WATER REQUIREMENTS OF BEDDING PLANTS: MANAGING SUBSTRATE WATER CONTENT AND STUDYING PHYSIOLOGICAL AND GROWTH RESPONSES OF PLANTS TO VARYING LEVELS OF WATER CONTENT

by

MURTHY NEMALI SAINATH KRISHNA

(Under the direction of Marc van Iersel)

ABSTRACT

Physiological and growth responses of bedding plants to substrate water content (θ) were studied after calibrating ECH_2O moisture sensors for water content, salinity, and temperature of the substrate, studying substrate (peat-perlite) water retention characteristics (SWRC), and developing an automated system for maintaining distinct set points of θ. Based on SWRC, the distinct θ treatments resulted in a broad range of water potentials (Ψ). Results indicate that maximum photosynthesis, Ψ, and quantum efficiency of leaves was highest at a θ of 0.22 or 0.32 $m^3 \cdot m^{-3}$ in all species. In a separate study, when the θ was allowed to dry down, the growth rate of a drought-tolerant (vinca) and -sensitive (salvia) species declined at a θ of 0.10 and 0.15 $m^3 \cdot m^{-3}$, respectively. Bedding plants responded to low θ by various mechanisms i.e., lower mesophyll resistance to CO_2 transfer (e.g., petunia), robust photosystem II (e.g., vinca, salvia, petunia), and lower leaf osmotic potential (e.g., vinca).

INDEX WORDS: A–Ci curves, automated watering system, chlorophyll fluorescence, dielectric constant, mesophyll resistance, pore-size distribution

WATER REQUIREMENTS OF BEDDING PLANTS: MANAGING SUBSTRATE WATER CONTENT AND STUDYING PHYSIOLOGICAL AND GROWTH RESPONSES OF PLANTS TO VARYING LEVELS OF WATER CONTENT

by

MURTHY NEMALI SAINATH KRISHNA

B.S., Acharya N. G. Ranga Agricultural University, India, 1992

M.S., The University of Georgia, 2002

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2005

WATER REQUIREMENTS OF BEDDING PLANTS: MANAGING SUBSTRATE WATER CONTENT AND STUDYING PHYSIOLOGICAL AND GROWTH RESPONSES OF PLANTS TO VARYING LEVELS OF WATER CONTENT

by

MURTHY NEMALI SAINATH KRISHNA

Major Professor: Marc W. van Iersel

Committee: David Radcliffe
Robert Teskey
Mark Rieger
Paul Thomas

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
December 2005

DEDICATION

I dedicate my dissertation to my wife (Aparna) and son (Ajay) whose unfailing support, love, and patience has brought me so far, and to my parents for their unconditional love, and always being there for me.

ACKNOWLEDGMENTS

I thank my major advisor Dr. Marc van Iersel, for supporting me and sharing his valuable scientific knowledge. His continued help and advisement were one of the primary reasons for the success of this project. I thank him for his valuable comments on my dissertation.

I thank Dr. Radcliffe for providing the lab facilities and equipment during my project. I thank Dr. Teskey for providing equipment for this project.

I thank Dr. Radcliffe, Dr. Teskey, Dr. Rieger, and Dr. Thomas, for serving in my committee, reviewing, and providing valuable comments on the dissertation.

I thank Mark Blonquist, Apogee Instruments, for his helpful comments on chapter 2.

I extend my special thanks to Francesco Monetsano, Università degli Studi di Bari, Italy, and all graduate students, research technicians, and staff members of UGA Horticulture for their help and providing a friendly working environment.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

Page

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Purpose of the study

Under optimal environmental conditions, the growth and quality of bedding plants largely depend on the amount of water and nutrients supplied during production. Nutrients for bedding plants are supplied with irrigation water, therefore irrigation management is among the most important operations controlling the growth and quality of bedding plants. In recent years, regulations on agricultural water use have become stricter (e.g., Maryland's Water Quality Improvement Act; Lea-Cox and Ross, 2001) due to increased urbanization, population growth, and decreased water resources for agriculture (including horticulture). In light of this, there has been an increased awareness among greenhouse growers to resort to more efficient irrigation practices to cope with government regulations and reduce wastage of good quality irrigation water. Presently, efficient irrigation management for bedding plants can be difficult owing to the following reasons:

(I) the information on minimal water content to be maintained in the substrate for normal plant growth (or avoiding drought stress) is lacking,

(ii) information on physiological responses of bedding plants to substrate water content is limited,

(iii) there is inadequate information on the water retention properties of soilless substrates,

(iv) affordable and reliable moisture sensors to accurately measure the water status in soilless substrates are unavailable,

(v) efficient irrigation systems that can supply water to substrate with the least amount of wastage are unavailable.

Hence, the current study was undertaken to identify solutions and develop information required for efficient irrigation management of bedding plants. Using the information from this research, it is hoped that greenhouse (particularly bedding plant) growers will irrigate crops with minimal water wastage and simultaneously reap the benefits of novel technology which can reduce labor costs and aid in growing good quality plants.

Water requirements of bedding plants

The scientific literature on bedding plants spans over a wide range of topics, including plug production, fertilizer requirements, environmental controls, growth regulation, and postharvest care. Water, as the medium for the metabolic reactions in plants, primarily controls plant growth. However, information pertaining to water requirements of bedding plants is limited in literature. This is perhaps due to two misassumptions, i.e., that the production scale of greenhouse crops is too small to affect water resources, and that the substrate for these high-value crops is mostly maintained at high water content. Neither assumption is realistic. Greenhouse growers in the western and southeastern US are facing water restrictions, because of increased

water-use. Water status in the shallow and small containers used for producing bedding plants fluctuates considerably to a point where water limitations usually affect plant growth (Milks et al., 1989).

Optimal irrigation not only supplies water in adequate amounts and at required intervals for maximum crop growth, but also maintains proper pore space and nutrients in the growing medium. Irrigation for bedding plants can be scheduled by determining either plant or substrate water status. Plant water status is mostly determined from leaf water potential, although water potential in the xylem and root cells can be determined as well. However, there are many limitations in using estimates of plant water status for scheduling greenhouse irrigation. Firstly, these techniques are time-consuming and destructive in nature. Secondly, they should be estimated with high accuracy using expensive equipment and preferably, by trained persons. Thirdly, they are not easy to interpret. Nonetheless, these measurements are highly valuable for scientific research. In contrast, irrigating bedding plants by assessing substrate water status is relatively simple and easy. Substrate water status can be assessed by both water potential and water content, between the two, water content is easier to measure and interpret.

Physiological responses of plants to substrate water content.

A knowledge of the effects of substrate water content on physiology of bedding plants is central to determining the water requirements of bedding plants. Photosynthesis, which is the primary mechanism for plant growth, is usually insensitive as substrate water content falls to a threshold level, below which the photosynthetic rate will drop quickly with further decrease in water content (McCree, 1986; Gindaba et al., 2005; Xu and Zhou, 2005). Stomates close in response to drought stress to reduce

transpiration (Sperry et al., 2002) and prevent subsequent death of plants due to dehydration, however, at the cost of decreased photosynthesis due to decreased CO_2 conductance (Jones, 1985). This implies that the primary limiting factor of photosynthesis in plants during drought is reduced CO_2 levels inside leaves due to decreased conductance through stomates. However, it appears that this is not completely true as it has been shown that, at severe drought stress, photosynthesis is limited not by low CO_2 levels inside leaves, but by low ATP levels (*Helianthus annuus* L.; Tezara et al., 1999). Increasing the CO_2 concentration had no effect on the photosynthetic rate of severely drought-stressed sunflower plants (*Helianthus annuus* L.; Tezara et al., 1999).

A decrease in ATP production during drought implies that the excitation energy gained from absorbed light is not channeled through photochemistry. To prevent damage to the photosynthetic apparatus due to excess energy that is not used in photochemistry, plants have developed mechanisms to dissipate the excess energy as heat or non-photochemical quenching process (Lawlor, 2002). Because of this, the quantum efficiency of photosystem II (ability to utilize the absorbed light) is usually not affected by drought when measured after the excess energy is completely dissipated, i.e. in the dark (Epron, 1997). However, a slight decrease in dark adapted quantum efficiency (Fv / Fm) of photosystem II can be seen after exposing plants (*Vitis vinifera* L.) to severe drought stress (Flexas et al., 1999). It is likely that, during severe drought stress, complete dissipation of excess energy is not possible, hence some damage to photosystem may be inevitable. Giardi et al. (1996) reported that the number of active photosystem II centers was reduced under long-term drought stress in plants.

Several researchers have addressed the issue of water requirements of plants by measuring leaf photosynthesis at different substrate water contents (Chapman and Augé, 1994; Arndt et al., 2002; Centritto et al., 2004; Inoue et al., 2004; Gindaba et al., 2005; Xu and Zhou, 2005; Zhang et al., 2005). The main reason for measuring leaf photosynthesis in plants to understand their water requirement is the fact that photosynthesis is the primary mechanism for carbon fixation in plants and that it is sensitive to changes in the substrate water content. It has been shown that leaf photosynthetic rate in plants will decrease below a threshold water content (Gindaba et al., 2005; Xu and Zhou, 2005) which can be used as the 'critical' level for metabolism in plants. However, the threshold water content for growth can not be accurately determined from leaf photosynthesis responses; for growth one has to measure whole-plant photosynthesis and respiration. Growth in plants is a consequence of the available carbon synthesized in photosynthesis after accommodating respiratory loss (Amthor 1984, Lawlor, 1995; Nemali and van Iersel, 2004). Owing to differences in photosynthetic rate of leaves at different positions in a canopy, leaf measurements can not predict plant growth (McCree, 1986; Nemali and van Iersel, 2004). However, leaf measurements are generally good to study plant responses and acclimation in plant responses. Photosynthesis – CO_2 responses are used to quantify stomatal and non-stomatal limitations to photosynthesis in plants at different levels of water content (Jones, 1985; Earl, 2002; Grassi and Magnani, 2005). Also, this technique can be useful to screen plant material for tolerance to drought or capability to withstand low substrate water contents (Earl, 2002).

Many other techniques and analysis tools are currently used in conjunction with regular photosynthesis measurements to study water requirements of plants. Chlorophyll fluorescence measurements are commonly used to study the efficiency of photosystem II to utilize the absorbed light under different substrate water contents (Giardi et al., 1996; Flexas et al., 1999; Epron, 1997; Tezara et al., 1999; Colom and Vazzana, 2003). As the fraction of absorbed light used in photochemistry is directly correlated with the rate of electron transport and photosynthesis, chlorophyll fluorescence measurements can indicate possible reasons for changes in photosynthesis under different levels of water content.

Acclimation in plants in response to drought stress.

Plants are plastic to changes in their environment and have evolved various physiological adaptations to endure periods of drought stress (Chapman and Augé, 1994). As photosynthetic mechanisms are plastic to changes in the soil water content (Chaves et al., 2003; Watkinson et al., 2003), accuracy of findings will depend on studying plant responses during a long-term exposure to different levels of water content. Watkinson et al. (2003) have indicated that under a 3 to 4 day (rapid) draw down cycle, photosynthetic acclimation occurred under mild drought stress and photosynthetic failure occurred under severe drought, which were correlated to changes in RNA transcript profiles. It was also shown that when plants were exposed to different water content for long periods extending to several days or weeks, they can acclimate by maintaining their growth rates within the range of mild to moderate drought stress (Olson et al., 2002; Wahbi et al., 2005; Zhang et

al., 2005), though the photosynthetic mechanism is severely affected under extreme drought conditions.

Osmotic adjustment is a common mechanism seen in drought-tolerant plants. In response to a low substrate water potential (Ψ_w), plants adjust osmotically (by synthesizing compatible solutes) and decrease leaf osmotic potential (Ψ_s). Osmotic adjustment decreases Ψ_w in leaves and roots, to maintain the gradient in Ψ_w required for movement of water into plants under conditions of low Ψ_w (drought) in the soil (Hsiao and Xu, 2000; Serraj and Sinclair, 2002). Plants can also protect cell organelles by synthesizing osmo-protectants inside cells (Serraj and Sinclair, 2002). Because turgor is maintained due to osmotic adjustment, plants can continue to photosynthesize, however osmotic adjustment can only aid in continuation of photosynthesis within a limited range of low soil Ψ_w (Serraj and Sinclair, 2002). The acclimation of photosynthetic process to low substrate water content can also occur due to improved uptake of water due to increased root growth (Frensch, 1997. Hsiao and Xu, 2000), and altered root hydraulic conductivity (Steudle, 2000).

Soilless substrates and their water retention characteristics

Soilless substrates used in bedding plant production are usually mixtures of several components. Peat and pine bark are the two primary organic components used in the substrate. Pine bark is coarse in texture and may contain air pockets or hold an inadequate amount of moisture (Bilderback and Lorscheider, 1995). In contrast, sphagnum peat has good water holding capacity but is difficult to rewet after drying out (Olson et al., 2002). Certain other components, like vermiculite and perlite, are

commonly added to the substrate. Vermiculite is a clay mineral which can improve the texture (adds weight to the substrate for supporting plants), hold water, and nutrients. Vermiculite is commonly added to peat-based media. Perlite (popped volcanic rock), on the other hand is added to improve the drainage and decrease the water-holding capacity of the substrate.

The five variables governing the physical properties of any soilless substrate are total porosity, container capacity, available water, unavailable water, and airspace (Milks et al., 1989). Total porosity of any growing medium is the total amount of space occupied by non-solid components of the growing medium, for a given bulk density. It can be equated to the volume of water contained in a medium at saturation. Container capacity is the volume of water retained in a medium after drainage from saturation, but before evaporation. Available water is the volume of water held at container capacity minus volume of unavailable water and air space is total porosity minus container capacity, for a given bulk density of the medium.

The type of the substrate used in production can affect the volume of water held at any moisture tension. Milks et al. (1989) compared the physical properties of peat-based (1 peat : 1 vermiculite) and bark-based (3 bark : 1 sand : 1 peat) media. Their results show not much difference in air space and unavailable water between the two media, however peat based medium had higher available water than the bark based medium due to greater container capacity resulting from higher total porosity of the peat-based substrate.

Permanent wilting point in soilless substrates.

The definition of 'permanent wilting point' is the lowest water potential of a soil at which plants can access water (Lambers et al., 1998). Although the water potential at the permanent wilting point is assumed to be -15330 cm (or -1500 kPa, -15 bars, -1.5 MPa), the actual water potential at wilting point will depend on species and soil type (Taiz and Zeiger, 2002). Usually, the amount of water held in a regular 'soil' medium at the permanent wilting point is low (~ 0.05 to 0.10 $cm^3 \cdot cm^{-3}$; Bachmann et al. 2002; Prunty and Cassy, 2002; Chan and Govindaraju, 2004). It is assumed that the water in a soil medium at water potential lower than -15330 cm is unavailable to plants as it is held tightly as a film on the surface of soil particles ('hygroscopic' as opposed to 'capillary' water). For this reason, structure and pore size distribution are considered unimportant for water availability in soils at tensions below -15330 cm and the end point of soil moisture retention curves is assumed to be -15330 cm (van Genuchten, 1980; Zurmühl and Durner, 1996). Research (Fonteno et al., 1981; Drzal et al., 1999; Sahin et al., 2002) on substrate moisture retention (SMR) curves has indicated that the total volume of water retained by soilless substrates at -15330 cm is approximately in the range of 0.20 to 0.30 $cm^3 \cdot cm^{-3}$. However, bedding plants in soilless substrates wilt when the substrate water content falls below 0.10 to 0.15 $cm^3 \cdot cm^{-3}$ (Olson et al., 2002; Nemali and van Iersel, 2005), which appears to be well below a tension of -15330 cm. Hence there is a need to determine SMR curves in soilless substrates in a broader range and below a tension of -15330 cm in soilless substrates. Currently, there are few studies which determined SMR curves in soilless substrates within the entire range of 0 to -15330 cm (Fonteno et al., 1981; Drzal et al., 1999). Most studies developed relations

among substrate water content and water potential within the range of 0 to -1000 cm (Bilderback et al., 1982; Bilderback and Fonteno, 1987; Ingram and Yeager, 1987; Tilt et al., 1987; Fonteno and Nelson, 1990).

Pore size distribution in soilless substrates.

Pore space is the most important physical characteristic of a soil or soilless substrate as it retains water (and nutrients in the water), oxygen, and allows root growth. A primary factor affecting water retention is pore-size distribution (Ahuja et al., 1998; Stange and Horn, 2005). Pore size distribution refers to the relative volume of different size pores existing in a soil at any particular time. Pore size distribution and water retention are mutually interactive, with pore size determining the extent of water retention, and conversely water/hydraulic pressure influencing the pore size (Stange and Horne, 2005).

Hillel (1982) has designated pores as inter-aggregate or macropores for water infiltration/drainage and intra-aggregate or micropores for water retention. Earlier reports on soilless substrates have indicated that 40 (e.g., peat, coir) to 90% (e.g., rock wool) of total water is usually retained in macropores between tensions 0 to -10 kPa (Bilderback et al., 1982; Bilderback and Fonteno, 1987; Fonteno and Nelson, 1980; Raviv et al., 2001). Most of the water retained in macropores of a soilless substrate is usually lost in drainage which lowers the substrate water status from saturation to container capacity. The easily available water for plants in soilless substrates is usually held at a tension range of -50 to -500 cm. In this range, water is retained mostly in the large micropores. At substrate moisture tensions below -500 cm, water is held in the ultra micropores (Drzal et al., 1999). However, there is little information on water

retention below a tension of -1000 cm. In spite of its importance, relatively little work has been done to categorize pore size distribution in soilless substrates, especially at tensions > -1000 cm. Pore size distribution can be estimated from SMR curves (Milks et al., 1989; Zurmühl and Durner, 1996; van Vliet et al., 1998; Drzal et al., 1999; Coppola, 2000). An understanding of the distribution of different sizes of pores will aid us to see the 'internal' structure of the soil or soilless substrate (Drzal et al., 1999).

Moisture sensors for soilless substrates

Why are available moisture sensors unsuitable?

Currently there are several moisture sensors available for measuring water potential and water content in substrates. Soil moisture sensors like tensiometers (Van Der Veken et al., 1982; Smajstrla and Locascio, 1996; Krüger et al.,1999), neutron probes (Black and Mitchell, 1968; Gear et al., 1977; McFall, 1978) and time domain reflectometry (TDR) probes (Topp and Davies, 1985) are popular. However, these moisture sensors are rarely used to control irrigation in potted bedding plant production. Potted bedding plants are commonly irrigated based on the visual appearance of the substrate or plants, or with the use of irrigation timers.

High costs, unsuitable size, and unreliable measurements of the available moisture sensors are the main reasons for not using moisture sensors to control irrigation in bedding plant production. For example, sensors like TDR probes can provide reliable measurements, but the required meter is expensive. To optimize space utilization, greenhouse crops are grown in small containers. This limits the suitability of sensors like neutron probes which require a large volume for installation and measurement.

Soilless substrates have high porosity and a large fraction of pores are filled with air. When a moisture sensor like a tensiometer is inserted into soilless substrates, a significant area of the sensor surface may be in contact with air. This could result in cavitation, causing erroneous and unreliable measurements. As it is difficult to hold the tensiometer firmly in a soilless substrate, the sensor is easily displaced which can cause a loss of contact between the tensiometer cup and the substrate. The ability of tensiometers to control irrigation in container production under high light and temperature conditions, and especially at low soil water potentials (more negative) was found to be substantially low (Hansen and Pasian, 1999). Moreover, tensiometers can only be used to measure matric potentials up to -80 kPa.

Screening new moisture sensors for suitability in soilless substrates.

Recently two moisture sensors, i.e., the ECH_2O dielectric aquameter (Decagon Devices, Pullman, WA, USA) and the theta probe ML2X (Delta–T devices, Cambridge, UK) have become available. The cost of these sensors is in the low to medium range. A set of five ECH_2O probes with a datalogger (EM 50, Decagon Devices) and software costs approximately $900 and ECH_2O probes are much cheaper when purchased in bulk. The price of a Theta probe ML2X with HH2 moisture meter for measurement is approximately $1000. These probes are available in convenient sizes. The sensor length for Theta probe is 6 cm and ECH_2O probes are currently available in 10 and 20 cm length. In spite of these advantages, these probes have never been tested for use in soilless substrates.

Both ECH_2O and Theta probes estimate the substrate water content by indirectly measuring the dielectric permittivity (or dielectric constant) of the substrate. Dielectric

permittivity can be understood as the inherent ability of any material to become polarized in an electromagnetic field. As water is a polar molecule, the dielectric permittivity of water is large (~80.4 at a temperature of 20 ^{o}C). Hence it is the main component contributing to the dielectric permittvity of a moist soilless substrates (called 'real permittivity'). Although it has been indicated that water is the major factor affecting dielectric permittivity of a moist substrate, other substrate related factors like air, solid matrix, electrical conductivity (EC) or salinity, temperature, and bulk density can affect bulk dielectric permittivity of a substrate. Of these factors, substrate air (~1) and matrix (~2 to 8) have a small contribution to bulk dielectric permittivity and their changes have a small overall effect on bulk dielectric permittivity. However, other factors like substrate temperature, EC, and bulk density can cause dielectric loss and potentially affect the permittivity of a substrate by raising or lowering the measured dielectric permittivity (called 'imaginary permittivity'). Usually in calibration of dielectric moisture sensors, an analytical relationship between changing water content and the associated change in dielectric permittivity is developed, and the volumetric water content of the substrate is estimated indirectly (Topp, 2003). However, if the contribution of imaginary permittivity is large, their affects have to be considered in estimating water content.

The details of the measuring technique for the Theta Probe can be obtained from Gaskin and Miller (1996). The Theta probe is equipped with an oscillator to send a 100 MHz signal (electromagnetic wave) into the built-in transmission line. The transmission line consists of an array of four coaxial rods and has an impedance (resistance) to the signal flow. The impedance of the transmission line depends on the medium surrounding the coaxial rods. When the transmission line is inserted into a soilless

substrate, the change in its impedance causes a proportion of the incoming signal to reflect back to the oscillator. The reflected signal interferes with the subsequent incoming signals to produce a standing wave along the transmission line. The amplitude of the standing wave is proportional to the impedance along the transmission line, which in turn is proportional to the dielectric permittivity of the substrate. The change in the amplitude can be measured as an analog voltage output. As the dielectric constant of the medium is proportional to the water content (Topp and Davies, 1985), changes in the volumetric water content cause changes in the amplitude of the standing wave or the analog voltage output. This principle is used in calibration of the probe.

The ECH_2O probes are capacitance probes equipped with a capacitor. Three copper plates run along the length of the probe (one of them connected to a positive terminal and the other two to a negative terminal), and form a parallel-plate capacitor. These plates are enclosed inside the body of the sensor. When the voltage is applied across the copper plates, an electromagnetic field is generated and charges the capacitor. The capacitance (amount of charge held at any voltage) of the capacitor changes when the sensor is inserted into a substrate. This is due to the interaction of the electromagnetic field with the substrate. When the sensor is inserted into a moist substrate (with a large dielectric constant), there is an increase in the capacitance of the capacitor and an increase in the time required to charge the capacitor. By keeping the applied voltage constant, and measuring the time required to charge the capacitor, its capacitance can be estimated. The dielectric constant can be estimated from the capacitance based on the area of and separation between the copper plates. The

region of measurement of the dielectric constant by ECH_2O probes lies in the fringed electromagnetic field in the substrate, which protrudes out of the body of the sensor (approximately 0.7 to 1 cm and runs along the both sides of the probe).

Greenhouse crops are supplied frequently with water-soluble fertilizers resulting in significant concentrations of ions of fertilizer salts in the substrate. The presence of charged particles, like ions of fertilizer salts, in the vicinity of the electromagnetic field generated by probes can attenuate the electromagnetic energy and influence the measurement of probes. Probe measurement can also be affected by substrate temperature. The dielectric permittivity of water decreases with increasing temperature (~0.4 K^{-1}). Temperature can also affect sensor electronics, in turn affecting the voltage output of probes. Fluctuations in the substrate temperature can be significant and may affect the measurement of the probes.

Efficient irrigation systems

Current automated irrigation systems.

Automated irrigation systems used currently in greenhouses are usually run by irrigation controllers set to a pre-determined schedule (e.g., to run at a particular time of the day and for a particular duration). Because the irrigation controllers water plants not based on the actual measurements of substrate water content, leaching and runoff from containers is a common phenomenon, and result in wastage of good quality irrigation water. To minimize water wastage from automated irrigation systems, there is a need to develop improved irrigation controllers which can irrigate based on substrate water status and to a desired substrate moisture level. Such controllers will aid greenhouse

growers to comply with stricter government regulations on water-use and fertilizer run-off (as bedding plants are mostly irrigated with a fertilizer solution).

Automated irrigation controller for imposing drought treatments in research.

The inability to irrigate the substrate to a desired water content imposes a limitation on the use of currently available irrigation systems in physiological experiments related to studying water requirements of plants. As it is required to maintain the substrate at desired levels to study plant responses at distinct water contents, experiments in the field of plant water relations are conducted by manually maintaining different substrate water contents. This method commonly involves weighing the containers in different water treatments daily and replenishing the fraction of water lost in transpiration (Sinclair and Ludlow, 1986; Ekanayake et al. 1993; Ray and Sinclair, 1988). This method is labor-intensive and in addition, changes in plant fresh mass are generally neglected. In some other studies, to overcome intensive labor work of the previously described technique, plant responses to substrate water content are studied by withholding irrigation and studying responses as substrate water content decreases. This is also not an ideal method as the rate at which drought stress develops after withholding water is usually faster in containers (due to the smaller volume of available water) than under natural conditions. Observed physiological responses in plants can be different for a rapidly-imposed and slowly-imposed drought stress (Cornic et al. 1987; Ludlow, 1987; Saccardy et al. 1996; Earl, 2003). In both these methods, it is not possible to have precise control over the rate at which drought stress is imposed (Earl, 2003). Hence, an irrigation controller which can water the substrate to a desired level will be useful in research related to plant-water relations.

Research objectives

Keeping these issues in mind, the following objectives were set in this research:

(I) to determine leaf physiological responses, particularly responses of photosynthesis, chlorophyll fluorescence, and water relations, of bedding plants to distinct levels of substrate water content,

(ii) to identify minimal substrate water content for normal growth of a drought–sensitive and drought–tolerant bedding plant species,

(iii) to develop SMR curves in soilless substrates in a broad range and well below -15330 cm to account for water retention from close to saturation to less than 0.10 $cm^{3} \cdot cm^{-3}$ in the substrate,

(iv) to calibrate the ECH_2O dielectric aquameter and Theta Probe ML2X for measuring the water content in soilless substrates and study the effect of substrate EC and temperature on probes,

(v) to develop a controller which can irrigate the substrate to a desired water content by measuring substrate water content.

Literature cited

Amthor, J.S. 1984. The role of maintenance respiration in plant growth. Plant Cell Environ. 7: 561-569.

Adams, W.W. III. and B. Demmig-Adams. 1992. Operation of xanthophyll cycle in higher plants in response to diurnal changes in incident sunlight. Planta 186:390-398.

Ahuja, L.R., F. Fiedler, G.H. Dunn, J.G. Benjamin, and A. Garrison. 1998. Changes in soil water retention curves due to tillage and natural reconsolidation. Soil Sci. Soc. Amer. J. 62: 1228-1233.

Arndt, S.K., S.C. Clifford, W. Wanek, H.G. Jones and M. Popp. 2110. Physiological and morphological adaptations of the fruit tree *Ziziphus rotundifolia* in response to progressive drought stress. Tree Physiol. 21: 705-715.

Augé, R.M., A.J.W. Stodola, J.L. Moore, W.E. Klingeman, and X.R. Duan. 2003. Comparative dehydration tolerance of foliage of several ornamental crops. Sci. Hort. 98: 511-516.

Bachmann, J., R. Horton, S.A. Grant, and R.R. van der Ploeg. 2002. Temperature dependence of water retention curves for wettable and water-repellant soils. Soil Sci. Soc. Amer. J. 66: 44-52.

Barker, D.H., L.R. Stark, J.F. Zimpfer, N.D. McLetchie, and S.D. Smith. 2005. Evidence of drought induced stress on biotic crust moss in the Mojave desert. Plant Cell Environ. 28: 939-947.

Baumhardt, R.L., Lascano, R.J., Evett, S.R., 2000. Soil material, temperature, and salinity effects on calibration of multisensor capacitance probes. Soil Sci. Soc. Amer. J. 64, 1940-1946.

Bilderback, T.E., and W.C. Fonteno. 1987. Effects of container geometry and media physical properties on air and water volumes in containers. J. Environ. Hort. 5:180-187.

Björkman, O. And B. Demmig. 1987. Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77K among plants of diverse origins. Planta 170:

489-504.

Bilderback, T.E., W.C. Fonteno and D.R. Johnson. 1982. Physical properties of media composed of peanut hulls, pine bark, and peat moss and their effects on Azalea growth. HortScience 107:522-525.

Bilderback, T.E. and M.R. Lorscheider. 1995. Physical properties of double processed pine bark: effects on rooting. Acta Hort. 101: 77-83.

Black, J.D.F., Mitchell, P.D., 1968. Near surface soil moisture measurement with neutron probe. J. Aust. Inst. Agric. Sci. 34, 181.

Centritto, M., S. Wahbi, R. Serraj and M.M. Chaves. 2005. Effects of partial root zone drying (PRD) on adult olive tree (*Olea europaea*) in field conditions under arid climate II. Photosynthetic responses. Agric. Ecosystems Environ. 106: 303-311.

Chan, T.P., and R.S. Govindaraju. 2004. Estimating soil water retention curve from particle size distribution data based on polydisperse sphere systems. Vadose Zone J. 3: 1443-1454.

Chapman, D.S. and R.M. Augé. 1994. Physiological mechanisms of drought resistance in four native ornamental perennials. J. Amer. Soc. Hort. Sci. 119:299-306.

Chapman, D.S. and R.M. Augé. 1994. Physiological mechanisms of drought resistance in four native ornamental perennials. J. Amer. Soc. Hort. Sci. 119:299-306.

Chaves, M.M., J.P. Maroco, and J.S. Pereira. 2003. Understanding plant responses to drought - from genes to the whole plant. Functional Plant Biol. 30: 239-264.

Close, D.C., C.L. Beadle and M.J. Hovenden. 2001. Cold-induced photoinhibition and

foliar pigment dynamics of *Eucalyptus nitens* seedlings during establishment. Aust. J. Plant Physiol. 28:1133-114.

Colom, M.R. and C. Vazzana. 2003. Photosynthesis and PSII functionality of drought resistant and drought sensitive weeping lovegrass plants. Environ. Exp. Bot. 49: 135-144.

Coppola, A. 2000. Unimodal and bimodal descriptions of hydraulic properties for aggregated soils. 64: 1252-1262.

Cornic, G., Papageorgiou, I., and Louason, G. 1987. Effect of a rapid and a slow drought cycle followed by rehydration on stomatal and non-stomatal components of photosyntheis in Phaseolus vulgaris L. J. Plant Physiol. 126: 309-318.

Drzal, M.S., W.C. Fonteno and K.D. Cassel. 1999. Pore fraction analysis: A new tool for substrate testing. Acta Hort. 481:43-54.

Earl, H.J. 2002. Stomatal and non-stomatal restrictions to carbon assimilation in soybean (Glycine max) lines differing in water use efficiency. Environ. Exp. Bot. 48: 237-246.

Earl, H.J. 2003. A precise gravimetric method for simulating drought stress in pot experiments. Crop Sci. 43: 1868-1873.

Ekanayake, I.J., De Datta, S.K., and Steponkus, P.L. 1993. Effect of water deficit stress on diffusive resistance, transpiration, and spikelet desiccation of rice (*Oryza sativa* L.) Ann. Bot. 72: 73-80.

Elliot, G. 1990. Reduce water and fertilizer with ebb- and- flow. Greenhouse Grower 8: 70-72, 74-75.

Epron, D. 1997. Effects of drought on photosynthesis and on the thermotolerance of

photosystem II in seedlings of cedar (*Cedrus atlantica* and *C. libani*). J. Expt. Bot. 48: 1835-1841.

Epron, D. 1997. Effecys of drought on photosynthesis and on the thermotolerance of photosystem II in seedlings of cedar (*Cedrus atlantica* and *C. libani*). J. Expt. Bot. 48: 1835-1841.

Evans, J.R. and H. Poorter. 2001. Photosynthesis acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. *Plant, Cell and Environ*. 24:755-767.

Flexas, J., J.M. Escalona, and H. Medrano. 1999. Water stress induces different levels of photosynthesis and electron transport rate regulation in grapevines. Plant Cell Environ. 22: 39-48.

Fonteno, W.C., D.K. Cassel, and R.A. Larson. 1981. Physical properties of three container media and their effect on poinsettia growth. J. Amer. Soc. Hort. Sci. 106:736-741.

Fonteno, W.C., and P.V. Nelson. 1990. Physical properties of and plant responses to rockwool-amended media. J. Amer. Soc. Hort. Sci. 115: 375-381.

Frensch, J. 1997. Primary responses of root and leaf elongation to water deficits in the atmosphere and soil solution. *J. Expt. Bot*. 48: 985-999.

Gaskin G.J., Miller J.D., 1996. Measurement of soil water content using a simplified impedance measuring technique. J.Agric. Res. 63, 153-160.

Gear, R.D., Dransfield, A.S., Campbell, M.D., 1977. Irrigation scheduling with neutron probe. J. Irrigation and Drainage Division-ASCE. 103, 291-298.

Giardi, M.T., A. Cona, B. Geiken, T. Kučera, J. Masojìdek, and A.K. Mattoo. 1996. Long-term drought stress induces structural and functional reorganization of photosystem II. Planta 199: 118-125.

Gindaba, J., A. Rozanov, and L. Negash. Photosynthetic gas exchange, growth and biomass allocation of two Eucalyptus and three indigenous tree species of Ethiopia under moisture deficit. Forest Ecol. Management 205: 127- 138.

Gong, Y., Cao, Q., Sun, Z., 2003. The effects of soil bulk density, clay content, and temperature on soil water content measurement using time-domain reflectometry. Hydrol. Proc. 17, 3601-3614.

Grassi, G. and F. Magnani. 2005. Stomata, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. Plant, Cell and Environ. 28: 834-849.

Hansen, R.C. and C.C. Pasian. Using tensiometers for precision micro irrigation of container grown roses. Applied Eng. Agric. 15: 15: 483-490.

Hsiao, T.C. and L.K. Xu. 2000. Sensitivity of growth of roots versus leaves to water stress: biophysical analysis and relation to water transport. J. Expt. Bot. 51: 1595-1616.

Hillel, D. 1982. Introduction to soil physics. Academic Press, Inc. San Diego.

Ingram, D.L. and T.H. Yeager, 1987. Effects of irrigation frequency and a water-absorbing polymer amendment on *Ligustrum* growth and moisture retention by a container medium. J. Environ. Hort. 5: 19-21.

Inoue, T., S. Inanga, Y. Sugimoto, P.An and A.E. Enenji. 2004. Effect of drought on ear and flag leaf photosynthesis of two wheat cultivars differing in drought resistance. Photosynthetica. 42: 559-565.

Jury, W.A., W.R. Gardner, and W.H. Gardner. 1991. Soil Physics. 5 th ed. Wiley, New York, p. 328.

Kay, B.D., and D.A. Angers. 2001. Soil structure. M.E. Summner (ed.). Handbook of soil science. CRC Press. Boca Raton, FL.

Krall, J.P. and G.E. Edwards. 1992. Relationship between photosystem II activity and CO_2 fixation in leaves. Physiol. Plantarum 86: 180-187.

Krüger, E., Schmidt,G., Brückner, U., 1999. Scheduling strawberry irrigation based upon tensiometer measurement and a climatic water balance model. Scientia Hort. 81, 409-424.

Lawlor, D.W. 1995. Photosynthesis, productivity and environment. J. Expt. Bot. 46: 1449-1461.

Lawlor, D.W. 2002. Limitation to photosynthesis in water stressed leaves: stomata vs metabolism and role of ATP. Ann. Bot. 89: 871-885.

Lambers, H., Chapin III, F. S., and T.L. Pons. 1998. Plant water relations, chapter 3, In: plant physiological ecology, Springer-Verlag New York Inc., pp. 158-162.

Laisk, A. And F. Loreto. 1996. Determining photosynthetic parameters from leaf CO_2 and chlorophyll fluorescence - ribulose 1,5- bisphosphate oxygenase specificty factor, dark respiration in the light, excitation distribution between photosystems, alternate electron transport rate, and mesophyll diffusion resistance. Plant Physiol. 110: 903-912.

Lea-Cox, J.D. and D.S. Ross. 2001. A review of the federal clean water act and the Maryland water quality improvement act: the rationale for developing a water and nutrient planning process for container nursery and greenhouse operations. J. Environ. Hort. 19:226-229.

Ludlow, M.M. 1987. Contribution of osmotic adjustment to maintenance of photosynthesis during water stress. P. 161-168. In J. Biggens (ed.) Progress in photosynthesis research. Vol. 4. Martinus Nijhoff Publ., Dordrecht, the Netherlands.

Marenco, R.A., J.F.C. Gonglaves and G. Vieira. 2001. Leaf gas exchange and carbohydrates in tropical trees differing in successional status in two light environments in central amazonia. Tree Physiol. 21:1311-1318.

Maxwell, K. And N. Johnson. 2000. Chlorophyll fluorescence - a practical guide. J. Exp. Bot. 345: 659-688.

McCree. K.J. 1986. Whole plant carbon balance during osmotic adjustment to drought and salinity stress. *Aust. J. Plant Physiol*. 13:33-43.

McFall, R.L., 1978. Irrigation scheduling with neutron probe. J. Irrigation and Drainage Division-ASCE 104, 245.

Mebrahtu, T. And J.W. Hanover. 1991. Leaf age effects on photosynthesis and stomatal conductance of black locust seedlings. Photosynthetica 25: 537-544.

Milks, R.R., W.C. Fonteno, and R.A. Larson. 1989. Hydrology of horticultural substrates. III. Predicting air and water content of limited volume plug cells. J. Amer. Soc. Hort. Sci. 114: 57-61.

Morvant, J.K., Dole, J.M., and Ellen, E. 1997. Irrigation systems alters distribution of roots, soluble salts, nitrogen and pH in the root medium. HortTechnology 7:156-160.

Naasz, R., Michel, J.-C., and Charpentier, S. 2005. Measuring hysteretic hydraulic properties of peat and pine bark using a transient method. Soil Sci. Soc. Amer. J. 69: 13-22.

Nemali, K.S. and M.W. van Iersel. 2004. Two new moisture sensors for soilless growing media. HortScience 39:763.

Nemali, K.S. and M.W. van Iersel. 2005. Water requirements and drought tolerance of potted bedding plants. HortScience 40:1115.

Nemali,K.S. and M.W. van Iersel. 2004. Light effects on wax begonia: photosynthesis, growth respiration, maintenance respiration, and carbon use efficiency. J. Amer. Soc. Hort. Sci. 129: 416-424.

Olson, D.L., R.D. Oetting, and M.W. van Iersel. 2002. Effect of potting media and water management on development of fungus gnats (Diptera: Sciaridae) and plant growth. HortScience 37:919-923.

Prunty, L., and F.X.M. Casey. 2002. Soil water retention curve description using a flexible smooth function. Vadose Zone J. 1: 179-185.

Naasz, R., Michel, J.-C., and Charpentier, S. 2005. Measuring hysteretic hydraulic properties of peat and pine bark using a transient method. *Soil Sci. Soc. Amer. J.* 69: 13-22.

Ray, J.D. and Sinclair, T.R. 1998. The effect of pot size on growth and transpiration of maize and soyabean during water deficit stress. J. Exp. Bot. 49: 1381-1386.

Raviv, M., J.H. Leith, D.W. Burger, and R. Wallach. 2001. Optimization of transpiration

and potential growth rates of ‘kardinal’ rose with respect to root-zone physical properties. J. Amer. Soc. Hort. Sci. 126: 638-643.

Roberts, J. 2000. The influence of physical and physiological characteristics of vegetation on their hydrological response. Hydrol. Process 14: 2885-2901.

Robinson,D.A., Gardner, C.M.K. Cooper. J.D., 1999. Measurements of relative permittivity in sandy soils using TDR, capacitance, and theta probes: comparison, including effects of ionic conductivity. J. Hydrol. 223, 198 -211.

Robinson, D.A., Jones, S.B., Wraith, J.M., Or, D., Friedman, S.P., 2003. A review of advances in dielectric and electrical conductivity measurements in soils using time domain reflectometry. Vadose Zone J. 2, 444-475.

Steudle, E. 2000. Water uptake by roots: effects of water deficit. *J. Expt. Bot*. 51: 1531-1542.

Saccardy, K., Cornic, G., Brulfert, J., and Reyss, A. 1996. Effect of drought stress on net CO_2 uptake by Zea leaves. Planta 199: 589-595.

Sahin, U., O. Anapali, S. Ericisli. 2002. Physico-chemical and physical properties of some substrates used in horticulture. Gartenbauwissenschaft. 67: 55-60.

Serraj, R. And T.R. Sinclair. 2002. Osmolyte accumulation: can it really help increase crop yield under drought conditions? Plant Cell Environ. 25: 333-341.

Sinclair, T.R., and Ludlow, M.M. 1986. Influence of soil water supply on the plant water balance of four tropical grain legumes. Aust. J. Plant Physiol. 13: 329-341.

