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UNROOTED STEM CUTTING PHYSIOLOGY; WATER USE AND LEAF GAS EXCHANGE OF SEVERED STEM CUTTINGS

Peter Alem
Clemson University, PALEM@CLEMSON.EDU

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Alem O. Peter
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Dr. Jim Faust, Committee Chair
Dr. Christina Wells
Dr. Dara Park
Dr. Patrick Gerard

ABSTRACT

Stem cuttings are one of the most frequently used and successful methods of vegetative plant propagation. The understanding of unrooted cutting physiology, especially gas exchange and water flux, is crucial for successful propagation of healthy plants. Prior to root initiation, water uptake is limited and leafy stem cuttings are most vulnerable to wilting.

Experiments were carried out in the greenhouse with poinsettia cuttings to determine water uptake, photosynthesis, transpiration and stomatal conductance from the time of severing to rooting, including time spent in storage. Water uptake through the severed stem was investigated in the laboratory and growth chamber. Cutting gas exchange (i.e., photosynthesis, stomatal conductance and transpiration) sharply declined after severing and gradually recovered in propagation after root initiation. CIRAS-2, with optional integrated Chlorophyll Fluorescence Module (CFM) was used to assess chlorophyll fluorescence and photosynthesis of cuttings at different stages: freshly severed cuttings (Day 1), 3 day-old cuttings, callused cuttings (Day 7 and 10), and rooted cuttings (Day 21 and 28). There was no significant difference in chlorophyll fluorescence between the cuttings and stock plants hence we inferred no significant damage to photosynthetic reaction centers as a result of severing. Unrooted cuttings had relatively low photosynthetic rates compared to rooted cuttings with water as a possible limiting factor. Our data also suggest that water use efficiency increases during the first week in propagation prior to root initiation. The mechanism for this improved water use appears to be due to a return in normal stomatal function during the first week in propagation

following severing the cutting from the stock plant. The results have implications for the management of mist in propagation environment.

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I would like to dedicate this work to my departed mother and father Akumu and Alem Limbe.

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CHAPTER ONE

LITRATURE REVIEW

Vegetative multiplication of plants is both a natural and an artificial process (Leakey, 1985). In nature, many plants by dividing through asexual structures to produce new plantlets for the next generation; they include corms, bulbils, runners and rhizomes, tuberous and bulbs (Bengtsson and Ceplitis, 2000). Artificial vegetative propagation of plants has been used for a long time and is still used by agriculturalists, horticulturalists, botanists and foresters, to propagate individual genotypes with preferred qualities such as color, compactness and quick maturity (Mudge and Brennan, 1999). The technique has also played an important role in conservation of rare and endangered species. Currently, it is widely applied to produce high demand plants like poinsettias and Christmas trees during the month of December (Lopez, 2008). Vegetative propagation of plants from stem cuttings has been used extensively in the horticulture industry for both herbaceous and woody species (Preece, 2003).

One of the challenges of producing plants from stem cuttings has been wilting and death of cuttings especially before they develop roots. Of the many causes of stem cutting wilting in propagation, one possible reason is failure to take up sufficient water through the stem or leaves (Loach and Whalley, 1978). Plants need to move water from roots to shoots to leaves and limit the amount of water they lose to the environment through transpiration to maintain favorable water potential in their tissues.

Water uptake by plants and plant parts is an important physiological process whose functions are key to plant growth and development. Plants need water for

translocation of nutrients, photosynthesis, cell turgidity and growth. Water deficiency is a serious environmental stress and the major constraint on plant productivity. It affects morphological, physiological, and molecular processes of plants such as delayed flowering, reduced dry matter accumulation and partitioning, and decreased photosynthetic capacity as a result of stomatal closure, metabolic limitations, and oxidative damage to chloroplasts (Muhammad et al., 2009).

Water potential

Water potential is comprised of three components: osmotic, gravity, matrix and pressure potentials. Water potential is measured in megapascals (MPa); reflects the energy level of water in the soil and plant tissues (Brodribb and Hill, 2000).

Pressure potential can be zero, negative or positive. Positive pressure occurs when a plant cell is fully hydrated (turgid). Negative pressure potential occurs in the xylem, wilted or water stressed living plant cells have a pressure potential near zero. Pressure potential enables water flow from soil to a plant and within plant tissues (Steudle and Peterson, 1998)

Osmotic potential is a consequence of reduction in energy of the water due to dissolved solute relative to that of pure water (Campbell, 1998). Leaf osmotic potential decreases as solutes accumulate in the tissues or when there is reduction in cell size due to environmental stress. Reduction in cell volume reduces the amount of water but not salts, hence the salt load per given volume of cytoplasm rises. This decrease in osmotic potential can correspond to decreases in total plant water potential, consequently maintaining favorable turgor pressures within the cells during drought (Cutler et al.,

1977, Osonubi and Davies, 1978). Water flow from soil to a plant and within plant tissues are driven by pressure and osmotic potential gradients. An osmotic flow occurs only in the presence of membranes and is important in hydraulic flows and water movement across cell membranes (Steudle and Peterson, 1998). Water flow across the cells (protoplast) is both due to water pressure and osmotic within the plant tissues gradient.

Another contributor of water potential is gravity; gravitational force causes water molecules to have energy (Bilskie, 2001). Energy due to gravitational force of the earth is referred to as gravitational potential. The influence of gravitational potential can be observed in cases where adhesive forces between water and soil are weaker than the gravitational forces acting on the water molecule and water moves downward. When it rains or when the soil is irrigated, water tends to flow downward due to gravity, from a region of more positive potential to less positive potential, until the force of gravity is balanced by that of capillarity.

The matric component of water potential represents the adsorptive and surface tension effects associated with solid surfaces of soil and plant tissues. Matric potential is as a result of the adhesion of soil solid particles with water, and cohesion of water molecules with each other each other (Campbell, 1998).

Pure water has a water potential of zero. The addition of solutes to water lowers its potential while increasing pressure increases water potential. Water moves from area of high (less negative) to low water potential. When the soil/media is dry (i.e., more negative water potential), the movement of water through the roots to the shoots of a plant is reduced (Gallego et al., 1994). If plant tissues have water potential more negative than the media onto which they are growing, water is pulled through the xylem to hydrate

the cells (Gerrit et al., 2009). Water potential in the soil is not stable, varies with time and space depending on prevailing weather conditions and soil attributes like texture, drainage, organic matter and structure. Plant water potential is affected by external abiotic and biotic factors. For instance, changes in stomatal conductance causes alterations in leaf water potential by its effect on transpiration rate (Farquhar and Sharkey 1982).

Stomatal behavior and functioning

Stomata are small pores on the surfaces of leaves and stems, bounded by a pair of guard cells that control the exchange of gases (most importantly water vapor and carbon dioxide) between the interior of the leaf and the atmosphere (Alistair et al., 2003), both water vapor and carbon dioxide diffuse in and out of the leaf tissues following a gradient. In this capacity, stomata play a major role in the ability of plants to control their water relations and to gain carbon. There is usually a trade-off between the need for plants to let in carbon dioxide needed for photosynthesis and control of water loss**.** The same stomata openings are used for letting in carbon dioxide and at the same time water vapor escapes from them. This balance is particularly critical in dry, hot and high-light conditions.

Stomatal distribution

In most plants, stomata can be found on both the upper and lower leaf surfaces with the majority of stomata found on the lower surface (Tichà, 1982). In some species (mostly woody trees), stomata are found only on the lower surface, while some aquatic

plants whose lower leaf surfaces are in contact with water surface have stomata only on the upper surface (Morison, 2003).

Stomatal movement is controlled by guard cells positioned on either sides of the stoma. Guard cells movement is very sensitive to external and internal factors, among which light is the most significant factor (Leng et al., 1998).

Factors responsible for opening and closing of stomata

Stomatal behavior is one of the most complex processes in plants. Numerous internal and external factors are known to influence stomatal aperture: light (Sharkey and Ogawa, 1987), CO_2 concentrations in the surrounding air and within the leaf (Morison, 1987), air humidity, soil drought (Schulze et al., 1987) and plant hormones like, abscisic acid (Raschke, 1987) and auxins (Davies and Mansfield, 1987). Abscisic acid (ABA)-induced stomatal closure is driven by a decrease in guard cell turgor pressure due to removal of K^+ and associated anions from the cytoplasm triggered by an increase in cytoplasmic Ca^{2+} concentrations (Ward and Schroeder, 1994).

Leaf stomatal conductance (g_s) can be defined as the maximum flow of water vapor or carbon dioxide across the stomatal pores. Variations in stomata aperture are under the control of a negative feedback associated with leaf water status (Barrs, 1971; Farquhar and Cowan, 1974). Stomatal movements respond to both the atmospheric and rhizospheric environments. The atmospheric environment comprises external variables like temperature, humidity, carbon dioxide, light and wind that directly impact the leaf or its boundary layer. The root environment includes factors that have an effect on root water status, hence has an influence on both xylem water potential and the production of

root-sourced chemical signals, such abscisic acid (ABA) (Gutschick and Simonneau, 2002).

Studies have been conducted in the past to illustrate the effects of light exposure on stomatal movement (Leng et al., 1998). The results from microelectrode and patch-clamping techniques used to measure a change in cell membrane voltage demonstrate that the main physiological effect of light is that to make guard cells' plasma membrane potential more negative. This hyperpolarized membrane potential activates voltage-gated K^+ channels and allows K^+ to enter the guard cells, altering the osmotic potential of the cytoplasm (Schroeder, 2003). Then, following the osmotic pressure gradient between guard and adjacent cells, water moves into the guard cells causing them to expand and open the stomata.

The increase in volume of both guard cells on the opposite sides of each stoma forces them to open. Due to the radial arrangement of cellulose fibrils in the cell wall, the expansion of kidney-shaped guard cells occurs mainly along the longitudinal axis hence creating tension between them and subsequent bending (Sharpe et al., 1987; Shope et al., 2003). The cross section of guard cells also changes from a flattened oval to a circular shape (Von, 1856), this opens the stomata pores.

