

A STUDY OF PROPAGULE PHYSIOLOGY DURING ADVENTITIOUS ROOT  
DEVELOPMENT (AND OTHER “INTERESTING” WORKS)

by

MICHAEL TERRY MARTIN, JR.

(Under the Direction of Matthew R. Chappell)

ABSTRACT

To remain competitive, nursery and/or greenhouse producers must be able to reduce cost and/or increase production. In vegetative propagation, this can be accomplished by increasing success rates (rooting percentages) of propagules, increasing the speed of propagule production and/or producing higher quality propagules. To accomplish these goals, a thorough understanding of the adventitious root development (*ARD*) process and propagule physiology is required. *ARD* allows for the asexual production of genetically uniform plant material, ensuring each propagule is genetically identical to its stock plant.

There are many knowledge gaps relating to *ARD* physiology. By attempting to fill these gaps, and gain an understanding of the physiological processes that are occurring during *ARD*, producers can potentially reduce cost while increasing production. This study examines how water potential and net photosynthesis of propagules relate to stock plants in an effort to discern how these two factors relate to *ARD* and what influence they may exert on the *ARD* process (or vice-versa). This study also attempts to identify the source of carbon utilized to form adventitious roots during *ARD*, with the goal of

improving carbohydrate availability during *ARD*. Additionally this study evaluates nutrient utilization in propagules during *ARD* to determine what nutrients may play a role in this process. Finally, this study endeavors to develop a propagation protocol for seed germination of *Amsonia tabernaemontana*, an underused, highly ornamental plant native to the southeastern U.S., with hopes of increasing its presence in the nursery trade.

From this research, it was concluded that propagules under intermittent misting successfully maintained water ( $\Psi$ ), osmotic ( $\Psi_s$ ) and turgor potential ( $\Psi_p$ ) values and were not water stressed. Additionally, net photosynthesis did occur in leafy propagules before adventitious roots appeared and increased in some species after adventitious roots appeared. Also, carbon utilized for *ARD* was initially provided by both reserve carbohydrates produced prior to propagule severance and newly produced photosynthates. Furthermore, N, P, K, S and Zn were utilized at greater quantities than other nutrients during *ARD*. Finally, an effective propagation protocol was developed for *Amsonia tabernaemontana*, including seed scarification by removal of one end of the seed coat combined with imbibition.

INDEX WORDS: Cutting propagation, Data logger, Eastern bluestar, Germination protocol, Imbibition, In-situ environment, Internal CO<sub>2</sub> concentration, Isotope labeling, Isotope-ratio mass spectrometry, Mechanical barrier, Nutrient translocation, Nutrient uptake, Psychrometers, Seed propagation, Stem propagule, Stomatal conductance, Vegetative propagation

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## DEDICATION

This dissertation is dedicated to three men that played very important roles in my life. All three valued hard work and diligence but placed a greater importance on education and knowledge. They were my mentors and my family, but most importantly my friends. They supported and encouraged me in my educational and professional endeavors. I am ever indebted to them.

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*“<sup>3</sup> I thank my God every time I remember you. <sup>4</sup> In all my prayers for all of you, I always pray with joy <sup>5</sup> because of your partnership in the gospel from the first day until now, <sup>6</sup> being confident of this, that he who began a good work in you will carry it on to completion until the day of Christ Jesus.”*

Philippians 1:3-6 (NIV)

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## CHAPTER 1

### INTRODUCTION AND LITERATURE REVIEW

Adventitious root development (*ARD*) allows for the asexual production of plant material, assuring that each asexually produced plant is genetically identical to its stock plant (de Klerk et al., 1999). This genetic uniformity allows for the maintenance of specific cultivars/genotypes in the ornamental horticulture industry. Physiologically, it is well documented that stock plant health is directly related to *ARD* success of propagules (Hassig, 1989 [*Pinus banksiana* Lamb]). But understanding how physiological measurements of propagules relate to those of stock plants is not well understood nor has it been reported. Understanding these relationships could provide valuable insight into *ARD* process, potentially increasing propagule success rates, decreasing propagule production times and/or improving the overall quality and health of propagules.

### WATER POTENTIAL

The practice of placing leafy stem propagules under intermittent mist or in a high humidity environment promotes high propagule *ARD* success rates by preventing propagule desiccation. Little has been reported on the underlying physiologic reasons why it is necessary to maintain these high humidity propagation environments, particularly the relationship between propagule water ( $\Psi$ ), osmotic ( $\Psi_s$ ) and turgor potential ( $\Psi_p$ ) and *ARD* in commercial production-style propagation environments. The lack of focus placed on propagule  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  in commercial settings could be related to

the expense or difficulty in accurately measuring these parameters and relating these factors to *ARD*. Despite a lack of knowledge on the roles and interactions among propagule  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$ , many suspect these factors are vital to the *ARD* process. LeBude et al.'s (2004) work with *Pinus taeda* L. demonstrated that maintaining a  $\Psi$  between -0.5 and -1.2 MPa is essential to successful *ARD*. LeBude et al. (2005) [*Pinus taeda* L.] further related a high rooting percentage ( $\geq 80\%$ ) to  $\Psi$  between -0.5 and -1.2 MPa, while propagules with  $\Psi$  outside this range rooted at lower percentages.

LeBude et al. (2004, 2005) [*Pinus taeda* L.], Slatyer (1960, 1967), Stirzaker et al. (1997) [*Solanum lycopersicum* L.] and Yates and Hutley (1995) [*Sloanea woollsii* F.Muell.] have demonstrated that unrooted propagules and severed branch sections have the ability to absorb water through leaf epidermal tissues or stomata, translocating this water throughout the propagule or branch section. These studies provide a means to (at least partially) explain how water enters into a propagule prior to *ARD*.

## PHOTOSYNTHESIS

An additional aspect of plant growth and development that warrants further study is propagule photosynthesis, and how it influences and is affected by *ARD*. It is widely accepted (Costa et al., 2001 [*Rosa hybrida* L. 'Madelon']) that propagules photosynthesize throughout the *ARD* process. It is still undetermined exactly what role photosynthesis and photosynthates play in the development of adventitious roots. Numerous authors (Davis and Potter, 1981 [*Pisum sativum* L.]; Hoad and Leakey, 1996 [*Eucalyptus grandis* (Maiden) W. Hill]; Rapaka et al., 2005 [*Pelargonium*  $\times$  *hortorum* L.H. Bailey 'Isabell']; Yue and Margolis, 1993 [*Picea mariana* (Mill.) B.S.P.]) suggest

that propagule photosynthesis provides photosynthates that are utilized during *ARD* and an inadequate supply of photosynthates can be a limiting factor in root initiation and development. Eliasson and Brunen (1980) [*Populus tremula* × *tremuloides* and *Salix caprea* × *viminalis* Willdenow] emphasized that providing the correct environmental conditions to allow propagule photosynthesis was essential for successful *ARD*. Despite providing ideal conditions for *ARD*, Fordham et al. (2001) [*Corylus maxima* Mill.] and Mesén et al (1997) [*Cordia alliodora* (Ruiz & Pavon) Oken] observed that, at severance from stock plant(s), leafy stem propagules immediately experienced shock, significantly decreasing propagule photosynthetic activity and stomatal control. Determining how environmental and morphologic factors influence net photosynthesis could prove highly beneficial to understanding the *ARD* process and how to increase *ARD* success rates.

### CARBON UTILIZATION

As carbohydrates are necessary for growth, they have been postulated to play a vital role in *ARD* (Davies, 1988). However, physiologic details regarding propagule carbohydrate storage and particularly carbohydrate allocation within asexual propagules, while in the rooting process, are contradictory. In previous work on this topic, several authors determined that stored carbohydrates are vitally important to the *ARD* process (Hassig, 1982, 1984 [*Pinus banksiana* Lamb]; Hoad and Leakey, 1996 [*Eucalyptus grandis* (Maiden) W. Hill]; Schaesberg and Lüdders, 1993 [*Mangifera indica* L.]). These authors' findings demonstrated the importance of carbohydrates stored in propagule tissues prior to removal from stock plants. These stored carbohydrates, or pre-severance carbohydrates, were produced via photosynthesis and stored while propagules were still

attached to the stock plant and subsequently provide propagules with a carbohydrate reserve post-severance, yet prior to *ARD*. Additionally, post-severance photosynthesis, or the production of new photosynthates (carbohydrates) by propagules, has also been associated with propagule *ARD* success (Davis, 1988; Druege et al., 1998 [*Dendranthema x grandiflorum* (Ramat.) Kitamura 'Puma']; Haissig, 1986; Pellicer et al., 2000 [*Larix x eurolepis* Henry]; Veierskov, 1988). Conversely, other studies found little to no evidence of the importance of post-severance carbohydrates in *ARD* (van Overbeek et al., 1946 [*Hibiscus rosa-sinensis* L.]; Reines and Bamping, 1962; Smalley et al., 1991 [*Acer rubrum* L.]).

Work by Hambrick et al. (1991) examined how propagule set date and propagule cane position (basal, medial, or apical) affected whole propagule carbon to nitrogen ratio in *Rosa multiflora* Thunb. 'Brooks 56' propagules. They demonstrated that propagules set between mid-November and mid-December, when carbohydrate reserves typically peak, had the highest *ARD* success rate. Lappalainen et al. (2000) investigated carbon allocation in *Betula pubescens* Ehrh. micropropagules; specifically the effect of nitrogen fertilization, defoliation, and nitrogen fertilization plus defoliation on carbon allocation. Nitrogen fertilization had a significant effect on carbon allocation, specifically decreasing root mass, negatively affecting the root to shoot ratio. Breen and Muraoka (1974) [*Prunus cerasifera x munsoniana* 'Marianna 2624'] examined how photosynthates produced by unrooted propagules, post-severance, were translocated throughout the propagule; finding that most photosynthates remained in the upper portions of the propagules. These results indicated that propagule photosynthesis had little effect on the carbohydrates found in the roots of propagules. However, Breen and Muraoka (1974)

were unable to determine the role of pre-severance carbohydrates in the *ARD* process, as they had no stock plant treatment in their study.

Pellicer et al. (2000) [*Larix x eurolepis* Henry] examined if stock plant derived carbon was the primary carbon source utilized for *ARD*. They treated stock plants with  $^{13}\text{C}$  and recorded a baseline  $^{13}\text{C}$  at the time of propagule harvest. Results indicated that the majority of carbon utilized during *ARD* was propagule-derived photosynthates and not re-allocated from stock plant reserves. Utilizing pulse-chase methodology and  $^{13}\text{C}$  labeling of stock plants and propagules pre- and post-severance, this study aims to acquire an increased understanding of what carbon source (stock plant or propagule produced carbon) is utilized to produce adventitious roots and when that carbon source is employed.

## NUTRIENT UTILIZATION

To better understand the physiological processes that occur during *ARD*, it is important to understand the nutrient requirements of this process. Little work has been conducted on deciphering the role of specific nutrients and their availability on vegetative propagule rooting during *ARD*; with the majority of work focusing on application of complete fertilizers or micronutrient fertilizers (Biernbaum et al., 1995; Carney and Whitcomb, 1983 [*Rhododendron* x 'Fashion', *Ilex crenata* Thunb. 'Hetzi', *Pyracantha coccinea* Roem 'Wyatti']; Gibson, 2003 [*Impatiens x hawkeri* Bull. 'Grenada', *Scaevola aemula* R. Br. 'Purple Fan', and *Bracteantha bracteata* (Vent.) A.A. Anderberg 'Florabella Pink']; Rowe and Cregg, 2002 [*Artemisia ludoviciana* Nutt. 'Valerie Finnis', *Gaura lindheimeri* Engelm. & Gray 'Whirling Butterflies', and *Nepeta x faassenii*

(Stearn.) Bergmans 'Six Hills Giant']; Santos et al., 2011 [*Petunia x hybrida* Vilm.) to propagules and subsequently measuring changes in biomass of propagules. The importance of specific macronutrients and micronutrients to the *ARD* process is less clear, yet has been documented in several studies. During the *ARD* process, deficiencies of mineral nutrients have been linked to an initial reduction in vegetative shoot growth over that of root growth (Eliasson, 1978 [*Pisum sativum* L.]), indicating that the roots act as a preferential sink during *ARD* development (van Overbeek et al., 1946 [*Hibiscus rosa-sinensis* L.]). The two mineral nutrients most noted as hindering root development when deficient in propagules and/or propagation substrate are Ca (Eliasson, 1978) [*Pisum sativum* L.] and B (Hemberg, 1951 [*Phaseolus vulgaris* L.]). Interestingly, Ca has been described as poorly translocated within propagules, particularly in phloem (Crafts and Crisp, 1971; Oertli and Richardson, 1970 [*Gossypium hirsutum* L.]). More recent work has indicated that, in addition to Ca and B; N, P, Mg, Mn, Zn (Anderson, 1986; Blazich, 1988) and Cu (Arnold et al., 1994 [*Betu pubescens* × *papyrzfera*]) are required for root initiation and/or elongation. Also, the rooting factor indole-3-acetic acid (IAA) has been shown to rely on the availability of Zn, Mn and B (Jarvis et al., 1984 [*Phaseolus aureus* Roxb.]; Salami and Kenefick, 1970 [*Zea mays* L.]; Takaki and Kushizaki, 1970; [*Zea mays* L.] Thomaszewski and Thimann, 1966 [*Pisum sativum* L. 'Alaska']). Furthermore, the rooting factor auxin has been shown to rely on Fe (Mengel and Kirkby, 1982), with the knockout of Fe uptake in mutant *Oryza sativa* L. lines resulting in significant reductions in root length of propagules (Qi et al., 2011).

Despite previous research on understanding the role of nutrient availability in *ARD* success and propagule health, little is known about the utilization (transport and

allocation) of nutrients in vegetative propagules post-severance from stock plants. Svenson and Davies (1995) were the first and only researchers to report utilizing Inductively Coupled Argon Plasma (ICAP) spectrometer technology to measure mineral elemental concentrations of propagules during *ARD*. Their work, utilizing *Euphorbia pulcherrima* (Klotzch.) Willd., propagules determined several overall trends in nutrient availability and allocation. By separating propagules into foliage and basal stem tissue (callus - roots), they to assessed changes in nutrient content over the *ARD* process. Overall, Fe, Cu and Mo concentrations increased in the basal portion of the propagule during callus formation, suggesting these nutrients may be important for callus formation and root initiation. After roots were observed, there was an increase in Mg, Mn, B and Zn, suggesting these nutrients may be vital to root elongation and maintenance. Conversely, there was a reduction in foliar concentrations of N, Fe and Mo. This reduction could be in part due to leaching of N and a concentration reduction due to increasing leaf biomass. However, Fe was apparently translocated from foliage to developing callus and roots.

It is hoped that undertaking this research will provide insight into how propagule water potential, photosynthesis, carbon allocation and nutrient usage and translocation affect or are affected by *ARD*.

## AMSONIA GERMINATION

In addition to the previously mentioned *ARD* physiology study, a study to improve the germination rates of *Amsonia tabernaemontana* was undertaken. The genus *Amsonia* is distributed worldwide with 16 recognized species and numerous subspecies

occurring in North America (USDA NRCS, 2009). Eastern bluestar (*Amsonia tabernaemontana* Walt. [Apocynaceae]) is a herbaceous perennial with a native range from southern NY to northern FL, west to eastern TX. *A. tabernaemontana* is an attractive plant with interesting ornamental characteristics, including pastel blue flowers in the spring and yellow fall foliage. *A. tabernaemontana* experiences few pest and disease problems, making it an ideal addition to the landscape. *A. tabernaemontana* is propagated primarily by seed, which are reported to be difficult to germinate in a production environment due to uneven germination time and low germination percentage (Nyárádi-Szabady et al., 1987; Scocco et al., 1998). Current production methods involve planting seed outdoors in fall (September and October), allowing seed to naturally scarify during winter months and germinate in late spring. In a commercial setting, this method of natural scarification is inefficient in its requirement for long-term use of production facilities (bench space). Additionally, variable germination times result in non-uniform liner size that can lead to a lack of uniformity later in container production. Several popular publications (Armitage, 2006; Cullina, 2000; Phillips et al., 1985) indicate that removing a portion of the seed coat increases germination percentage of *A. tabernaemontana*. The information provided by these sources is little more than anecdotal and providing no details as to the best method of seed scarification and if environmental requirements must be satisfied to facilitate germination. To date, no scientific study specifying a seed coat removal technique or stating how seed coat removal affects germination percentage has been published.

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## CHAPTER 2

### WATER, OSMOTIC AND TURGOR POTENTIAL OF INTERMITTENTLY MISTED LEAFY STEM PROPAGULES DURING ADVENTITIOUS ROOT DEVELOPMENT<sup>1</sup>

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## ABSTRACT

Understanding how intermittent misting affects water ( $\Psi$ ), osmotic ( $\Psi_s$ ) and turgor potential ( $\Psi_p$ ) of leafy propagules is fundamental to improving propagation success rates of nursery and greenhouse producers. The objectives of this study were to determine those environmental and/or morphological factor(s) that exert the greatest influence on leafy stem propagule  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  during adventitious root development (*ARD*); determine how propagule  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  are affected by adventitious root formation; and determine differences in stock plant and propagule  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  during the *ARD* process. Factors examined included morphological factors (propagule dry mass, propagule root length, propagule root dry mass) and environmental factors (maximum temperature, mean temperature, maximum photosynthetically active radiation and daily light integral). During the course of this study, three trends were observed: 1) Little fluctuation occurred in intermittently misted propagule  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$ . 2) Fluctuations in stock plant  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  measurements that are likely due to irrigation frequency and environmental influences on stock plants. 3) Lack of differences between stock plant and propagule  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  measurements indicate a lack of water stress in propagules. It is known that misting is required to maintain proper propagule water balance, a factor supported by this study in conjunction with photosynthesis research (Chapter 3) and carbon allocation research (Chapter 4).

### *Index words*

Data logger, in-situ environment, psychrometers, vegetative propagation

## INTRODUCTION

Placing leafy stem propagules under intermittent mist or in a high humidity environment promotes high propagule success rates by preventing propagule desiccation during adventitious root development (*ARD*). Little is known about the underlying physiologic reasons why it is necessary to maintain these high humidity propagation environments, particularly the relationship between propagule water ( $\Psi$ ), osmotic ( $\Psi_s$ ) and turgor potential ( $\Psi_p$ ) and *ARD* in commercial production-style propagation environments. The lack of focus placed on propagule  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  in commercial settings could be related to the expense or difficulty in accurately measuring these propagule parameters and difficulty relating these factors to *ARD*. Despite a lack of knowledge on the roles and interactions among propagule  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$ , many suspect these factors are vital to *ARD*. LeBude et al.'s (2004) work with *Pinus taeda* L. demonstrate that maintaining a  $\Psi$  between -0.5 and -1.2 MPa is essential to successful *ARD*. LeBude et al. (2005) [*Pinus taeda* L.] further related a high rooting percentage ( $\geq 80\%$ ) to  $\Psi$  between -0.5 and -1.2 MPa, while propagules with  $\Psi$  outside this range rooted at lower percentages.

LeBude et al. (2004, 2005) [*Pinus taeda* L.], Slatyer (1960, 1967), Stirzaker et al. (1997) [*Solanum lycopersicum* L.] and Yates and Hutley (1995) [*Sloanea woollsii* F.Muell.] have demonstrated that unrooted propagules and severed branch sections have the ability to absorb water through leaf epidermal tissues or stomata; translocating this water throughout the propagule or branch section based on osmotic gradients. These studies provide a means to (at least partially) explain how water enters into a propagule prior to *ARD*, but no research has made a comparison between propagule and stock plant

$\Psi$ ,  $\Psi_s$  and  $\Psi_p$  to determine how propagule values presented compare to values observed in stock plants. This study's objectives were threefold: first to determine the morphological factors (propagule dry mass, propagule root length, propagule root dry mass) and environmental factors (maximum temperature, mean temperature, maximum photosynthetically active radiation [PAR], daily light integral [DLI]) that exert the greatest influence on leafy stem propagule  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  during *ARD*. Second, to determine how  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  of propagules are affected by adventitious root formation. Third, determine differences in stock plant and propagule  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  during *ARD*.

## MATERIALS AND METHODS

### *Plant Material Preparation*

Stock plants of *Hibiscus acetosella* (Hiern.) Welw 'Panama Red' (PP20121), *Monarda* L. 'Petite Delight' (PP10784) and *Podocarpus macrophyllus* (Thunb.) Sweet were obtained as rooted liners. Stock plants were subsequently potted into 1671 cm<sup>3</sup> containers (Griffin Greenhouse & Nursery Supplies, Inc., Ball Ground, GA) filled with substrate (Fafard Nursery Mix, Conrad Fafard, Inc., Anderson, SC) and fertilized with 15 g of a 19-6-12 plus micros, 5-6 month slow release fertilizer (Harrell's LLC, Lakeland, FL). Stock plants were allowed to acclimate to new cultural conditions for 4 weeks prior to terminal, leafy propagules being harvested. *Podocarpus* measurements were collected between February and April while *hibiscus* and *monarda* measurements were collected between June and July. All propagules were set (inserted into rooting substrate) between 0900 and 1200 HR. *Hibiscus* propagules were five nodes in length and had all but the top two fully expanded leaves removed from the stem. Using a custom-made depth gauge,

propagules were set to a depth of 3 cm. Monarda propagules were six nodes in length with all but the top four fully expanded leaves removed; propagules were set to a depth of 2.5 cm. Podocarpus propagules were 10 cm in length with all but the top eight fully expanded leaves removed. Podocarpus propagules were wounded by removing a 2 mm by 10 mm strip of epidermal tissue to expose cambial tissues and quick dipped for 10 s in 6000 mg L<sup>-1</sup> water soluble IBA (Hortus IBA Water Soluble Salts, Phytotronics, Inc, Earth City, MO) to increase rooting percentages (Dirr, 2009) and were set to a depth of 3 cm. Podocarpus propagules were placed on 21 °C bottom heat 30 d after setting due to poor root initiation. All propagules were set in 72 cell flats, one propagule per cell, filled with substrate (Fafard 3B mix, Conrad Fafard, Inc., Anderson, SC). After propagules were collected from stock plants and set, they were placed on a mist bench, shaded with a 60% shade cloth (Griffin Greenhouse & Nursery Supplies, Inc., Ball Ground, GA) and misted from 0700 to 1900 HR every 10 min for 6 s.

#### *Water Potential Measurements*

Beginning on 0 d, when propagules were set,  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  measurements were taken every 3 d for 21 d for hibiscus and monarda propagules using individually calibrated thermocouple psychrometers (76-2V, J.R.D. Merrill Specialty Equipment, Logan, UT) and every 14 d for 70 d for podocarpus propagules using individually calibrated thermocouple psychrometers (75-2V, J.R.D. Merrill Specialty Equipment,). Five propagules per replication, among three replications, and three stock plants per replication among three replications were collected at each harvest date. Prior to collecting samples, propagule foliage was blotted dry with wipes (Kimwipes, Kimberly-Clark Corp, Neenah, WI). Hibiscus and monarda leaf disc samples were taken from

interveinal tissues. Podocarpus samples utilized entire leaf width due to narrow foliage. Water potential,  $\Psi_s$  and  $\Psi_p$  stock plant measurements were taken simultaneously to serve as a baseline measurement. A 5 mm biopsy punch (Miltex, Inc., York, PA) and rubber anvil were used to remove leaf discs from foliage. To collect a leaf disc, the rubber anvil was placed beneath an attached leaf and utilizing a rocking motion the biopsy punch was used to remove a leaf disc (Turner, et al., 2000). Utilizing a custom-built ejection mechanism, leaf discs were ejected from the biopsy punch in to the psychrometer chambers, which were sealed within 5 s of harvesting. Once discs were collected and placed in psychrometers, psychrometers were stored in a styrofoam cooler until they could be submerged in a 25 °C water bath (Neslab RTE-221, Thermo Fisher Scientific, Waltham, MA). Once submerged, psychrometers were allowed to equilibrate for 4 h at 25 °C, after which  $\Psi$  measurements were collected using a data logger (CR7, Campbell Scientific Inc, Logan, Utah). Once  $\Psi$  measurements were collected, psychrometers were placed in a -14 °C freezer for 12 h. At the conclusion of 12 h, psychrometers were again submerged in a 25 °C water bath and allowed to equilibrate for 4 h and re-measured to determine  $\Psi_s$ . Osmotic potential for each psychrometer was subtracted from the  $\Psi$  to determine  $\Psi_p$  of the leaf disc ( $\Psi - \Psi_s = \Psi_p$ ).

After leaf discs were collected, propagules were harvested. At this time, root lengths were measured and propagules were placed in a drying oven at 70 °C. After a minimum of 24 h in a drying oven, propagule dry mass and root mass dry mass were collected with a digital scale (AG204 Delta-Range, Mettler-Toledo Inc., Columbus, OH).

