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Submist as an Effective Method for the Rooting of Herbaceous and Woody Ornamental Plants by Stem Cuttings

Olivia Sanchez
University of Maine, olivia.sanchez@maine.edu

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By

Olivia Sanchez

B.S. University of Detroit Mercy, 1999

M.S. University of Maine, 2002

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Advisory Committee:

Stephanie E. Burnett, Associate Professor of Horticulture, Co-Advisor

Bryan J. Peterson, Assistant Professor of Environmental Horticulture, Co-Advisor

Matthew Wallhead, Extension Ornamental Horticulture Specialist and

Assistant Professor of Horticulture

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By Olivia Sanchez

Thesis Co-Advisor: Dr. Stephanie Burnett

Co-Advisor: Dr. Bryan Peterson

An Abstract of the Thesis Presented
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The primary method of vegetative propagation by stem cutting is to insert leafy cuttings into a solid soilless media and place the cuttings in an environment that provides moist, humid conditions to the foliage through fog or overhead intermittent mist. These systems reduce water loss through transpiration by reducing leaf temperature and provide moisture to the rooting media. However, excess water, both on the media and on the leaves can result in an increase incident of diseases. To alleviate some of these issues, research has found stem cuttings can be rooted in sub-mist systems that apply water solely to the base of cuttings without the need of an overhead mist system. With positive rooting results in these systems, including higher rooting percentage, longer roots and greater dry weight, there is limited data on how cuttings respond to being transplanted into a soilless media. In addition, while there is research reporting gas exchange measures during rooting of stem cuttings, it has focused on overhead mist. To gain insight into these inquires, two studies were conducted. The first compared the rooting and post-transplant performance of an ornamental herbaceous species, *Lantana camara* 'Dallas Red' (lantana) by stem cutting using an overhead mist (OM) and a submist system (SM). The second study compared the rooting and gas exchange measures of the woody ornamental plant *Syringa pubescens* ssp. p*atula* (manchurian lilac) by stem cutting using an OM, SM, and hybrid (HY) system.

Survival of lantana in both OM and SM was 99%; cuttings in the OM produced 45% more roots than the SM. By contrast, cuttings in the SM produced roots that were 73% longer. When comparing post-transplant performance, survival rate for plants grown from cuttings propagated under overhead mist was 53%, significantly lower than the submist cuttings with a 95% survival rate. The results of most measures of growth did not differ significantly in the remaining surviving cuttings.

In the second study, rooting percentages of manchurian lilac was significantly higher in the HY system (90%), while non-significant between OM and SM, at 68% and 62%, respectively. The systems utilizing submist (HY and SM) had significantly higher root count and root length and retained most of their leaves. Photosynthetic rate differences were non-significant between systems, but in all systems increased at root emergence. Stomatal conductance also increased as roots emerged in the cuttings but was more notable in the HY and OM systems. Stomatal conductance in the SM remained relatively constant even after root emergence and was significantly lower than both the HY and SM systems.

The use of systems utilizing submist, providing intermittent mist to the basal end of the cutting, have shown to be effective alternative methods to propagating both herbaceous and woody ornamental plants, with positive rooting performance in lantana and manchurian lilac.

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LITERATURE REVIEW

1.1. Current Methods of Vegetative Propagation

Vegetative propagation by stem cutting is not a modern discovery. In 1891, Liberty Bailey published his first edition of *The Nursery-Book: A complete guide to the multiplication and pollination of plants.* In this book, he includes a chapter titled "Cuttage" describing various methods of propagation by leaf, stem, and root cuttings. These types of cuttings are still used today, with the method of propagation remaining relatively unchanged as well.

The general method of propagation by stem cutting is to take the cutting from the stock plant and place it in a solid soilless media such a perlite, sand, peat, or a combination of those components. Trays of cuttings are then placed in a high humidity environment. This environment can be created using a fogging system or intermittent mist applied overhead to the foliage. This method of maintaining the water status of plants, while not new, has evolved over time. In 1852, Nathaniel Ward published his account of discovering an enclosed glass case could be used to maintain a humid environment conducive for plant growth, now known as the Wardian case, or more generally, a terrarium. In 1891, Bailey describes general methods of taking both hardwood and softwood cuttings, with propagation methods including setting them in "soil that is kept moist, bottom heated, in an enclosed case and sprinkling frequently to prevent wilt". This method, while effective, could also be labor and time intensive. In the 1940's and 1950's, the inventions of automated overhead irrigation controls changed plant production and efficiency. Harvey Templeton (1955) describes his design and use of the first electronic leaf and mist nozzles used to monitor and control water application to cuttings. During this same period, Charles Hess (1955) describes his invention and use of an electronic timer that further controlled when cuttings were watered. The combination of Templeton's mist nozzles and Hess' timer inventions set the standard method of providing water to cuttings during propagation and continues to

be used today. Modern plant propagators still use a type of overhead mist or fog system that can be controlled by timers or by controllers that monitor atmospheric conditions surrounding the cutting foliage to minimize heat or water stress. While overhead mist equipment modernized, and overtime have become more efficient, issues surrounding the use of overhead mist persist.