On the other hand, darkness depolarizes the guard cell membrane and K^+ exits from the guard cells, reducing their turgor and closing the and stomata.

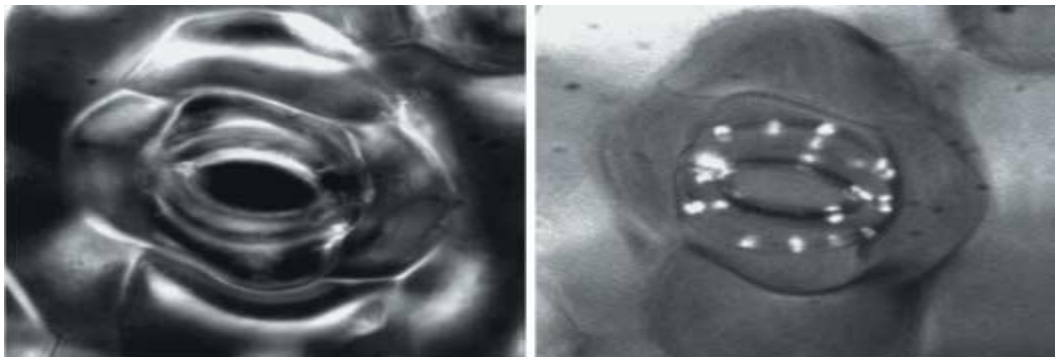

Figure 1.1 Reflected images of stomata from intact leaves of *Commelina communis* and steady-state fluorescence imaging (Lawson, 2008).

Stomatal closure occurs more rapidly than stomatal opening. The transpiration rate which is mainly a function of stomatal movement, increases with a velocity of 0.7 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the light as a result of stomatal opening, it decreases with a velocity of 1.9 $\mu mol\ m^{-2}\ s^{-1}$ after the application of ABA (Langer et al., 2004) a plant hormone which closes stomata. Stomatal opening is based on active transport which involves use of energy, while closure is based on the release of solutes along their concentration gradients which passive transport. Stomatal closure, therefore, may be dependent entirely on the activation of ion channels in the vacuolar and plasma membranes. K^+ ions will flow from the vacuole to the cytoplasm following a concentration gradient when its channels are activated in the vacuolar membrane (Roelfsema and Hedrich, 2005).

Stomata and transpiration

Stomata function is the continuous link between the plant and the atmospheric environment. Water movement along the soil-plant atmosphere continuum moves along a gradient; into the roots, a water potential difference between soil and roots cells drives transport across the membrane (Sperry et al., 2003). Negative hydrostatic pressure in the

xylem moves water from the roots to leaves. And finally, from leaves to the atmosphere, it is the vapour pressure gradient, which ultimately drives the whole process.

Models used to simulate water loss in plants through transpiration take into account stomatal conductance as the main outlet through which water is lost. The Penman Monteith equation commonly used to evaluate transpiration rates (Burman, 2003; Allen et al., 1998) and evaporation of water from the soil surface, combines the supply of energy form the sun and transport of water vapor from the canopy and effect of plant physiology is taken into account by introducing a stomatal conductance as factor regulating water loss from the leaves.

Factors affecting transpiration

The rate of water loss through transpiration from a leaf is determined by a driving force and a resistance of the pathway (Gates, 1968). Transpiration is the rate of evaporation of water into the atmosphere from given area of leaves and/or stems of plants per given time (i.e., the amount of water molecules lost from plants within given time per given area of a plant surface). The driving force is the difference in water vapor pressure within the sub-stomatal cavity; a hollow space immediately proximal to the stomatal aperture connected with intercellular air spaces that allow quick movement of carbon dioxide and water vapor found between mesophyll cells (i.e., loosely packed photosynthetic cells); and of the free air beyond the surrounding boundary layer (i.e., a zone of unstirred air) next to the leaf surface. The water vapor must diffuse following a gradient from the sub-stomatal cavity through the stomatal conduit and then through the boundary layer into the free air where the moisture is carried away by air movement

(Gates, 1968). This continues so long as the air above the boundary layer is not saturated with water vapor. In many cases, the relative humidity of that air is less than that of the sub-stomatal cavity, so at any given time we expect some extent of transpiration from a healthy leaf. The total resistance, of the diffusion pathway is given by an internal resistance, which is equal to the sum of sub-stomatal plus stomatal resistances and an external resistance, in the still air layer also known as the boundary layer around the leaf (Nobel and Jordan. 1983). Resistance to transpiration occurs due to physical factors like leaf geometry, stomatal aperture and environmental factors such as humidity, boundary layer conductance and temperature. It is crucial in controlling water loss from plants.

Transpiration is unavoidable consequence of photosynthesis; water is lost when stomata open for CO_2 uptake. Additionally, transpiration has been ascribed the functions of cooling leaves, driving root to shoot xylem transport and mass flow of nutrients through the soil to the rhizosphere and up to the shoot.

Light

Light affects transpiration indirectly by altering stomatal conductance. Transient response of stomatal conductance and photosynthetic rate under fluctuating light environment have been studied in the past (e.g. Chazdon, 1988; Knapp and Smith, 1990; Pearcy et al., 1990; Pearcy et al., 1994 and Tang, 1997). Even though the actual response is very complicated and many parts of the mechanism are still unknown (Jones, 1998), it is assumed that transpiration respond to changes in light intensity and specifically photosynthetic photon flux density (Ogata, 1998).

Stomatal opening is stimulated by light, including blue and red light wavelengths, and distinct mechanisms underlay stomatal opening in response to these different

wavelengths. Relative to blue light, red light has been found to stimulate photosynthesis but hinder stomatal conductance at nearly all photon irradiances (Zeiger and Field, 1982). Blue light is also responsible for opening of stomata at low light levels and may also explain why stomata opening precede transpiration and photosynthesis in the morning hours when blue light is most prevalent

Temperature

An increase in air temperature increases transpiration rate and leaf temperature for all values of internal diffusion resistance. The lower the internal resistance of the leaf to water diffusion, the more rapidly transpiration increases with air temperature. The capacity of the air surrounding the leaf and the boundary layer to accept more water vapor before saturation point is reached rises with an increase in temperature (Martin et al., 1999). Large size leaves experience massive changes of transpiration rate and leaf temperatures with variations of the amount of absorbed solar radiation (Gates, 1968). The variation is also caused by difference in leaf orientation, leaf architecture and color. The temperature difference between the leaf and the air is related to the magnitude of heat exchange by convection; heat transfer through a gas or liquid by circulation of currents from one region to another, thus, convection may have an influence on the rates of the metabolic processes occurring within the leaf. Leaf resistance to water loss is decreased with increasing air temperature (Ku et al., 1977).

Humidity

Stomatal closure is induced by a high air to leaf vapor pressure deficit and low leaf water potential (Mott and Parkhurst, 1991). In most plants, stomata close as the concentration of water vapor in the atmosphere declines while leaf temperature remains constant. This reduces the amount of water lost at low humidities, and in some species, stomatal closure at low humidities is sufficient to reduce transpiration rates below those observed at higher humidities (Sheriff, 1977). Most experimental studies and mathematical models that investigate stomatal responses to humidity use the difference between the mole concentration of water vapor in the air inside the leaf and the ambient air (VPD) as the independent variable. Vapor pressure refers to the amount of water molecules in a given volume of air at a given temperature. This reflects the dependence of transpiration on vapor pressure of ambient air (Brown and Jones, 1999). Vapor pressure deficit is the driving force of water loss through transpiration in plants. Ball and Berry (1991) have shown that stomatal responses to humidity can be predicted from the relative humidity of the air adjacent to the guard cells, i.e., beneath the boundary layer.

Soil water stress/ water potential

At low soil water potential the stomata in the upper leaves close early in the morning as the water potential in the leaf decreases and the bulk leaf turgor approaches a zero potential (Turner, 1974). Past studies have demonstrated that the critical leaf or soil water potential for stomatal closure and reduction in transpiration is not unique for any given species, cultivar; varies with stage of development, growth conditions, position in

the canopy, and previous stress experiences (Turner, 1975). Also, the development of plant water stress is influenced by aerial environment as well as the soil water status.

Stomatal closure is the first line of defense against desiccation since it is much quicker than changes that take place in the roots. Drought-tolerant species control stomatal function to conserve water but also allow some carbon fixation at stress, thus improving water use efficiency (i.e., the ratio of carbon fixed through photosynthesis to water lost the through transpiration) (Bucci et al., 2008). Some species open stomata rapidly when water deficit is relieved to fix carbon and replenish diminishing stored food resources. Stomatal closure usually occurs before inhibition of photosynthesis and restricts carbon dioxide availability for photosynthesis (Yordanov and Tsonev, 2000).

Water availability of rooting media

Propagation medium for the rooting of leafy cuttings should provide sufficient water and well aeration to the cuttings (Hartmann et al., 1990). Media of relatively high water content, such as sawdust, are associated with higher rates of water uptake (Loach, 1986) and consequently higher rooting percentages. However, water can present a major diffusion barrier to oxygen, and excess water may thereby result in anoxia within the cutting base (Loach, 1986). Reduced water absorption through the cutting base resulting from tissue death may explain reduced stomatal conductance and transpiration of cuttings propagated in sawdust (Grange and Loach, 1983) and other media with higher water holding capacities.

Relationship between photosynthesis and stomatal conductance

According to Tay et al., (2007), irrespective of the difference in mole water content between the air inside and the air outside the leaf (VPD), the relationship between photosynthesis and stomatal conductance is curvilinear for all measurement of different days and weather conditions. The relationship between these two parameters is influenced neither by vapor pressure deficit nor by the time of the day. Midday reduction in photosynthesis is a common occurrence in many plants subjected to high light stress and/or high temperature (Matos et al., 1998). The factors that either uniquely or simultaneously causes this phenomenon are stomatal closure and biochemical limitations (Niinemets et al., 2009).