### *Environmental Data Collection*

A PAR sensor (LI-190 Quantum Sensor, LI-COR Biosciences, Lincoln, NE) was used to measure PAR, and these data were used to calculate daily light integral (DLI) and determine daily maximum PAR. A temperature probe (107 Temperature Probe, Campbell Scientific Inc, Logan, Utah) was used to measure temperature, calculate daily mean temperature and determine daily maximum temperature. Sensors were connected to a data logger (CR10X, Campbell Scientific Inc, Logan, Utah) and sensor data was collected every 15 min for the duration of the study. During the course of the study, maximum temperature was  $26.74 \pm 0.75$  °C; mean temperature was  $23.82 \pm 1.12$  °C; maximum PAR was  $602 \pm 57$   $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; and DLI was  $13.1 \pm 2.3$   $\text{mol m}^{-2} \text{d}^{-1}$  (All errors are standard deviations).

### *Statistical Analysis*

The data were analyzed using a repeated measures, mixed model ANOVA with SAS 9.2 (SAS Institute Inc., Cary, NC) to determine differences between propagule  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  and stock plant  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  over time. The full model included tests for plant type (stock plant or propagule) and repeated measurements on plant type over time, each measurement date representing a unit of time. Replications were treated as a random effect. Pairwise t-tests were used to determine differences in plant type least square means within time periods. Multiple regression models were developed using step-wise methodology to determine correlations between propagule  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  and morphological factors (propagule dry mass, propagule root length, propagule root dry mass) and environmental factors (maximum temperature, mean temperature, maximum PAR, DLI).

## RESULTS AND DISCUSSION

During the study, monarda and podocarpus propagule  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  measurements (Mean propagule  $\Psi$ ,  $\Psi_s$  and  $\Psi_p \pm$  standard deviation [MPa]: Monarda  $\Psi = -0.68 \pm 0.22$ ,  $\Psi_s = -0.79 \pm 0.22$ ,  $\Psi_p = 0.16 \pm 0.18$ ; Podocarpus  $\Psi = -1.14 \pm 0.26$ ,  $\Psi_s = -1.46 \pm 0.16$ ,  $\Psi_p = 0.32 \pm 0.18$ ) showed little variation over time, whereas monarda and podocarpus stock plant  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  measurements (Mean stock plant  $\Psi$ ,  $\Psi_s$  and  $\Psi_p \pm$  standard deviation [MPa]: Monarda  $\Psi = -0.56 \pm 0.15$ ,  $\Psi_s = -0.71 \pm 0.24$ ,  $\Psi_p = 0.16 \pm 0.16$ ; Podocarpus  $\Psi = -1.11 \pm 0.19$ ,  $\Psi_s = -1.70 \pm 0.17$ ,  $\Psi_p = 0.61 \pm 0.21$ ) were highly variable. Psychrometer malfunctions prevented collection of hibiscus stock plant  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  preventing comparisons of hibiscus data. In this study, three distinguishable trends were evident in the data (Table 2.1).

Little fluctuation was observed in intermittent misted propagule  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$ . The formation of adventitious roots had no distinguishable effect on propagule  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  (Figs. 2.1, 2.2, 2.3). Puri and Thompson (2003) found that when observing unmisted *Populus x euramericana* (Dode) Guinier 'Robusta' propagules, newly formed adventitious roots were ineffective at absorbing and transporting water, producing little to no effect on  $\Psi$ . Puri and Thompson (2003) also demonstrated that after sufficient quantities of adventitious roots developed,  $\Psi$  stabilized. Expectations for this study were that propagule  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  would be significantly lower than stock plant  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  prior to adventitious root formation due to an inability to actively uptake the volume of water required to maintain  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$ . This was not the case, as only 4 of 42 propagule  $\Psi$ ,  $\Psi_s$  or  $\Psi_p$  measurements differed from simultaneously measured stock plant values (with two podocarpus propagule  $\Psi_s$  being higher and two podocarpus stock plant  $\Psi_p$

measurement were higher). This is likely the result of intermittent misting of propagules and passive uptake of water through the propagules' leaf epidermis and stomata due to an osmotic gradient created by maintaining a water film on propagule foliage. LeBude et al. (2004, 2005) concluded that mist increased *Pinus taeda* L. propagule  $\Psi$  through the uptake of water absorbed by the propagule's stomata. Slatyer (1960, 1967) found dew and fog affect plant leaf  $\Psi$  by entering directly into the leaf. Findings from Stirzaker et al. (1997) revealed that leaf  $\Psi$  of whole *Solanum lycopersicum* L. plants could be increased (made less negative) by misting propagule foliage. Yates and Hutley (1995) discovered similar movement of water into foliage of *Sloanea woollsi* F.Muell., as described by Limm et al. (2009), Slatyer (1960, 1967) and Stirzaker et al. (1997) [*Solanum lycopersicum* L.]. Of greater importance is Yates and Hutley's (1995) finding that water misted onto and taken up by foliage could be translocated to attached branch tissues. Once water had moved into the branch tissue it could then be translocated to unmisted foliage along a water potential gradient. The result of this physiological process is that whole cut branch section  $\Psi$  is brought into relative equilibrium over 6 h when placed under mist. This demonstrates water movement in a  $\Psi$  gradient from the leaf surface to the stem and back to deficient foliage.

Monarda propagules showed visible adventitious root formation after 9 d, yet this had no influence on propagule leaf  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  (Fig. 2.1a, 2.2a, 2.3a). Podocarpus propagules had visible adventitious root formation on 42 d, but again root formation had no influence on propagule leaf  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  (Fig. 2.1b, 2.2b, 2.3b). Hibiscus propagules rooted at 100%, monarda propagules rooted at 88% and podocarpus propagules rooted at 77%. These values are all similar to or greater than rooting percentages realized in

commercial production operations. Rooting percentages support the findings of LeBude et al. (2005) [*Pinus taeda* L.] that correlate  $\Psi$  between -0.5 and -1.2 MPa to high rooting percentages. The slightly lower rooting percentage of podocarpus (77%) could be related to a  $\Psi$  (-1.52 MPa on 0 d) which is outside this ideal range.

Regressions for propagule  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  were fitted to morphological and environmental data. No model with significant correlation could be developed. Previous studies of shade-grown *Hydrangea macrophylla* (Thunb.) Ser. daily water usage (O'Meara et al., 2011) found that DLI was consistently the major environmental factor in explaining stock plant daily water usage. In the current study, light and temperature were measured and subsequently utilized to develop  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  regression models. The resulting models were severely confounded by use of intermittent mist and secondarily by the use of shade cloth. Hess and Snyder (1955) and Langhans (1955) demonstrated propagule leaf temperatures decreased significantly under intermittent mist. Shading of propagules further cooled the leaf surface, producing a significant difference between air and leaf temperatures. While the initial expectation was that propagule  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  would exhibit a similar response to light as the daily water use in the study by O'Meara et al. (2011), it is possible that intermittent misting has a greater than expected confounding effect. Intermittent misting likely negated light and ambient air temperature effects on propagule  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$ , making them unresponsive to changing environmental conditions as well as provide an effective means to maintain  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$ .

Fluctuation in stock plant  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  measurements is due to irrigation frequency and environmental factors. There are numerous reports of the influence that soil moisture availability and environmental factors have on whole plant water potential.

Hellkvist et al. (1974) measured xylem pressure potential of field planted *Picea sitchensis* (Bong.) Carr. with a pressure chamber to examine vertical water potential gradients within trees. Results of their study found that as short-wave irradiance, vapor pressure deficit (VPD) and air temperature increase, tree  $\Psi$  became more negative. While they did not determine what factor had the greatest influence on tree  $\Psi$ , they did note typical interdependence of these factors. Specifically, as temperature increased, VPD increased, making it difficult to distinguish the effects of these correlated factors.

Additionally, Hicklenton and Cairns (1996) evaluated the effects of irrigation methods on plant water relations, specifically shoot water potential in containerized plants of *Cotoneaster dammeri* C.K. Schneid. 'Coral Beauty' and *Forsythia* Vahl 'Northern Gold'. Their study irrigated plants with subirrigation, drip, pulse or overhead methods and measured shoot  $\Psi$  at predawn, midday and dusk. They found that measurements of midday shoot  $\Psi$  provided an excellent measure of current day's water stress and was directly related to the VPD regardless of irrigation method. As VPD values increased, shoot  $\Psi$  decreased demonstrating environmental factors directly impact stock plant  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$ . Together these authors demonstrate how irrigation frequency and environmental factors influence and could potentially produce the fluctuation seen in stock plant  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  measurements in the current study.

Lack of significance between stock plant and propagule  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  measurements indicate a lack of water stress in propagules. While many studies have measured  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  in stock plants or propagules, to date no other study has simultaneously measured  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  in both stock plants and propagules grown under typical commercial conditions. As previously stated, there were only 4 of 42 total

measurements where propagule  $\Psi$ ,  $\Psi_s$  or  $\Psi_p$  differed from stock plant values. This lack of difference demonstrates a lack of water stress of propagules during *ARD*, with propagule values similar to those of stock plants. By maintaining propagule  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  at optimal levels, it appears that stomata remain open (Chapter 3), facilitating gas exchange necessary for photosynthesis. The idea of stomata remaining open is supported by the continuation of photosynthesis in propagules (Chapter 3). Without propagule photosynthesis, photosynthates that are utilized during *ARD* (Chapter 4) would not be available or greatly decreased in concentration. Lack of propagule photosynthesis would likely limit the propagules' ability to form new roots, subsequently diminishing the propagules' chances for successfully forming a new root system.

### *Summary*

This study determined that environmental and morphological factors measured in an in-situ commercial-style propagation environment have no significant correlation with propagule  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  of monarda and podocarpus. The lack of correlation was likely caused by intermittent misting and its effect on mist bench environmental conditions and propagule physiology; specifically the elimination of water stress. Adventitious root formation had no significant effect on  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  of monarda and podocarpus propagules. Initially, propagule  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  were expected to be significantly lower than values of corresponding stock plants. Based on these findings, intermittent misting potentially maintains leafy propagules  $\Psi$ ,  $\Psi_s$  and  $\Psi_p$  values until the time where roots have matured and vascular tissues are capable of translocating water, thereby preventing propagules from experiencing water stress that would be detrimental to their survival.

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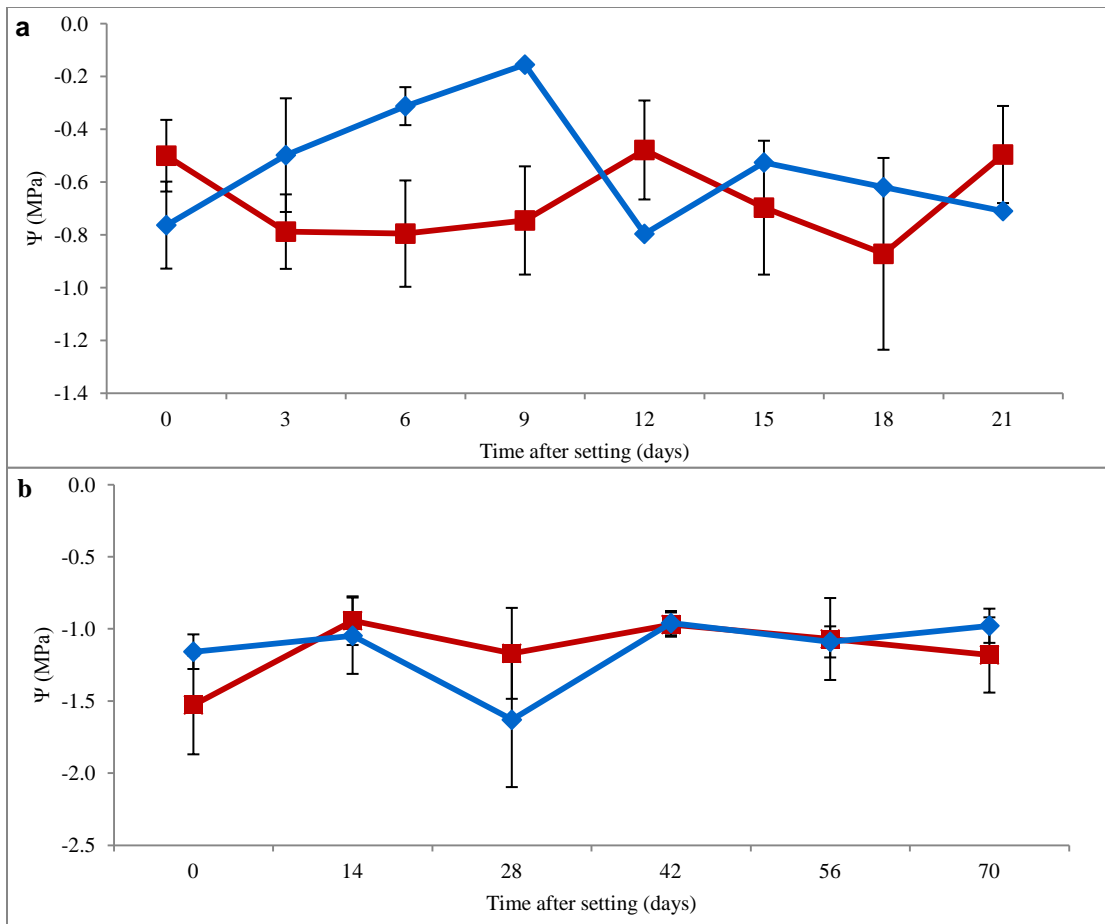


Fig. 2.1. Changes in water potential ( $\Psi$ ) of propagules and stock plants over time. Values are means with standard deviation . a. *Monarda* (n = 9), b. *Podocarpus* (n = 9). All date comparisons are non-significant.  $\blacklozenge$  = stock plants;  $\blacksquare$  = propagules

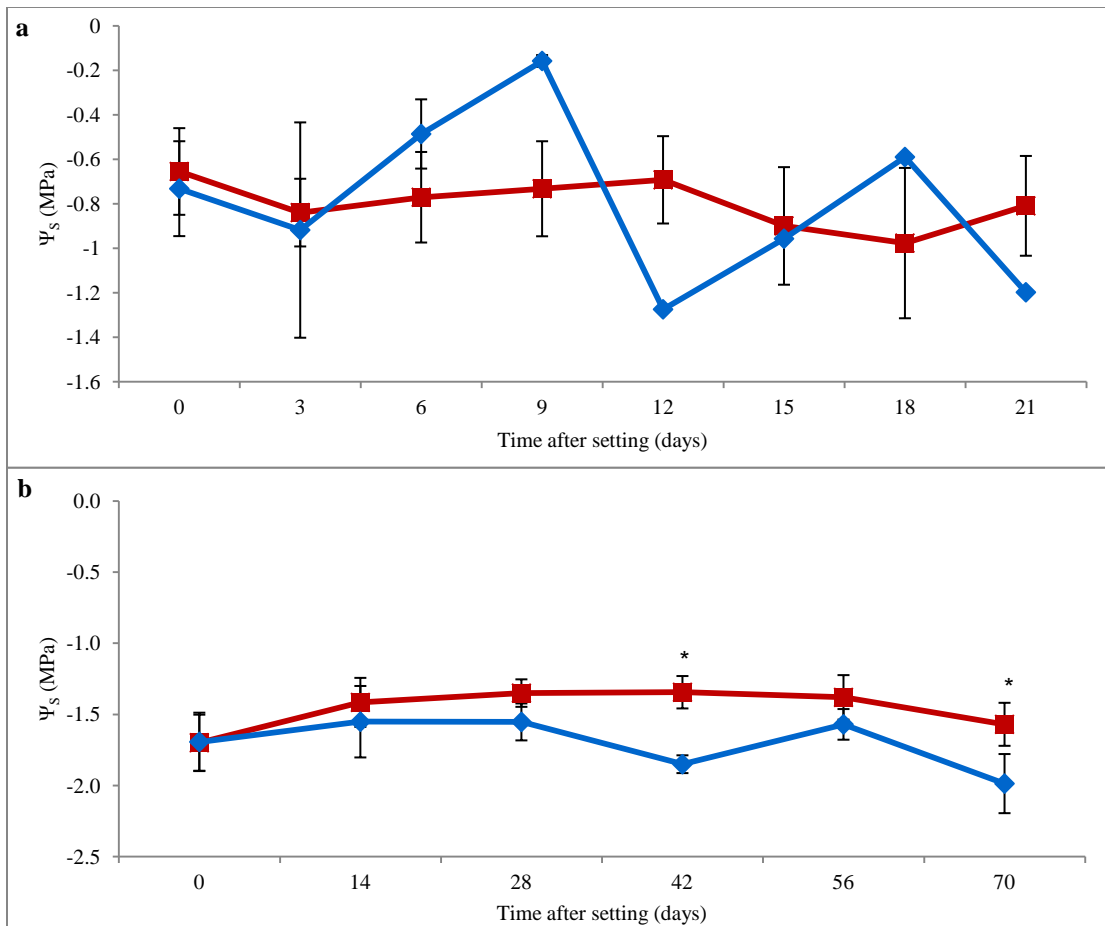


Fig. 2.2. Changes in osmotic potential ( $\Psi_s$ ) of propagules and stock plants over time. Values are means with standard deviation. a. *Monarda* ( $n = 9$ ), b. *Podocarpus* ( $n = 9$ ). \* =  $p < 0.05$ . All other date comparisons are non-significant.  $\blacklozenge$  = stock plants;  $\blacksquare$  = propagules

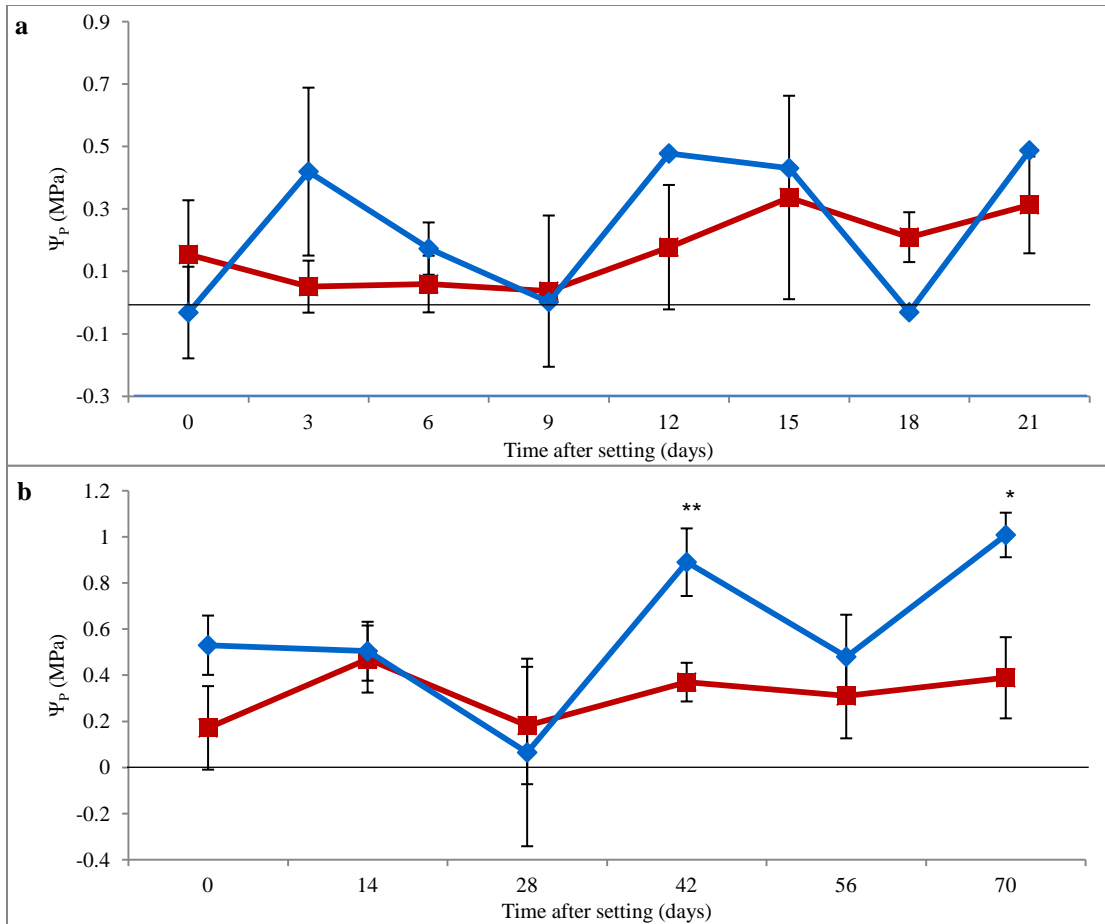


Fig. 2.3. Changes in turgor potential ( $\Psi_p$ ) of propagules and stock plants over time.

Values are means with standard deviation. a. *Monarda* (n = 9), b. *Podocarpus* (n = 9). \* =  $p < 0.5$ , \*\* =  $p < 0.01$ . All other date comparisons are non-significant.  $\blacklozenge$  = stock plants;  $\blacksquare$  = propagules

Species		Water Potential ( $\Psi$ )	Osmotic Potential ( $\Psi_s$ )	Turgor Potential ( $\Psi_p$ )
<i>Hibiscus</i>	Propagule	-0.49 $\pm$ 0.16 to -1.02 $\pm$ 0.15	-0.50 $\pm$ 0.38 to -0.99 $\pm$ 0.17	0.01 $\pm$ 0.04 to 0.16 $\pm$ 0.13
<i>Monarda</i>	Propagule	-0.48 $\pm$ 0.19 to -0.87 $\pm$ 0.36	-0.65 $\pm$ 0.19 to -0.98 $\pm$ 0.34	0.03 $\pm$ 0.24 to 0.34 $\pm$ 0.33
	Stock	-0.15 $\pm$ 0.01 to -0.79 $\pm$ 0.20	-0.16 $\pm$ 0.03 to -1.27	-0.08 $\pm$ 0.15 to 0.48
<i>Podocarpus</i>	Propagule	-0.94 $\pm$ 0.17 to -1.52 $\pm$ 0.34	-1.34 $\pm$ 0.11 to -1.70 $\pm$ 0.19	0.17 $\pm$ 0.18 to 0.47 $\pm$ 0.14
	Stock	-0.96 $\pm$ 0.08 to -1.16 $\pm$ 0.12	-1.55 $\pm$ 0.12 to -1.99 $\pm$ 0.20	0.07 $\pm$ 0.40 to 1.01 $\pm$ 0.09

Table 2.1. Maximum and minimum water potential ( $\Psi$ ), osmotic potential ( $\Psi_s$ ) and turgor potential ( $\Psi_p$ ) for hibiscus, monarda and podocarpus propagules and stock plants during course of study as presented in Fig. 2.1, 2.2, 2.3. All values in MPa, errors are standard deviations.

CHAPTER 3  
CHANGES IN LEAFY STEM PROPAGULE NET PHOTOSYNTHESIS DURING  
ADVENTITIOUS ROOT DEVELOPMENT <sup>1</sup>

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<sup>1</sup> Martin, Jr., M.T., M.R. Chappell and J.A. Porter. To be submitted to *Journal of the American Society for Horticultural Science*.

## ABSTRACT

An increased understanding of environmental and morphologic factors that influence adventitious root development (*ARD*) could help ornamental producers improve vegetative propagation success rates. The objectives of this study were to determine propagule net photosynthesis ( $P_n$ ), stomatal conductance ( $g_s$ ) and internal CO<sub>2</sub> concentration ( $C_i$ ) and how these factors affect *ARD* of *Hibiscus acetosella* (Hiern.) Welw 'Panama Red' (PP20121), *Monarda* L. 'Petite Delight' (PP10784) and *Podocarpus macrophyllus* (Thunb.) Sweet. Quantifying these factors will also determine if there are differences between propagule and stock plant  $P_n$ ,  $g_s$  and  $C_i$  during *ARD* as well as if and/or when propagule  $P_n$ ,  $g_s$  and  $C_i$  return to a rate similar to that of stock plants. By quantifying  $P_n$ ,  $g_s$  and  $C_i$  in conjunction with morphological and environmental factors; factors having the greatest influence on  $P_n$ ,  $g_s$  and  $C_i$  could be discerned. Stock plant  $P_n$  was significantly higher than propagule  $P_n$  for hibiscus and monarda and no significant difference among stock plant  $P_n$  and propagule  $P_n$  was observed for podocarpus. Stomatal conductance of propagules was typically lower and only being significant higher in hibiscus propagules. Rarely was  $C_i$  significantly different between propagule and stock plant and the  $C_i$  pattern over time did not follow a trend. Net photosynthesis and  $g_s$  of hibiscus propagules were positively correlated with propagule root length and daily light integral (DLI).

