and Simonneau, 1998). Direct effects of ABA and water deficit stress on photosynthesis have been shown to be negligible under mild conditions (Liang et al., 1997).

Although certain correlations exist, no single measure of water status can be expected to correlate with the numerous effects of water stress (Kramer, 1988). Ψ can be of value to describe both plant and soil water status on a common physiological basis, i.e. xylem water potential Ψ_x is a good indicator of water stress in lychee (Stern et al., 1998) and apple (Naor et al., 1995). However, plant metabolism may alter the cellular setting in which Ψ and its components act (Boyer, 1989) and other variables of water status could then be more appropriate, i.e. relative water content (RWC) or cell volume. Then, under moderate drought, stomatal responses can be more closely linked to soil drying than to leaf water status (Zhang and Davies, 1989) and stomatal closure or altered growth may then be more sensitive indicators of drought than loss of turgor (Chaves, 1991).

ABA and water deficit stress

It has previously been assumed that morphological and physiological adaptations to water deficit are transduced by cell turgor (Begg and Turner, 1976) and processes that aid in osmotic adjustment and important in maintaining growth through the maintenance of stomatal conductance, photosynthesis, leaf and root growth. Turner (1986) discussed the recent scepticism of the role of turgor, specifically leaf turgor, as sole transducer of the effects of water deficit on growth and photosynthesis and highlighted the possible role of plant growth substances. Zhang and Davies (1989, 1990) and Davies and Zhang (1991) implicated ABA in plant responses to water deficit. They hypothesised that in response to drying soil, roots produce ABA that is transported and accumulated in the leaves, resulting in decreased stomatal conductance and photosynthesis. ABA is also implicated in reduced growth in several plant tissues (Davies *et al.*, 1986).

Leaf water potential is the most commonly-used indicator of shoot water status and plant stress levels (Hsiao, 1973). Its appropriateness is questioned by Davies and Zhang (1991), since undisturbed turgor in plant tissues is sometimes accompanied by, simultaneous reductions in growth rates. Similarly, leaf water potentials are sometimes similar in stressed and unstressed plants, but they show differences in stomatal closure. Boyer (1989) claimed that roots could not be sensors of plant water deficit since they generally manifest higher water potential than shoots. Contrary to this general view at the time (Boyer, 1989), it seems as if leaf water potential is controlled by stomata (Levy, 1983; Davies and Zhang, 1991) and not vice versa. Roots may produce a stress signal, but water deficit in the soil is not required for stomatal closure and wilting, which may result from an unfavourable ambient environment (Kramer, 1988). Conversely, root signals may override the effects of the water status of the shoot and, for example, induce stomatal closure when leaf water potential is high (Passioura, 1988; Davies and Zhang, 1991).

Tardieu and Davies (1992) showed that leaf water status modulates the stomatal response to ABA. As leaf water potential decreases with increases in vapour pressure deficit and irradiance during the day, the guard cells are sensitised to ABA. Tardieu and

Davies (1993) postulated that the root message (ABA) provides the plant with a means to sense the conditions of water extraction, i.e. the water status as well as the resistance to water flux in the soil, on a daily time scale. The short-term plant responses to the root message would depend on the evaporative demand, influencing the current leaf water status.

Davies *et al.* (1994) tested models of stomatal control. A purely chemical model, negating stomatal sensitisation with increasing leaf water deficit, failed to control stomatal behaviour with the concentrations of ABA generally found in plants. A physical model predicts the drought-induced limitation of conductance but also predicts a re-opening of stomata in the afternoon, which is not observed in plants under natural conditions of soil drying (also noted by Tenhunen *et al.*, 1982). The authors caution that an observed relationship between chemicals in the xylem sap and stomatal conductance does not unequivocally prove chemical control. Tardieu (1995) demonstrated stomatal conductance and leaf water potential (Ψ_l) relationships to be physiologically non-significant. In anisohydric species (where Ψ_l decreases to a minimum at midday) stomatal control depends on the chemical signal alone and leaf water potential emerges as a consequence of stomatal conductance and water relations without any controlling effects. By contrast, leaf water potential has an effect *per se* in stomatal control of isohydric species (in which leaf water potential is constant during the day), in interaction with the chemical signal. Statistical relationships between leaf water potential and stomatal conductance are only observed in the cases where leaf water potential has no controlling action on stomata (Tardieu, 1995; Tardieu and Simonneau, 1998).

Another important change in perspective was that leaves do not necessarily function as a single unit. Non-uniform stomatal closure was shown to occur in response to ABA (Downton *et al.*, 1988). This implied a previous general over-estimation of the intercellular partial pressure of CO_2 (C_i), because gas exchange data did not reflect average C_i. Non-uniform stomatal closure may be especially important in plants with heterobaric leaves, in which lateral gas diffusion is non-uniform (Chaves, 1991; Syvertsen and Lloyd, 1994) such as in citrus. This means that stomatal closure may fully account for the inhibition of photosynthesis by ABA or drought (Downton *et al.*,

1988). Some recent controversy exists around the universality of non-uniform stomatal closure (Buckley *et al.*, 1999; Escalona *et al.*, 1999; Liang *et al.*, 1997).

Midday depression of photosynthesis and stomatal conductance

Midday depression of photosynthesis affects most plants living outside of a high humidity atmosphere and can have significant effects on crop yields (Xu and Shen, 1997). These authors indicate a midday depression of variable severity occurring in photosynthesis and stomatal conductance, depending on the soil water status and other environmental factors. Typical diurnal responses include:

- a one-peaked course without midday depression;
- a two-peaked course with a characteristic high peak in the late morning, a rather strong depression in the mid afternoon and a recovery phase during the late afternoon and
- a morning-peaked, severely-depressed course with no afternoon recovery.

The most important factors causing a midday depression in photosynthesis in woody perennials (from Xu and Shen, 1997) are high vapour pressure deficit and photosynthetic photon flux levels (as the influencing *environmental* factors). Their effects mediate the closure of stomata, and the over-exposure to light and the accompanying high temperatures cause physiological and biochemical depression. Stomatal closure and the enhancement of photorespiration during the midday hours are the major *physiological* causes of midday depression. Farquhar and Sharkey (1982) commented that stomatal closure could only be accepted as a cause of photosynthetic rate decline if the intercellular CO_2 concentration (C_i) decreases with A. This has been shown to be true, since Downton *et al.* (1988) explained the erroneous constancy in previously determined C_i levels with the demonstration of patchy stomatal closure. Photorepiration increases with temperature and further increases when stomata close and CO_2 becomes limited. The only *biochemical* factor of note is the photoinhibition of the photosynthetic apparatus that occurs in some woody plants, i.e. *Vitis vinifera* and *Camellia sinensis* as a result of the decline in photosystem II photochemical efficiency in high sunlight (Xu and Shen, 1997).

Further, Xu and Shen (1997) suggest possible mechanisms of midday depression. High photosynthetic photon flux density (PPFD) increases air temperature and vapour pressure deficit and decreases soil water potential that result in variations of biochemical and physiological factors. Xylem ABA is increased by soil water deficit that, combined with increased vapour pressure deficit, reduces stomatal conductance, decreasing net CO_2 assimilation. Increases in photorespiration and a downregulation of photosystem II photochemical efficiency may also be important (Chen and Zhang, 1994; Demmig-Adams et al., 1989).

A severe midday depression of photosynthesis (CO_2 exchange rate) has been observed in citrus (Sinclair and Allen, 1982). Although a role for direct effects of temperature on the photosystems or Calvin cycle could be considered, stomatal control mediated by vapour pressure deficit has been implicated as the main causal factor. In this study, maximum rates of transpiration remained constant with increasing vapour pressure deficit, implying that stomatal control restricted water loss. Khairi and Hall (1976) reported that increasing soil water deficit reduced photosynthesis more than transpiration during the afternoon. Continued high photosynthetic rates during the morning and late afternoon, together with closure of stomata during midday in response to vapour pressure deficit, should maintain a high water use efficiency (Turner, 1986).

Midday depression in papaya plants subjected to water deficit stress was more severe than in the control plants (Marler and Mickelbart, 1998), and it was argued that vapour pressure deficit and mild heat stress were the main causal factors of this midday depression. Prior et al. (1997) reported stronger stomatal conductance responses to vapour pressure deficit when predawn leaf water potential was moderately low compared to the correlation at high and very low predawn leaf water potential values. They also considered high leaf temperatures as the major cause of midday reductions in photosynthesis in young Eucalyptus trees. Chen and Zhang (1994) concluded that increased photorespiration, resulting from high midday temperatures, lowered C_i because of stomatal closure, and high light-induced photoprotection, are the main causal factors for the midday depression of photosynthesis and photosynthetic quantum efficiency in Citrus unshiu leaves.

Brakke (1989) studied midday depression in citrus in controlled environment chambers. Although considering vapour pressure deficit as a possible causal factor of midday depression, he argued that an increased mesophyll resistance induced by water deficit might be more significant and that midday depression would not occur in citrus as long as soil water is easily available. Sinclair and Allen (1982) also implicated the water supply rate as an important controlling factor of stomatal conductance and therefore also the midday depression of photosynthesis.

Tardieu and Simonneau (1998) recently reported that midday depression does not occur in plants that are not subjected to stress. They found that plants with a near zero xylem ABA concentration did not show vapour pressure deficit responses of stomatal conductance. This was consistent with Bunce (1996) who attributed the VPD-induced stomatal conductance response to ABA. A direct response of stomata to the transpiration rate has recently been demonstrated (Mott and Parkhurst, 1991; Monteith, 1995). Mott and Parkhurst (1991) showed, with measurements in a medium that allows for faster water diffusion, that stomata do not show direct sensing of vapour pressure at the leaf surface or to VPD between the leaf and air, but to the rate of water loss from the leaf. Monteith (1995) confirmed this with the re-analysis of work done on 16 species.

Correia et al. (1990) observed that stomata of well-watered grapevine plants closed under high temperature and vapour pressure deficit conditions. Tenhunen et al. (1980; 1981) proposed that the reduction in transpiration in Arbutus unedo plants around midday was not controlled by internal water status since stomatal closure were reported in droughted and well-watered plants. Stomata apparently responded primarily and reversibly to high leaf temperatures and vapour pressure deficit, and water deficit stress sensitised plants to it (Tenhunen et al., 1982). Increases in xylem ABA concentration relate to stomatal closure during midday (Pereira et al., 1989). Correia et al. (1990) speculated that leaf water deficits, even those too small to produce ABA, would lead to leaf ABA redistribution in the cellular components and between cells, possibly resulting from a high light-induced stress (i.e. cellular pH change), influencing stomatal conductance. Drought stress-induced increases in pH (Gollan et al., 1992) result in less ABA taken from the xylem stream into the mesophyll cells. More xylem-derived ABA is allowed to reach guard cells (Wilkinson and Davies, 1997), probably explaining part of

the interdependence of chemical (ABA) and hydraulic (Ψ_l) control of stomatal conductance (Tardieu, 1995; Tardieu and Simonneau, 1998).

On clear days, the differences in stomatal conductance, WUE and photosynthesis differences between stressed and unstressed papaya trees were reduced (Marler and Mickelbart, 1998), compared to overcast days in which the differences between the treatments were higher, despite the elevated levels of all the parameters. This is generally observed in plants showing midday depression (Xu and Shen, 1997). On clear days unstressed trees showed a two-peaked stomatal conductance response with the second peak (in the late afternoon) lower than the first (during mid-morning) but stressed trees did not recover after the initial midday decline, until the next morning (Marler and Mickelbart, 1998). Roessler and Monson (1985) reported an increase in the temperature and vapour pressure deficit at which midday depression occurred as the season progressed, probably due to biochemical and biophysical acclimation of guard cells.

Midday depression seems to be an adaptation to cope with environmental stresses, because midday stomatal closure and downregulation of photochemical efficiency are effective ways to avoid excess water loss and photodamage of photosynthetic apparatus under strong light and atmospherically dry conditions (Xu and Shen, 1997).

3   MEASUREMENT OF WATER DEFICIT

In this section, water potential, sap flow, gas exchange (relating to stomatal conductance, transpiration and photosynthesis) and photochemical efficiency of photosystem II (as measured by chlorophyll a fluorescence) will be discussed with regard to their use in identifying water deficit stress. This discussion does not cover the extent of the topic and should be seen as an overview. Recent reviews covering the topic and methodology will be cited.

3.1   Water Potential

Water potential (Ψ_w) is the thermodynamic parameter commonly used to describe the energy status of water in plants (Koide *et al.*, 1989; see this review for an introduction to concepts and measurement techniques). Boyer (1989) described water potential as the driving force of water movement through plants, bringing water into cells and generating the turgor required for enlargement and growth as well as stomatal regulation.

Water potential is a sensitive measure of plant water status (Landsberg et al., 1975). Predawn leaf water potential (Ψ_{pd}) is a useful measure of water availability to plants, since it integrates soil water potential (Ψ_s) over the root zone of the plant (Schulze and Hall, 1992) and decreases with a decrease in soil water potential (Tardieu and Simonneau, 1998). It closely represents soil water potential by equilibrating soil and plant water potential through the night when there is little or no transpirational water losses (Naor *et al.*, 1995; Tardieu and Simonneau, 1998). Leaf water potential at any given time of day is a result of the soil water potential (which determines the baseline leaf water potential in the absence of evaporative demand), the transpiration rate linked to the evaporative demand (Tardieu and Simonneau, 1998), and plant hydraulic conditions (Sinclair and Allen, 1982). Measurements at midday (Ψ_{md}) provide an indication of the extent of the plant water condition, being a combination of soil water supply and atmospheric demand, but at this stage usually dominated by the latter.

Measurement of plant water potential

Generally, two methods are used to determine water potential in higher plants under field conditions, i.e., the psychrometric and pressure chamber techniques (Koide *et al.*, 1989).

Thermocouple psychrometers are primarily used with excised tissue samples, although *in situ* leaf psychrometers are also available. In this technique, a tissue sample is placed in an enclosed, well-sealed psychrometer chamber, or a leaf surface with a partly abraded or dissoluted cuticle is sealed to the chamber, and held at constant temperature. This allows the water in the sample to equilibrate with the chamber

atmosphere and by measuring the equilibrium relative humidity (h) in the chamber; the water potential in the sample can be calculated (Koide *et al.*, 1989). Several methods of measuring h exist, and they form the major differences between apparatus. It is assumed that water loss from the sample to the chamber atmosphere does not affect the water potential of the sample.

Pressure chambers are widely used under field conditions since the technique is simple, reliable, and does not require precise temperature control. A leaf or shoot is cut with a razor blade and inserted into the pressure chamber with the cut surface protruding slightly through the rubber gasket. In the chamber, compressed air or nitrogen is used to gradually increase the pressure until xylem water first appears at the cut surface. A hand lens can aid in the determination of this end point. The chamber pressure at this point (taken as a negative value) equals the apoplasmic hydrostatic pressure in the leaf, and this in turn equals the symplasmic value of water potential under most conditions (Koide *et al.*, 1989). Describing the methodology used, Landsberg et al. (1975) remarked that pressure bomb measurements are highly variable, but they assumed that it gives a measure of the true water potential and that the variability is linked to within-tree positional and between-tree factors.

Water deficit and water potential

Severe water stress can result in large reductions in leaf water potential in citrus trees, with predawn water potential levels as low as –6.6 MPa (Fereres *et al.*, 1979), but under mild water deficit conditions relatively small reductions are the norm (Syvertsen and Lloyd, 1994). Mature 'Valencia' orange trees recovered within 12 hours after water deficit if predawn water potential did not fall lower than -3.0 MPa (Fereres *et al.*, 1979). Liang *et al.* (1997) showed that leaf water potential of -2.5 MPa had to be reached before non-stomatal limitations of photosynthesis (reduction in dark-adapted Fv/Fm values) played a role.

Tardieu (1995) suggested that plants can control gas exchange so that daytime leaf water status is unaffected by water deficits. This is termed isohydric behaviour and is characterised by feedforward control (i.e. stomatal conductance reduced at high leaf

water potentials, thereby preventing low water potentials), e.g. in poplar and maize (Tardieu and Simonneau, 1998). Alternatively, daytime leaf water potential decreases diurnally with increasing vapour pressure deficit and declines with decreasing soil water potential. This is termed anisohydric behaviour, characterised by feedback control (stomatal conductance reduced at low leaf water potentials), i.e. in sunflower, barley and wheat (Tardieu and Simonneau, 1998). Most fruit trees, including citrus, exhibit anisohydric behaviour (Jones et al., 1985) and it seems that leaf water status has very little controlling action on stomata in such species (Tardieu, 1995; Tardieu and Simonneau, 1998).

Tardieu and Simonneau (1998) showed that predawn water potential decreased in sunflower plants as soil water potential decreased. Midday water potential and stomatal conductance were both correlated to predawn water potential and decreased consequently. Stomatal closure provided plants with a coarse control against dehydration, but did not buffer leaf water potential against changes in evaporative demand. The use of midday xylem water potential as an indicator of stress in relation to apple fruit growth was proposed by Naor et al. (1995). Stern et al. (1998) suggested that midday xylem water potential correlated well with soil water content and that it could be used in irrigation scheduling, provided that evaporative demand is considered in determining the threshold for irrigation control. On the other hand, no relation between xylem water potential and soil water content were observed in cashew plants (Blaikie and Chacko, 1998) since xylem water potential actually increased in stressed plants, probably because of stomatal closure (Schaper et al., 1996). It was proposed that gas exchange measurements or sap flow should preferably be used in irrigation scheduling for cashew (Blaikie and Chacko, 1998).

Changes in plant water potential, and more specifically changes in turgor, may result in changes in ABA concentration, via synthesis or release (Pierce and Raschke, 1980; 1981). This may play an important role in stomatal control, both from the roots and leaves, since stomatal conductance is influenced by root-produced (and therefore xylem-derived) ABA as well as the production and accumulation of ABA in the leaves. In integrated models explaining stomatal behaviour, ABA-mediated stomatal closure was controlled by leaf water potential (Tardieu and Davies, 1993). The stomatal

response to vapour pressure deficit is influenced by soil water potential (possibly via ABA) in *Eucalyptus* saplings when the predawn water potential is moderately low (Prior et al., 1997) and the trees seem to behave isohydrically, having high leaf water potential but low photosynthetic rates in the afternoon due to a decline in stomatal conductance (Prior *et al.*, 1997). This has also been observed in poplar (Tardieu and Simonneau, 1998). Jones et al. (1985) reviewed the physiological control in water status in fruit trees. They showed stomatal conductance to control transpiration efficiently, at constant level in citrus and deciduous fruit trees, over many different production areas and climatic conditions. Stomatal conductance also controlled water potential by controlling transpiration rate (Jones *et al.*, 1985) and may even overcompensate in water deficit stressed trees after re-watering. Higher stomatal conductances in previously stressed trees compared to unstressed trees have been observed in both citrus and apple (Jones *et al.*, 1985).

The trend in diurnal water potential is similar in temperate and sub-tropical fruit trees (Jones *et al.*, 1985), being high at pre-dawn, then rapidly declining as soon as transpiration starts. There is a minimum just after midday when the vapour pressure deficit is lowest and a gradual increase as the day cools.

3.2 Sap Flow

The transpiration rate in whole branches and entire plants can be determined by measuring the xylem sap ascension rate in the stem. Sap flow measurement (theory and techniques reviewed by Smith and Allen, 1996) is a non-destructive method for determining continuous water use by entire plants in their natural environment. Eastham and Gray (1998) describe sap flow measurements as an integrated indicator of plant hydraulic responses to diurnal and daily fluctuations in transpiration.

Whole-plant transpiration determined by sap flow holds important advantages over the up-scaling of leaf-level transpiration measurement to canopies or tree crowns (see 3.3). Difficulties in aggregating transpiration to whole trees arise from variation in leaf age, boundary layer conductance and light penetration within canopies (Smith and Allen, 1996).

Measurement of sap flow

Four principal techniques are used to determine sap flow in woody stems and they all use heat as a tracer of sap movement (Smith and Allen, 1996). It is important to use the most appropriate method for a specific situation and to thoroughly take precautions against potential error sources. The techniques described by Smith and Allen (1996) are reported to be accurate within 10%. They are (1) the Granier thermal dissipation method, (2) the heat pulse method, (3) the stem heat balance method and (4) the trunk sector heat balance method.

For the **Granier thermal dissipation method**, two cylindrical probes are inserted one above the other, into the active xylem of the stem. The upper probe contains a continually-powered heater element as well as a thermocouple junction, referenced to another thermocouple in the lower probe. The temperature difference between the two probes is continually measured. The rate of heat dissipation depends on the rate of sap flow around the probes, and higher sap flow rates will dissipate the heat more quickly, thus decreasing the temperature difference. This method is relatively inexpensive, easy to install, has simple requirements for recording sensor outputs, and uses uncomplicated calculations to determine the volume of sap flow (Smith and Allen, 1996). The Granier method has been accurately applied in orchard research (Blaikie and Chacko, 1998).

The **heat pulse method** usually requires four sets of probes; each set in a different quadrant, and at a different radial depth of the stem. Three probes, a heater probe and two sensor probes with thermistors (one upstream and one downstream of the heater), comprise a set. The upstream probe is placed closer to the heater than the downstream probe and accurate spacing of the probes is essential. Continual monitoring of the sensor probes determines the velocity of the short (1 – 2 second) heat pulses that are periodically released from the heater probe, as they move with the sap stream. This placement ensures that the temperature at the closer upstream probe rises immediately after the heat pulse is released, due to conduction. The time required for convection (by the moving sap) to cool the upstream and heat the downstream probes to an equal temperature (t_e), is measured. Higher sap velocities reduce t_e. Sap

velocity equals heat pulse velocity in thermally homogeneous active xylem and subsequently the mass flow rate of sap can be determined, without previous calibration, from a set of calculations. Special care should be taken in the determination of wood homogeneity and to monitor possible wound reactions, since both these factors can seriously impair the accuracy of the technique (Smith and Allen, 1996).