Stomatal movements can change both the partial pressure of carbon dioxide at the sites of carboxylation and the rate of transpiration. The changes in transpiration can cause changes in the temperature and water potential of the leaf (Farquhar and Sharkey, 1982). Assimilation of carbon responds to changes in the partial pressure of carbon dioxide, leaf temperature, and transpiration rate. Steady photosynthetic rates are usually tightly coupled to stable stomatal conductance over a time period (Wong et al., 1979). However this relationship has an optimum limit beyond which further increase in stomatal conductance would not increase photosynthesis but will actually cause a reduction in carbon assimilation; Lu et al., (1998) observed a positive relationship between photosynthetic rate and stomatal conductance in the low range values, up to about 0.4 mol m^{-2} s^{-1}, but no apparent relation at higher conductance values.

Water movement in stem severed stems

Impaired water uptake in cuttings is normally associated with external stress indicators like flower or vegetative wilting, leaf curling and color change (Williamson and Milburn, 1995). The cause of water transport impairment in cut plant parts has been blamed on various factors like stomatal closure but notably stem occlusion by microorganism (e.g. Rasmussen and Carpenter, 1974; Put and Jansen, 1989) and other vessel plugging materials like resin and latex produced from wounds of some plant species (Burdett, 1970; Parups and Molnar, 1972; Lineberger and Steponkus, 1976). The idea of micro-organism stem occlusion is said to have some loop holes; measurable decline in water translocation is normally reported long before occlusion actually happens when a stem is cut from the mother plant (Durkin and Kuc, 1966; Mayak et al., 1974). In 1966, Durkin and Kuc (1966) reported that reduction in petal turgidity and fresh weight occurred after a decrease in water uptake rate and later Durkin (1979) proposed that xylem embolisms inhibited water uptake by cut stems, however, he remarked that xylem embolised channel would be difficult to recognize.

In the case of stem cuttings, availability of water is an important factor for activating root development, and cuttings have to maintain a favorable water status during root development otherwise they wilt (Loach, 1988). Rooting just like any other phenomenon of growth related physiological process will not progress adequately without sufficient water in the plant tissues to keep the cells turgid. Water status of cuttings is not the same as an intact plant. Cuttings lack root system to actively absorb water from a substrate, yet water loss continues through the leaves as environmental

factors influencing water loss like temperature remain constant. Cuttings must absorb water from the stem base and/ or leaves to prevent wilting.

There is limited knowledge about the maintenance and control of cuttings water potential during critical stages of initial root and leaf development (Sunil and Thompson, 2003). The present study was undertaken to examine the water relations and stomatal conductance of stem cuttings before rooting and after under controlled environmental conditions.

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CHAPTER TWO

WATER FLUX OF POINSETTIA CUTTINGS IN PROPAGATION

Introduction

The process of severing cuttings from stock plants results in the disruption of the leaf water status of the cutting and causes the water pressure potential of the severed shoot to decline (Gay and Loach, 1977; Smalley et al., 1991; Svenson et al., 1995). Following cutting removal water retreats from the wound and in the process a vacuum can be created within the vascular tissue which can result in an air bubble forming in the stem (Ieperen et al., 2002). The xylem also constricts as the hydrostatic pressure against the cell walls is reduced and can potentially collapse in severe cases, especially in herbaceous plants. This can happen when cuttings are kept warm after severing from the mother plant and extreme water deficit occurs (Gay and Loach, 1977). Keeping the cuttings alive after severing is largely dependent on the ability of the cutting to continue taking up water and to maintain a favorable water status, otherwise the cutting will wilt and die. To achieve this, the stem of the cutting is placed into a well-irrigated growing media under intermittent mist. The rate of water uptake from the growing media influences how quickly cuttings recover from severing. Stomatal conductance and photosynthesis decline following severing and then slowly recover as a favorable leaf water balance and turgidity is restored after root development (Svenson et al., 1995; Hoad and Leaky, 1996). The rate of recovery is a function how efficiently the cuttings are able to take up water and minimize water loss. Water loss through transpiration can be

minimized by providing a relatively low water vapor pressure deficit between the surrounding air and the leaves.

At the time of severing stem cuttings from the mother plant, stem sections exhibit a normal stem anatomy of xylem and phloem as an intact stem. The initial visible change on the stem during propagation is the formation of callus tissue that results from cell division occurring in the cortex tissue (Murthy et al., 2009). Within the callus tissue individual tracheid cells differentiate to form functional vascular vessels. The tracheid cells expand and eventually became continuous with the existing vascular tissue of the original cutting after which root primordia can be observed and then roots emerge (Anthony et al., 2004).

Poinsettia (*Euphorbia pulcherima*) normally begins to form callus tissue around the basal stem after one week in propagation. Callus formation is largely dependent on stem temperature, aeration of the growing media and moisture availability (Lopez, 2008). Root initiation starts after 10-14 days in propagation. After 17-21 days in propagation, poinsettia cuttings normally have a fully developed root system and are typically ready for transplant after 28 days.

Empirical observations of poinsettia wilting in propagation suggest that water demand by cuttings is reduced during the first week in propagation although there are no visual differences in stem anatomy during this time. Therefore, we hypothesized that this phenomenon could be caused by low initial cutting water content at the time of sticking the cutting in propagation and/ or an increase in the rate of water uptake through the stem or increased water use efficiency due to a change in stomatal functioning during the first seven days in propagation. Thus, the objectives of these experiments were: 1. to

investigate the effect of cutting age in propagation and PPF on water loss prior to wilting 2. to determine the effect the initial water status of poinsettia cuttings at the time of sticking in propagation has on cutting water uptake, 3. to quantify water uptake of poinsettia cuttings through the stem, 4. to quantify the rate of water loss through transpiration in propagation prior to root formation.

Materials and methods

Stem water uptake of un-rooted stem cuttings

Poinsettia (*Euphorbia pulcherima* 'Peterstar Red') stock plants were grown in 18 cm diameter plastic pots for one year in a peat-based growing medium (Fafard 3B, Fafard Inc., Anderson, SC). The stock plants were grown in a greenhouse (67-75% R.H, temperature 26-30°C, maximum PPF 1400 µmol $m^{-2}s^{-1}$) in Clemson, SC. Irrigation was done manually, and a water-soluble fertilizer (250 ppm N, 15-5-15 4Ca 2Mg; Scott's International) was applied with each irrigation.

Expt.1. Effect of cutting age in propagation and PPF on water loss prior to wilting

Ten poinsettia cuttings were harvested every other day over seven consecutive days, immediately stuck in a propagation medium (Oasis Wedges, Smithers-Oasis, Kent, OH) and placed under mist in a greenhouse environment (25-30 ºC, 75-85% R.H, maximum daily PPF 250-600 µmol $m^{-2}s^{-1}$). The cuttings were randomly harvested and assigned to the different days in propgattion. On the seventh day the cuttings had been in the propagation environment for 1, 3, 5, or 7 days. The cuttings were then removed from the propagation media, inserted into dry waterpiks (empty, 12 ml transparent plastic tubes

covered with rubber lids containing 2 mm diameter holes) and held upright. Then the cuttings were moved to a growth chamber (48% R.H, 26 ºC) under 0, 275, or 850 µmol $m^{-2}s^{-1}$ delivered by 500W metal halide lamps. Cutting fresh mass was initially measured when the cuttings were placed in the waterpiks and again after wilting. The time to wilting (curling and/or drooping of $\geq$ 2 fully expanded leaves) was recorded. The experiment was repeated four times. The number of times the experiment was repeated formed blocks for our data analysis hence we ended up with a split plot in randomized complete block design when 3 different levels of light was included.

Expt.2. Effect of cutting water status on water uptake through the stem (laboratory study)

Thirty poinsettia cuttings (Peterstar Red) were harvested from stock plants and taken to the laboratory (25 °C, RH 42%, PPF 10 µmol $m^{-2}s^{-1}$). The cuttings were the randomly assigned to one of each of the following treatments 0, 30, or 60 min. 150 µmol $m^{-2}s^{-1}$ PPF electric lamp hanged at 0.5 m above the cutting. This was done to manipulate the initial water status of the cuttings i.e., 100%, 85% and 75% respectively. Preliminary trials indicated that the cuttings wilted completely after 60 min under lamp; all expanded leaves curled and dropping. Ten cuttings were used for each treatment (duration of exposure to the lamp). The cuttings were then stuck into waterpiks filled with 8 ml of water. Rate of water uptake was determined by briefly removing the cutting from the waterpik and then individually recording the mass of the waterpiks and the cuttings after 2, 4, 6, and 8 h. cumulative water uptake was also determined after 24 h. This experiment was repeated 4 times and data analyzed as randomized complete block design. A similar experiment was also done in the greenhouse under mist and no mist conditions.

Expt.3. Rate of water uptake through the stem during the first week in propagation in the light and dark (Greenhouse study)

Ten cuttings were harvested each day at 10 a.m. from stock plants for seven consecutive days, stuck in Oasis wedges and placed in a greenhouse (23-30 ºC, 75-85% R.H, PPF 250-600 µmol $m^{-2}s^{-1}$) under a VPD-controlled mist system (Argus Control Systems Ltd., British Columbia, Canada). On the seventh day, the seven groups of ten cuttings were labeled by the number of days the cuttings had been on the propagation bench, e.g., Day 1, 2...7. Then the cuttings were removed from mist and transferred into waterpiks filled with 8 ml of water. The waterpiks and the cuttings were then taken in a growth chamber (48% R.H, 26 ºC) where two PPF treatments (0 or 375 µmol $m^{-2}s^{-1}$) were delivered from metal halide lamps (5000 W bulbs) positioned 0.9 m above the cuttings. The water uptake measurements were taken at 2, 4, 6, 8, and 24 h after the cuttings were moved to the growth chamber. This experiment was repeated four times and analyzed as split plot; light as the main plot and age of cutting as the sub-plot.