Although water usage can vary based on the type of nozzles used to apply mist, it can still be excessive and create sanitation issues in the root zone. A review of references on the propagation of woody species will show a greater emphasis on the type of soilless media to use to ensure adequate drainage and aeration to allow for root development. Hartman et. al. (2011) states the media should not be hydrophobic but be sufficiently porous to allow the influx of oxygen into the root zone. Aside from allowing for adequate aeration, a media that does not provide adequate drainage can result in excess water and an environment that is too wet, which, as well understood in the late 1800's and early 1900's, can lead to disease such as *Botrytis cinerea* affecting wounded cuttings or any one of *Pythium* species which can cause root rot (Preece, 2003; Hartman et. al., 2011, Nelson, 2012). Overhead mist can also lead to leaching of nutrients, which has been a known issue and noted by Nitche (1966). Despite disease concerns, contemporary propagation systems utilizing mist, fog, or enclosure function to provide a high humidity environment, because an unrooted cutting must remain hydrated until roots form (Bidlack and Jansky, 2017). When roots are severed, such as by the collection of leafy stem cuttings from stock plants, cuttings immediately begin to experience water stress. Hartman et al. (2011) emphasize the need to maintain both a humid environment around the leafy stem cutting and a moist medium to maintain cutting water status during root development. Although the stress responses of a cutting initiate the process of adventitious root formation, water-stressed cuttings in low moisture substrates may root more slowly and form fewer roots than cuttings provided ample substrate moisture (da Costa et al., 2013; Hartman et al., 2011).

1.2. Cutting physiology

The formation of roots in stem cuttings is often broken down into four stages: 1) dedifferentiation, 2) formation of root initials, 3) development of root primordia, and 4) emergence of root primordia and subsequent root growth (Hartman, et. al., 2011). This process results in wound-induced roots with the wound being from the initial cut from the stock plant, and, in the case of woody species, an intentional wound created along the length of the basal end of the stem. These wounds induce a hormonal response in the cutting where auxin is transported to the wound site where the root formation process begins. In addition, the additional wounding allows for better absorption of applied rooting hormones often applied prior to placing stem cuttings in the rooting media. During this time, the metabolism of the cutting changes in response to the severing of the stem from the stock plant vascular system. This includes a reduction in stomatal conductance and photosynthesis (Hartman, et. al., 2011).

As part of a plant's metabolic processes, the diffusion of gases into plant leaves through stomata is required for the assimilation of carbon dioxide (CO_2), a process that necessarily releases water to the atmosphere through open stomata (Bidlack and Janksy, 2017). The degree to which stomata open is dictated by the overall water status of a plant, as well as environmental factors like vapor pressure deficit, light, and temperature. Stomatal conductance of plants is decreased in soil with a low water potential. This limits water loss through transpiration, but also limits photosynthesis through reduced uptake of CO_2 (Taiz et al., 2015). Carvalho et al. (2016) grew grapevine plants in a variety of conditions to impose water, heat, and light stress, and combinations of stresses, such as water and heat stress. Plants under combinations of water stress and other stresses had significantly lower stomatal conductance than plants subjected light or heat stress alone.

In an effort to capture metabolic changes at key points during propagation, researchers have measured gas exchange parameters at the time of cutting, during the rooting phase, and when roots emerge. For example, Svenson et al. (1995) measured gas exchange rates in cuttings of *Euphorbia*

pulcherrima (poinsettia) propagated in perlite within a growth chamber. Physiological measurements of photosynthetic rate and stomatal conductance, recorded 8 times over a 24-d period, remained consistent through the first 10 d, during which no root formation was evident. Both the photosynthetic rate and stomatal conductance increased at d 13, when root primordia were visible, and continued to increase for the duration of the study. LeBude et al. (2005) reported the effect of overhead mist levels on gas exchange during rooting of *Pinus taeda* (loblolly pine) stem cuttings. Among a range of mist levels from 45 to 310 mL•m^{-2}, the higher mist levels increased both photosynthetic rate and stomatal conductance throughout the experiment. In addition, stomatal conductance did not increase significantly following 70 d after setting at mist levels between 45 to 75 mL•m^{-2} (LeBude et al., 2005).

Fordham et al. (2001) measured stomatal function in both old and young leaves of leafy stem cuttings and whole plants of *Corylus maxima* (purple filbert) under various moisture conditions. Stomatal conductance remained constant on older, fully expanded leaves over a 21 d period. In the younger leaves, stomatal conductance increased over the same period, prior to root emergence; stomatal conductance was measured through 21 d. Roots emerged between 24 to 30 d, however data after root emergence was not reported.

While these studies show relationships in gas exchange measures during rooting of leafy stem cuttings, cuttings in all of these studies were propagated in a solid, soilless medium with the relative humidity and leaf temperature affected by a growth chamber or overhead mist. Conversely, there is evidently no information in the literature on photosynthesis or stomatal conductance of cuttings propagated in a greenhouse environment without overhead mist.

1.3. Alternative Methods for Propagation by Stem Cutting

With ongoing research on the propagation of various plant species using the current method using overhead mist, research has been conducted using other options to reduce the moisture present on leaves of cuttings, such as varying the timing of mist or eliminating the use of an overhead mist

system altogether. One alternative propagation system that has been trialed is subirrigation, which uses a solid substrate in which cuttings are inserted. This system provides water to the basal ends of stem cuttings by allowing water to rise via capillary action through the substrate. Relatively coarse soilless media, such as perlite, are generally used in these systems, and the water level is maintained at a level below the basal end of the cutting. Giroux et al. (1999) used a subirrigation system with minimal mist (10 s every 64 min) to root softwood stem cuttings of nine different woody plants in a variety of media ranging from 1:4 peat:perlite to 100% perlite. The authors found that success varied based on the medium and species, but that subirrigation was a viable means of propagating plants (Giroux et al., 1999). Investigating the effects of air temperature, Graves and Zhang (1996) used 100% perlite in a subirrigation system to propagate stem cuttings of *Dendranthema grandiflorum* (chrysanthemum) and *Fuchsia hybrida* (fuchsia), and more quickly achieved 100% rooting for both taxa when the air temperature was held at a warmer temperature of 31°C instead of 23°C.