Smajstrla, A.G., Locascio, S.J., 1996. Tensiometer controlled drip irrigation scheduling of tomato. Applied Eng. Agric. 12, 315-319.

Sperry, J.S., U.G. Hacke, R. Oren, and J.P. Comstock. 2002. Water deficits and hydraulic limits to leaf water supply. Plant, Cell Environ. 25: 251-263.

Stange, C.F., and R. Horn. 2005. Modeling the soil water retention curve for conditions of variable porosity. Vadose Zone J. 4: 602-613.

Taiz, L. and E. Zeiger. 2002. Transport and translocation of water and solutes, unit 1. In: plant physiology 3rd edition. Sinauer associates inc., MA.

Tilt, K.M., T.E. Bilderback, and W.C. Fonteno. 1989. Particle size and container size effects on three ornamental species. J. Amer. Soc. Hort. Sci. 112: 981-984.

Tezara, W, V.J. Mitchell, S.D. Driscoll and D.W. Lawlor. 1999. Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. Nature 401:914-917.

Topp, G.C. 2003. State of the art of measuring soil water content. Hydrol. Process. 17, 2993-2996.

Topp, G.C., Davis, J.L., 1985. Measurement of soil water content using time domain reflectrometry (TDR): A field evaluation. Soil Sci. Soc. Amer. J. 49, 19-24.

Topp, G.C., Zegelin, S., White, I. 2003. Impacts of real and imaginary components of relative permittivity on time domain reflectometry measurements in soils. Soil Sci. Soc. Amer. J. 64, 1244 -1252.

Uva, W.L., Weiler, T.C., and Milligan, R.A. 1998. A survey on the planning and adoption of zero runoff subirrigation systems in greenhouse operations. HortScience 34:660-663.

Van Der Veken, L., Michels, P., Feyen, J., Benoit, F., 1982. Optimization of water application in greenhouse tomatoes by introducing a tensiometer controlled drip-irrigation system. Scientia Hort. 18, 9 -23.

Van Genuchten, M. 1980. A closed-form equation for predicting the hydraulic conductivity of unsaturated soils. Soil Sci. Soc. Amer. J. 44: 892-898.

van Iersel, M.W. 1996. Improving water and fertilizer efficiency in greenhouses. Georgia Floriculture 6:22-23.

van Iersel, M.W. and B. Bugbee. 2000. A multiple chamber, semicontinuous, crop carbon dioxide exchange system: Design, calibration, and data interpretation. J. Amer. Soc. Hort. Sci. 125: 86-92.

van Vliet, P.C. J., D.E. Radcliffe, P.F. Hendrix, and D.C. Coleman. 1998. Hydraulic conductivity and pore-size distribution in small microcosms with and without enchytraeids (Oligochaeta). 9: 277-282.

von Caemmerer, S and G.D. Farquhar. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta 153:376-387.

Wahbi, S., R. Wakrim, B. Aganchich et al., 2005. Effects of partial rootzone drying (PRD) on adult olive tree (Olea europaea) in field conditions under and climate - I. physiological and agronomic responses. Agric. Ecosystems and Environ. 106: 289-301.

Watkinson, J.I., A.A. Sioson, C. Vasquez-Robinet et al., 2003. Photosynthetic acclimation is reflected in specific patterns of gene expression in drought-stressed loblolly pine. Plant Physiol. 133: 1702-1717.

Whalley, W.R. 1993. Considerations on the use of time domain reflectometry (TDR) for measuring soil water content. J. Soil Sci. 44, 1 - 9.

White, I., Knight, J.H., Zegelin, S.J., and Topp, G.C. 1994. Comments on ' Considerations on the use of time domain reflectometry (TDR) for measuring soil water content' by W.R. Whalley. J. Soil Sci. 45, 503 - 508.

Xu, Z-Z., and G.S. Zhou. Effects of water stress and high nocturnal temperature on photosynthesis and nitrogen levels of perennial grass Leymus chinensis. Plant and Soil. 269: 131-139.

Yelanich, M.V. and Biernbaum, J.A. 1990. Effect of fertilizer concentration and method of application on media nutrient content, nitrogen runoff and growth of Euphorbia pulcherrima V-14 glory. Acta Hort. 272:185-189.

Zhang, X., N. Wu and C. Li. 2005. Physiological and growth responses of *Populus davidiana* ecotypes to different soil water contents. J. Arid Environ. 60: 567-579.

Zurmühl, T., and W. Durner. 1996. Modeling transient water and solute transport in a biporous soil. Water Res. Research 32: 819-829.

CHAPTER 2

CALIBRATION AND PERFORMANCE OF MOISTURE SENSORS IN SOILLESS SUBSTRATES: ECH$_2$O AND THETA PROBES [1]

[1] Nemali, K.S., F. Montesano, S.K. Dove, and M.W. van Iersel. To be submitted to *Scientia Horticulturae.*

Abstract

Reliable and affordable moisture sensors for measuring the water content in soilless substrates are limited. In this study, we examined the efficacy of two moisture sensors (ECH_2O–10 and Theta probe ML2X) for measuring water content in soilless substrates. We developed calibration equations and analyzed the effect of increasing electrical conductivity (EC) and substrate temperature on the voltage output of probes. We found that a single equation (one for each probe) could be used to adequately measure water content in different custom-made substrates maintained at low EC and a substrate temperature of ~23 ^{o}C. The calibration equation developed for Theta probe could also be used in two commercial substrates with high EC (2.0 to 5.0 $dS \cdot m^{-1}$). The output of the ECH_2O probe, but not the Theta probe, was significantly affected by substrate EC. Increasing the temperature of the substrate from 10 to 40 ^{o}C increased the voltage output of ECH_2O probes by 1.876 mV or or on an average 0.0022 $m^{3} \cdot m^{-3}$ water content per ^{o}C. There was no effect of increasing substrate temperature on the Theta probe. It was concluded that ECH_2O probes can be used in greenhouse operations requiring less measurement precision (like irrigation), however for accurate measurements of water content, the Theta probe is preferred.

Key words: Dielectric constant; Electrical conductivity; Greenhouse irrigation; Water content

Introduction

In recent years, regulations on agricultural water use have become stricter due to increased urbanization, population growth, and decreased water resources for agriculture (including horticulture). In light of this, there has been an increased awareness among greenhouse growers to irrigate crops judiciously. Potted plants are commonly irrigated based on the visual appearance of the substrate or plants, or with the use of irrigation timers. To irrigate potted plants (e.g., bedding plants) grown in soilless substrates with the right amount of water, it is important to accurately measure the substrate water content to decide when and how much irrigation is required. Mere visual observations of the substrate and/ or plants are not accurate and will not result in proper irrigation practices.

In spite of the availability of soil moisture sensors like tensiometers (Van Der Veken et al., 1982; Smajstrla and Locascio, 1996; Krüger et al.,1999), neutron probes (Black and Mitchell, 1968; Gear et al., 1977; McFall, 1978) and time domain reflectometry (TDR) probes (Topp and Davies, 1985), moisture sensors are rarely used to control irrigation in potted plant production. The main reasons for not using moisture sensors to control irrigation are high costs, unsuitable size, and unreliable measurements of the available moisture sensors. For example, sensors like TDR probes can provide reliable measurements, but the required meter is expensive. To optimize space utilization, greenhouse crops are grown in small containers. This limits the suitability of sensors like neutron probes which require a large volume for installation and measurement. Greenhouse crops are usually grown in soilless substrates with high porosity and a large fraction of pores are filled with air. When a moisture sensor like a tensiometer is

inserted into soilless substrates, a significant area of the sensor surface may be in contact with air. This could result in cavitation, causing erroneous and unreliable measurements. As it is difficult to hold the tensiometer firmly in a soilless substrate, the sensor is easily displaced which can cause a loss of contact between the tensiometer cup and the substrate. Hence, there is a need to screen and identify new moisture sensors suitable for soilless substrates.

Two moisture sensors, i.e., the ECH_2O dielectric aquameter (Decagon Devices, Pullman, WA, USA) and the theta probe ML2X (Delta–T devices, Cambridge, UK), have recently become available. A set of five ECH_2O probes with a datalogger (EM 50, Decagon Devices) and software costs approximately $900 and ECH_2O probes are much cheaper when purchased in bulk. The price of a Theta probe ML2X with HH2 moisture meter for measurement is approximately $1000. These probes are available in convenient sizes (sensor lengths are 6 cm for Theta probes and 10 and 20 cm for ECH_2O probes, respectively). In spite of these advantages, these probes have never been tested for use in soilless substrates.

Both ECH_2O and Theta probes estimate the substrate water content by indirectly measuring the dielectric permittivity (or dielectric constant) of the substrate. The main components of a soilless substrate that affect the dielectric constant are substrate water content, air, and solid matrix. The dielectric permittivity of water at a temperature of 20 ^oC is large ($\sim$80.4) compared to that of air ($\sim$1) or solid matrix ($\sim$2 to 8). Therefore, a change in the substrate water content can result in a significant change in the dielectric

permittivity of the substrate, and by developing an analytical relationship between changing water content and the associated change in dielectric permittivity, the volumetric water content of the substrate can be indirectly estimated (Topp, 2003).

The details of the measuring technique for the Theta Probe can be obtained from Gaskin and Miller (1996). The Theta probe is equipped with an oscillator to send a 100 MHz signal (electromagnetic wave) into the built-in transmission line. The transmission line consists of an array of four coaxial rods and has an impedance (resistance) to the signal flow. The impedance of the transmission line depends on the medium surrounding the coaxial rods. When the transmission line is inserted into a soilless substrate, the change in its impedance causes a proportion of the incoming signal to reflect back to the oscillator. The reflected signal interferes with the subsequent incoming signals to produce a standing wave along the transmission line. The amplitude of the standing wave is proportional to the impedance along the transmission line, which in turn is proportional to the dielectric of the substrate. The change in the amplitude can be measured as an analog voltage output. As the dielectric permittivity of the medium is proportional to the water content (Topp and Davies, 1985), changes in the volumetric water content cause changes in the amplitude of the standing wave or the analog voltage output. This principle is used in calibration of the probe.

The ECH_2O probes are capacitance probes equipped with three copper plates which run along the length of the probe (one of them connected to a positive terminal and the other two to a negative terminal), and form a parallel-plate capacitor. These plates are enclosed inside the body of the sensor. When the voltage is applied across the copper plates, an electromagnetic field is generated and charges the capacitor. The

capacitance (amount of charge held at any voltage) of the capacitor changes when the sensor is inserted into a substrate. This is due to the interaction of the electromagnetic field with the substrate. When the sensor is inserted into a moist substrate (with a large dielectric permittivity), there is an increase in the capacitance of the capacitor and an increase in the time required to charge the capacitor. By keeping the applied voltage constant, and measuring the time required to charge the capacitor, its capacitance can be estimated. The dielectric permittivity can be estimated from the capacitance based on the area of and separation between the copper plates. The region of measurement of the dielectric permittivity by ECH_2O probes lies in the fringed electromagnetic field in the substrate, which protrudes out of the body of the sensor. Although the extension of the fringed electromagnetic field is small (approximately 0.7 to 1 cm), it runs along the length (both sides) of the probe (10 or 20 cm, depending on the probe model).

Although it has been indicated that water content is the major factor affecting dielectric permittivity of a substrate (called 'real permittivity'), other substrate related factors like EC, temperature, and bulk density can cause dielectric loss and affect the permittivity of a substrate (called 'imaginary permittivity'). Greenhouse crops are supplied frequently with water-soluble fertilizers resulting in significant concentrations of ions of fertilizer salts in the substrate. The presence of charged particles, like ions of fertilizer salts, in the vicinity of the electromagnetic field generated by probes can attenuate the electromagnetic energy and influence the measurement of probes. Similarly, probe measurement can be affected by substrate temperature. The dielectric permittivity of water decreases with increasing temperature (~0.4 K^{-1}). Temperature can also affect sensor electronics, in turn affecting the voltage output of probes.

Fluctuations in the substrate temperature can be significant between day and night periods and may affect the measurement of the probe.

Keeping these issues in mind, our objectives were to calibrate the ECH_2O dielectric aquameter and Theta Probe ML2X for measuring the water content in soilless substrates and to study the effect of EC and substrate temperature on ECH_2O and Theta probe output.

Materials and methods

2.1 Calibration studies.

2.1.1. Methods. Probes were calibrated for custom-made substrates with different organic (peat and pine bark) and inorganic components (perlite and vermiculite) , while keeping the ratio of organic to inorganic components constant (60% organic), and for substrates with different ratios of organic to inorganic components. In both studies, substrate EC was maintained low (0.25 to 0.75 $dS \cdot m^{-1}$; substrate solution EC; measured with a SigmaProbe EC1, Delta -T, Burwell, Cambridge, UK). The calibration equations were tested for two commercial substrates having 60% peat and 40 % perlite (Fafard 2P, Fafard Inc., Anderson, S.C., USA) and 36% peat, 27% pine bark, 15% perlite, and 22% vermiculite (Fafard 4P), both having a high EC (2.0 to 5.0 $dS \cdot m^{-1}$; substrate solution EC).

Custom-made substrates were prepared by mixing different volumes of peat, pine bark, perlite, and vermiculite. Different organic compositions were peat (60% V/V), peat and bark in equal volumes (30% each), and pine bark (60%). For each organic composition, three inorganic compositions, i.e., perlite (40%), perlite and vermiculite

(20% each), and vermiculite (40%), were used, resulting in nine different substrates. The EC of the substrates was low as no fertilizer was added. To obtain a range of water contents in each substrate, from dry to near saturation, different volumes of deionized water were added to each substrate and mixed thoroughly to obtain uniformity. Substrates were then transferred into beakers (1.12 L).

To prepare substrates with different ratios of organic to inorganic components having a particular substrate composition, the peat to perlite ratio was altered to result in substrates with 80:20, 60:40, 40:60, and 20:80% each. Different substrate water contents for these four and two other commercial mixes were also prepared as mentioned above by mixing substrates with different volumes of deionized water.

2.1.2. Measurements. In total, we used 10 ECH_2O probes and one Theta probe in the study. Two ECH_2O probes were inserted into each beaker containing the substrate. The ECH_2O probes were inserted completely into the substrate to avoid measurement errors. A CR10X datalogger (Campbell Scientific, Inc., Logan, UT, USA) was used to excite and measure the output from the ECH_2O probes. The datalogger supplied 2.5 V of excitation to the ECH_2O probes and the output was measured as analog voltage in the range of 250 to 900 mV (dry to near saturation). The output from ECH_2O probes was measured at 2 s intervals. The measurement was recorded after attaining a stable voltage output. The Theta probe was inserted into the substrate in one beaker at a time for measurement after switching-off the ECH_2O probes. The transmission rods of the theta probe were completely inserted into the substrate for measurement. The output from Theta Probe was measured using a digital multimeter (DM 350A, A.W. Sperry, Hauppauge, N.Y.) or moisture meter (HH2, Delta -T, Burwell, Cambridge, UK).

Before inserting any probe, the initial weight of the beakers and substrate was determined. Probes were inserted carefully so as to not compress the substrate during insertion. After measuring the output from the probes, the substrate in the beakers was dried in a forced-air oven maintained at 80 °C. The substrate was weighed after drying, and used to determine the substrate water content. The substrate water content was determined by converting grams of water in the substrate to mL of water assuming that 1 g of water = 1 mL.

2.1.3. Analysis. There was one trial for different substrate types, however there were five trials for the two commercial substrates. Data were analyzed using PROC GLM of SAS (SAS institute, Cary, NC, USA). A mixed model comprising of both class and continuous variables was used in the analysis. Water content was tested as a dependent variable with probes and substrates as independent class variables, and voltage as independent continuous variable. Response of water content to changing voltage was determined by developing quadratic equations using regression procedures of SAS. Different equations used to describe the responses are indicated in the figure legends.

2.2 Electrical conductivity responses.

2.2.1. Methods. The effect of EC on probe output was studied in both solutions and substrates (or substrate with solution phase). Responses of probe output to increasing solution EC was determined by adding different volumes of a concentrated fertilizer solution

(15-5-15 Cal-Mag, The Scotts Co., Marysville, OH, USA) to deionized water and measuring probe output at each concentration.

ECH_2O and Theta probe responses to changing substrate EC were measured in a substrate comprising of 60% peat and 40% perlite. Initially, substrates with different water content were prepared as described earlier. To each substrate at a particular water content, concentrated fertilizer solution was added in incrementing volumes to increase the substrate EC. At any time, the total volume of concentrated fertilizer solution added was less than 1% of total volume of the substrate.

2.2.2. Measurements. Output of probes was measured at each EC level. Electrical conductivity was determined by inserting an EC meter in the substrate (substrate solution EC; Sigma probe) or solution (bulk EC, Field Scout soil EC probe, Spectrum Technologies, Plainfield, IL, USA). In both substrate and solution EC measurements, the EC probes were inserted completely into the substrate or solution. Because the Sigma probe cannot measure the EC in dry substrates, substrates with a water content > 0.25 $m^3 \cdot m^{-3}$ were used in this study. After measurements, the substrate in the beakers was dried in a forced-air oven maintained at 80 °C. The dry weight of the substrate was used to determine water content as described earlier.

2.2.3. Analysis. There were five replications in the solution and substrate studies. Similar to the analysis described in calibration procedure, a mixed model comprising of both class and continuous variables was used. Voltage was tested as a dependent variable and solution EC as independent continuous variable. In the substrate EC response study, voltage was tested as dependent variable and water content and EC were treated as independent continuous variables. Responses were studied using both

linear and nonlinear regression procedures of SAS.

2.3 Temperature responses.

2.3.1. Methods. To determine the effect of substrate temperature on probe measurement, beakers containing substrate (60% peat and 40% perlite) were placed in a growth chamber (Conviron CMP 4030, Winnipeg, Manitoba, Canada). The growth chamber was programmed to increase the chamber temperature from 10 to 40 °C in increments of 2 °C. The temperature in the growth chamber was raised only after the substrate temperature stabilized. During the entire measurement period, lights were turned off inside the growth chamber. Substrate temperature was measured using T-type thermocouples connected to a thermocouple thermometer (Digi-Sense 91100-50, Cole-Palmer instrument Co., Vernon Hills, IL, USA). The effect of substrate temperature on ECH_2O probe output was studied at three substrate water contents, i.e., 0.12, 0.25, and 0.35 $m^3 \cdot m^{-3}$, however for the Theta probe, temperature responses were studied only at a water content of 0.25 $m^3 \cdot m^{-3}$. The water content in the substrate was maintained constant for all measured substrate temperatures. This was accomplished by tightly fastening a paraffin film on top of each container after inserting the probes leaving no space between the substrate and paraffin to avoid evaporative water loss.

2.3.2. Measurements. Output of the probes was recorded at each substrate temperature after the substrate temperature has stabilized. At the end of the study, beakers were re-weighed and compared with their initial weight as a check against evaporative moisture loss during the experiment.

2.33. Analysis. Voltage was tested as the dependent variable with different water contents and temperature as independent continuous variable. Linear regression was

used to study responses.

2.4 Sensitivity of probes to dielectric discontinuity.

Dielectric discontinuity occurs when part of the sensor is inserted into a region of relatively high dielectric permittivity (e.g., water or wet substrate) while the rest of the sensor is in a region of low dielectric permittivity (e.g., air). Dielectric discontinuity can occur when part of the probe is inserted in either solutions or wet substrate. When a dielectric discontinuity occurs, enhancement of electromagnetic field will occur in the in the region of higher polarization or greater dielectric permittivity. Concentrating electrical energy in a small portion of the sensor could be advantageous under situations when a probe is used to measure θ in small containers, and only part of the probe can be inserted. As the Theta probe rods are bare (unlike ECH_2O sensors which are covered in fiberglass), it is likely that this sensor will be exposed to dielectric discontinuity when part of the bare rods are inserted into water. Because sensing part of ECH_2O probes (metal plates) is covered in fiber glass which acts as a barrier, it may be less sensitive to dielectric discontinuity.

To determine the response of probe output to dielectric discontinuity, and sensitivity of ECH_2O probes and Theta probe (bare or covered rods) along the length of the probes, the probes were inserted into a solution of deionized water in increments of 1 cm at a time. For each depth of insertion, voltage output of the probes was recorded. Effect of depth of insertion was studied by testing voltage as the dependent variable and depth of insertion as the independent continuous variable. To study the effect of covering bare Theta probe rods on the ability of the probe to detect dielectric discontinuity, a snugly fit plastic tubing was used to independently cover full length of

all four transmission rods.

Results and discussion

3.1 Calibration of probes in soilless substrates.

The response of ECH_2O and Theta probe output to increasing substrate water content was similar among the nine substrates having different organic and inorganic compositions. For both probe types, and in all nine substrates, there was an increase in the voltage output with increasing substrate water content in the studied range of 0 to 0.5 $m^{3}{\cdot}m^{-3}$ (Fig. 2.1). The fitted equations adequately ($0.95 < R^2 < 0.96$) described the response of voltage of both probes to increasing substrate water content. The nine different substrates studied had relatively low EC (0.25 $dS{\cdot}m^{-1}$ at high water content and 0.75 $dS{\cdot}m^{-1}$ at low water content). Though the type of organic and inorganic components varied among the substrates, the proportion of organic to inorganic matter remained constant (60:40). These results indicate that regardless of the type of the organic and inorganic components used in preparing substrate, a single calibration equation can be used to estimate substrate water content in these nine substrates with 60% organic and 40% inorganic components. This is a significant finding because the proportion of organic to inorganic components in many soilless greenhouse substrates is close to 60:40, and based on these results it appears that separate calibrations may not be necessary for ECH_2O and Theta probes in different soilless substrates having an EC < 1.0 $dS{\cdot}m^{-1}$.A single calibration equation was found to sufficiently describe the ECH_2O and Theta probe response when data from nine substrates was combined with data for all combinations of peat-perlite based substrates (i.e., varying ratios of organic

and inorganic components) studied (Fig. 2.2). This indicates that there was no significant effect of different ratios of organic and inorganic fractions on the dielectric permittivity of the substrate, and the change in dielectric permittivity is seen only due to an increase in substrate water content.

When the developed calibration equations for ECH_2O and Theta probes using the custom-made substrates (Figs. 2.1 & 2.2) were compared with the calibration equations for the two commercial substrates (Fafard 2P and 4P), only the response of the Theta probe was similar in all substrates (Fig. 2.3). Both commercial substrates contained starter fertilizer and the measured substrate EC at high to low water contents ranged from 2 to 5 $dS \cdot m^{-1}$, respectively. This indicated that a single calibration equation (Fig. 2.3) that can be used to adequately describe the response of Theta probe output to increasing water content for soilless substrates with different compositions and EC levels. However, the response of ECH_2O probes to increasing water content was different between the commercial and custom-made substrates (Fig. 2.3). Presence of fertilizer salts increased the apparent ECH_2O probe output at any substrate water content. Based on these results, the Theta probe seems to be insensitive to substrate EC, while the ECH_2O probe is not.

Robinson et al. (2003) indicated that the dielectric loss increases not only with increasing EC, but also with decreasing frequency of the propagation wave. Frequencies of 400-500 MHz were shown to be effective in decreasing dielectric losses due to ionic conductivity in clay soils (Topp et al., 2000). The maximum frequency of electromagnetic waves generated by ECH_2O and Theta probes were 20 and 100 MHZ, respectively. A lower frequency of the propagation wave perhaps makes ECH_2O

probes more vulnerable to increased dielectric losses in saline substrates than Theta probes. Although the frequency of the Theta probe was lower than that recommended as the 'effective' frequency to decrease ionic losses in the literature (*i.e.*, 400-500 MHz), it appears that Theta probe measurements were insensitive to substrate EC (Fig. 2.3).

3.1.1. Ready-to-use coefficients for the Theta probe.

The voltage of Theta probe can be linearly related to the square root of dielectric permittivity ($\sqrt{\varepsilon} = 1.1 + 4.44 \cdot V$, $r^2 = 0.99$, where ε is dielectric permittivity and V is voltage; http://www.delta-t.co.uk/; user manual for Theta probe ML2X). It has been shown that a simple universal linear relationship exits between dielectric permittivity and water content ($\sqrt{\varepsilon} = a_0 + a_1 \cdot \theta$; Whalley, 1993; White et al., 1994). In fact, these two equations are used to estimate water content from voltage output and pre-determined coefficient values (using dry and wet substrates respectively to obtain a_0 and a_1) by the HH2 moisture meter (Delta-T devices) of the Theta probe. The relationship between $\sqrt{\varepsilon}$ and V is developed by measuring voltage output in liquids of known ε. However, in soilless substrates ε is also affected by solid substrate components and air along with water (and their non-homogeneous nature). Because the contribution of the substrate and air to total ε changes with increasing water content (e.g., decrease in air-filled porosity with increasing water content), the increase in V with increasing water content cannot be related only to ε of water. Hence the measured water content using $\sqrt{\varepsilon}$ may not be exact. However, a reasonably good estimate can be obtained for practical purposes, since the ε of substrate components and air is low.

Our study indicated that one calibration could adequately describe the response of Theta probe in both commercial and custom-made substrates, we calculated values for coefficients a_0 and a_1 based on the equation in Fig. 2.3. The calculated values for a_0 and a_1 were 1.19 and 8.67 (see http://www.delta-t.co.uk/; user manual for Theta probe ML2X for description of calculation procedure). These values are close to those recommended by the manufacturer for organic soils (1.3 and 8.6, respectively for a_0 and a_1). As our values are based on different substrates, we recommend our coefficients for direct use with a HH2 moisture meter (Delta-T devices) to estimate water content in soilless substrates.

3.2 Effect of electrical conductivity on probe measurement.

Both ECH_2O and Theta probes were found to be sensitive to fertilizer salts or ions in solutions (Fig. 2.4). The response of probes to solution EC differed, with an increase in EC either causing a rise to a maximum output (ECH_2O probe) or a decrease to minimum (Theta probe). For both probes, the output changed rapidly with in the range of 0 to 3.0 $dS \cdot m^{-1}$ and at higher EC levels there was a relatively small change in the output. When the solution EC was increased from 0 to 3 $dS \cdot m^{-1}$, the output of ECH_2O probes increased by approximately 9.1% (~880 to 960 mV) and a further 2.4% increase in ECH_2O probe output was seen at an EC of 12 $dS \cdot m^{-1}$ compared to that at 3 $dS \cdot m^{-1}$ (Fig. 2.4). The Theta probe output decreased from 1145 mV to 1020 mV (10.9 %) with increasing solution EC from 0 to 3 $dS \cdot m^{-1}$ (Fig.2. 4). A further increase in EC decreased the output to 940 mV at an EC of 12 $dS \cdot m^{-1}$ (7.9% lower than that at 3 $dS \cdot m^{-1}$).

The different responses of probes to increasing solution EC can be attributed to the mechanism of operation of these probes. The presence of ions in the solution

surrounding the sensor will attenuate the electromagnetic signal which is a result of dissipation or loss of electric energy to the ions. In the case of the Theta probe, this attenuation (Gaskin and Miller, MLURI technical note, 2nd ed. (http://www.macaulay.ac.uk/MRCS/pdf/tprobe.pdf) results in an overall decrease in the amplitude of the electromagnetic wave traveling through the sensor, hence a decrease in analog voltage output with increasing solution EC. The response of ECH_2O probes is different because, due to the attenuation of the electromagnetic signal, it takes more time to charge the capacitor to the same level at a given applied voltage. As this time increases, the voltage output of the probe increases (http://www.ech2o.com/SupportFAQ.htm).

When the effect of EC of the substrate (more precisely substrate + solution) on ECH_2O probe output was tested at different substrate water contents, the probe response was affected by both water content and EC (Fig. 2.5). The ECH_2O probe output responded in a quadratic fashion to increasing EC of the substrate and this response was similar at different substrate water contents (Fig. 2.5). Similar to the effect seen in solution, the effect of increasing EC was greatest at low EC. The effect of increasing substrate EC on Theta probe output was not statistically significant (P = 0.153) at the three studied water contents (Fig. 2.5). Two possible reasons for finding a clear trend in Theta probe output to increasing solution EC but not with substrate EC are the higher sensitivity of the probe and homogeneity in the surrounding medium in solution as opposed to a substrate.

3.3 Effect of substrate temperature on probe measurement.

There was a linear increase in ECH_2O output with increasing temperature of the

substrate, independent of the substrate water content (1.88 $mV \cdot {}^{o}C^{-1}$) (Fig. 2.6). This translates to a change in estimated water content of 0.0018 to 0.0026 $m^{3} \cdot m^{-3} \cdot {}^{o}C^{-1}$ (from 23.2 to 24.2 ^{o}C) for a substrate at 0.12 and 0.34 $m^{3} \cdot m^{-3}$, respectively (based on the calibration equation for commercial mixes in Fig. 2.3). There was no change in the Theta probe output when the substrate temperature was increased from 10 to 40 ^{o}C (Fig. 2.6).

An earlier study by Baumhardt et al. (2000) indicated that under saturated soil conditions, increasing substrate temperature had a greater effect on capacitance probes than on TDR probes (estimated water content increased by 0.04 $m^{3} \cdot m^{-3}$ and 0.02 $m^{3} \cdot m^{-3}$ for a 15 ^{o}C change for capacitance and TDR probes, respectively). The rate of increase in voltage with increasing temperature for ECH_2O (capacitance) probe was approximately 0.033 $m^{3} \cdot m^{-3}$ for a 15 ^{o}C change in the substrate temperature in our study. Our results agree with those of Baumhardt et al. (2000) in that ECH_2O (capacitance) probe output was significantly affected but Theta probe output (uses wave reflection, like TDR, for estimating dielectric permittivity) was not affected by temperature. In other studies, a linear decrease in the water content measured by TDR probes with increasing substrate temperature, when the water content is above 0.30 $m^{3} \cdot m^{-3}$ and no change in measured water content with increasing temperature when the substrate water content is below 0.30 $m^{3} \cdot m^{-3}$ was noticed (Wraith and Or, 1999; Gong et al., 2003). Under conditions of low soil water content, a large fraction of the water is held by the solid surface as bound water which releases as free water when the temperature is increased. This increase in free water offsets the decrease in dielectric permittivity of water with increasing substrate temperature (‘thermodielectric effect’),

hence the net result is little or no change in the measured water content (Wraith and Or, 1999). As the substrate water content was 0.25 $m^{3}{\cdot}m^{-3}$ when the Theta probe response to substrate temperature was measured, it is likely that the lack of change in the output is due to the interplay between a decrease in dielectric permittivity and an increased release of bound water.

3.4 Sensitivity of probes to dielectric discontinuity.

When we sequentially measured the voltage output of ECH_2O and Theta probes while gradually inserting the sensors into deionized water, we found that the Theta probe output rapidly approached the maximum value (approximately 85% of the total voltage output) when only 1 cm of the sensor was inserted in deionized water (Fig. 2.7). This indicates that Theta probe is sensitive to dielectric discontinuity. This characteristic of Theta probe can be advantageous when the probe is used for measuring water content in small containers (e.g., plug cells used to grow seedlings of bedding plants). As the container volume may not be large enough to insert the entire length of the rods, there is a likely dielectric discontinuity as some portion of the Theta probe rods will be in the substrate and rest in the air. Even under these conditions, Theta probe output will be accurate, because most of the electrical energy used for measurement is concentrated in the portion with high dielectric permittivity (in this case, inside small volume substrate).

However, this will not be the case with an ECH_2O probe or likely with any covered sensor. The response of ECH_2O probes to sequential insertion in water was a gradual change with increasing depth of insertion (Fig. 2.7). This indicates that, to obtain an accurate measurement, entire length of the ECH_2O probe should be inserted into the

substrate. We verified the fact that, under conditions of a dielectric discontinuity, a covered sensor will not concentrate its electrical energy in the region of greatest dielectric permittivity by covering the rods of the Theta probe with a plastic tubing. When sequentially inserted into water, the response of the Theta probe with covered rods was different from that of bare rods, and was a gradual change in output with increasing depth of insertion, like the one seen in ECH_2O probe (Fig. 2.7).

3.5 Variation among ECH_2O probes.

Variability among different ECH_2O probes was tested in different experiments during the study (i.e., nine substrate calibration, commercial substrate calibration, solution EC response study). In all these experiments, statistical analysis indicated no significant differences among different ECH_2O probes. Hence it is inferred that probe-specific calibrations are not necessary for ECH_2O probes. This could not be verified for the Theta probe as we used only one probe in these studies.

Conclusions

Our objective was to calibrate ECH_2O and Theta probes for measuring water content of greenhouse substrates and study the effect of substrate EC and temperature on probe measurements. The following conclusions were drawn based on the results from this study:

(i) When the substrate EC levels are lower than 1.0 $dS \cdot m^{-1}$, ECH_2O probes can accurately measure the water content of the substrate. Substrate EC has the greatest effect on probe voltage between 1.0 to 3.0 $dS \cdot m^{-1}$. However, considering their low cost (~ \$60 if > 11 probes are purchased) and the fact that high accuracy may not be

required for irrigation purposes, ECH_2O probes can be recommended for greenhouse use, and especially for crops grown with low EC ($\leq$ 1.0 $dS \cdot m^{-1}$). At higher EC levels > 3.0 $dS \cdot m^{-1}$, a separate calibration using high EC in substrate can be used as there is little increase in the effect of substrate EC on probe output above 3.0 $dS \cdot m^{-1}$. Temperature compensation can be used to improve the performance of ECH_2O probe to minimize the effects of substrate temperature on probe output.

(ii) Substrate EC and temperature were shown to have little or no effect on Theta probe output. It is also shown that one calibration can be used to describe response of Theta probe in different soilless substrates. Hence, the Theta probe is suitable for precise measurements of water content**.**

Acknowledgments

We thank Mark Blonquist, technical service, Apogee Instruments, Logan, Utah, USA for his helpful comments on this manuscript.

References

Black, J.D.F., Mitchell, P.D., 1968. Near surface soil moisture measurement with neutron probe. J. Aust. Inst. Agric. Sci. 34, 181.

Baumhardt, R.L., Lascano, R.J., Evett, S.R., 2000. Soil material, temperature, and salinity effects on calibration of multisensor capacitance probes. Soil Sci. Soc. Amer. J. 64, 1940-1946.

Gaskin G.J., Miller J.D., 1996. Measurement of soil water content using a simplified impedance measuring technique. J.Agric. Res. 63, 153-160.

Gear, R.D., Dransfield, A.S., Campbell, M.D., 1977. Irrigation scheduling with neutron probe. J. Irrigation and Drainage Division-ASCE. 103, 291-298.

Gong, Y., Cao, Q., Sun, Z., 2003. The effects of soil bulk density, clay content, and temperature on soil water content measurement using time-domain reflectometry. Hydrol. Proc. 17, 3601-3614.

Krüger, E., Schmidt,G., Brückner, U., 1999. Scheduling strawberry irrigation based upon tensiometer measurement and a climatic water balance model. Scientia Hort. 81, 409-424.

McFall, R.L., 1978. Irrigation scheduling with neutron probe. J. Irrigation and Drainage Division-ASCE 104, 245.

Robinson,D.A., Gardner, C.M.K. Cooper. J.D., 1999. Measurements of relative permittivity in sandy soils using TDR, capacitance, and theta probes: comparison, including effects of ionic conductivity. J. Hydrol. 223, 198 -211.

Robinson, D.A., Jones, S.B., Wraith, J.M., Or, D., Friedman, S.P., 2003. A review of advances in dielectric and electrical conductivity measurements in soils using time domain reflectometry. Vadose Zone J. 2, 444-475.

Smajstrla, A.G., Locascio, S.J., 1996. Tensiometer controlled drip irrigation scheduling of tomato. Applied Eng. Agric. 12, 315-319.

Topp, G.C. 2003. State of the art of measuring soil water content. Hydrol. Process. 17, 2993-2996.

Topp, G.C., Davis, J.L., 1985. Measurement of soil water content using time domain reflectrometry (TDR): A field evaluation. Soil Sci. Soc. Amer. J. 49, 19-24.

Topp, G.C., Zegelin, S., White, I. 2003. Impacts of real and imaginary components of relative permittivity on time domain reflectometry measurements in soils. Soil Sci. Soc. Amer. J. 64, 1244 -1252.

Van Der Veken, L., Michels, P., Feyen, J., Benoit, F., 1982. Optimization of water application in greenhouse tomatoes by introducing a tensiometer controlled drip-irrigation system. Scientia Hort. 18, 9 -23.

Whalley, W.R. 1993. Considerations on the use of time domain reflectometry (TDR) for measuring soil water content. J. Soil Sci. 44, 1 - 9.

White, I., Knight, J.H., Zegelin, S.J., and Topp, G.C. 1994. Comments on ‘ Considerations on the use of time domain reflectometry (TDR) for measuring soil water content’ by W.R. Whalley. J. Soil Sci. 45, 503 - 508.

Figure 2.1. Relationship between water content of the substrate (θ) and the voltage output of ECH_2O and Theta probes in nine different substrates having 60% organic and 40% inorganic components. Although the fitted quadratic equation does not fit well at the low substrate water content, it was fitted for comparison with the fitted equation for ECH_2O probe (also note the improvement in the fit in Figs. 2.2 and 2.3 as more data were added from different substrates).

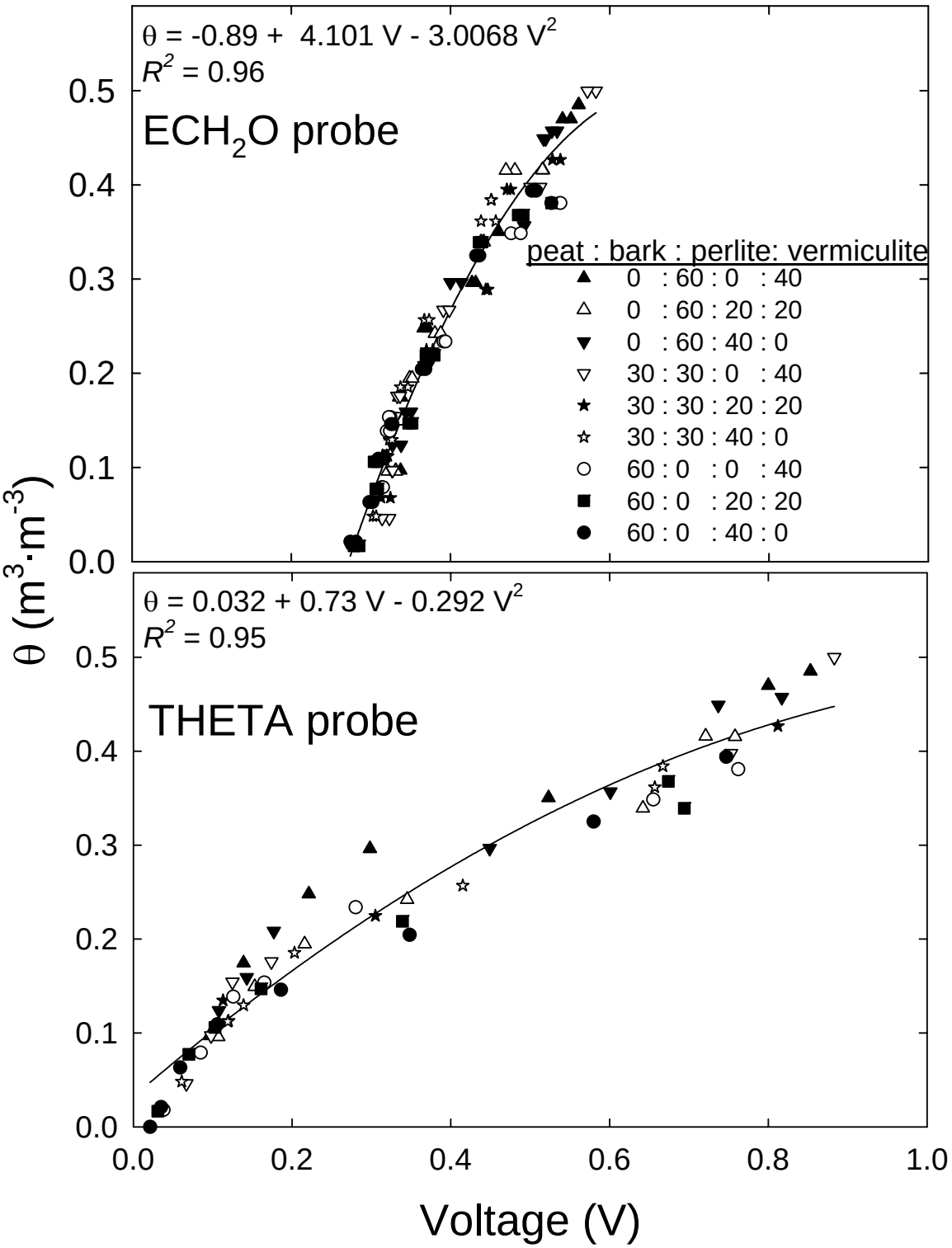

θ = -0.89 + 4.101 V - 3.0068 V2
R2 = 0.96
ECH2O probe
peat : bark : perlite: vermiculite
0 : 60 : 0 : 40
0 : 60 : 20 : 20
0 : 60 : 40 : 0
30 : 30 : 0 : 40
30 : 30 : 20 : 20
30 : 30 : 40 : 0
60 : 0 : 0 : 40
60 : 0 : 20 : 20
60 : 0 : 40 : 0
θ = 0.032 + 0.73 V - 0.292 V2
R2 = 0.95
THETA probe
θ (m3·m-3)
Voltage (V)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.8
1.0

Figure 2.2. Relationship between volumetric water content of the substrate (θ) and the voltage output of ECH_2O and Theta probes. Data include both nine substrates with different organic (60%) and inorganic components (40%) (see Fig. 2.1 for more details) and substrates having different proportions of peat and perlite. Fitted equations are for combined data.