### *Index words*

Internal CO<sub>2</sub> concentration, stem propagule, stomatal conductance, vegetative propagation

## INTRODUCTION

Asexual propagation of plants utilizing leafy stem propagules has long been at the foundation of horticultural and agroforestry industries that rely on maintaining genetic stability in elite cultivars. The ability to produce genetically identical plants ensures consistency in production and uniformity in appearance. As important as the adventitious root development (*ARD*) process is, many key physiologic mechanisms that induce this process and continue during *ARD* remain unexplained. More specifically, a fundamental aspect of plant growth and development is photosynthesis, but a thorough understanding of propagule photosynthesis and how it influences *ARD* is still warranted. It is widely accepted (Costa et al., 2001 [*Rosa hybrida* L. 'Madelon']) that propagules photosynthesize throughout the *ARD* process. It is still undetermined exactly what role propagule photosynthesis and propagule photosynthates play in the development of adventitious roots. Numerous authors (Davis and Potter, 1981 [*Pisum sativum* L.]; Hoad and Leakey, 1996 [*Eucalyptus grandis* (Maiden) W. Hill]; Rapaka et al., 2005 [*Pelargonium × hortorum* L.H. Bailey 'Isabell']; Yue and Margolis, 1993 [*Picea mariana* (Mill.) B.S.P.]) suggest that propagule photosynthesis provides photosynthates that are utilized during *ARD* and an inadequate supply of photosynthates can be a limiting factor in root initiation and development. Eliasson and Brunes (1980) [*Populus tremula × tremuloides* and *Salix caprea × viminalis* Willdenow] emphasized that providing the correct environmental conditions to allow propagule photosynthesis was essential for successful *ARD*. Despite providing ideal conditions for *ARD*, Fordham et al. (2001) [*Corylus maxima* Mill.] and Mesén et al (1997) [*Cordia alliodora* (Ruiz & Pavon) Oken] observed that, at severance from stock plant(s), leafy stem propagules immediately

experienced shock, significantly decreasing propagule photosynthetic activity and stomatal control. Determining how environmental and morphologic factors influence net photosynthesis ( $P_n$ ) could prove highly beneficial to understanding the *ARD* process and increasing propagule success rates.

Propagule  $P_n$  and effects of propagule  $P_n$  on *ARD* have been reported for numerous species. To date, these results have not been reported for *Hibiscus*, *Monarda*, or *Podocarpus*. The objectives of this study were to determine propagule  $P_n$  in three distinct species and the relationship between  $P_n$  and *ARD* for these species. It was expected that propagule  $P_n$  values would initially decline and begin increasing as *ARD* began, eventually returning to values similar to that of stock plants. In addition to  $P_n$ , this study measured two additional factors influencing  $P_n$ , stomatal conductance ( $g_s$ ) and internal  $\text{CO}_2$  concentration ( $C_i$ ). This study also concurrently compared and contrasted these propagule measurements to measurements of stock plant  $P_n$ ,  $g_s$  and  $C_i$ . Measuring  $P_n$ ,  $g_s$  and  $C_i$  concurrently allowed for more clarity in results and a better ability to correlate realistic production-based morphological factors (propagule dry mass, propagule root length, propagule root dry mass) and environmental factors (maximum photosynthetically active radiation [PAR], maximum temperature, mean temperature, daily light integral [DLI]) to propagule  $P_n$ ,  $g_s$  and  $C_i$ . All measurements in the current study occurred *in-situ*, unlike many previous studies (Okoro and Grace, 1976 [*Populus* × *euramericana* (Dode) Guinier]; Smalley et al., 1991 [*Acer rubrum* L.]; Svenson and Davies, 1990 [*Euphorbia pulcherrima* (Klotzsch) Willd.]) that removed propagules from the propagation environment to collect measurements, altering environmental conditions

(relative humidity, light levels, etc) that directly impact physiological processes and characteristics (stomatal opening) of the propagule.

## MATERIALS AND METHODS

### *Plant Material Preparation*

Stock plants of *Hibiscus acetosella* (Hiern.) Welw 'Panama Red' (PP20121), *Monarda* L. 'Petite Delight' (PP10784) and *Podocarpus macrophyllus* (Thunb.) Sweet were obtained as rooted liners. Stock plants were subsequently potted into 1671 cm<sup>3</sup> containers (Griffin Greenhouse & Nursery Supplies, Inc., Ball Ground, GA) filled with substrate (Fafard Nursery Mix, Conrad Fafard, Inc., Anderson, SC) and fertilized with 15 g of a 19-6-12 plus micros, 5-6 month slow release fertilizer (Harrell's LLC, Lakeland, FL). Stock plants were allowed to acclimate to new cultural conditions after potting for 4 weeks prior to terminal, leafy propagules being harvested. *Podocarpus* measurements were collected between February and April while hibiscus and monarda measurements were collected between June and July. All propagules were set (inserted into rooting substrate) between 0900 and 1200 HR. Hibiscus propagules were five nodes in length and had all foliage below the top two fully expanded leaves removed from the stem. Using a custom-made depth gauge, propagules were set to a depth of 3 cm. *Monarda* propagules were six nodes in length with all foliage below the top four fully expanded leaves removed; propagules were set to a depth of 2.5 cm. *Podocarpus* propagules were 10 cm in length with all foliage below the top eight fully expanded leaves removed. *Podocarpus* propagules were wounded by removing a 2 mm by 10 mm strip of epidermal tissue to expose cambial tissues and quick dipped for 10 s in 6000 mg L<sup>-1</sup> water soluble IBA

(Hortus IBA Water Soluble Salts, Phytotronics, Inc, Earth City, MO) to increase rooting percentages (Dirr, 2009) and were set to a depth of 3 cm. Podocarpus propagules were placed on 21 °C bottom heat 30 d after setting due to poor root initiation. All propagules were set in 72 cell flats, one propagule per cell, filled with substrate (3B mix, Conrad Fafard, Inc., Anderson, SC). After propagules were set they were placed on a mist bench, shaded with a 60% shade cloth (Griffin Greenhouse & Nursery Supplies, Inc., Ball Ground, GA) and misted from 0700 to 1900 HR every 10 min for 6 s.

*Net photosynthesis , stomatal conductance and intercellular CO<sub>2</sub> concentration measurements*

Beginning on 0 d (when propagules were set)  $P_n$ ,  $g_s$  and  $C_i$  measurements were taken every 3 d for 21 d for hibiscus and monarda and every 14 d for 70 d for podocarpus using a photosynthesis measurement system (LI-6400 Portable Photosynthesis System, LI-COR Environmental, Lincoln, NE). Prior to collecting measurements, propagule foliage was blotted dry with wipes (Kimwipes, Kimberly-Clark Corp, Neenah, WI). Net photosynthesis,  $g_s$  and  $C_i$  measurements (calculations) of propagules and their stock plants were made concurrently. Post-propagule severance, propagules and stock plants were slow to develop new foliage that was of a suitable size to measure. To compensate for this, the most recently fully expanded leaf of each propagule and stock plant was selected at the start of the study and these most recently fully expanded leaves were used for each subsequent measurement. All foliage utilized for measurements were harvested at the study's conclusion and scanned with a scanner (X6675 All-in-One Printer, Lexmark International, Inc., Lexington, KY). Once scanned, leaf area was determined using software (ImageJ, US National Institutes of Health, Bethesda, MD). Calculated leaf

area values were entered into LI-6400 Simulator Software v.5.3 (LI-COR Environmental, Lincoln, NE) to recalculate  $P_n$ ,  $g_s$  and  $C_i$  based on corrected leaf area. Measurements of  $P_n$ ,  $g_s$  and  $C_i$  took place between 1030 and 1400 HR. LI-6400 leaf chamber light level was set to  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$  for both propagule and stock plant to ensure both plant groups were being measured at similar rates and conditions. This light level,  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ , was the mean PAR measurement, measured for 30 d prior to study, measured using a PAR sensor (LI-190 Quantum Sensor, LI-COR Biosciences, Lincoln, NE), for propagules under shade cloth. This value provided enough PAR for photosynthesis of both propagules and stock plants but minimized potential damage to propagule photosystems. Leaf chamber air temperature was set to ambient temperature and  $\text{CO}_2$  concentration was supplemented to  $400 \mu\text{mol mol}^{-1}$ . Fan speed of leaf chamber was set to high and flow was  $500 \mu\text{mol s}^{-1}$ . Six propagules per three replications per species were utilized for  $P_n$ ,  $g_s$  and  $C_i$  measurements. While  $P_n$ ,  $g_s$  and  $C_i$  measurements were collected, the overhead mist system was turned off in 10 min intervals to prevent accidental misting of the LI-6400. At the conclusion of 10 min, the LI-6400 was removed from the mist bench area and propagules were misted to prevent desiccation.

#### *Environmental Data Collection*

A PAR sensor (LI-190 Quantum Sensor, LI-COR Biosciences, Lincoln, NE) was used to measure PAR, and these data were used to calculate daily light integral (DLI) and determine daily maximum PAR. A temperature probe (107 Temperature Probe, Campbell Scientific Inc, Logan, Utah) was used to measure temperature, calculate daily mean temperature and determine daily maximum temperature. Sensors were connected to a data logger (CR10X, Campbell Scientific Inc, Logan, Utah) and sensor data was

collected every 15 min for the duration of the study. During the course of the study, maximum temperature was  $26.74 \pm 0.75$  °C; mean temperature was  $23.82 \pm 1.12$  °C; maximum PAR was  $602 \pm 57$   $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; and DLI was  $13.1 \pm 23$   $\text{mol m}^{-2} \text{d}^{-1}$ . (All errors are standard deviations).

### *Statistical Analysis*

Data were analyzed using repeated measures, mixed model ANOVA with SAS 9.2 (SAS Institute Inc., Cary, NC) to determine differences between propagule  $P_n$ ,  $g_s$  and  $C_i$  values and stock plant  $P_n$ ,  $g_s$  and  $C_i$  values. The full model included tests for plant type (stock plant or propagule) and repeated measurements on plant type over time with each measurement date representing a unit of time. Replications were treated as a random effect. Pairwise t-tests were used to determine differences in plant type least square means within time periods. Pearson correlations and multiple regression models were developed to determine correlation effects between propagule  $P_n$ ,  $g_s$  and  $C_i$  values and morphological data (propagule dry mass, propagule root length, propagule root dry mass) and environmental data (maximum PAR, maximum temperature, mean temperature and DLI).

## RESULTS AND DISCUSSION

Propagule  $P_n$  values of this study (Mean propagules  $P_n \pm$  standard deviation [ $\mu\text{mol m}^{-2} \text{s}^{-1}$ ]: Hibiscus  $5.49 \pm 1.62$ ; Monarda  $3.78 \pm 2.69$ ; Podocarpus  $1.85 \pm 0.99$ ) (Table 3.1) were similar to those reported by Svenson and Davies (1990) who rooted *Euphorbia pulcherrima* (Klotzsch) Willd. propagules in growth chambers under varying light intensities and reported photosynthetic rate between 1.0 to 9.0  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Similar

propagule photosynthetic rate (0 to 6  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) are reported by Newton et al. (1992) who rooted propagules of *Terminalia spinosa* Engl. in humidity tents without overhead mist. Mesén et al. (1997) rooted leafy stem propagules of *Cordia alliodora* (Ruiz and Pav.) Oken using a similar propagation structure as Newton et al. (1992) and reported photosynthetic rate between 0.68 and 6.70  $\mu\text{mol m}^{-2} \text{s}^{-1}$ .

Formation of adventitious roots had no significant effect on monarda or podocarpus propagule  $P_n$ . *ARD* did have a significant effect on hibiscus propagule  $P_n$ . Adventitious roots were not visible on hibiscus until 9 d, but an increase in net photosynthesis occurred between 3 d and 6 d, similar to findings of Humphries and Thorne (1964) and Svenson and Davies (1990). Humphries and Thorne (1964) found that photosynthetic rate increased in *Phaseolus vulgaris* L. leaf propagules prior to visible *ARD*. Svenson and Davies (1990) found increases in photosynthetic rate of *Euphorbia pulcherrima* (Klotzsch) Willd. prior to visible *ARD*. Svenson and Davies (1990) noted that propagules with increasing photosynthetic rate prior to visible root formation had root initials and primordia present in microscopic rooting-zone analysis; possibly explaining the increase in  $P_n$  in the current study. There was a strong correlation ( $R = 0.72$ ,  $p < 0.0001$ ) between root mass (root dry mass) and propagule  $P_n$  in hibiscus species which rooted quickly and developed a large root mass, than in those species that either produced smaller root mass (monarda, no correlation) or rooted at slower rates (podocarpus  $R = 0.57$ ,  $p = 0.01$ ) (Fig. 3.1, Table 3.2)

No previous research has reported direct comparisons of stock plant  $P_n$  to propagule  $P_n$ . In the current study, when propagules were severed from stock plants, they showed an immediate decrease in  $P_n$  when compared to the stock plant from which they

were harvested (Figure 3.2, 0 d). This decrease was significant in hibiscus ( $p = 0.007$ ) but non-significant in monarda. Subsequent measurement of monarda propagules and stock plants showed a significant decrease in propagule  $P_n$  from 0 d to 3 d. Hibiscus propagule  $P_n$  was lower than stock plant  $P_n$  through 15 d, after which propagule  $P_n$  remained slightly higher than stock plant  $P_n$  until the study's conclusion. This decrease in hibiscus stock plant  $P_n$  is possibly the effect of leaf ageing. Similar trends of decreasing  $P_n$  were observed in *Gossypium hirsutum* L. 'Deltapine' plants as their foliage aged (Constable and Rawson, 1980). From 3 d, monarda propagule  $P_n$  was significantly lower than stock plant  $P_n$ . At no time was there any significant difference between podocarpus propagule and stock plant  $P_n$ . This trend of decreasing propagule  $P_n$  has been reported by numerous authors (Davis and Potter, 1981 [*Pisum sativum* L.]; Okoro and Grace, 1976 [*Populus × euramericana* (Dode) Guinier]; Smalley et al, 1991 [*Acer rubrum* L.]; Svenson and Davies, 1990 [*Euphorbia pulcherrima* (Klotzsch) Willd.]); a decrease that Loach (1988) related to stomatal closure from water stress experienced by propagules. However, this was not the case in this study, as propagules showed no water stress during this study (Chapter 1) and possibly a hormonal response.

Propagule  $g_s$  values in this study (Mean propagules  $g_s \pm$  standard deviation [mmol  $m^{-2} s^{-1}$ ]: Hibiscus  $157.65 \pm 78.65$ ; Monarda  $96.07 \pm 54.72$ ; Podocarpus  $20.36 \pm 9.75$ ) (Table 3.1) were similar to those reported by Fordham et al. (2001), who reported  $g_s$  values between 200 to 400 mmol  $m^{-2} s^{-1}$  for *Corylus maxima* Mill. propagules grown under intermittent fogging over a 21 d period. Smalley et al. (1991) reported  $g_s$  values between 4 to 262 mmol  $m^{-2} s^{-1}$  for *Acer rubrum* L. propagules grown under intermittent misting over 30 d and 56 d periods. Stomatal conductance values between 275 and 625

mmol m<sup>-2</sup> s<sup>-1</sup> were reported for single node propagules of *Cordia alliodora* (Ruiz & Pavon) Oken under no mist (Mesén et al. 1997). Severance of propagules from stock plants caused a reduction in  $g_s$  in hibiscus and monarda propagules (Fig. 3). As with  $P_n$ , an immediate and significant decrease in hibiscus propagule  $g_s$  was observed after setting, while monarda propagule  $g_s$  did not show a significant decrease until 3 d after setting. Gay and Loach (1977) observed a decrease in leaf conductance in *Cornus alba* L. within 2.25 h of setting propagules and within 6 h of setting *Rhododendron* L. propagules. These results agree with our findings that there are differences among species in response time to severance from stock plants. Stomatal conductance of monarda and podocarpus propagules remained lower than stock plant  $g_s$  throughout the study, yet the difference was rarely significant. Hibiscus propagule  $g_s$  was significantly lower than stock plant  $g_s$  between 0 d and 12 d but on 15 d there was no significant difference. On 18 d and 21 d hibiscus propagule  $g_s$  was significantly higher than stock plant  $g_s$ . The observed decrease in hibiscus stock plant  $g_s$  is likely the effect of leaf aging on stock plant foliage (Constable and Rawson, 1980 [*Gossypium hirsutum* L. 'Deltapine']).

Stomatal conductance of hibiscus and podocarpus begins to increase once *ARD* initiates, with stomatal conductance being correlated with root mass (root dry mass) for both species (Table 3.2: hibiscus  $R = 0.73$ ,  $p < 0.0001$ ; podocarpus  $R = 0.57$ ,  $p = 0.01$ ). Gay and Loach (1977) also noted increases of *Cornus* leaf conductance did not occur until after *ARD*. Observed  $g_s$  for both podocarpus propagules and stock plants were very low and remained low throughout the study, while also remaining very consistent throughout the study.

Intercellular CO<sub>2</sub> concentrations (Fig. 3.4) of hibiscus stock plants were significantly higher ( $p = 0.01$ ) than propagule  $C_i$  on 9 d. Hibiscus propagule  $C_i$  was significantly higher than stock plant  $C_i$  on 18 d ( $p = 0.003$ ) and 21 d ( $p < 0.0001$ ). This decline in hibiscus stock plant  $C_i$  on 18d and 21 d are possibly due to advanced leaf age; Constable and Rawson (1980) reported a decrease of  $g_s$  in *Gossypium hirsutum* L. ‘Deltapine’ foliage as they aged this decrease is similar to  $g_s$  decreases in hibiscus. This decrease in  $g_s$  could account for a decrease in  $C_i$  as a lack of conductance would result in lower  $C_i$  levels. Monarda propagule  $C_i$  was significantly higher ( $p = 0.03$ ) than stock plant  $C_i$  on 21 d of the study, with monarda propagule  $C_i$  for all other measurements similar to stock plant  $C_i$ . At no time was there a significant difference between podocarpus stock plant and propagule  $C_i$ .

Only two significant models relating physiological responses to morphological and environmental factors could be developed; hibiscus  $P_n$  and  $g_s$ . Net photosynthesis during *ARD* of hibiscus propagules were positively correlated (Fig. 3.5) ( $r^2 = 0.63$ ,  $p < 0.0001$ ) with propagule root length (Partial  $r^2 = 0.53$ ,  $p < 0.0001$ ) and DLI (Partial  $r^2 = 0.1$ ,  $p = 0.002$ ). Stomatal conductance of hibiscus propagules were also positively correlated with increasing propagule root length and increasing DLI. Partial  $r^2$  measures the contribution of one explanatory variable to the “total”  $r^2$  value. The positive correlation between  $P_n$  and root development supported by Davis and Potter (1981), who rooted *Pisum sativum* L. in growth chambers. Propagules were placed in assimilation chambers to measure net photosynthesis and their work demonstrated that as photoperiod increases, rooting increases, and that basal propagule portions had higher concentrations of sucrose and glucose. The importance of DLI in the *ARD* process is also supported by

Davis and Potter (1981), who found positive correlations between photosynthetic photon flux density and photoperiods and *ARD*. These two factors are utilized in the calculation of DLI. Gay and Loach (1977) also support the current study's findings of a positive effect of increasing DLI on  $P_n$  of propagules. Their work with *Cornus* propagules produced under various tenting, shading, misting and antitranspirant treatments in a greenhouse concluded *ARD* must depend upon reserve carbohydrates rather than newly produced carbohydrates; a conclusion based on decreased  $g_s$  during *ARD* (contrary to findings in Chapter 4).

In correlating root development and *A* (and associated photosynthetic products), the findings of Breen and Muraoka (1974) [*Prunus cerasifera* x *munsoniana* 'Marianna 2624'] contradict this study as well as those of Davis and Potter (1981) [*Pisum sativum* L.] and Gay and Loach (1977) [*Cornus alba* L. and *Rhododendron* L.]. Breen and Muraoka (1974) utilized  $^{14}\text{C}$  labeling of *Prunus cerasifera* x *munsoniana* 'Marianna 2624'. propagules to determine photosynthate production and translocation, finding that little  $^{14}\text{C}$  was translocated to the basal portion of the propagules. However, Breen and Muraoka (1974) only exposed the top most (youngest) propagule leaf to  $^{14}\text{CO}_2$ , potentially confounding their findings, as treated foliage may not have been fully expanded and photosynthesizing at a maximum rate. Additionally,  $^{14}\text{CO}_2$  exposure time may have been too brief to allow sufficient  $^{14}\text{CO}_2$  uptake, foliage exposed to  $^{14}\text{CO}_2$  was removed after 20 h. Additionally, leaf water potential ( $\Psi_{\text{LEAF}}$ ) (Chapter 1) of each species was compared against its respective propagule  $P_n$  (Dang et al., 1997 [*Pinus banksiana* Lamb., *Picea mariana* (Mill.) B.S.P., and *Populus tremuloides* Michx.]; Ehleringer and Cook, 1984 [*Encelia farinosa* Gray]; Hubbard et al., 1999 [*Pinus ponderosa* P.& C.

Lawson]; Reich and Hinckley. 1989 [*Quercus marilandica* Muenchh. and *Quercus rubra* Lam.]). There was no significant correlation between  $P_n$  and  $\Psi_{\text{LEAF}}$  for any of the species.

### *Summary*

This research further demonstrates that photosynthesis occurs during the *ARD* process (see  $^{13}\text{C}$  sequestering during *ARD*, Chapter 4), from 0 d after separation from the stock plant until the conclusion of the study. As expected, propagule  $P_n$  and  $g_s$  values initially declined but only in hibiscus and monarda. This decline was not related to water stress [see Chapter 2 for water ( $\Psi$ ), osmotic ( $\Psi_s$ ) and turgor potential ( $\Psi_p$ ) values during *ARD*]. *ARD* produced an increase in  $P_n$  of hibiscus propagules, but showed no effect on monarda and podocarpus propagules. There were significant differences between stock plant  $P_n$  and propagule  $P_n$  for hibiscus and monarda, but no significant difference between stock plant  $P_n$  and propagule  $P_n$  for podocarpus. Propagule  $P_n$  was typically lower than stock plant  $P_n$ .

$P_n$  returned to a rate similar to that of stock plants only in hibiscus, a fact that may be due to leaf aging on hibiscus stock foliage utilized in this study. Stomatal conductance of propagules was typically lower in propagules; with the difference being consistently and significantly lower only in hibiscus prior to *ARD* development. Rarely was  $C_i$  significantly different between stock plant and propagule. Podocarpus propagule and stock plant  $P_n$ ,  $g_s$  and  $C_i$  never differed significantly. Species that rooted quickly and produced a comparably larger root mass (hibiscus) demonstrated the greatest positive effect on propagule  $P_n$  late in the *ARD* process. Species that produced comparably smaller root mass or rooted more slowly had virtually no effect on propagule  $P_n$  at any point in the *ARD* process.

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Species		Net photosynthesis ( $P_n$ )	Stomatal Conductance ( $g_s$ )	Intercellular CO <sub>2</sub> Concentration ( $C_i$ )
<i>Hibiscus</i>	Propagule	1.63 ± 0.57 to 9.26 ± 0.68	23.03 ± 7.43 to 358.4 ± 43.7	240.3 ± 36.8 to 336.4 ± 5.1
	Stock	6.58 ± 2.01 to 13.07 ± 0.68	113.3 ± 32.1 to 341.3 ± 82.8	234.7 ± 24.8 to 310 ± 11.8
<i>Monarda</i>	Propagule	1.52 ± 0.59 to 14.75 ± 1.63	43.86 ± 22.2 to 270.8 ± 64.8	279 ± 18.5 to 328.1 ± 20.6
	Stock	5.95 ± 2.65 to 15.59 ± 3.95	102.9 ± 49.5 to 293.4 ± 68.7	263.5 ± 24.4 to 290.8 ± 20.5
<i>Podocarpus</i>	Propagule	-0.38 ± 1.27 to 3.13 ± 0.85	8.71 ± 1.39 to 25.45 ± 4.21	223.3 ± 39.3 to 291.7 ± 23.1
	Stock	2.01 ± 1.08 to 6.29 ± 3.68	12.11 ± 6.99 to 41.2 ± 21	177 ± 15.6 to 276 ± 57.9

Table 3.1. Maximum and minimum net photosynthesis ( $P_n$ ) ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), stomatal conductance ( $g_s$ ) ( $\text{mmol m}^{-2} \text{s}^{-1}$ ) and intercellular CO<sub>2</sub> concentration ( $C_i$ ) ( $\mu\text{mol mol}^{-1}$ ) for hibiscus, monarda and podocarpus propagules and stock plants during course of study as presented in Fig. 3.1, 3.2, 3.3. Values are means with standard deviation.