The **stem heat balance method** can be used in both woody and herbaceous plants with stems or branches varying in diameter, from 2 – 125 mm. The heat balance gauge comprises a flexible heater element, which wraps around the stem, enclosed in layers of cork, foam insulation and an aluminium-covered weather shield. Several pairs of connected thermocouple junctions are either embedded in the cork layer and measure the radial temperature away from the heater, or staggered above and below the heater element and measure components of the stem heat balance. The mass flow of sap can be obtained from the balance of heat fluxes into and out of the heated section of the stem, if the heat input is thoroughly limited to that applied by the heater. Accurate determination of the thermal conductance of the sheath surrounding the heater (K_{sh}), partly related to the stem diameter, is essential and can only be done when sap flow is zero. Error can also result from insufficient contact between the gauge and the stem, the condensation of water (or the seepage thereof) in the gauge and by extraneous thermal gradients across the stem, as well as from solar heating (Smith and Allen, 1996).

Sap flow in tree trunks with diameters larger than 120 mm can be determined with the **trunk sector heat balance method**. It is based on the same principle as the stem heat balance method, but heat is applied internally and only to a sector of the trunk. Five electrode plates are inserted, uniformly spaced, and parallel to each other, spanning the sapwood. Two parallel rows of four thermocouples in metal probes are inserted (with two being located midway across the central segments of the active xylem and two placed outside of the outer electrodes) level with the top of the electrodes and below the heated zone, respectively. The eight thermocouples are connected in series to give a measurement of the temperature increase in the heated sector, which is used to calculate the sap flow rate through the sector. As with the previous method, the

determination of the thermal conductance coefficient may be quite difficult, but is essential for accuracy (Smith and Allen, 1996).

Choosing a technique depends on the specific situation, i.e., stem diameter, xylem characteristics, length of the experiment, expertise and cost would all be determining factors.

Water deficit and sap flow

Few reports exist specifically relating sap flow to plant water deficits. Eastham and Gray (1998) have shown that sap flow measurements can elucidate transpiration differences between stressed and unstressed grapevines, as did Blaikie and Chacko (1998) in cashew trees. Both groups of researchers postulated a possible role for sap flow in irrigation scheduling.

Eastham and Gray (1998) showed that night time sap flow is increased under water deficit conditions. This has been confirmed for other species as well (Caspari *et al.,* 1993). Partial stomatal closure was suggested as a reason for night time sap flow, in kiwi-vines, since high vapour pressure deficits at night would facilitate night time transpiration. Conversely, Eastham and Gray (1998) argued that night time sap flow would be expected to be considerable in both well-watered and stressed plants if partial stomatal closure was involved, but they only observed it in grapevines experiencing water deficits. Caspari *et al.* (1993) interpreted this as stem, branch and leaf tissue rehydration, after daytime water loss. A time lag between leaf transpiration and sap flow may occur, resulting from capacitance in the stem or branches, that may store water utilised for early morning transpiration (Schulze *et al.,* 1985). Asynchronous changes in xylem and leaf water potentials during the day also illustrate the time lag between leaves and stems (Stern *et al.,* 1998).

3.3 Gas Exchange

It is generally accepted that stomatal conductance is regulated so as to optimise carbon gain relative to water loss, a ratio termed photosynthetic water use efficiency (Pearcy *et*

al., 1989). The specific mechanism of stomatal control is a very controversial topic as can be seen in the recent literature (Jarvis and Davies, 1998; Jones, 1998; Monteith, 1995; Tardieu and Simonneau, 1998). Stomata exhibit either mainly feed-forward control (direct response to light, temperature, vapour pressure deficit, etc.) or feedback control (in response to changes in intercellular CO_2 concentration, leaf water potential, transpiration, etc.) as noted by Syvertsen and Lloyd (1994). Recently, Mott and Parkhurst (1991) used modified atmospheres to show that stomata generally respond directly to the transpiration stream. Contrary to the general view, it seems as if Ψ_l is controlled by stomata (Davies and Zhang, 1991) in citrus (Levy, 1983) and apples (Jones, 1985) and not vice versa as in many other species (Hsiao, 1973). The data of Prior et al. (1997) is consistent with others (Monteith, 1995; Mott and Parkhurst, 1991; Sinclair and Allen 1982), i.e., the plant controls transpiration to achieve a relatively stable rate through the day. Sinclair and Allen (1982) even proposed a maximal transpiration rate mediated via the water supply to the leaves; therefore transpiration in trees may be limited by water supply (Bond and Kavanagh, 1999; Jones and Sutherland, 1991; Whitehead, 1998). Hydraulic conductivity is an important determinator of the rate of water flux through the plant and a role therefore was postulated concerning stomatal control in trees, as an alternative to ABA mediation (Jones and Sutherland, 1991; Bond and Kavanagh, 1999). Citrus transpiration rate is generally low, being between $6.0 - 8.0$ mmol m^{-2} s^{-1} at 30 °C whilst stomatal conductance at the same temperature is between 0.3 and 0.5 mol m^{-2} s^{-1}.

Many woody perennials have leaves that are hypo-stomatous i.e. stomata distributed only abaxially (Downton et al., 1988). A recent important change in perspective was the finding that leaves do not necessarily function as a single unit. Non-uniform stomatal closure was shown to occur in response to ABA (Downton et al., 1988), and Buckley et al. (1999) reported data consistent with recent work suggesting patchy stomatal closure might be common but unobserved. Eckstein et al. (1998) showed that ABA was not required for patchy stomatal closure and postulated that the variation in Ψ_l over the leaf surface is a key determinant. Patchiness implies a general over-estimation of the intercellular partial pressure of CO_2 (C_i), because gas exchange data does not reflect average C_i. Non-uniform stomatal closure may be especially important in plants with heterobaric leaves, in which lateral gas diffusion is non-uniform (Chaves, 1991;

Syvertsen and Lloyd, 1994) implying that stomatal closure may fully account for the inhibition of photosynthesis by ABA or drought (Downton et al., 1988). Some recent controversy exists around the universality of non-uniform stomatal closure (Buckley et al., 1999; Eckstein et al., 1998; Escalona et al., 1999; Liang et al., 1997).

Evergreen long-lived leaves, like citrus, have low photosynthetic CO_2 assimilation rates (typical values of 8 - 20 μmol CO_2 m^{-2} s^{-1}) compared to other woody perennials (typical values of 20 - 40 μmol CO_2 m^{-2} s^{-1}) (Vu, 1999; Lloyd et al., 1992). An explanation for this is the low level of internal diffusive conductance of CO_2 (g_i) in the thick leaves combined with the fact that stomata are restricted to the abaxial surface. Citrus limon, C. paradisi and Macadamia integrifolia have g_i levels (1.1 - 2.2 μmol CO_2 m^{-2} s^{-1} Pa^{-1}) only one third of those of Prunus persica (3.5 μmol CO_2 m^{-2} s^{-1} Pa^{-1}) and considerably lower than those of herbaceous crops (Lloyd et al., 1992). The evergreens, although having high leaf-N levels, use only 10% of available N in Rubisco (Lloyd et al., 1992). Peach trees use a higher percentage of leaf protein for Rubisco and photosynthesis, at any given N concentration on a leaf area basis, than evergreens do (Lloyd et al., 1992). However, citrus Rubisco activity is 10 times higher than needed for typical rates of photosynthesis in citrus, being 360 μmol CO_2 mg chlorophyll^{-1} hr^{-1}. Lloyd et al. (1992) noted that this rate was high enough to sustain photosynthetic rates of at least 30 μmol m^{-2} s^{-1}. Thus since no real biochemical limitations to photosynthesis exist, restricted CO_2 diffusive conductance must be the limiting factor to the photosynthetic rate, resulting in slow movement of CO_2 from the sub-stomatal cavity to the chloroplast and considerable drawdown along this path.

The CO_2 concentration difference from the sub-stomatal cavities (C_{st}) to the chloroplasts (C_c) can be substantial. Lloyd et al. (1992) calculated CO_2-partial pressure differences of 8.6 and 5.3 Pascal (Pa), in citrus and peach trees, respectively, with C_{st} = 19.8 Pa, C_c = 11.2 Pa for C. limon; and C_{st} = 23.1 Pa, C_c = 17.8 Pa for P. persica. Thus, in citrus the very low C_c explains the strong inhibition of photosynthesis above the level normally attributed to photorespiration in C_3 species (Syvertsen and Lloyd, 1994). Lloyd et al. (1987a) measured Rubisco activity and electron transport rates high enough to sustain net photosynthetic rates of at least 30 μmol CO_2 m^{-2} s^{-1}, but actual photosynthetic rates

are typically much lower for citrus. In their investigation, they found high levels of oxygen inhibition of photosynthesis in normal air, even when assimilation was CO_2 saturated. This implies a limitation of the photosynthetic rate by Rubisco, which has a high affinity for O_2. In Satsuma mandarin (*C. unshiu*) high rates of photorespiration have been considered as the causal factor for the midday depression of photosynthesis (Chen and Zhang, 1994). Blaikie and Chacko (1998) speculated that photorespiration might play an important role in droughted cashew trees, by maintaining electron flow when stomatal conductance and CO_2 assimilation decline.

Gas exchange measurements

Pearcy *et al.* (1989) reviewed the different techniques used to measure transpiration rate, describing sap flow systems (see 3.1), lysimetry, and porometry. Lysimetry is the most direct and accurate measure of whole plant water flux. It entails continuous weighing of the plant, in an enclosed pot or rooting chamber in the field. Lysimeters have been used to compare different methods and to calibrate transpiration models and estimates from small-scale transpiration measurements.

Stomatal conductance to water vapour is determined from the transpiration rate in a porometer leaf chamber and is proportional to the water flux through the leaf. Using this conductance, ambient leaf temperature and leaf to air vapour pressure deficit, it is possible to recalculate the transpiration rate associated with specific environmental conditions as well as the corresponding stomatal and boundary layer conductances. It is important to note whether leaves are hypostomatous or heterostomatous. Porometers measure relative humidity, leaf temperature and airflow. To accurately measure water vapour inside porometers, thin film capacitance sensors, dew point mirrors and infrared gas analysers are used. Different types of porometers exist of which steady state and constant flow porometers are the most important (Pearcy *et al.*, 1989).

Field *et al.* (1989) reviewed the principles and field techniques of photosynthesis measurements. Net CO_2 assimilation rates and water vapour exchange rates (transpiration) can be determined with closed gas exchange systems, periodically

measuring the CO_2 and water vapour concentrations in the leaf chamber. In steady state gas exchange systems air continually pass through the chamber. Compensating steady state systems keep the CO_2 concentration constant by CO_2 injection. Differential steady state systems measure the equilibrium CO_2 concentration, being the level of CO_2 when gas exchange reach a steady state under the specific conditions in the leaf chamber. The stomatal conductance to CO_2 can be calculated from the measured stomatal conductance to water vapour and the relationship between the diffusivity of CO_2 and water vapour in air.

Gas exchange and water deficit

Mild levels of water deficit generally have few effects on gas exchange, as already noted in 3.1. It is therefore important to note the level of water stress that plants are experiencing when gas exchange characteristics are considered. Strong water stress may have a profound influence on photosynthesis. However, specific sites of inhibition of photosynthetic CO_2 assimilation under water stress conditions are not fully elucidated and seem to be part of a complex reaction (Stuhlfauth *et al.*, 1990). Leaf photosynthetic reductions under water stress are known in many species (Farquhar and Sharkey, 1982) and can be ascribed to both stomatal closure and thus increased resistance to CO_2 diffusion, and non-stomatal factors related to photochemical or biochemical processes of photosynthesis (Chaves, 1991; Liang *et al.,* 1997). The relative importance of these factors as well as their modes of action is still under investigation. Under rapidly developing water stress conditions, stomata in citrus leaves close when leaf water potential levels of –2.5MPa are reached (Lloyd and Howie, 1989; Syvertsen and Lloyd, 1994). Other woody trees show similar responses (Liang *et al.* 1997).

Non-stomatal factors include a reduction in Rubisco concentration and activity under water-stressed conditions that may extend into the period after re-irrigation (Vu and Yelenosky, 1988). These reductions in Rubisco characteristics were, however, not severe enough to decrease even the highest photosynthetic rates measured in intact citrus leaves. It was concluded that partial or complete depression of afternoon photosynthetic rates were mostly due to partial or complete stomatal closure in well-watered and non-watered trees, respectively. Other non-stomatal factors involved

include reductions in hydraulic and gaseous conductivities in the stems and leaves (Lloyd et al., 1992), the effect of severely reduced leaf water potential (Hsiao, 1973), and decreasing photochemical efficiency of the photosynthetic apparatus under extreme circumstances (Liang et al., 1997).

It is well known that the ABA concentrations in both the xylem-sap and leaves increase as a result of water stress. Water potential (Fereres et al., 1979) and photosynthesis (Thompson et al., 1965) recovered more quickly than transpiration after a period of water deficit. No explanation is given in either case but one could speculate that this may be due to the elevated levels of ABA persisting in the plants and suppressing stomatal conductance. Tardieu and Simonneau (1998) concluded that stomatal control in anisohydric species (wheat, barley, sunflower, tree fruit) is largely independent of vapour pressure deficit, transpiration rate and ABA flux through plants since coupled values of stomatal conductance and xylem ABA concentration are similar at different transpiration rates. Stomatal control is also independent of leaf water potential because vapour pressure deficit variations result in large differences in leaf water potential, which had no apparent consequences on stomatal control. Gollan et al. (1985) found that, in a woody sclerophyllous species, vapour pressure deficit induced Ψ_l reductions, while roots controlled stomata via a chemical signal. Cornic and Miginiac (1983) showed a rapid direct non-stomatal influence of ABA on the photosynthetic rate in *Pharbitis* plants. They speculated that the increased concentration of leaf ABA during water stressed periods could be a factor involved in the reduction of the photosynthetic rate. The possible mode of action of the ABA was unknown to them but they hypothesised a physiological 'critical' point that, when reached, triggered the response. Raschke and Hedrich (1985) concluded from data of 16 species that because C_i remained constant over large ranges of stomatal conductance and photosynthetic rates, applied ABA had direct effects not only on the stomatal conductance but also the photosynthetic rates. Liang et al. (1997) found a reduction in photosynthetic rate and stomatal conductance in a tropical tree, *Leucaena leucocephala*, when very low leaf water potential (-2.5 MPa) had been reached. Zhang and Davies (1990) noted that the decrease in stomatal conductance caused by an increase in the ABA concentration in the xylem often preceded the decrease in leaf water potential. Thus ABA, and specifically its inhibitory effect on stomatal conductance, would effect a decrease in the photosynthetic rate, but

only after leaf water deficit develops. Direct effects on photosynthesis would be species-specific. Liang *et al.* (1997) deduced that the reduction of stomatal aperture was the most important factor reducing photosynthesis in tropical trees (*Acacia* and *Leucaena* spp.) under mild water deficit conditions (leaf water deficits ≤ 30 %). Recently this has also been found in various other species (Downton *et al.*, 1988; Renou *et al.*, 1990).

Commenting on earlier literature demonstrating direct non-stomatal effects of water stress on photosynthetic rate, Liang *et al.* (1997) mentioned that recent advances in the field of chlorophyll fluorescence and photosynthetic capacity assessment demonstrated the negligibility of mild water stress on the performance of the photosynthetic machinery (Krause and Weis, 1991). They also stressed the re-evaluation of the older literature in light of the recently described phenomenon of patchy stomatal closure (Downton *et al.*, 1988). This phenomenon may fully account for the inhibition of photosynthesis by ABA (Downton *et al.*, 1987) and various other internal and environmental factors (Stuhlfauth *et al.*, 1990). It does so by explaining the gross over-estimation of the internal CO_2 concentrations (C_i), leading to the erroneous reports of constant C_i with closed stomata (Raschke and Hedrich, 1985).

Citrus stomata exercise good control over transpiration and leaf water potential (Jones *et al.*, 1985; Syvertsen and Lloyd, 1994). As in other fruit trees, the rate of transpiration is kept relatively stable via stomatal control, and an 'overcompensation' for soil water deficits may even occur. Levy and Syvertsen (1981) showed stressed and afterwards re-watered citrus trees having higher water potentials than unstressed controls, as well as increases in leaf water potential after the midday closure of stomata occurred.

Stomatal conductance in many plants is correlated with xylem ABA concentration and in turn to the soil water content (Zhang and Davies, 1990). Liang *et al.* (1997) inferred from this that dry soil could reduce photosynthesis via short term (stomatal closure) or long-term effects (restriction of leaf area expansion). They cite many experiments as verification, i.e. their own research on deciduous tropical trees and research on vines and sunflowers by Downton *et al.* (1988).

The reduction in stomatal conductance as ABA concentration increases is also influenced by the current plant water status and atmospheric conditions (Tardieu and Davies, 1993). These authors conclude that: "... *the root message* (xylem ABA) *would provide the plant with a means to sense the conditions of water extraction (soil water status and resistance to water flux) on a daily time scale, while the short term plant response to this message would depend on the evaporative demand.*"

Citrus leaves show reductions in photosynthesis and stomatal conductance under high vapour pressure deficits (Syvertsen and Lloyd, 1994; Veste, 2000). Gas exchange is further reduced by concurrent environmental stresses of which high temperature is the most important. Soil water deficit plays a much lesser role, and an increase in soil water content will not reduce the midday depression in stomatal conductance nor enhance CO_2-uptake (Veste *et al.*, 2000).

In conclusion, reductions in stomatal aperture and high temperature inhibition may account for the water deficit-related reduction of photosynthesis, in many plants and specifically in *Citrus* (Downton *et al.*, 1988; Syvertsen and Lloyd, 1994; Veste *et al.*, 2000). It also seems that water deficit does not have a direct non-stomatal influence on photosynthesis in *Citrus*.

3.4 Chlorophyll Fluorescence

Radiation reaching a leaf can be transmitted, reflected or absorbed and generally, depending on specific plant and environmental characteristics, the ratios are 4% (ranging from 0% to 20%), 12% (ranging from 5% to 20%) and 84% (ranging from 70% to 90%), respectively (Salisbury and Ross, 1993). Chlorophyll molecules absorb light energy ($\lambda = 400 - 700$ nm) for use during the light-dependent stages of photosynthesis. These excited molecules are very unstable and the excitation energy is dissipated within 10^{-8}s (Bolhàr-Nordenkampf and Öquist, 1993). Several decay processes compete for this energy, i.e. charge separation as the primary photochemical step of photosynthesis; radiationless deactivation or heat; and fluorescence or the re-emission of the energy as a red light photon (Krause and Weis, 1991; Maxwell and Johnson, 2000).

Fluorescence constitutes a 1 – 2% fraction of the dissipated energy, but is coupled to the other dissipating pathways (Maxwell and Johnson, 2000). Its intensity will therefore be influenced by changes in both the photosynthetic rate and heat emissions. These variables are very dependent on the species, the plant's physiological condition and the environmental factors influencing it. Thylakoid membranes are very sensitive to stress factors such as chilling temperatures and freezing (Strand and Öquist, 1985), drought (Stuhlfauth et al., 1990), high temperatures and excessive radiation (Demmig et al., 1988). All these factors reduce the photosynthetic potential of the plants, albeit not directly, and a secondary light stress response can be observed, because as the photosynthetic rate is reduced the fluorescence signal is intensified (Bolhàr-Nordenkampf and Öquist, 1993).

Measurement of chlorophyll fluorescence

Chlorophyll a fluorescence can be used as a stress detection tool. This has been done during the past decade, since modulated fluorescence measuring systems became readily available for research purposes. In a recent review, Maxwell and Johnson (2000) describe the use of modulated fluorescence techniques in plant stress ecology, and van Kooten and Snel (1990) proposed definitions of the fluorescence parameters used in plant stress studies. The *maximum* quantum efficiency of photosystem II, measured as the ratio F_v/F_m, is a very useful parameter. The variable fluorescence (F_v) is defined as the difference in fluorescence yield between the maximum (F_m) and the minimum (F_0) fluorescence levels. F_m is the fluorescence yield of a dark-adapted leaf after exposure to a saturating light pulse (> 5000 μmol m^{-2} s^{-1}), i.e. when all the reaction centres are fully saturated. F_0 is achieved by a low intensity light (< 2 μmol m^{-2} s^{-1}), not allowing detectable photochemistry to occur. At F_0 all the reaction centres are open for primary photochemistry and the potential for photochemical quenching is maximal. Fv/Fm is generally between 0.75 and 0.85 and relates approximately linearly to the quantum yield of photosynthesis. It is a quick and easy measure of changes in photosynthetic capacity and has been used extensively in screening for plants with different levels of adaptation to, e.g., frost, chilling temperatures and pollution, or other characteristics e.g. grafting compatibility.

When a leaf is photosynthesising at a constant level of photosynthetic photon flux density (PPFD) the fluorescence yield stabilises at a level (F_s) between F_0 and F_m. When a saturating pulse of high intensity is applied to the leaf, the F'_m or maximum level of photochemical quenching at that specific light intensity (PPFD) is achieved, and if combined with previously attained levels of Fv/Fm, the extent of photochemical (q_P) and non-photochemical quenching (q_{NP}) can be determined. At any given point on the fluorescence induction curve both photochemical and non-photochemical activity quenches the fluorescence signal and these parameters provide additional information on the physiological condition of photosystem II. For the calculation of the quenching coefficients, the following equations are used:

Photochemical quenching $\qquad q_P \qquad = \dfrac{(F'_m - F_s)}{(F'_m - F'_0)}$

Non-photochemical quenching $\qquad q_{NP} \qquad = \dfrac{(F_m - F'_m)}{(F_m - F'_0)}$

$\qquad$ or $\qquad$ NPQ $\qquad = \dfrac{(F_m - F'_m)}{F'_m}$

The quantum efficiency of photosystem II at that level of PPFD (ΦPSII) can also be calculated and can serve as an important supplemental tool to the dark-adapted F_v/F_m quantum efficiency test. It provides information on photosynthetic capacity at specific light levels and indicates the stress level of a specific plant, relative to an unstressed equivalent or to a hypothetical plant with no photosynthetic limitations. Stress factors can be interactive and water stress can be translated into light stress. This increase in light stress can be assessed by a light limitation test. The so-called light limitation of a sample plant is the increased level of PPFD needed above the light intensity that an unstressed plant needs to achieve a corresponding rate of electron transport (J).

Multiplication of the specific light intensity and the quantum efficiency of photosystem II at that light intensity (ΦPSII) yield the relative J. This parameter can be used to approximate photosynthesis levels since an excellent correlation between J and quantum yield of CO_2 fixation exists.