While researchers investigated subirrigation, in which a solid, soilless medium is required, others investigated systems in which stem cuttings were suspended in aeroponic chambers without a solid medium. Soffer et al. (1988) compared the rooting performance of stem cuttings of *Ficus benjamina* (ficus) and *Chrysanthemum* × *morifolium* (garden mum) in an aero-hydroponic system and in solid, soilless media. In the aero-hydroponic system, the basal ends of cuttings were immersed in water, the middle portions of the stems were exposed to a water mist, and the upper portions were in air. The study compared rooting percentages when overhead mist was used and was not used. In the aero-hydroponic system, rooting percentages were over 60% in misted and unmisted systems, whereas the cuttings in the solid medium had maximum rooting percentages of 50%.

The study by Soffer et al. (1988) is one of only a limited number of studies investigating propagation systems without a solid medium. More recently, Peterson et al. (2018a) compared rooting performance of *Plectranthus scutellarioides* 'Wizard Mix' (coleus) in four different systems.

Subirrigation, submist, and subfog systems, in which water was applied only to the basal ends of cuttings, were compared to overhead mist. The submist system outperformed all the remaining systems in measures of root number, root length, and root dry weight. In addition, coleus rooted in the submist system and later transplanted into greenhouse pots filled with soilless media did not experience transplant shock and had post-transplant measures of root growth that were equal to, or exceeded, those of plants propagated by overhead mist. Peterson et al. (2018b) later investigated the use of submist to root *Syringa pubescens* ssp. *patula* (manchurian lilac) and *Ilex glabra* (inkberry). Cuttings of both woody species produced rooting percentages and root numbers in submist that were equal to, or greater than, those produced in overhead mist. These studies indicate that vegetative propagation of stem cuttings by the application of water to the basal ends of stem cuttings is a viable approach.

1.4. Further Investigations into Propagation by Stem Cuttings

Many of the studies using alternative methods of propagation have been single studies investigating rooting potential. It has been found that cuttings, both herbaceous and woody, placed in systems without the use of overhead mist have successfully rooted. What was not clear prior to the coleus study by Peterson et. al. (2018a) was whether cuttings rooted in a submist system could be transplanted into greenhouse media with no adverse effects. For the submist system to be considered a viable and effective method for propagation, post rooting performance is the next step to investigate further for both other herbaceous and woody species. For woody species, the type of cutting (softwood, semi-hard wood, hardwood) and time of season plays a role in the overall success of rooting. While successful rooting of a woody species can be initial proof of concept success, there is still a need to repeat the study over multiple seasons to show successful rooting using a new method when the window of obtaining cuttings at the most adventitious time can be narrow and vary from season to season. The ability for woody cuttings in various stages of growth to root in systems other than in solid media with overhead mist needs further investigation. Conducting additional studies using alternative

methods and comparing rooting success to the overhead mist system will also allow for other system comparisons.

Many of the physiological studies conducted on gas exchange measurements during rooting were conducted on cuttings placed in growth chambers or under overhead mist. While these have provided insight into the metabolic process under those conditions, it is hypothesized by this author that cuttings rooted in a submist system without the use of overhead mist are undergoing additional heat and water stress. To what degree this affects both stomatal conductance and photosynthetic rate compared to those under overhead mist is unknown. And would the use of a hybrid system in which both overhead mist and submist system are used, without the use of solid media, provide the cooling effect overhead mist provides that minimizes heat and water stress with the high rooting success of the submist system reported by others? The limited number of studies conducted on submist systems have shown it to be a potentially viable option for propagators, but there are still questions to be addressed. The studies presented here begin the process of gaining additional insight into these issues.

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CHAPTER 2

VEGETATIVE PROPAGATION OF *LANTANA CAMARA* 'DALLAS RED' BY SUBMIST AND OVERHEAD MIST

2.1. Abstract

Lantana camara 'Dallas Red' (lantana) was vegetatively propagated by stem cuttings using an overhead mist and a submist system to compare rooting effectiveness. Both systems applied mist for 10 s every 10 min**;** the overhead mist applied water to the leaves and the submist applied water to the basal ends of the cuttings. Cuttings propagated in both systems had a 99% survival rate. Cuttings in the overhead mist system produced 45% more roots than cuttings in the submist system. In contrast, cuttings in the submist system produced roots that were 73% longer. When comparing post-transplant performance, survival rate for plants grown from cuttings propagated under overhead mist was 53%, which was significantly lower than the 95% survival of plants grown from cuttings propagated by submist. Most measures of post-transplant growth did not differ significantly among surviving cuttings. Differences in post-transplant survival between systems may be attributed to transplant shock among cuttings rooting in overhead mist, transitioning from regular intermittent mist to a mist-free environment with a less frequent watering schedule. The use of a submist system, which provides intermittent mist only to the basal end of the cutting, was an effective alternative method to propagate lantana and produce cuttings that did not undergo obvious transplant shock.