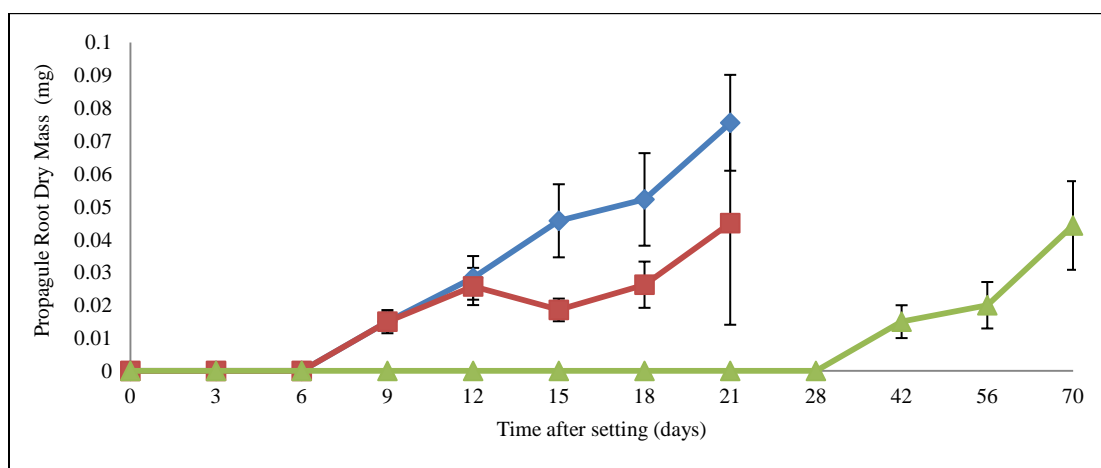


Fig. 3.1. Changes in propagule root dry mass over time. Values are means with standard deviation error bars ( $n = 9$ ).  $\blacklozenge$  = Hibiscus;  $\blacksquare$  = Monarda;  $\blacktriangle$  = Podocarpus

Species		Propagule Dry Mass	Root Length	Root Dry Mass	DLI	Maximum PAR	Maximum Temperature	Mean Temperature
<i>Hibiscus</i>	$P_n$	0.69 / < 0.0001	0.73 / < 0.0001	0.72 / < 0.0001	0.58 / < 0.0001	0.53 / < 0.0001	0.64 / < 0.0001	0.74 / < 0.0001
	$g_s$	0.53 / < 0.0001	0.76 / < 0.0001	0.73 / < 0.0001	0.46 / < 0.0001	0.41 / 0.0004	0.51 / < 0.0001	0.59 / < 0.0001
	$C_i$	0.21 / ns	0.68 / < 0.0001	0.64 / < 0.0001	0.29 / 0.01	0.26 / 0.03	0.2 / ns	0.18 / ns
<i>Monarda</i>	$P_n$	-0.55 / < 0.0001	0.08 / ns	0.1 / 0.5	-0.74 / < 0.0001	-0.78 / < 0.0001	-0.52 / < 0.0001	-0.28 / 0.02
	$g_s$	-0.41 / 0.0003	0.22 / 0.1	0.24 / 0.1	-0.63 / < 0.0001	-0.64 / < 0.0001	-0.35 / 0.002	-0.17 / ns
	$C_i$	0.24 / 0.04	0.14 / ns	0.15 / ns	0.15 / ns	0.2 / ns	0.3 / 0.01	0.25 / 0.03
<i>Podocarpus</i>	$P_n$	0.37 / 0.03	0.59 / 0.009	0.57 / 0.01	0.18 / ns	0.18 / ns	-0.06 / ns	-0.19 / ns
	$g_s$	0.14 / ns	0.63 / 0.005	0.67 / 0.002	-0.09 / ns	-0.06 / ns	0.13 / ns	0.02 / ns
	$C_i$	-0.1 / ns	-0.42 / 0.08	-0.32 / ns	0.14 / ns	0.09 / ns	0.14 / ns	0.3 / ns

Table 3.2. Correlation of hibiscus, monarda and podocarpus propagule net photosynthesis ( $P_n$ ), stomatal conductance ( $g_s$ ) and intercellular CO<sub>2</sub> concentration ( $C_i$ ) to environmental and morphologic factors. Results are R values / p-values. ns = non-significant.

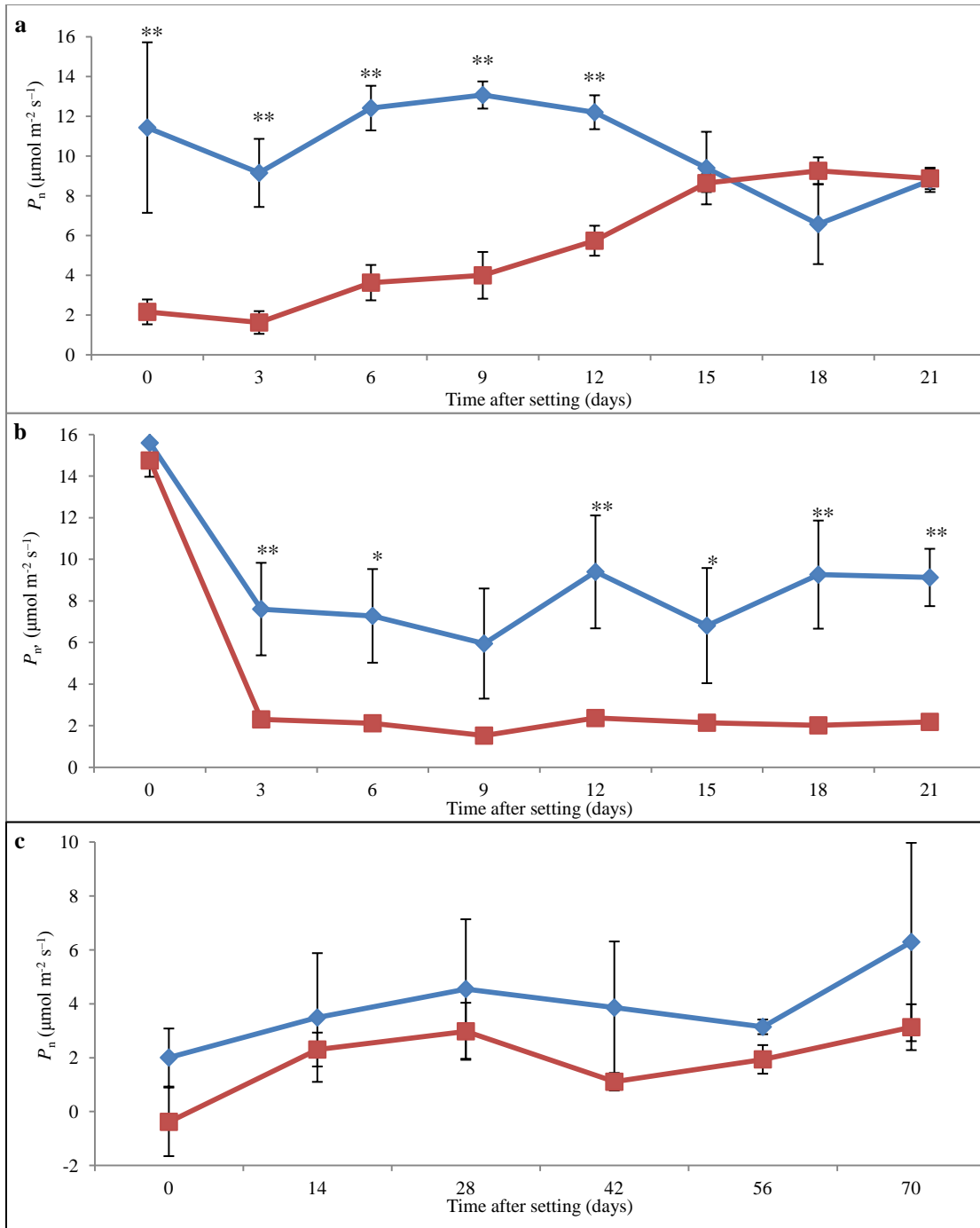


Fig. 3.2. Changes in net photosynthesis ( $P_n$ ) of propagules and stock plants over time.

Values are means with standard deviation error bars. a. Hibiscus (n = 9), b. Monarda (n =

9), c. Podocarpus (n = 6), no significance for Podocarpus. \* =  $p < 0.05$ , \*\* =  $p < 0.01$ .  $\blacklozenge$

= stock plants;  $\blacksquare$  = propagules

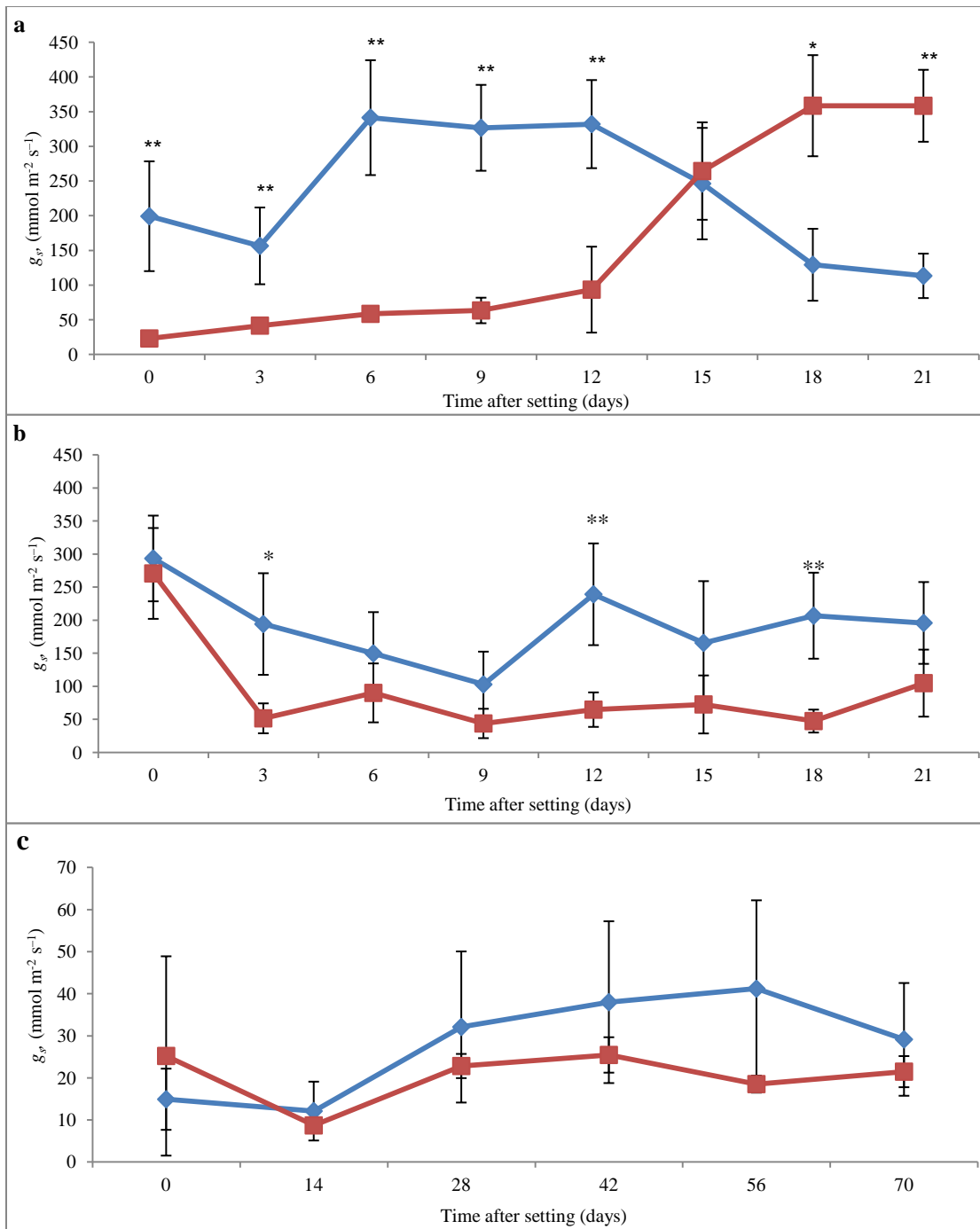


Fig. 3.3. Changes in stomatal conductance ( $g_s$ ) of propagules and stock plants over time. Values are means with standard deviation error bars. a. Hibiscus (n = 9), b. Monarda (n = 9), c. Podocarpus (n = 6), no significance for Podocarpus. \* =  $p < 0.5$ , \*\* =  $p < 0.01$ .  $\blacklozenge$  = stock plants;  $\blacksquare$  = propagules

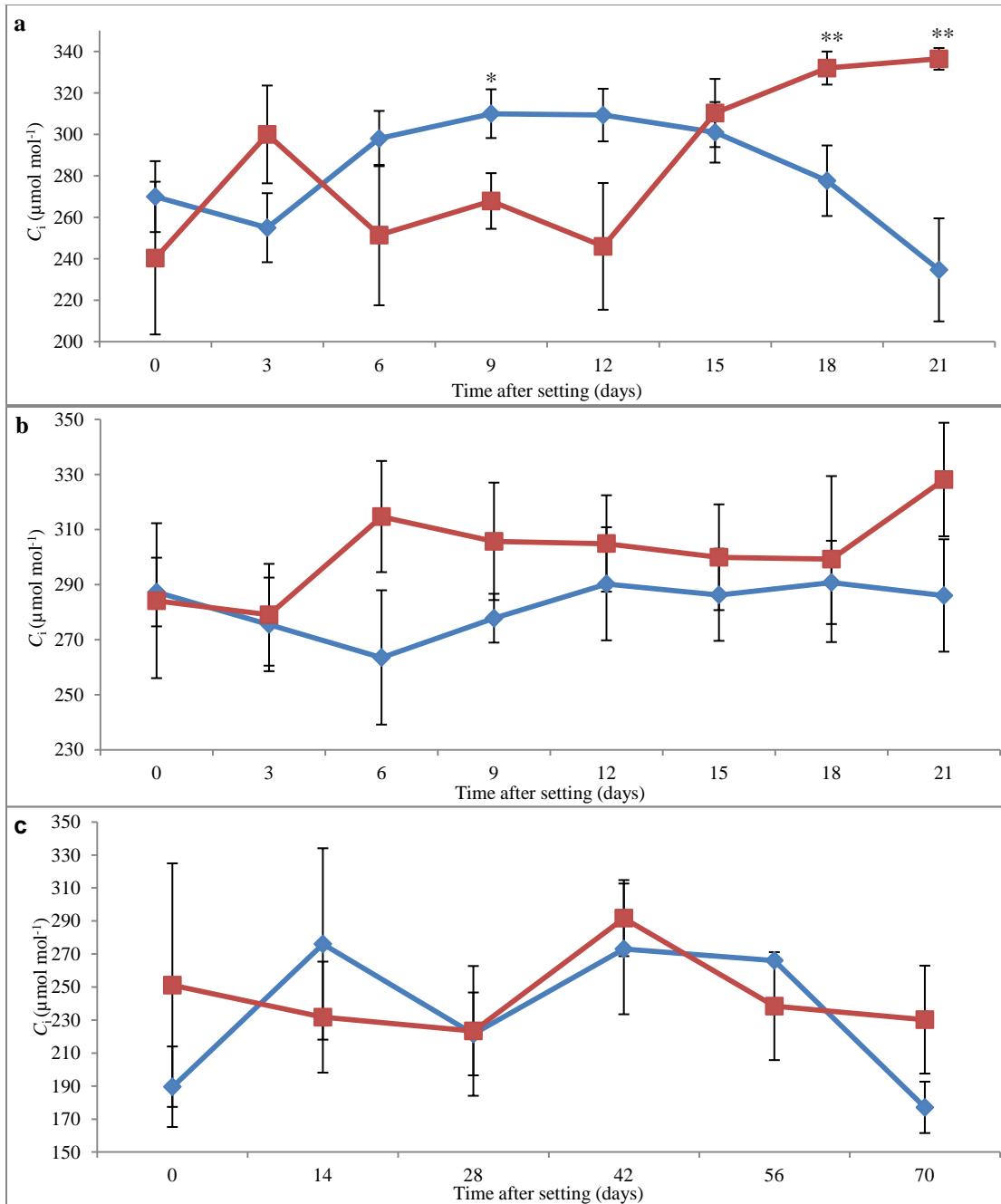


Fig. 3.4. Changes in intercellular CO<sub>2</sub> concentration ( $C_i$ ) of propagules and stock plants over time. Values are means with standard deviation error bars. a. Hibiscus (n = 9), b. Monarda (n = 9), c. Podocarpus (n = 6), no significance for Monarda and Podocarpus. \* =  $p < 0.5$ , \*\* =  $p < 0.01$ .  $\blacklozenge$  = stock plants;  $\blacksquare$  = propagules

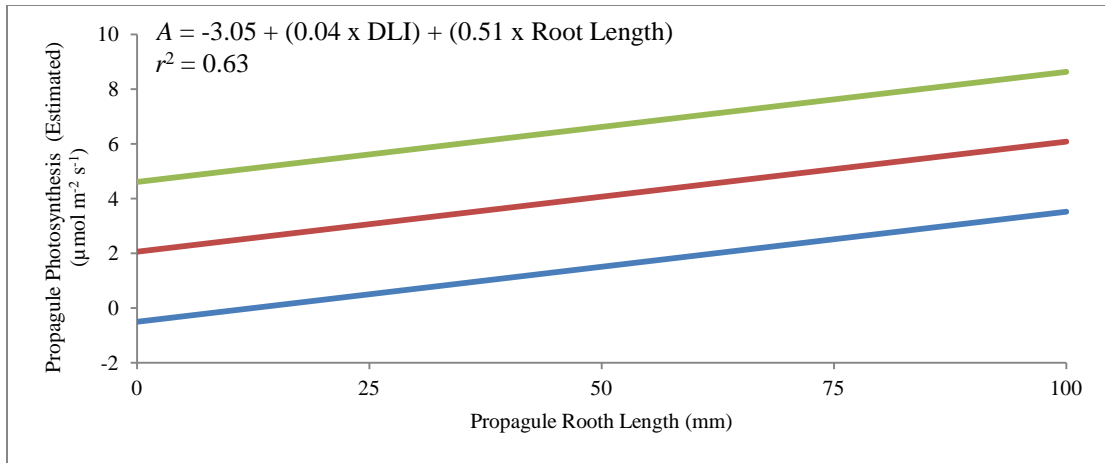


Fig. 3.5. Hibiscus propagule photosynthesis multiple regression, describing correlation of propagule root length and daily light integral ( $\text{mol m}^{-2} \text{d}^{-1}$ ) on propagule photosynthesis ( $P_n$ ). Daily Light Integral values ( $\text{mol m}^{-2} \text{d}^{-1}$ ):  $\blacklozenge = 5$ ;  $\blacksquare = 10$ ;  $\blacktriangle = 15$

CHAPTER 4  
DETERMINING CARBON SOURCE FOR ADVENTITIOUS ROOT DEVELOPMENT  
USING  $^{13}\text{C}$  CARBON ISOTOPE LABELING <sup>1</sup>

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<sup>1</sup> Martin, Jr., M.T., M.R. Chappell and T.R. Maddox. To be submitted to *Journal of the American Society for Horticultural Science*.

## ABSTRACT

Adventitious root development (*ARD*) during asexual propagation is a foundation of commercial horticulture production. Popular theory suggests a major source of carbon for adventitious root formation for vegetative propagules originates in leaf and stem tissues prior to propagule severance from the stock plant. Previous studies to identify the carbon source utilized in vegetative propagation have not included “continuous” measurement of both environmental factors and propagule physiology. Utilizing  $^{13}\text{C}$  isotopic pulse labeling methodology, this study utilized continuous measurements to determine the source of carbon utilized in carbohydrate formation during the *ARD* process and also at what time the carbon was utilized in this process. This study also determined the time frame that propagules are able to photosynthesize and more importantly if asexual propagules are capable of photosynthesizing prior to callus formation and subsequent *ARD*. Early in the *ARD* process, there was no difference in  $^{13}\text{C}$  concentrations in roots between vegetative propagules that were treated with and assimilated  $^{13}\text{C}$  as stock plants (prior to severance) and those propagules that were treated with and assimilated  $^{13}\text{C}$  as vegetative propagules (post-severance). This trend indicates that both propagule produced carbon and stock plant produced carbon are important to the *ARD* process. This also indicates a critically important factor in vegetative propagation; That propagules continue to actively photosynthesize immediately after severance from the stock plant through *ARD*. This has been supported by the presence of  $^{13}\text{C}$  at higher than natural abundance concentrations in the foliage of propagules being treated with  $^{13}\text{C}$  post severance.

*Index words*

Isotope labeling, isotope-ratio mass spectrometry, photosynthesis, stem propagule, vegetative propagation

## INTRODUCTION

Adventitious root development (*ARD*) allows for the asexual production of plant material, assuring that each asexually produced plant is genetically identical to its stock plant (de Klerk et al., 1999). This genetic uniformity allows for the maintenance of specific cultivars/genotypes in the ornamental horticulture industry. Physiologically, it is well documented that stock plant health is directly related to *ARD* success of propagules (Hassig, 1989 [*Pinus banksiana* Lamb]). As carbohydrates are necessary for growth, they have been postulated to play a vital role in *ARD* (Davies, 1988). However, physiologic details regarding propagule carbohydrate storage and particularly carbohydrate allocation within asexual propagules, while in the rooting process, are contradictory. In previous work on this topic, several authors determined that stock plant produced carbohydrates are vitally important to the *ARD* process (Hassig, 1982, 1984 [*Pinus banksiana* Lamb]; Hoad and Leakey, 1996 [*Eucalyptus grandis* (Maiden) W. Hill]; Schaesberg and Lüdders, 1993 [*Mangifera indica* L.]). These authors' findings demonstrate the importance of carbohydrates stored in propagule tissues prior to removal from stock plants. These stored carbohydrates, or pre-severance carbohydrates, were produced via photosynthesis and stored while propagules were still attached to stock plant and subsequently provide propagules with a carbohydrate reserve post severance. Additionally, post-severance photosynthesis, or the production of new photosynthates (carbohydrates) by propagules,

has also been associated with propagule *ARD* success (Davis, 1988; Druege et al., 1998 [*Dendranthema x grandiflorum* (Ramat.) Kitamura 'Puma']; Haissig, 1986; Pellicer et al., 2000 [*Larix x eurolepis* Henry]; Veierskov, 1988). Conversely, other studies found little to no evidence of the importance of post-severance carbohydrates in *ARD* (van Overbeek et al., 1946 [*Hibiscus rosa-sinensis* L.]; Reines and Bamping, 1962; Smalley et al. 1991 [*Acer rubrum* L.]).

Work by Hambrick et al. (1991) examined how propagule set date and propagule cane position (basal, medial, or apical) affected whole propagule carbon to nitrogen ratio in *Rosa multiflora* Thunb. 'Brooks 56' propagules. They demonstrated that propagules set between mid-November and mid-December, when carbohydrate reserves typically peak, had the highest *ARD* success rate. Lappalainen et al. (2000) investigated carbon allocation in *Betula pubescens* Erhr. micropropagules; specifically the effect of nitrogen fertilization, defoliation, and nitrogen fertilization plus defoliation on carbon allocation. Nitrogen fertilization had a significant effect on carbon allocation, specifically decreasing root mass, negatively affecting the root to shoot ratio. Breen and Muraoka (1974) [*Prunus cerasifera x munsoniana* 'Marianna 2624'] examined how photosynthates produced by unrooted propagules, post-severance, were translocated throughout the propagule; finding that most photosynthates remained in the upper portions of the propagules. These results indicated that propagule photosynthesis had little effect on carbohydrates found in propagules' roots. However, Breen and Muraoka (1974) were unable to determine the role of pre-severance carbohydrates in the *ARD* process as they did not analyze stock plants in their study.

Pellicer et al. (2000) worked with *Larix x eurolepis* Henry to examine if stock plant derived carbon was the primary carbon source utilized for *ARD*. To do so, they treated stock plants with  $^{13}\text{C}$  and recorded a baseline  $^{13}\text{C}$  at the time of propagule harvest. Propagule carbon assimilation and subsequent translocation and allocation during the *ARD* process was assumed based on dilution of  $^{13}\text{C}$  in above-ground and below-ground propagule sections over time compared to a baseline value. Results indicated that the majority of carbon utilized during *ARD* was propagule-derived photosynthates and not re-allocated from stock plant reserves.

By utilizing  $^{13}\text{C}$  pulse-chase methodology to label both stock plants and propagules post-severance, with the goal of acquiring an increased understanding of what carbon source (stock plant or propagule produced carbon) is utilized to produce adventitious roots could prove useful in increasing propagule success in a commercial or conservation setting. Research in this area could also lead to improved treatment of stock plants to ensure optimal carbohydrate reserves for *ARD* in vegetative propagules or  $\text{CO}_2$  treatments, specifically timed, to treat propagules with supplemental  $\text{CO}_2$  to improve propagule *ARD* success.

The objectives of this study were to apply  $^{13}\text{C}$  isotopic pulse-chase methodology, to  $^{13}\text{C}$  label stock plants only, propagules only, both stock plants and propagules, or stock plants and propagules grown at  $^{13}\text{C}$  natural abundance, to determine the carbon source utilized during *ARD* and determine if carbon allocation during *ARD* can be quantified using  $^{13}\text{C}$  concentrations and subsequent dilution between  $^{13}\text{C}$  pulses.

## MATERIALS AND METHODS

### *Stock Plant Preparation*

Stock plants of *Hibiscus acetosella* (Hiern.) Welw 'Panama Red' (PP20121), *Monarda* L. 'Petite Delight' (PP10784) and *Podocarpus macrophyllus* (Thunb.) Sweet were obtained as rooted liners. Stock plants were subsequently potted into 1671 cm<sup>3</sup> containers (Griffin Greenhouse & Nursery Supplies, Inc., Ball Ground, GA) filled with substrate (Fafard Nursery Mix, Conrad Fafard, Inc., Anderson, SC) and fertilized with 15 g of a 19-6-12 plus micros, 5-6 month slow release fertilizer (Harrell's LLC, Lakeland, FL). Stock plants were allowed to acclimate to new cultural conditions for 4 weeks prior to terminal, leafy propagules being harvested. Podocarpus measurements were collected between February and April while hibiscus and monarda measurements were collected between June and July.