Water deficit and chlorophyll fluorescence

Although chlorophyll fluorescence has been widely used as an indicator of water deficit stress in some species (e.g. grapes; Flexas *et al.*, 1999), it is not always effective as a stress quantifier in others. This might be because the photosynthetic light-harvesting apparatus is less sensitive to water stress than previously thought (Demmig-Adams *et al.*, 1989; Flexas *et al.*, 1999). In some cases the quantum efficiency of photosystem II as well as the quantum efficiency of oxygen evolution is not affected by dehydration until a very high level of water deficit occurs. This has been found in certain herbaceous plants, i.e. *Phaseolus vulgaris* (Cornic *et al.*, 1989) and *Spinacia oleracea* (Kaiser, 1987), in which a leaf water loss of 30% was needed before photosynthetic capacity decreased. In some woody evergreen subtropical species water stress has no influence on the photosynthetic capacity e.g. cashew (Blaikie and Chacko, 1998) or citrus (Lloyd *et al.* 1987) and other sub-tropical trees (Liang *et al.*, 1997). This does not mean photosynthesis is unaffected by water stress, because gas exchange measurements do indicate decreases thereof, due to increases in stomatal limitation (Flore and Lakso, 1989). A more fitting explanation would be that the plants are very well adapted to high light conditions under water deficit and can dissipate excess light very effectively, even under conditions of low photosynthetic light use. Photodamage is prevented by very effective systems of photoprotection (Demmig-Adams and Adams, 1992) in situations where photosynthetic light use is low (Chaves, 1991). These include photo-oxidation, CO_2-recycling and the xanthophyll cycle.

Photooxidation is the consequence of the reduction of O_2, which is an effective acceptor of photosynthetic energy (Cornic *et al.*, 1989). Firstly, direct O_2 reduction is mediated through the electron transport chains and other components of both photosystems II and I (Foyer *et al.*, 1994). Secondly, there is the photorespiratory pathway (Salisbury and Ross, 1993) that starts with ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco - the first enzyme of the Calvin CO_2-fixation cycle) catalysing the oxygenation of ribulose-1,5-bisphosphate, rather than its carboxylation. The affinity of Rubisco for both CO_2 and O_2 has important implications. As the stomata close in response to water deficit or any other factor, the intercellular CO_2 concentration is reduced albeit only after a while. This would shift Rubisco's affinity even more to O_2 (Salisbury and Ross, 1993),

as would conditions of high temperatures (Syvertsen and Lloyd, 1994). In *Citrus unshiu*, both the decline in net photosynthesis rate and quantum efficiency in the late morning have been attributed to an increase in photorespiration (Xu and Shen, 1997). Photorespiration may protect the photosynthetic apparatus against photoinhibition when stomatal conductance and CO_2 assimilation decline in drying cashew trees, by maintaining electron flow (Blaikie and Chacko, 1998). Foyer *et al.* (1994) considered such protection as the principle role of photorespiration. The increase in photorespiration could be a response to increased temperatures, high light or the decline in C_i due to the midday closure of stomata.

The recycling of CO_2 (Stuhlfauth *et al.*, 1990) also protects the photosynthetic apparatus. Internally-produced CO_2 (i.e. by photorespiration and dark respiration) is used to consume excess light energy by its re-fixation. During mild water stress stomatal aperture is usually more reduced than the Calvin cycle activity, because CO_2 is recycled, and in this way the light energy trapped by the light harvesting complexes can still be channelled from them. The reaction centres also quench excess light by photosystem II electron cycling and the antennae by means of the xanthophyll cycle (Foyer *et al.*, 1994). Demmig-Adams and Adams (1990; 1992) have described the mechanism and properties as well as the importance of the xanthophyll cycle.

Excess light energy might damage the photosynthetic apparatus if it is not dissipated. Such excess may occur under normal conditions with high light intensities, or under unfavourable conditions that reduce photosynthesis and indirectly reduce light dissipation. Most of the photoprotective measures do, however, come at a cost, of which the most notable is the evolution of radical oxygen species. These compounds present a great risk to the photosynthetic apparatus, cell membranes and other vital components of living cells (Foyer *et al.* 1994).

In conclusion, chlorophyll a fluorescence is not a very good indicator of water stress in evergreen sub-tropical fruit crops like citrus. No effect of soil water deficit on chlorophyll a fluorescence was found in citrus (Syvertsen and Lloyd, 1994). Veste *et al.* (2000) confirmed this, showing citrus plants under extreme drought conditions without reductions in their photosystem II quantum efficiency. This has been attributed to high

levels of photorespiration, especially during the midday stomatal closure (Veste *et al.*, 2000). Citrus plants are well-adapted to high light conditions and do not show secondary light stress in response to water deficits.

4 CONCLUSIONS

Woody perennials

Water deficit stress is the biggest factor limiting crop yield (Hsiao, 1973) and it affects vegetative growth, tree size as well as fruit quality in citrus (Fereres et al., 1979; Peng and Rabe, 1995) and most other plants (Begg and Turner, 1976). Trees are irrigated to enhance productivity (Fereres et al., 1979; Kriedemann and Barrs, 1981). Irrigation increases fruit set, fruit size and fruit quality in citrus and numerous other fruit crops (Kriedemann and Barrs, 1981). Plant and specifically fruit growth is mediated by cell turgor and these processes are the first to decline under conditions of water deficit stress (Hsiao, 1973). Plants growing under unstressed conditions will have high pre-dawn water potentials (implying optimal cell turgor throughout the plant) and photosynthetic rates not limited by water deficits, and will therefore have high growth rates. Mantell (1977) showed that the photosynthetic water use efficiency in citrus is higher than some other C_3 species because of its high stomatal and mesophyll resistances. Citrus trees show very low stomatal response (Syvertsen and Lloyd, 1994). Bielorai and Mendel (1969) and Sinclair and Allen (1982) found no increase in photosynthetic water use efficiency in citrus under mild water deficit, because stomatal conductance is not decreased low enough to decrease transpiration. Low leaf conductance to CO_2 and high hydraulic resistances, combined with high levels of photorespiration, predispose these plants to low photosynthetic rates. These low rates can be reduced even further under severe water deficit stress.

Blaikie and Chacko (1998) and Syvertsen and Lloyd (1994), working on cashew and citrus, respectively, and others (cited by Chaves, 1991) have recently published data showing that many woody plants are very well-adapted to drought. Although reductions in gas exchange and sap flow develop with water stress, their photosystems are

resistant to photodamage and they show rapid recovery of photosynthesis, sap flow and Fv/Fm after re-watering. It has been proposed that photorespiration protects citrus (Chen and Zhang, 1994) and cashew plants (Blaikie and Chacko, 1998) against photoinhibition.

Midday depression

Many crop plants, including evergreen subtropical trees, experience midday depressions of photosynthesis and resulting decreases in plant productivity. High midday temperatures also result in high rates of photorespiration, which was shown to play a role in the midday depression of *Citrus unshiu* (Chen and Zhang, 1994). The specific mechanism of midday photosynthetic reduction is not always clear, but it may represent a strategy to cope with environmental stresses (Xu and Shen, 1997). Under strong light and atmospheric drought conditions, excess water loss can be avoided by stomatal closure and by downregulation of photosynthesis and photochemical efficiency. Photodamage of the photosynthetic apparatus can thus be limited.

Alleviation of the midday depression could increase crop productivity. Stomatal conductance and photosynthesis were increased with mist irrigation around midday in many experiments cited by Xu and Shen (1997). Brakke (1989) reported that a midday depression of photosynthesis was not observed in citrus plants when soil water was easily available in a controlled atmosphere environment. Such interpretations should be applied with care, specifically when comparisons are drawn to field situations in subtropical climates. It has been proposed that irrigation during the daylight hours would decrease the midday depression in citrus (Woods, 1999), but this has not been confirmed in the scientific literature. The role of ABA in the control of the midday depression should be further elucidated.

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ARTICLE 1 - COMPARISON OF STOMATAL CONDUCTANCE, SAP FLOW, XYLEM WATER POTENTIAL AND CHLOROPHYLL A FLUORESCENCE BETWEEN DAILY DAYLIGHT FERTIGATED AND CONVENTIONALLY IRRIGATED CITRUS TREES

ABSTRACT

In daily daylight fertigation (DDF) systems, nutrient and pH balanced fertigation solutions a re a pplied d aily v ia drip irrigation during the daylight hours. 'Midknight' Valencia (*Citrus sinensis* Osb.) trees at Addo and Citrusdal, South Africa, under DDF and conventional micro-jet irrigation with broadcast fertilisation regimes were monitored, comparing the relative stress levels as manifested in sap flow, stomatal conductance, xylem water potential and chlorophyll a fluorescence. We observed no difference in sap flow between the treatments. Stomatal conductance in the DDF treatment was found to be higher relative to the conventional micro-jet irrigation treatment in the early morning in one trial, but during the late morning and afternoon the values were similar. Pre-dawn xylem water potential was higher in the DDF treatment at Addo, although both treatments showed relatively unstressed conditions. We conclude that plants under DDF regimes perform better than plants under conventional micro-jet irrigation during the early morning, because soil water conditions are optimal. Later in the day, however, this difference is not maintained, emphasising that in woody evergreen crops like citrus, the atmospheric conditions, specifically vapour pressure differences, determine transpiration and sap flow during daytime, rather than the soil water potential. The midday depression of stomatal conductance was not alleviated by daily fertigation. Chlorophyll a fluorescence did not show any treatment effects, affirming citrus' high level of resistance to drought-induced light stress. The data suggests that producers should optimise morning conditions, e.g. by early irrigation, so that plants can perform optimally at this time of highest physiological activity.

INTRODUCTION

The use of open hydroponic systems (OHS) in citrus orchard management recently became an option for producers. As a sensitive nutrient and moisture management system, OHS exercises a high degree of control over the development of the crop (Stassen *et al.*, 1999). Specific water and nutritional requirements for specific phenological phases of the plants are incrementally applied via daily fertigation. The nutrient solution being carefully balanced and pH as well as EC controlled, through a drip irrigation system.

Some controversy exists concerning the ascribed benefits of the use of OHS rather than conventional micro- and drip-irrigation systems. Stassen *et al.* (1999) compares OHS to pot experiments in which crop development can be controlled to a large degree. Since the field system is not hydroponics as such, it is preferable to refer to it as daily daylight fertigation (DDF). Daily daylight fertigation involves drip fertigation (fertilisation applied with and by irrigation) of the plants, on a daily basis, during the daylight hours. Sensitive crop management can be achieved in such a system, because the nutrient medium, rather than the soil, creates the environment on which root growth is dependent (Stassen *et. al.*, 1999). This makes open hydroponic or daily daylight fertigation systems particularly important tools in situations with low quality soils and poor water quality.

Citrus and other fruit trees respond strongly to environmental conditions, and especially so when stressed for water. Tree water potential usually decreases as soil water stress develops and this may result in impaired physiological activity. Concurrently, a midday depression in stomatal conductance and photosynthesis may develop, when conditions of high light, temperatures and vapour pressure deficits prevail (Xu and Shen, 1997). In some daily daylight fertigation systems, the daily water requirements are divided into portions, each pulse given at a specific increase in vapour pressure deficit, thereby attempting to compensate for the increases in atmospheric stress. Root irrigation, however, does not reduce atmospheric stress. In conventional irrigation systems, trees are usually irrigated only with regards to the soil water status.

Water deficit stress' influence on physiological activity can be assessed by various techniques. Eastham and Gray (1998) used sap flow to measure transpiration differences between stressed and unstressed grapevines, as did Blaikie and Chacko (1998) in cashew trees. Both groups of researchers postulated a possible role for sap flow monitoring in irrigation scheduling. Predawn xylem water potential is a useful measure of water availability to plants, since it integrates the soil water potential over the root zone of the plant (Schulze & Hall, 1982) and decreases with a decrease in soil water potential (Tardieu & Simonneau, 1998). In many plants, stomatal conductance is correlated with xylem abscisic acid concentration and in turn with the soil water content (Zhang & Davies, 1990). Dry soil could reduce photosynthesis via reductions in stomatal aperture (Liang *et al.*, 1997) and this decline in stomatal conductance may fully account for the water deficit-related reduction of photosynthesis (Downton *et al.*, 1988). Chlorophyll a fluorescence techniques are considered a sensitive tool which may provide early signs of developing stress (Maxwell & Johnson, 2000) since fluorescence will be higher in plants experiencing a higher level of stress.

There is a need to evaluate the efficacy of the new technology relative to conventional irrigation systems. Standard horticultural evaluation of orchard management practices is very time consuming. We opted for physiological studies comparing plant stress levels in an attempt to quantify plant performance under each irrigation system. These studies may provide quicker answers than conventional evaluation and the techniques could be used to timeously predict plant performance under the differing management systems.

In this study, citrus trees under daily daylight fertigation and conventional microjet and drip-irrigated regimes were monitored to establish plant stress levels as indicated by sap flow, xylem water potential, stomatal conductance and chlorophyll a fluorescence.

MATERIALS AND METHODS

Site description, and plant material

Two experimental sites were used: (i) Addo in the Eastern Cape Province, having rainfall peaks in spring and summer (dry subtropical climate) and a silt loam (Oakleaf form) soil. The 'Midknight' Valencia trees were planted in 1991 on rough lemon rootstock, in a north/south row direction, and at 6.6 x 4.0 m tree spacing. The trees are part of a fertigation trial and the irrigation treatments are arranged in a randomised block design with four replicates and five trees per replicate. One of the three centre trees in each replicate was used for the measurements. Trees under DDF were only recently (November 1998) converted to drip, from growing under a micro-jet-irrigated regime.

No statistically laid out irrigation trial sites were available in the Western Cape Province, but to compare the data collected in Addo to another environment, data was also collected in (ii) Citrusdal (South Western Cape), with predominantly winter rainfall (dry Mediterranean climate) and sandy soil (< 5% clay; Kroonstad form). Four trees in each of two adjacent blocks with rows in the north/south direction were chosen randomly. Irrigation methods and plant material in the two blocks differed. The one block has 'Midknight' Valencia trees, planted in 1997 on 'Troyer' citrange rootstock, at a 5.0 x 2.0 m spacing, growing from planting under a daily daylight fertigated regime. The other orchard has micro-irrigated 'Nules' Clementine trees planted in 1995 on 'Troyer' citrange rootstock, at a 5.0 x 2.0 m spacing.

Irrigation methods

The irrigation treatments were micro-jet-irrigated and broadcast-fertilised; and daily daylight fertigation (DDF). In one experiment done at Addo, stomatal conductance of a conventional drip and weekly fertigated treatment was compared to that of the conventional and DDF treatments. Specific fertilizer applications are not described in detail.

Micro-jet irrigation with broadcast fertilisation: Irrigation scheduling was done according to neutron moisture probe measurements of the soil water content (irrigation to field capacity after depletion of 40 % of the easily available soil water), and macro element fertiliser was broadcast according to conventional fertilisation scheduling.

Daily daylight fertigation: A balanced nutrient solution (as prescribed by OHS Africa) containing macro and micro elements at a specific pH was applied daily as determined by soil water content measurements (Addo – neutron probe; Citrusdal – EnviroSCAN capacitance probe), keeping the rooting volume as close as possible to field capacity. The nutrient solution was applied either as a single pulse (Addo) or divided into three to five pulses during the day (Citrusdal).

Conventional drip fertigation: The daily irrigation requirement as determined by neutron moisture probe measurements was applied in one pulse and fertigation of a macro element nutrient solution was applied once a week.

Measurements

Sap flow rates were measured during November 1999 (Table 1A) at Addo. A sap flow measuring system utilising a heat balance equation (Dynagage, Dynamax, Houston, USA) was used. Four sensors monitored two trees of each treatment. Branches chosen were usually northwest facing (the warm side) at 1.0 to 1.5 m above ground level. Total leaf area of the measured branches was determined with a leaf area meter (LAI3200, LiCor, Lincoln, USA). The leaf area data was used to express sap flow rates on a leaf area basis.

Stomatal conductances were measured diurnally during August and November 1999 (Table 1A) and February 2000 (Table 1B) in Addo with a porometer (EGM 2.0, PP Systems, Hertfordshire, UK). Five sun-exposed leaves, of similar size, from the most recently-hardened flush, were chosen around the north-western side of each of three replicate trees per treatment. Measuring started as soon as all visible moisture evaporated from the foliage and continued into the late afternoon.

Water potential: Xylem water potential was monitored with a pressure chamber (PCI 600, PMS, Corvallis, Oregon, USA), as described by Koide *et. al* (1989), during November 1999 (Table 1A) and February 2000 (Table 1B), at Addo, and during March and December 2000 (Table 1C) at Citrusdal, usually as pre-dawn measurements. It was assumed (i) that shoots were more accurate indicators of xylem water potential than leaves and (ii) that shoots equilibrate to the xylem water potential of the tree during the night and that accurate approximation of xylem water potential could be determined by measuring the values in the immediate hours before dawn. Two or three shoots, from the most recent hardened flush, were randomly selected around each tree about 1.5 m above ground level, cut and immediately measured.

Fluorescence characteristics were measured at Addo during November 1999 (Table 1A) and February 2000 (Table 1B) with a modulated fluorescence measuring system (FMS2, Hansatech, Norfolk, UK). The instrument provided photosynthetic photon flux density (PPFD) at 94 and 1220 μmol m^{-2} s^{-1}. The fluorescence characteristics analysed were as follows (defined by van Kooten and Snel (1990) and Bolhàr-Nordenkampf and Öquist (1993)): the maximum quantum efficiency of photosystem II (Fv/Fm); the antennae efficiency of photosystem II (Fv'/Fm'); the quantum efficiency of photosystem II (ΦPSII); the photochemical quenching coefficient (qP) and the non-photochemical quenching coefficient (qNP). Three sun-exposed leaves of similar size, from the most recently-hardened flush, were measured per tree.

Separate but similar leaves (in terms of size, appearance, age and position on the tree) were used for the measurement stomatal conductance, leaf water potential and chlorophyll a fluorescence.

The Agricultural Research Council (Infruitec, Stellenbosch, South Africa) provided the weather data for Addo and Citrusdal. Daily average temperatures, minimum and maximum temperatures, as well as wind speed, light hours and precipitation was provided. The general linear model procedure (SAS Inc., 1990) was used in the analysis of variance. Data was considered significant at $P \leq 0.05$.

Concurrently to this study, 'Midknight' Valencia root development under different irrigation regimes was researched (Pijl, 2001). Separate profile pits being 2 m wide and in direction with as well as against tree rows, were dug directly underneath drippers or micro-jets (depending on the irrigation regime), in conventionally micro-jet-irrigated, conventionally drip irrigated and daily daylight fertigated irrigation regimes, at Addo and at Citrusdal. The soil profile was divided in 100 cm^2 square grid blocks and active feeder roots were counted and plotted.

RESULTS AND DISCUSSION

August and November 1999, Addo

Weather conditions as well as the dates on which specific measurements were taken are provided in Table 1A. Sap flow was marginally lower in the daily daylight fertigation treatment than in the micro-jet irrigated treatment (Figure 1), however the general trend was similar.

This lower level of sap flow in the daily daylight fertigation treatment is not understood and could be due to experimental error due to the lack of sufficient replications.

Measurements of stomatal conductance during winter (27 August 1999) showed a significant time effect (Figure 2). The stomatal conductances were high in the early morning (134 mmol m^{-2} s^{-1}) and moderately low during mid-morning (around 30 - 40 mmol m^{-2} s^{-1}). A secondary peak around midday (concurrent with a temporary drop in leaf temperature to about 31 °C) was followed by very low levels around 14h00 (7 mmol m^{-2} s^{-1}; leaf temperature = 35 °C). Recovery to moderately low levels occurred during mid-afternoon (32 mmol m^{-2} s^{-1}) occurred. The time*treatment interaction was non-significant.

During summer, stomatal conductance showed a significant time*treatment interaction (Figure 3). Early in the morning the daily daylight fertigation treatment

had a high stomatal conductance (105 mmol m^{-2} s^{-1}), whilst the micro-jet-irrigation treatment showed a low (41 mmol m^{-2} s^{-1}) conductance. Thereafter, stomatal conductance of the micro-jet-irrigation treatment rose to levels similar to those of the daily daylight fertigation treatment. At about 09h00 a rapid drop in stomatal conductance (to around 30 - 50 mmol m^{-2} s^{-1}) occurred in both treatments. A small, but significant difference between the treatments was observed during the afternoon.

Stomatal conductance values and the diurnal trend of conductance were similar between the daily daylight fertigation and conventional micro-jet irrigation treatments. The daily daylight fertigation treatment had significantly higher stomatal conductances in the early morning (in November 1999), compared to the conventional micro-jet-irrigation treatment. A root zone at field capacity could account for this observation, since the plant water potential would equilibrate to it during the night and facilitate high stomatal conductances and transpiration as soon as the day starts heating up.

It could be that the smaller root zone of the daily daylight fertigation treatment would be under stress to provide enough water to meet the atmospheric demand later in the day. The conventional micro-jet irrigation treatment compared to the daily daylight fertigation treatment showed higher stomatal conductance values in the mid-afternoon (August and November 1999). This could be due to the more extensive root systems of the conventional micro-jet irrigated trees. All trees showed a mid-morning decrease in stomatal conductance.

Xylem water potential was measured pre-dawn and in the late afternoon on 23 November 1999. The time*treatment interaction was significant, with the daily daylight fertigation treatment having a slightly higher pre-dawn xylem water potential than the micro-jet-irrigation treatment, but in the late afternoon there were no significant differences between the treatments (Figure 4).

The higher pre-dawn xylem water potential of the daily daylight fertigation treatment could be due to a high soil water potential, providing unstressed root conditions to which the plant water potential equilibrates during the night. Such unstressed root conditions could probably explain the higher levels of stomatal conductance during

the early morning in the daily daylight fertigation treatment as well. This difference in xylem water potential disappeared during the afternoon, probably because the trees regulate their stomatal conductance to provide an upper limit to water loss via transpiration (Bond and Kavanagh, 1999). Under these moderate levels of water stress, such a limit could account for the similar levels of xylem water potential during the afternoon. Atmospheric influences override soil water status very soon after sunrise.

Chlorophyll fluorescence characteristics were measured on 21 November 1999 (Table 2). Measurements were made from mid-morning to mid-afternoon. Dark-adapted Fv/Fm readings were not significantly different between the treatments. At PPFD = 94 μmol m^{-2} s^{-1}, qP showed a time effect, being lower later during the day; the other fluorescence characteristics (i.e., Fv'/Fm'; ϕPSII and qNP) did not show this trend. There were no treatment differences regarding chlorophyll fluorescence characteristics at PPFD = 94 or 1220 μmol m^{-2} s^{-1} and a time effect was not observed at PPFD = 1220 μmol m^{-2} s^{-1}.