2.2. Introduction

Over the past century, researchers have investigated the use of different systems to propagate plants by stem cuttings. Initial propagation studies involved inserting cuttings in a solid medium and using various methods to maintain a high-humidity environment. The use of small glass enclosures dates to 1829 (Ward, 1852; Hershey, 1996); Bailey (1896) and Corbett (1902) later used such enclosures to propagate plants from stem cuttings. However, the high moisture in the enclosed case resulted in cutting rot and increased incidences of diseases, which, given the low air flow in those cases, is now

thought to be *Botrytis cinerea* (Preece, 2003). Preece (2003) described the first recorded research using overhead intermittent mist and fog systems dating back to around 1940. This was followed by numerous studies through the 1950's, all demonstrating the effectiveness of overhead fog or mist systems to root stem cuttings. Such systems continue to be used today for commercial propagation (Hartman et al., 2011).

Despite the widespread use of overhead mist, concerns identified in the early days of the technology persist today. While the use of mist minimizes transpiration by decreasing leaf temperature, evaporative cooling also creates a cooling effect in the root zone (Hartman et al., 2011). On leaves, excess water can create unsanitary conditions leading to disease and leaching of foliar nutrients, while insufficient water or air currents can lead to inadequate mist coverage (Preece, 2003). Because of these concerns, some have investigated other methods of vegetative propagation that do not use overhead mist.

Research has demonstrated that leafy stem cuttings can be propagated in environments that provide adequate moisture solely through the base of the cuttings, without the need for overhead mist or enclosures. Graves and Zhang (1996) used a subirrigation system to propagate a variety of herbaceous and woody plants. While investigating the effects of different photoperiods and temperature on rooting success, they found that a subirrigation system, without the use of overhead mist, was a viable means for propagation for the two ornamental crops they evaluated. Peterson et al. (2018a) also successfully propagated coleus in subirrigation and submist systems, which produced cutting rooting percentages higher than those rooted in overhead mist systems. Manchurian lilac and inkberry have both been successfully propagated from stem cuttings using a submist system, which produced rooting percentages equal to or greater than rooting of cuttings propagated in overhead mist (Peterson et al., 2018b).

The objective of this study was to determine if the submist system is an effective method to propagate herbaceous stem cuttings of *Lantana camara* 'Dallas Red'. To accomplish this, the rooting success of stem cuttings was compared between overhead mist and submist systems, after which rooted cuttings from both systems were evaluated for post-transplant performance.

2.3. Materials and Methods

On 2 May 2017, 200 two-node stem cuttings of lantana were received from a commercial propagator (Ball Horticulture Company; West Chicago, IL). These cuttings were propagated in either an overhead or submist system. For the overhead mist system, twenty cells of a fifty-cell propagation flat (Dillen-ITML, Middlefield, OH) were filled with a soilless medium containing peat moss, perlite, and vermiculite (Fafard Germination Mix, Sun Gro Horticulture, Agawam, MA). The cuttings were arranged in an alternating pattern, leaving an empty cell between adjacent cuttings. The mist system consisted of a single low-pressure nozzle (Vibro-Spreader; Rain-Tal, Or-Akiva, Israel) mounted on the top of a 57-cm tall polyvinyl chloride (PVC) riser. Mist was turned on for 10 s every 10 min using a normally closed 24-V AC solenoid valve (Netafim, Fresno, CA) controlled by an electronic timer (Gemini 6A; Phytotronics, Earth City, MO).

The fifty-cell propagation flats were also used in the submist system, but with open bottoms created by removing the lower ¾ of each cell. Each cutting was held in a 2-inch diameter neoprene cutting holder (xGarden; Amazon.com) inserted and arranged in the same alternating pattern as the overhead mist, leaving a cell containing just a neoprene holder between cuttings. Each submist system consisted of 16 mist nozzles (Botanicare 330 Micro Sprayer; American Agritech, Chandler, AZ) tapped into to a 3/4-inch, 33 x 56 cm PVC manifold that was placed in a 74 x 52 x 37 cm plastic tub (Commander 27-Gallon Black Tote; Centrex Plastics, Findlay, OH). Water was pumped through the manifold using a submersible pump (Eco-plus ECO-396; Sunlight Supply, Vancouver, WA) controlled by a timer (Titan Controls Apollo 12 Timer; Sunlight Supply) which turned the pump on every 10 min for 10 s. Water was

checked weekly and added as needed to maintain a consistent volume of approximately 13 gal through the duration of the experiment.

The experiment was conducted in a triple-layer polycarbonate glazed quonset-style greenhouse, where temperature and photosynthetically active radiation (*PAR*) were measured using a weather station (Watchdog 1450 micro station; Spectrum Technologies, Aurora, IL). The average daily temperature during the experiment was 23.7 °C, with a maximum instantaneous temperature of 45.3°C and instantaneous *PAR* reading of 1175.5 $\mu mol \cdot m^{-2} \cdot d^{-1}$. The daily light integral (DLI) was computed by multiplying *PAR* readings by 0.0864. The average DLI during the experiment was 4.2 $mol \cdot m^{-2} \cdot s^{-1}$.