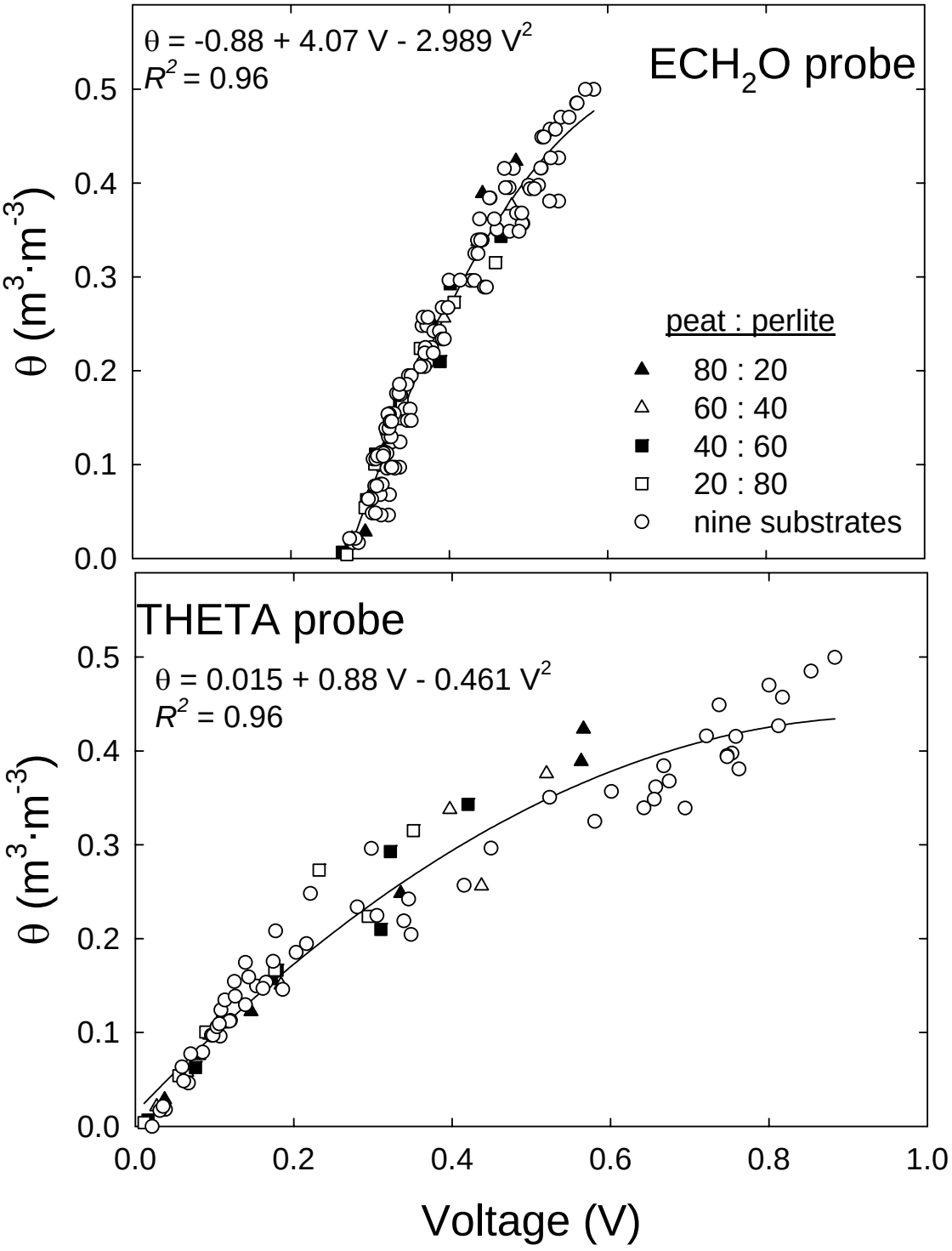

θ = -0.88 + 4.07 V - 2.989 V²
R² = 0.96
ECH2O probe
peat : perlite
80 : 20
60 : 40
40 : 60
20 : 80
nine substrates
θ (m³·m⁻³)
THETA probe
θ = 0.015 + 0.88 V - 0.461 V²
R² = 0.96
θ (m³·m⁻³)
0.0
0.1
0.2
0.3
0.4
0.5
0.0
0.2
0.4
0.6
0.8
1.0
Voltage (V)

Figure 2.3. ECH_2O and Theta probe calibration equations for two commercial substrates (P-P indicates peat-perlite and P-B-P-V indicates peat-bark-perlite-vermiculite) with high EC, and 13 substrates with low EC (see Fig. 2.1 and 2.2 for more details on these 13 substrates). The equation for the ECH_2O probe is for two commercial substrates with high EC only. The equation for the Theta probe is for all 15 substrates combined.

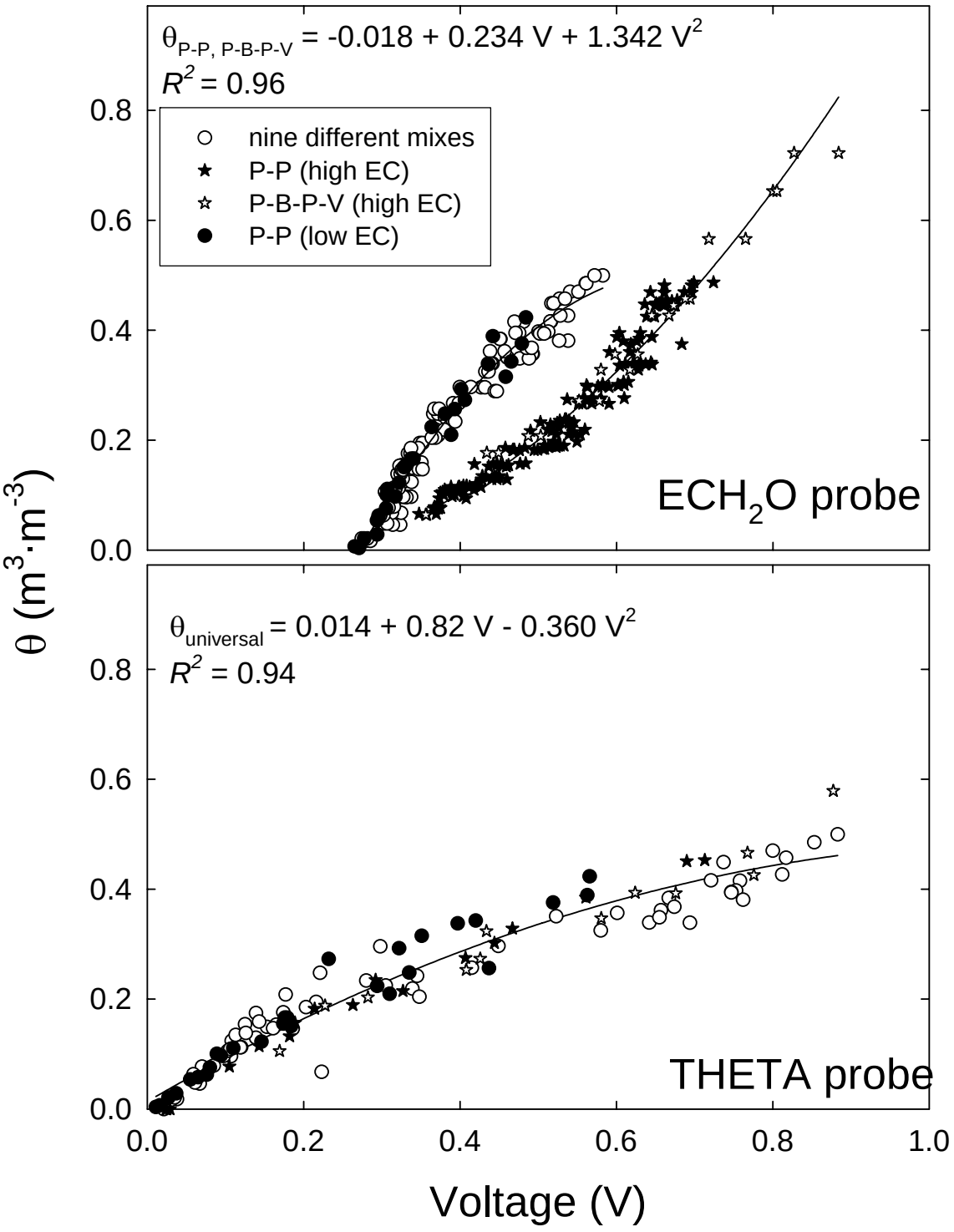

θP-P, P-B-P-V = -0.018 + 0.234 V + 1.342 V2
R2 = 0.96
nine different mixes
P-P (high EC)
P-B-P-V (high EC)
P-P (low EC)
ECH2O probe
θuniversal = 0.014 + 0.82 V - 0.360 V2
R2 = 0.94
THETA probe
θ (m3·m-3)
Voltage (V)
0.0
0.2
0.4
0.6
0.8
1.0

Figure 2.4. Effect of increasing electrical conductivity of fertilizer solution ($EC_{solution}$) on the output of ECH_2O and Theta probes. The sensing parts of the probes were completed submerged in the solutions.

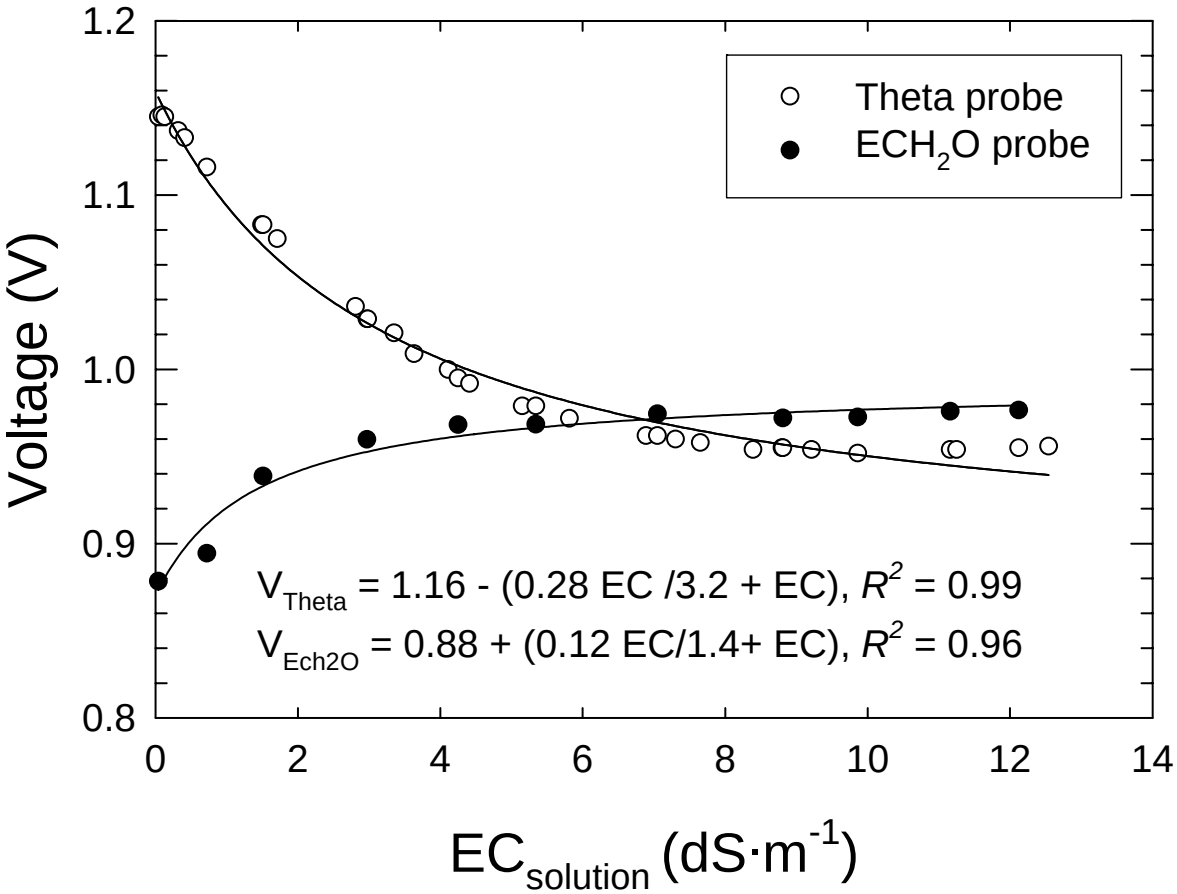

Theta probe
ECH2O probe
Voltage (V)
1.2
1.1
1.0
0.9
0.8
0
2
4
6
8
10
12
14
VTheta = 1.16 - (0.28 EC /3.2 + EC), R2 = 0.99
VEch2O = 0.88 + (0.12 EC/1.4+ EC), R2 = 0.96
ECsolution (dS·m-1)

Figure 2.5. Effect of increasing electrical conductivity of the substrate ($EC_{substrate}$) at different substrate water contents on the output of ECH_2O and Theta probes. There was no significant effect of EC on Theta probe output.

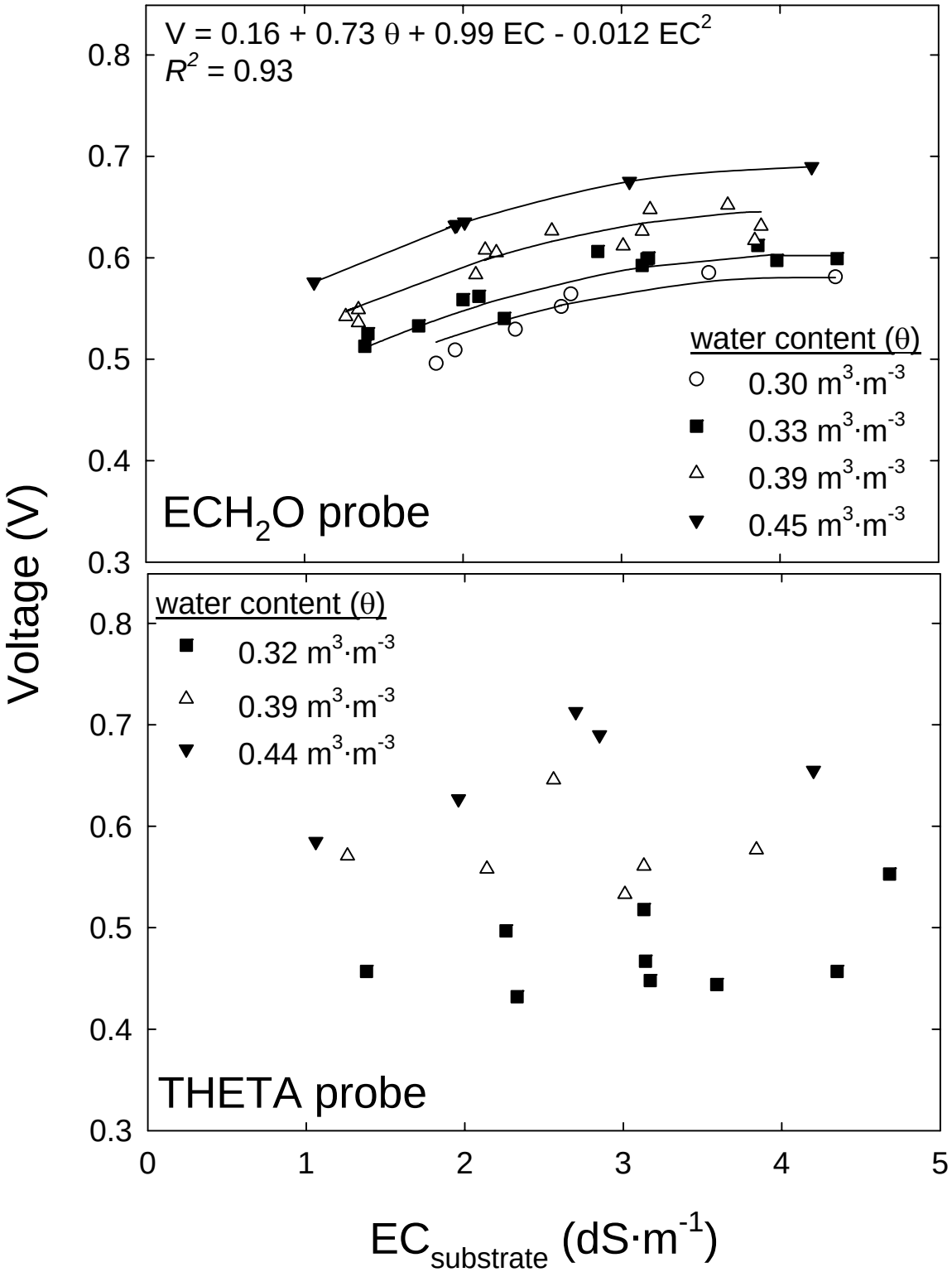

V = 0.16 + 0.73 θ + 0.99 EC - 0.012 EC2
R2 = 0.93
water content (θ)
0.30 m3·m-3
0.33 m3·m-3
0.39 m3·m-3
0.45 m3·m-3
ECH2O probe
water content (θ)
0.32 m3·m-3
0.39 m3·m-3
0.44 m3·m-3
THETA probe
Voltage (V)
ECsubstrate (dS·m-1)
0.3
0.4
0.5
0.6
0.7
0.8
0
1
2
3
4
5

Figure 2.6. Effect of increasing substrate temperature ($Temperature_{substrate}$) on the output of ECH_2O and Theta probes. Responses were measured at three water contents for ECH_2O probes, whereas response of the theta probe was measured at one water content (0.25 $m^{3\cdot\cdot}m^{-3}$).

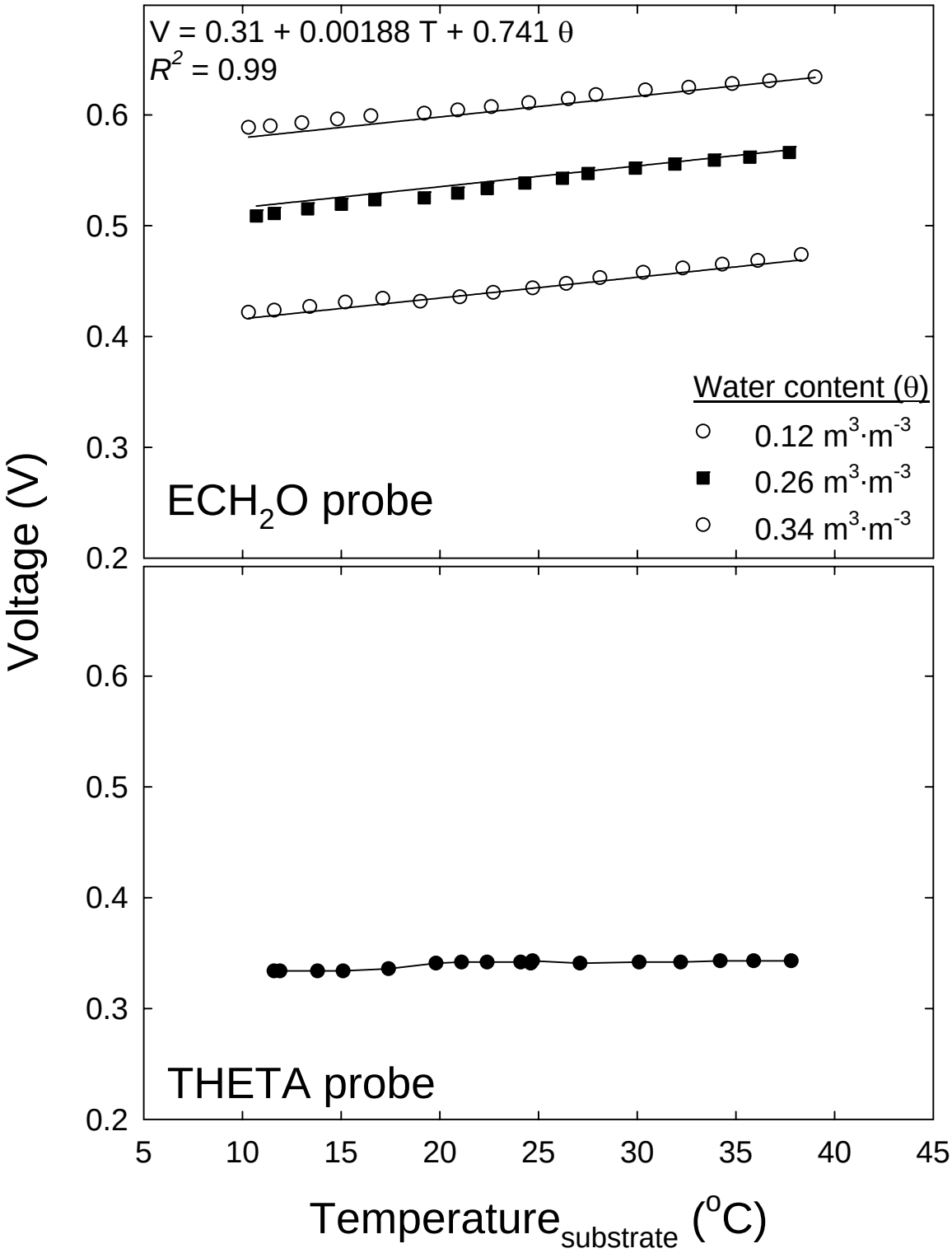
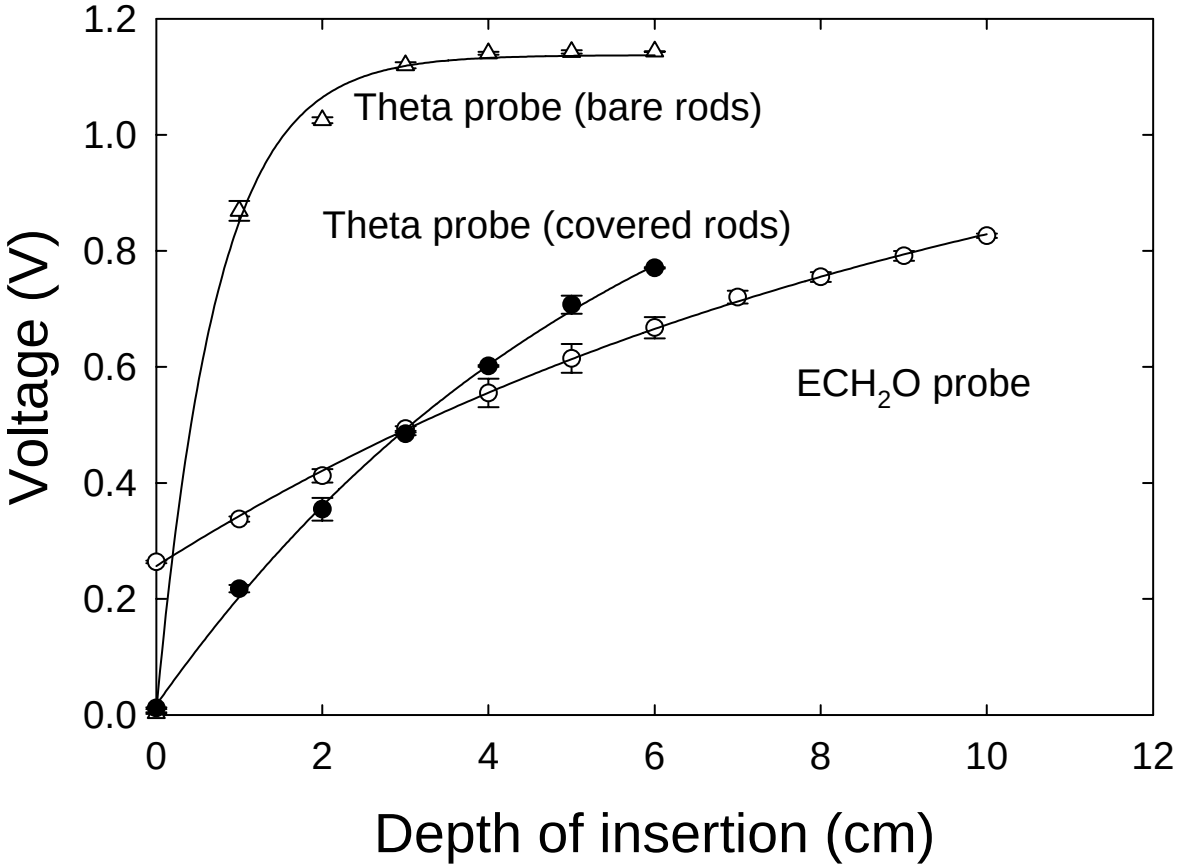

V = 0.31 + 0.00188 T + 0.741 θ
R^2 = 0.99
Water content (θ)
0.12 m³·m⁻³
0.26 m³·m⁻³
0.34 m³·m⁻³
ECH2O probe
THETA probe
Voltage (V)
Temperature substrate (°C)
0.2
0.3
0.4
0.5
0.6
5
10
15
20
25
30
35
40
45

Figure 2.7. Response of ECH_2O and Theta probe output to increasing depth of insertion in deionized water. Error bars represent standard deviation of the mean (bars not visible are within the limit of the symbol). $Theta_{covered} = 0.017 + 1.73 \cdot (1 - \exp(-0.173 \cdot depth))$, $ECH_2O = 0.256 + 0.9 \cdot (1 - \exp(-0.100 \cdot depth))$, $Theta_{bare} = 0.006 + 1.32 \cdot (1 - \exp(-0.137 \cdot depth))$. $R^2 = 0.99$ for all equations.


Voltage (V)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
12
Depth of insertion (cm)
Theta probe (bare rods)
Theta probe (covered rods)
ECH2O probe

CHAPTER 3

A NOVEL IRRIGATION CONTROLLER FOR WATERING AND SIMULATING DROUGHT STRESS IN POTTED PLANTS [1]

---

[1] Nemali, K.S. and M.W. van Iersel. To be submitted to *Scientia Horticulturae*

Abstract

Efficient watering systems which can irrigate substrate to a desired level and supply plants with just the amount of water required for normal plant growth are currently not available. These systems, if developed, can reduce wastage of irrigation water due to excess application, and subsequent leaching and runoff, and aid growers to cope up with the ever increasing restrictions on water-use by many state governments in US. In this study, we developed an irrigation controller that irrigates substrate to a set-point (volumetric water content, θ) and maintains θ close to set-point for several weeks. The controller uses calibrated ECH$_2$O moisture sensors (Decagon Devices, Pullman, WA) interfaced with a CR10x datalogger and solenoid valves connected to SDM CD16 AC/DC controller. The datalogger measures the θ of the substrate every 20 min. When the θ of the substrate drops below the set-point, the controller opens a solenoid valve, which results in irrigation. Substrate volumetric water content is maintained near a constant level as the datalogger is programmed to increase θ by 2 to 3 % during each irrigation. Using this controller with bedding plants *(Salvia splendens, Catharanthus roseus, Petunia hybrida,* and *Impatiens walleriana)* , we were able to maintain four distinct levels of θ for a prolonged period (40 days), regardless of changes in plant size and environmental conditions. The daily average θ maintained was slightly higher (within 2 to 3% on any particular day) than the set-point. When the θ measured and maintained by ECH$_2$O probe was tested in a separate experiment using measurements by another ECH$_2$O probe placed in the same container, the θ measured by both probes was found to be statistically not different..

Keywords: Bedding plants, ECH_2O dielectric sensor, Transpiration water-use, Watering system

Introduction

Increased labor costs, stricter environmental regulations, and increased competition for water resources from urban areas provide strong motivation for greenhouse and nursery growers to opt for efficient irrigation systems which can reduce labor costs and wastage of water. Overhead irrigation systems like sprinkler-, boom-, and drip -irrigation, and subirrigation systems like ebb-and-flow and flooded floor irrigation are automated, hence can reduce labor costs on irrigation, with subirrigation systems having an additional advantage of minimizing leaching losses from the substrate (Elliiot, 1990; Yelanich and Biernbaum,1990; van Iersel, 1996; Morvant et al., 1997; Uva et al., 1998). However, the potential weakness with these automated systems is their inability to irrigate the substrate to a desired moisture level or in the minimal amounts for normal growth.

Automated irrigation systems are usually run by controllers set to a pre-determined irrigation schedule (e.g., to run at a particular time of the day and for a particular duration) and not based on the actual measurements of θ. Often times, automated systems irrigate the substrate close to saturation regardless of plant water requirement and result in wastage of good quality irrigation water through leaching and runoff. To minimize water wastage from automated irrigation systems, there is a need to develop improved irrigation controllers which can irrigate the substrate to a desired level. Such controllers will aid greenhouse growers to comply with stricter government regulations on water-use and fertilizer run-off.

An irrigation controller which can wet the substrate to a desired level also will be useful in research on plant water relations. The inability to irrigate the substrate to a desired moisture level imposes a limitation on the use of currently available irrigation systems in physiological experiments related to studying water requirements of plants. As it is required to maintain the substrate at desired levels to study plant responses at distinct water contents, experiments in the field of plant water relations are conducted by manually maintaining different substrate water contents. This method commonly involves weighing the containers daily and replenishing the fraction of water lost in transpiration (Sinclair and Ludlow, 1986; Ekanayake et al. 1993; Ray and Sinclair, 1988). This method is labor-intensive and in addition, changes in plant fresh mass are generally neglected. In some other studies, to overcome intensive labor work of the previously described technique, plant responses to substrate water content are studied by withholding irrigation and studying responses as substrate water content decreases. This is also not an ideal method as the rate at which drought stress develops after withholding water is usually faster in containers (due to the smaller volume of available water) than under natural conditions. Observed physiological responses in plants can be different for a rapidly-imposed and slowly-imposed drought stress (Cornic et al. 1987; Ludlow, 1987; Saccardy et al. 1996; Earl, 2003).

In both the above methods, it is not possible to have precise control over the rate at which drought stress is imposed (Earl, 2003). An irrigation controller which can irrigate the substrate to a desired level will be also useful in research related to plant-water relations. Using these new irrigation controllers, it may be possible to study the plant response at distinct and precisely controlled levels of θ.

In the present study, we developed an irrigation controller that can be used to irrigate and maintain the substrate close to a desired θ for prolonged periods. The controller is a datalogger (CR10X, Campbell Scientific, Logan, UT, USA) which uses dielectric moisture sensors (ECH_2O probes, Decagon, Pullman, WA, USA), a relay driver (SDM-CD16AC/DC, Campbell Scientific), and solenoid valves to irrigate and maintain substrate close to a desired level.

The objectives of the present study were:

(i) to test whether the controller can maintain the θ of the substrate at a constant level and close to a set–point for a long period and within an acceptable range of the targeted value,

(ii) to test whether fluctuations in greenhouse environment and variations in plant size can affect the performance of the controller to irrigate and maintain substrate close to a desired θ level,

(iii) to test the reliability of θ maintained in the substrate by the controller.

Materials and methods

2.1 Watering system.

Details of the watering system are shown in figure 3.1. Frequent measurements of the θ of the substrate were accomplished using calibrated [$\ln(\theta) = -6.99 + 1.58 \cdot 10^{-2} \cdot mV - 9.91 \cdot 10^{-6} \cdot mV^2$, $R^2 = 0.91$] ECH_2O dielectric soil moisture sensors. A total of 16 ECH_2O moisture sensors were used in the study. The ECH_2O moisture sensors were inserted at an angle into the substrate. As we used 10 cm ECH_2O moisture sensors, they extended almost to three-fourth of the depth of the container. The ECH_2O moisture sensors were

connected in a single-ended fashion to a multiplexer (AM25T, Campbell Sci.), which in turn was connected to a datalogger (CR10X, Campbell Sci.) to measure the sensor output. Type-T thermocouples were used to measure the temperature of the substrate. The thermocouples were connected to the multiplexer along with ECH_2O moisture sensors. The datalogger was programmed to automatically measure ECH_2O output once every 20 minutes, calculate and compensate θ for changes in substrate temperature (i.e., above or below 23.2 ^oC, the temperature at which the sensors were calibrated) based on a pre-determined relationship between substrate temperature and probe output (θ estimated changed by 0.003 $m^3 \cdot m^{-3}\ ^oC^{-1}$, Nemali and van Iersel, 2004).

To control irrigation, 16 solenoid valves (X-13551-72, Dayton electric company, Niles, IL, USA), connected to a 16 port relay driver (SDM-CD16 AC/DC controller, Campbell Sci.), were used. Each solenoid and port of the relay driver were related to one of the 16 containers used in the study and irrigated the substrate in the respective containers. The solenoids were constantly supplied with irrigation water from a pressure-regulated water source. In their regular position, the solenoid valves remained closed, hence no water passed through solenoids. When the datalogger measured a lower θ than the set-point in any container, it was programmed to close a specific port of the relay driver related to that container. By closing the relay related to the container, the corresponding solenoid valve was powered and opened, and the substrate in the container was irrigated. Flexible PVC tubing (Bev-a-Line IV, 3.2 mm i.d., Cole-Palmer, Vernon hills, IL) connected to the outlet of solenoid valve supplied water to containers.

The duration of irrigation was controlled by programming the datalogger to supply power to the solenoid valve for a specific period (one minute) when the ECH_2O moisture sensor measured a lower θ than the set-point. As the datalogger measured θ once every 20 min, there was a period of 19 min for the water to equilibrate and uniformly wet the substrate before the next possible irrigation. The volume of water supplied to the substrate during each irrigation was controlled using pressure compensated drip emitters (Rain-Bird irrigation, Tucson, AZ, USA). The drip emitters were connected to the outlet tubing from each solenoid. The irrigation water was applied on top of the surface using 30 cm dribble rings with 7 holes (Dramm, Manitowoc, WI). The amount of water supplied to different containers during each irrigation was measured before the experiment (approximately 100 mL/minute).

2.2 Methods.

To study the first two objectives, data were separately collected from a larger experiment conducted on bedding plant species, [impatiens (Impatiens walleriana Hook. f), petunia (Petunia xhybrida Vilm.), salvia (Salvia splendens Sellow ex Roemer & J.A. Schultes) and vinca (Catharanthus roseus (L.) G. Don)]. In brief, seedlings were grown for four weeks from seed in 96-cell plug flats and seedlings belonging to all four species were transplanted (one plant from each species per container) into large plastic containers (17.5 L) filled with a soilless substrate [Fafard 2P mix; 60% peat and 40% perlite (v/v)]. All four species were grown together in one container to ensure that all species were exposed to the same θ. Approximately 22.5 g of a slow-release fertilizer (Osmocote 14-14-14, Scotts Co., Marysville, OH, USA) was thoroughly mixed with the substrate in each container before transplanting to meet nutrient requirements of the

plants during the experiment. Seedlings were irrigated normally (a $\theta > 0.4$ $m^3 \cdot m^{-3}$) for a week before subjecting them to water treatments. Treatments comprised of four distinct levels of θ corresponding to irrigation set points of 0.09, 0.15, 0.22, and 0.32 $m^3 \cdot m^{-3}$.

To study the third objective, another experiment was conducted using the same setup and with substrate (Fafard 2P mix) in 15 cm plastic containers (1.76 L). Two ECH_2O moisture sensors were inserted into each container along with two respective thermocouples for temperature compensation of probe output. Similar to earlier experiment, the datalogger maintained water content in each container based on a set-point using the measurement from one of the two ECH_2O moisture sensors (first sensor), while datalogger also measured the output of the second ECH_2O moisture sensor. The second ECH_2O moisture sensor was used as a cross-check to test the reliability of θ maintained by the controller using the first ECH_2O moisture sensor. There were four set-points (0.09, 0.15, 0.22, and 0.32 $m^3 \cdot m^{-3}$) maintained in the substrate during the study. Because the volume of the container was smaller compared to that used in studying first two objectives, the irrigation interval was changed to 60 min (as opposed to 20 min. in the earlier study). The total volume of water supplied in each irrigation was approximately 100 mL.

2.3 Measurements

Two quantum sensors (Apogee instruments, Logan, UT) and an aspirated temperature/ RH sensor (HTO-45R, Rotronic instruments, Huntington, NY) were connected to the datalogger to measure environmental conditions. Environmental data were collected by the datalogger once every 2 min. to obtain hourly and daily averages, and daily minimum and maximum values. Daily light integral (DLI, $mol \cdot m^{-2} \cdot s^{-1}$) was

calculated by integrating the photosynthetic photon flux measurements of the quantum sensors throughout each day. Vapor pressure deficit (VPD, kPa) was calculated as the difference between saturation vapor pressure and actual vapor pressure. Saturation vapor pressure (VP_{sat}, kPa) and actual vapor pressure (VP) were calculated from mean daily temperature (t,°C) and RH (%) as follows:

$VP_{sat} = 0.614 \cdot exp[(17.52 \cdot t) / (240.97 + t)]$ and,

$VP = VP_{sat} \cdot (RH/100)$

Volumetric water content of the substrate was measured by the datalogger once every 20 min. (or 60 min. depending on irrigation interval) to obtain hourly and daily averages, and minimum and maximum values during a day.. The datalogger also measured the number of times each container was irrigated.

Total evapotranspiration (L) from each treatment was estimated from the number of irrigations, θ before imposing treatments ($\theta_{initial}$), and θ in the substrate at the end of the experiment (θ_{final}):

$total\ evapotranspiration = (number\ of\ irrigations \cdot 100/1000) + [(\theta_{initial} - \theta_{final}) \cdot 15],$

where 100 is the mL of water added in each irrigation, dividing by 1000 converts mL to L, and 15 is the approximate volume (L) of the substrate in the containers. Shoot dry mass of the plants in different treatments was determined at the end of the study. Total shoot dry mass from any container was determined by summing shoot dry mass of all four species in any container. Evapotranspirational water-use [volume (mL) of water lost per gram of dry matter produced] in any treatment was estimated as the ratio of total evapotranspiration and total shoot dry weight. This equation ignores the initial shoot dry weight of plants before transplanting and differences in growth rate of species.

2.3. Design and analyses.

The design was a randomized complete block with two replications in both experiments. The experimental unit consisted of a single container at any set-point. Variability in actual θ measured in different set-points (experiment with plants) was shown as standard error of the mean. Data for shoot dry mass and evapotranspirational water-use were analyzed with using 'Proc GLM' of statistical analysis software (SAS, SAS systems, Cary, NC). Means were separated using Tukey's HSD. A $P < 0.05$ was considered to be statistically significant. Significant differences between two ECH_2O probes in any experimental unit in experiment 2 was tested using ANOVA.

Results and discussion

3.1 Experiment 1.

Large variations were seen in the mean DLI and VPD inside the greenhouse (Fig. 3.2) during the 40 days of the experimental period (study with plants). The temperature inside the greenhouse was controlled, hence it did not show large variations during the experiment (Fig. 3.2). The minimum, maximum, and mean values during the experiment for DLI and VPD were 0.44, 11.26, and 4.03 $mol \cdot m^{-2} \cdot d^{-1}$ and 0.19, 1.66, 0.78 kPa, respectively. Corresponding values for temperature were 18.7, 24.1, and 20.8 °C, respectively. This indicates that large fluctuations were noticed in greenhouse environment (DLI and VPD) during the 43 day study.

The shoot dry mass was different among the four θ levels maintained in the study (Fig. 3.3). Shoot dry mass of the two wetter treatments (0.22 and 0.32 $m^3 \cdot m^{-3}$) was significantly higher than that of the two drier treatments (0.09 and 0.15 $m^3 \cdot m^{-3}$). However, shoot dry mass was not different between the two drier or wetter treatments. This indicates that differences existed in plant size among different treatments. This also indicates that plants grew at different rates in different treatments. The average number of irrigations to maintain set-point s of 0.09, 0.15, 0.22, and 0.32 $m^3 \cdot m^{-3}$ were 11, 48, 136, and 137 respectively.

Evapotranspirational water-use was different among the four θ levels maintained in the study (Fig. 3.4). It was highest in the wettest treatment (0.32 $m^3 \cdot m^{-3}$), lowest in the driest treatment (0.09 $m^3 \cdot m^{-3}$), and was not different between 0.15 and 0.22 $m^3 \cdot m^{-3}$. Evapotranspirational water-use at 0.15 and 0.22 $m^3 \cdot m^{-3}$ was also not different from both the wettest and driest treatments. Hence there were differences in water needs among plants grown at different set-point s of water content.

As we were interested to study the effects of variations in greenhouse environment, plant size, and water needs of plants grown at different set-points on the efficacy of the controller to maintain θ in different treatments, we compared the daily average water content to set-points in different treatments. In the two wetter treatments (0.22 and 0.32 $m^3 \cdot m^{-3}$), the controller started to maintain θ immediately after the start of the experiment. As both of the drier treatments were started at a higher θ than the target level, it took several days for θ in these treatments to dry down to the target level (data not shown) before the set-point was maintained. However, there were at least 20 days during which the system maintained θ in the drier treatments (Fig. 3.5). The irrigation controller

maintained θ at 2 to 3% above the set-point in all treatments. The average θ was never below any set-point . The mean and standard error of θ measured in set-points of 0.09, 0.15, 0.22, and 0.32 $m^3 \cdot m^{-3}$ were 0.104 ± 0.0008, 0.168 ± 0.0017, 0.231 ± 0.0026, and 0.331 ± 0.0003 $m^3 \cdot m^{-3}$. In general, the daily variability in measurement was more pronounced in the two drier (0.09 and 0.15 $m^3 \cdot m^{-3}$) than the wetter treatments (Fig. 3.5). The average θ fluctuated on several days (though it was never > 3% on any particular day) in the two drier treatments.