Statistical Analysis

Data were subjected two-way analyses of variance, split plot in RCB (Expt. 1 & 3), RCB (Expt. 2), (Proc GLM), and means were compared with Fisher's protected least significant difference (LSD) at $P = 0.05$; SAS software (Version 9.1; SAS Institute, Cary, NC, USA) was used**.**

Results and Discussion

In Expt.1, Day 1, 3, 5, and 7 cuttings were removed from the propagation media and left to wilt under three different PPF. Cuttings lost approximately 0.27 ml/cutting of water or 4% of total cutting mass when wilting occurred, regardless of PPF and cutting age (Fig. 2.1). However, PPF and cutting age significantly affected the length of time before wilting occurred (Fig. 2.2). Day 7 cuttings took the longest time to wilt both in darkness and under light while Day 1 cuttings took the shortest time. The mean time to wilt in dark increased from 72 to 322 minutes as the age of cutting increased from 1 to 7 days in propagation. Time to wilt also decreased as PPF increased, e.g., Day 1 cuttings wilted in 72 min. in the dark and 26 min. at 850 μmol $m^{-2}s^{-1}$. This experiment demonstrates the phenomenon that cuttings wilt less quickly as time passes throughout the first week in propagation.

In Expt. 2 a. cutting water stress was simulated by placing the cuttings in front of an incandescent lamp for 0, 30 or 60 min.. Wilting was observed on the cuttings at approximately 60-minute exposure time. The mass of the cuttings following the 0, 30 and 60 min. exposure was 100, 85, and 75% of the initial mass, respectively. The results showed that the lower the initial mass of the cuttings, the higher the cumulative water uptake during the first 24 h in propagation (Fig. 2.3). The rate of water uptake was greatest in first 2 h in propagation and then declined over the following 8 h (Fig. 2.4). The results suggest that a low initial water content that might result from desiccation during the postharvest environment does not hinder water uptake through the stem and, in fact, increases water uptake over the first 24 h (Fig. 2.3).

Expt. 2 b. showed that mist does not alter the effect of initial water status on water uptake by cuttings in the first 24 h in propagation. The results suggested a significant difference ($p<0.0001$, Table 2.1) in the rate and cumulative water taken up by cuttings of different initial water status prior to propagation. The initial water status of cuttings and mist conditions (mist or no mist) after sticking influenced the rate and amount of water taken up of the cuttings (Table 2.1). However, there was no interaction between the conditions of propagation (Mist or No mist) with the initial water stress ($p<0.429$); regardless of the conditions after sticking i.e. under mist or no mist. Cutting water status significantly ($p<0.0001$) and consistently influence rate and amount of water uptake through the stem. Irrespective of mist conditions driest cuttings (75% of their original weight at harvesting) took more water than less dry cuttings (i.e., 85 & 100% weight at harvesting). Rate of water uptake under mist had the similar trend as in the laboratory and under no mist in the greenhouse. However, the rate of water take was lower as compared to the two locations (Fig. 2.5).

Expt. 3 demonstrated no significant ($p<0.889$) difference in water uptake among different aged cuttings under light (375 µmol $m^{-2}s^{-1}$): the rate of water uptake was approximately 1.4 ml/cutting over the first 24 h in propagation (Fig. 2.6). However, in the dark the rate of water uptake decreased as cutting age increased, e.g., water uptake decreased from 1.2 ml/cutting on Day 1 to 0.8 ml/cutting on Day 7 (Fig. 2.6). These data suggest that stomata do not close normally in the dark on Day 1 but stomatal regulation of water loss does improve over the first week in propagation.

Empirical observations of cuttings in propagation by commercial growers suggest that water use by cuttings is reduced during the first week in propagation despite no

visible root initiation. Our experiments clearly demonstrate that this is, in fact, an accurate observation. This was illustrated in our experiment where Day 7 cuttings in propagation took longer time to wilt than Day 1 when removed from the misting bench and let to wilt under different light levels in the growth chamber (Fig. 2.2).

One possible explanation can be that Day 7 cuttings lose more water before wilting than Day 1 cuttings, however this we found not to be true, since all unrooted cuttings lost equal amount of water before wilting both in light and dark (Figure 2.1). Another possibility could be that the initial water status is low on Day 1 and this inhibits water uptake so younger cuttings are closer to the wilting point. However, our data clearly show that cuttings with low initial water content, i.e., wilting, actually have higher rates of water uptake through the stem (Fig. 2.3 & 2.4). The difference in wilting could also occur if older cuttings (Day 7) can take up more water than young cuttings (Day 1); however, we found the reverse to be true (Fig. 2.6). From our results, we think that reduced water use by cutting during the first week in propagation is due to improved stomatal regulation of water loss. It appears that stomatal regulation declines as a result of severing cuttings from stock plants. This may be due to decreased synthesis of ABA due to lack of roots. During the first week in propagation, stomatal regulation is improved so there is less water loss during darkness or during periods of water stress. Thus, as cuttings age in propagation they are less likely to wilt. These results have implications for mist control systems which attempt to compensate for and reduce water loss through the application of water in the form of mist to the cuttings in propagation.

Conclusion

There is no significant difference in stem water uptake under light in the first week in propagation. Water use of unrooted cuttings decreases with time in propagation and the rate of water uptake does not increase before root initiation. Therefore it appears that stomatal functioning of unrooted cutting changes in propagation. Callused cuttings are able to respond to stress and close their stomata tighter to limit water loss. Our data suggest that young cuttings (1-3 days in propagation) and drought-stressed cuttings do not wilt more quickly due to limited water uptake. In fact they take up more water and at a faster rate than older cuttings and non-drought stressed cuttings therefore, our data suggest that reduced mist requirements during the first week in prop is not due to an increase in rate of water uptake as cuttings sit in prop but rather an improvement in water regulation and water use efficiency which imply that stomatal functioning changes, i.e., there is less water loss per given time rather than increased water uptake.

Table 2.1 Effect tests for initial water content, mist conditions (mist or no mist) and interaction (Expt. 2b). df; degree of freedom, prob>F; level of significance. Significance value (Alpha; $p<0.05$).

Source	df	Sum of squares	F.ratio	Prob.>F
Cuttings water status	2	6.30330	9.9977	<0.0001
Mist/No mist	1	2.9880	9.4786	<0.0023
Water status* mist/no mist	2	0.53515	0.8488	<0.4290

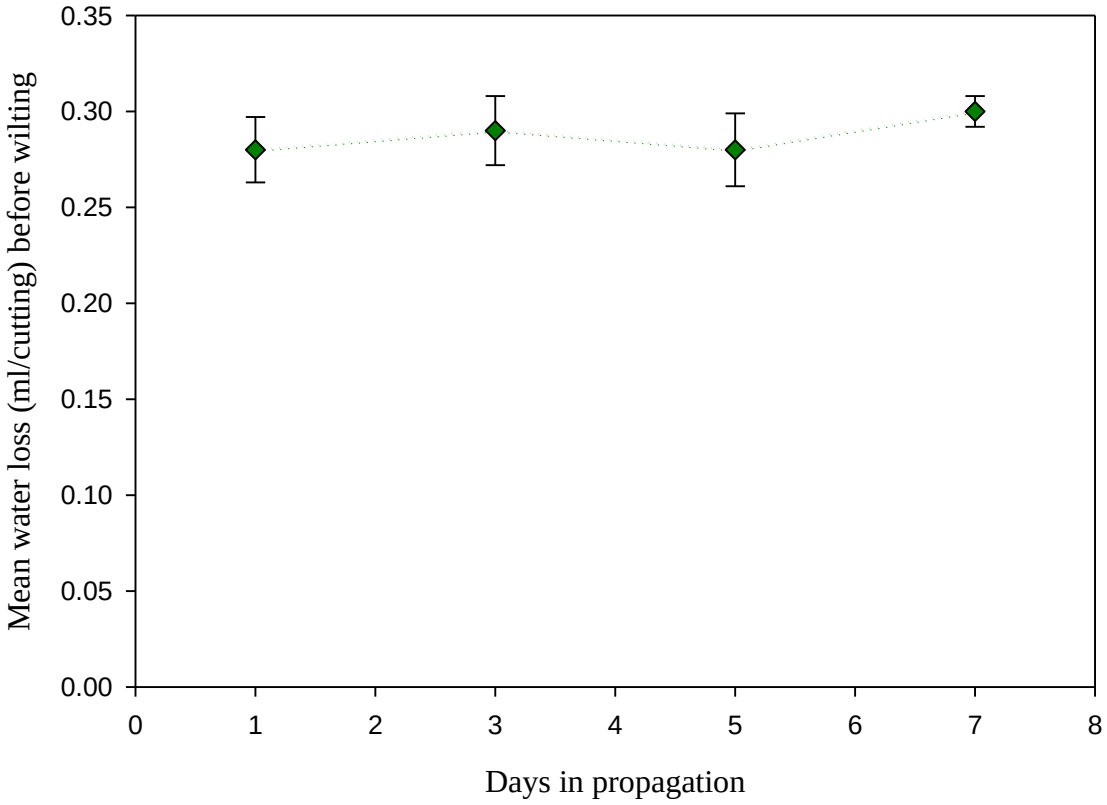

Figure 2.1 Mean water loss during drought stress before wilting of unrooted cuttings (Expt. 1). Ten cuttings each of 1, 3, 5 & 7 days old in propagation were removed from propagation media and left to wilt in the dark and under light (275 & 850 µmol $m^{-2}s^{-1}$) in a growth chamber. Cutting time in propagation and PPF did not significantly affect cutting water loss at wilting ($P \leq 0.78$), ANOVA (α=0.05). Error bars indicate mean±SE.