It has been established that citrus trees under mild water deficit conditions do not show photo-inhibitory stress (Syvertsen and Lloyd, 1994) and no significant differences in fluorescence characteristics were detected between the treatments. The time effect observed in qP could be expected since a decrease in air temperature and PPFD would result in a decreased rate of photochemistry. The midday depression of photosynthesis would also be expected to contribute to this reduced level of photochemical quenching in the afternoon hours.

February 2000, Addo

Weather conditions as well as the dates on which specific measurements were taken can be seen in Table 1B. On 11 February, significant time and treatment main effects were showed for stomatal conductance (Figure 5). The trees receiving daily daylight fertigation had significantly higher stomatal conductances than the trees receiving micro-jet irrigation. Stomatal conductance was relatively high (>40 mmol m^{-}

^{2} s^{-1}) and stable for most of the morning, but lower levels were observed during mid-morning and a general decline in conductance occurred after midday.

Stomatal conductance was again measured on 18 February, this time two days after the last irrigation of the micro-jet irrigation treatment. Trees receiving conventional drip fertigation were also measured. A significant time*treatment interaction was observed (Figure 6). No differences were observed early in the morning, but at mid-morning, trees receiving daily daylight fertigation and conventional micro-jet irrigation had stomatal conductances double those of trees receiving conventional drip-irrigation. The conductances in all the treatments declined rapidly after mid-morning.

The influence of vapour pressure deficit on stomatal conductance can clearly be seen in the data from these two days. The lower total evaporation (see Table 1B), implying therefore a higher relative humidity on 11 February, facilitated higher average stomatal conductance rates (51 mmol m^{-2} s^{-1}) than on 18 February (37 mmol m^{-2} s^{-1}), w hen h igher t otal e vaporation a nd l ower r elative h umidity p revailed. The evaporative demand as well as leaf temperature was lower on 11 February than on 18 February; therefore, the transpiration rates on these days differed as well, being 1.08 and 1.46 mmol m^{-2} s^{-1}, respectively. The higher level of water loss on 18 February resulted in stronger stomatal control relative to that on 11 February. Days with lower vapour pressure deficits usually lead to less pronounced midday depressions in photosynthesis and stomatal conductance (Marler and Mickelbart, 1998; Xu and Shen, 1997). This can also be seen in our data, with a severe midday depression absent on 11 February, but present on 18 February. Daily daylight fertigation did not differ from the conventional systems in this respect.

The treatment differences on 11 February (Figure 5) can be explained by more favourable soil water conditions under daily daylight fertigation, which played a larger role in the absence of severe atmospheric demand. The conventional micro-jet irrigation t reatment r eceived i ts last irrigation three days prior to the measurement date, with very high vapour pressure deficits and temperatures prevailing during the following three days (31.0, 34.5 and 39.5 °C respectively for 8, 9 and 10 February). Although conductances were lower in the conventional micro-jet irrigated plants, the

general level of stomatal conductance in these plants indicated relatively unstressed conditions on 11 February.

The mid-morning maximum stomatal conductance on 18 February (Figure 6) did not differ between the daily daylight fertigation and conventional micro-jet irrigation treatments (average being 90 mmol m^{-2} s^{-1}), but were significantly lower in the conventional drip irrigation treatment (52 mmol m^{-2} s^{-1}). The reason for this low level in the conventional drip irrigated treatment is not known. Severe midday depressions in stomatal conductance were observed in all three treatments, but levels were slightly lower in the conventional irrigation regimes than in the daily daylight fertigation treatment during late morning but not thereafter. A mid-afternoon secondary peak in stomatal conductance occurred in the conventional micro-jet irrigated treatment but not in the two drip-irrigated treatments. The latter did not recover from the midday depression. This peak could be explained by a more extensive root system and larger rooting volume under the micro-jet irrigation regime, possibly resulting in higher hydraulic conductivity and better water delivery to the canopy, under conditions of high evaporative demand.

Pre-dawn xylem water potential was measured on 11, 12, 14 and 15 February, starting 3 days after the last irrigation in the micro-jet-irrigation treatment. A significant treatment effect was found, with the daily daylight fertigation treatment having higher pre-dawn xylem water potential than the micro-jet irrigation treatment (Figure 7).

The increase in pre-dawn xylem water potential in the conventional micro-jet irrigation treatment, between 11 and 12 February, can be ascribed to rainfall (5.5 mm). A similar effect of the rain was not observed in the daily daylight fertigation treatment, because the soil water potential was already optimal in this treatment. The significantly higher pre-dawn xylem water potential in the daily daylight fertigation treatment, relative to the conventional micro-jet irrigation treatment could be explained by more favourable water potential levels in the rooting volume of the plants under the daily daylight fertigation regime. Pre-dawn xylem water potentials were high in both treatments and indicated relatively unstressed conditions.

Chlorophyll fluorescence characteristics were measured from mid-afternoon to late afternoon (Table 3) on 16 February. Dark-adapted Fv/Fm readings did not differ significantly between the treatments. At both light intensities no significant differences were found between the irrigation treatments or between time periods for any of the chlorophyll fluorescence characteristics, except Fv'/Fm', which was higher in the late afternoon, compared to the earlier afternoon.

Treatment effects in afternoon chlorophyll fluorescence characteristics were absent on 16 February, as in November 1999. The increased level of Fv'/Fm' in the late afternoon compared to the mid-afternoon, under the high light treatment (PPFD = 1220 μmol m^{-2} s^{-1}) could be explained by a reduction in leaf temperature and vapour pressure deficit to more favourable levels, which would enhance the potential photon harvesting of the antennae.

March and December 2000, Citrusdal

Weather conditions as well as the dates on which the measurements were taken can be seen in Table 1C. A significant time*treatment interaction was observed in the diurnal xylem water potential. High xylem water potentials were measured at pre-dawn, early morning and in the late afternoon, with no differences between the treatments at these times (Figure 8). At midday the xylem water potential decreased, with the daily daylight fertigation treatment having a significantly higher xylem water potential (-1.3 MPa) than the micro-jet irrigation treatment (-1.7 MPa). These levels of midday xylem water potential are not unusual in fruit trees and leaves recover quickly later in the day.

The significantly higher xylem water potential at midday and in the mid-afternoon, under daily daylight fertigation could indicate a lower level of stress in these plants, at this time of day, compared to that of the plants under the conventional micro-jet irrigation regime. No significant differences in pre-dawn xylem water potential, between the treatments or over a period of one week, were observed during December 2000 (Figure 9), and the levels were very high. This indicates that there

were no differences in water deficit stress experienced by trees of different treatments.

Root development under the daily daylight fertigation regime was excellent, but limited to a strip adjacent to and between the drip onions. Roots of plants under the micro-jet irrigation treatment were well developed, but much more scattered compared to the daily daylight fertigation treatment and distributed over the whole soil profile (Pijl, 2001).

CONCLUSION

The sap flow data indicated a marginally higher level of transpiration in the conventional micro-jet irrigated plants than what was observed in the daily daylight fertigated plants. The observation could be due to experimental error from lack of replications. These trees are still less stressed than conventionally-irrigated trees judging from xylem water potential and stomatal conductance measurements.

The pre-dawn xylem water potential and stomatal conductance data indicate a trend that plants under daily daylight fertigation performed more optimally in the early morning compared to plants under conventional micro-jet irrigation. In the afternoon, however, the plants were also under stress, showing midday depressions in stomatal conductance and little recovery in the late afternoon. Later in the day, plants under conventional micro-jet irrigation generally had similar or higher stomatal conductances than plants under daily daylight fertigation, and these were sometimes accompanied by higher xylem water potentials.

We conclude that plants under a daily daylight fertigation regime have excellent soil water conditions in their rooting volume, and therefore experience negligible baseline levels of stress. The limited root volume does not, however, supply water to the same extent as a larger root volume in response to high atmospheric demand. Therefore, the trees experience midday depressions in stomatal conductance, similar to trees under conventional regimes. It appears as if a larger rooting volume enhances recovery from the midday depression.

Generally, trees under a daily daylight fertigation regime experience lower baseline stress levels in comparison to conventional irrigation regimes. They are not, however, better buffered against short-term daytime effects of high atmospheric demand during the afternoon. It is recommended that producers optimise the morning hours, by early irrigation, so that plants can function optimally, whilst environmental conditions are most favourable, resulting in the highest physiological activity.

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TABLE 1A. Weather conditions and measurements taken during August and November 1999 at Addo.

Parameters	Aug 26	Aug 27	Aug 28	Nov 20	Nov 21	Nov 22	Nov 23	Nov 24
Max Temp (°C)	23.6	29.4	31.2	29.4	28.6	29.6	20.7	33.6
Min Temp (°C)	7.1	8.1	8.5	12.5	17.6	14.4	18	16
Ave Temp (°C)	15.4	18.8	19.9	21	23.1	22	19.4	24.8
Precipitation (mm)	0	0	0	0	0	0	0	1.5
Light Hours (hrs)	9.0	9.9	7.6	7.4	9.9	11.4	8.7	
Sap flow						Sap flow		
Stomatal conductance		g_s					g_s	
Water potential							Pre-dawn	
Water potential							Midday	
Chlorophyll fluorescence				Fluorescence				

TABLE 1B. Weather conditions and measurements taken during February 2000 at Addo.

Parametrs	Feb 10	Feb 11	Feb 12	Feb 13	Feb 14	Feb 15	Feb 16	Feb 17	Feb 18
Max Temp (°C)	39.5	31	29	21.5	31.5	30	27	34.5	41.5
Min Temp (°C)	18	19	20	20.5	17	17	12.5	11.5	14.5
Ave Temp (°C)	28.8	25	24.5	21.0	24.3	23.5	19.8	23	28
Precipitation (mm)	0	0	5.5	0.4	0	0	0	0	0
Evaporation (mm)	6.5	3	3	1.4	5	7.5	5.5	12.5	6.5
Light Hours (hrs)	12.0	2.7	2.4	0.6	11.5	9.9	11.7	12.0	8.3
Stomatal conductance		g_s							g_s
Water potential		Pre-dawn	Pre-dawn		Pre-dawn	Pre-dawn			
Chlorophyll fluorescence							Fluorescence		

TABLE 1C. Weather conditions and measurements taken during March and December 2000 at Citrusdal.

Parametrs	Mrc 18	Dec 23	Dec 24	Dec 25	Dec 26	Dec 27	Dec 28	Dec 30	Dec 31
Max Temp (°C)	33.70	34.3	28.96	30.04	34.09	30.44	29.06	29.36	29.96
Min Temp (°C)	14.34	17.9	15.82	12.48	13.26	15.14	15.34	13.36	14.34
Ave Temp (°C)	24.02	25.42	21.89	21.16	24.36	22.61	22.02	21.49	20.85
Precipitation (mm)	0	0	0	0	0	0	0	0	1.8
Radiation (MJ/m^2)	20.68	30.03	21.88	30.05	30.34	29.2	26.15	29.62	28.36
Water potential	Diurnal		Pre-dawn		Pre-dawn				Pre-dawn

TABLE 2. Chlorophyll fluorescence characteristics (dark adapted and PAR = 94 and 1220 μmol m^{-2} s^{-1}) measured on 21 November 1999 in Addo. Measurements were made at four time periods during the day and treatments were daily daylight fertigation (irrigated daily) and conventional micro-jet irrigation (trees irrigated on 20 November).

21 November 1999		94 μmol m^{-2} s^{-1}				1220 μmol m^{-2} s^{-1}			
Period	Fv/Fm	Fv'/Fm'	ϕPSII	qP	qNP	Fv'/Fm'	ϕPSII	qP	qNP
11h30 - 12h00	0.792 ± 0.013	0.678 ± 0.025	0.642 ± 0.025	0.946**a** ± 0.006	0.501 ± 0.056	0.352 ± 0.016	0.202 ± 0.032	0.576 ± 0.032	0.923 ± 0.040
12h00 - 12h45	0.819 ± 0.013	0.685 ± 0.025	0.649 ± 0.025	0.946**a** ± 0.006	0.538 ± 0.056	0.361 ± 0.016	0.190 ± 0.032	0.523 ± 0.032	0.925 ± 0.040
13h00 - 13h30	0.823 ± 0.013	0.630 ± 0.025	0.595 ± 0.025	0.944**a** ± 0.006	0.626 ± 0.056	0.362 ± 0.016	0.229 ± 0.032	0.495 ± 0.036	0.895 ± 0.040
16:30 - 17h00	0.829 ± 0.013	0.705 ± 0.025	0.646 ± 0.025	0.915**b** ± 0.006	0.451 ± 0.063	0.391 ± 0.016	0.177 ± 0.032	0.454 ± 0.032	0.848 ± 0.040
DDF	0.820 ± 0.009	0.689 ± 0.018	0.646 ± 0.018	0.938 ± 0.004	0.486 ± 0.040	0.375 ± 0.011	0.217 ± 0.023	0.514 ± 0.024	0.901 ± 0.028
Micro	0.812 ± 0.009	0.660 ± 0.018	0.619 ± 0.018	0.938 ± 0.004	0.572 ± 0.042	0.358 ± 0.011	0.182 ± 0.023	0.510 ± 0.023	0.894 ± 0.028
Source	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F
Time	0.2471	0.2219	0.3988	*0.0071*	0.2214	0.3669	0.7122	0.0982	0.5045
Trt	0.5762	0.2552	0.2875	0.9475	0.1457	0.3192	0.2930	0.8963	0.8651
Time*Trt	0.2528	0.4528	0.3686	0.0647	0.8065	0.7858	0.5171	0.8438	0.3206

TABLE 3. Chlorophyll fluorescence characteristics (dark-adapted, PAR = 94 and 1220 μmol m^{-2} s^{-1}) measured on 16 February 2000 in Addo. Measurements were made during three time periods and treatments were daily daylight fertigation (irrigated daily) and conventional micro-jet irrigation (trees irrigated on this day).