We can not associate the fluctuations in θ in the two drier treatments to the effects of greenhouse environment or plant size as fluctuations of this nature were not seen in wetter treatments which required more irrigations and where plants were larger. Peat-based substrates have a lower hydraulic conductivity with decreasing water content (Naasz et al., 2005). It is possible that in the two drier treatments hydraulic conductivity was low, and because of slow movement of water in the substrate, the applied irrigation water did not equilibrate evenly in the substrate within 20 minutes resulting in variability in measurements. Based on these results, it can be inferred that environmental fluctuations, plant-size, and plant water need had a minimal effect on the performance of the controller. If it is important to maintain θ closer to the set point, less water could be applied per irrigation, either by decreasing the duration of each irrigation interval, or by using emitters with a lower flow rate.

3.2 Experiment 2.

There were no significant differences in the average θ (pooled across 7 days) measured by both ECH_2O moisture sensors. In fact, the θ measured by the second ECH_2O moisture sensor closely tracked that of the first ECH_2O sensor (Fig. 3.6) during

different days. As the θ measured by the first ECH_2O moisture sensor was used to maintain θ above the set-points, and that this θ was similar to that measured by second ECH_2O moisture sensor during different days, it can be inferred that the θ values maintained in the substrate by the controller in both experiments were reliable.

Up to a θ level of 0.22 $m^3 \cdot m^{-3}$ there was practically no leaching during both experiments. Only slight leaching was noticed in the wettest treatment (0.32 $m^3 \cdot m^{-3}$). This shows the superiority of this system compared to other automated irrigation systems. Unlike automated systems which result in leaching losses and run-off, our system had little or no wastage of water. The system required little maintenance during the study. Regardless of the time of the day, the system irrigated the plants when the substrate moisture fell below the target level. On a commercial scale this would result in significant decrease in labor costs of irrigation if automated irrigation is not practiced. The system can be set to maintain a high θ set-point that would result in leaching on any day when it is desired to leach the excess fertilizer salts from the substrate, thereby preventing their accumulation in the substrate.

Conclusions

The automated irrigation controller has potential use in the irrigation of greenhouse crops and studies related to plant water relations. The following conclusions were drawn from this study:

(i) The watering system was able to maintain θ for a long period within an acceptable range of the set-point despite large variations in the environment and plant size.

(ii) As opposed to the dry-down or frequent weighing technique for imposing drought stress, this system maintained θ close to the set-point with minimal or no effect of environment and plant size or effort.

(iii) The validation study confirmed that the θ maintained by the controller was reliable.

This system can be used as the basis for future generation automated irrigation controllers to achieve significant reductions in labor costs and water wastage, and also in studies related to substrate-plant-water relations. We used large volume containers (17.5 L) in studying the system. When containers having smaller volume compared to those in our study were used, it is likely that the system can maintain set-points more efficiently. Water can equilibrate more uniformly and quickly in small containers and probe measurement is more accurate in small volume containers as the ratio of measured volume to total volume of the substrate increases with decreasing volume of the container. However, the irrigation interval and water equilibration time have to be assessed for small containers.

References

Cornic, G., Papageorgiou, I., and Louason, G. 1987. Effect of a rapid and a slow drought cycle followed by rehydration on stomatal and non-stomatal components of photosyntheis in Phaseolus vulgaris L. J. Plant Physiol. 126: 309-318.

Earl, H.J. 2003. A precise gravimetric method for simulating drought stress in pot experiments. Crop Sci. 43: 1868-1873.

Ekanayake, I.J., De Datta, S.K., and Steponkus, P.L. 1993. Effect of water deficit stress on diffusive resistance, transpiration, and spikelet desiccation of rice (Oryza sativa L.) Ann. Bot. 72: 73-80.

Elliot, G. 1990. Reduce water and fertilizer with ebb- and- flow. Greenhouse Grower 8: 70-72, 74-75.

Ludlow, M.M. 1987. Contribution of osmotic adjustment to maintenance of photosynthesis during water stress. P. 161-168. In J. Biggens (ed.) Progress in photosynthesis research. Vol. 4. Martinus Nijhoff Publ., Dordrecht, the Netherlands.

Morvant, J.K., Dole, J.M., and Ellen, E. 1997. Irrigation systems alters distribution of roots, soluble salts, nitrogen and pH in the root medium. HortTechnology 7:156-160.

Naasz, R., Michel, J.-C., and Charpentier, S. 2005. Measuring hysteretic hydraulic properties of peat and pine bark using a transient method. Soil Sci. Soc. Amer. J. 69: 13-22.

Nemali, K.S. and M.W. van Iersel. 2004. Two new moisture sensors for soilless growing media. HortScience 39:763.

Ray, J.D. and Sinclair, T.R. 1998. The effect of pot size on growth and transpiration of maize and soyabean during water deficit stress. J. Exp. Bot. 49: 1381-1386.

Saccardy, K., Cornic, G., Brulfert, J., and Reyss, A. 1996. Effect of drought stress on net CO_2 uptake by Zea leaves. Planta 199: 589-595.

Sinclair, T.R., and Ludlow, M.M. 1986. Influence of soil water supply on the plant water balance of four tropical grain legumes. Aust. J. Plant Physiol. 13: 329-341.

Uva, W.L., Weiler, T.C., and Milligan, R.A. 1998. A survey on the planning and adoption of zero runoff subirrigation systems in greenhouse operations. HortScience 34:660-663.

van Iersel, M.W. 1996. Improving water and fertilizer efficiency in greenhouses. Georgia Floriculture 6:22-23.

Yelanich, M.V. and Biernbaum, J.A. 1990. Effect of fertilizer concentration and method of application on media nutrient content, nitrogen runoff and growth of Euphorbia pulcherrima V-14 glory. Acta Hort. 272:185-189.

Figure 3.1. Schematic diagram showing various parts of the watering system. 1. pressure regulated water source, 2. water line from source, 3. inlet tubing for solenoids, 4. solenoid valve, 5. outlet tubing, 6. pressure-compensated emitter, 7. ECH_2O sensor, 8. thermocouple, 9. drip emitter (ring), 10. CR10x datalogger, 11. AM25T multiplexer, 12. SDM-16AC/DC controller (relay driver), 13. power supply to solenoids, 14. to main power supply, 15. connecting wires between CR10x and AM25T, 16. connecting wires between CR10x and SDM-16AC/DC controller. Only one container is shown in detail although 16 containers can be irrigated.

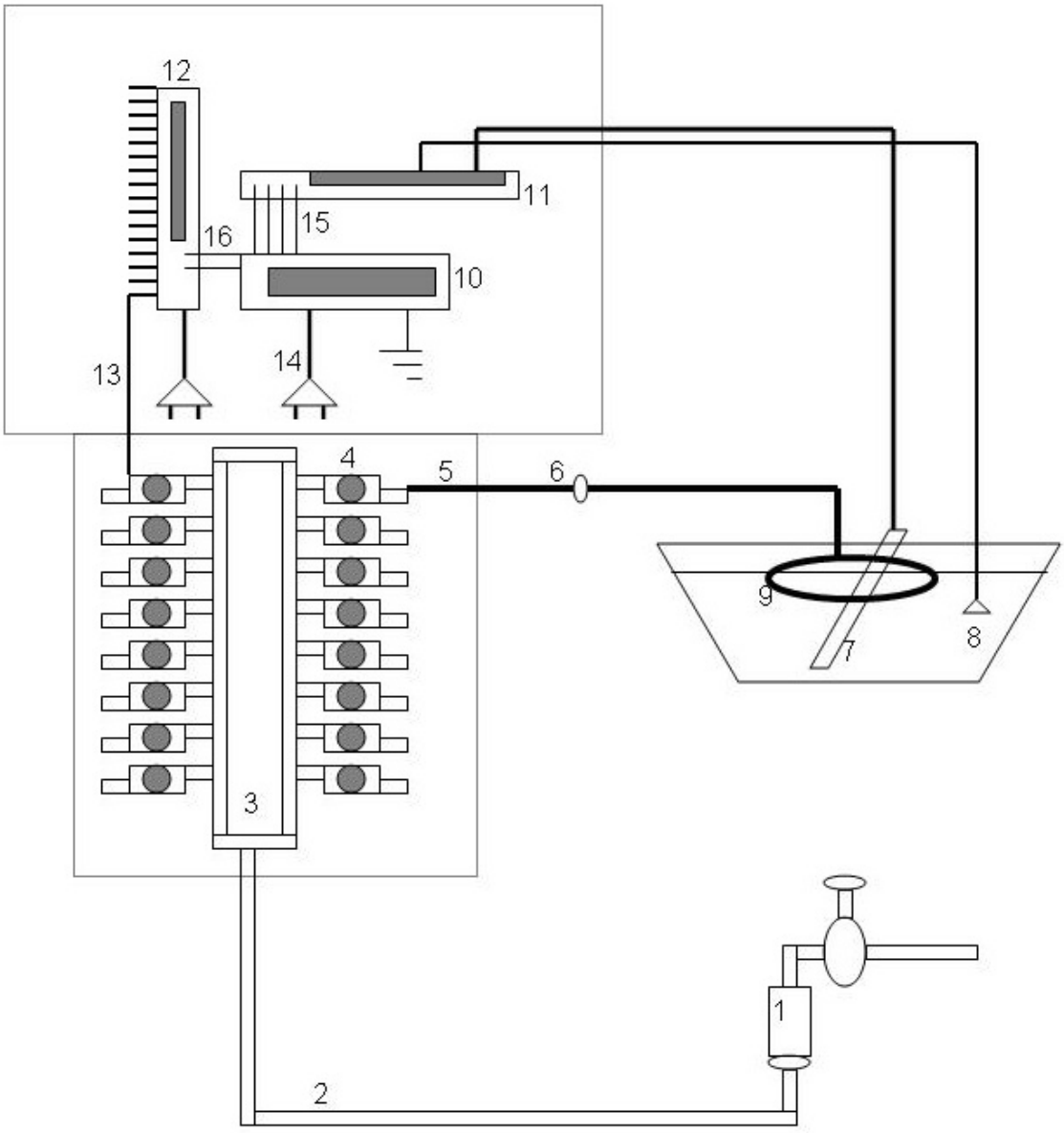

12
11
15
16
10
13
14
4
5
6
9
7
8
3
1
2

Figure 3.2. Changes in average daily light integral (DLI), vapor pressure deficit (VPD), and temperature inside the greenhouse during experiment 1.

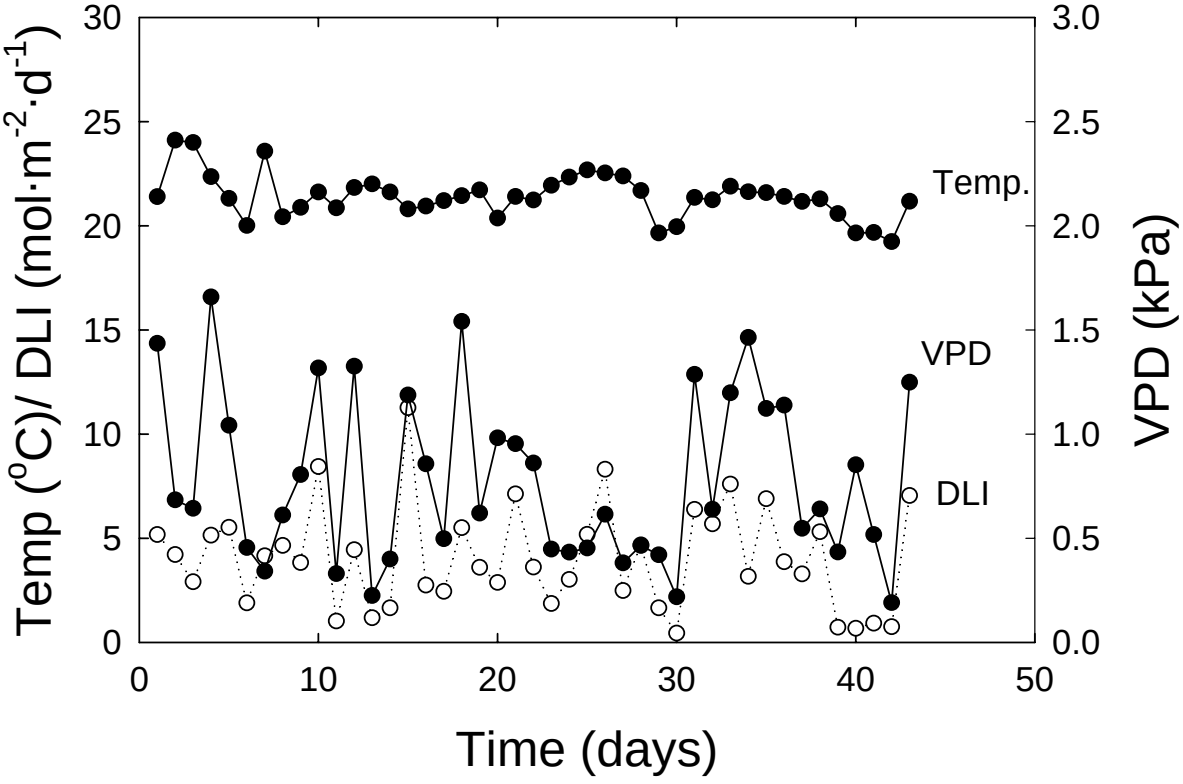

Temp (°C)/ DLI (mol·m-2·d-1)
VPD (kPa)
Time (days)
Temp.
VPD
DLI
0
5
10
15
20
25
30
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0
10
20
30
40
50

Figure 3.3. Mean (n = 2) shoot dry mass in different treatments during experiment 1.

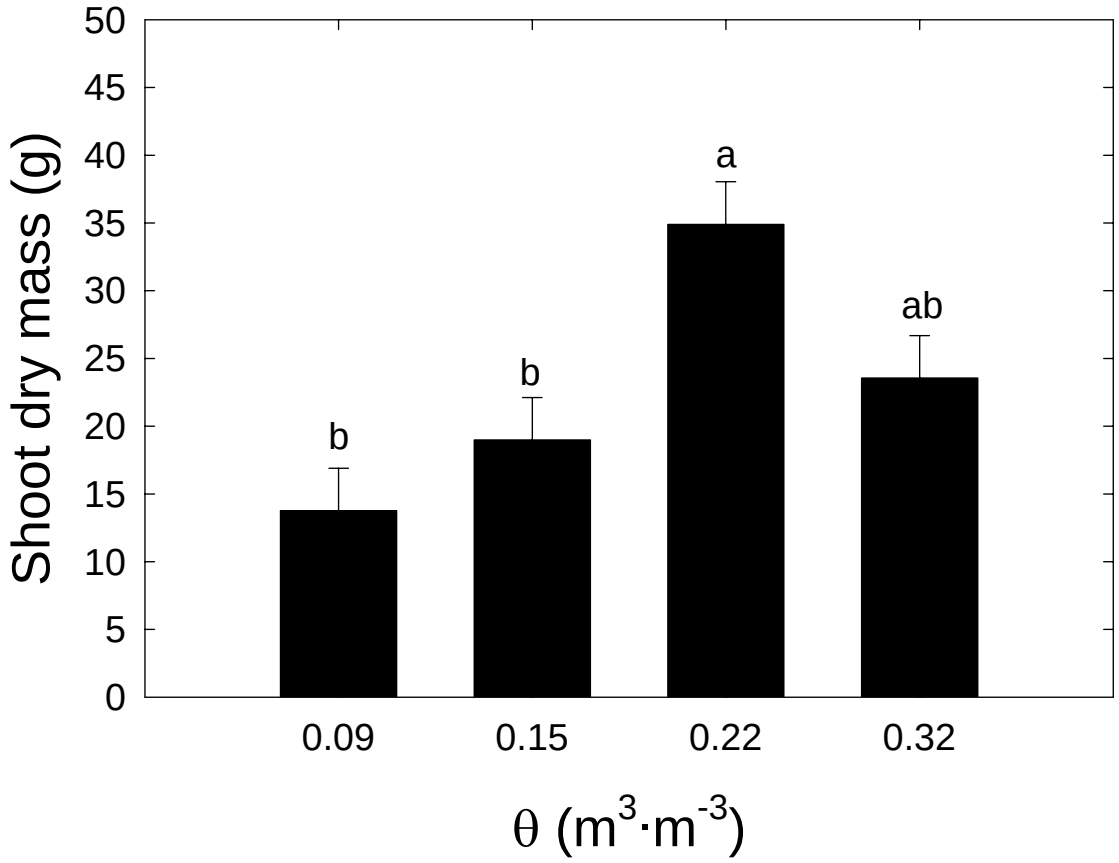

Shoot dry mass (g)
50
45
40
35
30
25
20
15
10
5
0
a
ab
b
b
0.09
0.15
0.22
0.32
θ (m3·m-3)

Figure 3.4. Mean evapotranspirational water-use (TWU) in different treatments during experiment 1.

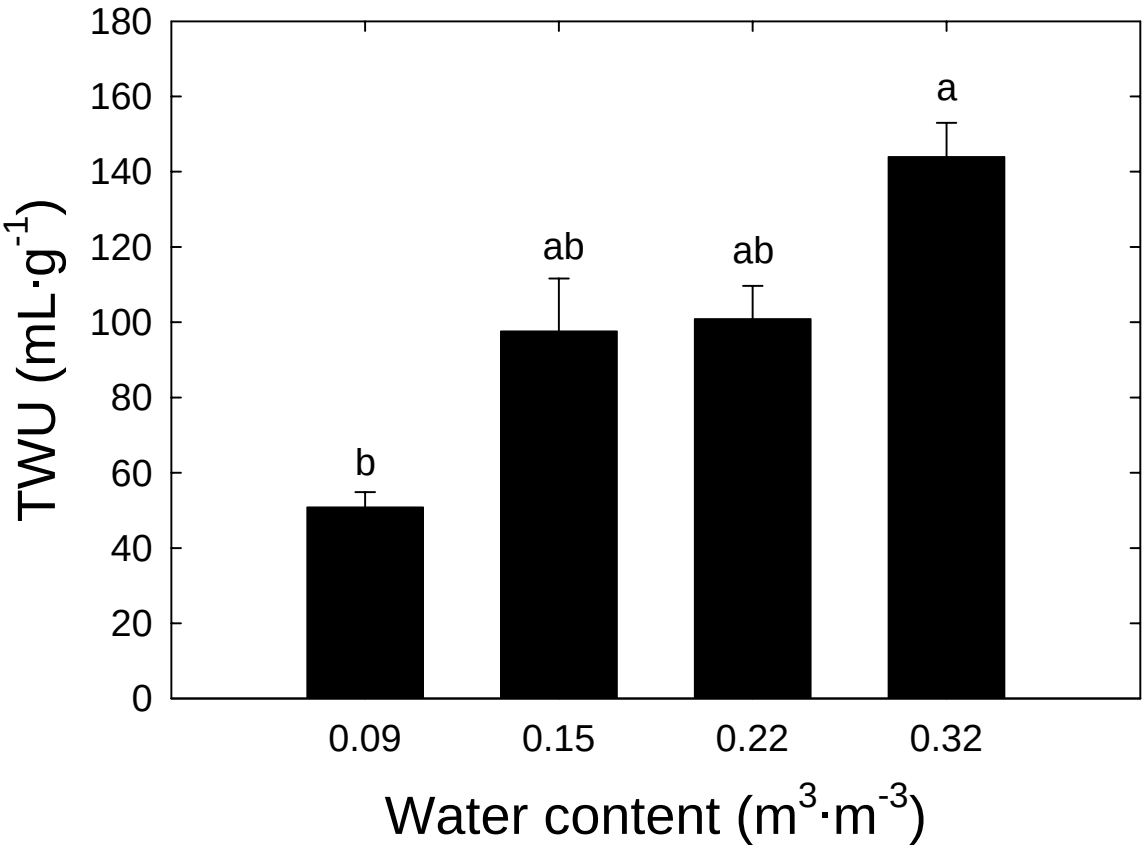

180
160
140
120
100
80
60
40
20
0
TWU (mL·g-1)
b
ab
ab
a
0.09
0.15
0.22
0.32
Water content (m3·m-3)

Figure 3.5. Average (n = 2) daily volumetric water content of the substrate (θ) maintained in different treatments during experiment 1. Error bars represent standard deviation of the mean.

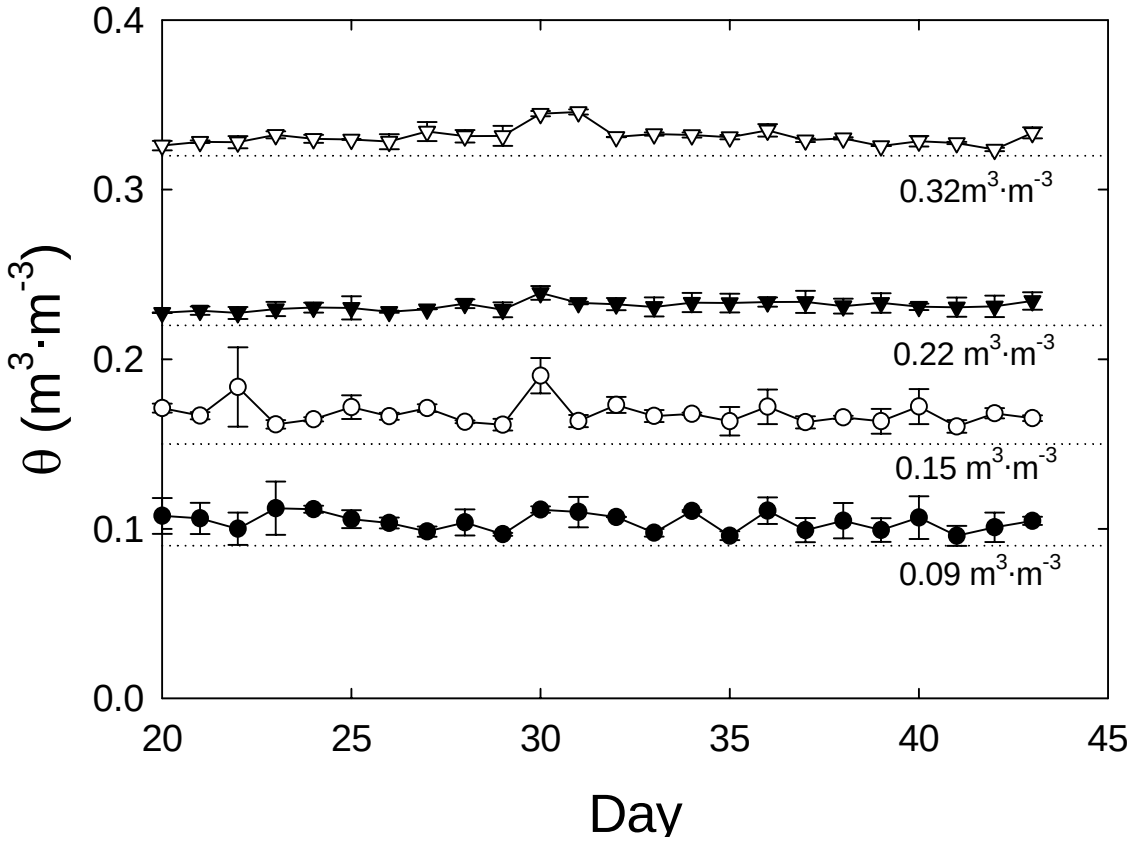
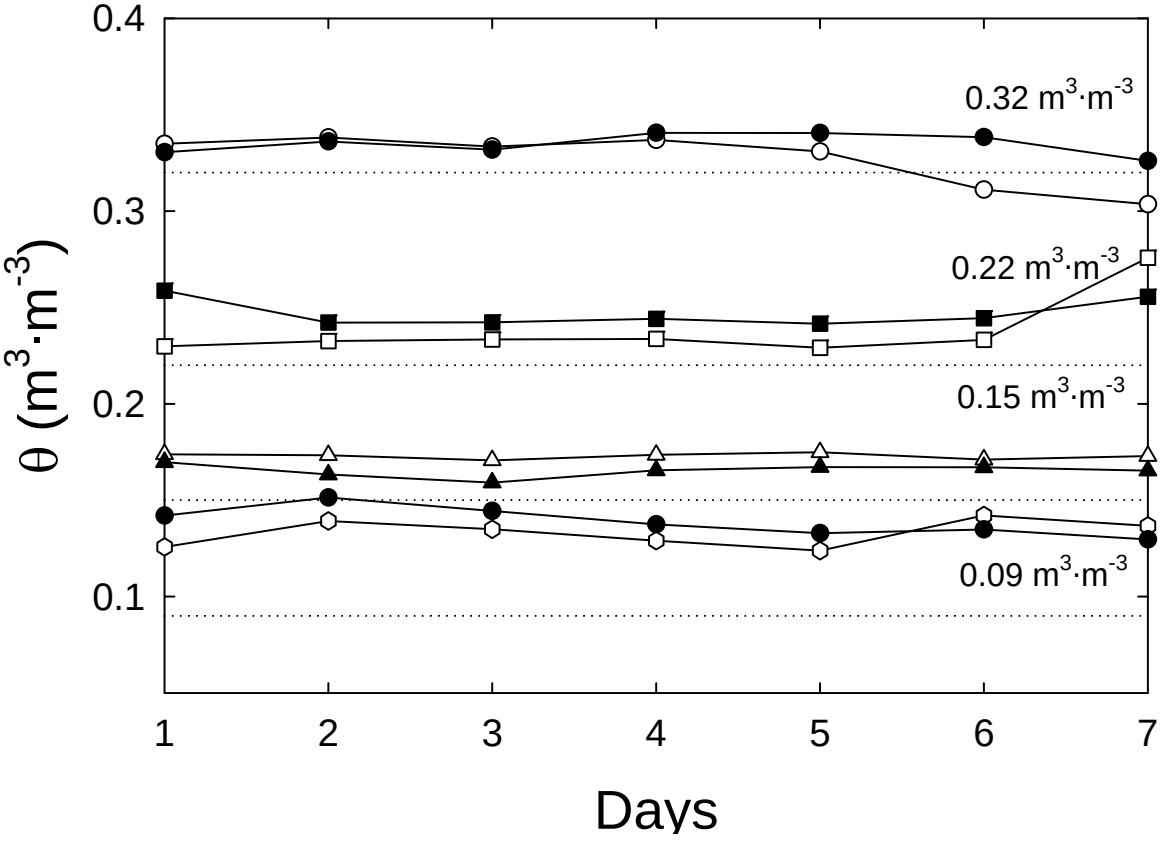

0.4
0.3
0.2
0.1
0.0
θ (m3·m-3)
0.32m3·m-3
0.22 m3·m-3
0.15 m3·m-3
0.09 m3·m-3
20
25
30
35
40
45
Day

Figure 3.6. Average (n = 2) daily volumetric water content of the substrate (θ) in different treatments during different days in experiment 2. Closed symbols represent θ measured and maintained by first ECH_2O sensor, while open symbols represent θ measured by second ECH_2O sensor, which was used for validation.


0.4
0.3
0.2
0.1
θ (m3·m-3)
0.32 m3·m-3
0.22 m3·m-3
0.15 m3·m-3
0.09 m3·m-3
1
2
3
4
5
6
7
Days

CHAPTER 4

MOISTURE RETENTION CURVES AND PORE-SIZE DISTRIBUTION IN SOILLESS SUBSTRATES [1]

---

[1] Nemali, K.S., D.E. Radcliffe, and M.W. van Iersel. To be submitted to *HortScience*

Moisture Retention Curves and Pore-Size Distribution in Soilless Substrates

Additional Index Words. Chilled-mirror dew point hygrometer, peat, perlite, pine bark, pressure chamber, Tempe cells, vermiculite

Abstract

Currently, there is little information available on plant available water and pore-size distribution in soilless substrates. In this study, we developed substrate moisture retention (SMR) curves in the range of 0 to -50000 cm of capillary head (-5 MPa or -50 bars of water potential) and determined pore-size distribution for peat + perlite (P-P) and peat + bark + perlite + vermiculite (P-B-P-V) based substrates. We also compared the efficacy of chilled mirror dew point hygrometer and pressure chamber to determine water retention characteristics in soilless substrates in the tension range of -1000 to 50000 cm. When the water potential was maintained between -5000 to -14500 cm in both substrates using a pressure chamber, the volume of water retained was higher than that retained at a much higher water potential (-950 cm) maintained in the substrate using Tempe cells. This anomaly could be due to the discontinuity in pores between the substrate and ceramic plate of the pressure chamber. However, no anomalies were noted while using Tempe cells and hygrometer. We were able to see significant changes in water retention at tensions between -1000 to -50000 cm using a hygrometer. Based on our results obtained by combining measurements from Tempe cells and hygrometer, there was little water (0.09 $m^{3} \cdot m^{-3}$) retained in a P-P substrate that would

drain in the range of -1000 to -15330 cm, whereas there was large volume of water retained (0.29 $cm^{3}{\cdot}cm^{-3}$) in the same range in a P-B-P-V substrate . Because of this, water potential would drop rapidly from -1000 to -15330 cm in a P-P substrate and gradually in a P-B-P-V substrate. At a substrate water content of ~0.20 $cm^{3}{\cdot}cm^{-3}$, the water potential was five-fold lower in the P-P substrate than the P-B-P-V substrate. Our results also indicated that large volume of water (0.19 $cm^{3}{\cdot}cm^{-3}$) can be drained from a P-P substrate with in the tension range of -15330 to -50000 cm. Based on our results, there were many pore-sizes in both substrates in the ultra micropore range, indicating the importance of ultra micropores in storage, availability, and transport of water in soilless substrates.

It is important to know the plant available water of potting media or soilless substrates. Plant available water is the difference between the container capacity (drained upper limit) and permanent wilting point. The definition of 'permanent wilting point' is the lowest water potential of a soil at which plants can access water (Lambers et al., 1998). Although the water potential at the permanent wilting point is assumed to be -15330 cm (or -1500 kPa, -15 bars, -1.5 MPa), the actual water potential at wilting point will depend on species and soil type (Taiz and Zeiger, 2002). It is also possible that other factors like plant age and history may influence the wilting point. For these reasons, assuming permanent wilting point in soils to be -15330 cm may not be accurate. Usually, the amount of water held in a regular 'soil' medium at the permanent wilting point is low (~ 0.05 to 0.10 $cm^{3}{\cdot}cm^{-3}$; Bachmann et al. 2002; Prunty and Cassy, 2002; Chan and Govindaraju, 2004). It is assumed that the water in a soil medium at water potential

lower than -15330 cm is unavailable to plants as it is held tightly in a film on the surface of soil particles ('hygroscopic' as opposed to 'capillary' water). For this reason, structure and pore size distribution are considered unimportant for water availability in soils at tensions below -15330 cm and the end point of soil moisture retention curves is assumed to be -15330 cm (van Genuchten, 1980; Zurmühl and Durner, 1996). Moisture retention curves often are determined by either using Tempe cells or tension plates up to tensions of -1000 cm and using a pressure chamber for measuring water retention at a tension of -15330 cm (Drzal et al., 1999).

Several studies (Fonteno et al., 1981; Drzal et al., 1999; Sahin et al., 2002) on SMR curves have indicated that the total volume of water retained by the substrate at -15330 cm is approximately in the range of 0.20 to 0.30 $cm^{3} \cdot cm^{-3}$. However, bedding plants in soilless substrates wilt when the substrate water content falls below 0.10 to 0.15 $cm^{3} \cdot cm^{-3}$ (Olson et al., 2002; Nemali and van Iersel, 2005), which appears to be well below a tension of -15330 cm. There are few studies which determined SMR curves in soilless substrates within the entire range of 0 to -15330 cm (Fonteno et al., 1981; Drzal et al., 1999). Most studies developed relations among substrate water content and water potential within the range of 0 to -1000 cm (Bilderback et al., 1982; Bilderback and Fonteno, 1987; Ingram and Yeager, 1987; Tilt et al., 1987; Fonteno and Nelson, 1990). Hence there is a need to determine SMR curves in soilless substrates in a broader range and below a tension of -15330 cm in soilless substrates.

Moisture retention curves can differ among different soilless substrates due to differences in pore-size distribution. Pore size distribution refers to the relative volume of different size pores existing in a substrate at any particular time. The volume of water

retained at any water potential will depend on several factors of which pore-size distribution is most important (Ahuja et al., 1998; Stange and Horn, 2005). Pore size distribution and water retention are mutually interactive, with pore size determining the extent of water retention, and conversely water/hydraulic pressure influencing the pore size (Stange and Horne, 2005). Pore size distribution can be estimated from SMR curves (Milks et al., 1989; Zurmühl and Durner, 1996; van Vliet et al., 1998; Drzal et al., 1999; Coppola, 2000). An understanding of the distribution of different sizes of pores will aid us in seeing the 'internal' structure of the substrate (Drzal et al., 1999).

Pore space is the most important physical characteristic of a soilless substrate as it retains water (and nutrients in the water), oxygen, and allows root growth. Hillel (1982) has designated substrate pores as inter-aggregate or macropores for water infiltration/drainage and intra-aggregate or micropores for water retention. Earlier reports have indicated that 40 (e.g., peat, coir) to 90% (e.g., rock wool) of total water is usually retained in macropores between tensions 0 to -10 kPa (Bilderback et al., 1982; Bilderback and Fonteno, 1987; Fonteno and Nelson, 1980; Raviv et al., 2001). Most of the water retained in macropores is usually lost in drainage which lowers the substrate water status from saturation to container capacity. Water is taken up easily by plants in soilless substrates within a tension range of -50 to -500 cm. In this range, water is retained mostly in the large micropores. At substrate moisture tensions below -500 cm, water is held in the ultra micropores (Drzal et al., 1999). In spite of its importance, relatively little work has been done to categorize pore size distribution in soilless substrates, especially at tensions below -1000 cm.

Two objectives were set for this experiment,

(i) develop SMR curves for peat-perlite and peat-bark-perlite-vermiculite media (both having approximately 60% organic and 40% inorganic content) in a broad range and well below -15330 cm to account for water retention from close to saturation to less than 0.10 $cm^{3}{\cdot}cm^{-3}$ in the substrate, and

(ii) determine pore-size distribution in both substrates using the SMR curves.

Materials and Methods

Materials:

Two commercially-available soilless substrates i.e., Fafard 2P (60% peat and 40% perlite; hereafter P-P) and Fafard 4P (36% peat, 27% processed pine bark, 15% perlite, and 22% vermiculite; hereafter P-B-P-V) were used in the study. Substrate moisture retention curves in the range of 0 to -1000 cm of capillary head (hereafter water potential) were determined using Tempe cells (Soil Moisture Equipment Corp., Santa Barbara, Calif.). A pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, Calif.) was used to determine SMR curves in the range of -5000 to -14500 cm (as the maximum pressure that can be retained was -15 bars or -15330 cm in the available pressure chamber) and a hygrometer (Dewpoint Potentiometer; model WP4, Decagon devices, Pullman, Wash.) was used to determine SMR curves in the range of -2000 to -50000 cm.

Methods:

a. Tempe cells. The bottom of the metallic rings used to hold the substrate was covered with a nylon porous cloth before filling rings with substrate. The nylon cloth was always fastened to the metallic rings to hold the substrate. Care was taken not to compress the substrate while filling the rings. The rings containing the substrate and ceramic plates (air entry value of 1 bar or 1022 cm) of the Tempe cells were soaked in 0.01 M $CaCl_2$ solution and allowed to saturate over a period of 24 h (to prevent dispersion of substrate particles and saturate the substrate without any air gaps). After soaking thoroughly, the ceramic plates were fastened to the bottom of the Tempe cells using O-rings. The saturated substrate inside the rings was placed on the ceramic plates. Water was sprayed on the ceramic plate before placing the rings to improve the contact between the substrate and ceramic. The top of the Tempe cell was assembled by inserting an O-ring in the cap and pressing it onto the brass ring. The weight of the saturated substrate along with that of the Tempe cell was measured prior to subjecting them to any air pressure.

An air line was connected to the cap of the Tempe cell and a water manometer was connected to the air line to monitor pressures. For a given pressure setting, Tempe cells were weighed daily until the weight loss did not change over a 24 h period. This weight of the Tempe cell was noted as the equilibrium weight and subsequently air pressure was increased to the next level. The process was repeated and equilibrium weights were noted for each pressure setting. Air pressure settings were selected to result in a substrate water potential of -50, -100, -300, -500, -700, and -950 cm. After the last pressure setting, the Tempe cells were disassembled, and the substrate was oven dried

at 105 °C. The oven dry weight of the substrate was used in the estimation of the volumetric water content (θ in cm^3 cm^{-3}) at each pressure setting. One gram of water was assumed to be 1 mL to obtain volume of water in the substrate. Volume of substrate was equated to the volume of the ring.

b. Pressure chamber. A pressure chamber was used to determine the water content retained in the substrates at tensions of -5000, -10000, and -14500 cm. Metallic rings were filled with the substrate as described earlier and soaked in 0.01 M $CaCl_2$ along with a ceramic pressure plate (air entry value of 15 bars or 15330 cm) for 24 h. The ceramic plate was placed in a pressure chamber and rings containing substrate were placed on the ceramic plate after sprinkling water on the plate to improve contact between the substrate and ceramic plate. The pressure chamber was connected to a hose to deliver required air pressure. There was an outlet tube for water to drain from the substrates at any pressure setting. After reaching equilibrium (usually 3 days), the substrate along with the metallic ring was weighed. The substrate was dried in an oven at 105 °C before determining the oven dry weight of the substrate. A similar procedure was repeated at each desired air pressure setting.

c. Hygrometer. Prior to making any measurements, standard cleaning and calibrations procedures were followed as described in the manual. The hygrometer calibration was verified using standard KCl solution of known osmotic potential. Later, substrate samples were prepared by adding different volumes of water to the substrate. Samples were weighed prior to placing inside the hygrometer. Sampling cups were filled with a known volume (5 mL) of the substrate. The water potential of the sample was measured by inserting the cup containing the sample inside the hygrometer. After

measurement, the samples were dried in an oven at 105 °C before determining the oven dry weight.

Water content ($cm^3 \cdot cm^{-3}$) retained at any pressure setting for the above three methods was calculated after subtracting the oven dry weight of the substrate (and equipment weight) from recorded equilibrium weight of the substrate (and equipment/ container weight) at any pressure setting.

Statistical Analysis:

Data were analyzed using SAS (statistical analysis software, Cary, N.C.) with $P < 0.05$ considered significant. Two separate non-linear equations were fitted to develop SMR curves for Tempe cells together with pressure chamber data and Tempe cells together with hygrometer data. Non-linear equations were fitted using the regression procedure of SAS (Proc Nlin; $P < 0.05$). We used these equations as opposed to the conventional unimodal equation (van Genuchten, 1980) as they gave a better fit to the data, especially at water potentials below -15330 cm.

For Tempe cells + pressure chamber data,

$$\theta(h) = a \cdot exp(b \cdot h) + c \cdot exp(d \cdot h) \quad \text{[eq.1]}$$

For Tempe cells + hygrometer data,

$$\theta(h) = y_0 + a \cdot exp(b \cdot h) + c \cdot exp(d \cdot h) \quad \text{[eq. 2]}$$

where y_0, a, b, c, and d are regression coefficients and h is substrate water potential (cm head).

Capacitance [the rate at which water is released or the amount of retained water decreases with decreasing water potential, C(h)] values were estimated for SMR curves developed from Tempe cells + hygrometer data. Capacitance was calculated as the

derivative of the fitted equation. For both equations, the derivative is

$$d\theta / dh = - a \cdot b \cdot exp(-b \cdot h) - c \cdot d \cdot exp(-d \cdot h) \quad \text{[eq. 3]}$$

The radius (r, cm) of the largest water-filled pore at each water potential (matric head, cm) was estimated using the capillary rise equation (Jury et al., 1991):

$$r(h) = - 2\sigma \cdot \cos\alpha / \rho_l \cdot g \cdot h \quad \text{[eq. 4]}$$

where σ is the surface tension (71.9 ergs· cm^{-2} at 25 ^{o}C), α is the contact angle (assumed to be 0^{o}), ρ_l is the density of water (0.99708 g·cm^{-3}), g is the acceleration due to gravity (980 $cm.s^{-2}$) and h is the water potential in cm. Pore volume (v) was calculated as the volume of pores of a given radius divided by the total pore volume or porosity. The relationship between pore volume and pore radius was described by fitting the following double hyperbolic equation as it resulted in the best fit:

$$v(r) = [(a \cdot r) / (b + r)] + [(c \cdot r) / (d + r)] \quad \text{[eq. 5]}$$

where a, b, c, and d are regression coefficients, and r is the radius of the pore. The prediction intervals (95%) for the fitted hyperbolic functions were obtained from the SAS analysis.

Pores were categorized into different classes based on the Soil Science Society of America pore size classification system (Kay and Angers, 2001). Based on this, macropores were considered to have a radius greater than 0.0075 cm, mesopores to have a radius in the range of 0.003 to 0.0075 cm, micropores to have a radius in the range of 0.003 to 0.0003 cm, and ultra micropres to have a radius less than 0.0003 cm. As total porosity can be equated to volume of water at saturation (~ 1 cm capillary head), the upper end of the macropores was designated as pores that would drain at a water potential of 1 cm (radius ~ 0.147 cm). Volume of solids was determined as 1 - total

volume of pores having a radius smaller than 0.147 cm. Volume of macropores in the substrate was determined by subtracting the volume of pores with radius smaller than 0.0075 cm from the volume of pores with radius smaller than 0.147 cm. Similarly, volume of mesopores and micropores was determined by considering 0.0075 cm/0.003 cm and 0.003 cm/0.0003 cm as the upper/lower end radii, respectively and substituting the values in the fitted equation. The volume of ultra micropores was determined as the volume of remaining pores below a radius of 0.0003 cm by solving the fitted equation for $r \leq 0.0003$ cm.