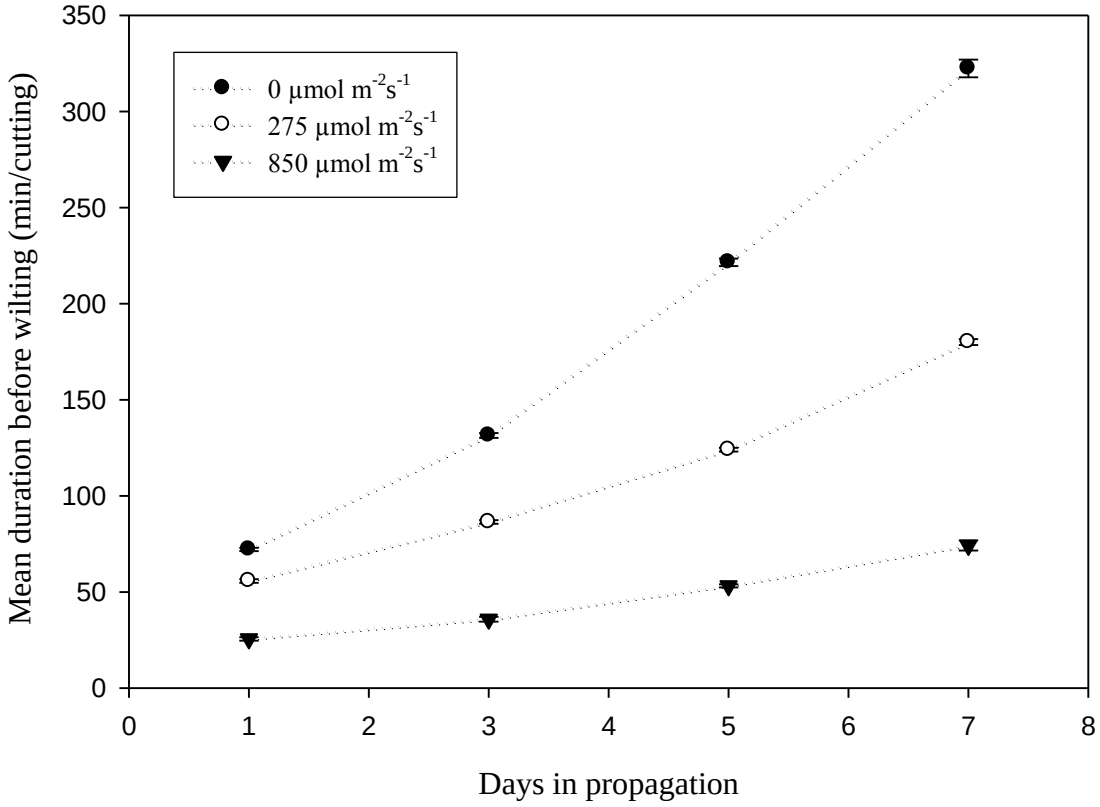

Figure 2.2 Mean time (min.) before wilting of unrooted cutting of 1, 3, 5 or 7 days old in propagation in the dark and in light (275 & 850 µmol $m^{-2}s^{-1}$). Error bars indicate the mean ± SE (n = 10) (Expt. 1). Mean duration (min.) before wilting under 3 diffent light intensities was significantly different from Day 1-7 ($P \leq 0.0001$), ANOVA ($\alpha=0.05$).

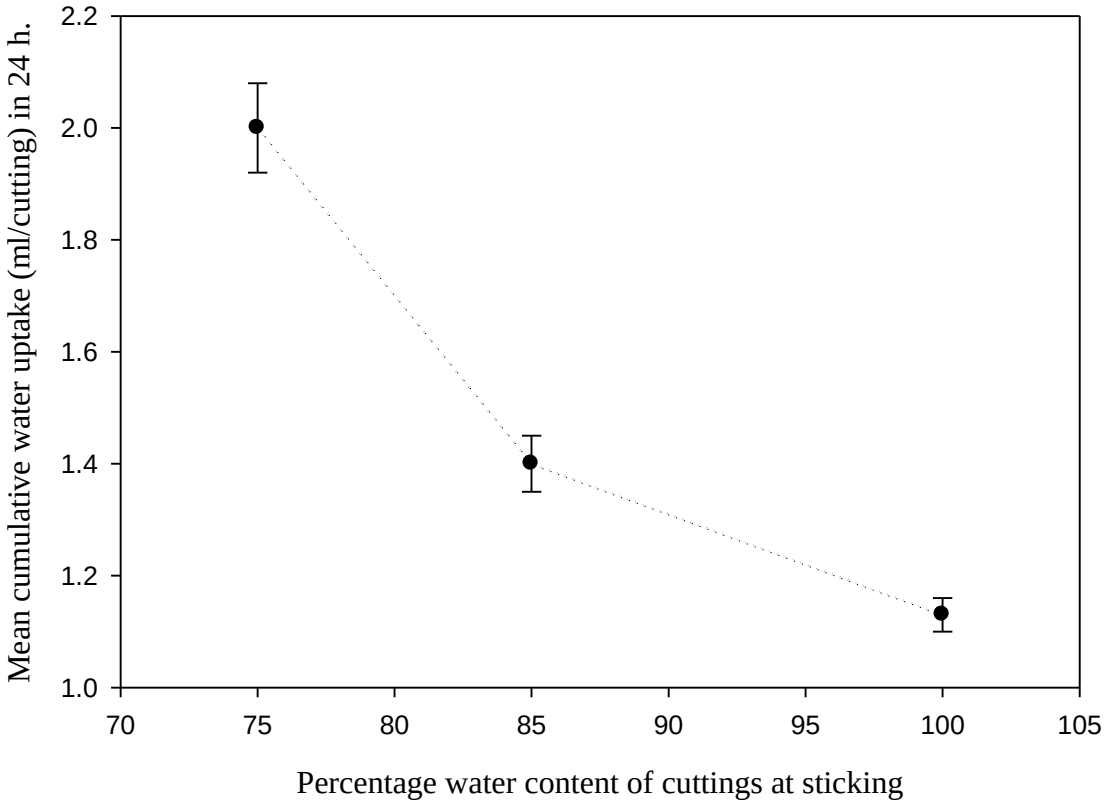

Figure 2.3 The effect of the initial water content of the cutting on the mean cumulative water uptake over 24 h (Expt. 2 a). The initial water content of the cuttings was manipulated by placing the cuttings in front of a lamp supplying 150 umol $m^{-2}s^{-1}$ at a distance of 0.5 m for 0, 30 or 60 min. ANOVA, $\alpha=0.05$, $P\leq0.0001$.

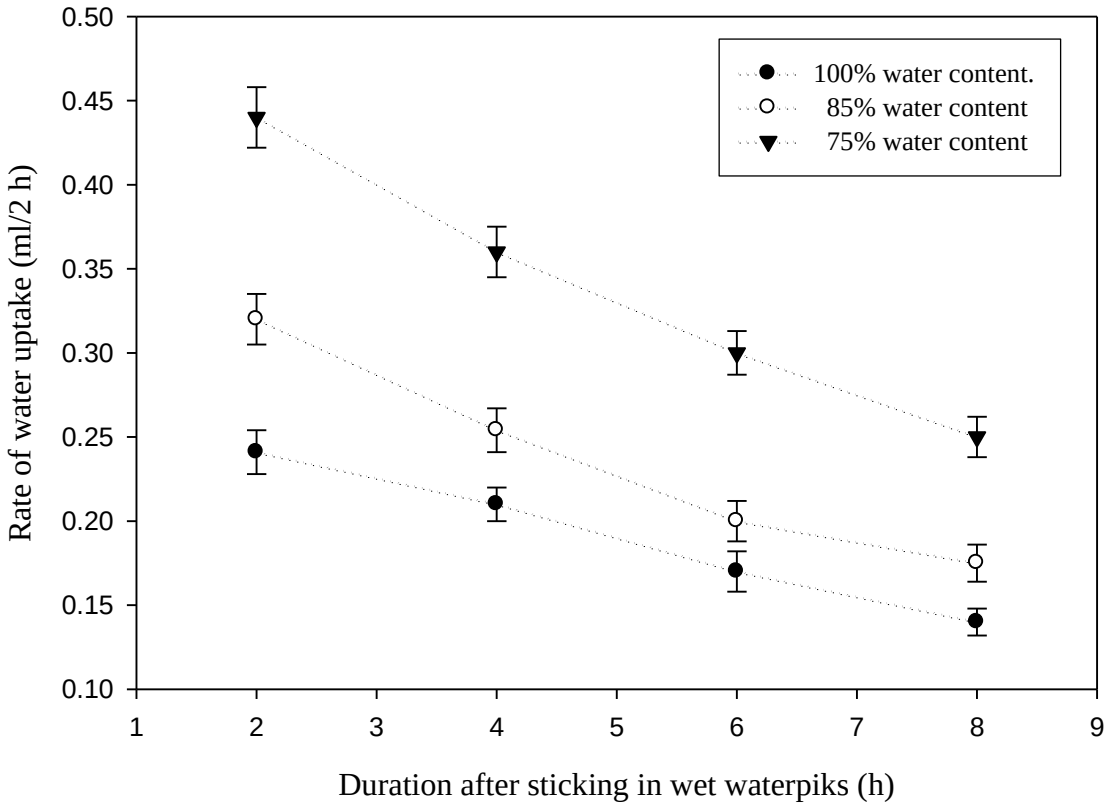

Figure 2.4 The effect of initial cutting water content on the rate of water uptake (ml/2h) during the first 8 hours after placing the cutting in propagation (Expt. 2 a). Error bars indicate ±SE (n = 10).