### *Treatments*

This study utilized four treatments groups (Fig. 4.1). 1) A natural CO<sub>2</sub> abundance (NA) treatment, with stock plants and their propagules treated with ambient air having a CO<sub>2</sub> concentration of approximately 390 ppm. This treatment served as a baseline for <sup>13</sup>C concentration during the study. 2) A propagule carbon (PC) treatment with stock plants treated with ambient air and their propagules treated with <sup>13</sup>C. This treatment was utilized to determine if propagules were photosynthesizing after severance from stock plants (Chapter 2) yet prior to *ARD* and if propagule produced photosynthates/carbohydrates were utilized in the production of adventitious roots. 3) A stock plant <sup>13</sup>C (SC) treatment with stock plants treated with <sup>13</sup>C and their propagules treated with ambient air. This treatment was utilized to determine if stock plant produced photosynthates/carbohydrates

were utilized in the production of adventitious roots. 4) A double  $^{13}\text{C}$  (DC) treatment with stock plants treated with  $^{13}\text{C}$  and their propagules treated with  $^{13}\text{C}$ . This treatment was utilized to determine if a dose effect of  $^{13}\text{C}$  could be induced in the propagules by double exposing them to  $^{13}\text{C}$ .

#### *Stock Plant Pulse-Labeling*

Hibiscus, monarda and podocarpus stock plants were randomly placed into two groups (Fig. 4.1); Group 1 received ambient air treatments while Group 2 received  $^{13}\text{CO}_2$  (Sigma-Aldrich Co., St. Louis, MO) pulse-labeling treatments. Prior to pulse-labeling, 12 propagules were randomly harvested from each treatment group to use as a baseline comparison for  $^{13}\text{C}$  content. Group 2 of hibiscus and monarda were pulsed-labeled with  $^{13}\text{CO}_2(\text{gas})$  every 6 d for 24 d for a total of four pulse-labeling events (24, 18, 12, 6 d before setting) and Group 2 of podocarpus was pulsed-labeled every 7 d for 28 d for a total of four pulse-labeling events (28, 21, 14, 7 d before setting).

Pulse-labeling took place in 64.8 cm x 64.8 cm x 85.7 cm (359.9 L) Plexiglas chambers. Chambers were shaded with 50% shade cloth (Aluminet Cool Shade, Green-Tek Inc. Janesville, WI) to prevent overheating of plant material within chambers. To facilitate air circulation and uniform distribution of treatment gas within chambers, 120 mm fans were placed in each chamber. Once chambers were sealed, starting at 0 min, chambers containing Group 2 plants were injected with 99 atom%  $^{13}\text{CO}_2(\text{gas})$  every 20 min for a 60 min period for a total of four injections, as modified from Simard et al. (1997). Each injection was 45 ml of 99 atom%  $^{13}\text{CO}_2(\text{gas})$  for a total of 180 ml on each treatment date.

Following final  $^{13}\text{CO}_2$  (gas) injection, 30 additional minutes elapsed to allow maximum  $^{13}\text{CO}_2$  (gas) uptake. After 90 min chambers were opened and allowed to vent. To prevent potential contamination of Group 1 stock plants with  $^{13}\text{C}$ , Group 1 and Group 2 plants were kept in separate greenhouse bays and rotated between bays every 6 d.

### *Propagule Preparation*

Six days after the final pulse-labeling of hibiscus and monarda stock plants and 7 d after the final pulse-labeling of podocarpus stock plants occurred, 288 hibiscus, 288 monarda and 216 podocarpus propagules were collected from each group of stock plants and placed in their respective propagule treatments. All propagules were set (inserted into rooting substrate) between 0900 and 1200 HR. Hibiscus propagules were five nodes in length and had all but the top two fully expanded leaves removed from the stem. Using a custom-made depth gauge, propagules were set to a depth of 3 cm. Monarda propagules were six nodes in length with all but the top four fully expanded leaves removed; propagules were set to a depth of 2.5 cm. Podocarpus propagules were 10 cm in length with all but the top eight fully expanded leaves removed. Podocarpus propagules were wounded by removing a 2 mm by 10 mm strip of epidermal tissue to expose cambial tissues and quick dipped for 10 s in 6000 mg L<sup>-1</sup> water soluble IBA (Hortus IBA Water Soluble Salts, Phytotronics, Inc, Earth City, MO) to increase rooting percentages (Dirr, 2009) and were set to a depth of 3 cm. Podocarpus propagules were placed on 21°C bottom heat 30 d after setting to enhance root development. All propagules were set in 72 cell flats, one propagule per cell, filled with substrate (Fafard 3B mix, Conrad Fafard, Inc., Anderson, SC). After propagules were set, they were placed on a mist bench, shaded

with a 60% shade cloth (Griffin Greenhouse & Nursery Supplies, Inc., Ball Ground, GA) and misted every 10 min for 6 s from 0700 to 1900 HR.

Treatments were arranged along the bench (Fig. 4.2) to provide an upwind gradient in the following order: natural abundance (NA), stock plant carbon (SC), propagule carbon (PC), and double  $^{13}\text{C}$  (DC), with 30 cm separating each subgroup. This arrangement was to minimize possible contamination of the NA treatment with  $^{13}\text{C}$  released during respiration.

#### *Propagule Pulse-Labeling and Harvest*

Once propagules had been removed from stock plants (0 d), they were pulse-labeled every 6 d for 18 d, with a total of four pulse-labeling events (0, 6, 12, 18 d) for hibiscus and monarda propagules and every 14 d for 70 d, with a total of five pulse-labeling events (0, 14, 28, 42, 56 d) for podocarpus propagules. The pulse-labeling process was similar to that described for stock plants.

Beginning at 0 d, propagules were harvested every 3 d for 21 d for hibiscus and monarda and every 14 d for 70 d for podocarpus. Propagules were harvested for  $^{13}\text{C}$  sampling prior to any pulse-labeling scheduled for a harvest date. Six propagules per replication among three replications per treatment were randomly collected at each harvest date. Each propagule was separated into four groups by plant part; foliage, above ground stems, below ground stems, and callus plus roots. Below ground stem tissue was defined as the basal 3 cm of stem tissue for hibiscus and podocarpus propagules and the basal 2.5 cm of monarda propagule stem tissue. Plant parts were bulked by replication for each treatment for isotope ratio analysis. Substrate was washed from propagules' roots. After bulking, samples were placed in a freeze-dryer for 48 h.

### *Propagule Sample Preparation*

Bulked samples were removed from the freeze dryer and ball mill ground to less than 250  $\mu\text{m}$  particle size. 1.5-2.5 mg samples were weighed ( $\pm 0.001 \mu\text{g}$ ) into 5 x 9 mm tin capsules (Cole-Parmer, Vernon Hills, IL) and analyzed at the Stable Isotope/Soil Biology Laboratory of the University of Georgia's Odum School of Ecology using an elemental analysis isotope ratio mass spectrometer (EA-IRMS). EA-IRMS determines the percent total carbon of the samples and the percent  $^{13}\text{C}$  of the total amount of carbon. Percent values were converted to milligrams of  $^{13}\text{C}$  per gram of sample by:

$$\left( \frac{\left( \text{Sample Weight} \left( \frac{\text{Total \%C}}{100} \right) \right) \times \left( \frac{\text{Atom \% } ^{13}\text{C}}{100} \right)}{\text{Sample Weight}} \right) \times 1000$$

### *Statistical Analysis*

Data were analyzed using repeated measures, mixed model ANOVA with SAS 9.2 (SAS Institute Inc., Cary, NC) to determine differences between  $^{13}\text{C}$  values of NA, PC, SC and DC treatments. The full model included tests of  $^{13}\text{C}$  treatments and repeated measurements of  $^{13}\text{C}$  treatments over time with each measurement date representing a unit of time. Replications were treated as a random effect. Tukey's HSD was used to determine differences in  $^{13}\text{C}$  treatments least square means within time periods. Analysis was conducted on each plant part (foliage, above ground stems, below ground stems and roots) separately.

## RESULTS AND DISCUSSION

Propagules of hibiscus and monarda in the PC treatment were first pulsed with  $^{13}\text{C}$  immediately after severing them from stock plants. The presence of  $^{13}\text{C}$  at greater

than NA concentrations in the foliage of PC propagules on 3 d (Fig. 4.3a, 4.4a) indicate that propagules of hibiscus ( $p = 0.0006$ ) and monarda ( $p = 0.0001$ ) were photosynthesizing prior to *ARD*. Adventitious roots appeared in propagules of hibiscus and monarda 9 d after setting (Figs. 4.3d and 4.4d) [Chapter 3, Fig. 3.2]. The presence of  $^{13}\text{C}$  at higher than NA concentrations in the roots of PC propagules on 9 d (Fig. 4.3d, 4.4d) demonstrates that propagules of hibiscus ( $p = 0.0003$ ) and monarda ( $p = 0.0024$ ) had assimilated sufficient  $^{13}\text{C}$  post-severance and translocated this carbon to the developing callus and roots to increase  $^{13}\text{C}$  concentrations of adventitious roots during *ARD*. These findings that leafy stem propagules photosynthesize prior to *ARD* are further supported by the “real time” photosynthesis measurements of this same group of propagules (Chapter 3). Additionally, others authors (Davis and Potter, 1981 [*Pisum sativum* L.]; Loach, 1988; Okoro and Grace, 1976 [*Populus × euramericana* (Dode) Guinier]; Smalley et al, 1991 [*Acer rubrum* L.]; Svenson and Davies, 1990 [*Euphorbia pulcherrima* (Klotzsch) Willd.]) have reported propagule photosynthesis during *ARD*.

On 9 d and 12 d there was no difference in  $^{13}\text{C}$  concentrations between propagule produced carbon (PC) and stock produced carbon (SC) treatments (hibiscus: 9 d,  $p = 0.74$ ; 12 d,  $p = 0.30$ ; monarda: 9 d,  $p = 0.56$ ; 12 d,  $p = 0.56$ ). For simplicity, the DC treatment is not reported, as the DC treatment provided no further insight into propagule carbon allocation. In podocarpus (Fig. 4.5d), adventitious roots did not appear until 42 d. While PC and SC  $^{13}\text{C}$  concentrations were greater than NA  $^{13}\text{C}$  concentrations in roots on 42 d, differences between NA, PC and SC  $^{13}\text{C}$  concentrations were not significant (42 d, NA:PC  $p = 0.45$ ; NA:SC  $p = 0.07$ ; PC:SC  $p = 0.92$ ). This lack of significance could be related to podocarpus' low photosynthesis rates (reported in Chapter 3). The trend

among all three species indicated that both propagule produced carbon and stock produced carbon were utilized during the early *ARD* process, as no differences in  $^{13}\text{C}$  concentrations of the roots were observed among propagules derived from SC and PC treatments. After the callus stage of *ARD*, when roots were observed (9 d in hibiscus and monarda, 42 d in podocarpus), there was a distinct shift from utilizing both propagule produced carbon and stock produced carbon to utilizing predominantly propagule produced carbon (Figs. 4.3, 4.4, 4.5). This is the point at which the propagules become “self-sustaining”.

Breen and Muraoka's (1974) work with leafy stem propagules of *Prunus cerasifera x munsoniana* 'Marianna 2624' showed translocation of  $^{14}\text{C}$  throughout the entire propagule shortly after propagule foliage was exposed to  $^{14}\text{CO}_2$ . Much like this study, they were also able to show that as *ARD* occurred, a small portion of  $^{14}\text{C}$  was translocated to the basal area of the propagule and utilized for subsequent root development. While Breen and Muraoka (1974) did not examine the utilization of existing (stock plant produced) carbon, their findings do support that propagule produced carbon is partially utilized in the development of adventitious roots. This basipetal translocation of photosynthates may provide a means of nutrient translocation via phloem to basal portions of propagules (Chapter 5). Tissue et al. (1995) treated attached foliage of *Tipularia discolor* (Pursh) Nutt. corms with  $^{14}\text{CO}_2$  and found that carbohydrates labeled with  $^{14}\text{C}$  were present throughout the entire plant within 7 d of  $^{14}\text{C}$  labeling. These results concur with this study, whereby  $^{13}\text{C}$  was detected in all propagule tissues 3 d (hibiscus, monarda) or 7 d (podocarpus) after pulsing  $^{13}\text{C}$ . Approximately 6 to 9 months after  $^{14}\text{C}$  labeling, Tissue et al. (1995) harvested newly produced foliage, roots and

corns, finding  $^{14}\text{C}$  present in these tissues, demonstrating the importance of stored carbon to the production of new tissue. Tissue et al. (1995) did not simultaneously measure newly produced and existing carbon utilization for the production of new growth after *ARD*, yet their work provided a foundation for current research to build upon. Pellicer et al. (2000) conducted a  $^{13}\text{C}$  labeling of *Larix x eurolepis* Henry similar to the current study, yet a propagule labeling treatment was omitted. They found that propagule produced carbon accounted for 83 to 90% of the carbon utilized in the formation of adventitious roots. Utilization of a species having an extended rooting period (*Larix*) of 36 to 57 d between set date and first harvest to measure root  $^{13}\text{C}$  concentrations likely reduced the fine-time scale ability to track  $^{13}\text{C}$  during *ARD*. Results of podocarpus in the current study exemplify the confounding effects that long rooting periods can have on determining time-sensitive differences in the *ARD* process; particularly early *ARD*.

This study demonstrates that a narrow window of time exists during the *ARD* process whereby a change in carbon utilization occurs within a propagule. As seen in hibiscus and monarda, ideally, tissue would be sampled at this time. If this time does not coincide with a sampling, it is likely that the researcher would be unable to pinpoint the exact timing whereby propagules switch from utilizing stock plant and propagule produced carbon to predominately utilizing propagule produced carbon. In both Pellicer et al.'s (2000) study and the podocarpus portion of this study, more frequent sampling could provide a finer scale measurement, alleviating any time-sensitivity issues and hopefully providing a better understanding of carbon source utilization in this (and other) species that require long periods to develop adventitious roots. In contrast, two of three species [hibiscus and monarda (Fig. 4.3d, 4.4d)] utilized in this study were selected based

on their ability to quickly generate roots during the *ARD* process. Both had predominately become self-sufficient by 15 d, switching from utilizing stock plant derived carbon in the early *ARD* process to primarily using propagule carbon for post-*ARD* root growth and development.

In hibiscus and monarda foliage and stem tissues, an initial increase in  $^{13}\text{C}$  concentration in the PC treatment was seen in the measurement immediately following the  $^{13}\text{C}$  pulse on 3 d (3, 9, 15, 21 d). The next measurements (6 d after  $^{13}\text{C}$  pulse) typically showed a decrease in  $^{13}\text{C}$  concentration. While a quantitative means of determining carbon allocation could not be determined, a qualitative means of measuring carbon allocation based on post-pulse  $^{13}\text{C}$  concentrations and subsequent dilutions was successfully developed. Greater rates of  $^{13}\text{C}$  dilution could be due to three processes. First, between  $^{13}\text{C}$  pulses,  $^{12}\text{C}$  is also being allocated within a sampled part of the plant (e.g. foliage). This allocation of  $^{12}\text{C}$  decreases the  $^{13}\text{C}$  total carbon ratio and subsequently dilutes the amount of  $^{13}\text{C}$  in that portion of the plant. While this methodology does not account for carbon loss via respiration; it does provide an effective indication of carbon allocation within the propagules. The second means of greater rates of  $^{13}\text{C}$  dilution is that  $^{13}\text{C}$  is lost due to respiration; causing an overall decrease in measured  $^{13}\text{C}$ . Last,  $^{13}\text{C}$  can be translocated to another sampled portion of the propagule (e.g. roots).

In PC treatment of hibiscus, small decreases in  $^{13}\text{C}$  concentration were observed on 12 d and 18 d in foliage and above ground stems and below ground stems (Fig. 4.3a,b,c), indicating that a small amount of newly produced carbohydrates were allocated to these parts when compared to decreases of  $^{13}\text{C}$  concentration in the roots (4.3d). In hibiscus, on 18 d there is a significant decrease in  $^{13}\text{C}$  concentration in propagule roots

due to dilution by  $^{12}\text{C}$ ; signifying a large portion of the newly produced carbohydrates were allocated to developing roots. In monarda foliage (Fig. 4.4a) there were decreases in  $^{13}\text{C}$  concentration on 6, 12 and 18 d. In monarda above ground stems (Fig. 4.4b) there were decreases in  $^{13}\text{C}$  concentration on 12 and 18 d. Monarda and podocarpus propagules showed little decreases in  $^{13}\text{C}$  concentration during the study (Fig. 4.4 and 4.5), which could be due to the length of time (14 d) between  $^{13}\text{C}$  pulses and measurements. The length of time between measurements may have masked any effects of increasing or decreasing  $^{13}\text{C}$  concentration or low photosynthesis and slow growth rates could have reduced the amount of  $^{13}\text{C}$  uptake making it difficult to determine carbon allocation.

### *Summary*

This research indicates that both stock plant carbon and propagule produced carbon are initially utilized during *ARD*. Furthermore, propagule produced carbon becomes important to successful *ARD*, providing the majority of *ARD* carbon and resulting in a 'self-sufficient' propagule. This research also fundamentally demonstrates  $^{13}\text{C}$  labeling as a viable method to determine carbon allocation in propagules. This is especially important when considering fine- time scale measurements of carbon assimilation, translocation and respiration of propagules. This can lead to substantial cost and materials savings in commercial horticulture operations by decreasing propagation and overall production times that could lead to increased production (plants per square meter per year) by allowing more propagation cycles per year. Reducing propagation time also can decrease input costs associated with maintaining propagules in a propagation environment.

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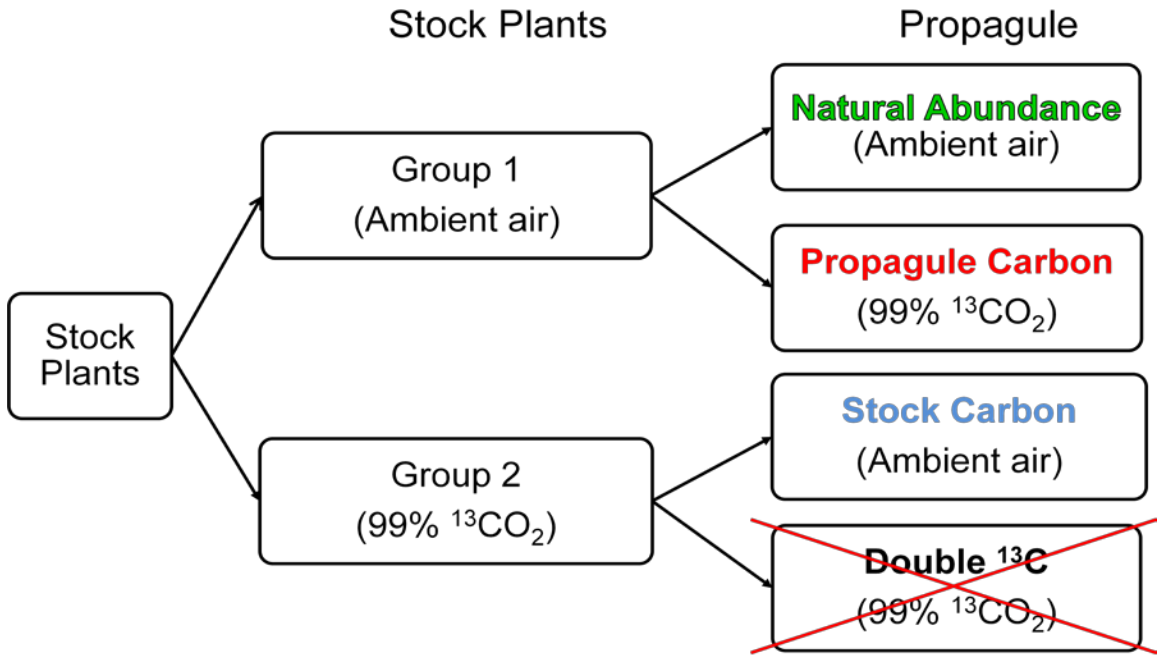


Fig. 4.1 Breakdown of <sup>13</sup>CO<sub>2</sub> and ambient air labeling treatments of stock plants and their subsequent propagules.

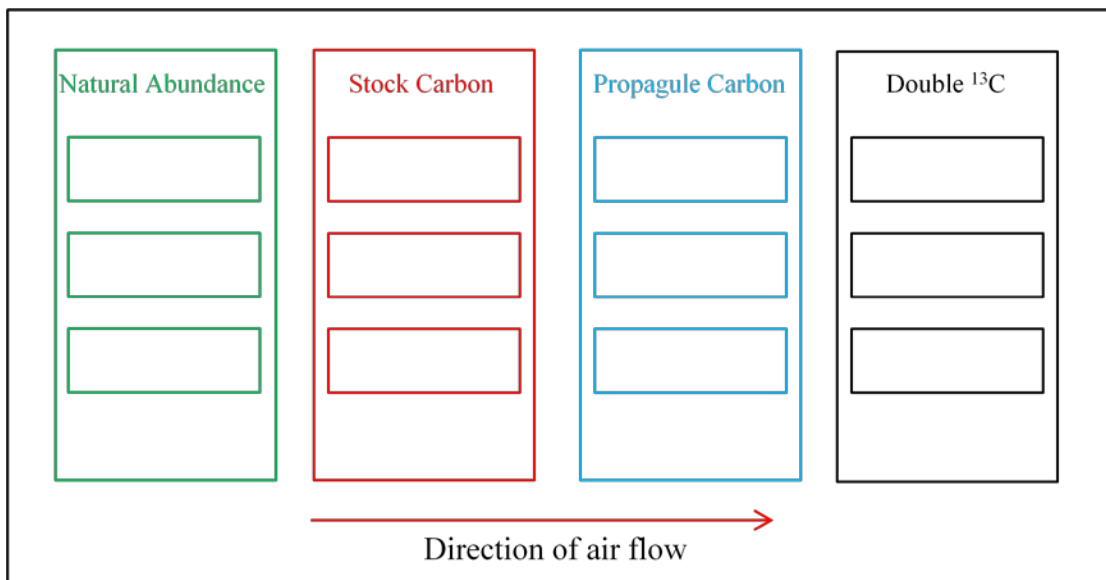


Fig. 4.2 Treatments arranged along greenhouse bench providing increasing upwind gradient to prevent contamination of natural abundance propagules and stock carbon propagules by potential <sup>13</sup>C respiration of propagule carbon and double <sup>13</sup>C treatments.

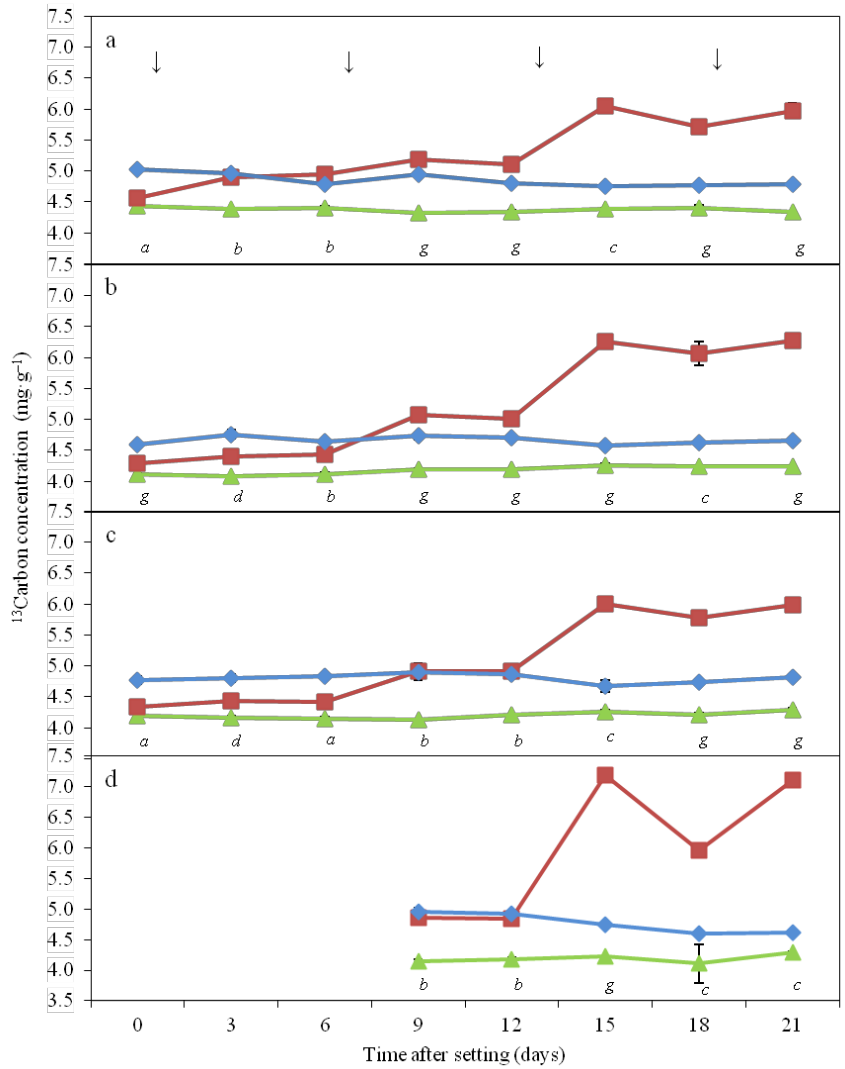


Fig. 4.3. Changes in hibiscus propagule  $^{13}\text{C}$  concentration (milligram  $^{13}\text{C}$  per gram of  $\text{C}^{\text{Total}}$ ) over time. Values are means with standard deviation error bars. a. Hibiscus foliage (n = 6), b. Hibiscus above ground stems (n = 6), c. Hibiscus below ground stems (n = 6), d. Hibiscus roots (n = 6).  $\blacktriangle$  = natural abundance;  $\blacksquare$  = propagule produced carbon;  $\blacklozenge$  = stock plant produced carbon.  $\downarrow$  = propagule exposure to  $^{13}\text{C}$ .