Data are represented in *CGS* units as opposed to *SI* units as it is conventional to represent water potential as cm of head and pore radius in cm. Hence, the dependent variables i.e., water volume, capacitance, and pore volume were also represented in *CGS* units. For the benefit of readers, conversions to *SI* units are shown in table 1.

Results and discussion

Soil moisture retention curves. The substrate comprised of P-B-P-V retained more water at saturation than that comprised of P-P (Fig. 4.1). This indicates that the total porosity of the P-B-P-V substrate was higher than that of the P-P substrate. The calculated dry bulk density of P-P and P-B-P-V substrates was 0.094 and 0.139 $g \cdot cm^{-3}$. Usually, a regular soil medium with low bulk density will have more porosity as porosity is inversely related to bulk density [porosity = 1- (bulk density/particle density); a constant value of 2.65 $g \cdot cm^{-3}$ is assumed for particle density of soil particles]. However, the relationship between porosity and bulk density can be affected in soilless substrates as the substrates are comprised of a large fraction of particles with a low particle density,

like perlite (~0.032 g.cm^{-3}; www.schundler.com/coatings.htm). The P-P substrate contained 40% perlite, whereas P-B-P-V contained only 15% perlite.

There was a steep decrease in the water retention capacity of both substrates as the water potential decreased to -100 cm (Fig. 4.1). The water content in P-P and P-B-P-V substrates dropped from saturation to 0.28 and 0.32 cm^3·cm^{-3} when water potential dropped to -300 cm. This indicates that a large volume of water (~0.30 cm^3·cm^{-3}) was held in pores which drained at suctions $\leq$ -300 cm. The substrate composed of P-B-P-V retained more water than that of P-P at any water potential up to -1000 cm. Earlier reports have also indicated that significant volume of water is retained in soilless substrates within a water potential range of 0 to -300 cm (Fonteno et al., 1981; Bilderback et al., 1982; Ingram and Yeager, 1987; Fonteno and Nelson, 1990).

The hygrometer was found to be more effective in both soilless substrates for measuring water retention than the pressure chamber below tensions of -1000 cm (Fig. 4.1). Our results indicate that an approximately three-fold decrease in water potential from -5000 to -14500 cm using a pressure chamber did not result in any change in the water retained by both substrates (Fig. 4.1). In fact, there was a discontinuity of data, when pressure chamber measurements were combined with measurements from Tempe cells, as the water content retained was higher at -5000 to -14500 cm (pressure chamber) compared to -1000 cm (Tempe cells). There are only a few studies (Fonteno et al., 1981; Drzal et al., 1999; Sahin et al., 2002) which describe the relationship between water content and water potential in soilless substrates in the tension range of -1000 cm to -15330 cm. All these studies used a pressure chamber to determine water retention at a tension of -15330 cm. Earlier reports in a bark-based substrate indicated

either no change (Drzal et al., 1999) or a slight decrease of 0.10 $cm^3 \cdot cm^{-3}$ (Fonteno et al., 1981) in water content between -1000 and -15330 cm as measured by a pressure chamber. The results from our study suggest that there are no pores which could empty in the range of -5000 to -14500 kPa, which seems unlikely. A likely reason for finding anomalies in water content data between -1000 and -15330 cm in our study could be the lack of continuity of pores between the substrate and tension plate or among the smaller pores within the substrate, which could have potentially blocked the transport of water to the exterior of the pressure chamber when air pressure was increased inside a pressure chamber. Hence, the water contents of ~0.28 and 0.34 $cm^{3} \cdot cm^{-3}$ retained in P-P and P-B-P-V substrates at -15330 cm (Fig. 4.1) respectively, may not be true and appear to be an artifact of discontinuity of pores. This was not noticed when the data from the hygrometer were combined with those of the Tempe cells.

As the data obtained from the Tempe cells and hygrometer did not show discontinuity (Fig. 4.1) when combined, the combined data was used to develop SMR curves. The rest of this discussion is based on SMR curves developed using Tempe cells and hygrometer. The relationship between substrate water potential and water content in the two substrates was different below a tension of -1000 cm. There was approximately 0.09 $cm^{3} \cdot cm^{-3}$ decrease (0.28 to 0.19 $cm^3 \cdot cm^{-3}$) in the water content of the substrate comprised of P-P, whereas the substrate comprised of P-B-P-V lost approximately ~ 0.29 $cm^{3} \cdot cm^{-3}$ water (0.34 to 0.05 $cm^{3} \cdot cm^{-3}$) when the water potential was decreased from -1000 to -15330 cm (Fig. 4.1). These results indicate that there will be little water in the substrate comprised of P-B-P-V at a water potential of -15330 cm. So, unlike other earlier reports (Fonteno et al., 1981; Drzal et al., 1999; Sahin et al.,

2002), water content at a tension of -15330 cm was low in the P-B-P-V substrate and close to 0.05 $cm^{3} \cdot cm^{-3}$.

The finding that only 0.09 $cm^{3} \cdot cm^{-3}$ water was released in P-P substrate in the range of -1000 to -15330 cm indicates that the SMR curve was relatively flat in this range (Fig. 4.1). In contrast, a substrate comprised of P-B-P-V drained gradually in the same range. There are perhaps few pores in the P-P substrate that drain in this tension range. It is likely that a substrate comprised of P-P will change its water potential rapidly from -1000 to -15330 cm as the substrate dries or plants take up a relatively small volume of water. Unlike the P-B-P-V substrate, the total volume of water retained in the P-P substrate was high (0.19 $cm^{3} \cdot cm^{-3}$) when the substrate water potential was -15330 cm. When both substrates were compared at a water content of ~0.20 $cm^{3} \cdot cm^{-3}$, the water potential of the P-P substrate (-15000 cm) was five-fold lower than that of the P-B-P-V substrate (-3000 cm). There was a steep decrease in the water content of the P-P substrate with a further decrease in water potential from -15330 to -50000 cm, suggesting the presence of many pores in this tension range (Fig. 4.1). At a substrate water potential of -45000 cm, the total volume of water retained in P-P substrate was close to 0.05 $cm^{3} \cdot cm^{-3}$.

Capacitance was highest and similar in both substrates between -1 to -100 cm (Fig. 4.2). This indicates that the rate at which water releases from these substrates with decreasing water potential was highest in the range of 0 to -100 cm. This also indicates that the pores draining in this tension range are similar between the two substrates. The capacitance function was higher in P-B-P-V than P-P substrate between the tension range of -300 to -10000 cm, indicating a higher rate of change or presence of more pores in the P-B-P-V substrate which can drain in this tension range. Below a water

potential of -15330 cm, the capacitance function in the P-B-P-V substrate quickly dropped to a negligible value, indicating little water released in this range. In the same tension range, the slope of the SMR curve was higher for a substrate comprised of P-P, which indicates the presence of more pores and water in this tension range (Fig. 4.2).

Pore-size distribution. The volume of pores having a radius smaller than 0.0003 cm (ultra micropores) was higher in P-P (0.33 $cm^3 \cdot cm^{-3}$) than P-B-P-V (0.28 $cm^3 \cdot cm^{-3}$) substrate (Fig. 4.3 & 4.4). Drzal et al. (1999) have reported that bark- and peat-based media contained 0.29 and 0.22 $cm^{3} \cdot cm^{-3}$ of ultra micropores, respectively. The values of P-B-P-V substrate are close to the results obtained from other bark-based substrates (Drzal et al., 1999). As ultra micropores comprise a large fraction of the total pore space and retain a large volume of water, and releases water at tensions below the permanent wilting point, it is likely that they are important for plant processes. The reason why both substrates contain a large volume of pore space as ultra micropores could not be determined here, however it is speculated that a large fraction of the ultra micropores are present inside the fibrous peat particles, and ultra micropores could have been more prevalent in the P-P than the P-B-P-V substrate because of the higher peat content of the former substrate. The volume of pores with radii from 0.003 to 0.0003 cm (micropore range) was higher in the P-B-P-V (0.18 $cm^3 \cdot cm^{-3}$) than the P-P (0.13 $cm^3 \cdot cm^{-3}$) substrate (Fig. 4.4). Put together, both micropores

and ultra micropores account for 0.40 an 0.43 $cm^3 \cdot cm^{-3}$ pore space in P-B-P-V and P-P substrates, respectively.

The volumes of mesopores and macropores were similar between the two substrates (Fig. 4.4). Mesopores accounted for 0.085 and 0.082 $cm^3 \cdot cm^{-3}$ of pore space in P-B-P-V and P-P substrates, respectively. On the other hand, the volume of macropores accounted for 0.11 and 0.12 $cm^3 \cdot cm^{-3}$ of pore space, respectively in P-B-P-V and P-P substrates (Fig. 4.4). The volume of solid components in P-B-P-V and P-P substrates was 0.30 and 0.39 $cm^3 \cdot cm^{-3}$, respectively.

Conclusions

The following conclusions can be drawn from this study:

(i) In the tension range of -1000 to -15330 cm, the P-B-P-V substrate released more water than the P-P substrate. The P-P substrate released more water than P-B-P-V substrate at tensions lower than -15330 cm. This is due to the presence or absence of pores draining in the respective tension ranges, with more water being released when there are many pores present,

(ii) Ultra micropores contribute a significant volume of the total pore space and these pores can hold a significant amount of water,

(iii) In both soilless substrates, the hygrometer was more sensitive in the range of -1000 to -50000 cm than the pressure chamber.

References

Ahuja, L.R., F. Fiedler, G.H. Dunn, J.G. Benjamin, and A. Garrison. 1998. Changes in soil water retention curves due to tillage and natural reconsolidation. Soil Sci. Soc. Amer. J. 62: 1228-1233.

Bachmann, J., R. Horton, S.A. Grant, and R.R. van der Ploeg. 2002. Temperature dependence of water retention curves for wettable and water-repellant soils. Soil Sci. Soc. Amer. J. 66: 44-52

Bilderback, T.E., and W.C. Fonteno. 1987. Effects of container geometry and media physical properties on air and water volumes in containers. J. Environ. Hort. 5:180-187.

Bilderback, T.E., W.C. Fonteno and D.R. Johnson. 1982. Physical properties of media composed of peanut hulls, pine bark, and peat moss and their effects on Azalea growth. HortScience 107:522-525.

Coppola, A. 2000. Unimodal and bimodal descriptions of hydraulic properties for aggregated soils. 64: 1252-1262.

Chan, T.P., and R.S. Govindaraju. 2004. Estimating soil water retention curve from particle size distribution data based on polydisperse sphere systems. Vadose Zone J. 3: 1443-1454.

Drzal, M.S., W.C. Fonteno and K.D. Cassel. 1999. Pore fraction analysis: A new tool for substrate testing. Acta Hort. 481:43-54.

Fonteno, W.C., D.K. Cassel, and R.A. Larson. 1981. Physical properties of three container media and their effect on poinsettia growth. J. Amer. Soc. Hort. Sci. 106:736-741.

Fonteno, W.C., and P.V. Nelson. 1990. Physical properties of and plant responses to rockwool-amended media. J. Amer. Soc. Hort. Sci. 115: 375-381.

Hillel, D. 1982. Introduction to soil physics. Academic Press, Inc. San Diego.

Ingram, D.L. and T.H. Yeager, 1987. Effects of irrigation frequency and a water-absorbing polymer amendment on *Ligustrum* growth and moisture retention by a container medium. J. Environ. Hort. 5: 19-21.

Jury, W.A., W.R. Gardner, and W.H. Gardner. 1991. Soil Physics. 5 th ed. Wiley, New York, p. 328.

Kay, B.D., and D.A. Angers. 2001. Soil structure. M.E. Summner (ed.). Handbook of soil science. CRC Press. Boca Raton, FL.

Lambers, H., Chapin III, F. S., and T.L. Pons. 1998. Plant water relations, chapter 3, In: plant physiological ecology, Springer-Verlag New York Inc., pp. 158-162.

Milks, R.R., W.C. Fonteno, and R.A. Larson. 1989. Hydrology of horticultural substrates. III. Predicting air and water content of limited volume plug cells. J. Amer. Soc. Hort. Sci. 114: 57-61.

Nemali. K.S. and M.W. van Iersel. 2005. Water requirements and drought tolerance of potted bedding plants. *HortScience* 40:1115.

Olson, D.L., R.D. Oetting, and M.W. van Iersel. 2002. Effect of potting media and water management on development of fungus gnats (Diptera: Sciaridae) and plant growth.

HortScience 37:919-923.

Prunty, L., and F.X.M. Casey. 2002. Soil water retention curve description using a flexible smooth function. Vadose Zone J. 1: 179-185.

Raviv, M., J.H. Leith, D.W. Burger, and R. Wallach. 2001. Optimization of transpiration and potential growth rates of 'kardinal' rose with respect to root-zone physical properties. J. Amer. Soc. Hort. Sci. 126: 638-643.

Stange, C.F., and R. Horn. 2005. Modeling the soil water retention curve for conditions of variable porosity. Vadose Zone J. 4: 602-613.

Sahin, U., O. Anapali, S. Ericisli. 2002. Physico-chemical and physical properties of some substrates used in horticulture. Gartenbauwissenschaft. 67: 55-60.

Taiz, L. and E. Zeiger. 2002. Transport and translocation of water and solutes, unit 1. In: plant physiology 3rd edition. Sinauer associates inc., MA.

Tilt, K.M., T.E. Bilderback, and W.C. Fonteno. 1989. Particle size and container size effects on three ornamental species. J. Amer. Soc. Hort. Sci. 112: 981-984.

Van Genuchten, M. 1980. A closed-form equation for predicting the hydraulic conductivity of unsaturated soils. Soil Sci. Soc. Amer. J. 44: 892-898.

van Vliet, P.C. J., D.E. Radcliffe, P.F. Hendrix, and D.C. Coleman. 1998. Hydraulic conductivity and pore-size distribution in small microcosms with and without enchytraeids (Oligochaeta). 9: 277-282.

Zurmühl, T., and W. Durner. 1996. Modeling transient water and solute transport in a biporous soil. Water Res. Research 32: 819-829.

Table 4.1. CGS and *SI* units of different parameters used in the study and their inter-conversions.

Parameter	**CGS units**	***SI* units**	**Conversion**
Water content	$cm^3 \cdot cm^{-3}$	$m^3 \cdot m^{-3}$	1 $cm^3 \cdot cm^{-3}$ = 1 $m^3 \cdot m^{-3}$
Water potential	cm	MPa	1 MPa = 10220 cm
Surface tension	ergs	Joules	1 joule = 10^7 ergs
Bulk density / density of water	$g \cdot cm^{-3}$	$kg \cdot m^{-3}$	1 $g \cdot cm^{-3}$ = 1000 $kg \cdot m^{-3}$
acceleration due to gravity	$cm \cdot s^{-2}$	$m \cdot s^{-2}$	1 $m \cdot s^{-2}$ = 100 $cm \cdot s^{-2}$
radius	cm	m	1 m = 100 cm

Figure 4.1. Water content measured in a peat +bark + perlite + vermiculite (open symbols) and peat + perlite (closed symbols) at different water potentials (log scale) as measured by Tempe cells (circles), a pressure chamber (squares), and a *Hygrometer* (triangles). The fitted equations by considering together the data from Tempe cells and pressure chamber were $\theta(h) = 0.372 \cdot exp(-0.0154 \cdot h) + 0.342 \cdot exp(-1.4 \cdot 10^{-13} \cdot h)$ (R^2 = 0.88) for peat +bark + perlite + vermiculite substrate and $0.342 \cdot exp(-0.0174 \cdot h) + 0.281 \cdot exp(-1.84 \cdot 10^{-13} \cdot h)$ (R^2 = 0.88) for peat + perlite substrate. The fitted equations by considering together the data from Tempe cells and hygrometer were $\theta(h) = -0.195 + 0.352 \cdot exp(-0.0165 \cdot h) + 0.467 \cdot exp(-1.356 \cdot 10^{-5} \cdot h)$ (R^2 = 0.95) for peat + perlite substrate and $-0.0552 + 0.358 \cdot exp(-0.0158 \cdot h) + 0.3003 \cdot exp(-1.88 \cdot 10^{-4} \cdot h)$ (R^2 = 0.97) for peat +bark + perlite + vermiculite substrate. The dotted line indicates -15330 cm of capillary head, *i.e.* the 'permanent wilting point'.

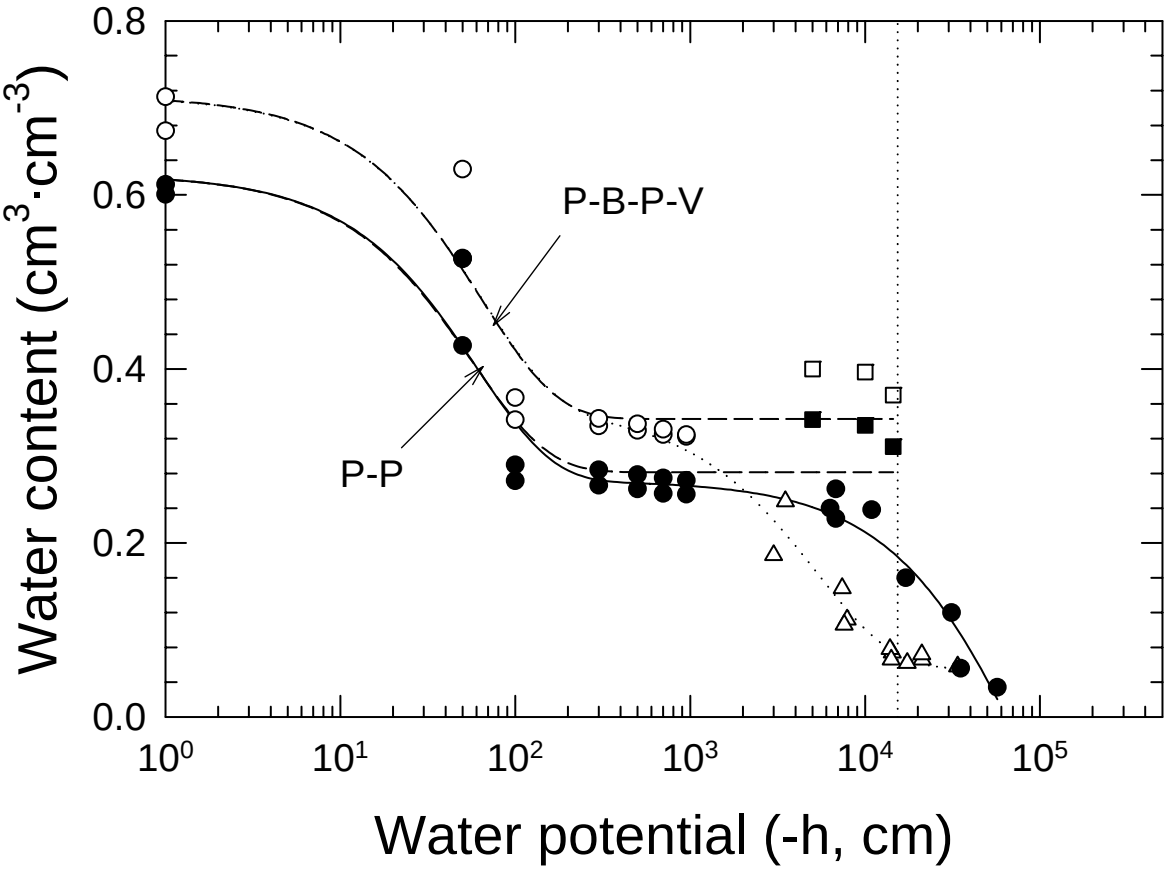

0.8
0.6
0.4
0.2
0.0
Water content (cm3·cm-3)
P-B-P-V
P-P
10^0
10^1
10^2
10^3
10^4
10^5
Water potential (-h, cm)

Figure 4.2. Slope of the soil moisture retention curve (capacitance) with decreasing substrate water potential (-h) for peat +bark + perlite + vermiculite and peat + perlite substrates determined from Tempe cells and hygrometer measurements. The dotted line indicates -15330 cm of capillary head.

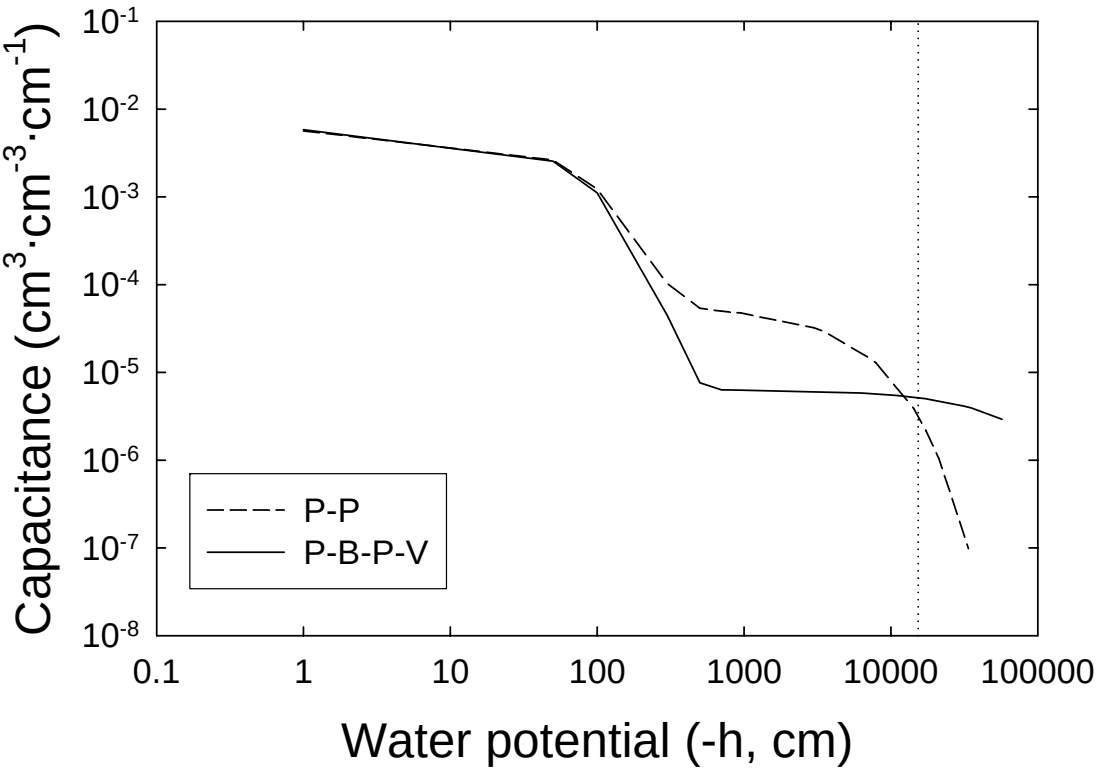

Capacitance (cm3·cm-3·cm-1)
10-1
10-2
10-3
10-4
10-5
10-6
10-7
10-8
0.1
1
10
100
1000
10000
100000
Water potential (-h, cm)
P-P
P-B-P-V

Figure 4.3. Relationship between cumulative pore volume (*V*) and radius of pore for peat + bark + perlite + vermiculite (open circles) and peat + perlite (closed circles) substrates. The fitted equations are $V = [(0.2572 \cdot r) / (5.314 \cdot 10^{-6} + r)] + [(0.3658 \cdot r) / (0.0041 + r)]$, $R^2 = 0.91$ for peat + perlite substrate and $V = [(0.3284 \cdot r) / (2.943 \cdot 10^{-5} + r)] + [(0.3815 \cdot r) / (0.0033 + r)]$, $R^2 = 0.96$ for peat +bark + perlite + vermiculite substrate. Confidence band (5 to 95%) is shown as dashed lines for both functions.

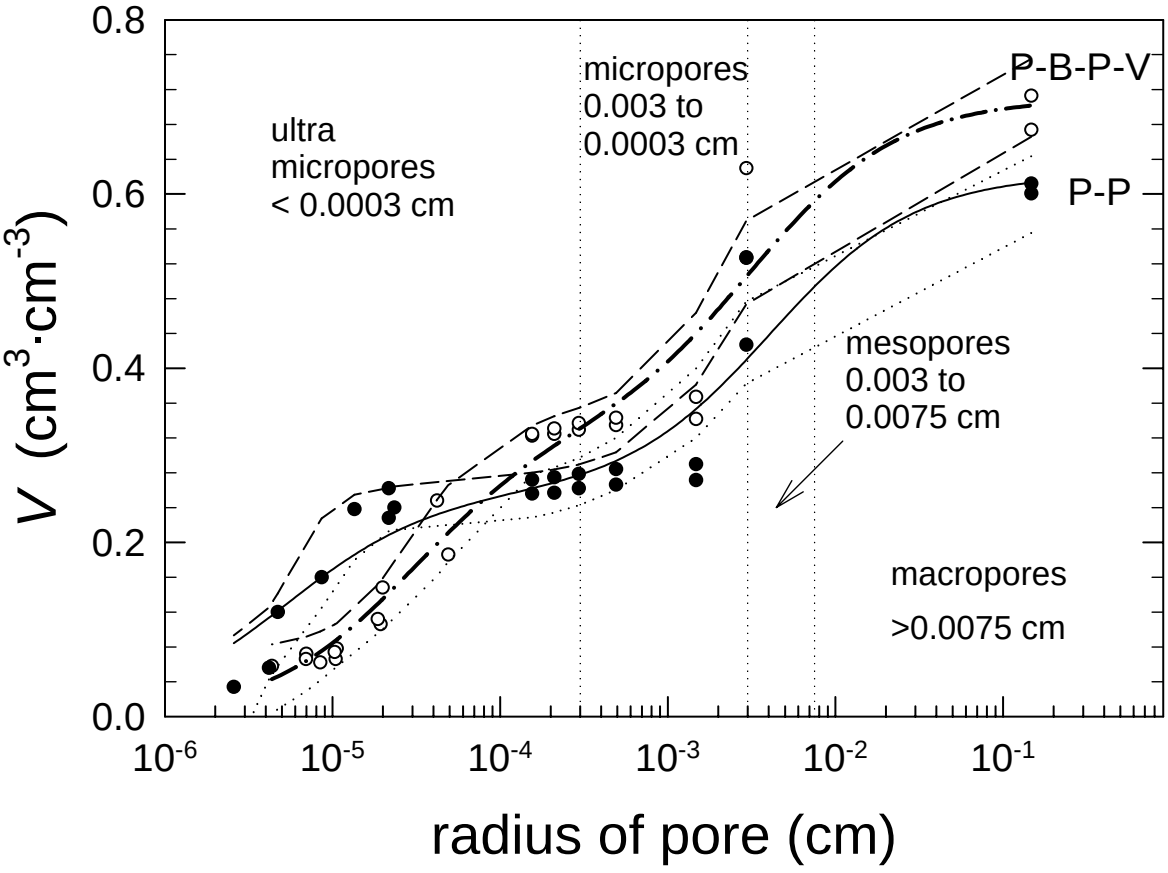
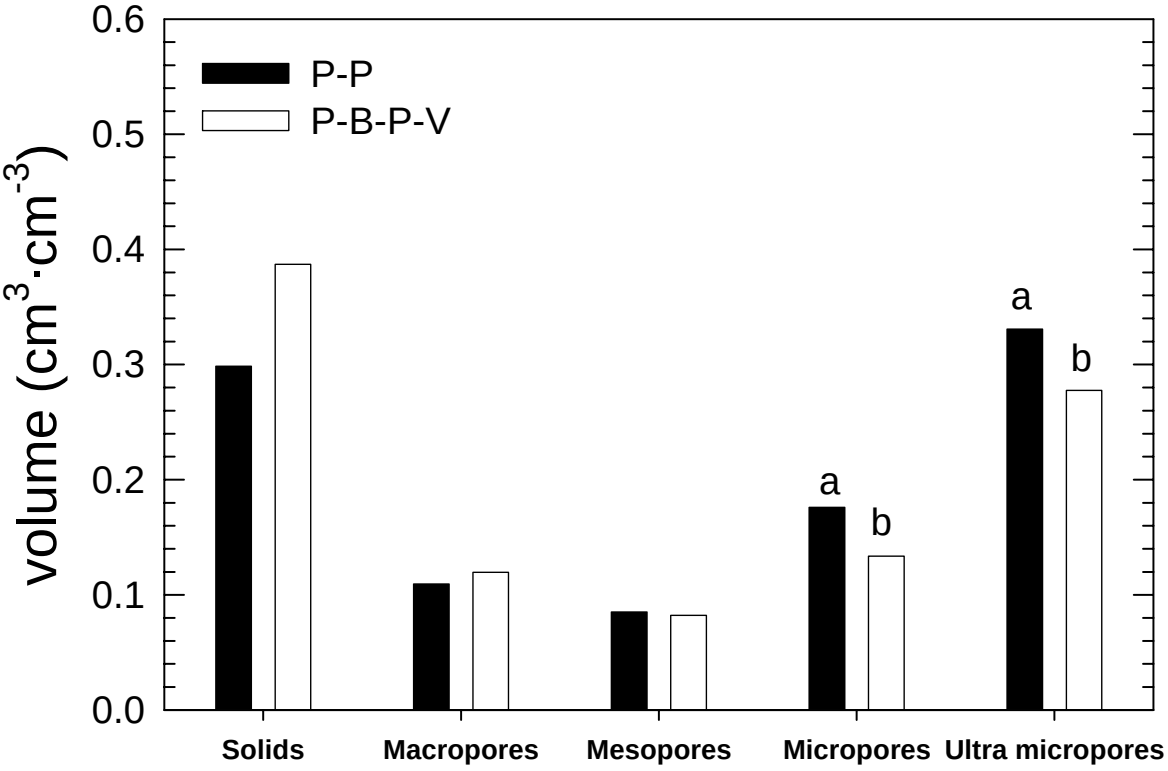

0.8
0.6
0.4
0.2
0.0
V (cm3·cm-3)
10-6
10-5
10-4
10-3
10-2
10-1
radius of pore (cm)
ultra
micropores
< 0.0003 cm
micropores
0.003 to
0.0003 cm
mesopores
0.003 to
0.0075 cm
macropores
>0.0075 cm
P-B-P-V
P-P

Figure 4.4. Volume of different components in a peat +bark + perlite + vermiculite and peat + perlite substrates. Confidence band in figure 4.3 was used to detect significant differences at different regions of the nonlinear functions describing the relationship between pore volume and radius of pore for a peat +bark + perlite + vermiculite (dotted lines) and peat + perlite (solid line) substrates. A different letter indicates statistical significance.


0.6
0.5
0.4
0.3
0.2
0.1
0.0
volume (cm3·cm-3)
P-P
P-B-P-V
a
b
a
b
Solids
Macropores
Mesopores
Micropores
Ultra micropores

CHAPTER 5

LEAF GAS EXCHANGE, CHLOROPHYLL FLUORESCENCE, AND COMPONENT LIMITATIONS TO PHOTOSYNTHESIS IN BEDDING PLANTS GROWN UNDER DIFFERENT SUBSTRATE WATER CONTENTS [1]

---

[1] Nemali, K.S. and M.W. van Iersel. To be submitted to *J. Amer. Soc. Hort. Sci.*

Subject category: Soil-Plant-Water relations

Leaf Gas Exchange, Chlorophyll Fluorescence, and Component Limitations to Photosynthesis in Bedding Plants Grown Under Different Substrate Water Contents

Additional Index Words: A_n : C_i response curves; leaf water potential; leaf osmotic potential; mesophyll resistance; quantum efficiency; stomatal and non-stomatal limitations

Abstract

We studied physiological responses of bedding plants to substrate volumetric water content (θ, $m^3 \cdot m^{-3}$) by growing plants under four constant levels of θ (0.09, 0.15, 0.22, and 0.32 $m^3 \cdot m^{-3}$). Impatiens (*Impatiens walleriana* Hook F.) , salvia *(Salvia splendens* Sellow ex Roemer & J.A. Schultes), petunia (*Petunia hybrida* Hort ex. Vilm.), and vinca (*Catharanthus roseus* (L.) G. Don) were grown from seed and transplanted into large containers (17.5 L) filled with a soilless substrate. All four species were transplanted in each container to expose all species to similar θ in each treatment. Results indicated that mean leaf water potential (Ψ_W) of all species was lowest at a θ of 0.09 $m^3 \cdot m^{-3}$ and did not differ among 0.15, 0.22, and 0.32 $m^3 \cdot m^{-3}$. Mean maximum photosynthetic rate (A_{max}), stomatal conductance (g_s), and light period quantum efficiency (Φ_{PSII}) were highest at a θ of 0.22 or 0.32 $m^3 \cdot m^{-3}$ for all species. However, for petunia, A_{max} was not different

among θ levels of 0.15, 0.22, and 0.32 $m^3 \cdot m^{-3}$. When stomatal and non-stomatal limitations to photosynthesis in petunia and salvia grown at a θ of 0.15 and 0.22 $m^3 \cdot m^{-3}$ were quantified and compared, petunia recorded lower mean (pooled over θ levels) mesophyll (r_*) resistance than salvia. Because of lower r_*, petunia maintained a lower substomatal CO_2 concentration (C_i, $\mu mol \cdot mol^{-1}$) compared to salvia, despite no significant differences in g_s between the species indicating a higher water use efficiency in petunia compared to salvia.

Research on greenhouse irrigation has gained much importance in recent years due to new state laws that have been passed to regulate the amount of runoff from agriculture, including floriculture (e.g., Maryland's Water Quality Improvement Act; Lea-Cox and Ross, 2001). Added to this, competition for water from population growth and increased urbanization may decrease the water resources available to the greenhouse sector. In light of these events, it is important to irrigate greenhouse crops more efficiently to comply with state government regulations and, at the same time, conserve the available water. A disjuncture in the path of developing efficient irrigation guidelines for bedding plants is the unavailability of information on optimal water requirements of bedding plants.

Several researchers have addressed the issue of optimal water content for plants by measuring plant photosynthesis at different substrate water contents (Chapman and Augé, 1994; Arndt et al., 2002; Centritto et al., 2004; Inoue et al., 2004; Gindaba et al., 2005; Xu and Zhou, 2005; Zhang et al., 2005). This is because photosynthesis is one of

the primary mechanisms for growth in plants and that it is sensitive to changes in the substrate water content. It has been shown that the photosynthetic rate in plants is not affected within a broad range of relatively high water content, however it will decrease sharply below a threshold θ (McCree, 1986; Gindaba et al., 2005; Xu and Zhou, 2005). This threshold θ can be used as a critical value for metabolism in plants without experiencing drought stress.

The photosynthetic mechanism in plants is plastic to changes in the θ and drought stress will result in different levels of acclimation in plants (Chaves et al., 2003; Watkinson et al., 2003). The level of acclimation response seen in plants to drought will depend on the rate and severity of drought stress experienced by plants. For example, Watkinson et al. (2003) have indicated that under a 3 to 4 d (rapid) drying cycle, photosynthetic acclimation occurred under mild drought stress but not under severe drought, which was correlated to changes in RNA transcript profiles. It was also shown that when plants were exposed to different θ for long periods extending to several days or weeks, they can acclimate and maintain their growth rates when exposed to moderate drought stress (Wahbi et al., 2005; Zhang et al., 2005), though the photosynthetic mechanism may be affected under severe drought conditions. The acclimation of the photosynthetic process to low θ can occur due to many processes, viz., osmotic adjustment or synthesis of osmoprotectants (McCree, 1986; Serraj and Sinclair, 2002), improved uptake of water due to increased root growth (Frensch, 1997; Hsiao and Xu, 2000), and altered root hydraulic conductivity (Steudle, 2000).

A primary mechanism affecting the rate of photosynthesis during drought is the rate of CO_2 transfer through stomatal and leaf mesophyll regions before CO_2 reaches the

sites of Rubisco for carboxylation. Hence, quantifying relative stomatal and nonstomatal limitations to photosynthesis at different levels of θ can aid in assessment of different factors and their levels affecting rate of photosynthesis in plants (Jones, 1985; Earl, 2002; Grassi and Magnani, 2005). Also, this technique can be useful to screen plant material for tolerance to drought or ability to withstand low substrate water contents (Earl, 2002). Another technique, chlorophyll fluorescence, is commonly used to study the efficiency of photosystem II to utilize the absorbed light under different substrate water contents (Giardi et al., 1996; Flexas et al., 1999; Epron, 1997; Tezara et al., 1999; Colom and Vazzana, 2003). The fraction of absorbed light used in photochemistry is affected when drought stress causes damage to photosystems, thereby decreasing the rate of electron transport and photosynthesis in plants. Hence, chlorophyll fluorescence measurements can indicate possible reasons for changes in photosynthesis under different levels of θ.

Studies involving detailed investigations of different levels of θ on the photosynthetic rate of bedding plants are limited. To the best of our knowledge, the effect of different levels of θ on photochemical efficiency and extent of drought stress experienced by plants have never been quantified. Hence, the objective of the present study were:
(i) determine the minimal θ to be maintained in the substrate that would result in normal rate photosynthesis in bedding plants,
(ii) study the effect of different levels of θ on the efficiency of photosystem II by measuring chlorophyll fluorescence,
(iii) quantify the stomatal and nonstomatal limitations in bedding plants at different levels of θ.

Materials and methods

Plant material.

Seeds of *Salvia splendens* ‘Bonfire Red’, *Catharanthus roseus* ‘Cooler Peppermint’, *Petunia hybrida* ‘Lavender White’, and *Impatiens walleriana* ‘Cherry Pink’ were sown in 128 cell plug-flats in September, 2004. The seeds were germinated under a mist system. Approximately four weeks after germination, seedlings were transplanted into large plastic containers (30.4 cm · 45.7 cm · 17.0 cm; 17.5 L) filled with a soilless substrate (Fafard 2P, Fafard, Anderson, S.C., USA) containing 60% peat and 40% perlite. All four species were transplanted in each container with one seedling per species per container. This was done to expose all four species to similar levels of θ in different treatments. Prior to transplanting, 22.5g of a 14.0N- 6.16P-11.62K slow release fertilizer (Osmocote 14-14 -14, Scotts Co. Marysville, Ohio) was incorporated into the substrate to meet the nutrient requirements of the plants. No other fertilizer was added during the experiment after the initial incorporation into the substrate. The pore water conductivity (EC) was measured using a digital EC meter (Sigma probe EC1, Delta -T, Burwell, Cambridge, UK) before the start of the treatments and at harvest. At both times the substrate was thoroughly wetted before making EC measurements. The average EC maintained in different treatments was close to 1.1 $dS \cdot m^{-1}$.

Greenhouse environment.

Seedlings were grown inside a temperature-controlled glass greenhouse. An aspirated temperature-RH sensor (HTO-45R, Rotronic instruments, Crawley, UK) and two quantum sensors (QSO-Sun, Apogee instruments Inc., Logan, U.T.) were installed 0.6 m above the plants and interfaced to a datalogger to measure environmental

conditions during the experiment. Vapor pressure deficit fluctuated from as low as 0.19 kPa to as high as 1.66 kPa during the experiment with an average VPD close to 0.77 kPa (Fig. 5.1A). Fluctuations in incident *PPF* during the experiment period resulted in large variation in the total amount of light received per day by plants (Fig. 5.1A). Daily light integral ranged from 1 to 10 $mol \cdot m^{-2} \cdot d^{-1}$ during different days and averaged 4.5 $mol \cdot m^{-2} \cdot d^{-1}$ during the experiment. As the temperature was controlled, mean daily temperature remained close to the greenhouse set-point of 22 ^{o}C (Fig. 5.1A).

Watering system.

Seedlings were watered with a drip irrigation system controlled by a irrigation controller which was programmed to maintain different set points of θ in the substrate. Details about this irrigation controller are described elsewhere (Nemali and van Iersel, 2005). In brief, the irrigation controller monitored the θ of the substrates in eight containers once every 20 min using ECH_2O dielectric moisture sensors (Decagon devices, Pullman, W.A.). When the θ dropped below a set point in any container, the controller opened a solenoid valve specific to that container, which resulted in irrigation. The controller was programmed to open the solenoid valve for a short period (1 min), hence irrigation happened for a short period during which a small volume (approximately 100 mL) of water was added to the substrate. This increased the θ by a small fraction (2 to 3%) after each irrigation. In order to equilibrate the θ in the entire container after each irrigation, sufficient time (20 min.) was allowed between irrigations. This was achieved by programming the controller to measure θ only once every 20 min. Hence, there was a period of 19 min. for θ to equilibrate before another irrigation happened. In the present study, the θ was maintained high (0.32 ± 0.02 $m^3 \cdot m^{-3}$) for a period of two weeks after

transplanting seedlings to allow seedling establishment. Then the set points in the controller were changed to the respective treatment levels.

Treatments and measurements.

Plants were grown under four θ set points corresponding to 0.09, 0.15, 0.22, and 0.32 $m^3 \cdot m^{-3}$. Treatments consisted of 4 species, -each grown under four θ levels. In total, eight containers were used in the study (four containers representing four θ levels in each replication, see below). Weekly measurements included A_{max}, g_s, Ψ, and Φ_{PSII} of plants in all treatments. Measurements were taken from a fully grown leaf at the top of the canopy. At any time, measurements were taken from one leaf per experimental unit. Photosynthesis and g_s measurements were taken after exposing leaves to a *PPF* of 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$, cuvette temperature and RH of 25 ^oC and 70%, and CO_2 concentration of 400 $\mu mol \cdot mol^{-1}$ for at least 20 minutes using a leaf photosynthesis system (CIRAS I, PP systems Inc., Amesbury, Mass.) equipped with a LED light unit.