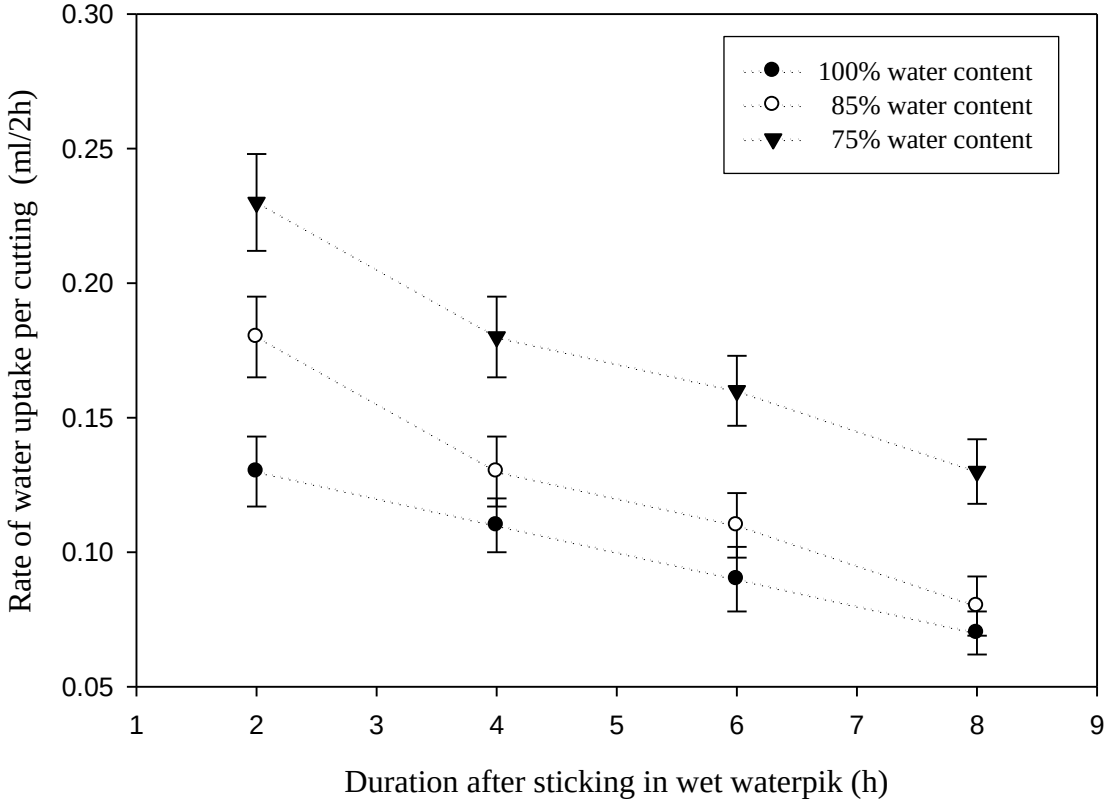

Figure 2.5 The effect of initial cutting water content on the rate of water uptake under mist (ml/2h) during the first 8 hours after placing the cutting in propagation (Expt. 2b). Error bars indicate ±SE (n = 10).

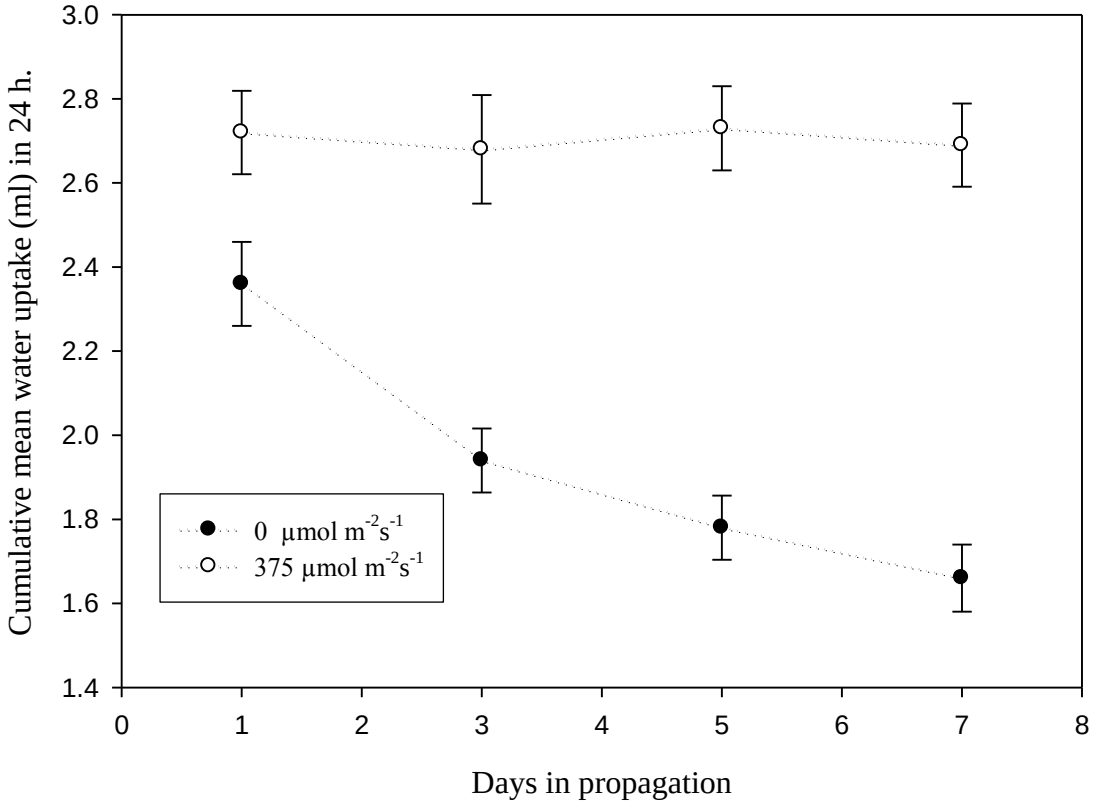

Figure 2.6 Mean cumulative water uptake through the stem of unrooted cuttings (1-7 days in propagation) during exposure to light (375 µmol $m^{-2}s^{-1}$) and in the dark for 48 h (Expt. 4). In light, there was no significant difference between cuttings of 1-7 days old in propagation, ANOVA; $P \leq 0.889$ & $\alpha=0.05$. Water uptake in the dark was significantly different ($P \leq 0.0001$). Error bars indicate mean ± SE (n=20).

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CHAPTER THREE

GAS EXCHANGE OF POINSETTIA LEAVES FROM THE STOCK PLANT THROUGH THE PROPAGATION ENVIRONMENT

Introduction

Poinsettias are propagated from vegetative stem cuttings placed under mist. The cuttings are produced on stock plants that are primarily grown in Mexico and Guatemala for the North American market. Immediately after harvesting the cuttings, they are put in cold storage at 10-12ºC to minimize water loss and respiration. The cuttings are then transported via air and land freight over two to three days. After delivery to the propagator the cuttings are stuck in a propagation medium and put under intermittent mist to promote root initiation and growth before transplanting approximately four weeks later. Cuttings quality is affected by water loss and subsequent wilting in postharvest (Wills et al., 1989). Plants lose the majority of their water through open leaf stomata; however, little is known about the response of stomata to severing and postharvest handling practices (Thomson, 2005).

Stomata respond to both internal and external stimuli. In intact plants, stomatal opening and closing can be gradual or rapid depending on the level of environmental stress. Disturbances like severing a shoot from a mother plant limits water uptake to the shoot and reduces gas exchange (Jones, 1998). Stomata respond to maintain a balance between water loss through transpiration and carbon gain for photosynthesis, hence preventing water potential from declines to an extend of xylem embolism (Tyree and Sperry, 1988 and Nardini and Salleo, 2000). The mechanisms of rapid stomatal response to water deficits are not yet fully understood. Past studies have concluded that stomatal conductance is reduced within a few minutes following a perturbation of the water

potential gradient in the xylem suggesting that stomata respond directly to hydrostatic signals (Whitehead, 1998). However, other researchers think that stomata do not respond directly to changes in hydraulic conductivity, but rather to changes in water status within the leaf as a result of the limitation to water flow (Saliendra et al., 1995 and Hubbard et al., 2001). The continuous hydraulic pressure stream that drives water to the shoot through the stem is cut off suddenly and fluids in the xylem and phloem tissues abruptly retreat up the stem. Moreover, the vapor pressure deficit in the stock plant environment is relatively large, so the cuttings are immediately wrapped in moistened paper and placed in a cooler as fast as possible in order to minimize further water loss.

Considerable work has been done on the gas exchange of intact plants but little research has been undertaken concerning the gas exchange of severed cuttings in storage and propagation. Understanding the changes in the gas exchange of leafy stem cuttings is important in attempting to keep severed plant parts alive until they can be successfully propagated.

Mist is provided to poinsettia cuttings in propagation in order to minimize the water stress that might occur prior to the cutting forming new roots. Excess water can result in increased pathogen pressure and physiological problems, so the propagator's goal is to provide just enough mist to meet the water demand of the cuttings. The standard practice is for commercial producers to gradually decrease the mist frequency and duration over time in propagation. Mist programs will vary between growers and locations; however, the following is an example of an intermittent mist program for a static timer used to propagate poinsettias during the sunny days of the peak summer season. On the first day in propagation the cuttings are misted at a 4-minute interval and

misting continues throughout the night at a lower frequency, e.g., 30-minute interval. The second day will have a 6-minute interval and will continue at a lower frequency throughout the night, e.g., 60-minute interval. The third and fourth days will continue with a 6-minute mist frequency, but no mist will be delivered during the night throughout the remainder of time in propagation. The mist frequency will be reduced to an 8-minute interval during the 5, 6 & 7^{th} days in propagation, a 10-minute interval on the 8^{th}-10^{th} days, and a 16-minute interval on the 11^{th}-14^{th}day in propagation. From Day 15 to 21, mist can be reduced to a 30-60-minute interval or may be terminated altogether depending on the degree and uniformity of rooting. Once the cuttings are uniformly rooted, the mist is turned off and irrigation schedules are initiated.