The cuttings were harvested on 9 June 2017. Of the 20 cuttings in each system, 10 were destructively harvested. For each of these cuttings, a subjective root rating was assigned (0 = no roots; 5 = prolific roots), the longest root length was measured, and number of roots was counted. The roots were then separated from the stem, placed in unbleached cone coffee filters (Melitta USA; Clearwater, FL) and dried in a 68°C heated room for four weeks prior to being weighed. The stem and leaves were also placed in unbleached cone coffee filters, dried and weighed.

The remaining 10 cuttings were transplanted into 6-inch plastic pots filled with a soilless medium containing peat moss, perlite, vermiculite, dolomitic limestone and a wetting agent (Fafard 1-PV; Sun Gro Horticulture, Agawam, MA). They were top dressed with 12 g of controlled-release fertilizer containing 14% N, 6.1% P_2O_5, and 11.7% K_2O (Osmocote Pro 14-14-14; Everris, Dublin, OH). They were grown in the quonset greenhouse on a single bench, where they were watered every 3-4 days as needed, and allowed to grow until 4 August 2017. The average temperature during the two-month period was 27.0°C, with an instantaneous maximum temperature of 38.5°C, instantaneous *PAR* of 1203 $\mu mol \cdot m^{-2} \cdot d^{-1}$ and DLI of 4.3 $mol \cdot m^{-2} \cdot s^{-1}$. The plants were harvested by gently rinsing the substrate off the roots, assigning a subjective root rating as described above, measuring the longest root and counting the number of roots for each cutting. In addition, the total leaf area was measured for twelve plants

originally rooted in submist and twelve plants originally rooted in overhead mist using a portable leaf area meter (Li-Cor LI-3000A; Li-Cor, Inc.; Lincoln, NE). For these cuttings, the roots, stem, and leaves were dried and weighed separately as described above.

The experimental design consisted of five blocks with each block consisting of one overhead mist system and one submist system. Twenty cuttings, serving as subsamples, were placed in each system (n=5; N=20). To analyze the data, all subsamples per replicate system were averaged to obtain a single value for each experimental unit. RStudio Version 1.0.136 with the *agricolae* package (RStudio, Inc.; Boston, MA) was used to analyze the data by two-way analysis of variance to account for the randomized complete block design, at an alpha of 0.05. Because the two-way ANOVA procedure is equivalent to a paired two-sample t-test when two treatments are investigated, additional tests were not necessary for means separation. Only four of the five blocks were analyzed due to a system malfunction in one of the blocks early in the experiment.

2.4. Results

Rooting outcomes differed among cuttings propagated in the two systems, but the ranking between systems varied among dependent variables. The overhead mist system produced cuttings with almost twice the number of roots and a higher root quality than the submit system, but roots were 73% shorter and weighed 33% less than roots produced by the submist system (Table 2.1).

Table 2.1. Root rating, root number, root length, root dry weight, and stem dry weight of *Lantana camara* 'Dallas Red' cuttings after 38 d in overhead mist and submist propagation systems.

	Overhead Mist	Submist	*P*-value
Root rating (0-5)[a]	2.85	2.07	5.87E-05
Roots (No.)	13.25	7.24	2.21E-10
Root length (cm)[b]	12.31	45.85	2.00E-16
Root dry weight (mg)[c]	0.068	0.102	5.47E-03
Stem dry weight (mg)	0.158	0.109	7.63E-06

[a] 0 (no roots) to 5 (robust rooting).
[b] Length of the longest root present on the cutting; 1 cm = 0.3937 inch.
[c] 1 mg = $3.5274 \cdot 10^{-5}$ oz.

In contrast to differences observed following propagation, plants grown for 56 d in pots filled with soilless media did not differ significantly in most measures of growth. Plants propagated in submist produced roots that were 42% longer than those of plants propagated in the overhead mist system, but P-values for other measures exceeded alpha (Table 2.2).

Table 2.2. Root number, root length, root dry weight, stem dry weight, leaf area, and leaf dry weight of *Lantana camara* 'Dallas Red' propagated by submist or overhead mist and grown for 56 days in 6-inch pots filled with soilless media.

	Overhead Mist	Submist	*P*-value
Survival (%)	53	95	7.8E-06
Roots (No)[a]	6.6	5.4	0.0501
Root length [b]	35.6	61.8	6.05E-07
Root dry weight [c]	2.6	2.9	0.210
Stem dry weight [c]	4.5	4.5	0.943
Leaf Area [d]	1020	1046	0.775
Leaf dry weight [c]	4.4	4.2	0.588

[a] 0 (no roots) to 5 (robust rooting).
[b] Length of the longest root present on the cutting; 1 cm = 0.3937 inch.
[c] 1 mg = 3.5274 · 10–5 oz.
[d] Total area of leaves on cutting (cm^2); 1 cm^2 = 0.155 in^2

2.5. Discussion

2.5.1 Propagation

The results of this study show stem cuttings of lantana can be rooted in the submist system. Although having almost 50% fewer roots than cuttings propagated in the overhead mist system, cuttings from the submist system had longer roots with a higher root dry mass (Table 1). Roots emerged (longer than one mm) from the cuttings in submist by 8 d after placement, and within two weeks, root tips reached the water in the reservoir. Within the same timeframe, roots from cuttings in the overhead mist system emerged from the bottoms of the cells. There is limited information on the timing for root emergence in this species, but propagation time has been reported to be 3-4 weeks in softwood cuttings of lantana (Dole et al., 2005).