Water potential measurements were taken at noon on any measurement day using leaf cutter thermocouple psychrometers (Model 76, J.R.D. Merril, Logan, Utah) after equilibration at 25 °C for four hours. The osmotic potential was measured from lysed leaf discs after placing the psychrometers overnight inside a freezer. Turgor potential was calculated by subtracting water potential from osmotic potential.

The quantum efficiency of photosystem II was calculated from maximum fluorescence in light (F_m') obtained after exposing leaves to a *PPF* of 1000 - 1100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for a period of 3 minutes (to obtain steady state fluorescence, F_s) and subjecting to a saturating pulse for 0.8 s as $(F_m' - F_s)/ F_s$, using a portable chlorophyll fluorometer (Mini-PAM, Heinz Walz GmbH, Effeltrich, Germany).

At the end of the experiment, response of leaf photosynthesis to internal CO_2 concentration (hereafter, A_n - C_i) was measured for petunia and salvia grown at a θ of 0.15 and 0.22 $m^3 \cdot m^{-3}$ as these treatments were found to show significant differences in A_{max} from the analysis of the weekly measurements. The A_n - C_i responses were measured after initial exposure of individual leaves to a *PPF* 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and CO_2 concentration (ambient) of 400 $\mu mol \cdot mol^{-1}$ for a period of at least 40 min and subsequently after exposing the leaf to different CO_2 concentrations for a period of 3 to 5 min. The *PPF* was maintained at 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at any CO_2 concentration. Measurements were taken at distinct levels by initially decreasing the CO_2 concentration in the cuvette in decrements of 100 $\mu mol \cdot mol^{-1}$ until it dropped to a low value of approximately 25 $\mu mol \cdot mol^{-1}$. Then measurements were taken at distinct levels by increasing the CO_2 concentration in the cuvette in increments of 100 $\mu mol \cdot mol^{-1}$ to a high concentration of 1200 $\mu mol \cdot mol^{-1}$. This was done to prevent any feedback inhibition due to sudden exposure of leaves to a low CO_2 concentration. At each CO_2 concentration, measurements were taken after the photosynthetic rate stabilized.

The component limitations to photosynthesis were quantified using the differential approach described by Jones (1985). This approach has the potential advantage that it does not require a hypothetical ‘elimination’ of one component (stomatal limitation) by extrapolating to unrealistic values (Earl, 2002). The differential approach uses relative sensitivities of both stomatal and non-stomatal components by calculating their responses to a small increase in C_i at the ‘operating point’ or ambient CO_2 concentration, in this case assumed to be 400 $\mu mol \cdot mol^{-1}$.

Experimental design and statistical analyses.

The experimental design was a split‐plot design with repeated measures and two replications. The main plot consisted of a container with four species under a particular moisture level with each species in a container as the split. Hence an experimental unit consisted of a particular plant at a particular moisture level. Data were collected 1 to 2 times during the study depending on the parameter. Data collected between day 20 to 40 were used in the analyses since the moisture levels in the different treatment was steady during this period.

Main and interaction effects of moisture level, species, and time of measurement on measured parameters were analyzed with ANOVA ($P < 0.05$ considered significant) using SAS (SAS institute, Cary, N.C.). Means of the main effects were separated using Tukey's HSD and means of interaction effects were separated using Fisher's protected LSD ($P < 0.05$). In analyzing the interaction effects, means were compared between two species or measurement times at a particular moisture level or a particular species / measurement time at different moisture levels.

In the A_n-C_i analysis, a nonlinear regression {rectangular hyperbola: $A = A_0 + [(a \cdot C_i) / (b + C_i)]$, where A_0 is the photosynthetic rate when C_i is 0, A_0 + a is the maximum attainable A, and b is C_i when A is A_{max} / 2} was fitted, to describe the response of photosynthesis to changing internal CO_2 concentration.

The CO_2 compensation point (Γ, μmol·mol^{-1}) was calculated from the fitted rectangular hyperbolic equation as C_i when photosynthetic rate was zero:

$\Gamma = - (A_0 \cdot b) / (A_0 + a)$

The carboxylation efficiency (α, $mol \cdot m^{-2} \cdot s^{-1}$) was calculated as the slope of the A_n - C_i response curve at the compensation point by substituting $C_{i,compensation}$ in the following equation:

$$\alpha = (a \cdot b) / (b + I)^2$$

Gas phase resistance (r_g; $m^2 \cdot s^1 \cdot mol^{-1}$) was calculated as (Jones, 1985):

$$-(1/r_g) = A_{OP} / (C_a - C_i)$$

where A_{OP} is the photosynthetic rate at operating point and C_a and C_i are the ambient and internal CO_2 concentrations.

Non-gas phase or mesophyll resistance (r*; $m^2 \cdot s^1 \cdot mol^{-1}$) was calculated as slope of A_n - C_i curve at the operating point as:

$$1/r^* = a \cdot b / (b + C_i)^2.$$

Relative gas phase resistance (l) was calculated as the ratio of r_g and (r_g + r*).

Results and discussion

Volumetric water content of the substrate.

Regardless of the environmental fluctuations (RH, DLI) and changes in plant size during the experiment, the irrigation controller maintained the θ close to the set point (2 to 3% higher than the set point) in different treatments (Fig. 5.1B). The mean and standard deviation of actual θ measured in set-points of 0.09, 0.15, 0.22, and 0.32 $m^3 \cdot m^{-3}$ during different days were 0.104 ± 0.0008, 0.168 ± 0.0017, 0.231 ± 0.0026, and 0.331 ± 0.0003 $m^3 \cdot m^{-3}$. There were relatively more fluctuations from the set point in the drier treatments (0.09 and 0.15 $m^3 \cdot m^{-3}$) compared to the wetter treatments (0.22 and 0.32 $m^3 \cdot m^{-3}$) during the experiment. This could be due to the lower hydraulic conductivity of

the peat-based substrate in the drier treatments (Naasz et al., 2005). Because of the lower hydraulic conductivity in drier treatments, the small volume of water applied in each irrigation may not

have properly equilibrated in the substrate. This could have resulted in variability in measurements.

Leaf water status in different moisture treatments.

Mean Ψ_w was affected by species and θ treatment, but not their interaction. Pooled over different θ treatments and measurement times, Ψ_w was lower (more negative) in vinca compared to other species. Leaf water potential of salvia was not different from that of petunia and lower than that of impatiens. No significant differences in the Ψ_w were noted between petunia and impatiens (Fig. 5.2A). Pooled over different species, and measurement times, Ψ_w was significantly lower at 0.09 $m^3 \cdot m^{-3}$ compared to other θ levels. Leaf water potential was not significantly different among θ set points of 0.15, 0.22, and 0.32 $m^3 \cdot m^{-3}$ (Fig 2, B). Wahbi et al. (2005) indicated that midday Ψ_w of olive tree (*Olea europea* L.) was not significantly different among plants irrigated with amounts varying from 50 to 100% of evapotranspiration rate. Leaf osmotic potential was lower in vinca compared to that of petunia and impatiens (Fig. 5.3). We did not find differences in Ψ_s among θ treatments (-0.91, -0.63, -0.73, and -0.53 MPa at 0.09, 0.15, 0.22, and 0.32 $m^3 \cdot m^{-3}$, respectively) and Ψ_p among species (0.08, 0.20, 0.32, 0.33 MPa for impatiens, petunia, salvia, and vinca, respectively) or θ treatments (0.03, 0.28, 0.35, 0.28 MPa at 0.09, 0.15, 0.22, and 0.32 $m^3 \cdot m^{-3}$, respectively).

Substrate water content and maximum leaf photosynthetic rate.

The effect of different θ set points on A_{max} depended on species (P = 0.01). For impatiens, salvia, and vinca, A_{max} was significantly higher at a θ of 0.22 $m^3 \cdot m^{-3}$ compared to 0.09 $m^3 \cdot m^{-3}$ and 0.15 $m^3 \cdot m^{-3}$ (Fig. 5.4) and A_{max} of salvia was found not to be different between θ set points of 0.09 and 0.15 $m^3 \cdot m^{-3}$. There was a slight but significant decrease in the A_{max} of impatiens, salvia, and vinca at 0.32 $m^3 \cdot m^{-3}$ compared to 0.22 $m^3 \cdot m^{-3}$. In case of petunia, A_{max} was significantly lower at 0.09 $m^3 \cdot m^{-3}$ but was not different between 0.15 , 0.22, and 0.32 $m^3 \cdot m^{-3}$ (Fig. 5.4).

The type of response of photosynthesis to different levels of θ seen in this study, with a threshold θ below which there is a significant decrease and above which no or small differences were noted, seems to be conservative in nature among a broad range of species. Photosynthetic responses similar to those in our study were also seen in perennial rhizome grass (*Leymus chinensis* Trin. Tzvelev; Xu and Zhou, 2005), sorghum (*Sorghum bicolor* (L.) Moench; McCree, 1986), and eucalyptus (*Eucalyptus camaldulensis* Dehnh. and *E. globulus* Labill.; Gindaba et al., 2005).

Stomatal conductance.

The three-way interaction among species, θ set points, and measurement time was significant for g_s (P = 0.0138). In general, response of g_s to θ for a particular species depended on the measurement time, with a lower g_s during the second measurement time for all species compared to the first (Fig. 5.5). The first and second measurement were taken 20 and 27 d after imposing the θ levels in different treatments. As the decrease in g_s during the second cycle was noted even in some of the wetter treatments, it can not be solely attributed to drought stress. In our study, we measured g_s on fully-

expanded leaves at the top of the canopy. Leaf ageing could be among other factors causing reduction in g_s during the second cycle (Mebrahtu and Hanover, 1991; Roberts, 2002). Within each measurement period, response of g_s to θ set point depended on species (Fig. 5.5).

At both times, g_s of vinca was not different between set points of 0.09 and 0.15 $m^3 \cdot m^{-3}$, highest at a θ of 0.22 $m^3 \cdot m^{-3}$, and decreased in the wettest treatment. A similar response was seen in impatiens during the second measurement time. At the first measurement time, no significant differences were seen in g_s of impatiens at a θ of 0.09 and 0.15 $m^3 \cdot m^{-3}$. During the second measurement time, g_s in impatiens was higher at a θ of 0.15 than 0.09 $m^3 \cdot m^{-3}$. We did not consider the g_s in impatiens at a θ of 0.22 $m^3 \cdot m^{-3}$ during the first measurement time as the values were so high that they appeared to be out of range. At the first measurement time, g_s of salvia was highest at a θ of 0.32 $m^3 \cdot m^{-3}$ followed by 0.22 $m^3 \cdot m^{-3}$, and did not differ between 0.15 and 0.09 $m^3 \cdot m^{-3}$. The g_s in salvia during second measurement time was different between the wetter and drier treatments, but not between the two drier or wetter treatments. In the case of petunia, g_s was lower in the two drier treatments than the two wetter treatments and was not different between the two wetter treatments during the first measurement cycle. During the second measurement time in petunia, g_s was lower at 0.09 $m^3 \cdot m^{-3}$ and was not significantly different among 0.15, 0.22, and 0.32 $m^3 \cdot m^{-3}$.

Regardless of species, significant reductions in g_s were noticed after an exposure period of one week at a θ of 0.09 $m^3 \cdot m^{-3}$. Xu and Zhou (2005) reported that g_s of *Leymus chinensis* was only significantly lower under severe drought stress (< 35% field capacity). In general, g_s was not different between the two drier treatments during the first

measurement time. However, g_s of impatiens and petunia at a set point of 0.15 $m^3 \cdot m^{-3}$ was higher than 0.09 $m^3 \cdot m^{-3}$ during the second measurement time. In both species, g_s remained unaffected between the two measurement times at 0.15 $m^3 \cdot m^{-3}$ but decreased at 0.09 $m^3 \cdot m^{-3}$ during the second measurement, hence differences were noticed between 0.09 and 0.15 $m^3 \cdot m^{-3}$ at the second measurement time. Stomatal conductance of all species was either higher at a θ of 0.22 compared to 0.32 $m^3 \cdot m^{-3}$ or not different between 0.22 and 0.32 $m^3 \cdot m^{-3}$. The response of g_s of petunia during the second measurement time is consistent with A_{max}, which was not significantly different among 0.15, 0.22, and 0.32 $m^3 \cdot m^{-3}$.

Quantum efficiency in light.

There was a significant main effect of species and interactive effect of θ level and time on Φ_{PSII}. Our results indicated that all four species had different Φ_{PSII} when data were pooled for θ treatments and measurement times (Figure 5.6A). Quantum efficiency in light measures the proportion of light absorbed by chlorophyll associated with photosystem II that is used in photochemistry. It is important to realize that Φ_{PSII} measured in light will be lower than Φ_{PSII} measured in dark, as non-photochemical quenching is active in light. Quantum efficiency in light is linearly related to the photosynthetic performance of plants (Maxwell and Johnson, 2000). The Φ_{PSII} values measured for different species in this study were consistent with the photosynthetic rates. Vinca and petunia with higher A_{max} also recorded high Φ_{PSII} values, especially in the two drier treatments. It has been reported that shade-loving species will have lower photosynthetic capacity under high *PPF* mainly due to lower Rubisco levels (Adams and Demmig-Adams, 1992; Evans and Poorter, 2001; Marenco et al., 2001; Close et al.,

2001). As impatiens is a shade-loving species, the low Φ_{PSII} in impatiens can be related to its inherent low photosynthetic capacity.

There was an interactive effect of θ treatment and measurement time on Φ_{PSII} of plants (pooled over all species). During the first measurement period, Φ_{PSII} was highest at a θ of 0.22 $m^3 \cdot m^{-3}$, and was not different between 0.32 and 0.15 $m^3 \cdot m^{-3}$ or 0.15 and 0.09 $m^3 \cdot m^{-3}$. Quantum efficiency decreased in all treatments, except the wettest treatment, from the first to the second measurement period (Fig. 5.6B). However, even during the second measurement, Φ_{PSII} was higher at θ of 0.32 and 0.22 $m^3 \cdot m^{-3}$ compared to θ of 0.15 and 0.09 $m^3 \cdot m^{-3}$.

These results are consistent with the photosynthesis measurements and suggests that the ability to utilize the captured light was highest at θ of 0.22 or 0.32 $m^3 \cdot m^{-3}$ depending on species. A decrease in Φ_{PSII} during drought stress was also reported by Flexas et al. (1999) and Colom and Vazzana (2003). However, Epron (1997) has reported a slight reduction in Φ_{PSII} only under severe drought stress. It is important to realize that drought stress in the above cited experiments were given by withholding irrigation, as opposed to constant θ levels maintained in this study. We noticed a decrease in g_s in all treatments during the second measurement time. It is possible that the decrease in Φ_{PSII} during the second measurement time could be due to leaf ageing.

Photosynthesis-internal CO_2 response curves.

Responses were studied for plants grown under 0.15 and 0.22 $m^3 \cdot m^{-3}$ as a decrease in A_{max} due to drought stress was noticed at a θ of 0.15 $m^3 \cdot m^{-3}$ in some species and highest A_{max} was seen at a θ of 0.22 $m^3 \cdot m^{-3}$ for all species. Because of the different A_{max} response in petunia (not different between 0.15 and 0.22 $m^3 \cdot m^{-3}$) compared to other

species (e.g., salvia; differences in A_{max} noted between 0.15 and 0.22 $m^3 \cdot m^{-3}$), we studied stomatal and non-stomatal limitations to photosynthesis in petunia and salvia at a θ of 0.22 and 0.15 $m^3 \cdot m^{-3}$. The fitted hyperbolic function adequately described the response of photosynthesis to increasing C_i in all treatments ($0.88 < R^2 < 0.94$). The response of A_n in petunia to increasing C_i was similar between the two θ levels of 0.15 and 0.22 $m^3 \cdot m^{-3}$. In salvia, the response of A_n to increasing C_i was different at θ levels of 0.15 and 0.22 $m^3 \cdot m^{-3}$; plants grown at a θ of 0.15 $m^3 \cdot m^{-3}$ constantly had lower A_n than at 0.22 $m^3 \cdot m^{-3}$ at all levels of C_i. This is consistent with the earlier finding that A_{max} of salvia was lower at a θ set point of 0.15 than at 0.22 $m^3 \cdot m^{-3}$ (Fig. 5.7).

In salvia, α was significantly lower at a θ of 0.15 (0.066 ± 0.022 $mol \cdot m^{-2} \cdot s^{-1}$) than at a θ of 0.22 $m^3 \cdot m^{-3}$ (0.199 ± 0.049 $mol \cdot m^{-2} \cdot s^{-1}$), however, in petunia α was not significantly different between a θ of 0.15 (0.171 ± 0.069 $mol \cdot m^{-2} \cdot s^{-1}$) and 0.22 $m^3 \cdot m^{-3}$ (0.156 ± 0.039 $mol \cdot m^{-2} \cdot s^{-1}$). A drought-imposed decrease in α was earlier reported by Tezara et al., 1999. At low values of C_i, photosynthesis is mainly limited by resistance to CO_2 transfer from ambient air to carboxylation sites and not directly related to down regulation of any process in the dark cycle (in fact, *Ribulose bis-phosphate* is at saturating level under these conditions, von Caemmerer and Farquhar, 1981). The estimated values of CO_2 compensation points for petunia at a θ of 0.15 and 0.22 $m^3 \cdot m^{-3}$ were 54 and 49 $\mu mol \cdot mol^{-1}$ respectively, and for salvia at a θ of 0.15 and 0.22 $m^3 \cdot m^{-3}$ were 45, 37 $\mu mol \cdot mol^{-1}$, respectively.

Component limitations to photosynthesis.

The differential method of quantifying stomatal and non-stomatal limitations to photosynthesis indicated no significant differences among species (32.6 ± 6.6 $m^2 \cdot s \cdot mol^{-1}$

for petunia and 50.9 ± 18.4 $m^2 \cdot s \cdot mol^{-1}$ for salvia) or θ set points (32.1 ± 6.1 at 0.15 $m^3 \cdot m^{-3}$ and 17.5 ± 2.7 at 0.22 $m^3 \cdot m^{-3}$) in stomatal limitation (or gas phase resistance). Significant differences between species were noted in C_i at the operating point (sub-stomatal CO_2 concentration when the ambient CO_2 concentration in the measuring chamber is 400 $\mu mol \cdot mol^{-1}$) and non-stomatal limitation (or non-gas phase / mesophyll resistance) to CO_2 transfer in petunia and salvia. There was no effect of θ treatment on operating C_i and non-stomatal limitation. Averaged across the two θ set points, operating C_i was lower (Fig. 5.8A) in petunia (158 ± 8 $\mu mol \cdot mol^{-1}$) compared to salvia (194 ± 8 $\mu mol \cdot mol^{-1}$). Averaged across two θ set points, mesophyll resistance to CO_2 transfer during photosynthesis was significantly lower (Fig. 5.8, B) in petunia (14.8 ± 0.9 $m^2 \cdot s \cdot mol^{-1}$) compared to salvia (30.9 ± 2.3 $m^2 \cdot s \cdot mol^{-1}$). The relative gas phase resistance was not different between species and θ set points. Averaged across two θ set points, it was 0.62 ± 0.06 for petunia and 0.57 ± 0.18 for salvia. Averaged across two species, it was 0.60 ± 0.07 at 0.15 $m^3 \cdot m^{-3}$ and 0.43 ± 0.11 at 0.22 $m^3 \cdot m^{-3}$. Mean A_n at the operating point for petunia and salvia were 9.16 (± 0.64) and 7.60 (± 0.65) $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively.

The earlier reported responses of stomatal and nonstomatal limitations to CO_2 transfer during drought stress are not consistent among different studies. No difference in mesophyll resistance between the control and drought treatments of two cultivars of soybean (*Glycine max* Merr.) was reported, however two soybean cultivars differed in their gas phase resistance (Earl, 2002). These results (Earl, 2002) are consistent with our results for mesophyll resistance but not with those of gas phase resistance. In an other study, an increase in both stomatal and mesophyll resistance was proposed in

response to drought stress in ash (*Fraxinus* sp. Marshall) and oak trees (*Quercus* sp. L.) (Grassi and Magnani, 2005). It was also reported that response of stomatal resistance to drought stress depended on species, when drought stress resulted either in a decrease or increase in gas phase resistance (Jones, 1985).

It is important to realize that the following interpretations were based on pooled data from both θ set points for salvia and vinca. In spite of no significant differences in gas-phase resistance to CO_2 transfer between petunia and salvia, the operating C_i was lower and operating A_n was higher in petunia compared to salvia. This can be attributed to the lower mesophyll resistance to CO_2 transfer in petunia compared to salvia. Due to the lower mesophyll resistance in petunia, the CO_2 concentration drop between sub-stomatal and biochemical sites is smaller than in salvia (Jones, 1985). This results in higher rates of CO_2 fixation by RuBisCO, which subsequently draws more CO_2 from the sub-stomatal regions. The net result is a lower C_i in the sub-stomatal region of petunia compared to salvia. The slower rate of CO_2 draw-down from the sub-stomatal region in salvia due to higher mesophyll resistance might have resulted in an accumulation of CO_2 in the sub-stomatal region when the ambient CO_2 concentration was increased sequentially in the study. This could be the reason for C_i levels above 1000 $\mu mol \cdot mol^{-1}$ in salvia as opposed to petunia where C_i values seldom reached above 600 $\mu mol \cdot mol^{-1}$ even at an ambient CO_2 concentration of 1200 $\mu mol \cdot mol^{-1}$. Our results could have been strengthened if we could have separated mesophyll resistance among θ treatments and for individual species. The lower operating C_i in petunia than salvia when gas phase conductance (inverse of 'resistance' term) was not different between species indicates that petunia is more efficient in water use than salvia (Earl, 2002). Based on our results, the

physiological mechanism of higher water use efficiency in petunia is due to increased photosynthetic capacity as a lower C_i was noticed due to greater mesophyll conductance in petunia when limitation through stomates was not different between two species.

Conclusions

These results indicate that A_{max}, g_s, and Φ_{PSII} were highest at a θ of 0.22 or 0.32 $m^3 \cdot m^{-3}$ in all species. The mean Ψ_W of all species was not significantly different between θ set points of 0.22 and 0.32 $m^3 \cdot m^{-3}$. It appears that photosynthesis in petunia will not be significantly affected by maintaining a lower θ (0.15 $m^3 \cdot m^{-3}$), perhaps due to improved conductance or small resistance to CO_2 transfer in the non-gas or liquid phase of transport from sub-stomatal regions to biochemical sites of CO_2 fixation and higher carboxylation at the sites of RuBisCO.

References

Adams, W.W. III. and B. Demmig-Adams. 1992. Operation of xanthophyll cycle in higher plants in response to diurnal changes in incident sunlight. *Planta* 186:390-398.

Arndt, S.K., S.C. Clifford, W. Wanek, H.G. Jones and M. Popp. 2110. Physiological and morphological adaptations of the fruit tree *Ziziphus rotundifolia* in response to progressive drought stress. *Tree Physiol*. 21: 705-715.

Björkman, O. And B. Demmig. 1987. Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77K among plants of diverse origins. *Planta* 170: 489-504.

Centritto, M., S. Wahbi, R. Serraj and M.M. Chaves. 2005. Effects of partial root zone drying (PRD) on adult olive tree (*Olea europaea*) in field conditions under arid climate II. Photosynthetic responses. *Agric. Ecosystems Environ*. 106: 303-311.

Chapman, D.S. and R.M. Augé. 1994. Physiological mechanisms of drought resistance in four native ornamental perennials. *J. Amer. Soc. Hort. Sci*. 119:299-306.

Chaves, M.M., J.P. Maroco, and J.S. Pereira. 2003. Understanding plant responses to drought - from genes to the whole plant. *Functional Plant Biol*. 30: 239-264

Close, D.C., C.L. Beadle and M.J. Hovenden. 2001. Cold-induced photoinhibition and foliar pigment dynamics of *Eucalyptus nitens* seedlings during establishment. Aust. *J. Plant Physiol*. 28:1133-1141.

Colom, M.R. and C. Vazzana. 2003. Photosynthesis and PSII functionality of drought resistant and drought sensitive weeping lovegrass plants. *Environ. Exp. Bot.* 49: 135-144.

Earl, H.J. 202. Stomatal and non-stomatal restrictions to carbon assimilation in soybean (Glycine max) lines differing in water use efficiency. *Environ. Exp. Bot.* 48: 237-246.

Epron, D. 1997. Effecys of drought on photosynthesis and on the thermotolerance of photosystem II in seedlings of cedar (*Cedrus atlantica* and *C. libani*). *J. Expt. Bot.* 48: 1835-1841.

Evans, J.R. and H. Poorter. 2001. Photosynthesis acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. *Plant, Cell and Environ*. 24:755-767.

Flexas, J., J.M. Escalona, and H. Medrano. 1999. Water stress induces different levels of photosynthesis and electron transport rate regulation in grapevines. *Plant Cell Environ.* 22: 39-48.

Frensch, J. 1997. Primary responses of root and leaf elongation to water deficits in the atmosphere and soil solution. *J. Expt. Bot*. 48: 985-999.

Giardi, M.T., A. Cona, B. Geiken, T. Kučera, J. Masojìdek, and A.K. Mattoo. 1996. Long-term drought stress induces structural and functional reorganization of photosystem II. *Planta* 199: 118-125.

Gindaba, J., A. Rozanov, and L. Negash. Photosynthetic gas exchange, growth and biomass allocation of two Eucalyptus and three indigenous tree species of Ethiopia under moisture deficit. *Forest Ecol. Management* 205: 127- 138.

Grassi, G. and F. Magnani. 2005. Stomata, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. *Plant, Cell Environ*. 28: 834-849.

Hsiao, T.C. and L.K. Xu. 2000. Sensitivity of growth of roots versus leaves to water stress: biophysical analysis and relation to water transport. *J. Expt. Bot.* 51: 1595-1616.

Inoue, T., S. Inanga, Y. Sugimoto, P.An and A.E. Enenji. 2004. Effect of drought on ear and flag leaf photosynthesis of two wheat cultivars differing in drought resistance. *Photosynthetica.* 42: 559-565.

Jones, H.J. 1985. Partitioning stomatal and non-stomatal limitations to photosynthesis. *Plant, Cell and Environ*. 8: 95-104.

Jones, H.J. 1992. Plants and microclimate: a quantitative approach to environmental plant physiology. Second ed., Camb. Univ. press, Cambridge

Krall, J.P. and G.E. Edwards. 1992. Relationship between photosystem II activity and CO2 fixation in leaves. *Physiol. Plantarum* 86: 180-187.

Laisk, A. And F. Loreto. 1996. Determining photosynthetic parameters from leaf CO_2 and chlorophyll fluorescence - ribulose 1,5- bisphosphate oxygenase specificty factor, dark respiration in the light, excitation distribution between photosystems, alternate electron transport rate, and mesophyll diffusion resistance. *Plant Physiol*. 110: 903-912.

Lea-Cox, J.D. and D.S. Ross. 2001. A review of the federal clean water act and the Maryland water quality improvement act: the rationale for developing a water and nutrient planning process for container nursery and greenhouse operations. *J. Environ. Hort.* 19:226-229.

Marenco, R.A., J.F.C. Gonglaves and G. Vieira. 2001. Leaf gas exchange and carbohydrates in tropical trees differing in successional status in two light environments in central amazonia. *Tree Physiol*. 21:1311-1318.

Maxwell, K. And N. Johnson. 2000. Chlorophyll fluorescence - a practical guide. *J. Exp. Bot.* 345: 659-688.

McCree. K.J. 1986. Whole plant carbon balance during osmotic adjustment to drought and salinity stress. *Aust. J. Plant Physiol*. 13:33-43.

Mebrahtu, T. And J.W. Hanover. 1991. Leaf age effects on photosynthesis and stomatal conductance of black locust seedlings. *Photosynthetica* 25: 537-544.

Naasz, R., Michel, J.-C., and Charpentier, S. 2005. Measuring hysteretic hydraulic properties of peat and pine bark using a transient method. *Soil Sci. Soc. Amer. J.* 69: 13-22.

Roberts, J. 2000. The influence of physical and physiological characteristics of vegetation on their hydrological response. *Hydrol. Process* 14: 2885-2901.

Serraj, R. And T.R. Sinclair. 2002. Osmolyte accumulation: can it really help increase crop yield under drought conditions? *Plant Cell Environ*. 25: 333-341.

Steudle, E. 2000. Water uptake by roots: effects of water deficit. *J. Expt. Bot*. 51: 1531-1542.

Tezara, W, V.J. Mitchell, S.D. Driscoll and D.W. Lawlor. 1999. Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. *Nature* 401:914-917.

von Caemmerer, S and G.D. Farquhar. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* 153:376-387.

Wahbi, S., R. Wakrim, B. Aganchich et al., 2005. Effects of partial rootzone drying (PRD) on adult olive tree (Olea europaea) in field conditions under and climate - I. physiological and agronomic responses. *Agric. Ecosystems and Environ*. 106: 289-301.

Watkinson, J.I., A.A. Sioson, C. Vasquez-Robinet et al., 2003. Photosynthetic acclimation is reflected in specific patterns of gene expression in drought-stressed loblolly pine. *Plant Physiol*. 133: 1702-17.

Xu, Z. -Z., and G.-S. Zhou. Effects of water stress and high nocturnal temperature on photosynthesis and nitrogen level of a perennial grass *Leymus chinenesis*. *Plant and Soil.* 269: 131-139.

Zhang, X., N. Wu and C. Li. 2005. Physiological and growth responses of *Populus davidiana* ecotypes to different soil water contents. *J. Arid Environ.* 60: 567-579.

Figure 5.1. (A). Daily light integral (DLI), daily average vapor pressure deficit (VPD), and daily average (day and night) temperature (temp.) during the experiment. (B). Mean (n = 2) daily volumetric water content (θ) in different moisture treatments (indicated by '----' lines) during different days after the start of the treatments. Symbols △, ▼, •, and ○ indicate θ of 0.09, 0.15, 0.22, and 0.32 $m^3 \cdot m^{-3}$, respectively.

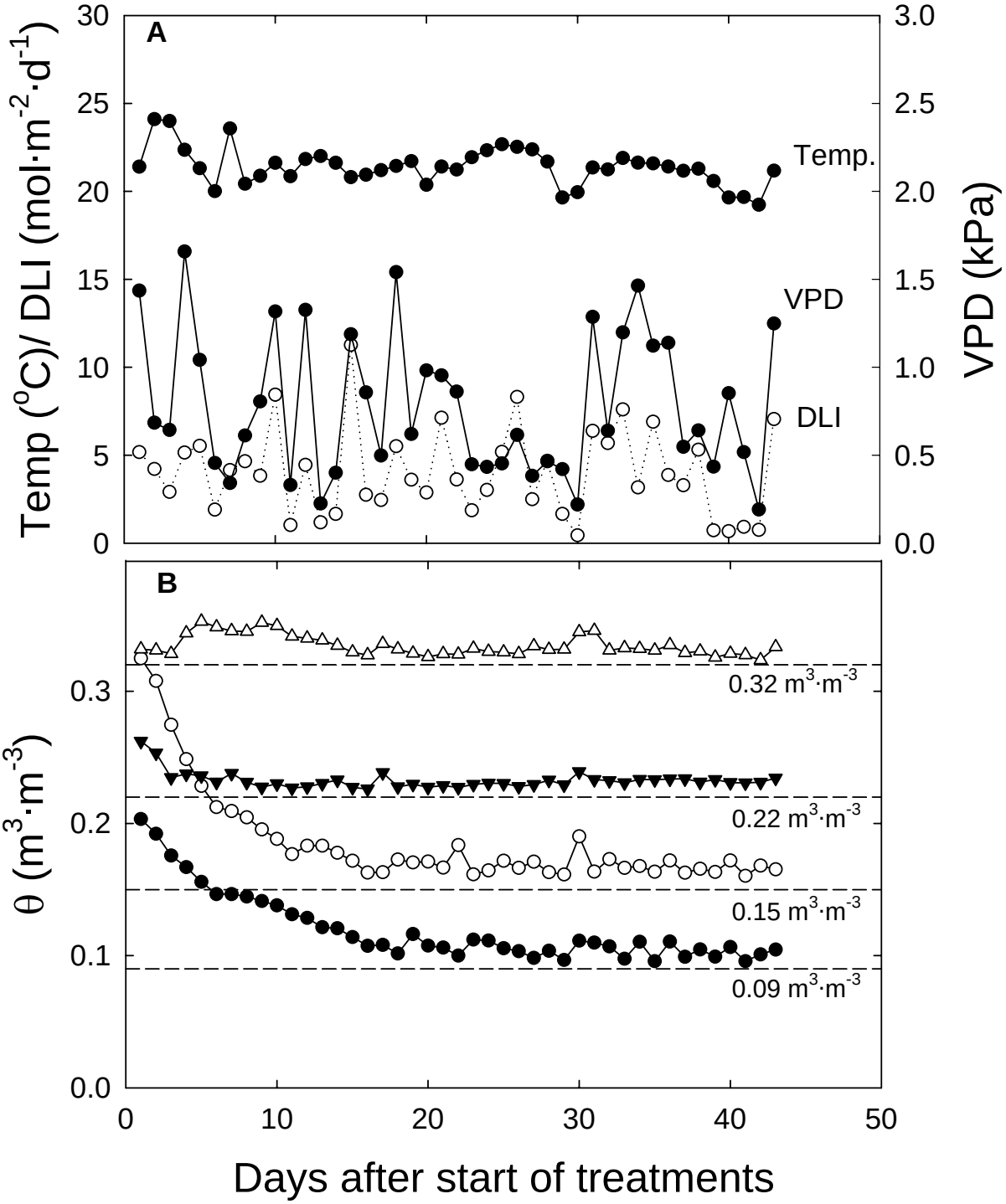
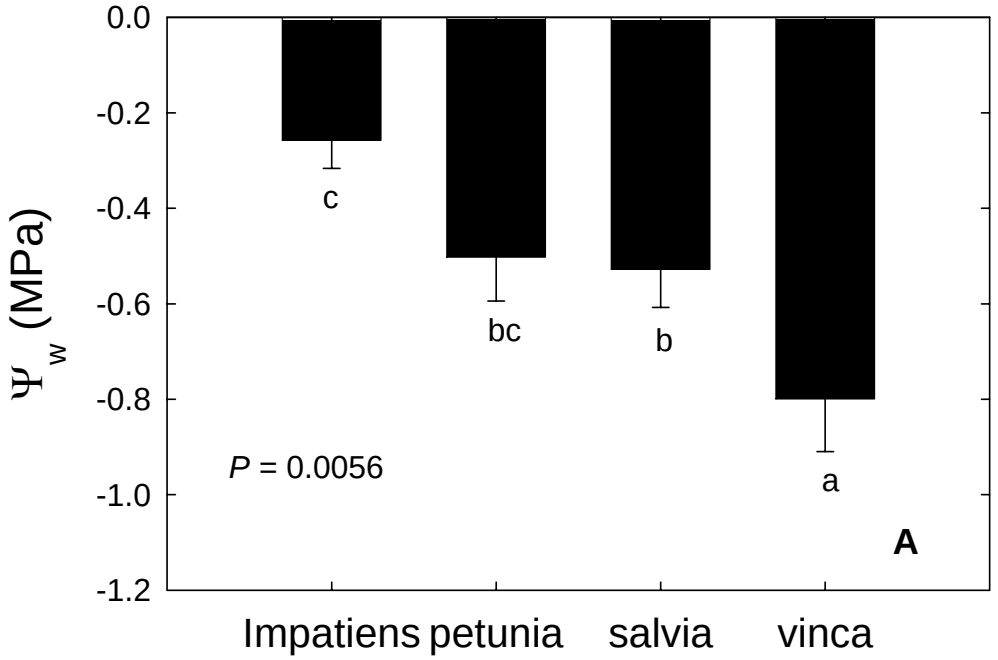

A
B
Temp.
VPD
DLI
Temp (°C)/ DLI (mol·m-2·d-1)
VPD (kPa)
θ (m3·m-3)
0.32 m3·m-3
0.22 m3·m-3
0.15 m3·m-3
0.09 m3·m-3
Days after start of treatments

Figure 5.2. Mean (n = 16) leaf water potential (Ψ_w) in (A) different species and (B) different θ set points during the experiment. Means were separated using Tukey's HSD ($P < 0.05$) and means with the same letter are not significantly different. Error bars represent the standard error of the mean.


0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
Ψw (MPa)
c
bc
b
a
P = 0.0056
A
Impatiens
petunia
salvia
vinca

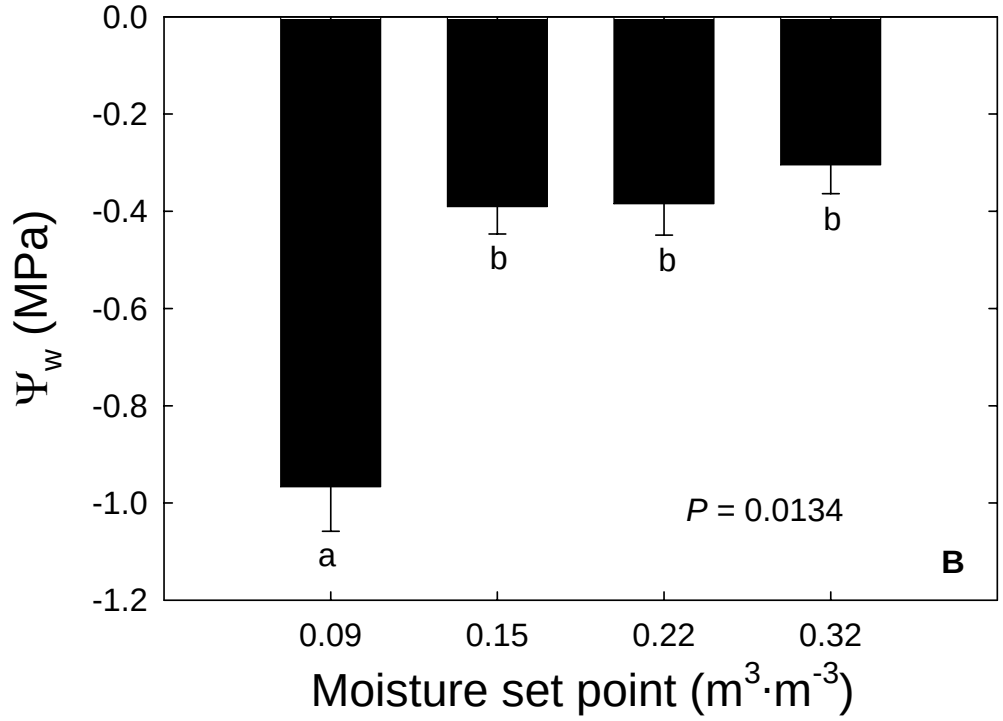

0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
Ψw (MPa)
a
b
b
b
P = 0.0134
B
0.09
0.15
0.22
0.32
Moisture set point (m3·m-3)

Figure 5.3. Mean (n = 16) leaf osmotic potential (Ψ_s) in different species during the experiment. Significant differences among means were indicated by different alphabet. Means were separated using Tukey's HSD ($P < 0.05$). Error bars represent the standard error of the mean.

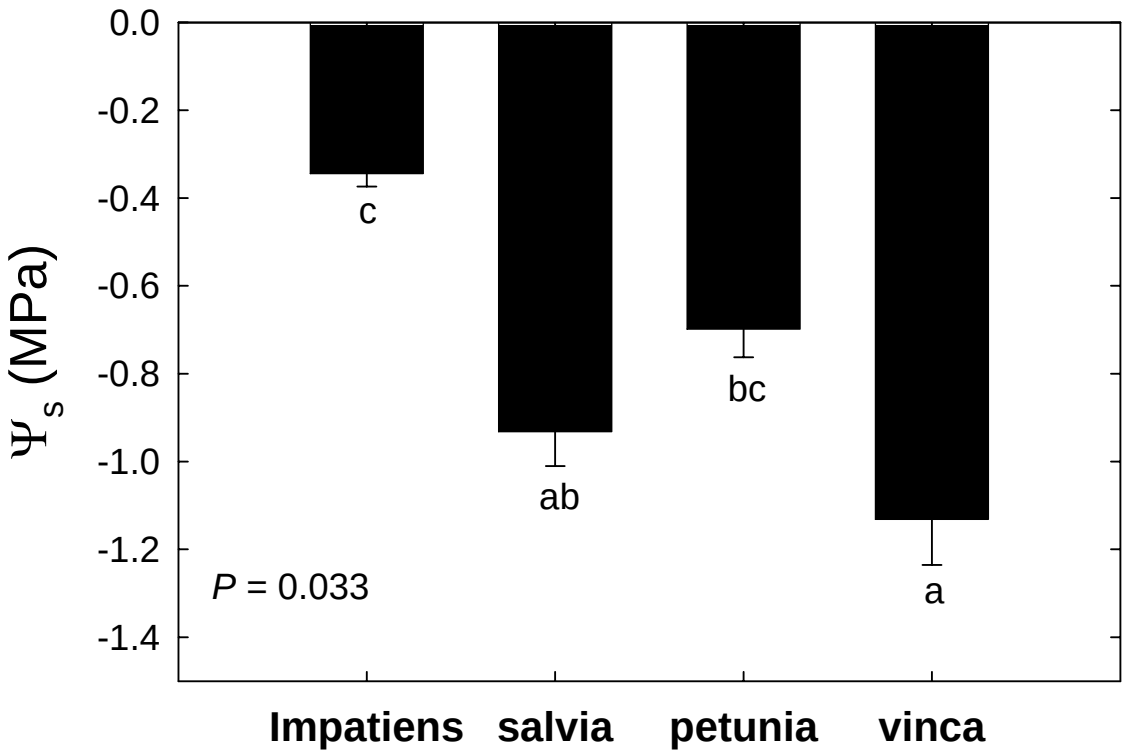

0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
Ψs (MPa)
c
ab
bc
a
P = 0.033
Impatiens
salvia
petunia
vinca

Figure 5.4. Interactive effects of species and θ set points on the mean (n = 4) photosynthetic rate (A_{max}) at a photosynthetic photon flux of 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ The error bar indicates Fisher's protected least significant difference (LSD) ($P < 0.05$). The $LSD_{0.05}$ was used to compare means among all species in one moisture treatment or among all moisture treatments within one species.