**Treatment Significance Key**

- a = [Propagule produced carbon : Natural abundance = ns; Propagule produced carbon : Stock plant produced carbon = p <0.05; Natural abundance : Stock plant produced carbon = p <0.05]
- b = [Propagule produced carbon : Natural abundance = p <0.05; Propagule produced carbon : Stock plant produced carbon = ns; Natural abundance : Stock plant produced carbon = p <0.05]
- c = [Propagule produced carbon : Natural abundance = p <0.05; Propagule produced carbon : Stock plant produced carbon = p <0.05; Natural abundance : Stock plant produced carbon = ns]
- d = [Propagule produced carbon : Natural abundance = ns; Propagule produced carbon : Stock plant produced carbon = ns; Natural abundance : Stock plant produced carbon = p <0.05]
- g = [Propagule produced carbon : Natural abundance = p <0.05; Propagule produced carbon : Stock plant produced carbon = p <0.05; Natural abundance : Stock plant produced carbon = p <0.05]

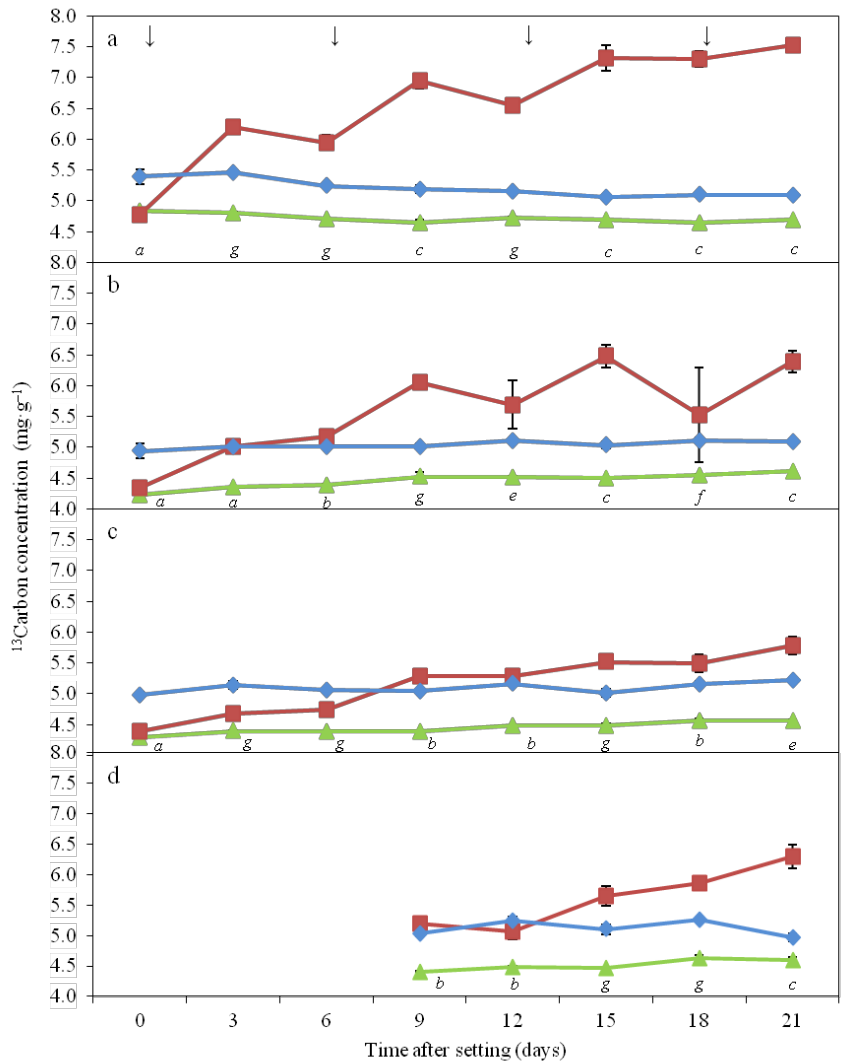


Fig. 4.4. Changes in monarda propagule  $^{13}\text{C}$  concentration (milligram  $^{13}\text{C}$  per gram of  $\text{C}^{\text{Total}}$ ) over time. Values are means with standard deviation error bars. a. Monarda foliage (n = 6), b. Monarda above ground stems (n = 6), c. Monarda below ground stems (n = 6), d. Monarda roots (n = 6).  $\blacktriangle$  = natural abundance;  $\blacksquare$  = propagule produced carbon;  $\blacklozenge$  = stock plant produced carbon.  $\downarrow$  = propagule exposure to  $^{13}\text{C}$ .

#### Treatment Significance Key

- a = [Propagule produced carbon : Natural abundance = ns; Propagule produced carbon : Stock plant produced carbon = p <0.05; Natural abundance : Stock plant produced carbon = p <0.05]
- b = [Propagule produced carbon : Natural abundance = p <0.05; Propagule produced carbon : Stock plant produced carbon = ns; Natural abundance : Stock plant produced carbon = p <0.05]
- c = [Propagule produced carbon : Natural abundance = p <0.05; Propagule produced carbon : Stock plant produced carbon = p <0.05; Natural abundance : Stock plant produced carbon = ns]
- e = [Propagule produced carbon : Natural abundance = p <0.05; Propagule produced carbon : Stock plant produced carbon = ns; Natural abundance : Stock plant produced carbon = ns]
- f = [Propagule produced carbon : Natural abundance = ns; Propagule produced carbon : Stock plant produced carbon = ns; Natural abundance : Stock plant produced carbon = ns]
- g = [Propagule produced carbon : Natural abundance = p <0.05; Propagule produced carbon : Stock plant produced carbon = p <0.05; Natural abundance : Stock plant produced carbon = p <0.05]

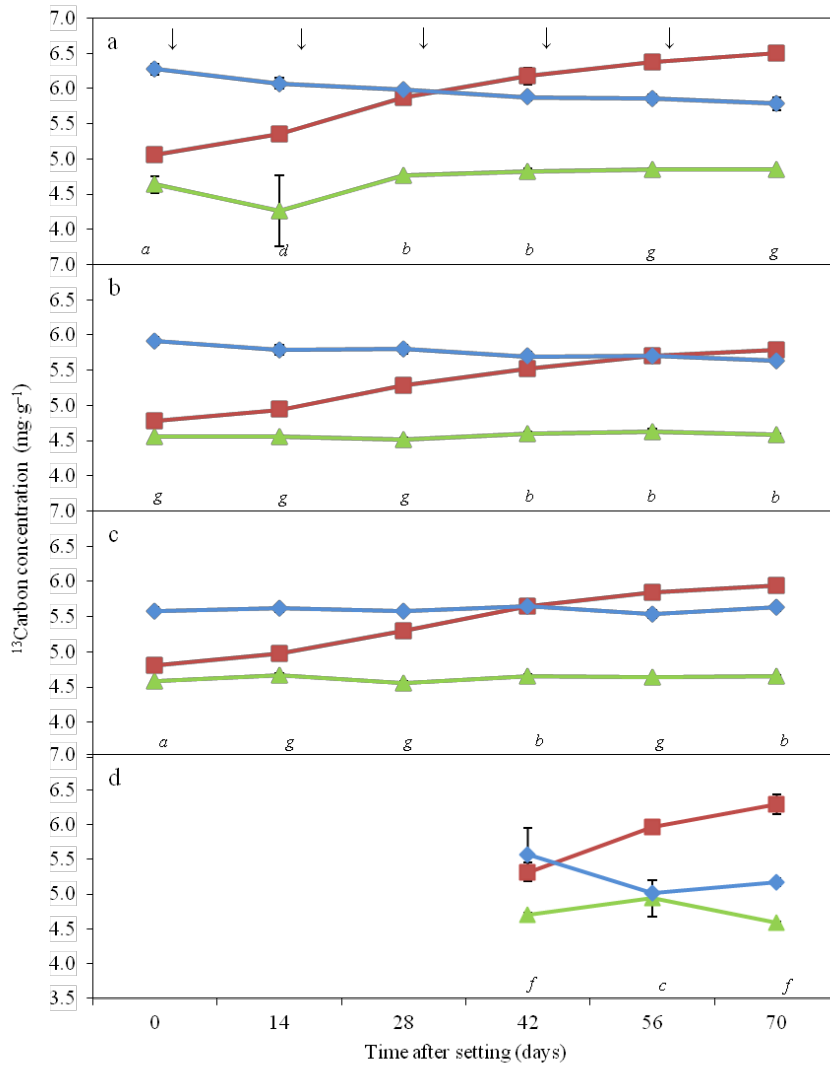


Fig. 4.5. Changes in podocarpus propagule  $^{13}\text{C}$  concentration (milligram  $^{13}\text{C}$  per gram of  $\text{C}^{\text{Total}}$ ) over time. Values are means with standard deviation error bars. a. Podocarpus foliage (n = 6), b. Podocarpus above ground stems (n = 6), c. Podocarpus below ground stems (n = 6), d. Podocarpus roots (n = 6).  $\blacktriangle$  = natural abundance;  $\blacksquare$  = propagule produced carbon;  $\blacklozenge$  = stock plant produced carbon.  $\downarrow$  = propagule exposure to  $^{13}\text{C}$ .

#### Treatment Significance Key

a = [Propagule produced carbon : Natural abundance = ns; Propagule produced carbon : Stock plant produced carbon = p <0.05; Natural abundance : Stock plant produced carbon = p <0.05]  
 b = [Propagule produced carbon : Natural abundance = p <0.05; Propagule produced carbon : Stock plant produced carbon = ns; Natural abundance : Stock plant produced carbon = p <0.05]  
 c = [Propagule produced carbon : Natural abundance = p <0.05; Propagule produced carbon : Stock plant produced carbon = p <0.05; Natural abundance : Stock plant produced carbon = ns]  
 d = [Propagule produced carbon : Natural abundance = ns; Propagule produced carbon : Stock plant produced carbon = ns; Natural abundance : Stock plant produced carbon = p <0.05]  
 f = [Propagule produced carbon : Natural abundance = ns; Propagule produced carbon : Stock plant produced carbon = ns; Natural abundance : Stock plant produced carbon = ns]  
 g = [Propagule produced carbon : Natural abundance = p <0.05; Propagule produced carbon : Stock plant produced carbon = p <0.05; Natural abundance : Stock plant produced carbon = p <0.05]

CHAPTER 5  
MINERAL ELEMENTAL CONCENTRATIONS WITHIN VARIOUS PROPAGULE  
SECTIONS DURING ADVENTITIOUS ROOT DEVELOPMENT <sup>1</sup>

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## ABSTRACT

Perpetuating genotypes with preferred ornamental traits is a cornerstone of the ornamental horticulture industry. Key to this process is adventitious root development (*ARD*) of asexual (vegetative) propagules. Little work has been conducted on deciphering the role of specific nutrients and their availability in vegetative propagule rooting during *ARD*. By decreasing the interval between measurements, compared to previous studies, and separating propagule tissue into four sections, this study afforded the ability to determine fine-scale changes in tissue mineral elemental concentrations and mineral allocation within various propagule sections during *ARD*. *Hibiscus acetosella* (Hiern.) Welw. 'Panama Red' (PP20121) propagules were harvested every 3 d for 21 d. Propagules were separated (by plant part) into foliage, above-ground stems, below-ground stems and callus plus roots. Mn, Fe, B, Cu, Zn, Ni, Mo, P, K, Ca, and Mg concentrations were analyzed using an inductively coupled argon plasma (ICAP) spectrometer, S was analyzed using a carbon, nitrogen and sulfur analyzer and N was measured using an elemental analysis isotope ratio mass spectrometer (EA-IRMS). Changes in nutrient concentration during nutrient analysis studies are attributed to one of three causes: dilution, uptake or translocation. Utilizing this information, three groups of nutrient use, likely due to nutrient mobility and source-sink gradients, became apparent. The first group, including N, P, K, S and Zn, are mobilized/utilized in larger quantities. The second group, including Ca, B and Mg are mobilized/utilized in comparatively smaller quantities. The third group, including Mn, Fe, Cu, Ni and Mo, has no appreciable change or pattern of use during the *ARD* process. Research of this nature is extremely important as it provides information concerning nutrient mobility and utilization during

the *ARD* process. With a better understanding of nutrient utilization during *ARD*, it is possible to increase the specific nutrients either by nutrient loading stock plants or providing a nutrient solution via misting to supplement these requirements with the objective of improving propagule health and subsequent rooting success.

*Index words*

Nutrient uptake, nutrient translocation, vegetative propagation

## INTRODUCTION

Adventitious root development (*ARD*) is a cornerstone of the ornamental horticulture industry, allowing for the perpetuation of genotypes with preferred ornamental traits. Hartmann et al. (1990) divided the *ARD* process into two distinct stages: 1) Propagule tissue dedifferentiation and redifferentiation into root initials and 2) root growth and further root tissue differentiation into mature roots. Little work has been conducted on deciphering the role of specific nutrients and their availability on vegetative propagule rooting during *ARD*, with the majority of work focusing on application of complete fertilizers or micronutrient fertilizers (Biernbaum et al., 1995; Carney and Whitcomb, 1983 [*Rhododendron* x 'Fashion', *Ilex crenata* Thunb. 'Hetzi', *Pyracantha coccinea* Roem 'Wyatti']; Gibson, 2003 [*Impatiens* x *hawkeri* Bull. 'Grenada', *Scaevola aemula* R. Br. 'Purple Fan', and *Bracteantha bracteata* (Vent.) A.A. Anderberg 'Florabella Pink']; Rowe and Cregg, 2002 [*Artemisia ludoviciana* Nutt. 'Valerie Finnis', *Gaura lindheimeri* Engelm. & Gray 'Whirling Butterflies', and *Nepeta* x *faassenii* (Stearn.) Bergmans 'Six Hills Giant']; Santos et al., 2011 [*Petunia* x *hybrida* Vilm.] to

propagules and subsequently measuring changes in biomass of propagules. The importance of specific macronutrients and micronutrients to the *ARD* process is less clear, yet has been documented in several studies. During the *ARD* process, deficiencies of mineral nutrients have been linked to an initial reduction in vegetative shoot growth over that of root growth (Eliasson, 1978) [*Pisum sativum* L.], indicating that the roots act as a preferential sink during *ARD* development (van Overbeek et al., 1946 [*Hibiscus rosa-sinensis* L.]). The two mineral nutrients most noted as hindering root development when deficient in propagules and/or propagation substrate are Ca (Eliasson, 1978 [*Pisum sativum* L.]) and B (Hemberg, 1951 [*Phaseolus vulgaris* L.]). Interestingly, Ca has been described as poorly translocated within propagules, particularly in phloem (Crafts and Crisp, 1971; Oertli and Richardson, 1970 [*Gossypium hirsutum* L.]). More recent work has indicated that in addition to Ca and B; N, P, Mg, Mn, Zn (Anderson, 1986; Blazich, 1988) and Cu (Arnold et al., 1994 [*Betu pubescens* × *papyrzfera*]) are required for root initiation and/or elongation. Also, the rooting factor indole-3-acetic acid (IAA) has been shown to rely on the availability of Zn, Mn and B (Jarvis et al., 1984 [*Phaseolus aureus* Roxb.]; Salami and Kenefick, 1970 [*Zea mays* L.]; Takaki and Kushizaki, 1970 [*Zea mays* L.]; Tomaszewski and Thimann, 1966 [*Pisurm sativuirn* L. 'Alaska']). Furthermore, the rooting factor auxin has been shown to rely on Fe (Mengel and Kirkby, 1982), with the knockout of Fe uptake in mutant *Oryza sativa* L. lines resulting in significant reductions in root length of propagules (Qi et al., 2012).

Despite previous research on understanding the role of nutrient availability on *ARD* success and propagule health, little is known about the utilization (transport and allocation) of nutrients in vegetative propagules post-severance from stock plants.

Svenson and Davies (1995) were the first and only researchers to report utilizing Inductively Coupled Argon Plasma (ICAP) spectrometer technology to measure mineral elemental concentrations of propagules during *ARD*. Their work, utilizing propagules of *Euphorbia pulcherrima* (Klotzch.) Willd., determined several overall trends in nutrient availability and allocation in vegetative propagules of poinsettia. By separating propagules into foliage and basal stem tissue (callus and/or roots), they were able to assess changes in nutrient content during the *ARD* process. Overall, Fe, Cu and Mo concentrations increased in the basal portion of the propagule during callus formation, suggesting these nutrients may be important for callus formation and root initiation. After roots were observed there was an increase in Mg, Mn, B and Zn; suggesting these nutrients may be vital to root elongation and maintenance. Conversely, there was a reduction in foliar concentrations of N, Fe and Mo. This reduction could be in part due to leaching of N and a concentration reduction due to increasing leaf biomass. However, Fe was purportedly translocated from foliage to developing callus and roots. This is interesting, considering Fe has been suggested to be an immobile nutrient.

This study was conducted to build on the work of Svenson and Davies (1995) [*Euphorbia pulcherrima* (Klotzsch) Willd.] by decreasing the time between measurements from 13 d to 3 d. Fine scale measurements can affect the ability to observe small changes in cutting physiology, especially during early *ARD* (Chapter 4). Additionally, the current study has separated propagule tissue into four sections; foliage, above-ground stem tissue, below ground stem tissue and callus and roots. The combination of reducing time between harvests and separating propagules into smaller units should allow for increases in the ability to determine fine-scale changes in tissue

mineral elemental concentrations and mineral allocation within various propagule sections during *ARD*. Specifically, if basipetal translocation of nutrients occurs during *ARD*, particularly nutrients classically considered immobile.

## MATERIALS AND METHODS

### *Stock Plant and Propagule Preparation*

Stock plants of *Hibiscus acetosella* (Hiern.) Welw. 'Panama Red' (PP20121), were obtained as rooted liners. Stock plants were subsequently potted into 1671 cm<sup>3</sup> containers (Griffin Greenhouse & Nursery Supplies, Inc., Ball Ground, GA) filled with substrate (Fafard Nursery Mix, Conrad Fafard, Inc., Anderson, SC) and fertilized with 15 g of a 19-6-12 plus micros, 5-6 month slow release fertilizer (Harrell's LLC, Lakeland, FL). Stock plants were allowed to acclimate to new cultural conditions for 4 weeks prior to terminal, leafy propagules being harvested. Propagules were harvested from stock plants and set (inserted into rooting substrate) between 0900 and 1200 HR. Propagules were five nodes in length and had all but the top two fully expanded leaves removed from the stem. Using a custom-made depth gauge, propagules were set to a depth of 3 cm. Propagules were set in 72 cell flats, one propagule per cell, filled with substrate (Fafard 3B mix, Conrad Fafard, Inc., Anderson, SC). After propagules were harvested and set they were placed on a mist bench, shaded with a 60% shade cloth (Griffin Greenhouse & Nursery Supplies, Inc., Ball Ground, GA) and misted from 0700 to 1900 HR every 10 min for 6 s.

### *Propagule Harvest*

Beginning at 0 d, hibiscus propagules were harvested every 3 d for 21 d. Six propagules per replication among three replications per treatment were randomly collected at each harvest date. Propagules were separated by plant part into foliage, above ground stem, below ground stem, and callus plus roots. Below ground stem tissue was defined as the basal 3 cm of stem tissue. Plant parts were bulked by replication for each treatment for subsequent nutrient analysis. Substrate was washed from the propagules' roots. After bulking, samples were placed in a freeze-dryer for 48 hours.

### *Propagule Sample Preparation*

Bulked samples were removed from the freeze-dryer, ball-mill ground to less than 250  $\mu\text{m}$  particle size and submitted to the University of Georgia Agricultural and Environmental Services Laboratories for analysis. 250 mg ( $\pm 2$  mg) of each tissue sample were weighed and placed into a microwave digestion vessel (CEM Corporation, Matthews, NC). 10 ml  $\text{HNO}_3$  was added to each vessel and the mixture allowed to sit for 15 min. Samples were then placed in a microwave digestion system (CEM Mars 5, CEM Corporation, Matthews, NC) for acid digestion of samples. Once samples were cooled, samples were placed in 100 ml volumetric flasks and diluted with deionized water. This solution was used for the analysis of Mn, Fe, B, Cu, Zn, Ni, and Mo. This solution was further diluted, one to ten with deionized water, for the analysis of P, K, Ca, and Mg. Once solutions were prepared, samples were analyzed using an inductively coupled argon plasma (ICAP) spectrometer (Thermo Jarrell-Ash Enviro I, Thermo Jarrell-Ash, Franklin, MA). To determine S content of samples, 150 to 200 mg of each sample were weighed out and analyzed using a carbon, nitrogen and sulfur analyzer (CNS 2000, LECO

Corporation, St. Joseph, MI). Nitrogen was measured by weighing 1.5-2.5 mg of sample ( $\pm 0.001 \mu\text{g}$ ) into 5 x 9 mm tin capsules (Cole-Parmer, Vernon Hills, IL) and analyzed at the Stable Isotope/Soil Biology Laboratory of the University of Georgia's Odum School of Ecology using an elemental analysis isotope ratio mass spectrometer (EA-IRMS).

### *Statistical Analysis*

Data were analyzed using repeated measures, mixed model ANOVA with SAS 9.2 (SAS Institute Inc., Cary, NC) to determine differences between plant parts. The full model included tests of plant parts and repeated measurements of plant parts over time with each measurement date representing a unit of time. Replications were treated as a random effect. Tukey HSD was used to determine differences in plant parts least square means within time periods.

## RESULTS AND DISCUSSION

The only study of this nature was Svenson and Davies' (1995) work examining nutrient concentration changes during *ARD* in *Euphorbia pulcherrima* (Klotzsch) Willd. propagules. However, since no statistical analysis was conducted by Svenson and Davies (1995), direct comparisons between the two studies are not possible. The current study was conducted to build upon their research by decreasing measurement intervals and increasing the number of plant parts measured; with the expectation of determining nutrient concentrations of various propagule parts at various propagule rooting stages. Changes in nutrient concentration during nutrient analysis studies are usually attributed to one of three causes: dilution, uptake or translocation. To fully comprehend what is occurring during this study, an understanding of dilution, uptake and translocation is

vitaly important. Dilution, or dilution effect, is a decrease in nutrient concentration caused by an increase in growth, expressed as dry mass. Examples include Aronsson and Elowson (1980), who concluded that decreases of macronutrient concentrations in needles of *Pinus sylvestris* L. [whole tree study] resulted from rapid shoot and needle growth prior to tissue nutrient analysis. White (1954) noted that dilution of nutrient concentrations in needles of *Pinus resinosa* Aiton [whole tree study] were affected by their canopy position. Needles lower in the canopy photosynthesized at lower rates, decreasing carbohydrate production and reducing nutrient dilution rates. Nutrient uptake is the process of moving nutrients from the exterior of the plant, to “inside” the plant for utilization in plant growth and development processes. Nutrient uptake, as characterized by White (2012), has three distinctive components: 1. nutrient selectivity where a plant preferentially selects for or discriminates against uptake of a particular nutrient. 2. Nutrient accumulation when cellular sap nutrient concentrations are at higher concentrations compared to the external solution. 3. Genotypic difference, that each plant species will have unique uptake characteristics. While uptake most commonly occurs via roots, it can also occur via foliage, even in unrooted propagules, as Santos et al. (2009) demonstrated with foliar nutrient applications of *Petunia x hybrida* Vilm. propagules. Translocation or nutrient mobility is the internal movement of a nutrient based on a source-sink relationship. Examples include the work of Hu and Brown (1997) who demonstrated translocation of B, utilized in cellular wall components of *Helianthus annuus* L., along a concentration gradient. Also, a severe Mg deficiency in mature *Pinus radiata* D. Don foliage was shown by Will (1968) as being induced by translocation of Mg from mature foliage to new foliage. Mg uptake was limited by a spring drought and

heavy pruning further decreased Mg reserves, both reducing the Mg source from which the new foliage could utilize, ultimately causing the deficiency.

During this study, the *ARD* process was divided into four distinct stages based on either physical observations or physiological (photosynthetic) measurements (based on the findings of Chapter 3). The first stage was dedifferentiation that occurred between 0 and 6 d. The second stage was visible root formation, the time when root initials began to appear, that occurred between 6 and 9 d. The third stage was root elongation, when root initials began to elongate, that occurred between 9 and 15 d. The fourth stage was propagule self-sufficiency, based on plateauing of photosynthetic data (Chapter 3), indicating that hibiscus propagules had reached a self-sufficient state, that occurred between 15 and 21 d.

Based on visual observations, there was no appreciable growth of foliage, above ground stems or below ground stems of hibiscus propagules during dedifferentiation. As there was no appreciable growth during this time, changes in nutrient concentrations of foliage, above ground stems or below ground stems cannot be due to dilution. Additionally, during dedifferentiation, there was no root system for effective uptake of nutrients. Therefore, any changes in nutrient concentrations during this time were likely an effect of translocation. Furthermore, during root elongation and propagule self-sufficiency stages, root dry mass greatly assisted with determining if changes in nutrient concentrations were caused by dilution, uptake or translocation.