In this study, the overhead mist system produced cuttings with almost twice as many roots as cuttings propagated in submist. Peterson et al. (2018a) reported that the submist system produced

cuttings of coleus with a significantly higher root count, which was not the case for lantana (Table 2.1). A consistent result between this study and Peterson et al. (2018a) is the markedly longer roots produced in the submist system compared to the overhead mist system (Table 2.1). Studies conducted using similar systems, often referred to generally as aeroponic systems, have produced similar results. Souret and Weathers (2000) found *Crocus sativis* L. (saffron) bulbs grown in an aeroponic system had 60% longer roots than those grown in a hydroponic system. Soffer and Burger (1988) found that roots of *Chrysanthemum x morifolium* (garden mum) grew 66% and 98% longer in an aero-hydroponic system than in a perlite/vermiculite mix and a sand/peat/bark mix, respectively, in misted environments.

Both Souret and Weathers (2000) and Soffer and Burger (1988) made use of nutrient solutions in their systems, and the latter also treated cuttings with K-IBA. In this study, lantana cuttings were not treated with rooting hormones, and both the sub-mist and overhead mist systems used only tap water. While it is not known if results from this study would have differed if the cuttings were treated with rooting hormone and/or a nutrient solution, other propagation studies have produced successful rooting without the use of nutrients or hormones.

Mehandru et al. (2014) investigated the use of an aeroponic system to propagate three medically important plants*, Caralluma edulis* (Paimpa), *Leptadenia reticulata* (Jeewanti), and *Tylophora indica* (Burm.f.). While the focus was on the effects of different auxin concentrations applied to the cuttings, for all three plants, those rooted in the aeroponic system with no auxin applied had 50-100% longer roots than those grown in soil with no auxin applied. Srikanth et al. (2016) propagated a variety of *Brassica* species in an aeroponic system without the use of hormones or nutrients and successfully root all six species trialed. More recently, Peterson et al. (2018) found no significant difference in root number, root length, and root dry weight in coleus cuttings rooted in a submist system using plain tap water when compared to a submist system using a nutrient solution.

2.5.2. Transplanting

Although survival rates after transplanting were 95% for cuttings of lantana propagated in the submist system and 53% for the overhead mist cuttings, none of the post-transplant measures of rooting differed between systems (Table 2.2). The longer roots on plants originally propagated in the submist system were not unexpected, given that they were longer when transplanted from submist into solid media. Similar results were found by Peterson et al. (2018b), who compared rooting effectiveness and transplant success in manchurian lilac; roots on cuttings from the submist system were 62.5% longer after the propagation period, and 64% longer 88 d after transplanting into soilless media, than roots of cuttings from the overhead mist system.

Limited research has investigated growth of cuttings transplanted into a greenhouse medium following propagation in submist. Peterson et al. (2018a) rooted coleus cuttings in several propagation systems, transplanted them into a medium and grew them for 34 d, after which cuttings rooted in submist systems had 40% greater root dry weights than cuttings rooted in overhead mist. Peterson et al. (2018b) investigated manchurian lilac and inkberry cuttings in overhead mist and submist systems; they reported 80% post-transplant survival among cuttings of manchurian lilac rooted in the submist system, compared to 40% among cuttings rooted in the overhead mist system.

2.6. Conclusion

The results of this study continue to validate the use of the submist system as an effective method to root cuttings of herbaceous ornamental crops and demonstrate that those cuttings do not appear to suffer transplant shock when transplanted into greenhouse media. The use of a submist system to root cuttings without a solid substrate or applications of overhead mist could mean greater savings in substrate and water usage. Further studies need to be conducted on a larger scale (e.g., more cuttings, additional species, and commercially realistic spacing of cuttings), which would permit the

quantification of actual water savings. Other measures of rooting and transplant success should also be evaluated, such as time required to root and to reach marketable size and condition.

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CHAPTER 3

VEGETATIVE PROPAGATION OF *SYRINGA PUBESCENS SSP. PATULA* BY SUBMIST, OVERHEAD MIST, AND A HYBRID SYSTEM

3.1. Abstract

Vegetative propagation of *Syringa pubescens* ssp. p*atula* (manchurian lilac) from stem cuttings was conducted using overhead mist (OM), submist (SM), and hybrid (HY) propagation systems. Physiological measurements were recorded to assess whether differences in rooting could be attributed to differences in photosynthesis or stomatal conductance among systems. Rooting percentages were significantly greater in the HY system (90%), while OM and SM produced similar rooting percentages at 68% and 62%, respectively. The systems utilizing submist (HY and SM) had significantly greater root counts and root lengths and retained most of their leaves. Photosynthetic rates were initially low and did not differ among systems,but increased when roots emerged within all three systems. Stomatal conductance also increased with root emergence in the cuttings, but the degree of increase was more notable in cuttings within the HY and OM systems. Stomatal conductance in the SM system remained relatively constant, even after root emergence. The use of systems that provide intermittent mist to the basal end of the cutting were effective for propagating manchurian lilac. The results of this study suggest that differences in rooting observed among cuttings in these systems are not related to differences in photosynthesis, because the rate of photosynthesis was similar among cuttings in the three systems.