Chlorophyll fluorescence has been employed in the investigation of plant responses to various environmental stresses, e.g., water, light and temperature stress. Using the CIRAS-2 (PP Systems Inc., Amesbury, MA U.S.A.) and a cuvette-mounted chlorophyll florescence module, the following parameters were either measured or calculated: a minimum fluorescence (F_o) under darkness adaptation, a maximum fluorescence (F_m) when chloroplasts are exposed to saturating light for a short time. The difference between maximum and minimum fluorescence is known as F_v, the electron transfer rate. F_v/F_m is the parameter that expresses the maximum quantum yield of photosystem which can be used to estimate the efficiency of photosystem II.

We hypothesized that stomata lose their normal functionality when cuttings are severed from stock plants, i.e., they do not close properly in response to dark or environmental stress and then regain their function gradually in propagation. Therefore, our objective was to determine the changes of stomatal conductance, transpiration and

photosynthesis on the leaves of stem cuttings after severing and placing in storage, and finally during propagation and to find out the effects of severing cuttings on leaf photosynthesis and light use efficiency.

Materials and Methods

Poinsettia stock plants (Peterstar Red) were grown in the greenhouse (26-30 ºC, 67-75% R.H and a maximum PPF of 1400 µmol $m^{-2}s^{-1}$) and provided with a constant liquid fertilization program consisting of 250 ppm N from a 15-5-15 4Ca 2Mg (Peters Professionals, Scotts International B.V., Scotts Professional, Geldermalsen, The Netherlands). The stock plants were maintained in a vegetative state by providing night-interruption lighting with incandescent lamps.

Expt.1. Effect of severing and time in propagation on gas exchange of stem cuttings

Stomatal conductance (g_s), transpiration (E), leaf temperature (T_i) and internal CO_2 concentration (C_i) were measured on one leaf each of ten stock plants using a CIRAS-2 Portable Photosynthesis System with a broad leaf cuvette in a greenhouse (26-30 ºC, 67-75% R.H and PPF of 800-1000 µmol $m^{-2}s^{-1}$) at 11 a.m.

The shoots were then severed to make standard stem cuttings, which were then taken to the laboratory (10 µmol $m^{-2}s^{-1}$, 50% R.H and 24-26 ºC) where the gas exchange measurements were taken 5 min. after severing on the same leaves that were measured on the stock plants. The cuttings were then packed into plastic bags (10 cuttings per bag) and stored in a cold room (10 ºC & 40% R.H.). The gas exchange measurements were repeated in the dark on Day 1, 2 & 3 of cold storage at 11 a.m. The cuttings were then

stuck in a propagation media (Oasis Wedges, Smithers-Oasis North America, Kent, OH) and put under mist in a propagation environment (23-30 ºC, 75-85% R.H, maximum daily PPF 250-600 $\mu mol \cdot m^{-2} \cdot s^{-1}$). For the gas exchange measurements during propagation, cuttings were removed from the mist bench and taken to a growth chamber (48% R.H, 26 ºC and PPF of 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$) from where measurements were made after 1, 3, 7, 14, 21 and 28 days in propagation, i.e., 4, 7, 11, 18, 25 and 31 days after harvesting the cutting, respectively. The experiment was repeated twice; this resulted in randomized block (2 blocks) with repeated measurements design.

Expt.2. The Effect of Time in propagation on the photosynthesis efficiency of cuttings (Chlorophyll fluorescence- F_v/F_m)

Ten poinsettia (Peterstar Red) cuttings were harvested each day from stock plants grown in a green house and stuck in a propagation media (Oasis Wedges) for 29 consecutive days. Cuttings from seven specific days (Day 1, 2, 3, 5, 7, 21 and 28 after removal from the stock plant) were selected for the experiment; ten cuttings per day.

The CIRAS-2 with an integrated Chlorophyll Fluorescence Module (CFM) unit (PP Systems, Amesbury, MA) mounted on an automatic universal leaf cuvette (PLC6 (U)) was used to measure chlorophyll fluorescence of each of the seven different aged cutting as well as on leaves on the stock plant. The measured chlorophyll fluorescence parameters were: F: fluorescence intensity at any time, $F_{o:}$ minimal fluorescence (dark); F_m: maximum fluorescence (dark); $F_v = F_m - F_o$: variable fluorescence. This experiment was repeated five times (blocks) with ten cuttings per treatment.

Expt.3. The Effect of Time in propagation on light use efficiency (Light response curves)

Photosynthesis measurements were performed on the most newly-expanded, intact leaves of poinsettias (Peterstar Red) stem cuttings after 1, 3, 5, 7, 14, 21 and 28 days in a propagation environment and on stock plants using a CIRAS-2 Portable Photosynthesis System adapted with an external LED light unit mounted on the leaf cuvette. Leaves were pre-adapted to darkness for 30 min. and then subjected to a sequence of increasing light intensity, rising stepwise from 0 to 100, 200, 400, 600, 800 and 1000 µmol $m^{-2}s^{-1}$ for 45 sec at each intensity. Gas exchange measurements were automatically recorded at each PPF step and stored in the CIRAS-2 memory. This experiment was carried out in the laboratory (42-52% R.H, 23-26 ^{0}C and 10 µmol $m^{-2}s^{-1}$). The photosynthetic light response curves were determined at 300 ppm CO_2. The experiment was repeated five times, with 5 cuttings per treatment (cutting age); randomized complete block design.

Statistical analysis

Data were subjected to one-way and two-way analyses of variance (ANOVA), and means were compared with Fisher's protected least significant difference (LSD) at *P* = 0.05; SAS software (Version 9.1; SAS Institute, Cary, NC, USA) was used**.**

Results and Discussion

Severing shoots from the stock plant led to a reduction in stomatal conductance, transpiration and photosynthesis within five minutes (data not shown). For example, five minutes after severing the cutting from the stock plant the stomatal conductance decreased from 298 to 173 mmol $m^{-2}s^{-1}$, transpiration decreased from 2.67 to 1.3 mmol $m^{-2}s^{-1}$ and photosynthesis decreased from 7.47 to -3.7 µmol $m^{-2}s^{-1}$. The gas exchange parameters decreased slightly during the three days in the 10 ºC storage room and then significantly increased on the first day in propagation (Fig. 3.1). Stomatal conductance and photosynthesis values increased gradually throughout the time in propagation; however, they did not return to the stock plants values even when they become fully rooted after 28 days (Fig. 3.1A & B). Transpiration followed the same trend; however, transpiration did recover to the stock plant levels after 28 days in propagation (Fig. 3.1C).

Svenson et al., (1995) also reported a reduction in stomatal conductance and photosynthesis of poinsettia cuttings after severing from the stock plants. This reduction appears to be a result of the break in water flow from the roots; however, water continues to be lost through transpiration due to an apparent loss of stomatal functioning (Chapter 2). Once cuttings are inserted into the propagation medium, the water column is quickly reestablished, however transpiration remains significantly lower than on the stock plant due to the reduced water transport resulting from the lack of roots. As cuttings stay in propagation under mist, stomatal functioning returns so water use efficiency improves and the cuttings can respond in the event of water stress. Once a significant root system has been reestablished (Day 28 in propagation), transpiration returned to a similar rate as occurs on the stock plant, however photosynthesis rates did not fully recover by Day 28.

Chlorophyll fluorescence (F_v/F_m) of the stock plant and the cuttings throughout the 28 days in propagation displayed no significant trends (Fig. 3.2). Johnson et al., (1993) reported that healthy plants for most species have chlorophyll fluorescence values near 0.83. The chlorophyll fluorescence measurements in this study ranged from 7.7 to 7.9 close to those of a healthy intact plant. This suggests that the process of harvesting and propagating cuttings does not affect the non-photochemical quenching capacity of photosystem II. Enfield (unpublished data) demonstrated that cuttings experiencing chilling temperature stress during the postharvest environment have significantly reduced chlorophyll fluorescence which would potentially impact photosynthesis and consequent rooting in propagation. Our data suggest that photosynthesis is not limited by the efficiency of the photosynthetic apparatus under normal propagation environments, so this does not explain the relatively low rates of photosynthesis that occur on cuttings during propagation.

Unrooted cuttings, i.e., Days 1, 3, and 7 in propagation, did not have significantly different photosynthesis rates, while photosynthesis of rooted cuttings, i.e., Days 21 and 28 in propagation, increased significantly (Fig. 3.3). Day 28 cuttings in propagation had similar photosynthesis rates like stock plants at PPF $\leq$800 µmol $m^{-2}s^{-1}$; however at higher PPF, the stock plants performed at a higher photosynthetic rate than the Day 28 cuttings. These data suggest that photosynthesis of a fully rooted cutting (Day 28) nearly recovered to a similar capacity as the stock plants. A reduction in photosynthesis before rooting and then recovery after rooting supports the hypothesis of water stress as the limiting factor to photosynthesis in unrooted cuttings (Svenson et al., 1995).

Conclusion

Leafy herbaceous cuttings depend on current photosynthates produced while the cuttings are in the propagation environment in order for root initiation and growth to occur (Leakey, 2004); however, photosynthesis rates of unrooted cuttings were very low compared to shoots on the stock plants. Photosynthesis does not appear to be limited by stomatal aperture as stomata do not appear to be responsive to environmental stimuli immediately following severing from the stock. Also, photosynthesis does not appear to be limited by any damage that may have occurred to photosystem II during the cutting harvest and shipping process. Therefore, we conclude that photosynthesis if primarily limited by water uptake by the severed stem. Photosynthesis does recover significantly as root growth occurs on the cutting. This suggests that light management in propagation should be driven by the stage of rooting of the cuttings.