| 16 February 2000		94 μmol m^{-2} s^{-1}					1220 μmol m^{-2} s^{-1}			
	Fv/Fm	Fv'/Fm'	ϕPSII	qP	qNP	Fv'/Fm'	ϕPSII	qP	qNP	
15h20 -	0.801	0.678	0.637	0.939	0.433	0.369**b**	0.154	0.418	0.879	
16h00	± 0.013	± 0.015	± 0.020	± 0.013	± 0.056	± 0.016	± 0.015	± 0.035	± 0.015	
16h00 -	0.818	0.682	0.639	0.936	0.438	0.375**b**	0.171	0.456	0.891	
16h30	± 0.013	± 0.015	± 0.020	± 0.013	± 0.056	± 0.016	± 0.015	± 0.035	± 0.015	
17h30 -	0.837	0.717	0.646	0.900	0.434	0.443**a**	0.154	0.352	0.844	
18h10	± 0.013	± 0.015	± 0.020	± 0.013	± 0.056	± 0.016	± 0.015	± 0.035	± 0.015	
DDF	0.807	0.681	0.621	0.912	0.448	0.400	0.151	0.386	0.858	
	± 0.011	± 0.012	± 0.016	± 0.011	± 0.045	± 0.013	± 0.012	± 0.029	± 0.013	
Micro	0.831	0.704	0.660	0.937	0.423	0.391	0.169	0.432	0.884	
	± 0.011	± 0.012	± 0.016	± 0.011	± 0.045	± 0.013	± 0.012	± 0.029	± 0.013	
Source	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	
Time	0.1918	0.1775	0.9505	0.1001	0.9982	*0.0094*	0.6632	0.1474	0.1280	
Trt	0.1392	0.2187	0.1136	0.1234	0.7031	0.6601	0.3257	0.2832	0.1650	
Time*Trt	0.3422	0.6042	0.8108	0.4614	0.7431	0.4683	0.7519	0.7600	0.7369	
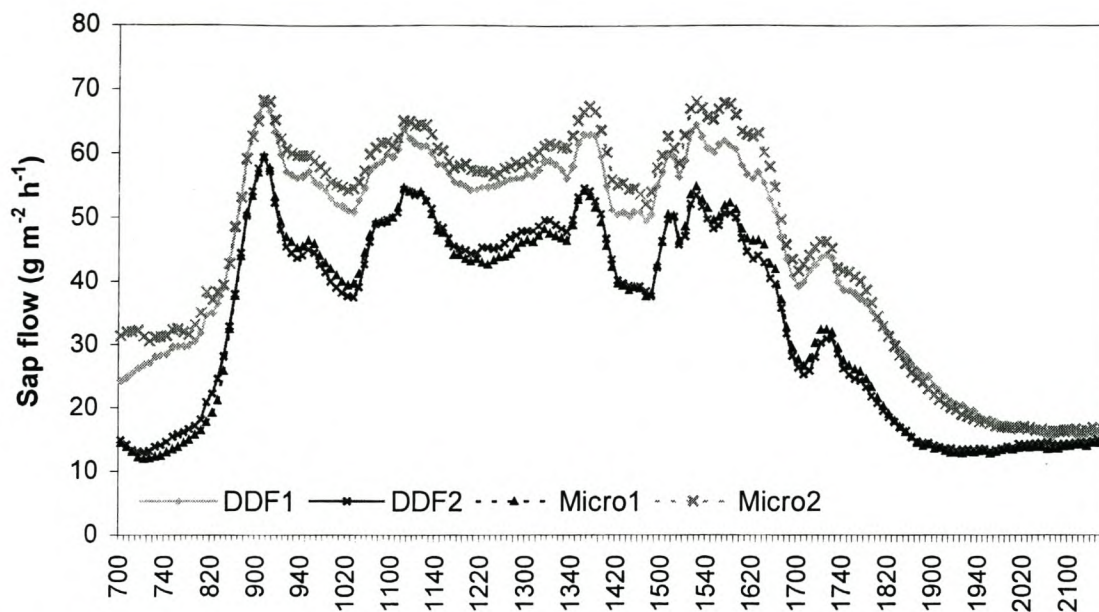
FIGURE 1. Diurnal sap flow measurements made on 22 November 1999 at Addo. Trees were given daily daylight fertigation (irrigated on a daily base; being DDF1 and DDF2) or conventional micro-jet irrigation (irrigated two days previously, on 20 November; being Micro1 and Micro2). A branch form the warm quadrant (north west facing) from two trees in each of the treatments was measured.
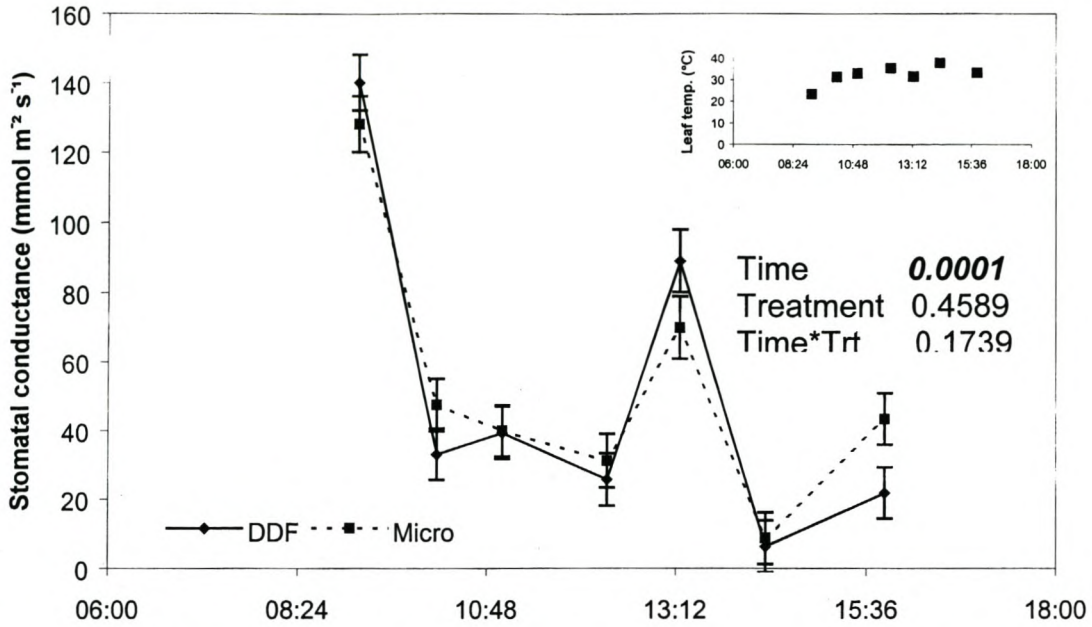
FIGURE 2. Stomatal conductance measured diurnally on 27 Augustus 1999 at Addo. Measurements were taken at seven times throughout the day and treatments were daily daylight fertigation (irrigated daily) and conventional micro-jet irrigation. Values are means (n = 15) with standard error bars. Diurnal leaf temperature is shown as insert. Significant differences were only observed over time, and not between treatments or in the time*treatment interaction.
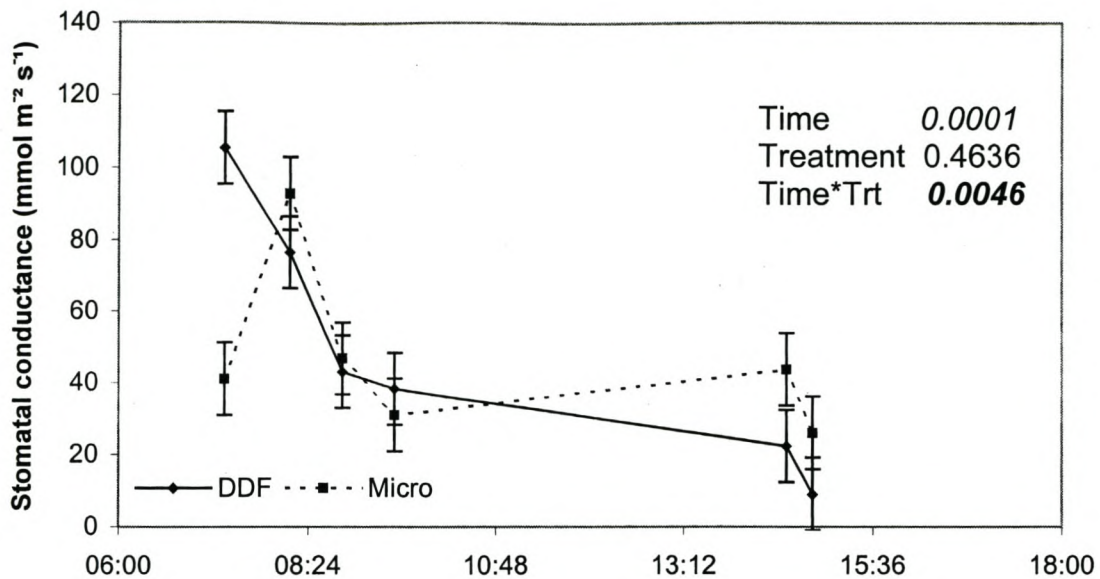
FIGURE 3. Stomatal conductance measured on 23 November 1999 at Addo. Measurements were taken at six times throughout the day and treatments were daily daylight fertigation (irrigated daily; DDF) and conventional micro-jet irrigation (irrigated three days previously, on 20 November; Micro). Values are means (n = 15) with standard error bars. The time*treatment interaction is significant, with daily daylight fertigation treatment showing higher values during the morning and lower values during the later afternoon, compared to the conventional treatment.
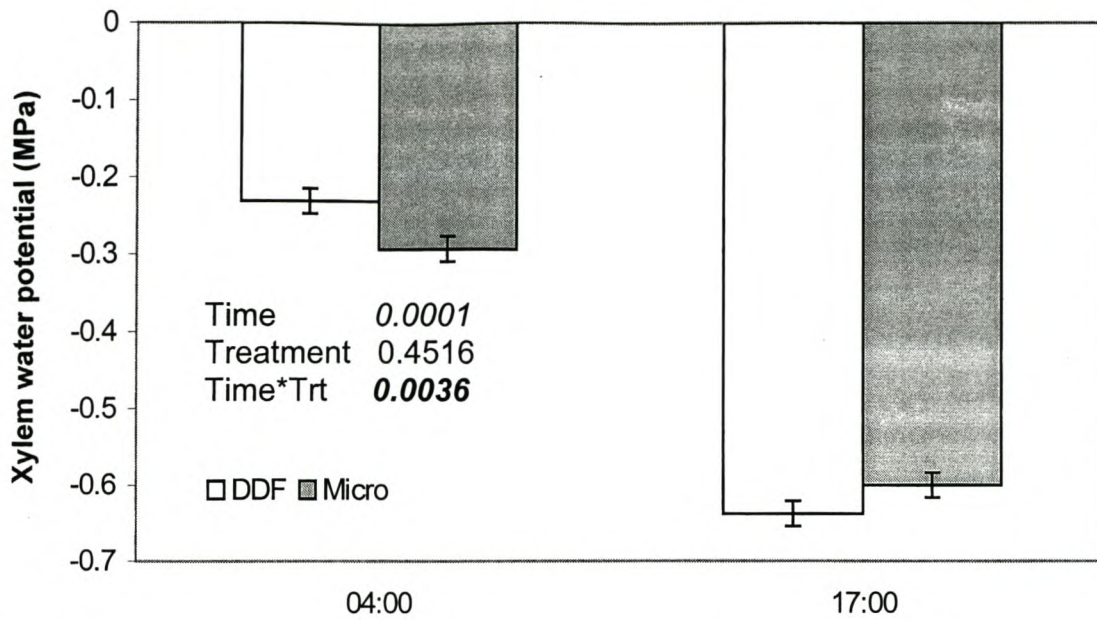
FIGURE 4. Water potential measured at Addo on 23 November 1999. Measurements were made predawn and in the late afternoon. The treatments were daily daylight fertigation (irrigated daily; DDF – the left bar at every measurement time) and conventional micro-jet irrigation (trees irrigated three days previously, on 20 November; Micro). Values are means (n = 18) with standard error bars and the time*treatment interaction was significant.
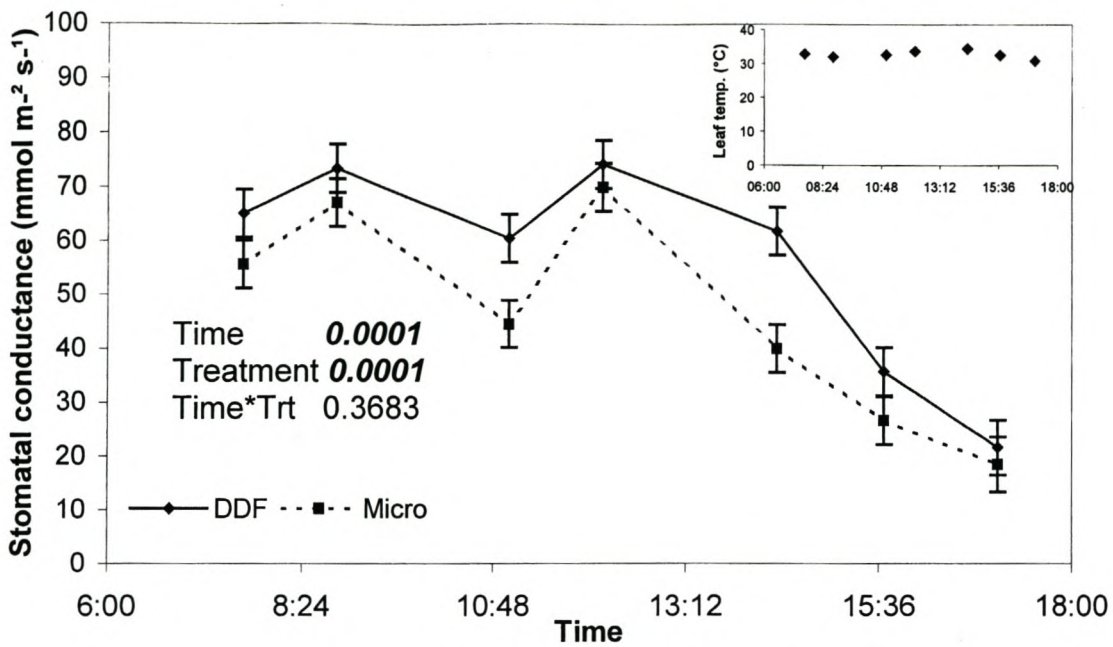
FIGURE 5. Stomatal conductance measured on 11 February 2000 at Addo. Measurements were taken at seven times throughout the day and treatments were daily daylight fertigation (irrigated daily; DDF) and conventional micro-jet irrigation (trees irrigated three days previously, on 8 February; Micro). Values are means (n = 15) with standard error bars. Significant differences were observed in both time and treatment. Diurnal leaf temperature is shown as insert.
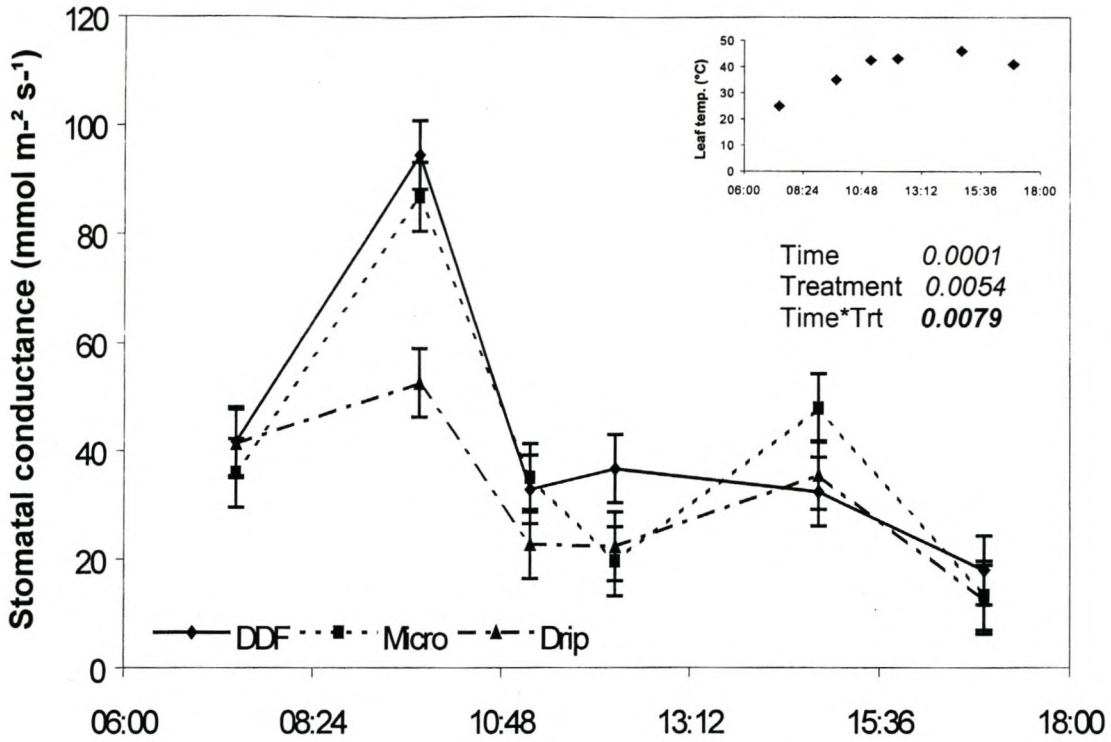
FIGURE 6. Stomatal conductance measured on 18 February 2000 at Addo. Measurements were taken at six times throughout the day. Treatments were daily daylight fertigation (irrigated daily; DDF), conventional micro-jet irrigation (trees irrigated two days previously, on 16 February; Micro) and conventional drip irrigation (irrigated daily; Drip). Values are means (n = 15) with standard error bars and the time*treatment interaction is significant. Diurnal leaf temperature is shown as insert.
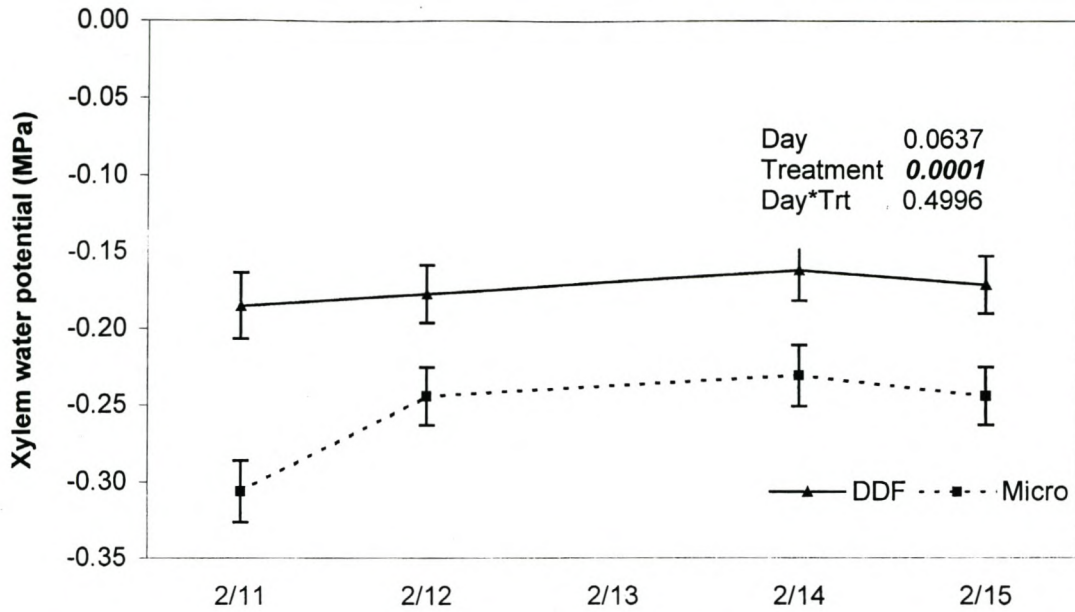
FIGURE 7. The pre-dawn water potential measured at Addo on 11, 12, 14 and 15 February 2000. Treatments were daily daylight fertigation (irrigated daily; DDF) and conventional micro-jet irrigation (irrigated on 8 February, three days prior to the start of the measurements, and 5.5 mm rain fell on 12 February; Micro). Values are means (n = 18) with standard error bars and significant differences between the treatment.
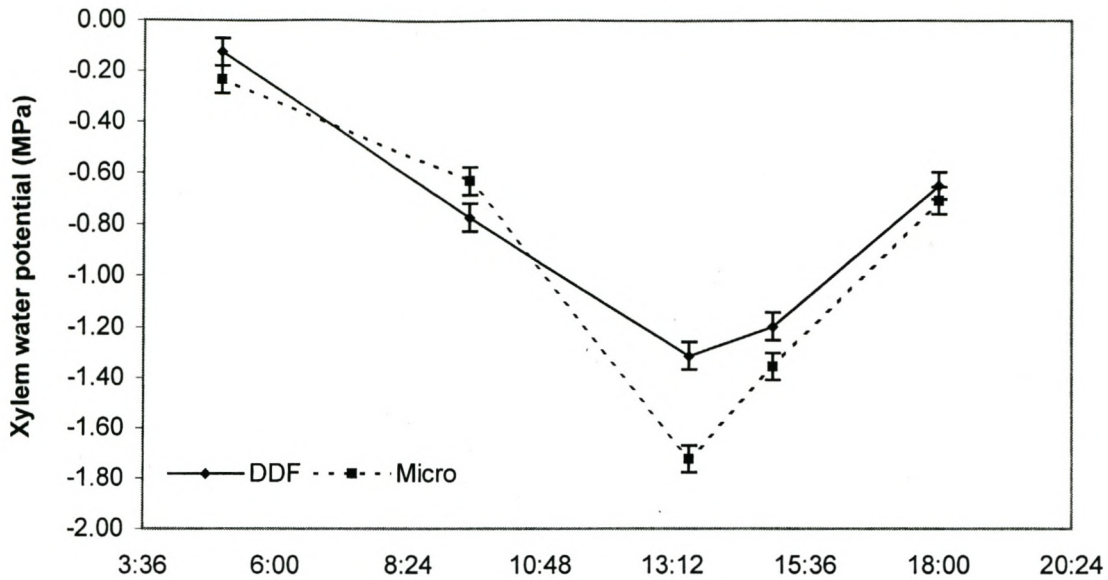
FIGURE 8. Xylem water potential measured at Citrusdal on 18 March 2000. Measurements were taken at five times throughout the day. The treatments were daily daylight fertigation (irrigated daily; DDF) and conventional micro-jet irrigation (Micro). Values are means (n = 18) with standard error bars.
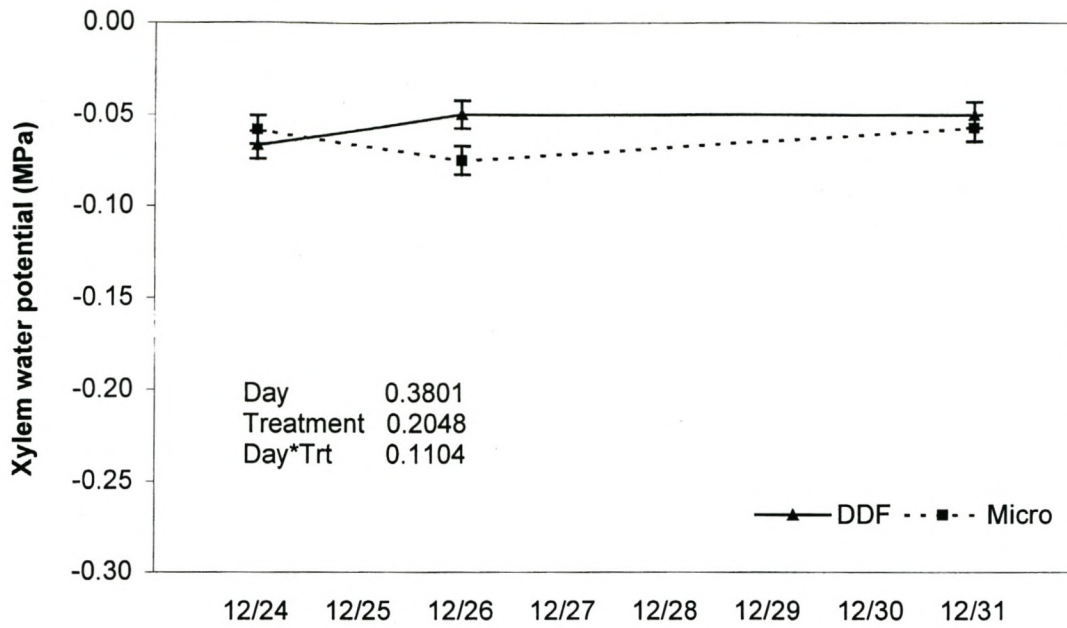
FIGURE 9. The pre-dawn water potential measured at Citrusdal. Measurements were made on 24, 26 and 31 December 2000. Treatments were daily daylight fertigation (irrigated daily; DDF) and conventional micro-jet irrigation (Micro). Values are means (n = 18) with standard error bars. No significant differences were observed.

ARTICLE 2 - SAP FLOW, STOMATAL CONDUCTANCE, XYLEM WATER POTENTIAL AND CHLOROPHYLL A FLUORESCENCE OF DAILY DAYLIGHT FERTIGATED CITRUS TREES DURING AN ARTIFICIAL SOIL DRYING EVENT

ABSTRACT

Valencia orange trees (*Citrus sinensis* cv. 'Midknight') under daily daylight fertigation (DDF) were subjected to soil drying treatments at Addo and Citrusdal, South Africa. This was done in an attempt to assess the effect of withholding water from trees adapted to a daily daylight fertigation regime, assess the risk involved with short-term water deficits in trees adapted to this regime, as well as the usefulness of physiological techniques for identifying water stress. We assessed the effect of water deficit stress on sap flow, stomatal conductance, xylem water potential and chlorophyll a fluorescence. Sap flow was relatively stable in all experiments, as the soil dried, confirming that transpiration is determined primarily by atmospheric factors, notably, vapour pressure deficit, and not by soil water content under moderate droughting. Xylem water potential and stomatal conductance decreased progressively as drying treatments proceeded. Midday depressions in stomatal conductance were observed in all treatments. Chlorophyll a fluorescence characteristics showed no consistent treatment differences. Water deficit did not increase light inhibition of photosynthesis, indicating that the photosynthetic apparatus of citrus trees is drought tolerant. Xylem water potential was a good indicator of relative water deficit, as it was one of the first parameters to show decreases during a drying treatment. Trees under DDF regimes show enhancements in xylem water potential and stomatal conductance when fertigated daily, but little or no enhancement effect is observed in sap flow and chlorophyll a fluorescence characteristics.

INTRODUCTION

The use of open hydroponic systems (OHS) in citrus orchard management recently became an option for producers. As a sensitive nutrient and moisture management system, OHS exercises a high degree of control over the development of the crop (Stassen *et al.*, 1999). Specific water and nutritional requirements for specific phenological phases of the plants are incrementally applied via daily fertigation. The nutrient solution being carefully balanced and pH as well as EC controlled, through a drip irrigation system.

Some controversy exists concerning the ascribed benefits of the use of an OHS rather than conventional micro- and drip-irrigation systems. Stassen *et al.* (1999) compares OHS to pot experiments in which crop development can be controlled to a large degree. Since the field system is not hydroponics as such, it is preferable to refer to it as daily daylight fertigation (DDF). Daily daylight fertigation involves drip fertigation (fertilisation applied with and by irrigation), on a daily basis, during the daylight hours. Sensitive crop management can be achieved in such a system, because the nutrient medium, rather than the soil, creates the environment on which root growth depends (Stassen *et. al.*, 1999). This makes open hydroponic or daily daylight fertigation systems particularly important tools in situations with low quality soils and poor water quality.

Unconfirmed reports state that sap flow in citrus trees decreases rapidly with time after irrigation in the conventional irrigation systems (R. Valero-Martinez, unpublished). As the soil water potential decreases, water is less available to plants and photosynthetic productivity is expected to decrease accordingly (Woods, 1999). This reduction in photosynthetic productivity is due to stomatal as well as non-stomatal factors (e.g. effects on metabolic processes due to a loss in turgor, changes in quantum efficiency and carboxylation efficiency). Stomatal conductance generally decreases in response to developing water stress, thus reducing the availability of CO_2 for photosynthesis. Daily fertigation (DDF), according to current perceptions, is expected to keep the sap flow more constant and plant stress levels lower, making more energy available for production purposes.

Water deficit stress' influence on physiological activity can be assessed by various techniques. Eastham and Gray (1998) used sap flow to measure transpiration differences between stressed and unstressed grapevines, as did Blaikie and Chacko (1998) in cashew trees. Both groups of researchers postulated a possible role for sap flow monitoring in irrigation scheduling. Predawn xylem water potential is a useful measure of soil water availability to plants, since it integrates the soil water potential over the root zone of the plant (Schulze and Hall, 1982) and decreases with a decrease in soil water potential (Tardieu and Simonneau, 1998). In many plants, stomatal conductance is correlated with xylem abscisic acid concentration and in turn with the soil water content (Zhang and Davies, 1990). Dry soil could reduce photosynthesis via reductions in stomatal aperture (Liang *et al.*, 1997) and this decline in stomatal conductance may fully account for the water deficit-related reduction of photosynthesis (Downton *et al.*, 1988). Chlorophyll a fluorescence techniques are considered a sensitive tool which may provide early signs of developing stress (Maxwell and Johnson, 2000) since fluorescence will be higher in plants experiencing a higher level of stress.

The objectives of our trials were to assess the effect of withholding water from trees adapted to a daily daylight fertigation regime. This was done in an attempt to assess the risk involved with short-term water deficits in trees adapted to this regime, as well as the usefulness of physiological techniques for identifying water stress. Sap flow, stomatal conductance, xylem water potential and chlorophyll fluorescence were followed through various artificial soil drying treatments in two differing climatic regions.