3.2. Introduction

Over the past century, researchers have investigated various systems to propagate plants by stem cuttings. Initial propagation studies involved inserting cuttings in a solid medium and using methods to maintain a high-humidity aerial environment. The use of small glass enclosures dates to 1829 (Ward, 1852; Hershey, 1996); Bailey (1896) and Corbett (1902) later used such enclosures to

propagate plants from stem cuttings. However, the high moisture in the enclosed case resulted in cutting rot and increased incidences of diseases, which, given the low air flow in those cases, is now thought to be *Botrytis cinerea* (Preece, 2003). Preece (2003) describes the first recorded research using overhead intermittent mist and fog systems dating back to around 1940. This was followed by numerous studies through the 1950's, all demonstrating the effectiveness of using overhead fog or mist systems to root stem cuttings. Generally, such methods continue to be used today (Hartman et al., 2011).

Research has demonstrated that leafy stem cuttings of woody plants can be propagated in environments that provide adequate moisture solely through the base of the cuttings, without the need of overhead mist or enclosures. Graves and Zhang (1996) used a subirrigation system to propagate a variety of herbaceous and woody plants. While investigating the effects of different photoperiods and temperatures on rooting success, they reported that the subirrigation system, without the use of overhead mist, was a viable means of propagation for the two ornamental crops trialed. Zhang and Graves (1995) later compared rooting success in *Acer rubrum* 'Franksred' (red maple) between subirrigation and overhead mist and found similar results. Cuttings of red maple propagated in a subirrigation system had a 95% rooting percentage, compared to 33% for cuttings propagated in an overhead mist system (Zhang and Graves, 1995). In the same study, 22% of *Syringa reticulata* (Japanese tree lilac) cuttings were rooted using subirrigation, while zero percent of cuttings rooted under mist. Manchurian lilac and inkberry have both been successfully propagated by stem cuttings using a submist system, with equal or greater rooting percentages compared to overhead mist (Peterson et al., 2018). Soffer and Burger (1988) propagated *Ficus benjamina* (ficus) in an aero-hydoponic system, using basal mist with and without overhead mist. In this aero-hydroponic system, rooting percentage was 60% when overhead mist was applied, which was significantly lower than the 95% rooting percentage of cuttings propagated without overhead mist. Rooting percentages in ficus cuttings propagated in soilless

media where significantly lower than in cuttings propagated within the aero-hydroponic system, in both misted and unmisted environments. In a study of propagation by subirrigation, Aiello and Graves (1998) found reductions in the number of leaves retained on 5 out of 6 woody species when they were rooted in a subirrigation system instead of an overhead mist system. Desiccation of leaves on cuttings of korean lilac was observed in a previous study working with submist, indicating that water stress is likely greater in submist systems than in overhead mist systems (Peterson, personal communication).

While there is increasing research on rooting performance of cuttings in propagation systems that provide water only to the basal ends of cuttings, there is limited information on the physiological responses of cuttings propagated in environments without overhead mist. Carvalho et al. (2016) found significant differences in stomatal conductance in whole plants of *Vitis vinifera* 'Touriga National' and 'Trincadeira' (grape) when they were subjected to heat stress, water stress, or heat stress plus water stress compared to unstressed control plants. When investigating effects of overhead mist on both net photosynthesis and stomatal conductance of *Pinus taeda* (loblolly pine) stem cuttings in a solid medium, LeBude et al. (2005) found both measures were low at low overhead mist volumes. Humphries and Throne (1964) measured net photosynthesis in detached *Phaseolus vulgaris* (dwarf bean) leaves in a nutrient solution and found that photosynthesis readings remained relatively flat until the emergence of roots, when readings increased. Machida et al. (1977) reported increases in photosynthesis during root formation in *Forsythia suspensa* (weeping forsythia), *Camellia japonica* (Japanese camellia), and *Chrysanthemum x morifolium* (garden mum), all rooted in solid media. Smalley et al. (1991), Fordham et al. (2001), and LeBude et al. (2005) investigated gas exchange measurements in stem cuttings of woody plants and found similar trends in photosynthesis. In addition, these three studies reported a trend of increasing stomatal conductance with the emergence of roots. However, these studies investigated propagation of cuttings only under mist or high humidity environments.

This study had three objectives. The first objective was to validate previously obtained results propagating manchurian lilac in submist to confirm the reliability of this system over seasons. With limited additional information on leaf status of cuttings rooted with and without overhead mist, a second objective of this study was to determine if a hybrid system, which applied overhead mist to cuttings placed in a submist system, would reduce leaf desiccation. The third objective was to evaluate the possible roles of photosynthesis and stomatal conductance in the rooting responses produced by cuttings in each system.

3.3. Materials and Methods

On 12 July 2018, 150 semi-hardwood terminal stem cuttings of the same manchurian lilac used by Peterson et al. (2018a) were taken from established plantings on the University of Maine campus in Orono, ME. The stem cuttings consisted of two nodes, with 2 leaves at each node. The cuttings were wounded at the basal end by gently scraping the bark on one side using a razor blade. They were treated with 8000 $mg \cdot L^{-1}$ K-IBA (Sigma Chemical Co., St. Louis, MO) by dipping the basal one inch of each cutting in the solution for 10 s and allowing them to air dry before placing them in one of three propagation systems: overhead mist, sub-mist, and a combination system. For the overhead mist system, ten cells of a fifty-cell propagation flat (Dillen-ITML, Middlefield, OH) were filled with 50:50 peat:perlite (Fafard Canadian Sphagnum, Sun Gro Horticulture, Agawam, MA; Super Coarse Horticultural Perlite, Whittemore Co., Lawrence, MA). The cuttings were placed in the second and fourth rows of the flat, with five cuttings per row, leaving an empty cell between adjacent cuttings within rows.