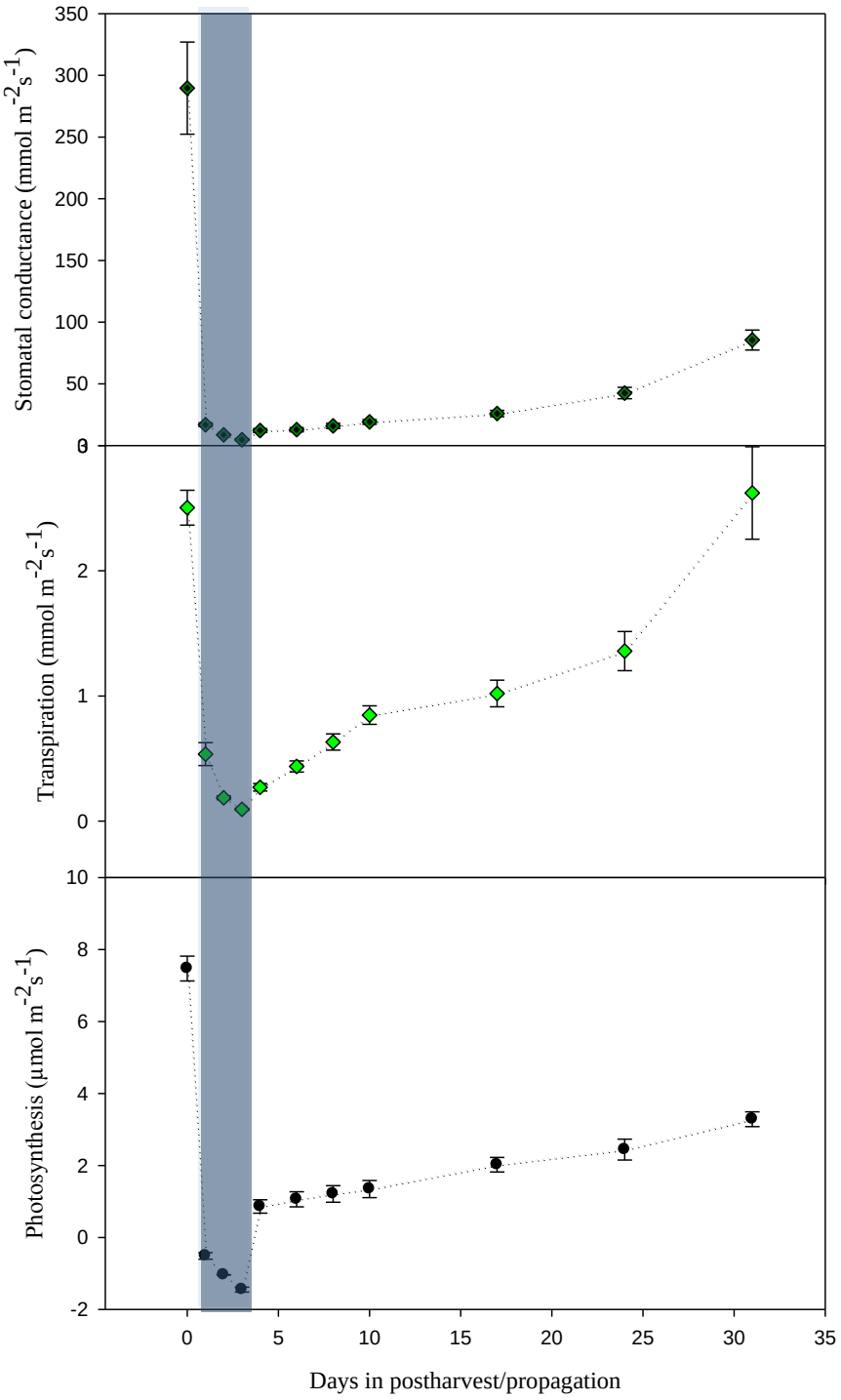

Figure 3.1 Gas exchange measurements included A) stomatal conductance, B) transpiration and C) photosynthesis from stock plant (Day 0) and cuttings (Days 1-3; in 10ºC cold, dark storage; Days 4-28 in propagation). Measurements made in dark storage are indicated by the shaded area. Error bars represent mean±SE.

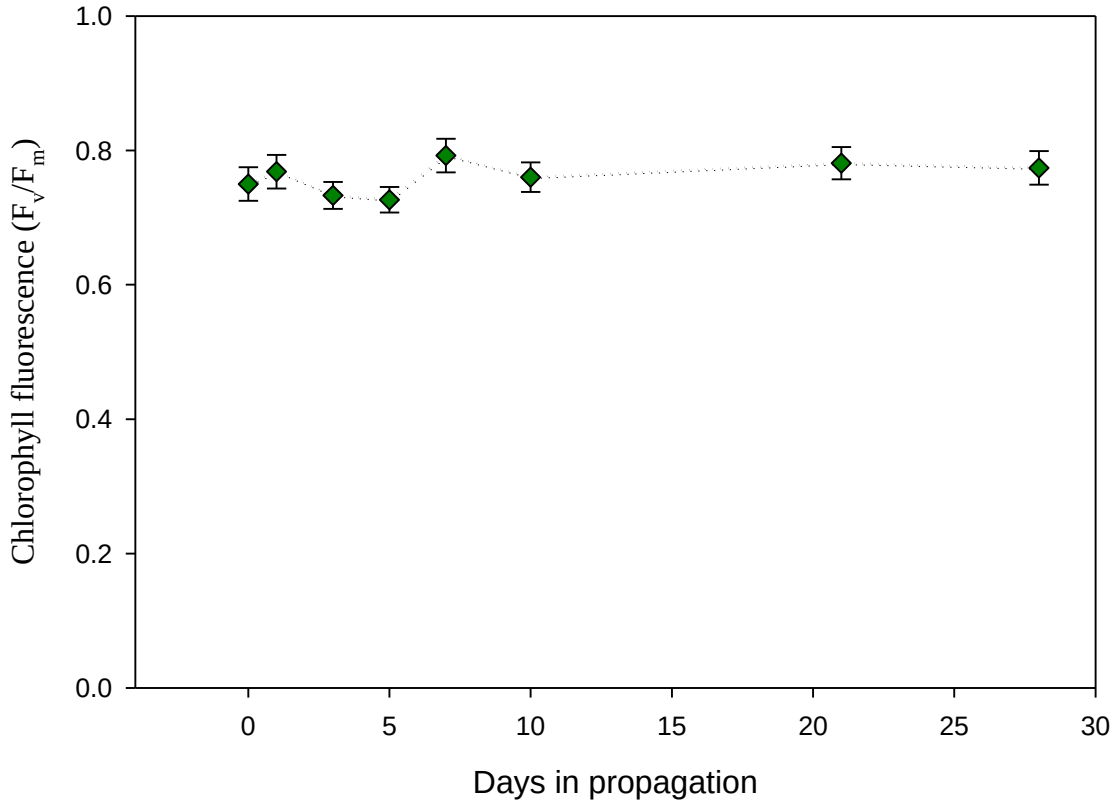

Figure 3.2 Chlorophyll fluorescence (F_v/F_m) measured on stock plants (Day 0), and cuttings in propagation (Day1, 3, 5, 7, 21 or 28). Error bars indicate ± SE.

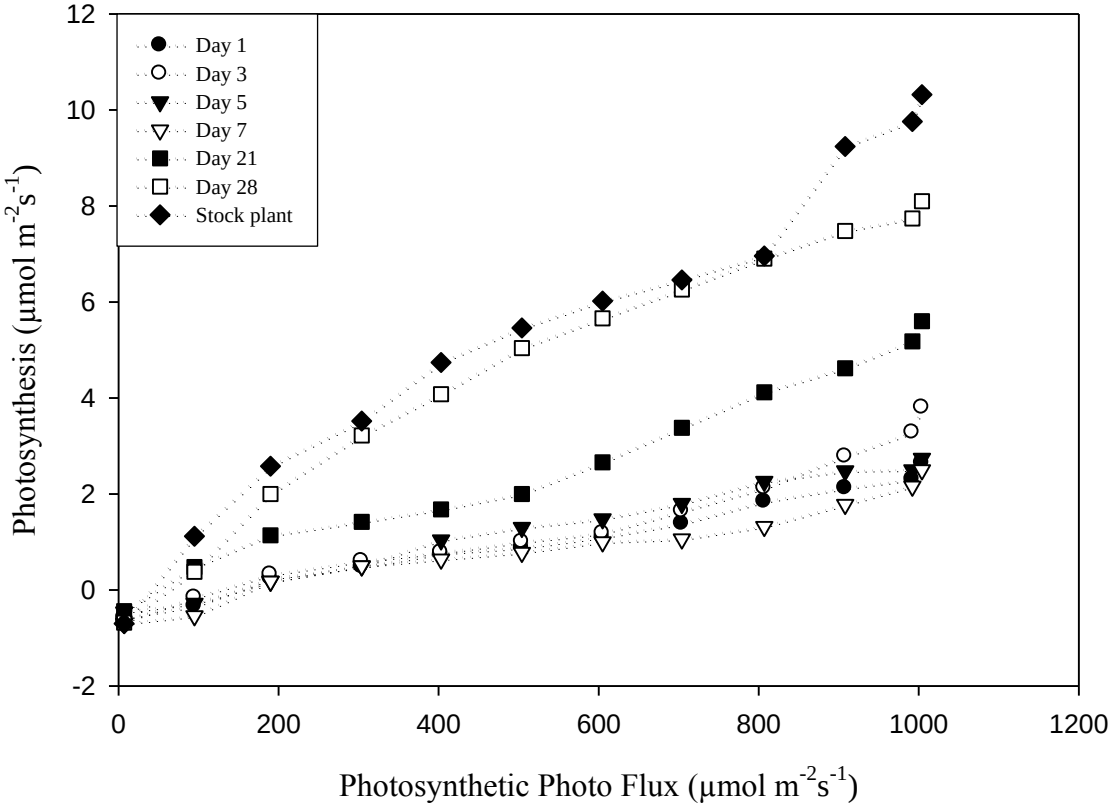

Figure 3.3 Photosynthesis response curves generated by a chlorophyll fluorescence module mounted on the CIRAS-2 System. ANOVA ($P<0.0001$); $\alpha=0.05$. Means compared with Fisher's protected LSD showed unrooted cuttings (Days 1-7) had no significant difference.

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