MATERIALS AND METHODS

Sites, plant material and treatments

Valencia orange trees, cv. Midknight were used at two sites: (i) Citrusdal, in the South Western Cape Province, with predominantly winter rainfall (dry Mediterranean climate) a nd s andy s oil (< 5% clay; Kroonstad form) and (ii) Addo, in the Eastern Cape Province, having rainfall peaks in spring and summer (dry subtropical climate)

and silt loam soil (Oakleaf form). The trees in Citrusdal were planted in 1997 on 'Troyer' citrange rootstock, at 5.0 x 2.0 m spacing with rows in the north/south direction. A daily daylight fertigation regime was employed from the time of planting. Over the past few years, changes to the fertigation scheduling were introduced, i.e. commencing earlier in the morning, and more frequent shorter fertigation pulses during the day (usually 4 or 5, depending on the specific tree water requirement for the day), rather than one single pulse over the midday period. In Addo, trees were planted in 1992 on 'Carrizo' citrange rootstock, in a north/south row direction, at 6.0 x 3.0 m tree spacing. Daily daylight fertigation scheduling commenced in September 1998 after switching from a micro-jet irrigation regime installed at planting. This explains why the trees had more extensive root systems than expected for a DDF regime (Pijl, 2001). The trees in Addo receive a single fertigation pulse during the day.

Representative trees were selected randomly for trials in November 1999 at Addo and Citrusdal, February 2000 at Addo and December 2000 at Citrusdal. Trees were either artificially stressed by withholding water, by closing drip-line drippers for the extent of the trial period, or not artificially stressed, receiving their daily fertigation as normal (control). In most cases there were three replicates in each treatment, except for the sap flow measurements, where four sensors were available, thus using two trees per treatment. Three adjacent trees per replicate were droughted by blocking their drip-line drippers during the early morning of the starting day of each drying treatment (SD-0). The centre tree was used as measuring unit. Descriptions of the specific treatments, measurements taken and weather conditions during each experiment are presented in Table 1A (for November 1999 in Citrusdal), Table 1B (for December 2000 in Citrusdal), Table 1C (for November 1999 in Addo) and Table 1D (for February 2000 in Addo).

Concurrently to this study, 'Midknight' Valencia root development under different irrigation regimes was researched (Pijl, 2001). Separate profile pits being 2 m wide and in direction with as well as against tree rows, were dug directly underneath drippers or micro-jets (depending on the irrigation regime), in conventionally micro-jet-irrigated, conventionally drip irrigated and daily daylight fertigated irrigation

regimes, at Addo and at Citrusdal. The soil profile was divided in 100 cm^2 square grid blocks and active feeder roots were counted and plotted.

Instruments and measurements

Sap flow rates were measured during November 1999 in Citrusdal, and November 1999 and February 2000 in Addo. A sap flow measuring system utilising a heat balance equation (Dynagage, Dynamax, Houston, Texas, USA) was used. Four sensors were used to monitor two trees per treatment. Branches chosen were north-west facing at 1.0 to 1.5 m above ground level. Total leaf area of the measured branches was determined with a leaf area meter (LAI3200, Li-Cor, Lincoln, Nebraska, USA). The leaf area data was used to express the sap flow rates on a leaf area basis. Average daytime sap flow rates (08:00 - 16:00) are presented as relative values, by assigning the sap flow rate on the day on which the stress treatment started (SD-0; stress day 0), the value of 100%. The sap flow rates during the following days of increasing water stress, (SD-1; one full day of stress, etc.) are then expressed as a percentage of the initial value.

Stomatal conductances were measured diurnally during November 1999 and February 2000 in Addo and during November 1999 in Citrusdal, with a porometer (EGM 2.0, PP Systems, Hertfordshire, UK). Five sun-exposed leaves, of similar size, from the most recently-hardened flush, were chosen around the north-western side of each tree, and there were three trees per treatment. Measuring started as soon as all visible moisture evaporated from the foliage and continued into the late afternoon.

Water potential: Xylem water potential was monitored with a pressure chamber (PCI 600, PMS, Corvallis, Oregon, USA), as described by Koide *et. al* (1989), during all experiments, except during November 1999 at Citrusdal, usually as pre-dawn measurements. It was assumed (i) that shoots were more accurate indicators of xylem water potential than leaves and (ii) that shoots equilibrate to the xylem water potential of the tree during the night and that accurate approximation of xylem water potential could be determined by measuring the values in the immediate hours before dawn. Two or three shoots, from the most recent hardened flush, were randomly

selected around each tree about 1.5 m above ground level, cut and immediately measured.

Fluorescence characteristics were measured during November 1999 (at both Addo and Citrusdal) and February 2000 (at Addo) with a modulated fluorescence measuring system (FMS2, Hansatech, Norfolk, UK). The instrument provided photosynthetic photon flux density (PPFD) at 94 and 1220 $\Box$mol m^{-2} s^{-1}. The fluorescence characteristics used were: the maximum quantum efficiency of photosystem II (Fv/Fm); the antennae efficiency of photosystem II (Fv'/Fm'); the quantum efficiency of photosystem II ($\Box$PSII); the photochemical quenching coefficient (qP) and the non-photochemical quenching coefficient (qNP), as defined by Van Kooten and Snel (1990) and Bolhàr-Nordenkampf and Öquist (1993). Three sun-exposed leaves of similar size, from the most recently-hardened flush, were measured per tree.

Fruit growth was monitored at Addo during February 2000. 20 fruit per treatment (from three trees) were tagged and measured daily for the duration of the experiment. Fruit diameter was measured during the early evening (18h00 – 19h00) using a digital calliper.

Weather data was obtained from the Agricultural Research Council (Infruitec, Stellenbosch, South Africa), from their weather stations in the Addo Elephant Park, and at the Citrusdal experimental farm, close to study sites. The general linear models procedure (SAS Inc., 1990) was used in the statistical analysis. Data was considered to differ significantly at P $\leq$ 0.05.

RESULTS AND DISCUSSION

November 1999, Citrusdal

The diurnal sap flow of the droughted and control trees is shown in Figure 1. During very hot days SD-1 and SD-3 the sap flow of stressed trees was 8 - 10% lower than in control trees, but on the cooler day (SD-2), the sap flow of the treatments was similar. This pattern is the same for both average daytime and average midday sap

flow. On SD-1, control trees had 108% of SD-0 sap flow, whilst the sap flow of the stressed trees remained similar to that on SD-0. The sap flow on SD-2 is lower than the other days (60% of SD-0), due to the lower temperatures and cloud cover during the morning. Control trees exhibited midday sap flow on SD-3 that was 88% of that on SD-0, due to the lower temperature on this day. Thus, on this sandy soil, one very hot day without water lowered the sap flow of the stressed trees to 90% of that of control trees, but this was not observed on the cool day.

Fluorescence characteristics (dark-adapted and at PPFD = 300 μmol m^{-2} s^{-1}) measured on SD-1 (Table 2) showed no significant differences between the treatments during early morning or midday. Time differences were observed in PSII and qP, being lower in the early morning than during midday. It is most likely a temperature effect, an increase in photosynthetic rate and photosynthetic efficiency as the temperature rises. On 5 November, measurements were taken during the morning (08:30 - 10:00) and late morning (10:30 - 12:00), but no time effect was recorded (Table 3). The treatments differed in relation to Fv/Fm, being lower after three days of stress compared to two days of stress, but not different from control trees.

December 2000, Citrusdal

Stressed trees had significantly lower pre-dawn xylem water potential than the control trees (Figure 2). It must be noted that the baseline xylem water potential for the stressed trees were not measured. The pre-dawn xylem water potential values for the unstressed control trees remained between -0.04 and -0.08 MPa whilst those of the stressed trees decreased to about -0.14 MPa after three days and remained relatively constant thereafter. None of the values were outside of the physiologically acceptable range for citrus, since enzymatic inhibition occurs only at water potentials lower than –2.5 MPa (Fereres *et al.* 1979; Jones et. al., 1985; Syvertsen, 1981).

November 1999, Addo

Sap flow in stressed and control trees followed similar patterns, falling to 81% of SD-0 flow on SD-1 and SD-2 (Figure 3). No differences were observed between the

treatments, either in the daily, or the midday sap flow averages. The higher water holding capacity of the soils in Addo compared to Citrusdal, and therefore a slower stress development, could explain these lack of differences.

A significant time*treatment interaction was found for stomatal conductance as measured on 18 November 1999, being high in the early morning, but decreased thereafter, ending very low in the evening (Figure 4). The stressed trees' stomatal conductance values were significantly lower for the mayor part of the day. Average diurnal leaf temperatures was low in the early morning, increased and remained relatively constant as soon as direct sunlight started shining on the leaves, and a rapid decrease followed in the late afternoon. The large difference in stomatal conductance between control and stressed trees was, however, not accompanied by any differences in the sap flow. Water in some tree tissues (e.g. the bark, fruit) plays a buffering role, termed capacitance, and can supply transpirational water-requirements, moderating the effect of soil water deficit.

Xylem water potential as measured on 19 and 20 November 1999, showed a significant time*treatment interaction (Figure 5). Compared to the trees that did not receive irrigation for two days, the less stressed trees had significantly higher xylem water potential values during mid-morning to mid-afternoon. The diurnal water potential over the cycle was as follows: high at pre-dawn, low at midday and recovering in the late afternoon. None of the measurements were out of the physiologically normal range for citrus (Jones et. al., 1985).

The initial high values for stomatal conductance during the early morning were accompanied by high values for xylem water potential and the lowest values for stomatal conductance were recorded concurrently with the lowest values for xylem water potential, i.e. during late morning. Although a recovery in xylem water potential values was recorded during the latter part of the day, it was not observed in stomatal conductance values. Recovery of stomatal conductance in trees previously subjected to water deficit, often lag behind recovery in water potential and photosynthesis.

A significant treatment effect was observed in Fv/Fm, being higher in the more stressed trees (Stressed-2 and Stressed-3). At PPFD = 28 mmol m^{-2} s^{-1} all fluorescence characteristics differed significantly over time (Table 4). The quantum yield of photochemistry ($\square$PSII) gives a measure of the photosynthetic capacity of the leaf. It was the lowest in the early morning (8:00 - 9:30) and late afternoon (16:30 - 18:00), had a peak in midmorning (10:00 - 11:30), a dip over midday (12:30 - 14:00) and a second peak in the afternoon (15:00 - 16:00). The component of the fluorescence signal that is quenched by photochemical activity, photochemical quenching (qP), showed a trend similar to $\square$PSII. Non-photochemical quenching (qNP), the component of the fluorescence signal quenched by activity not directly related to photochemistry, reacted exactly opposite, being high in the early morning and late afternoon, and lower during the rest of the day. $\square$PSII at high light intensity (PPFD = 1220 mmol m^{-2} s^{-1}) was influenced by the treatment as well, being highest in control trees and lower in the stressed trees. The time*treatment interaction was significant for qP at PPFD = 1220 mmol m^{-2} s^{-1}, with no differences between the treatments during the day until the late afternoon, when the trees in the Control treatment had the lowest values (Figure 6).

February 2000, Addo

On SD-4, average daily sap flow was about 25% less than on SD-1 in both control and stressed trees. After re-irrigation it was 20 and 40% higher than SD-1, respectively, in control and stressed trees (Figure 7). Average midday sap flow was even more exaggerated, being 35 and 60% higher than on SD-1, respectively. These higher levels in stressed compared to control could be ascribed to high uptake and flow rates, after being deprived of water for such a long time. The relatively higher levels on this day compared to SD-0 could also be ascribed to a higher temperature on this day than on SD-0. Sap flow is determined primarily by the atmospheric demand, i.e. the rate of transpiration is high when the atmospheric demand is high, in turn determined by the humidity and temperature. Stomatal conductance also plays a very important role, keeping the sap flow rate relatively constant, so as not to exceed a limit where-after xylem cavitation occurs.

There was a significant time*treatment interaction for stomatal conductance, being highest during the mid-morning in control trees and decreasing rapidly towards midday and thereafter. The stressed trees had high stomatal conductance values only in the early morning, decreasing faster than the Control trees, but reaching the same level around midday. Stomatal closure regulates transpiration to be relatively constant through the day (Syvertsen and Lloyd, 1994). Conductances are high when mild atmospheric conditions prevail, i.e. during the morning when mild temperatures and vapour pressure deficits limit transpirational water loss. The typical clear-day leaf temperature changes can be seen in the insert in Figure 8.

As seen in figure 9, significant differences were found in xylem water potential values between the treatments, with control trees (irrigated daily) having a higher pre-dawn xylem water potential than stressed trees (stressed for 6 days). Pre-dawn xylem water potential decreased gradually with duration of water stress until the lowest value was reached at the peak of the stress treatment. Following re-irrigation, the xylem water potential recovered to a value corresponding to the initial value within one day. None of the values are out of the normal range for pre-dawn xylem water potential for citrus. Diurnal xylem water potential was measured on SD-7. The treatment*time interaction was significant (Figure 10) with the less stressed trees continually having higher xylem water potential values and the diurnal cycle was as follows: high at pre-dawn, low at midday and recovering in the late afternoon.

Pre-dawn and midday xylem water potentials were measured on 4 and 8 February in stressed (without water for 3 and 7 days respectively) and control (irrigated daily) trees. Treatments were significantly different, with the control trees having higher xylem water potential than the stressed trees (not illustrated). The day*time interaction was significant (Figure 11). The midday xylem water potential values on 4 February were lower than on 8 February because the latter was a cooler day with cloud cover and thus a less severe VPD (31°C → 8°C less than SD-3). None of the measurements were out of the physiologically normal range for citrus.

No significant differences were observed in any of the fluorescence characteristics (Table 5) as measured on 8 February. This was the peak of the drying treatment. The day was cloudless and had a maximum temperature of 31°C. It is significant

that on this day no differences were observed: it is unlikely that differences existed for the duration of the droughting, if not measurable on this day. Syvertsen and Lloyd (1994) reported this previously for 'Valencia' trees, from unpublished work by the same authors, in which stressed trees showed symptoms of reduced photosynthesis, but no reduction in chlorophyll a fluorescence characteristics. Two days after re-irrigation commenced, the fluorescence measurements were repeated (Table 6). Fv/Fm was significantly higher in stressed than in control trees. This has been observed previously (Table 3; 4). qNP changed with time at both light intensities, being higher in the early afternoon than later, as would be expected, since non-photochemical quenching is correlated to day temperatures (Bolhàr-Nordenkampf and Öquist, 1991). □PSII changed conversely, being higher in the later afternoon.

No significant differences in initial fruit size were found, but control trees had a significantly faster relative fruit growth rate than droughted trees (Figure 12).

CONCLUSION

Sap flow was very responsive to ambient weather conditions, but did not give a good indication of the stress level of the plants. Sap flow is relatively stable in woody plants, many of which operate at stable transpiration levels, mediated by stomatal control in the dynamically changing environment. Xylem cavitation may even sometimes occur to maintain stomatal conductance and thereby enhancing photosynthesis (Bond and Kavanagh, 1999). Ambient conditions (i.e. VPD and diurnal temperature) generally determine the measured parameters and may override the effects of soil conditions (Jones et al., 1985; Flore and Lakso, 1989). A sap flow treatment effect was observed in February 2000 when the stressed trees had a higher level of sap flow after re-irrigation.

Daily irrigation generally did not mitigate the midday depression in gas exchange, xylem water potential or chlorophyll fluorescence characteristics, although in some cases a degree of alleviation did occur. Complete mitigation can only occur when atmospheric conditions do not induce such a depression. No consistent treatment differences were found concerning the fluorescence characteristics, and there were

none on the peak of the drying cycle during February 2000. Water stress seemed to have little effect on these parameters. This observation has recently been reported in citrus (Syvertsen and Lloyd, 1994, Veste et al., 2000) and other subtropical crops, i.e. papaya (Marler and Mickelbart, 1998) and cashew (Blaikie and Chacko, 1998) as well as in apple (Fernandez et al., 1997). Treatment effects were absent because citrus leaves are well adapted to high light conditions (Syvertsen and Lloyd, 1994). Increased light limitation by mild drought stress conditions does not enhance photo-inhibition, because excess light is dissipated away from the photosynthetic apparatus. This is done via a range of dissipation pathways, of which photorespiration has been identified to play an important role in citrus (Chen and Zhang, 1994) and cashew (Blaikie and Chacko, 1998). The midday reduction in photosynthetic efficiency (as seen in reductions in qP and □PSII and the increase in qNP) is typical of leaves in high light and high temperature conditions (Blaikie and Chacko, 1998; Marler and Mickelbart, 1998). The Fv/Fm values measured in these trials are not out of the typical range for unstressed evergreen crops (Bolhàr-Nordenkampf and Öquist, 1991). Other indications, e.g. stomatal conductance and xylem water potential, demonstrate that plants start to show decreases in their physiological activity after stress initiated, compared to daily-irrigated trees.

Stomatal conductance and xylem water potential indicated water stress sooner than the other physiological parameters. This reduction in stomatal conductance or xylem water potential is not necessarily observed as a large value on the first day after irrigation stopped and it does not imply a continuing trend of decreasing activity. It can, however, indicate an environment beginning to have stressful effects on the trees as could be seen in the reduction in fruit growth when the trees were subjected to water deficit stress.

Citrus trees seem to be relatively insensitive to water deficit stress as measured by sap flow and chlorophyll a fluorescence. The stressed and unstressed control trees in these trials had similar levels of these parameters. Sap flow is buffered by tree capacitance, and although mediated via stomatal conductance, atmospheric conditions and not the soil water content primarily determine it. Daily fertigation is applied to trees under DDF regimes and they exhibit more optimal levels of xylem water potential and stomatal conductance, compared to trees from which water is

withheld. Although alleviating it, daily irrigation did not mitigate the midday depression in these values. Seen over a season, even small enhancements of stomatal conductance (and with it photosynthesis and possibly, growth) and xylem water potential, could incrementally produce higher yields.

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TABLE 1A. Weather conditions and measurements taken during November 1999 at Citrusdal. The drying treatment started on 2 November, during the early morning, whilst control trees continued to be irrigated daily. Additional drip-line drippers were closed on 4 November, so that on 5 November treatments consisted of control, Stressed-1 (being stressed one day) and Stressed-3 (being stressed three days).

Parameter	Nov 1	Nov 2	Nov 3	Nov 4	Nov 5	Nov 6
Max Temp (°C)	32.5	34.2	36.9	23.6	29.8	36.0
Min Temp (°C)	14. 4	14.1	15.0	13.0	9.6	14.5
Ave Temp (°C)	23.4	24.6	25.6	17.6	20.2	25.0
Precipitation (mm)	0	0	0	0.6	0	0
Radiation (MJ/day)	26.0	26.7	26.9	15.1	27.3	27.5
Sap flow		Sap flow	Sap flow	Sap flow	Sap flow	
Stomatal conductance		g_s				
Chlorophyll Fluorescence			Fluorescence		Fluorescence	
Drying treatment		stress day 0	stress day 1	stress day 2	stress day 3	

TABLE 1B. Weather conditions and measurements taken during December 2000 at Citrusdal. Pre-dawn xylem water potential was measured. The drying treatment started on 18 December and lasted 13 days until 31 December, with the control irrigated daily. Rain fell on 31 December (1.8 mm) in the late morning, not influencing the pre-dawn measurement of that day.

Parameter	Dec 19	Dec 20	Dec 21	Dec 22	Dec 23	Dec 24	Dec 25	Dec 26	Dec 27	Dec 28	Dec 30	Dec 31
Max Temp (°C)	33.5	32.6	35.9	39.8	34.3	29.0	30.0	34.1	30.4	29.1	29.4	30.0
Min Temp (°C)	16.8	15.1	16.5	18.3	17.9	15.8	12.5	13.3	15.1	15.3	13.4	14.3
Ave Temperature (°C)	24.7	23.6	26.7	29.5	25.4	21.9	21.2	24.4	22.6	22.0	21.5	20.9
Precipitation (mm)	0	0	0	0	0	0	0	0	0	0	0	1.8
Radiation (MJ/day)	30.2	29.4	27.6	29.6	30.0	21.9	30.1	30.3	29.2	26.2	29.6	28.4
Water potential		Pre-dawn		Pre-dawn		Pre-dawn		Pre-dawn				Pre-dawn

TABLE 1C. Weather conditions and measurements taken during November 1999 at Addo. The drying treatment was applied from 17 November to 20 November. On every consecutive day of the drying treatment, before the daily fertigation commenced, drip-line drippers under the trees that formed part of the stress treatment, were closed. Subsequently, on SD-2 there were 3 treatments (control, being irrigated daily; stressed-1 and stressed-2; with stressed-1 being stressed one day, etc.) and on SD-3, four treatments (control; stressed-1; stressed-2 and stressed-3).

Parameter	Nov 16	Nov 17	Nov 18	Nov 19	Nov 20	Nov 21
Max Temp (°C)	34.3	27.6	26.2	25.8	29.4	28.6
Min Temp (°C)	19.5	15	18.5	9.5	12.5	17.6
Ave Temp (°C)	27	21.3	22.4	17.7	21	23.1
Precipitation (mm)	0.7	0	0	0	0	0
Light Hours (hrs)	2.4	9.8	10.6	10.3	7.4	9.9
Sap flow		Sap flow	Sap flow	Sap flow		
Stomatal conductance			g_s			
Water potential		Diurnal	Diurnal	Diurnal		
Chlorophyll Fluorescence					Fluorescence	
Drying treatment	stress day 0	stress day 1	stress day 2	stress day 3	stress day 4	

TABLE 1D. Weather experienced and measurements taken during February 2000 at Addo. In this experiment the stress treatment commenced on 2 February and irrigation resumed on 10 February (first day of the recovery cycle; RD-0). Control trees were irrigated daily. Recovery was monitored until 14 February.

Date	Drying treatment	Max Temp (°C)	Min Temp (°C)	Ave Temp (°C)	Precip- itation (mm)	Light Hours (hrs)	Sap flow	Chlorophyll fluorescence	Stomatal conductance	Water potential	Water potential	Fruit growth
Feb 01	stressday0	26	18	22	0	2.1						
Feb 02	stressday1	25	13.5	19.3	0	2	Sap flow					frtgrwth
Feb 03	stressday2	31	18.5	24.8	0	11.9					predawn	frtgrwth
Feb 04	stressday3	39.5	16	27.8	0	-999				midday	predawn	frtgrwth
Feb 05	stressday4	27	20.5	23.8	0	4.3	Sap flow					frtgrwth
Feb 06	stressday5	28.5	19.5	24	0	-999						frtgrwth
Feb 07	stressday6	35	17	26	0	-999					predawn	frtgrwth
Feb 08	stressday7	31	20	25.5	0	10.6		fluorescence		diurnal	predawn	frtgrwth
Feb 09	re-irr0	34.5	19	26.8	0	10.2			g_s			frtgrwth
Feb 10	re-irr1	39.5	18	28.8	0	0						frtgrwth
Feb 11	re-irr2	31	19	25	0	2.7					predawn	frtgrwth
Feb 12	re-irr3	29	20	24.5	5.5	2.4	Sap flow	fluorescence				frtgrwth
Feb 13	re-irr4	21.5	20.5	21	0.4	0.6						
Feb 14	re-irr5	31.5	17	24.3	0	11.5					predawn	
Feb 15	re-irr6	30	17	23.5	0	9.9						

TABLE 2. Fluorescence characteristics (dark-adapted and at PPFD = 300 μmol m^{-2} s^{-1}) measured on 3 November 1999 at Citrusdal. Measurements were made in the early morning and at midday and the treatments were Control (irrigated daily) and Stressed (trees without water for one day).