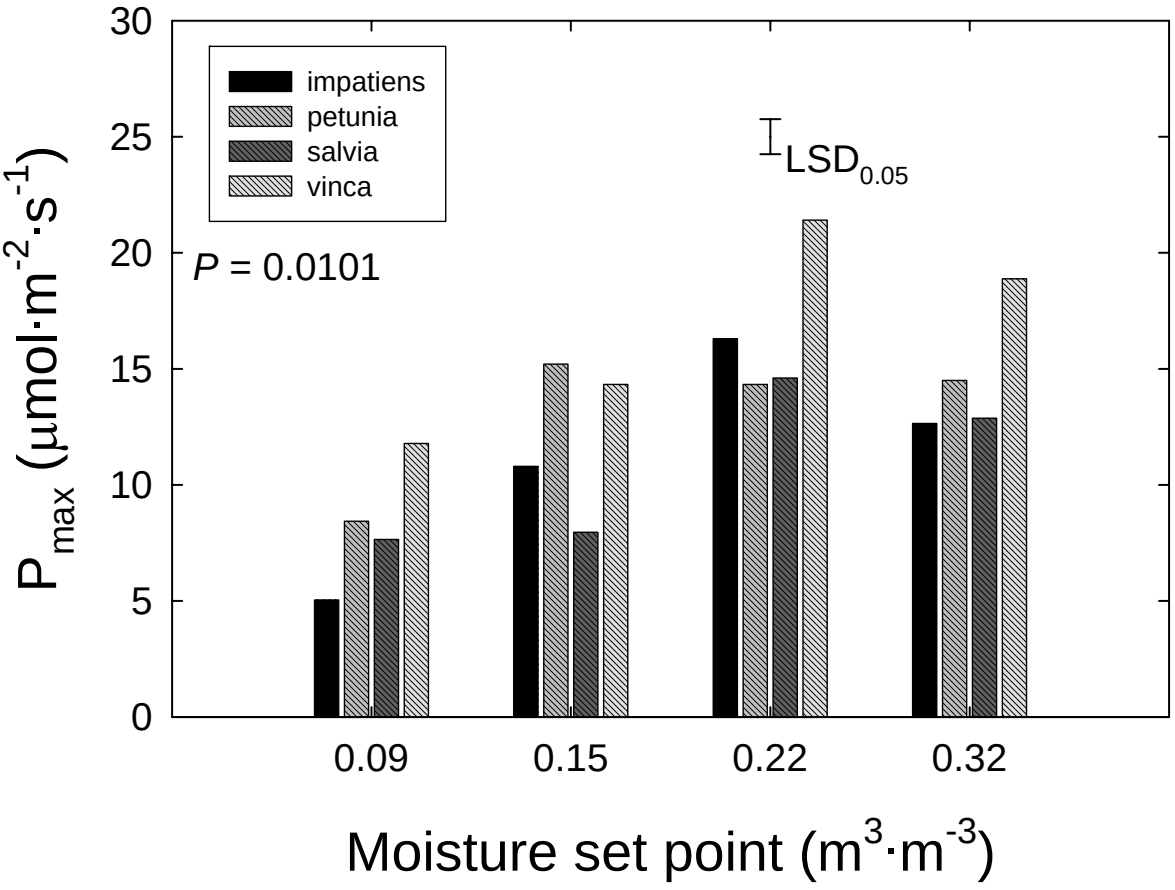

30
25
20
15
10
5
0
impatiens
petunia
salvia
vinca
LSD0.05
P = 0.0101
Pmax (µmol·m-2·s-1)
0.09
0.15
0.22
0.32
Moisture set point (m3·m-3)

Figure 5.5. Interactive effects of species, θ set points , and measurement time on stomatal conductance (g_s) of plants (n = 2). The error bar indicates Fisher's protected least significant difference (LSD) (P = 0.05). The $LSD_{0.05}$ was used to compare means among all species in one moisture treatment at a particular measurement time or among all moisture treatments in one species at a particular measurement time or between two measurement times for one species at a particular moisture treatment.

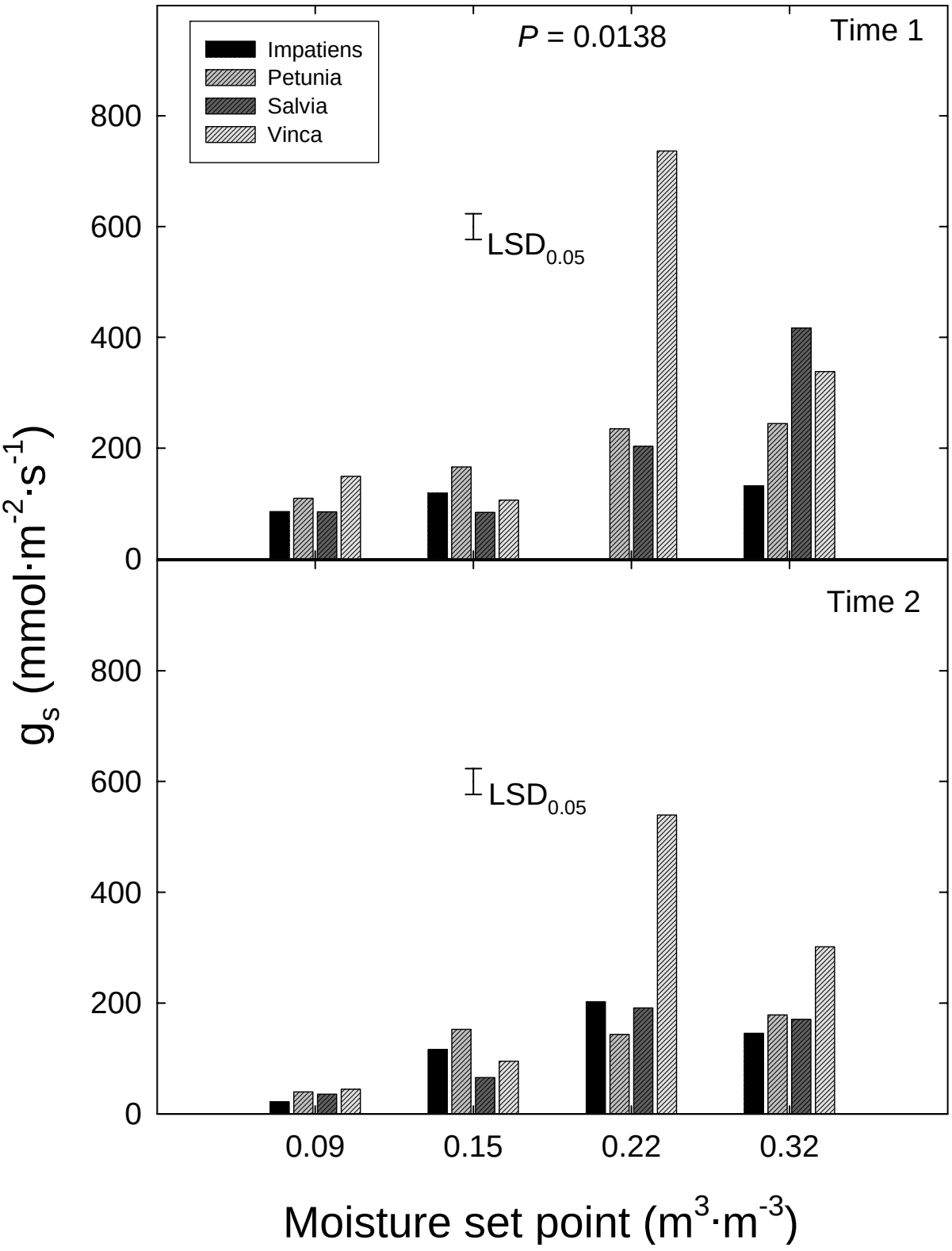
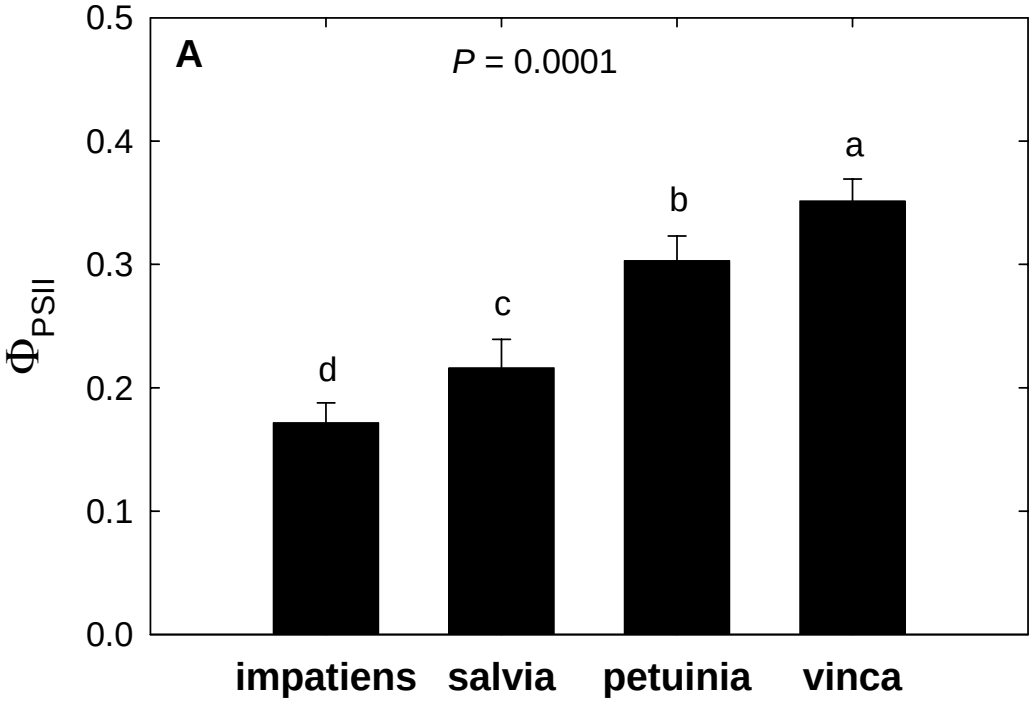

Impatiens
Petunia
Salvia
Vinca
P = 0.0138
Time 1
LSD0.05
Time 2
LSD0.05
800
600
400
200
0
0.09
0.15
0.22
0.32
gs (mmol·m-2·s-1)
Moisture set point (m3·m-3)

Figure 5.6. (A). Mean (n = 16) quantum efficiency of photosystem II (Φ_{PSII}) in light (*PPF* 1000 to 1100 $\mu mol \cdot m^{-2} \cdot s^{-1}$) in different species during the experiment. Means were separated using Tukey's HSD ($P < 0.05$). Error bars represent the standard error of the mean. (B). Interactive effect of θ set points and measurement time (n = 8) on quantum efficiency of photosystem II (Φ_{PSII}) in light. The error bar indicates Fisher's protected least significant difference (LSD) ($P < 0.05$). The $LSD_{0.05}$ was used to compare means between two measurement times in one moisture treatment or among all moisture treatments at one measurement time.


A
P = 0.0001
0.5
0.4
0.3
0.2
0.1
0.0
ΦPSII
a
b
c
d
impatiens
salvia
petuinia
vinca

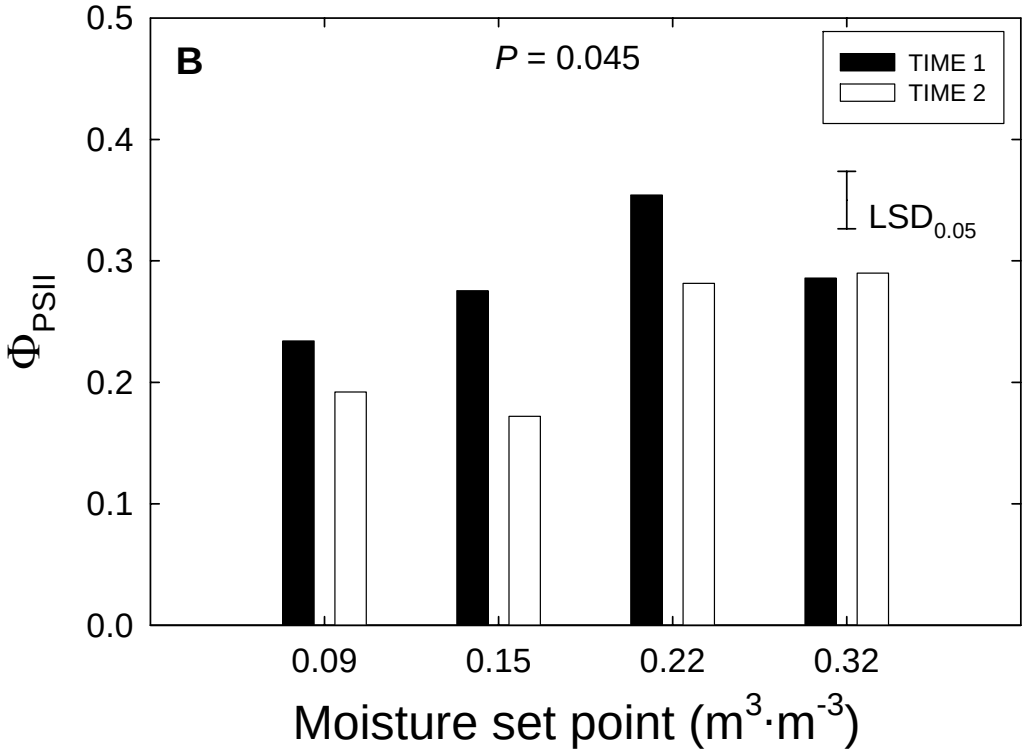

B
P = 0.045
TIME 1
TIME 2
LSD0.05
0.5
0.4
0.3
0.2
0.1
0.0
ΦPSII
0.09
0.15
0.22
0.32
Moisture set point (m3·m-3)

Figure 5.7. Response of net photosynthesis at a *PPF* of 1000 µmol·m^{-2}·s^{-1} ($A_{n,1000}$) to increasing CO_2 concentration in the sub-stomatal region (C_i). A rectangular hyperbola was fitted to describe the response in all treatments. For petunia at 0.22 and 0.15 m^3·m^{-3}, $A_{n,1000} = -8.5 + [(43.9 \cdot C_i) / (211.4 + C_i)]$ and $-7.7 + [(50.2 \cdot C_i) / (271.8 + C_i)]$, respectively. For salvia at 0.22 and 0.15 m^3·m^{-3}, $A_{n,1000} = -7.8 + [(30.7 \cdot C_i) / (96.0 + C_i)]$ and $-2.9 + [(29.0 \cdot C_i) / (369.3 + C_i)]$, respectively.

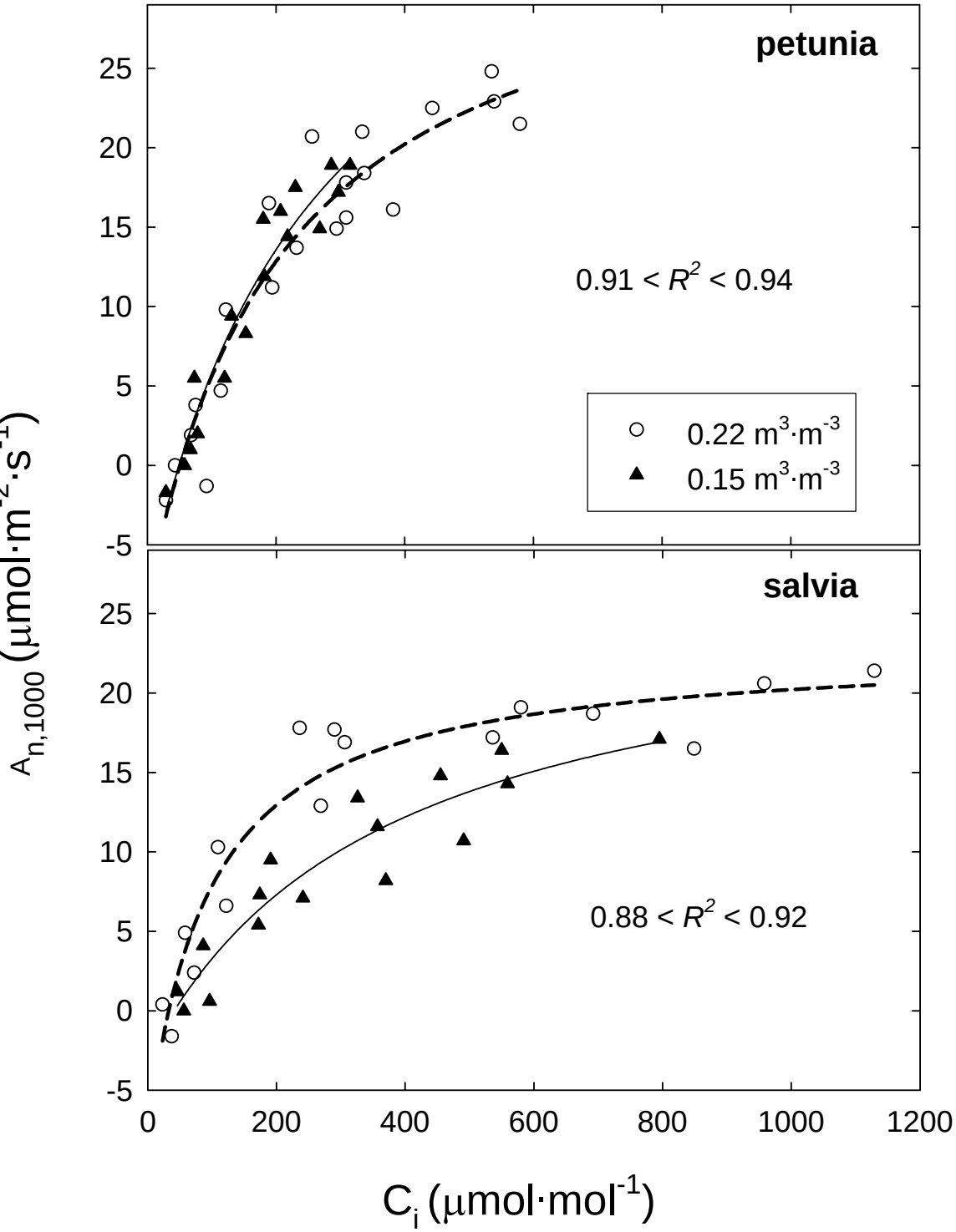
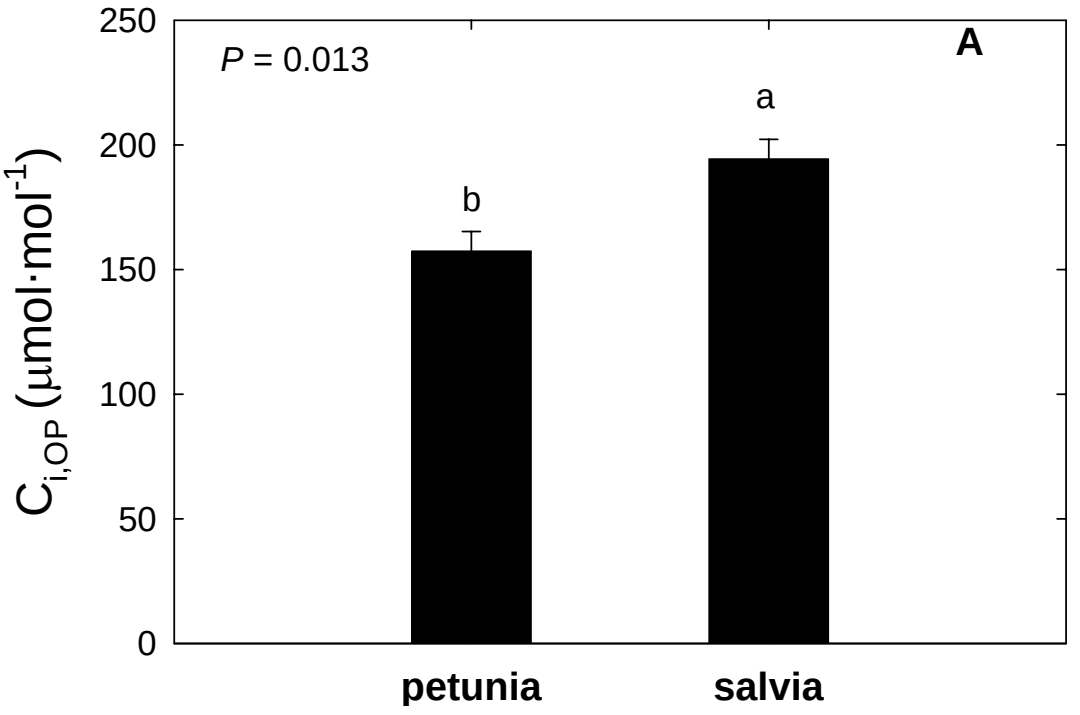
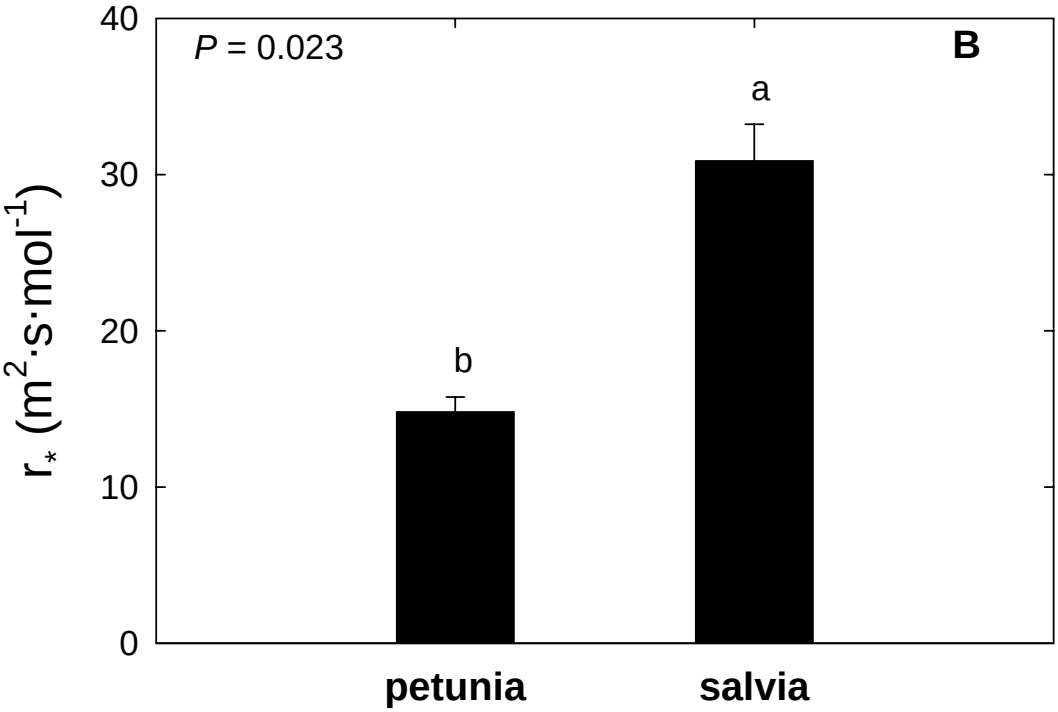

petunia
0.91 < R2 < 0.94
0.22 m3·m-3
0.15 m3·m-3
salvia
0.88 < R2 < 0.92
An,1000 (µmol·m-2·s-1)
Ci (µmol·mol-1)
25
20
15
10
5
0
-5
0
200
400
600
800
1000
1200

Figure 5.8. Mean (n = 4) (A) sub-stomatal CO_2 concentration at the operating point ($C_{i,OP}$) and (B) non-gas phase resistance to CO_2 transfer (r_*) in petunia and salvia during the experiment. Means were separated using Tukey's HSD ($P < 0.05$). Error bars represent the standard error of the mean.


A
P = 0.013
250
200
150
100
50
0
C i,OP (µmol·mol-1)
a
b
petunia
salvia


B
P = 0.023
40
30
20
10
0
r* (m2·s·mol-1)
a
b
petunia
salvia

CHAPTER 6

GROWTH AND PHYSIOLOGY OF SALVIA AND VINCA SUBJECTED TO DECREASING SUBSTRATE WATER CONTENT [1]

---

[1] Nemali, K.S. and M.W. van Iersel. To be submitted to *J. Amer. Soc. Hort. Sci.*

Subject category: Soil-Plant-Water Relationships

Growth and Physiology of Salvia and Vinca Subjected to Decreasing Substrate Water Content

Additional index words. Chlorophyll fluorescence, dark-adapted quantum efficiency (F_V / F_M,), ECH_2O probes, leaf water potential, osmotic adjustment, whole-plant gas exchange

Abstract

We investigated the effects of decreasing substrate water content (θ) at two temperatures (21 and 27 °C) on the daily carbon gain (DCG) of salvia (*Salvia splendens* Sellow ex Roemer & J.A. Schultes) and vinca (*Catharanthus roseus* (L.) G. Don) to identify the minimal θ that would result in normal growth of plants. The substrate (60% peat, 40% perlite) was allowed to dry down inside whole-plant gas exchange chambers until plants wilted after a thorough initial wetting. Whole-plant CO_2 exchange rate (CER) and θ were simultaneously measured during the dry-down cycle. To further investigate the mechanism of drought tolerance or sensitivity in both species, we measured leaf water (Ψ_w), osmotic (Ψ_s), and turgor (Ψ_p) potential, leaf chlorophyll concentration, and dark-adapted quantum efficiency (F_v / F_m) in fully turgid leaves before and after the dry down cycle. The results indicate that moderate (10%) decrease in DCG of vinca and salvia was seen at a θ of 0.08 and 0.12 $m^3 \cdot m^{-3}$, respectively. Our results also indicate that vinca maintained a higher Ψ_p and F_v / F_m than salvia. Leaf chlorophyll concentration

decreased in salvia, but not in vinca, after subjecting plants to drought stress. We conclude that salvia should be grown at a higher θ than vinca and that maintenance of lower Ψ_s, higher Ψ_p, robust photosystem II, and higher leaf chlorophyll concentration are possible reasons for higher DCG in vinca compared to salvia at low θ.

Efficient irrigation management for bedding plants is difficult owing to two reasons i.e., the water requirements of bedding plants are not well documented and the prevalence of the misconception that the effects of excess irrigation are not deleterious to crops. Because of these two reasons, often times, good quality irrigation water is wasted as leachate and/or runoff. The regulations on water-use and environmental impacts due to leaching and runoff from greenhouses are becoming stricter (e.g., Maryland's Water Quality Improvement Act; Lea-Cox and Ross, 2001). To comply with regulations, bedding plant growers should resort to efficient irrigation practices which will avoid wastage of water. One way to avoid wastage of irrigation water is to supply plants with just the amount of water required for their normal growth. To do this, growers will need information on the minimal θ to be present in the substrate, below which plant growth is affected. However, such information is currently lacking for bedding plants.

A knowledge of the physiological effects of low substrate θ on bedding plants and responses seen in bedding plants when exposed to drought stress is central to determining the water requirements of bedding plants. Photosynthesis, which is the primary mechanism for plant growth, is usually insensitive as substrate θ falls to a threshold level, below which the photosynthetic rate will drop quickly with further decrease in θ (McCree, 1986; Gindaba et al., 2005; Xu and Zhou, 2005). Stomates

close in response to drought stress to reduce transpiration (Sperry et al., 2002) and prevent subsequent death of plants due to dehydration, however, at the cost of decreased photosynthesis due to decreased CO_2 conductance. This implies that the primary limiting factor of photosynthesis in plants during drought is reduced CO_2 levels inside leaves due to decreased conductance through stomates. However, it appears that this is not completely true as it has been shown that, at severe drought stress, photosynthesis of sunflower (*Helianthus annuus* L.) is limited not by low CO_2 levels inside leaves, but by low ATP levels. Increasing the CO_2 concentration had no effect on the photosynthetic rate of severely drought-stressed sunflower plants (Tezara et al., 1999).

A decrease in ATP production during drought implies that the excitation energy gained from absorbed light is not channeled through photochemistry. If the absorbed excitation energy is not completely used, it could damage the photosystem II. To prevent damage to the photosystem II due to excess energy that is not used in photochemistry, plants have developed mechanisms to dissipate the excess energy as heat or non-photochemical quenching processes (Lawlor, 2002). However, as the capacity to dissipate excess energy can be limited, some damage to photosystem II is inevitable during severe drought stress. Because the quantum efficiency of photosystem II is maximum and close to 0.83 in dark adapted leaves (F_v / F_m), its measurement can provide an extent of damage to photosystem II (Epron, 1997). A slight decrease in F_v / F_m of photosystem II was seen after exposing grapes (*Vitis vinifera* L.) to severe drought stress (Flexas et al., 1999). Giardi et al. (1996)

reported that the number of active photosystem II centers was reduced under long-term drought stress in plants.

Plants are plastic to changes in their environment and have evolved various physiological adaptations to endure periods of drought stress (Chapman and Augé, 1994). Osmotic adjustment is a common physiological mechanism seen in drought-tolerant plants. Osmotic adjustment lowers Ψ_w in leaves and roots, to maintain the gradient in Ψ_w required for movement of water and subsequent maintenance of leaf turgor in plants under conditions of low Ψ_w (drought) in the soil or substrate (Hsiao and Xu, 2000; Serraj and Sinclair, 2002). Plants can also protect cell organelles by synthesizing osmo-protectants or compatible solutes inside cells (Serraj and Sinclair, 2002). Because turgor is maintained due to osmotic adjustment, plants can continue to photosynthesize, however osmotic adjustment can only aid in continuation of photosynthesis within a limited range of low soil Ψ_w (Serraj and Sinclair, 2002).

The objectives of this study were to identify the minimal substrate θ to avoid drought stress in drought-sensitive (*Salvia splendens*) and drought-tolerant (*Catharanthus roseus*) species and to understand the mechanism of drought tolerance, if seen in either of the two species. Physiological responses to drought were studied from whole-plant gas exchange of salvia and vinca. Plant responses to decreasing substrate θ can be studied nondestructively using gas exchange measurements, as opposed to destructive growth analysis.

Materials and methods

Plant material and environment.

Seeds of salvia ‘Bonfire Red’ and vinca ‘Cooler Peppermint’ were sown in standard flats (0.55 m · 0.2 m · 0.06 m) filled with a soilless substrate (Fafard 2P, Fafard, Andersen, S.C.) and germinated under a mist system. The seeds were sown at different times as replications (see below) were spaced over time. At each time, one flat was sown with for each species. Each time, two more flats were filled with the same soilless substrate and left unsown. Approximately a week after germination, seedlings were thinned to 32 per flat (8 rows · 4 seedlings per row) and trays shifted to a greenhouse. Plants were subirrigated with a 15N:2.2P:12.5K water-soluble fertilizer (Peters 15-5-15 Cal-Mag; Peat-lite special, The Scotts Co., Marysville, Ohio) at a rate of 1 to 1.5 $dS \cdot m^{-1}$ (150 to 225 ppm N) in an ebb-and-flow system until the leaf canopy covered the trays. The mean and standard deviation of temperature, RH, and daily light integral (DLI) inside the greenhouse were 21.5 ± 0.9 ^{o}C, 44.7 ± 11.3 %, and 7.38 ± 2.24 $mol \cdot m^{-2} \cdot d^{-1}$, respectively.

Plants inside gas exchange chambers.

After canopy coverage, the trays containing plants were shifted into whole-plant gas exchange chambers (van Iersel and Bugbee, 2000) arranged inside a growth chamber. The unsown trays were also shifted into gas exchange chambers to correct gas exchange rates for microbial respiration from the substrate during the study. Presence of roots in the substrate will influence the respiration from the substrate. This system does not separate root and shoot respiration but accounts for both. Microbial respiration resulting from carbon exudates into the substrate is also accounted for by this system.

The C exudates from roots are important as they decrease the carbon gained by plants. Prior to shifting trays into gas exchange chambers, the substrate in all trays was thoroughly wetted. Inside the gas exchange chambers, the substrate was allowed to dry until plants started to show wilting signs. The time between placing trays inside gas exchange chambers and wilting in plants constituted an irrigation cycle. After an irrigation cycle, the trays were taken out of the gas exchange chambers, the substrate was again thoroughly wetted, and trays were placed back inside the gas exchange chambers. There were two irrigation cycles for all trays. The first cycle was intended to acclimate the plants to conditions inside the growth chamber (acclimation cycle) and measurements from the second cycle were used for analysis (measurement cycle).

Environment control inside gas exchange chambers.

The growth chamber was programmed to a light-dark cycles of 4 and 2 h, respectively. During the gas exchange study, the DLI during 16 h of light was maintained similar to the DLI received by plants inside the greenhouse by adjusting the photosynthetic photon flux (*PPF*) incident on plants inside the gas exchange chambers. The instantaneous photosynthetic photon flux (*PPF*) maintained inside the growth chamber was approximately 130 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The RH inside the gas exchange chambers was measured using a humidity sensor (HTO-45R, Rotronic instruments, Crawley, UK). In general, RH inside gas exchange chambers depended on the substrate water content and on an average varied between 75 to 30% as the water content depleted from high to low θ. The temperature inside the gas exchange

chambers was regulated by heater strips controlled by a datalogger (CR10T, Campbell Scientific, Logan, Utah) and maintained at 21 or 27 °C.

Treatments.

Two species, vinca (drought-tolerant) and salvia (drought-sensitive), were grown in the study. Both species were grown under similar environmental conditions inside the greenhouse. Each species was grown at two different temperatures (21 and 27 °C) inside the gas exchange chambers. Two temperatures were selected because the rate of substrate drying can be faster at a higher temperature and plant response to decreasing θ might vary with rate of drying. Therefore, there were four treatments in total and plants in each treatment were grown inside one gas exchange chamber. The chambers with unsown trays were also set at 21 and 27 °C.

Measurements.

The volumetric water content of the substrate was continuously measured once every 10 minutes using dielectric aquameters (ECH_2O-10, Decagon devices Inc., Pullman, WA) connected to a datalogger (CR10, Campbell Sci). The datalogger measured θ based on a substrate specific calibration equation which uses the voltage output of the probes [$ln(\theta) = -6.99 + 1.58 \cdot 10^{-2} \cdot mV - 9.91 \cdot 10^{-6} \cdot mV^2$, $R^2 = 0.91$]. More details about the calibration of ECH_2O moisture sensors are described elsewhere (Nemali et al., 2005). The whole-plant gas exchange system measured the gas exchange rate of each group plants once every 10 minutes. Prior to placing plants inside the gas exchange chambers, leaf chlorophyll concentration, Ψ_w, Ψ_s, Ψ_p, and F_v / F_m of plants were measured. Leaf water potential was measured five times viz., at the start (T1, turgid leaves) and end (T2, flaccid leaves) of the acclimation cycle, the start (T3, after plants

had been rewatered, turgid leaves) and end (T4, flaccid leaves) of measurement cycle, and after rehydrating the plants from the measurement cycle (T5, turgid leaves). Leaf Ψ_s, Ψ_p, F_v / F_m, and leaf chlorophyll concentration were measured at times T1, T3, and T5 (on turgid leaves). Measurements on leaves were performed on the uppermost fully-developed leaves.

Leaf chlorophyll concentration was measured with a non-destructive meter (SPAD-502, Minolta, Osaka, Japan) on five random leaves in each treatment and averaged to obtain a representative value. Leaf Ψ_w was measured using leaf-cutter thermocouple psychrometers (Model 76, J.R.D. Merril, Logan, UT) after equilibration at 25 °C for four hours. The psychrometers were kept in a freezer overnight to kill the leaf samples after Ψ_w measurements to determine Ψ_S from lysed cells. Measurements of Ψ_s were taken after equilibrating the psychrometers at 25 °C for four hours again. Leaf Ψ_P was calculated as difference between Ψ_S and Ψ_w. Plants were dark-adapted for 40 to 60 minutes before exposing leaf portions to a saturating pulse (> 8000 $\mu mol \cdot m^{-2} \cdot s^{-1}$) to obtain a dark-adapted measure of quantum efficiency of photosystem II (F_v/F_m) (mini PAM, Walz, Effeltrich, Germany).

The carbon dioxide exchange rate (CER) of plants ($\mu mol\ CO_2 \cdot s^{-1}$) was corrected for microbial respiration from the substrate by subtracting the CER from unsown trays . Photosynthetic and respiration rates of plants ($\mu mol \cdot s^{-1}$) during each cyclic photoperiod were estimated as the mean CER during the light and dark periods, respectively. The daily average photosynthetic (P_n, $\mu mol \cdot s^{-1}$) and respiration (R_d, $\mu mol \cdot s^{-1}$) rates were estimated as the mean of four light or dark periods. Data were the total CER of 32 plants in each treatment. Daily carbon gain (DCG, $\mu mol \cdot d^{-1}$; µmol of carbon gained by a group

of 32 plants in a day; a measure of growth rate of plants) was estimated as follows:

$DCG = [(P_n \cdot 3600 \cdot 16) - (R_d \cdot 3600 \cdot 8)]$

where 3600 refers to the number of seconds per hour, 16 and 8 are total hours of light and dark in a day.

Experimental design and statistical analysis.

The experiment consisted of four treatments (2 species · 2 temperatures) and three replications in a randomized complete block design. The replications were spaced over time. Daily carbon gain data were analyzed using the non-linear regression procedure of SAS (proc NLIN, SAS systems, Cary, N.C.). The following regression was fitted to describe the response of DCG to decreasing substrate θ:

$DCG = a \cdot exp[-exp -((\theta - \theta_0) / b)]$

where θ is the substrate moisture content, a is the maximum DCG (asymptote), b and θ_o are regression parameters. The asymptote values were used to normalize DCG (expressed as percentage of asymptote value) to correct for differences in leaf area of plants among replications. A confidence interval (5 to 95%) was used to show differences in response of DCG to decreasing water content among treatments. The threshold θ for DCG to decrease by 10 and 50% in DCG was estimated from the fitted equations.

Main and interactive effects of species, temperature, and time on Ψ_w, Ψ_s, Ψ_P, F_v / F_m, and leaf chlorophyll concentration were tested using proc ANOVA of SAS ($P < 0.05$). Means were separated using Tukey's HSD.

Results and discussion

Whole-plant CO_2 exchange rate.

The representative CO_2 exchange rate during light (photosynthesis) and dark (respiration) periods was shown in figure 6.1. Both P_n and R_d were high at the start of any irrigation cycle ($\theta > 0.25$ m^3 m^{-3}). Daily mean photosynthetic rate remained unaffected as the θ dropped, however beyond a species-dependent threshold θ (Fig. 6.2), P_n decreased sharply with decreasing θ. This was noted during both the acclimation and measurement cycles (Fig. 6.2) and all treatments. Similar responses of leaf photosynthesis were reported for grasses (perennial rhizome grass, *Leymus chinensis* Trin. Tzvelev; Xu and Zhou, 2005), field crops (sorghum,*Sorghum bicolor* L.; McCree, 1986), and trees (eucalyptus, *Eucalyptus camaldulensis* Dehnh. and *E. globulus* Labill.; Gindaba et al., 2005). The response of R_d to decreasing θ was different than that seen for P_n. The dark respiration rate gradually decreased (i.e., closer to zero) with decreasing θ and a similar response was seen in both acclimation and measurement cycles (Fig. 6.2) and all treatments.

The confidence intervals used to distinguish the relationship describing the response of DCG to decreasing θ at any temperature and for a particular species were not different (data not shown). This indicates that there was no effect of temperature on the response of DCG to decreasing θ in both species. An earlier study by Xu and Zhou (2005), who measured the effect of θ and temperature on leaf photosynthesis of *Leymus chinensis* (Trin.) Tzvel, indicated that the decrease in leaf photosynthesis with decreasing θ was more pronounced at higher night time temperature (25 ^{o} opposed to 20 ^{o}C). However, they did not report about the response of growth rate as they measured

photosynthesis at leaf scale and did not measure the response of respiration to decreasing θ in plants at different temperatures. As plant growth is the result of carbon gained after accommodating respiratory loss (Amthor 1984, Lawlor, 1995; Nemali and van Iersel, 2004), it may not be appropriate to assume the response of plant growth rate (or DCG, as in this study) to be similar to that observed for leaf photosynthesis (more pronounced decrease at high temperature with decreasing θ) as in the Xu and Zhou (2005) study.