Utilizing this information, three groups of nutrient use, likely due to nutrient mobility and source-sink gradients, became apparent. The first group includes nutrients that are mobilized/utilized in comparatively larger quantities. The second group includes

nutrients that are mobilized/utilized in comparatively smaller quantities. The third group includes nutrients that have no appreciable change or pattern of use during the *ARD* process.

The nutrients mobilized/utilized in comparatively larger quantities were N, P, K, S and Zn (Table 5.1). N was basipetally translocated from foliage to below ground stems during dedifferentiation, with decreases in N leaf concentration coinciding with increased concentration in below ground stems. This basipetal movement is likely due to importance of N in amino acid, protein and enzyme formation and activity, as well as utilization in DNA and RNA in developing tissues (Garnica et al., 2010 [*Triticum aestivum* L. ‘Amarok’]; Hawkesford et al., 2012; Inoue et al., 2001 [*Arabidopsis thaliana* (L.) Heynh.]; Mills and Jones, 1996; Miyawaki et al., 2004 [*Arabidopsis thaliana* (L.) Heynh.]). N foliar concentrations also decreased during visible root formation stage but thereafter remained unchanged to the study’s conclusion.

Foliar concentrations of P decreased, with slight but non-significant concentration increases in above ground stems and below ground stems during dedifferentiation, signifying a downward movement of P toward the base of the propagule. Similar to N foliar concentrations, P foliar concentration also decreased during Visible Root Formation. Above ground stem and below ground stem P concentrations also continuously decrease between dedifferentiation stage to the study’s conclusion. Given the physiological activity that occurs during *ARD*, particularly new cell formation and division, it was expected P would have been translocated at a noticeable measure, considering its importance in cellular energy (ATP), as a major of constitute of nucleic acids (DNA and RNA) and cellular wall formation (Assuero et al., 2004 [*Zea mays* L.

‘Cecilia’]; Doerner, 2008; Dong et al., 1999 [*Arabidopsis thaliana* (L.) Heynh.]; Hawkesford et al., 2012; Mills and Jones, 1996).

K concentrations had the greatest decrease in foliar concentration of any nutrient reported during the dedifferentiation stage. K was obviously translocated during this stage but its sink/destination is not readily obvious when comparing K concentration in above ground stems or below ground stems. It is postulated that K was basipetally translocated for use in cell wall formation, specifically cellulose synthesis (Hawkesford et al., 2012; Mills and Jones, 1996; Pflüger and Cassier, 1977; Suelter, 1970). After the dedifferentiation stage, K concentrations continued to decrease in foliage, above ground stems and below ground stems until the study’s conclusion. During the propagule self-sufficiency stage, root uptake of K did occur based on increasing K root concentrations as well as increasing actual root content of K, but no apical translocation was observed.

During the dedifferentiation stage, S was basipetally translocated from foliage to below ground stems. This basipetal translocation was likely a result of S importance in amino acid formation and enzymatic activity (Benning, 1998; De Kok et al., 2002; Hawkesford et al., 2012; Hell, 1997; Leustek and Saito, 1999; Mills and Jones, 1996). After the dedifferentiation stage, S concentrations continued to decrease in foliage, above ground stems and below ground stems until study’s conclusion. As observed in K, apical translocation of S was not observed during the propagule self-sufficiency stage, but root uptake did occur based on increasing concentrations and actual root content of S.

Zn concentration decreased in all plant parts during dedifferentiation, yet as with K, its sink/destination is not readily obvious. Zn is very important for RNA synthesis (Broadley et al., 2012; Mills and Jones, 1996; Prask and Plocke, 1971 [*Euglena gracilis*

Klebs]; Sousa et al., 2009) and therefore would be in great demand in the propagule basal *meristematic region during ARD*. This decrease in Zn concentration continued throughout the study in above ground stems. This concentration decrease plateaus after the dedifferentiation stage in foliage and plateaus in below ground stems during the propagule self-sufficiency stage. Root uptake of Zn did occur during the propagule self-sufficiency stage based on increasing concentrations and actual root content of Zn, but as with K and S, there was no observed apical translocation of Zn.

Those nutrients mobilized/utilized in smaller quantities are Ca, B and Mg (Table 5.2). Calcium concentration during dedifferentiation stage decreased and continued this decrease in concentration in below ground stems while showing little change in concentration in foliage or above ground stems. Ca was expected to translocate in higher amounts given its importance in cellular wall composition and utilization during cell division to stabilize mitotic spindles (Cole and Fowler, 2006; Hawkesford et al., 2012; Mills and Jones, 1996; Shear, 1975; White and Broadley, 2003). This lack of translocation could be a sign of Ca immobility or that local Ca reserves were sufficient for these processes to occur.

B concentration in below ground stems decreased during the dedifferentiation stage. B concentrations in foliage and above ground stems did not begin decreasing until visible root formation stage. Thereafter, B concentration in foliage, above and below ground stems decreased until the end of root elongation stage and beginning of propagule self-sufficiency stage. During the propagule self-sufficiency stage, root uptake of B began, with concentrations of B in below ground stems increasing; possibly demonstrating apical translocation of B. While increases in B concentration were

observed in foliage and above ground stems, they are not significant increases. B is extremely important for meristematic growth, cell differentiation, maturation, division and elongation (Broadley et al., 2012; Loomis and Durst, 1992; Mills and Jones, 1996; Lange et al. 1987 [*Picea abies* (L.) Karst.]; Rao et al. 1987 [*Helianthus annuus* L. 'IS 894'] ) and would be in great demand for root development during *ARD* and new shoot growth after a propagule has rooted.

Mg concentration in above ground stems decreased during the dedifferentiation stage, while concentration in below ground stems decreased between the dedifferentiation stage and the visible root formation stage. Mg is utilized to transfer phosphryl groups between ATP and ADP (Hawkesford et al., 2012; Mills and Jones, 1996; Nyomora et al., 2000 [*Prunus dulcis* (Mill.) D.A. Webb 'Ruby', 'Butte', and 'Mono']; Tanada, 1982 [*Vigna radiata* (L.) R. Wilczek]). Of greater interest is that Mg foliar concentration decreased during the dedifferentiation and visible root formation stages. This foliar concentration began to increase during the root elongation and propagule self-sufficiency stages. This increase in foliar concentration coincides with root uptake of Mg and demonstrates apical translocation.

The third group of nutrients had no appreciable change or pattern of use during the *ARD* process. These nutrients include Mn, Fe, Cu, Ni and Mo. It is possible that these nutrients are: 1) completely immobile and cannot be translocated from existing tissues to the basal portion of the stem or 2) they are required in such minute amounts they are "locally" available in sufficient quantity as not to require translocation. Of additional interest is the abnormally high Fe concentration seen in this study. It is hypothesized the high anthocyanin content of 'Panama Red' cultivar utilized in this study could have

skewed this value as a result of anthocyanin molecular chemistry and synthesis (Brouillard et al, 2010).

More data, specifically dry mass of foliage, above and below ground stems would assist in drawing more definitive conclusions. Research of this nature is extremely important as it provides information concerning nutrient mobility and utilization during the *ARD* process. With a better understanding of nutrient utilization during *ARD* it is possible to increase the specific nutrients either by nutrient loading stock plants or providing a nutrient solution via misting to supplement these requirements to hopefully improve rooting success and propagule health. Based on this work, it is suggested N, P, K, S and Zn are vitally important to *ARD* while Ca, B and Mg are also needed during the rooting process, albeit at lower quantities. Propagation specific fertilizers with these nutrients may significantly improve the overall health and rooting ability of vegetative propagules.

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Day	N (g·kg <sup>-1</sup> )	P (g·kg <sup>-1</sup> )	K (g·kg <sup>-1</sup> )	S (g·kg <sup>-1</sup> )	Zn (mg·kg <sup>-1</sup> )	Ca (g·kg <sup>-1</sup> )	B (mg·kg <sup>-1</sup> )	Mg (g·kg <sup>-1</sup> )
<b>Foliage</b>								
0	27.54 ± 1.00 a	2.73 ± 0.09 a	11.29 ± 0.09 a	1.92 ± 0.04 a	79.23 ± 8.96 a	9.58 ± 0.48 ab	27.47 ± 0.42 a	4.09 ± 0.07 ab
3	24.58 ± 1.68 ab	2.36 ± 0.11 ab	10.00 ± 0.00 b	1.80 ± 0.02 ab	37.84 ± 0.49 b	8.89 ± 0.67 ab	21.55 ± 2.23 ab	3.75 ± 0.07 ab
6	22.65 ± 1.50 b	2.22 ± 0.12 b	8.38 ± 0.32 c	1.79 ± 0.10 abc	33.55 ± 0.59 b	6.69 ± 0.06 b	20.54 ± 0.93 ab	3.21 ± 0.06 b
9	18.79 ± 0.83 c	1.57 ± 0.12 c	7.12 ± 0.27 cd	1.52 ± 0.07 bcd	31.69 ± 2.56 b	7.52 ± 0.26 ab	15.06 ± 3.59 b	3.36 ± 0.01 b
12	18.78 ± 1.71 c	1.53 ± 0.04 c	6.09 ± 0.25 de	1.46 ± 0.02 bcd	30.50 ± 0.93 b	7.96 ± 0.52 ab	17.00 ± 0.71 b	3.46 ± 0.15 b
15	17.78 ± 0.94 c	1.52 ± 0.06 c	5.55 ± 0.10 e	1.50 ± 0.03 bcd	41.04 ± 10.57 b	8.03 ± 0.74 ab	16.89 ± 1.03 b	3.76 ± 0.19 ab
18	17.56 ± 1.19 c	1.35 ± 0.11 c	5.09 ± 0.34 e	1.34 ± 0.12 d	20.54 ± 2.09 b	8.40 ± 0.85 ab	18.35 ± 2.42 ab	3.83 ± 0.45 ab
21	16.63 ± 0.78 c	1.59 ± 0.05 c	6.06 ± 0.19 de	1.44 ± 0.02 cd	26.42 ± 0.88 b	10.29 ± 0.21 a	22.09 ± 0.66 ab	4.56 ± 0.09 a
<b>Above Ground Stems</b>								
0	14.43 ± 0.78 a	2.55 ± 0.15 ab	24.62 ± 1.19 a	1.69 ± 0.04 a	63.63 ± 3.46 a	8.98 ± 0.34 a	23.45 ± 1.00 a	4.36 ± 0.06 a
3	13.98 ± 1.55 a	2.45 ± 0.15 abc	23.96 ± 0.67 a	1.73 ± 0.04 a	44.71 ± 0.50 b	8.98 ± 0.55 a	19.44 ± 0.72 ab	3.90 ± 0.03 a
6	12.38 ± 1.28 ab	2.65 ± 0.03 a	22.79 ± 0.34 ab	1.64 ± 0.02 a	42.30 ± 0.85 bc	6.96 ± 0.41 ab	20.64 ± 0.42 ab	3.19 ± 0.11 b
9	10.92 ± 1.69 bc	1.98 ± 0.13 bcd	22.79 ± 0.98 bc	1.33 ± 0.04 b	39.47 ± 0.37 bc	6.84 ± 0.25 ab	18.09 ± 0.97 b	2.66 ± 0.06 c
12	10.09 ± 1.29 bcd	1.87 ± 0.10 cde	17.77 ± 0.77 cd	1.16 ± 0.02 bc	34.81 ± 0.73 cd	6.76 ± 0.41 ab	16.98 ± 1.11 b	2.44 ± 0.11 c
15	8.13 ± 0.58 cd	1.66 ± 0.17 de	15.61 ± 0.31 cd	1.07 ± 0.06 c	29.78 ± 1.37 de	6.56 ± 0.31 b	16.84 ± 0.39 b	2.39 ± 0.10 c
18	7.91 ± 2.00 d	1.56 ± 0.02 de	16.39 ± 0.29 cd	1.09 ± 0.07 c	24.26 ± 2.47 e	7.12 ± 0.53 ab	19.29 ± 1.35 ab	2.55 ± 0.15 c
21	7.32 ± 0.31 d	1.24 ± 0.07 e	14.37 ± 0.52 d	0.94 ± 0.04 c	26.92 ± 0.72 de	6.80 ± 0.25 ab	18.72 ± 1.16 ab	2.59 ± 0.06 c
<b>Below Ground Stems</b>								
0	7.82 ± 0.62 bc	1.52 ± 0.12 abc	20.00 ± 1.00 ab	1.14 ± 0.09 b	50.05 ± 4.55 a	7.62 ± 0.23 a	21.69 ± 0.96 a	3.51 ± 0.13 a
3	9.56 ± 1.16 ab	1.74 ± 0.08 ab	20.92 ± 1.12 a	1.74 ± 0.06 a	39.02 ± 0.81 b	7.49 ± 0.11 a	16.78 ± 0.55 b	3.63 ± 0.18 a
6	10.59 ± 1.26 a	1.95 ± 0.109 a	20.42 ± 0.36 ab	1.91 ± 0.08 a	36.55 ± 0.96 b	6.19 ± 0.11 b	13.26 ± 0.20 bcd	3.44 ± 0.20 a
9	7.36 ± 0.73 c	1.39 ± 0.17 abcd	16.49 ± 0.65 bc	1.19 ± 0.07 b	36.54 ± 0.34 b	5.37 ± 0.23 bc	12.77 ± 0.77 bcd	2.67 ± 0.14 b
12	6.58 ± 0.74 cd	1.19 ± 0.03 bcd	14.22 ± 0.62 cd	1.04 ± 0.02 bc	29.23 ± 1.14 bc	5.04 ± 0.14 c	11.48 ± 1.03 d	2.46 ± 0.08 b
15	5.20 ± 0.49 de	1.21 ± 0.13 bcd	12.85 ± 0.44 cd	0.92 ± 0.02 bc	22.65 ± 2.72 c	4.69 ± 0.14 c	12.27 ± 0.43 cd	2.29 ± 0.03 b
18	5.06 ± 0.37 de	0.99 ± 0.08 cd	11.54 ± 0.59 d	0.76 ± 0.05 c	21.86 ± 1.19 c	5.09 ± 0.26 c	16.39 ± 1.31 bc	2.51 ± 0.12 b
21	4.31 ± 0.22 e	0.90 ± 0.02 d	11.67 ± 0.42 d	0.78 ± 0.03 c	24.39 ± 1.28 c	5.11 ± 0.04 c	17.07 ± 0.33 b	2.67 ± 0.02 b
<b>Roots</b>								
0	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-
9	14.73 ± 1.05 a	2.37 ± 0.20 a	16.29 ± 1.08 c	2.87 ± 0.41 bc	60.44 ± 4.39 a	6.65 ± 0.31 a	10.15 ± 2.65 b	4.01 ± 0.32 c
12	14.69 ± 1.02 a	2.22 ± 0.14 a	21.42 ± 1.40 b	2.51 ± 0.11 c	42.36 ± 2.93 b	6.11 ± 0.30 a	34.73 ± 4.51 a	4.23 ± 0.36 c
15	14.44 ± 0.72 a	2.25 ± 0.14 a	30.53 ± 0.48 a	3.94 ± 0.13 ab	28.34 ± 2.46 b	4.25 ± 0.21 b	50.32 ± 3.68 a	5.97 ± 0.23 b
18	13.15 ± 0.82 a	2.02 ± 0.03 a	32.43 ± 0.28 a	5.03 ± 0.14 a	30.36 ± 1.37 b	3.39 ± 0.08 b	39.54 ± 1.45 a	8.60 ± 0.15 a
21	11.19 ± 0.22 b	1.76 ± 0.04 a	31.51 ± 0.54 a	5.01 ± 0.10 a	29.45 ± 1.79 b	3.11 ± 0.14 b	43.60 ± 1.32 a	8.82 ± 0.18 a
<b>Roots, Actual Nutrient Content</b>								
Day	N (mg)	P (mg)	K (mg)	S (mg)	Zn (µg)	Ca (mg)	B (µg)	Mg (mg)
0	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-
9	3.59 ± 0.25 e	0.58 ± 0.05 d	3.98 ± 0.27 e	0.70 ± 0.10 d	14.76 ± 1.08 d	1.63 ± 0.08 d	2.48 ± 0.65 d	0.98 ± 0.08 e
12	8.92 ± 0.59 d	1.35 ± 0.09 c	12.99 ± 0.85 d	1.52 ± 0.07 d	25.70 ± 1.78 cd	3.71 ± 0.18 c	21.08 ± 2.74 c	2.57 ± 0.22 d
15	20.15 ± 0.97 c	3.14 ± 0.19 b	42.60 ± 0.68 c	5.49 ± 0.18 c	39.55 ± 6.85 bc	5.93 ± 0.29 b	70.23 ± 5.13 b	8.33 ± 0.32 c
18	22.27 ± 1.33 b	3.42 ± 0.06 b	54.92 ± 0.48 b	8.52 ± 0.24 b	51.42 ± 2.32 b	5.74 ± 0.14 b	66.96 ± 2.45 b	14.57 ± 0.25 b
21	26.64 ± 0.50 a	4.19 ± 0.09 a	75.03 ± 1.29 a	11.94 ± 0.24 a	70.12 ± 4.27 a	7.40 ± 0.33 a	103.82 ± 3.13 a	21.01 ± 0.42 a

Table 5.1. Nutrient concentrations in plant parts, of nutrients mobilized/utilized during the adventitious root development process of *Hibiscus acetosella* 'Panama Red' propagules and actual nutrient content of roots.

Day	Mn (mg·kg <sup>-1</sup> )	Fe (mg·kg <sup>-1</sup> )	Cu (mg·kg <sup>-1</sup> )	Ni (mg·kg <sup>-1</sup> )	Mo (mg·kg <sup>-1</sup> )
<b>Foliage</b>					
0	72.80 ± 12.27 a	1141 ± 159.4 a	10.38 ± 0.97 a	93.47 ± 16.21 a	9.14 ± 1.51 a
3	70.28 ± 13.72 a	1255 ± 463.9 a	10.92 ± 1.84 a	109.03 ± 47.89 a	9.21 ± 1.45 a
6	71.31 ± 12.68 a	545.0 ± 78.26 a	7.31 ± 0.51 a	41.80 ± 7.19 a	7.53 ± 1.33 ab
9	45.25 ± 6.53 a	763.4 ± 544.2 a	6.99 ± 0.31 a	60.47 ± 23.74 a	5.56 ± 0.92 abc
12	41.51 ± 3.84 a	406.3 ± 13.29 a	5.89 ± 0.47 a	29.57 ± 1.81 a	2.92 ± 0.24 bc
15	55.30 ± 4.04 a	450.3 ± 90.08 a	5.83 ± 1.01 a	33.87 ± 8.84 a	2.09 ± 0.48 c
18	43.46 ± 4.79 a	373.3 ± 19.14 a	6.08 ± 1.64 a	28.40 ± 2.59 a	1.96 ± 0.19 c
21	42.69 ± 0.99 a	450.3 ± 35.20 a	6.63 ± 0.42 a	35.77 ± 4.88 a	2.17 ± 0.19 bc
<b>Above Ground Stems</b>					
0	39.96 ± 2.67 ab	894.8 ± 29.32 ab	8.63 ± 0.50 ab	71.07 ± 3.00 ab	8.29 ± 0.70 a
3	48.55 ± 8.41 a	1085 ± 232.9 a	10.20 ± 1.61 a	94.20 ± 24.00 a	9.18 ± 1.95 a
6	40.21 ± 5.07 ab	485.3 ± 41.43 bc	6.34 ± 0.54 abc	38.63 ± 3.33 b	6.71 ± 0.28 ab
9	25.89 ± 0.71 b	554.7 ± 80.31 bc	6.90 ± 0.49 abc	49.70 ± 8.07 ab	5.24 ± 0.93 abc
12	20.75 ± 0.94 b	383.7 ± 36.25 bc	5.28 ± 0.40 bc	30.67 ± 4.15 b	2.69 ± 0.60 bc
15	21.60 ± 1.88 b	403.67 ± 14.79 bc	4.34 ± 0.15 c	30.00 ± 0.66 b	1.41 ± 0.08 c
18	19.44 ± 1.85 b	347.77 ± 51.04 c	3.66 ± 0.72 c	27.27 ± 4.77 b	2.39 ± 0.42 bc
21	20.10 ± 2.20 b	399.3 ± 47.74 bc	4.21 ± 0.53 c	34.50 ± 4.09 b	2.38 ± 0.38 bc
<b>Below Ground Stems</b>					
0	42.09 ± 4.56 a	1331 ± 106.6 a	8.22 ± 0.64 ab	101.57 ± 9.90 ab	9.24 ± 0.68 a
3	34.93 ± 1.84 ab	1100 ± 11.82 ab	6.35 ± 0.34 ab	89.13 ± 7.66 ab	9.01 ± 0.27 a
6	35.25 ± 2.05 ab	1017 ± 144.8 ab	7.69 ± 0.35 ab	84.30 ± 6.89 ab	8.46 ± 0.13 ab
9	33.74 ± 1.86 ab	1215 ± 45.58 ab	8.76 ± 0.30 a	111.10 ± 4.49 a	7.29 ± 0.63 abc
12	26.41 ± 0.82 b	996.3 ± 58.75 ab	6.72 ± 0.34 abc	83.83 ± 2.81 ab	4.48 ± 0.52 cd
15	31.38 ± 2.24 ab	1224 ± 82.62 ab	6.66 ± 0.97 abc	104.87 ± 7.80 ab	5.78 ± 0.99 bcd
18	22.59 ± 1.67 b	794.7 ± 95.33 b	4.26 ± 0.14 bc	69.43 ± 7.13 b	3.74 ± 0.43 d
21	27.61 ± 1.74 b	974.0 ± 99.05 ab	5.39 ± 0.71 c	90.43 ± 8.93 ab	4.98 ± 0.37 cd
<b>Roots</b>					
0	-	-	-	-	-
3	-	-	-	-	-
6	-	-	-	-	-
9	94.23 ± 4.20 a	4375 ± 159.2 a	22.49 ± 1.21 a	377.63 ± 24.09 a	21.06 ± 1.02 a
12	62.13 ± 6.31 b	2751 ± 216.5 b	14.42 ± 1.06 b	220.00 ± 17.72 b	11.60 ± 0.34 b
15	74.58 ± 7.17 ab	2509 ± 292.9 bc	14.78 ± 1.87 b	222.73 ± 30.81 b	14.15 ± 2.81 ab
18	58.77 ± 5.88 b	1644 ± 206.3 cd	11.11 ± 1.66 b	133.97 ± 18.52 b	10.29 ± 0.61 b
21	59.68 ± 5.85 b	1409 ± 72.56 c	9.41 ± 0.32 b	130.37 ± 7.44 b	10.17 ± 0.38 b
<b>Roots, Actual Nutrient Content</b>					
Day	Mn (µg)	Fe (µg)	Cu (µg)	Ni (µg)	Mo (µg)
0	-	-	-	-	-
3	-	-	-	-	-
6	-	-	-	-	-
9	23.02 ± 1.03 b	1069 ± 38.87 c	5.49 ± 0.29 b	92.25 ± 5.89 b	5.14 ± 0.25 b
12	37.69 ± 3.83 b	1669 ± 131.3 bc	8.75 ± 0.64 b	133.47 ± 10.76 b	7.04 ± 0.21 b
15	104.08 ± 9.99 a	3502 ± 408.89 a	20.63 ± 2.61 a	310.83 ± 42.98 a	19.74 ± 3.92 a
18	99.53 ± 9.95 a	2784 ± 349.4 ab	18.82 ± 1.97 a	226.90 ± 31.34 ab	17.43 ± 1.03 a
21	142.09 ± 13.92 a	3354 ± 172.8 a	22.40 ± 0.77 a	310.40 ± 17.71 a	24.22 ± 0.89 a

Table 5.2. Nutrient concentrations in plant parts, of nutrients with no appreciable change or pattern of use during the adventitious root development process of *Hibiscus acetosella* ‘Panama Red’ propagules and actual nutrient content of roots.

CHAPTER 6  
SCARIFICATION AND GERMINATION OF AMSONIA TABERNAEMONTANA  
(WALT.) SEEDS <sup>1</sup>

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<sup>1</sup> Martin, Jr., M.T. , M.R. Chappell and L.R. Opie. Submitted to *Journal of Environmental Horticulture*, 7/20/2012.

## ABSTRACT

The ability to germinate seeds of *Amsonia tabernaemontana* Walt. [Apocynaceae] at a high percentage was demonstrated in this study. Using the best seed treatment protocol developed in this study, *A. tabernaemontana* germinated at a rate of 70.4% during a 28 d period. This protocol included mechanical scarification followed by imbibition for 48 hr at 24° C (72° F). Seeds receiving no scarification and no imbibition germinated at 0% during this same 28 d period. Numerous seeds were dissected to determine the best method for seed coat scarification. These dissections showed embryos were arranged longitudinally within the seeds and that mechanically removing 1 - 2 mm (0.04 - 0.08 in) of the seed coat terminus to expose the embryo was the best method of scarification. Imbibition curves were developed for scarified and control (unscarified) seeds to explain the dormancy mechanism for *A. tabernaemontana* seeds. Water was imbibed at a similar rate ( $P = 0.5217$ ) for treatments, implying seed coat or endosperm act as a mechanical barrier to germination. Regardless of imbibition treatment, mechanically scarified seeds germinated at rates higher than unscarified seeds as shown in germination curves.