		300 μmol m^{-2} s^{-1}		
	Fv/Fm	ΦPSII	qP	qNP
8:00	0.744	0.342b	0.536b	0.499
	± 0.032	± 0.028	± 0.035	± 0.035
12:00	0.747	0.434a	0.708a	0.493
	± 0.041	± 0.036	± 0.045	± 0.045
Control	0.759	0.410	0.641	0.457
	± 0.038	± 0.034	± 0.042	± 0.042
Stressed	0.733	0.366	0.603	0.535
	± 0.035	± 0.031	± 0.039	± 0.039
Source	Pr>F	Pr>F		
Time	0.8156	0.0478	0.0085	0.8145
Treatment	0.4316	0.2511	0.4397	0.1905
Time*Trt	0.2403	0.4233	06663	0.9351

TABLE 3. Fluorescence characteristics (dark-adapted and at PPFD = 300 μmol m^{-2} s^{-1}) measured on 5 November 1999 at Citrusdal. Measurements were made in the early and mid-morning and treatments were Control (irrigated daily), Stressed-1 (trees without water for one day) and Stressed-2 (trees without water for three days).

	Fv/Fm	300 μmol m^{-2} s^{-1}		
		ΦPSII	qP	qNP
08h30 -	0.699	0.281	0.493	0.467
10h00	± 0.035	± 0.028	± 0.035	± 0.049
10h30 -	0.684	0.304	0.512	0.496
12h00	± 0.035	± 0.028	± 0.035	± 0.049
Control	0.671**ab**	0.306	0.505	0.432
	± 0.042	± 0.034	± 0.043	± 0.060
SD-1	0.786**a**	0.292	0.481	0.597
	± 0.042	± 0.034	± 0.043	± 0.060
SD-2	0.618**b**	0.280	0.523	0.415
	± 0.042	± 0.034	± 0.043	± 0.060
Source	Pr>F	Pr>F		
Time	0.7728	0.5692	0.7046	0.6808
Treatment	***0.0440***	0.8612	0.7930	0.0965
Time*Trt	0.1045	0.7963	0.5918	0.8096

TABLE 4. Fluorescence characteristics (PPFD = 28 and 1220 μmol m^{-2} s^{-1}) measured on 20 November 1999 at Addo. Measurements were made during five time periods and treatments were Control (irrigated daily), Stressed-1 (trees without water for one day), Stressed-2 (trees without water for two days) and Stressed-3 (trees without water for three days).

		28 μmol m^{-2} s^{-1}			1220 μmol m^{-2} s^{-1}		
	Fv/Fm	ΦPSII	qP	qNP	ΦPSII	qP	qNP
8:00	0.831	0.615c	0.879c	0.5765a	0.117 b	0.284	0.894
	± 0.008	± 0.014	± 0.009	± 0.037	± 0.008	± 0.018	± 0.012
10:00	0.833	0.688a	0.919ab	0.412c	0.172 a	0.389	0.890
	± 0.008	± 0.014	± 0.009	± 0.037	± 0.008	± 0.018	± 0.012
12:30	0.826	0.652b	0.928a	0.486b	0.171 a	0.435	0.906
	± 0.008	± 0.014	± 0.009	± 0.037	0.008	± 0.018	± 0.012
15:00	0.842	0.670ab	0.913ab	0.460bc	0.166 a	0.401	0.900
	± 0.008	± 0.015	± 0.009	± 0.039	± 0.008	± 0.018	± 0.012
16:30	0.847	0.619c	0.895c	0.568a	0.124 b	0.292	0.876
	± 0.008	± 0.014	± 0.009	± 0.037	± 0.008	± 0.019	± 0.013
Control	0.824b	0.661	0.915	0.475	0.165a	0.370	0.870
	± 0.007	± 0.013	± 0.008	± 0.035	± 0.007	± 0.017	± 0.011
Stressed-1	0.827b	0.635	0.908	0.500	0.140bc	0.348	0.895
	± 0.007	± 0.012	± 0.008	± 0.033	± 0.007	± 0.016	± 0.011
Stressed-2	0.842a	0.652	0.900	0.474	0.155ab	0.378	0.903
	± 0.007	± 0.012	± 0.008	± 0.033	± 0.007	± 0.016	± 0.011
Stressed-3	0.849a	0.646	0.905	0.552	0.140bc	0.346	0.905
	± 0.007	± 0.012	± 0.008	± 0.033	± 0.007	± 0.016	± 0.011
Source	Pr>F		Pr>F			Pr>F	
Time	0.2726	0.0019	0.0015	0.0121	0.0001	0.0001	0.7663
Treatment	0.0375	0.4991	0.5115	0.3049	0.0305	0.2937	0.2247
Time*Trt	0.9879	0.1526	0.5799	0.3005	0.0967	0.0391	0.3076

TABLE 5. Fluorescence characteristics (dark-adapted and at PPFD = 28 and 1220 μmol m^{-2} s^{-1}) measured on 8 February 2000 at Addo. Measurements were made during the mid-afternoon (15:30) treatments were Control (irrigated daily) and Stressed (trees without water for six days).

| | Fv/Fm | 28 μmol m^{-2} s^{-1} | | | 1220 μmol m^{-2} s^{-1} | | |
		ΦPSII	qP	qNP	ΦPSII	qP	qNP
Control	0.852	0.721	0.940	0.340	0.177	0.425	0.876
	± 0.013	± 0.020	± 0.007	± 0.026	± 0.010	± 0.026	± 0.010
Stressed	0.834	0.721	0.956	0.340	0.162	0.433	0.899
	± 0.013	± 0.020	± 0.007	± 0.026	± 0.010	± 0.026	± 0.010
Source	Pr>F	Pr>F			Pr>F		
Treatment	0.3680	0.9932	0.1531	1.000	0.3282	0.8469	0.1469

TABLE 6. Fluorescence characteristics (dark-adapted and at PPFD = 28 and 1220 μmol m^{-2} s^{-1}) measured on 12 February 2000 at Addo. Measurements were made at midday and at mid-afternoon and treatments were Control (irrigated daily) and Stressed (trees had received irrigation for two days on this day after being stressed for seven days).

		28 μmol m^{-2} s^{-1}			1220 μmol m^{-2} s^{-1}		
	Fv/Fm	ΦPSII	qP	qNP	ΦPSII	qP	qNP
13:00	0.830	0.623b	0.927	0.508a	0.139	0.380	0.876a
	± 0.009	± 0.014	± 0.008	± 0.047	± 0.010	± 0.024	± 0.018
16:00	0.832	0.675a	0.909	0.326b	0.153	0.338	0.827b
	± 0.007	± 0.012	± 0.006	± 0.039	± 0.008	± 0.019	± 0.014
Control	0.817b	0.662	0.923	0.421	0.136	0.342	0.847
	± 0.007	± 0.014	± 0.008	± 0.048	± 0.008	± 0.019	± 0.014
Stressed	0.844a	0.635	0.913	0.413	0.156	0.376	0.857
	± 0.009	± 0.012	± 0.006	± 0.039	± 0.010	± 0.024	± 0.018
Source	Pr>F	Pr>F			Pr>F		
Time	0.5285	*0.0080*	0.1506	*0.0070*	0.1814	0.2354	*0.0340*
Trt	*0.0213*	0.1016	0.3891	0.8829	0.1119	0.2855	0.5541
Time*Trt	0.8127	0.3183	0.3941	0.9520	0.7893	0.9317	0.4257

TABLE 7. Fruit growth rates measured during February 2000 at Addo. Daily measurements were taken in the early evening and treatments were Control (irrigated daily) and Stressed (drought stressed from 2 to 8 February followed by recovery from 9 to 12 February).

Treatment	Y-axis intercept (mm)	Gradient (mm day^{-1})
Control	54.045	0.285b
	± 0.872	± 0.011
Stressed	55.861	0.231a
	± 0.872	± 0.011
Source	Pr>F	
Treatment	0.1493	*0.0019*
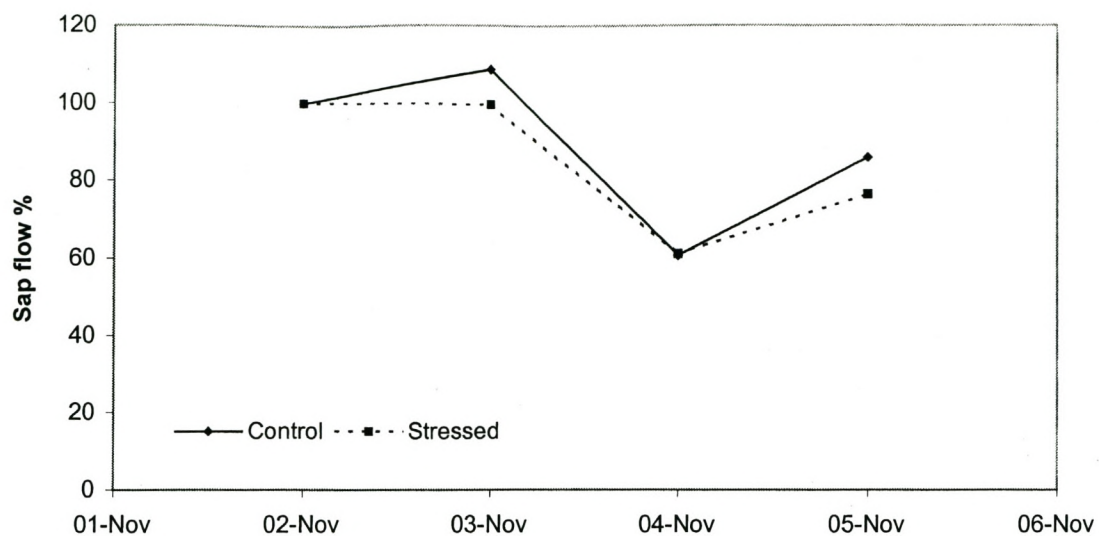
FIGURE 1. Relative average daily sap flow during a drying cycle in November 1999 at Citrusdal. Control trees were irrigated daily and Stressed trees were not irrigated from the morning of 2 November (SD-0) until 5 November (SD-3)
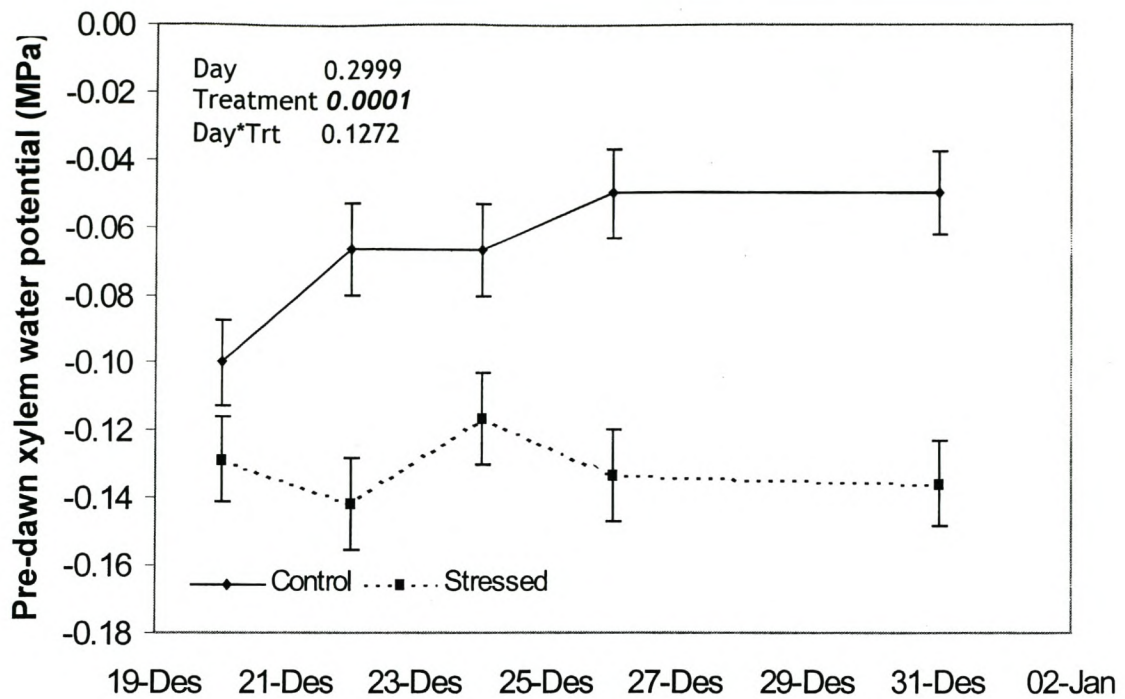
FIGURE 2. Pre-dawn water potential measured at Citrusdal during December 2000. The treatments were Stressed (trees not irrigated as from 18 December) and Control (trees irrigated daily).
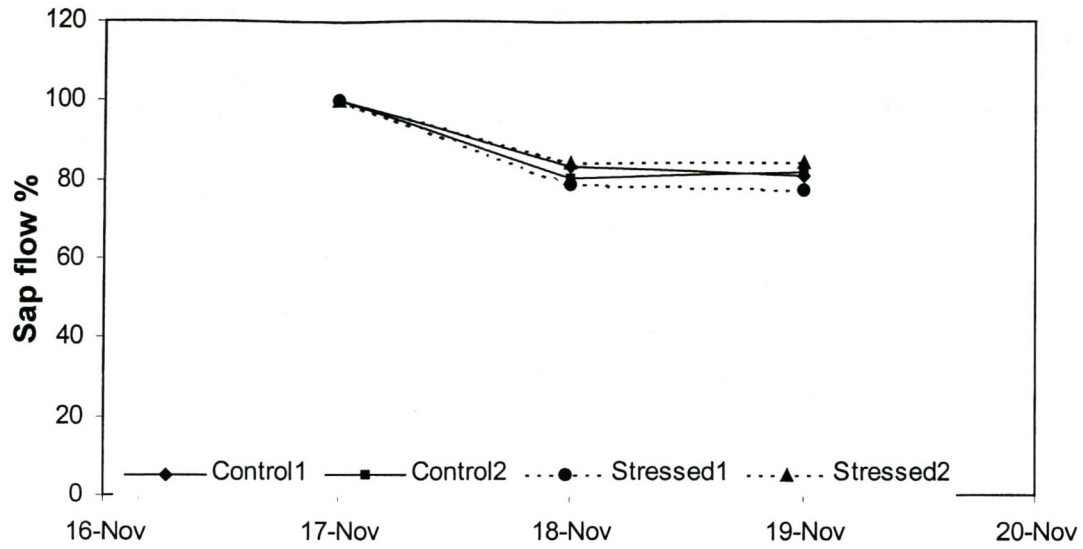
FIGURE 3. Relative daytime sap flow during a drying treatment in November 1999 at Addo. Control trees were irrigated daily. The drying treatment commenced on the morning of 17 November.
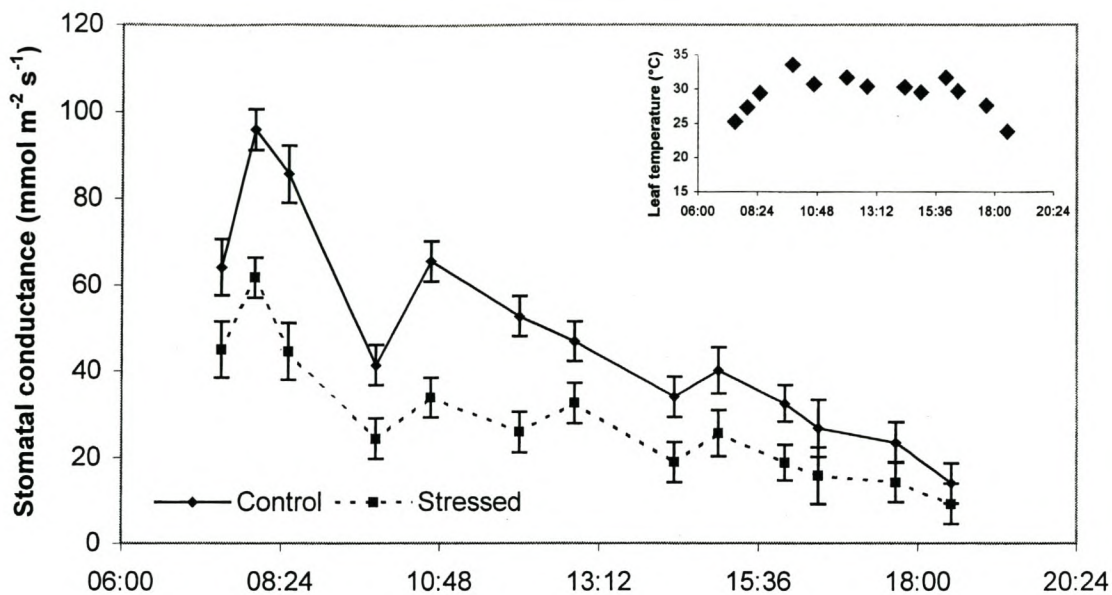
FIGURE 4. Stomatal conductance measured on 18 November 1999 at Addo. Measurements were taken diurnally and treatments were Control (trees irrigated daily) and Stressed (trees without water for one day). Values are means (n = 15) with standard error bars. Average diurnal leaf temperature is shown as insert.
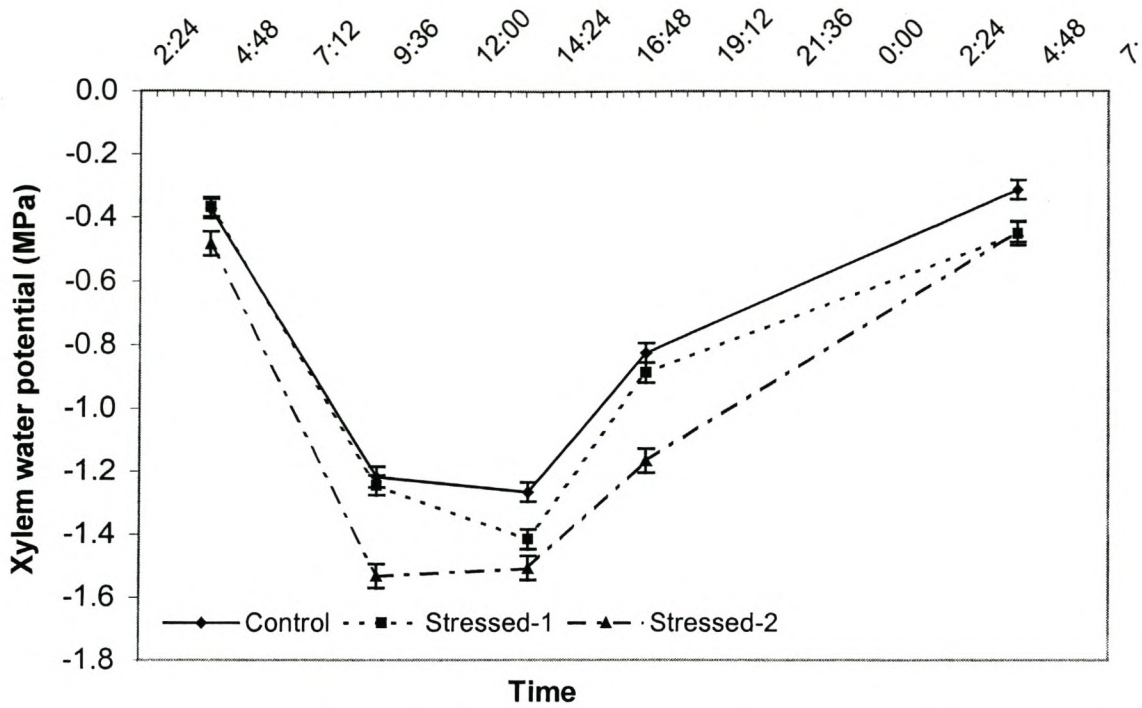
FIGURE 5. Xylem water potential measured at Addo on 19-20 November 1999. Measurements were made five times throughout the day. The treatments were Control (trees irrigated daily), Stressed-1 (trees without water for one day), Stressed-2 (trees without water for two days).
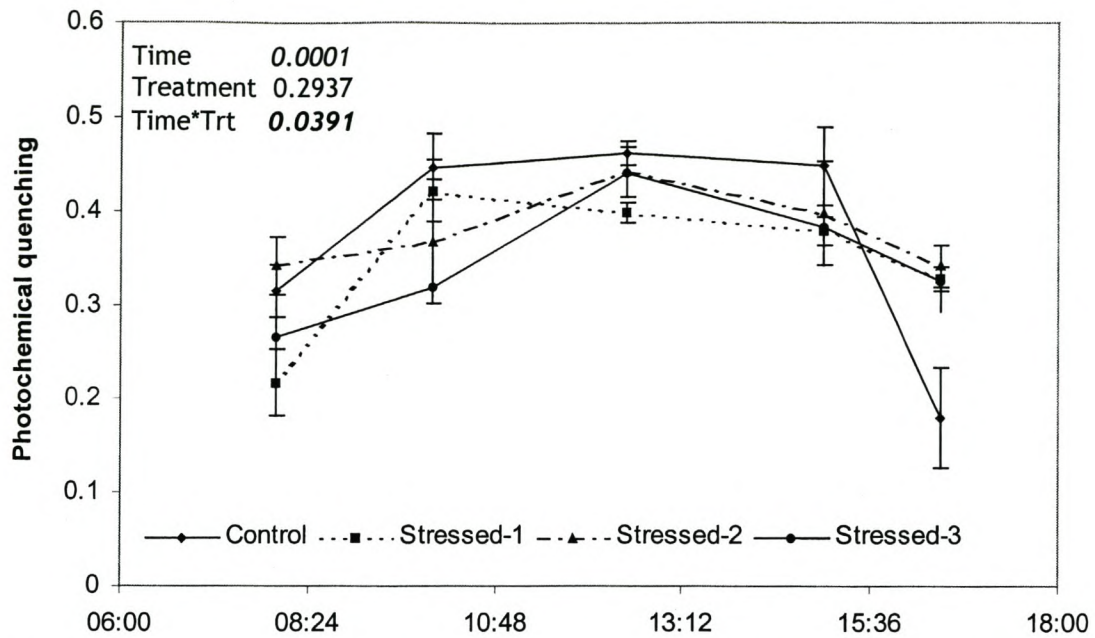
FIGURE 6. The time-treatment effect observed in photochemical quenching (PPFD = 1220 mmol m^{-2} s^{-1}) measured on 20 November 1999 at Addo. Measurements were made at five times and treatments were Control (trees irrigated daily), Stressed-1 (trees without water for one day), Stressed-2 (trees without water for two days) and Stressed-3 (trees without water for three days).
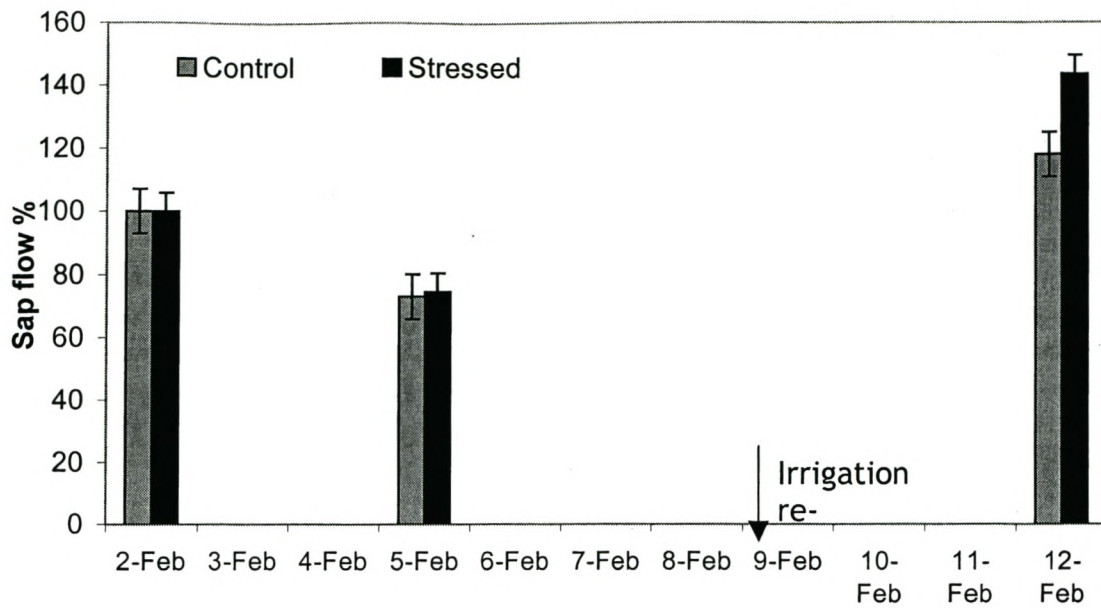
FIGURE 7. Relative average daily sap flow during a drying cycle in February 2000 at Addo. Control trees were irrigated daily, Stressed trees were not irrigated from 2 – 9 November, re-irrigation commence as from 10 February.
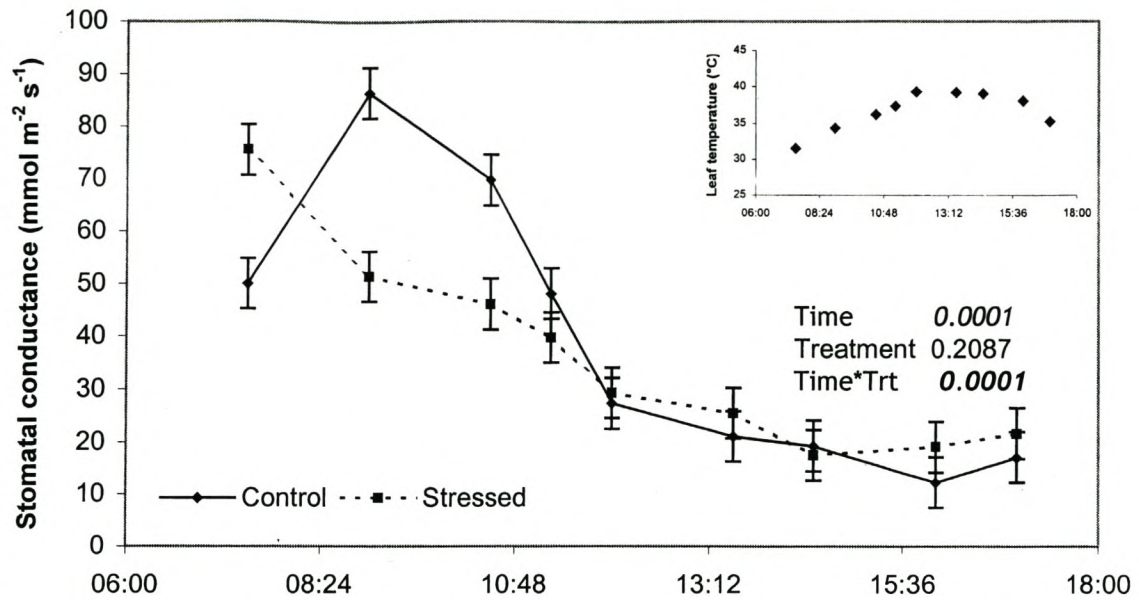
FIGURE 8. Stomatal conductance measured diurnally on 9 February 2000 at Addo. M easurements w ere t aken t hroughout the day and treatments were Control (irrigated daily) and Stressed (trees without water for seven days). Values are means (n = 15) with standard error b ars. A verage d iurnal l eaf temperature is shown as insert.
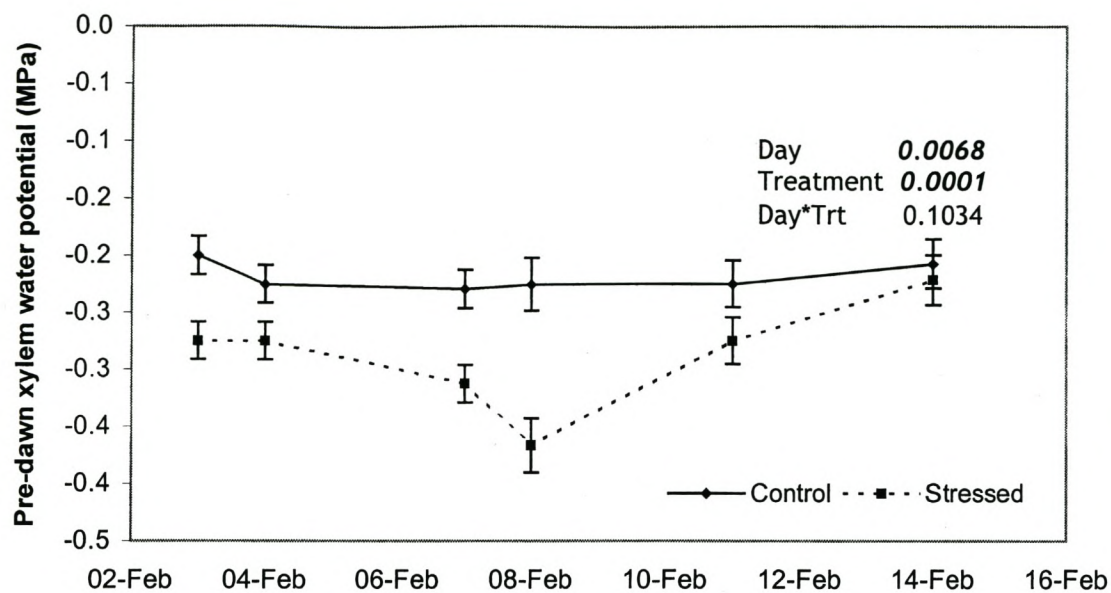
FIGURE 9. Pre-dawn water potential measured at Addo on 3 - 14 February 2000. The treatments were Stressed (trees not irrigated as from 2 February and irrigation re-commenced on 9 February) and Control (trees irrigated daily).
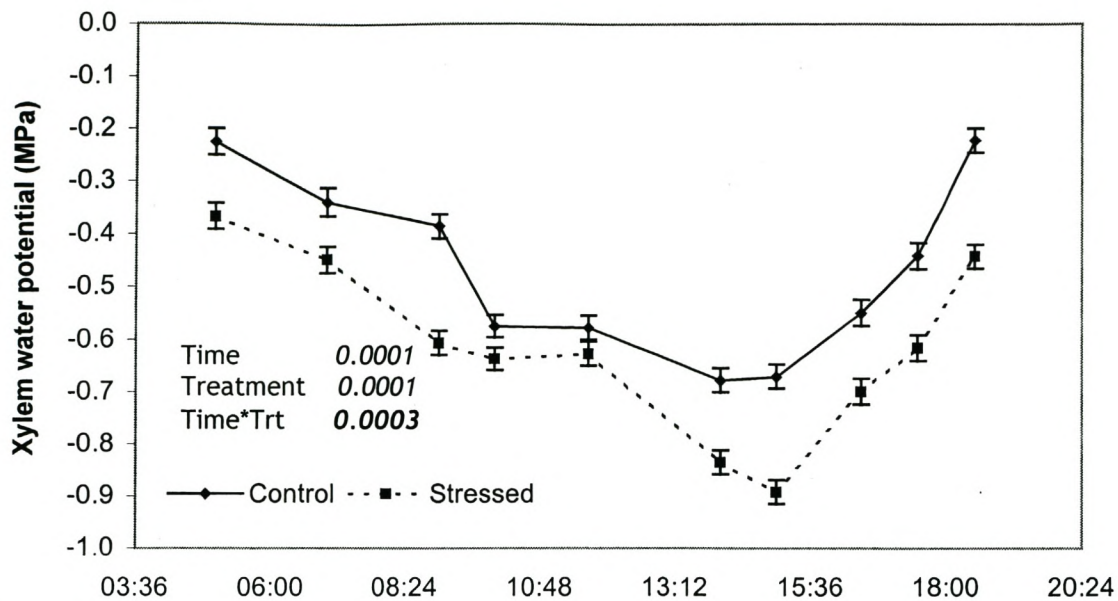
FIGURE 10. Diurnal water potential measured at Addo on 8 February 2000. The treatments were Control (trees irrigated daily) and Stressed (trees not irrigated for six days).
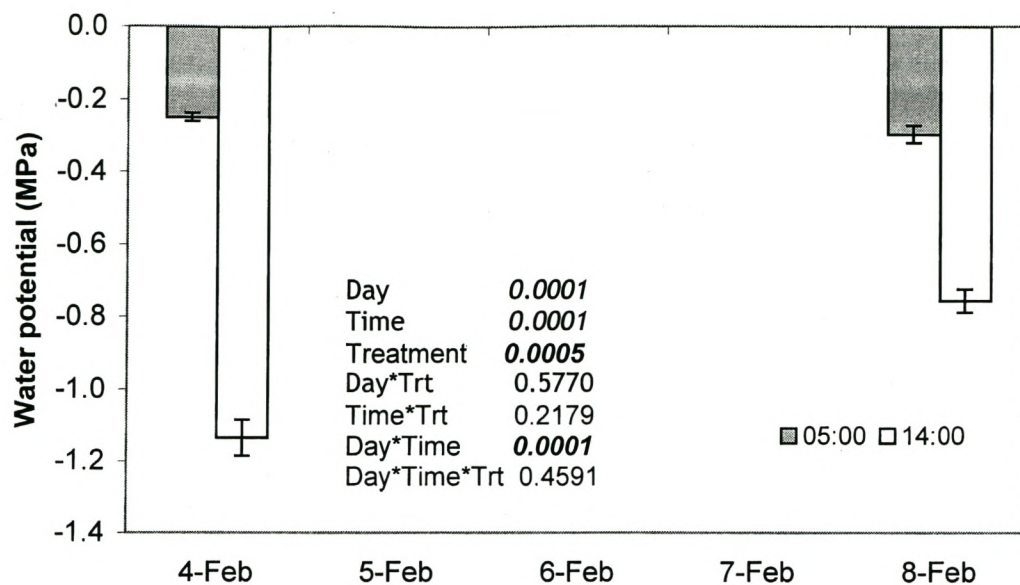
FIGURE 11. Day*Time interaction of xylem water potential measured at Addo on 4 and 8 February 2000. Measurements were made at 05h00 and 14h00.