When the data from both temperatures were pooled, significant differences in the response of DCG to decreasing θ were observed between the two species (Fig. 6.3). The fitted non-linear equation described the response of DCG of both species to decreasing θ with reasonable accuracy ($0.77 < R^2 < 0.86$). As the θ decreased, the DCG of plants in all treatments was mostly unaffected until a θ of approximately 0.15 m^3 m^{-3} (or 15% v/v). Shoot dry mass of chrysanthemum (*Chrysanthemum* x *morifolium.* Ramat.) was not affected until θ dropped to a low value (15% of that at saturation; Olson et al., 2002). A further depletion in θ to 0.12 $m^3 \cdot m^{-3}$ in our study resulted a 10% decrease in DCG of salvia (Fig. 6.3) and the θ at which vinca started to show a 10% decline in DCG was lower compared to that of salvia at approximately 0.08 $m^3 \cdot m^{-3}$. The water content at which severe drought stress (50% decrease in DCG) was noticed in salvia and vinca was 0.084 and 0.06 $m^3 \cdot m^{-3}$, respectively. This indicates that, when exposed to similar drought stress, the growth of salvia is inhibited earlier than that of vinca. Thus, to prevent drought stress, substrate water content for salvia and vinca should be maintained above 0.15 and 0.10 $m^3 \cdot m^{-3}$, respectively.

Leaf water, osmotic, and turgor potential.

There was no effect of species or temperature, but there was an effect of time of measurement on the Ψ_w of the plants (Fig. 6.4). As expected, Ψ_w was lower at times T2 and T4 (wilted plants, low θ) compared to T1, T3, and T5 (turgid leaves, high θ). However, Ψ_w was slightly, but significantly, lower at T1 compared to T3 and T5, and Ψ_w was not different between T3 and T5 (turgid leaves, after acclimation and measurement cycle, Fig. 6.4). There was only a significant species effect on Ψ_s and Ψ_p. It is important to note that values of Ψ_S and Ψ_p shown here are from fully turgid leaves at times T1, T3, and T5. Osmotic potential of the rehydrated leaves of vinca was significantly lower (more negative) than that of salvia (Fig. 6.5). Mean Ψ_p of the rehydrated leaves of vinca was higher than that of salvia (Fig. 6.5).

Because there were no differences in Ψ_s of turgid leaves at times T1, T3, and T5, it is concluded that no osmotic adjustment had occurred in leaves of either species after two drying cycles. These results are surprising and do not agree with the general phenomenon of occurrence of osmotic adjustment during drought [e.g., as seen in *Rudbeckia fulgida* (Chapman and Augé, 1994) and salvia (Augé et al., 2003)]. However, it is possible that other mechanisms, like osmotic adjustment in root cells, leading to improved root water uptake (Hsiao and Xu, 2000) could have contributed to drought tolerance in vinca compared to salvia. The higher leaf Ψ_p measured in vinca compared to salvia was mainly due to the lower leaf Ψ_s in vinca than salvia. Although osmotic adjustment was not noticed in leaves, it is possible that the lower Ψ_s (measured on turgid leaves) maintained in vinca compared to salvia is one possible reason that vinca can uptake water from a lower substrate water potential (provided that Ψ_w also remains low

for vinca under low θ) and continue to maintain growth rate under lower θ (< 0.15 m^3 m^{-3}) than salvia.

Leaf chlorophyll concentration and chlorophyll fluorescence in dark adapted leaves.

There was an interactive effect of species · time on leaf chlorophyll concentration. Leaf chlorophyll concentration was not significantly different between the turgid leaves of both species either before or after the measurement cycle. But there was a significant decrease in leaf chlorophyll concentration of turgid leaves of salvia after subjecting plants to drought (Fig. 6.6). This was not noticed in vinca, whose leaf chlorophyll concentration was not significantly different between both measurement times. Our results support the findings of Barker et al. (2005) (and references therein) that drought stress caused chlorosis in leaves of desert grasses. Quantum efficiency of dark-adapted leaves of salvia was significantly lower than that of vinca (Fig. 6.7A). Pooled across both species and measurement times, F_v / F_m was slightly higher at 27 than at 21 ^oC (Fig. 6.7B). We noticed more leaf abscission in salvia compared to vinca at both temperatures.

Leaf chlorophyll concentration was lower in salvia, but not in vinca, after subjecting the plants to a drought stress. In turn, this could be a possible reason for the lower quantum efficiency of photosystem II efficiency in dark-adapted leaves of salvia compared to vinca. It is also possible that there was a damage to the photosystem II during drought stress (Flexas et al., 1999) in salvia but not in vinca. However, the possibility that the inherent capacity of intrinsic photochemical efficiency in salvia is lower than that of vinca can not be disregarded.

Conclusions

In conclusion, our results indicate that the minimal θ for normal growth of salvia and vinca for a 60% peat - 40% perlite substrate is 0.15 and 0.10 $m^3 \cdot m^{-3}$, respectively. These findings (lower threshold θ for DCG to decrease, higher Ψ_p, and F_v / F_m) suggest that vinca is able to withstand drought stress better than salvia, although this could depend on the cultivars used in this study. To the best of our knowledge, our study is the first to use a quantitative approach to nondestructively and continuously monitor growth rate (or DCG) with decreasing substrate water content. In this study we not only identified the optimal substrate water content for vinca and salvia but also some of the physiological mechanisms responsible for drought tolerance in vinca. This study could be a model for future studies aimed at identifying optimal substrate moisture content for other species.

References

Amthor, J.S. 1984. The role of maintenance respiration in plant growth. Plant Cell Environ. 7: 561-569.

Augé, R.M., A.J.W. Stodola, J.L. Moore, W.E. Klingeman, and X.R. Duan. 2003. Comparative dehydration tolerance of foliage of several ornamental crops. Sci. Hort. 98: 511-516.

Barker, D.H., L.R. Stark, J.F. Zimpfer, N.D. McLetchie, and S.D. Smith. 2005. Evidence of drought induced stress on biotic crust moss in the Mojave desert. Plant Cell Environ. 28: 939-947.

Chapman, D.S. and R.M. Augé. 1994. Physiological mechanisms of drought resistance in four native ornamental perennials. J. Amer. Soc. Hort. Sci. 119:299-306.

Epron, D. 1997. Effects of drought on photosynthesis and on the thermotolerance of photosystem II in seedlings of cedar (*Cedrus atlantica* and *C. libani*). J. Expt. Bot. 48: 1835-1841.

Flexas, J., J.M. Escalona, and H. Medrano. 1999. Water stress induces different levels of photosynthesis and electron transport rate regulation in grapevines. Plant Cell Environ. 22: 39-48.

Giardi, M.T., A. Cona, B. Geiken, T. Kučera, J. Masojìdek, and A.K. Mattoo. 1996. Long-term drought stress induces structural and functional reorganization of photosystem II. Planta 199: 118-125.

Gindaba, J., A. Rozanov, and L. Negash. Photosynthetic gas exchange, growth and biomass allocation of two Eucalyptus and three indigenous tree species of Ethiopia under moisture deficit. *Forest Ecol. Management* 205: 127- 138.

Hsiao, T.C. and L.K. Xu. 2000. Sensitivity of growth of roots versus leaves to water stress: biophysical analysis and relation to water transport. *J. Expt. Bot.* 51: 1595-1616.

Lawlor, D.W. 1995. Photosynthesis, productivity and environment. J. Expt. Bot. 46: 1449-1461.

Lawlor, D.W. 2002. Limitation to photosynthesis in water stressed leaves: stomata vs metabolism and role of ATP. Ann. Bot. 89: 871-885.

Lea-Cox, J.D. and D.S. Ross. 2001. A review of the federal clean water act and the Maryland water quality improvement act: the rationale for developing a water and nutrient planning process for container nursery and greenhouse operations. *J. Environ. Hort.* 19:226-229.

McCree. K.J. 1986. Whole plant carbon balance during osmotic adjustment to drought and salinity stress. Aust. J. Plant Physiol. 13:33-43.

Nemali,K.S. and M.W. van Iersel. 2004. Light effects on wax begonia: photosynthesis, growth respiration, maintenance respiration, and carbon use efficiency. J. Amer. Soc. Hort. Sci. 129: 416-424.

Olson, D.L., R.D. Oetting, and M.W. van Iersel. 2002. Effect of potting media and water management on development of fungus gnats (Diptera: Sciaridae) and plant growth. HortScience 37:919-923.

Serraj, R. And T.R. Sinclair. 2002. Osmolyte accumulation: can it really help increase crop yield under drought conditions? Plant Cell Environ. 25: 333-341.

Sperry, J.S., U.G. Hacke, R. Oren, and J.P. Comstock. 2002. Water deficits and hydraulic limits to leaf water supply. Plant, Cell Environ. 25: 251-263.

Tezara, W, V.J. Mitchell, S.D. Driscoll and D.W. Lawlor. 1999. Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. *Nature* 401:914-917.

van Iersel, M.W. and B. Bugbee. 2000. A multiple chamber, semicontinuous, crop carbon dioxide exchange system: Design, calibration, and data interpretation. J. Amer. Soc. Hort. Sci. 125: 86-92.

Xu, Z-Z., and G.S. Zhou. Effects of water stress and high nocturnal temperature on photosynthesis and nitrogen levels of perennial grass Leymus chinensis. Plant and Soil. 269: 131-139.

Figure 6.1. Carbon dioxide exchange rate (CER) during a typical day. The measurements were collected from vinca grown at a temperature of 21 °C (data for plants in other treatments looked similar) during four light-dark cycles on a typical day. Positive and negative CER were designated as whole-plant photosynthesis and respiration rates, respectively.

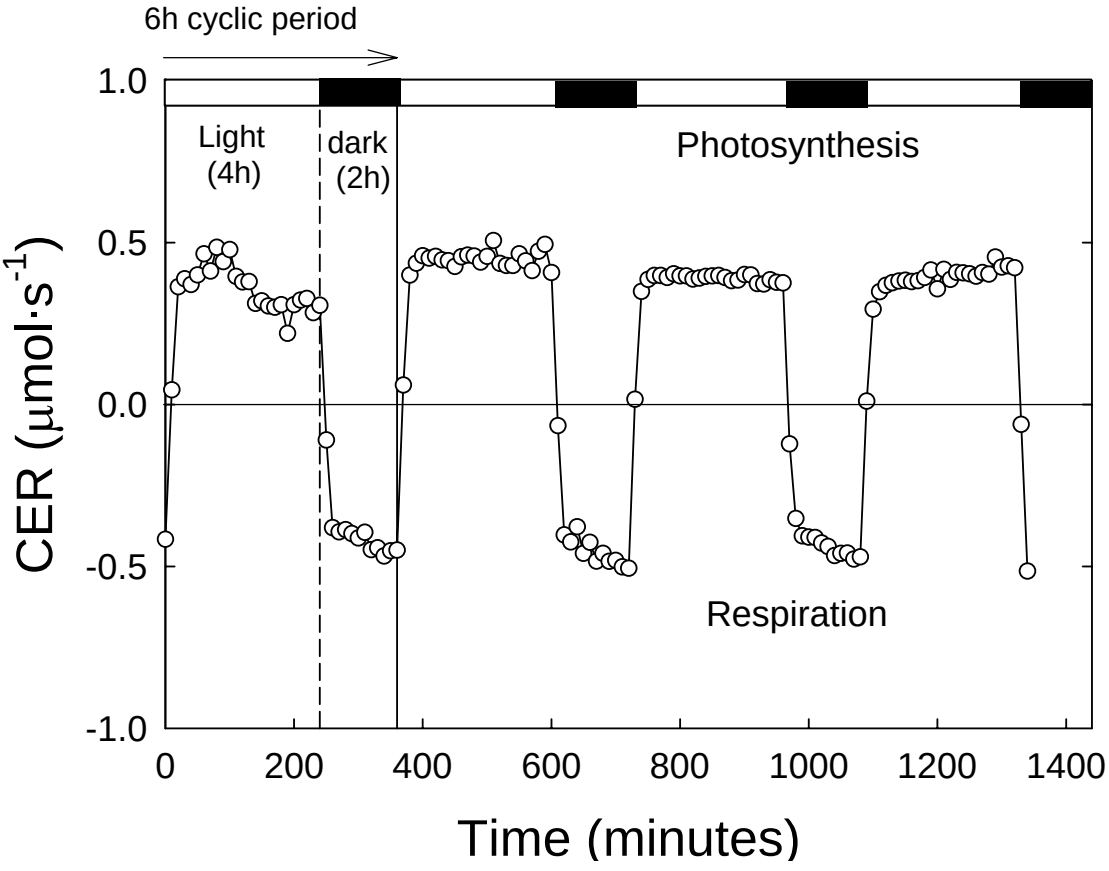

6h cyclic period
1.0
0.5
0.0
-0.5
-1.0
Light
(4h)
dark
(2h)
Photosynthesis
Respiration
CER (µmol·s-1)
0
200
400
600
800
1000
1200
1400
Time (minutes)

Figure 6.2. Average water content, photosynthesis, and respiration during different days for a typical acclimation and measurement cycle. The data were collected from vinca grown at a temperature of 27 °C (data for plants in other treatments looked similar). The time of irrigation (after wilting) is indicated with a vertical dashed line separating acclimation and measurement cycles. The substrate was wetted to a lower water content during the measurement cycle to reduce the duration of the cycle, however the water content was always higher than the level at which DCG decreased in all treatments.

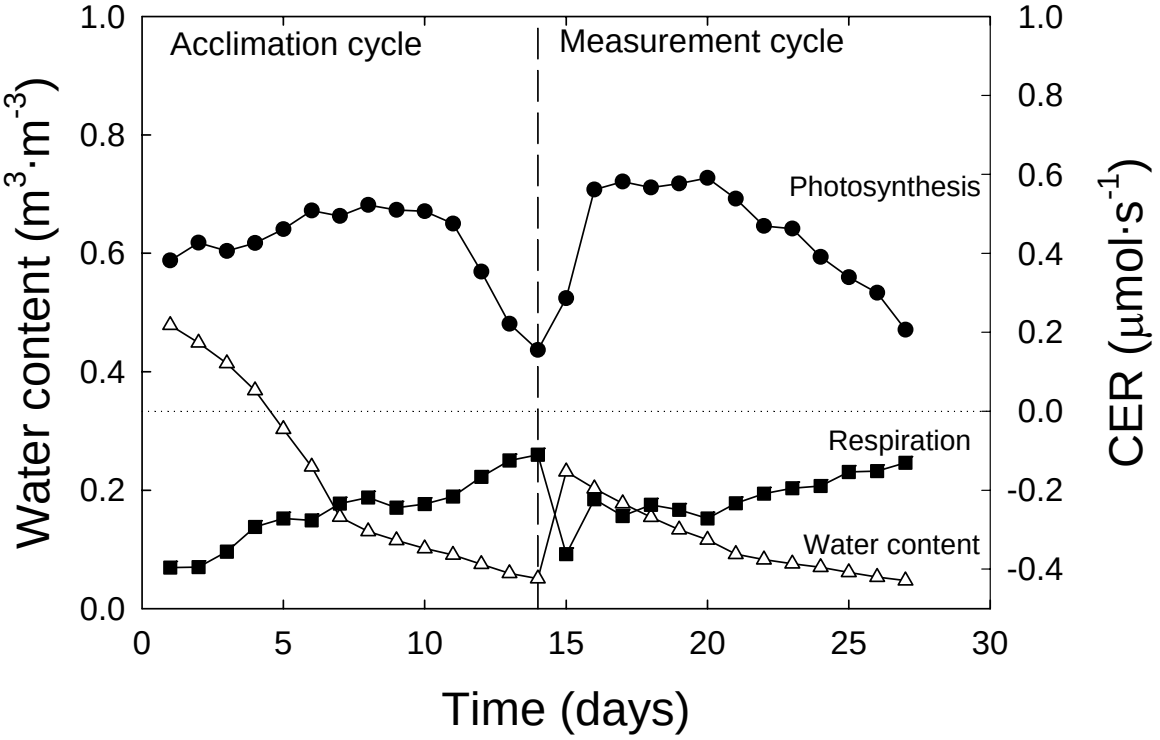

Acclimation cycle
Measurement cycle
Photosynthesis
Respiration
Water content
Water content (m3·m-3)
CER (µmol·s-1)
Time (days)
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
0
5
10
15
20
25
30

Figure 6.3. Response of daily carbon gain (DCG, expressed as % of asymptote) in salvia and vinca to decreasing substrate water content. Data were collected during the measurement cycles. Nonlinear functions were used to describe the response; $DCG_{vinca} = 100 \cdot [exp(-exp(-(\theta - 0.0549) / 0.0133)]$ (R^2 = 0.86), $DCG_{salvia} = 100 \cdot [exp(-exp(-(\theta - 0.0768) / 0.0193)]$, (R^2 = 0.77). Significant differences in the response of DCG to water content between two species was indicated by confidence bands (5 to 95%; dotted lines). The threshold water contents for 10 percent DCG decrease (S or $V_{10\%}$), and 50 percent DCG decrease (S or $V_{50\%}$) are indicated by arrows.

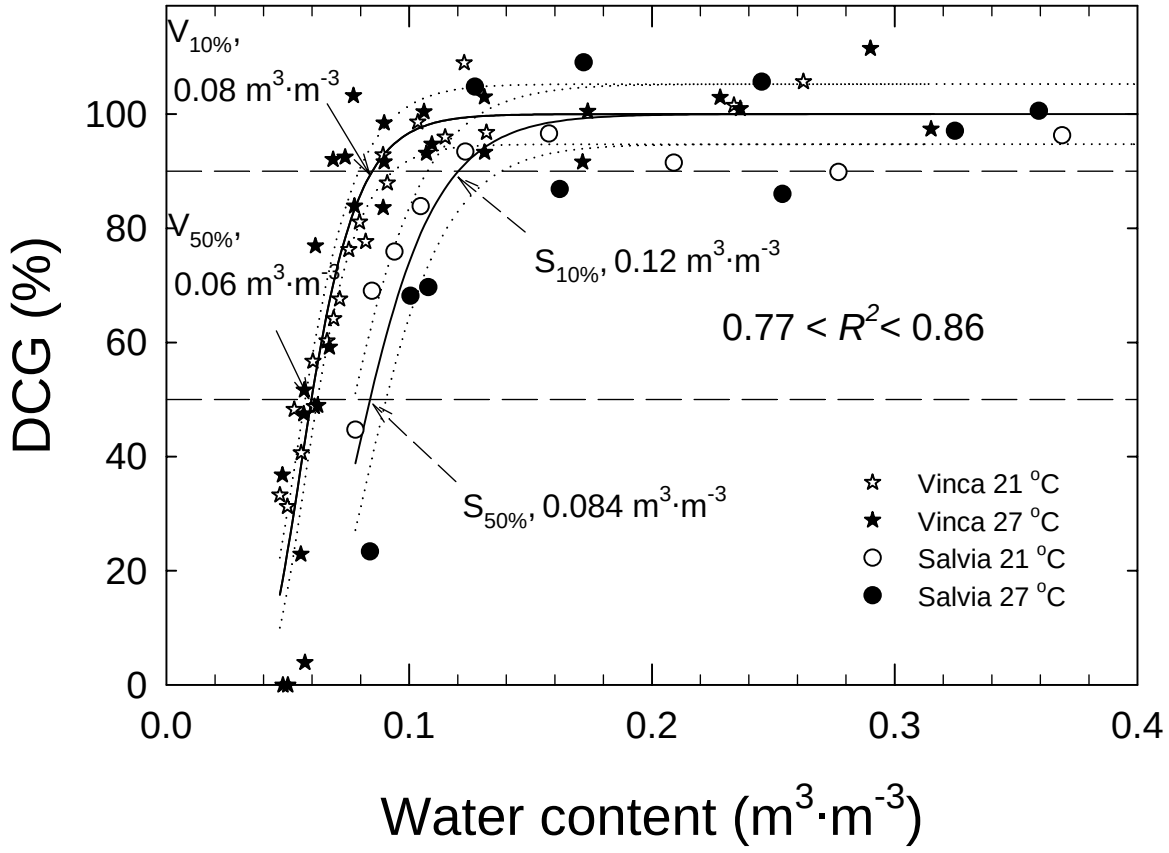

V10%,
0.08 m3·m-3
V50%,
0.06 m3·m-3
S10%, 0.12 m3·m-3
S50%, 0.084 m3·m-3
0.77 < R2< 0.86
Vinca 21 oC
Vinca 27 oC
Salvia 21 oC
Salvia 27 oC
DCG (%)
Water content (m3·m-3)
0
20
40
60
80
100
0.0
0.1
0.2
0.3
0.4

Figure 6.4. Mean leaf water potential (Ψ_w) of plants (n = 12) during different times. Data were pooled from different treatments and replications. Times T1 and T2 correspond to start (turgid leaves) and end (flaccid leaves) of the acclimation cycle, whereas times T3 and T4 correspond to start (turgid leaves) and end (flaccid leaves) of the measurement cycle, and T5 correspond to rehydrated leaves after the measurement cycle. Error bars represent the standard error of the mean. Mean separation by Tukey's HSD.

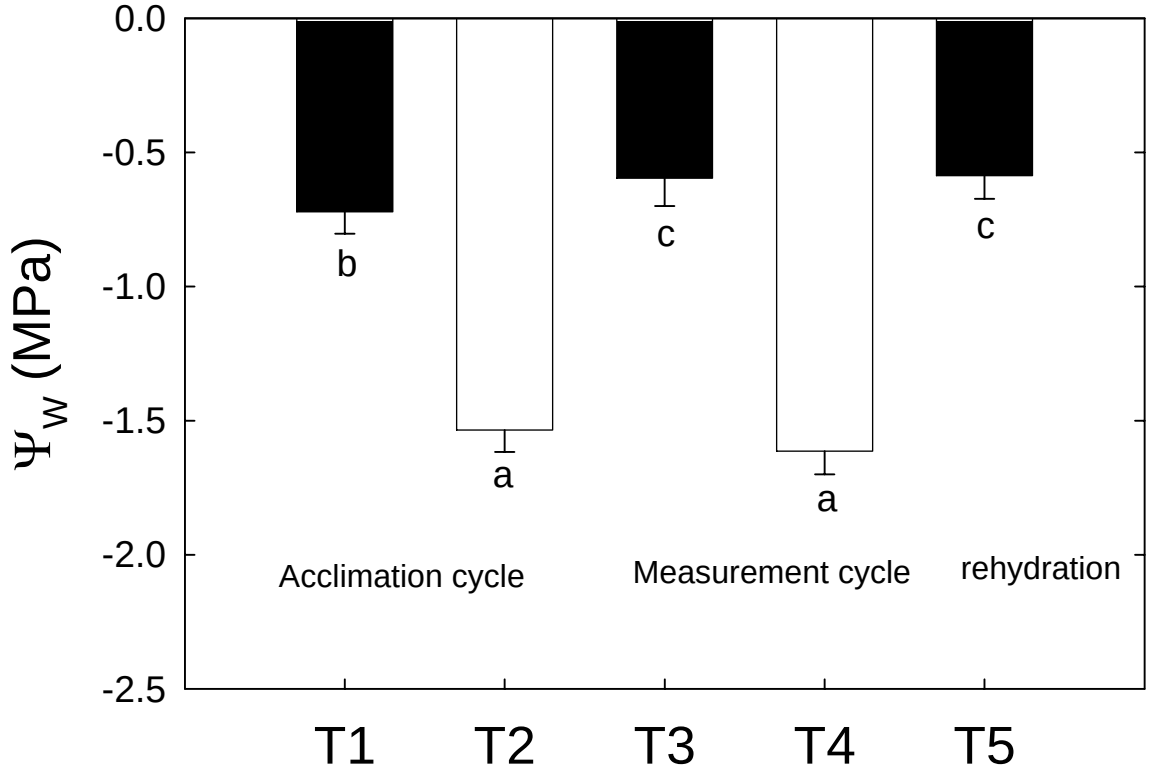

0.0
-0.5
-1.0
-1.5
-2.0
-2.5
Ψ_w (MPa)
b
a
c
a
c
Acclimation cycle
Measurement cycle
rehydration
T1
T2
T3
T4
T5

Figure 6.5. Mean (n = 18) osmotic (Ψ_s) and turgor potential (Ψ_p) of salvia and vinca. Data were pooled from different temperatures, measurement times, and replications. Osmotic and turgor potentials were measured on fully turgid leaves (times T1, T3, and T5, see legend of figure 6.4 for details). Error bars represent the standard error of the mean. Mean separation by Tukey's HSD.

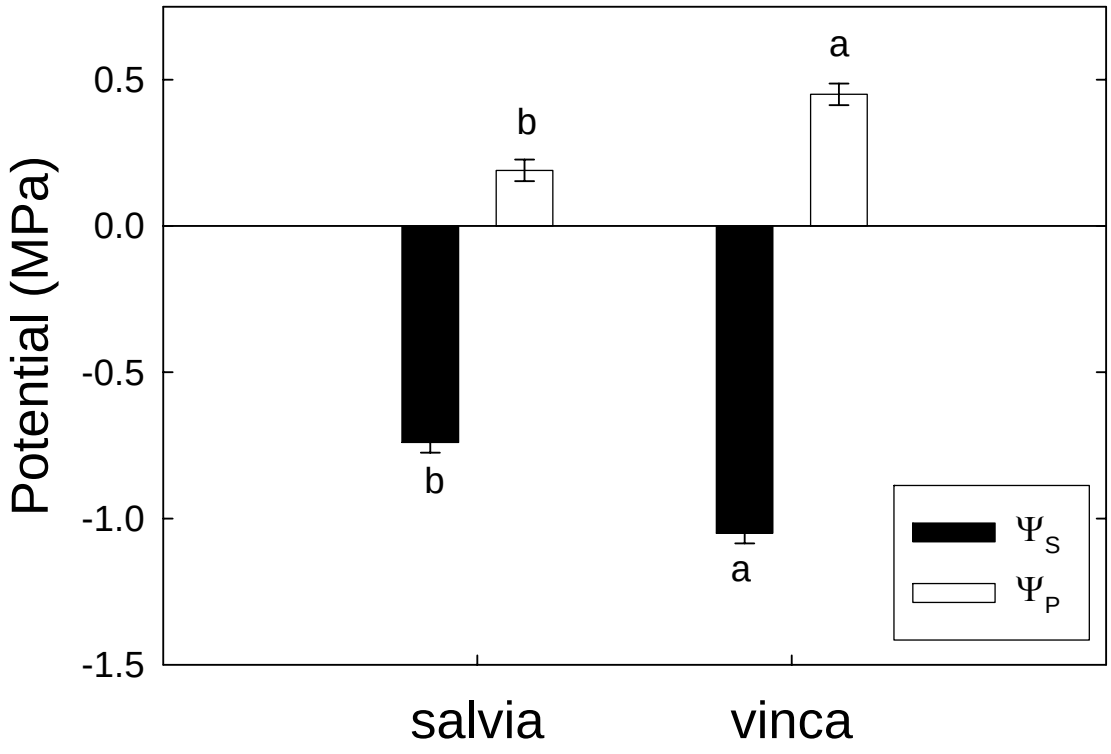

Potential (MPa)
0.5
0.0
-0.5
-1.0
-1.5
b
b
a
a
Ψ_S
Ψ_P
salvia
vinca

Figure 6.6. Interactive effect (n = 6) of species · time on leaf chlorophyll concentration. Data are pooled from different temperatures and replications. Leaf chlorophyll concentration was measured on turgid leaves (times T1, T3, and T5, see legend of figure 6.4 for details). Error bars represent standard error of mean. Capital letters were used to separate means between species at any time and small letters were used to separate measurement times for one species. Mean separation by interactive LSD.

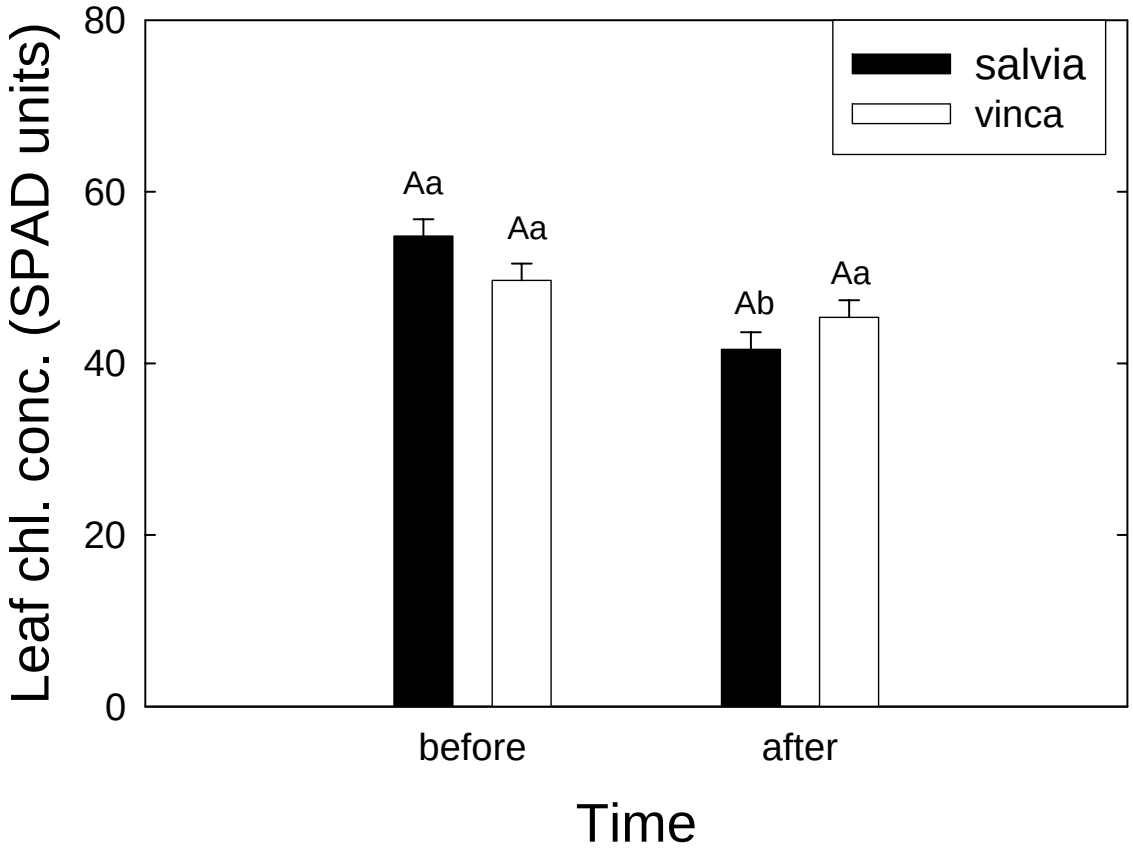
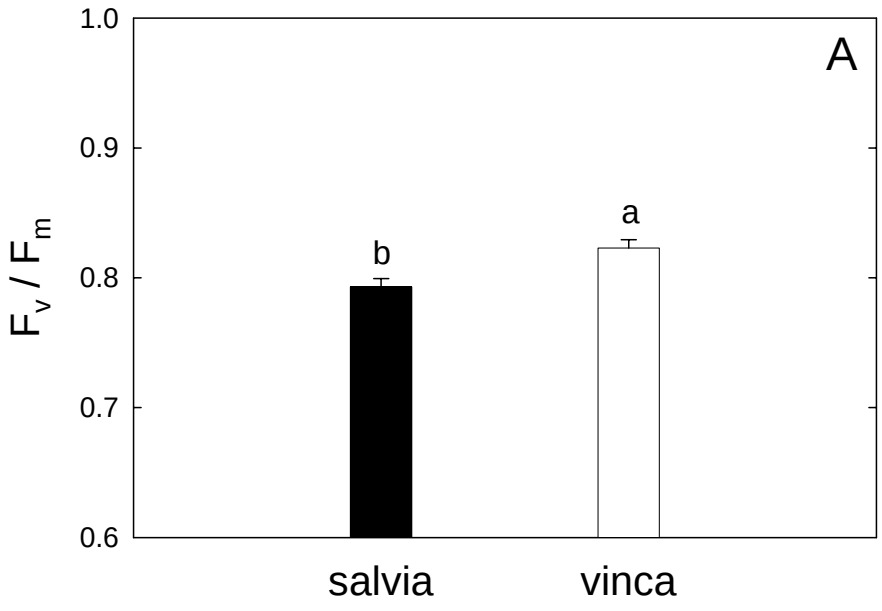
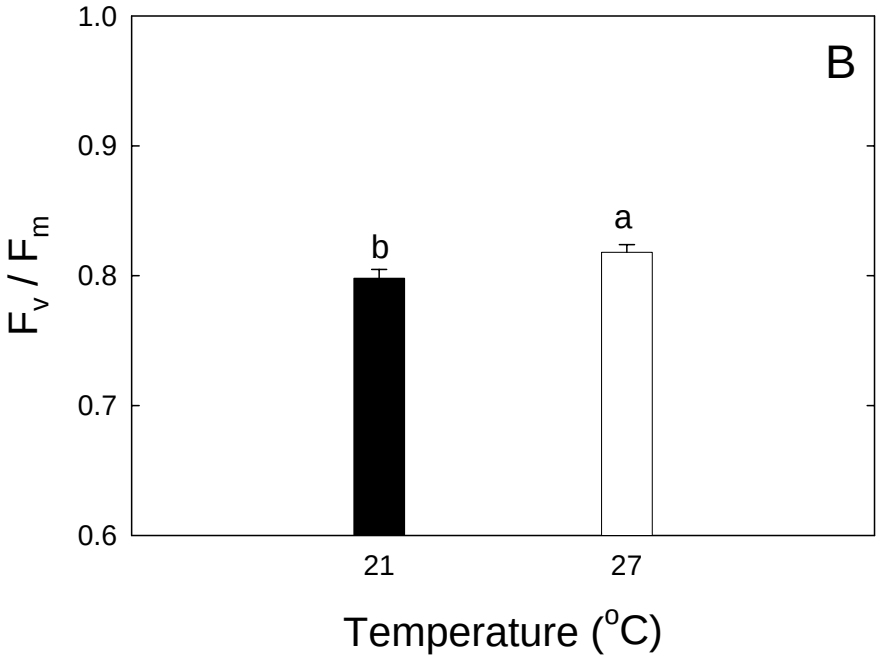

Leaf chl. conc. (SPAD units)
80
60
40
20
0
salvia
vinca
Aa
Aa
Ab
Aa
before
after
Time

Figure 6.7. A. Mean (n = 12) quantum efficiency of photosystem II in dark-adapted leaves of salvia and vinca. Data were pooled from different temperatures, measurement times (times T1, T3, and T5, see legend of figure 6.4 for details), and replications, and B. mean (n = 12) quantum efficiency of photosystem II in dark-adapted leaves at different temperatures. Data were pooled from different species, measurement times, and replications. In both figures, measurements were taken on fully turgid leaves. Error bars represent standard error of the mean. Mean separation by Tukey's HSD.


A
1.0
0.9
0.8
0.7
0.6
F_v / F_m
b
a
salvia
vinca


B
1.0
0.9
0.8
0.7
0.6
F_v / F_m
b
a
21
27
Temperature (^oC)

CHAPTER 7

CONCLUSIONS

The purpose of this research was to develop information on the minimal water content to be present in a peat (60%) and perlite (40%) substrate that would result in normal physiological and growth responses in bedding plants. The other objectives were to study water retention characteristics of the peat-perlite substrate, identify reliable and affordable moisture sensors for soilless substrates, and interface moisture sensors to a datalogger to build an automated irrigation controller which can irrigate the substrate to a desired level and maintain the desired water content for a prolonged period in the substrate. I approached these objectives by first identifying suitable moisture sensors for soilless substrates, studying water retention characteristics of the peat-perlite substrate, and building an automated watering system. Then I used the watering system to develop information on minimal substrate water content resulting in normal physiological and growth responses in bedding plants.

I calibrated ECH_2O and Theta probes for measuring water content in soilless substrates and studied the effect of substrate EC and temperature on measurement of both probes. This study has shown that under conditions of low substrate EC (< 1.0 $dS \cdot m^{-1}$), water content can be measured accurately using voltage responses of ECH_2O probe. However, this was not the case under conditions of high substrate EC. Increasing substrate EC increased ECH_2O probe output. The substrate EC had its greatest effect

on probe output up to 3.0 $dS \cdot m^{-1}$ and a relatively small effect at higher EC levels. However, considering their low cost (~ $60 each if > 11 probes are purchased) and the fact that high accuracy is not required for irrigation purposes, ECH_2O probes can be recommended for greenhouse use. Our study has shown that, using ECH_2O probes, there was an increase in the estimated water content by 0.0018 and 0.0026 $m^3 \cdot m^{-3} \cdot {}^oC^{-1}$ (from 23.2 to 24.2 ^oC) for a substrate at 0.12 and 0.34 $m^3 \cdot m^{-3}$, respectively. Temperature compensation can be used to improve the performance of ECH_2O probes for minimizing the effects of substrate temperature on probe output. The voltage output of the Theta probe was found to be not affected by changes in substrate EC, temperature, and composition. My study has shown that a single equation can be used to measure water content in 15 different substrates having EC from low to high in range. Hence, the Theta probe is preferred when accurate measurements of substrate water content are required and price is not a constraint.

My study on water retention characteristics of peat-perlite substrate has shown that the water content treatments selected in this research for studying physiological responses of bedding plants spanned a broad range of water potentials in the substrate. This research has also shown that most of the water retained in soilless substrate was held mainly in the two tension ranges i.e., 0 to to -0.01 MPa and below -0.4 MPa. There was little water in the substrate between these two tension ranges. This was also confirmed from pore fraction analysis. Whereas mesopores accounted for only 0.08 $cm^3 \cdot cm^{-3}$ of pore space, put together, micropores and ultra micropores comprised nearly 0.40 $cm^3 \cdot cm^{-3}$ of pore space. Most of the water retained in the tension range of 0 to to -0.01 MPa will be lost in drainage (gravitational water), which indicates that water retained

in the tension range below -0.4 MPa or in micro- and ultramicro pores is important for plant uptake.

The automated irrigation controller developed for this research has proved to be highly useful. This controller has shown potential for use in greenhouse irrigation. Using the watering system, it was possible to maintain distinct set points of water content in the substrate for a prolonged period. The validation study confirmed that the set point maintained by the controller was reliable. The performance of the controller was not affected by large variations in environmental conditions and plant size. We hope that this system can be used as a basis for future generation, automated irrigation controllers for greenhouses to achieve significant reductions in irrigation water wastage and labor costs. The irrigation controller also has potential use in studies related to plant water relations. As opposed to the dry–down or frequent weighing techniques for studying plant water relations, plant responses can be studied at distinct water contents using this system / controller.

Physiological responses of bedding plants to varying substrate water content were studied at both leaf and whole-plant (or group of plants) scales. Leaf responses like maximum photosynthetic rate, leaf water status (or potential), quantum efficiency of leaves was highest at a substrate water content of 0.22 or 0.32 $m^3 \cdot m^{-3}$ in all species. This is important because my research has shown that normal physiological responses can be seen in plants even when the substrate water content is well below the container capacity in soilless substrates (normally greenhouse plants are irrigated to container capacity). These findings were also confirmed from whole-plant responses, when the growth rate of a drought-tolerant (vinca) and drought-sensitive (salvia) species

experienced a moderate decline only at a substrate water content of 0.10 and 0.15 $m^3 \cdot m^{-3}$, respectively. To the best of our knowledge, the study conducted at the whole-plant scale was the first to use a quantitative approach to continuously monitor growth rate (or carbon gain per day) with decreasing substrate water content. My research has shown different physiological mechanisms in bedding plants in response to low substrate water content, i.e., maintenance of a lower mesophyll resistance to CO_2 transfer to maintain photosynthetic rate (e.g., petunia), a robust photosystem II (e.g., vinca, salvia, petunia), and / or lower osmotic potential to retain turgor and growth rate (e.g., vinca).

Before making final remarks, it is important to point out some possible topics for future research. In my view, these topics will broaden the information developed from the current research. These topics are outlined below:

(i) the irrigation controller with ECH$_2$O probes should be tested in a real world situation to determine its true potential for greenhouse irrigation. Water savings should be quantified by interfacing this controller with currently available automated irrigation systems like sprinkler–, drip–, and boom–irrigation.

(ii) Information should be developed on the number of ECH$_2$O moisture sensors required to effectively irrigate one acre of greenhouse space. A key objective is to strike a balance between economics of probe purchase and efficiency of irrigation in deciding the optimum number of moisture sensors. An experiment can be planned to study the effect of different number of sensors on measurement error. The average water content measured by probe(s) can be compared to the average water content of all containers irrigated by the probe(s). Error in measurement of water content can be used to

determine the optimal number of probes, however economics of probe purchase will largely depend on the individual grower.

(iii) the irrigation system maintains a particular water content in the substrate, thereby allowing physiological processes in plants to acclimate to a particular water content. This may have a positive effect when the plants are transplanted in the landscape. The positive effect can be exemplified from the information obtained on petunia or vinca from this study. As these species are capable of withstanding low substrate water content (~0.15 $m^3 \cdot m^{-3}$), it would be interesting to study the interaction between temperature and water content on physiological responses of species. It has been shown that plants grown at low substrate water content can synthesize osmoprotectants (glycine betaine, trehalose, fructans etc.,) that could potentially help plants when exposed to supra- or suboptimal temperatures. For example, the critical temperature for decline in quantum efficiency was shown to be higher for a drought stressed plant than unstressed plant. A hypothesis that could be tested is that petunia and vinca grown at low and constant substrate water content could better withstand high temperature after the production phase without subsequent hardening compared to those grown using current irrigation practices. If this hypothesis is true, it would indicate that these species can be hardened while in production by growing them at low and constant substrate water content, and no additional hardening will be required after production and before transplanting.