### *Index words*

Eastern bluestar, imbibition, mechanical barrier, seed propagation,

### *Species Used in this Study*

*Amsonia tabernaemontana* Walt. [Apocynaceae] (Nomenclature: USDA NRCS, 2011)

## INTRODUCTION

Eastern bluestar (*Amsonia tabernaemontana*), is an herbaceous perennial with a native range from southern NY to northern FL east to eastern TX. The genus *Amsonia* is distributed worldwide with 16 recognized species and numerous subspecies occurring in North America (13). *A. tabernaemontana* is an attractive plant with interesting ornamental characteristics, including pastel blue flowers in the spring and yellow fall foliage. *A. tabernaemontana* experiences few pest and disease problems, making it an ideal addition to the landscape. *A. tabernaemontana* is propagated primarily by seed and are reported to be difficult to germinate in a production environment due to uneven germination time and low germination percentage (9, 12). Current production methods involve planting seed outdoors in fall (September and October), allowing seed to naturally scarify during winter months and germinate in late spring. In a commercial setting this method of natural scarification is inefficient in its requirement for long-term use of production facilities (bench space). Additionally, variable germination times result in non-uniform liner size that can lead to a lack of uniformity later in container production.

Several popular publications (10, 4, 1) indicate that removing a portion of the seed coat increases germination percentage of *A. tabernaemontana*. The information provided by these sources is little more than anecdotal notes and provide no details as to the best method of seed scarification and if environmental conditions must be satisfied to facilitate germination. To date no scientific study specifying a technique for seed coat removal or stating a rate (percentage) that seed coat removal affects germination percentage has been published. A series of seed treatments, including the current

commercial method of no scarification or imbibition, were developed to determine how scarification and imbibition affected *A. tabernaemontana* germination. Two imbibition temperatures were utilized; 24° C (72° F) and 34° C (93° F). Each temperature was paired with both non-scarified and scarified seed treatments. Using 24° C (72° F), room temperature, as the base temperature it was hoped that raising the imbibition temperature 10° C to 34° C (93° F) would provide a Q<sub>10</sub> effect (8) increasing embryo enzymatic activity and thereby decreasing germination time. Additionally, a scarified without imbibition treatment was included to discern the effect of imbibition on germination. The objectives of this study were to determine the best method for seed coat removal and develop an overall germination protocol for *A. tabernaemontana*.

## MATERIALS AND METHODS

Mature seeds were collected in August 2008 from wild populations of *A. tabernaemontana* in Oconee County, SC. After collecting, seeds were cleaned, sealed in polyethylene bags and stored at room temperature (24° C; 72° F) until the study began. To determine the best method for cutting (scarifying) *Amsonia* seed coats, 10 seeds were cut longitudinally to determine how embryos were positioned within seed (Figure 1D, 1E). Additionally, seed length and width were measured for 60 seeds. Observations lead to the conclusion that carefully removing 1 - 2 mm (0.04 - 0.08 in) of either end of the seed coat with a surgical scalpel would not severely damage embryo. Using a method of removing thin slices of seed coat until embryo was exposed further minimized embryo damage; this methodology was used for treatments that had their seed scarified. Imbibition curves for control and scarified seeds (30 seeds in each group) were developed

for seeds submerged in 24° C (72° F) water for 48 hr. Dry weight of each seed was taken at time 0 and reweighed at 1, 2, 3, 4, 6, 12, 24 and 48 h intervals. After imbibition was initiated, seeds were blotted dry prior to weighing. Once a proper scarification technique had been developed, the following treatments were developed to simulate both natural and ideal germination situations. Treatments were: 1) control (no scarification or imbibition), 2) seed coat scarified, no imbibition, 3) no scarification, imbibed for 48 h at 24° C, (72° F) 4) seed coat scarified, imbibed for 48 h at 24° C (72° F), 5) intact seed coat, imbibed for 48 h at 38° C (100° F) and 6) seed coat scarified, imbibed for 48 h at 38° C (100° F). Seeds for treatments 3 through 6 were imbibed in 40 ml (1.35 oz) of deionized water using two Conviron CMP3244 plant growth chambers (Controlled Environments Limited, Winnipeg, Manitoba, Canada). Use of growth chambers ensured that treatment temperatures were maintained during the entire imbibition period. Each treatment consisted of 4 replications of 38 seeds (152 seeds per treatment), planted at a depth of 15 mm (0.59 in) in a 195 cm<sup>3</sup> (11.9 in<sup>3</sup>) 38-cell seedling tray (Landmark Plastic Corp., Akron, OH) filled with Fafard 52 media mix (Conrad Fafard, Inc., Agawam, MA).

After planting, seeds were placed under a mist bench to maximize germination potential. The mist system used Netafim Violet Vibro-Mist Misters (Netafim USA, Fresno, CA) spaced at 1 m (3.28 ft) intervals along the length of the bench and placed at a height of 0.75 m (2.46 ft) from the bench surface. The system operated from 7:00 AM to 8:00 PM daily and activated every 6 min for 6 sec using a 1A Single Zone Controller (Phytotronics, Inc., Earth City, MO). The output of each misting head ranged from 650 to 700 ml (21.9 to 23.6 oz) per minute. Seed germination was measured daily for 28 d; a seed was considered germinated if the cotyledon had emerged from the soil surface. A 28

d germination period was selected to correspond with standard commercial propagation protocols for seed germination. All data were analyzed using SAS 9.2 (SAS Institute Inc. Cary, NC) proc GLM. Seed imbibition data were analyzed using a student t-test to determine differences between imbibition for each treatment group. Germination percentage data were analyzed using a general liner model with Tukey's HSD for means separation. A significance of  $P = 0.05$  was used for all testing.  $T_{50}$  values and germination values were calculated for all treatments.

## RESULTS AND DISCUSSION

Seeds of *A. tabernaemontana* used in this study were  $9.92 \pm 1.25$  mm ( $0.39 \pm 0.05$  in) in length and  $2.47 \pm 0.25$  mm ( $0.1 \pm 0.01$  in) in diameter. Dissection of *A. tabernaemontana* seeds showed the longitudinal arrangement (Figure 1D) of embryos within seed. It was determined that a cross-sectional cut near the seed middle or a longitudinal cut through the seed would cause embryo mortality. Based on this observation, removing 1 - 2 mm of the seed coat end to expose the embryo (Figure 1C) was the preferred seed scarification method. Ideally, this scarification cut would be made on cotyledon end of the seed but distinguishing one end of the seed from the other has proven impossible. This inability to distinguish ends caused concern that scarification cuts may damage root radicals, ultimately proving fatal to embryos. This concern proved unwarranted as seed scarified on radical end germinated similarly to seeds whose cotyledon ends were scarified (Table 1). Adventitious roots were observed on germinated seed where radicals had been damaged during scarification and no difference in seedling performance was observed.

Imbibition curves (Figure 2) of control and scarified seeds show that water uptake was not influenced by scarification, as there was no difference between values for the two treatments throughout imbibition period ( $P = 0.5217$ ). After 48 hr, non-scarified seeds had increased their mass by 88% and scarified seeds had increased their mass by 90%. This indicates seed coats of *A. tabernaemontana* are permeable. Seeds that were scarified imbibed water and had embryo emergence from seed as seen in Figure 1E. Given that control seed showed no sign of embryo emergence but imbibed similar quantities of water, it is probable that the seed coat acts as a mechanical barrier to germination (mechanical dormancy). This differs from physical dormancy, as defined by Baskin and Baskin (3) and Finch-Savage and Leubner-Metzger (7), who defines physical dormancy as the prevention of water uptake by the seed coat or endosperm. This mechanical dormancy may explain poor and uneven germination of seeds allowed to naturally scarify during winter months. Unscarified seed would require a combination of natural abrasion or microbial action to weaken seed coat and an increase of embryo turgor pressure to break through the seed coat so germination could occur. Washitani and Masuda (14) conducted germination studies on *Amsonia elliptica* [(Thunberg ex Murray) Roemer & Schultes], an Asian *Amsonia* species, utilizing increasing or decreasing temperature regimes (stratification). Washitani and Masuda (14) found an increasing temperature regime following “a long moist chilling” yielded a study high of 10% germination for *A. elliptica*. This study did not report on the imbibition of water prior to germination or the effect of mechanical scarification on germination, so it is impossible to discern if there is a physical barrier to water uptake or physiologic barrier to germination in *A. elliptica*; or simply a mechanical barrier as described in this study with *A. tabernaemontana*.

Nonetheless, Baskin and Baskin (2) interpreted Washitani and Masuda's (14) results to be a form of physiological dormancy, yet noted this classification was inferred. It is possible that the dormancy exhibited by *A. tabernaemontana* in this study is a form of nondeep physiological dormancy that is met after a short period in storage and that storage of seeds at room temperature (24° C; 72° F) met requirements to break this dormancy. Regardless, this work has demonstrated that the dominant form of seed dormancy in *A. tabernaemontana* is mechanical dormancy imposed by the seed coat. From a commercial producer's prospective, this mechanical dormancy is the most difficult to overcome and hence should be the focus of any seed treatment in *A. tabernaemontana*.

Germination curves (Figure 3) showed mechanically scarified seeds germinated at rates higher than seeds that were not mechanically scarified. Germination curves also showed that imbibition and imbibition temperatures influenced germination rates of scarified seed. Treatments whereby seeds were not mechanically scarified germinated at rates between 0 and  $4.61 \pm 1.66\%$  (Table 1). These low rates of germination were likely due to mechanical dormancy caused by the seed coat. Scocco and others (12) found that untreated seeds germinated at rates between 80 and 82.7% for seeds collected previous growing season and germinated in Petri dishes during a 30 d period. This implies a lack of mechanical dormancy and is in direct contradiction to findings of present study (Table 1) and the low germination percentage observed anecdotally by commercial growers and many trusted resources (10, 4, 1). Additionally, Scocco et al. (12) found that seeds soaked for 24 h in 10 mM GA<sub>3</sub> germinated at rates between 96.0-98.7%. Differences in germinations rates between the two studies could be explained by differing seed

morphology, specifically seed coat properties as related to seed provenience or population.

Scarified seeds imbibed at 38° C (100° F) germinated at lower rate (36.18 ± 3.78%) than scarified seeds that received no imbibition or were imbibed at 24° C (72° F). This lower germination rate is possibly due to high temperature embryo damage or increased respiration rates exhausting endosperm reserves prior to germination. Scarified seeds that received no imbibition germinated at a high rate, 57.24 ± 3.46%. This moderate germination rate is due to imbibition of soil moisture once seed were planted. While this rate is lower than a 24° C (72° F) imbibition treatment, it is note-worthy that scarified seed, when provided with adequate soil moisture (germination conditions) will germinate at a rate near commercially acceptable levels. Further, imbibition of scarified seeds may act as a primer, increasing germination rates and possible decreasing time to maximum germination rates. Based on these results, the following protocol is recommended for *Amsonia tabernaemontana* seed germination: scarification of one end of the seed and imbibition for 48 h at 24° C (72° F).

T<sub>50</sub> values indicate *Amsonia tabernaemontana* seed scarified and imbibed for 48 h at 24 °C and seed that were scarified but with no imbibition had similar germination rates. For the two stratification treatments, seed scarified and imbibed for 48 h at 24 °C and seed scarified but with no imbibition, stratification decreased the number of days to 50% germination. It is likely that the high imbibition temperatures (38 °C) of the third stratification treatment significantly reduced the germination of seeds in this treatment. All unscarified treatments as well as seed scarified and imbibed at 38 °C did not obtain a T<sub>50</sub> value during the course of this 28 day study (Table 6.1). Furthermore, germination

value, a combined index of speed and completeness of germination (11) based on peak germination percent and mean daily germination (5, 6) was calculated. Germination values indicate that *A. tabernaemontana* seed that were scarified and imbibed at 24 °C germinated at a rate nearly twice that of the next closest treatment of seed that were scarified with no imbibition. Germination values of unscarified treatments were much lower than those of scarified treatments. A direct comparison of unscarified and scarified seeds both imbibed at 24 °C showed that scarification increases germination values by 160 times, demonstrating the importance of mechanical scarification to germination of *A. tabernaemontana* seeds.

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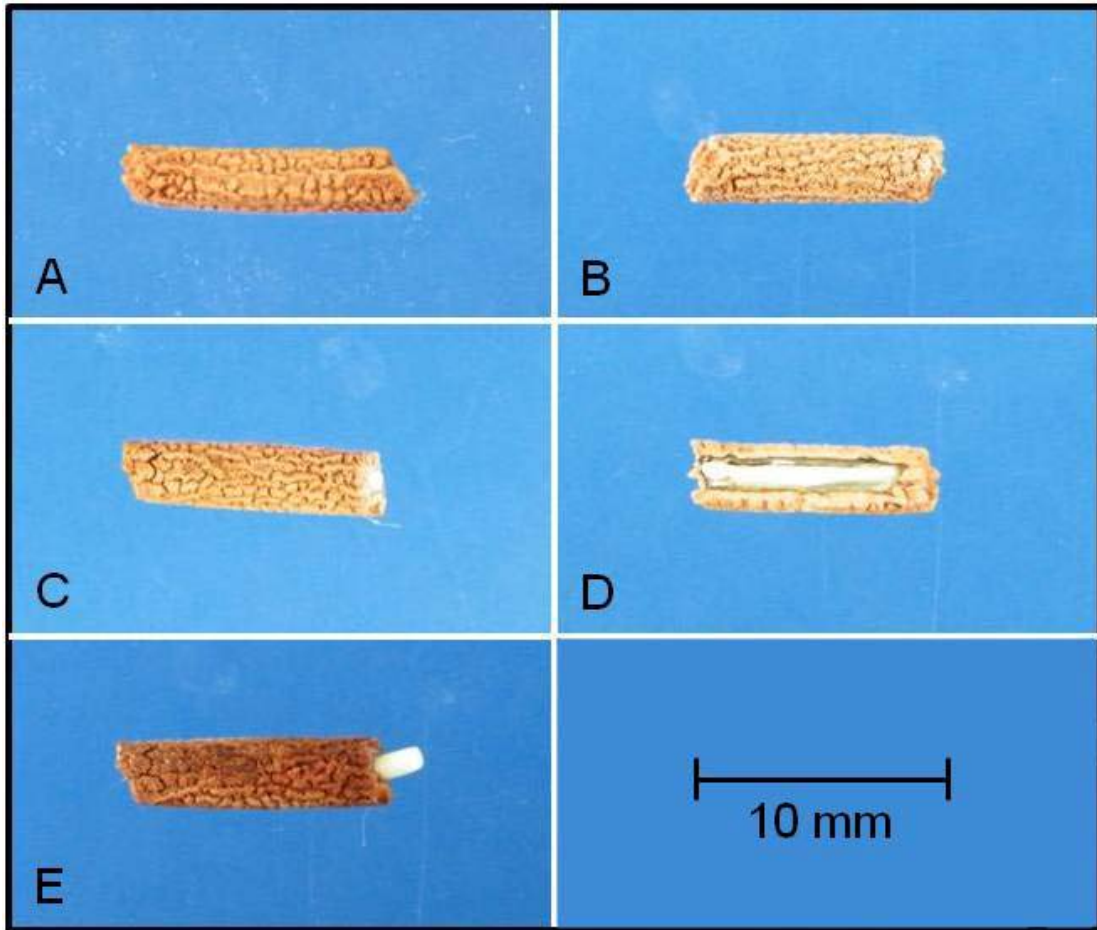


Figure 6.1. Progression of seed scarification treatments. A, B. seeds with no scarification. C. Seed A with 2mm of seed coat removed from end of seed (cut made on right side). D. Seed B cut longitudinally. E. Seed A imbibed for 48 h at 24 °C after scarification, with partial embryo emergence.

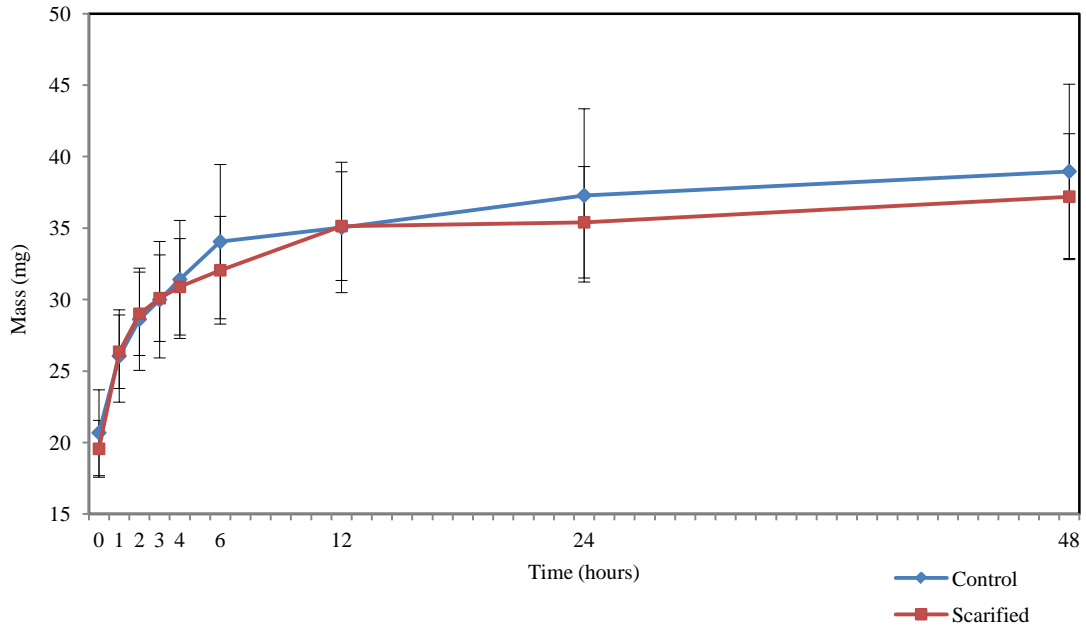


Figure 6.2. Imbibition curves for control and scarified *A. tabernaemontana* seeds.

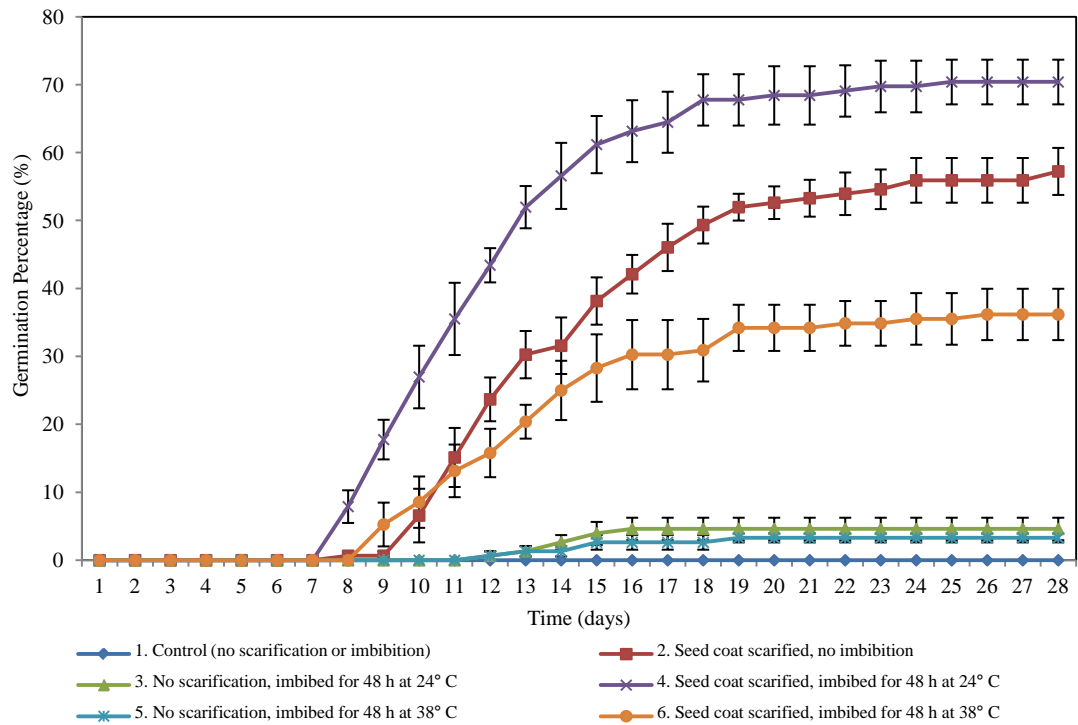


Figure 6.3. Germination curves for control and combinations of imbibition and scarification treatments of *A. tabernaemontana* seeds.

Treatment	Days to 50%	Germination (%)	Germination value
1. Control (no scarification or imbibition)	*	0 ± 0 d	0 ± 0 c
2. Seed coat scarified, no imbibition	13.25 a	57.2 ± 3.46 b	13.04 ± 1.46 b
3. No scarification, imbibed for 48 h at 24 °C	*	4.61 ± 1.66 d	0.14 ± 0.06 c
4. Seed coat scarified, imbibed for 48 h at 24 °C	13.50 a	70.4 ± 3.29 a	22.37 ± 2.62 a
5. Intact seed coat, imbibed for 48 h at 38 °C	*	3.29 ± 0.66 d	0.06 ± 0.02 c
6. Seed coat scarified, imbibed for 48 h at 38 °C	*	36.2 ± 3.78 c	5.65 ± 1.22 c

Table 6.1. Mean days to 50% germination, final germination percentage, and final germination value for *A. tabernaemontana* seeds after scarification and imbibition treatments. Errors represent standard deviations, letters are means separation. \* = treatment did not obtain 50% germination.

## CHAPTER 7

### CONCLUSIONS

Adventitious root development (*ARD*) is an extremely complex morphological and physiological process. The goal of this research was to gain a better understanding of basic vegetative propagule physiology and how water relations, environmental factors under mist, photosynthesis, carbon utilization and nutrition affect basic propagule physiology and *ARD* success. While this study successfully answered the questions set forward by these objectives, it has also led to additional questions.

Propagules under intermittent misting successfully maintained water ( $\Psi$ ), osmotic ( $\Psi_s$ ) and turgor potential (P) values (Chapter 2) and appeared not to experience water stress that could be detrimental to *ARD*, propagule survival and propagule quality. The maintenance of  $\Psi$ ,  $\Psi_s$  and P demonstrated the likelihood of an “inverse” water potential gradient, with water entering via the stomata or leaf surface and traveling down the vascular tissue toward the developing roots. This “downward” movement of water could also provide a means for basal translocation of some nutrients via xylem (Chapter 4).

Despite a lack of water stress observed in propagules (Chapter 2), stomatal closure was observed in propagules immediately following severance from stock plants. This stomatal closure subsequently led to decreases in net photosynthesis of propagules (Chapter 3). From this, the question arises: What triggers stomatal closure in propagules if not water stress? Answering this question could lead to maintaining net photosynthesis similar to that of stock plants and potentially speeding up the process of *ARD*.

Results indicated that photosynthesis did occur in leafy propagules before adventitious roots appeared. This is supported by both real-time photosynthetic measurements (Chapter 3) and  $C^{13}$  sequestering on 0 d, evident in propagules harvested on 3 d (Chapter 4). The production of new photosynthates by propagules is important to the *ARD* process, as these photosynthates are basally translocated (Chapter 4). This basipetal translocation of photosynthates likely demonstrates a means of transporting selected nutrients via phloem (Chapter 6) to the basal portion of propagules. The relationship between propagule photosynthesis and *ARD* warrants further research. Specifically with hibiscus, propagule net photosynthesis (Chapter 3) and  $C^{13}$  sequestering rates (Chapter 4) greatly increased after adventitious roots appeared. This seems to indicate that while photosynthesis is important for initial *ARD*; increased propagule net photosynthesis are dependent upon some factor related to *ARD* such as increased water or nutrient availability.

For nursery and/or greenhouse producers, this research demonstrates the importance of adequate misting to maintain proper water potential status of propagules to increase *ARD* success (Chapter 2). Atmospheric  $CO_2$  enrichment may not be cost effective until net photosynthesis increase in propagules. More work should be conducted on when to initiate  $CO_2$  enrichment, as this study indicates propagule net photosynthesis do not increase until roots become visually apparent (Chapter 3 and 4). Carbohydrate and nutrient “loading” of stock plants may be beneficial to propagule success (Chapter 4) and development of propagule specific nutrient mist to improve propagule success rates (Chapter 5) may be warranted.

In a separate study, development of an effective means of seed propagation of *Amsonia tabernaemontana* (Chapter 6) will hopefully increase the use and sale of this herbaceous ornamental taxa in the nursery trade. For effective seed scarification, research indicated that embryo arrangement warranted removal of one end of the seed coat. Imbibition curves showed that water uptake was not influenced by scarification, indicating seed coats were water-permeable; and that seed coat acts as a mechanical barrier to germination (mechanical dormancy). Further, this propagation method needs to be applied to other *Amsonia* species to test its effectiveness across the genus. If effective in other *Amsonia* species, it could be applied to threatened and endangered *Amsonia* species or used to germinate seeds produced via interspecific hybridization.