CONCLUDING REMARKS: **OPEN HYDROPONIC FERTIGATION, A GENERAL DISCUSSION**

In this report I will give a general overview of the open hydroponic concept and follow that with a discussion of the work we did on the physiological measurements on these plants and proposed future research.

OPEN HYDROPONICS

Open hydroponics as alternative orchard irrigation and fertilisation regime was introduced in South Africa during the early 1990's. The concept was developed by Prof. Valero-Martinez and is a paradigm shift away from conventional systems such as micro-irrigation and broadcast-fertilised orchards and even from drip fertigation (Woods, 1999). Open hydroponics is a sensitive nutrient and moisture management system with which a high degree of control over the development of the crop can be exercised (Stassen *et al.*, 1999). Water and nutritional requirements are applied daily via a nutrient solution (fertigation), specific for the phenological phase of the plants, carefully balanced and pH as well as EC controlled.

After the initial introduction, a number of alternative systems based on the same principles were developed. We use the term daily daylight fertigation (DDF) to include the alternative systems in our discussion and because the true hydroponics imply an inert medium, and in these systems the soil does still play a role, although much smaller than in conventional systems.

Drip irrigation facilitated the general use of fertigation. It became possible to target apply the nutrient solution to a limited root zone. By daily fertigation, the roots develop close to and in the wetted zone, thereby keeping the rooting volume small relative to trees under conventional micro irrigation regimes with large rooting volumes (50 – 80 times smaller; Martinez, pers. comm.). Flooding of the whole root system does not occur. Therefore the roots are always well aerated. Plants are often ridged for quicker leaching, aerating the soil and higher soil temperatures, and causing better nutrient uptake. A small

rooting volume theoretically enables easy tree manipulation. Water stress can be introduced quickly, if necessary, to e.g. enhance the sugar concentration in the late maturation phase. Trees can react quickly to changes in the root environment and easy treatment of root diseases is possible (all the roots are within close reach of the irrigation system). Expensive fertilisers, additives and phytosanitary chemicals can be used since relatively small quantities will reach the greater part of the root system and when scheduling is done well, very little is leached beyond the root zone.

Vegetative growth is the first parameter to decline in response to increasing water stress. Photosynthesis starts to decline only at very low water potentials. Therefore, by letting plants operate at mild levels of water stress, the vegetative growth can be kept at a minimum without decreasing photosynthesis, thereby selecting for reproductive growth, distributing sugars to fruit. By variation of the nutrient mixture levels, phonological stages can also be manipulated.

As already stated, additives, e.g. amino acids, sugars and plant growth regulators can be applied to the roots via the irrigation system with the macro- and microelements.

Irrigation and fertigation scheduling is done in a novel way. Firstly, the annual water use (by evapotranspiration levels) and nutrient requirements (by nutrient element removal per ton of fruit) are calculated for a specific crop size. The tree phenology is then used as base for the scheduling of element quantities partitioned for the specific phase (e.g. re-activation, flowering, fruit set, fruit growth, maturation and post-harvest). Daily portions for each phase are calculated and given over the day with the required water quantity as determined by evapotranspiration, scheduled in pulses from the early morning through midday and late afternoon.

By keeping the EC constant in the soil with daily fertigation roots are not required to adapt to lower soil water potentials as soil drying increases and thereby soil EC increases. Water is therefore always available at the same

levels of matrix and osmotic potential. The nutrient solution is balanced, implying that even poor quality water can be used. High levels of sodium would not be taken up if high enough levels of potassium are applied, etc. By keeping the pH at a constant optimum level, nutrient and micro-element uptake is optimal and root growth is not impaired. This is possible because water is the rooting medium and it can be easily adjusted by buffering.

Healthy, long living and supposedly very efficient root hairs develop under daily daylight fertigation. Irrigation scheduling is done as an integration of all environmental and cultural practices in a measurement of relative growth. Trees in the three monitor rows are irrigated normally, double normal rate and half normal rate: fruit growth or shoot growth are measured weekly to determine which irrigation regime is most accurate. This method is very crude and should be refined, but could act as a good additional check.

In citrus species sap flow is low, but fairly constant since transpiration is relatively stable at mild levels of water deficit. The plant's hydraulic properties and the atmospheric demand (vapour pressure deficit) determine the rate of transpiration. Stomata close to keep the rate constant for the tree and will only decrease at very low soil water potentials.

It is speculated that trees in daily daylight fertigation orchard situations constantly sense a degree of stress, even when irrigated at optimal levels. It is proposed that trees are in simulated split root system pots, constantly having roots in both the wet drip zone as well as the dry zone between the drip onions. Such roots may produce ABA in response to the water deficit stress in the dry areas and may increase the base level of stress sensed by the tree. Such small root systems could also be subject to quick soil drying.

On days with low humidities, trees experience midday depression in photosynthesis and stomatal conductance, regardless of the soil water content. It is proposed that producers optimise plant productivity during the morning with its natural climax in these factors during the mid/late morning. An unstressed tree will probably have higher rates of photosynthesis and stomatal conductance since it operates at optimal conditions. As soon as the

midday depression starts, the differences disappear between these and trees under conventional irrigation regimes.

The basic requirements of a DDF system (Rabe 2002, unpublished) is firstly pressure controlled drippers delivering 1.5 to 4.0 litres of water per hour at a 0.6 to 1.5 meter spacing. The balanced fertigation solution containing all the essential elements for plant growth is applied in two or more pulses during the day, therefore a pump and irrigation system able to create pressure on the laterals within a very short time is necessary. The fertigation solution must also be pH and EC controlled. The mixture is done from three to four tanks containing:

Tank 1 -   N/P/K/Mg and micro-elements (1-5 L/m^3 water)

Tank 2 -   Ca/Mg (0.5-4 L/m^3 water)

Tank 3 -   A pH control (acid or alkali at 0.05 – 2 L/m^3 water)

Tank 4 -   An acid (or alternative) cleaning solution (alternatively can be included in tank 1 if pH needs reduction)

Logging of pH, EC, the flow rate and irrigation time is necessary.

A comparison of the data on DDF and conventional irrigation systems from Article 1, 2 and the literature and the popularly accepted dogma are summarised as follows:

Parameter	Accepted dogma	Research data	
		DDF system	Conventional system
Sap flow	Dramatic decrease with time after irrigation (e.g. decrease of 50% of initial sap flow on second day after irrigation).	Little difference over time, even after four days of stress. Sap flow is determined by tree hydraulic characteristics and leaf to air VPD.	Data over time not available.
Xylem water potential	A decrease with time as the soil dries out.	Observed to be a very sensitive parameter to water deficit stress.	Observed to be a very sensitive parameter to water deficit stress.
Stomatal conductance	Midday depression in stomatal conductance (and photosynthesis) primarily a result of soil drying and mainly in water-stressed trees.	Midday depression in stomatal conductance occurs in all trees primarily because of high VPD and ambient temperatures and secondarily because of soil water deficit.	Midday depression in stomatal conductance occurs in all trees primarily because of high VPD and ambient temperatures and secondarily because of soil water deficit.
Chlorophyll fluorescence	Indication of physiological stress (e.g. heat, water, cold, herbicide damage, ozone, etc.).	Recent literature shows high sensitivity in many fields, but not for water deficit stress in some woody evergreens (i.e. citrus), since a variety of light dissipation pathways protect the photosystems from photo-inhibition (i.e. photorespiration and the xanthophyll cycle).	Recent literature shows high sensitivity in many fields, but not for water deficit stress in some woody evergreens (i.e. citrus), since a variety of light dissipation pathways protect the photosystems from photo-inhibition (i.e. photorespiration and the xanthophyll cycle).

PHYSIOLOGICAL MEASUREMENTS OF OPEN HYDROPONIC CITRUS TREES

A)   We assessed the ascribed benefits of the use of OHS rather than conventional micro- and drip-irrigation systems. Citrus trees under daily daylight fertigation and conventional micro-jet and drip-irrigated regimes were monitored to establish plant stress levels as indicated by sap flow, xylem water potential, stomatal conductance and chlorophyll a fluorescence

We concluded that plants under a daily daylight fertigation regime have excellent soil water conditions in their rooting volume, and therefore experience negligible baseline levels of stress. The limited root volume does not, however, supply water to the same extent as a larger root volume in response to high atmospheric demand. Therefore, the trees experience midday depressions in stomatal conductance, similar to trees under conventional regimes. It appears as if a larger rooting volume enhances recovery from the midday depression. It is recommended that producers optimise the morning hours, by early irrigation, so that plants can function optimally, whilst environmental conditions are most favourable, resulting in the highest physiological activity.

B)   The effect of withholding water from trees adapted to a daily daylight fertigation regime, by artificial drying treatments, was evaluated in an attempt to determine the risk involved with short-term water deficits in trees adapted to this regime, as well as the usefulness of physiological techniques for identifying water stress. These studies were just the beginning of the research on the physiological implications open hydroponic systems on citrus trees. More research is necessary, specifically regarding the influence of physiological events on horticultural characteristics.

Stomatal conductance and xylem water potential indicated water stress sooner than the other physiological parameters. Citrus trees seem to be relatively insensitive to water deficit stress as measured by sap flow and chlorophyll a fluorescence. Sap flow is buffered by tree capacitance, and

although mediated via stomatal conductance, atmospheric conditions and not the soil water content primarily determine it. Daily fertigation is applied to trees under DDF regimes and they exhibit more optimal levels of xylem water potential and stomatal conductance, compared to trees from which water is withheld. A lthough alleviating it, daily irrigation did not mitigate the midday depression in these values. Seen over a season, even small enhancements of stomatal conductance (and with it photosynthesis and possibly, growth) and xylem water potential, could incrementally produce higher yields.

FUTURE RESEARCH

Research into horticultural differences between conventional and daily daylight fertigated orchards is necessary, specifically fruit and yield characteristics in relation to the observed physiological differences. More research i s a lso n eeded i nto d aily d aylight fertigated plants' photosynthesis and their water relations, including the effect of capacitance. M easures o f mitigating the midday depression are needed.

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