The overhead mist (OM) system consisted of a single low-pressure nozzle (Vibro-Spreader; Rain-Tal, Or-Akiva, Israel) mounted on the top of a 57-cm tall polyvinyl chloride (PVC) riser. Mist was turned on for 10 s every 10 min using a normally closed 24-V AC solenoid valve (Netafim, Fresno, CA) controlled by an electronic timer (Gemini 6A; Phytotronics, Earth City, MO).

A germination tray (T.O. Plastics, Clearwater, MN) was used in each submist (SM) system, with ten 2-inch diameter holes drilled in the bottom matching the location of the cells in the propagation flat used in the OM system. Each hole held a 2-inch diameter neoprene cutting holder (xGarden; Amazon.com) in which the cuttings were inserted. A ½-inch layer of perlite was placed in the tray to prevent light from entering the root zone through the drainage holes in the tray. The submist system consisted of 16 mist nozzles (Botanicare 330 Micro Sprayer; American Agritech, Chandler, AZ) tapped into to a 3/4-inch, 33 x 56 cm PVC manifold that was placed in a 74 x 52 x 37 cm plastic tub (Commander 27-Gallon Black Tote; Centrex Plastics, Findlay, OH). Water was pumped through the manifold using a submersible pump (Eco-plus ECO-396; Sunlight Supply, Vancouver, WA) controlled by a timer (Titan Controls Apollo 12 Timer; Sunlight Supply; Vancouver, WA) that turned the pump on every 10 min for 10 s. Water in the reservoir was checked weekly and added as needed to maintain a volume of 13 gal through the duration of the experiment.

Each hybrid (HY) system consisted of the submist system described above with the addition of a single mist nozzle mounted on a PVC riser, identical to those used in the overhead mist system. Cuttings were placed as described for the submist system. A 1/8-inch diameter hole was drilled on one side of the tub midway up the side to allow drainage of excess water from the overhead mist to maintain a water volume of 13 gal. The application of overhead mist and submist in the hybrid system was controlled by separate controllers, as described for the submist and overhead mist systems.

The experiment was conducted in a triple-layer polycarbonate glazed quonset-style greenhouse, where temperature and photosynthetically active radiation (*PAR*) were measured using a weather station (Decagon VP-4, Decagon Devices, Pullman, WA). The average daily temperature during the experiment was 27.3 °C, with a maximum instantaneous temperature of 39.6°C and instantaneous *PAR* reading of 1157 $\mu mol \cdot m^{-2} s^{-1}$. The daily light integral (DLI) was computed by multiplying *PAR* readings by 0.0864. The average DLI during the experiment was 4.2 $mol \cdot m^{-2} d^{-1}$. A temperature and humidity sensor

(Omega OM-92, Omega Engineering, Norwalk, CT) was placed at leaf level over each system in all blocks to record environmental conditions at the system level.

Using a portable open-flow photosynthetic system (Li-Cor 6400; Li-Cor, Inc., Lincoln, NE), the net leaf photosynthetic rate (A_N), instantaneous stomatal conductance (g_s), and leaf vapor pressure deficit (vpd-l) were measured from the uppermost fully expanded leaf from one representative cutting per system per block (n=5, N=15). A 2 cm x 3 cm leaf chamber was clamped on the leaf, ensuring the entire 6-in^2 chamber area was covered by the leaf. The readings were taken three days per week between 11:00 am and 1:00 pm. Data was logged at a rate of 1 sample every 5 s for five min. The environmental conditions (CO_2 and *PAR*) in the leaf chamber were set to match the ambient conditions of the greenhouse each time the measurements were taken. CO_2 concentrations ranged from 250-300 μmol CO_2 ·mol and *PAR* ranged from 250-1150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ over the 69 d rooting period.

The cuttings were harvested on 18 September 2018. Of the 10 cuttings in each system, 5 were destructively harvested. For each of these cuttings, a subjective root rating was assigned (0 = no roots; 5 = prolific roots), the longest root length measured, and number of roots and leaves counted. The roots were then separated from the stem, placed in unbleached cone coffee filters (Melitta USA; Clearwater, FL) and dried in a room heated to 68°C for two weeks and weighed.

The remaining cuttings were potted in 6-inch plastic pots (Kord, Toronto, Canada) filled with a soilless medium of 55% peat moss, 30% bark, and 15% perlite (Fafard 3B; Sun Gro Horticulture; Agawam, MA). Potted cuttings remained in the quonset greenhouse on a single bench and were watered as needed. They were grown until 20 December 2018. The average temperature during the two-month period was 20.6°C, with an instantaneous maximum temperature of 35.4°C, maximum instantaneous *PAR* of 737 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and DLI of 2.7 $\mu mol \cdot m^{-2} \cdot d^{-1}$.

The experimental design consisted of five blocks, each with one overhead mist system, one submist system, and one hybrid system serving as experimental units. Ten cuttings, serving as

subsamples, were placed in each system (n=5; N=15). To analyze the morphological data, all subsamples per replicate system were averaged to obtain a single value for each experimental unit. To obtain a single value for physiological data for each experimental unit, the logged values for each parameter were averaged over a 30 s period once the Li-Cor readings stabilized. The data were analyzed using RStudio Version 1.0.136 with the *agricolae* package (RStudio, Inc.; Boston, MA). We used analysis of variance with an alpha of 0.05 to test for an effect of systems, after which Tukey's HSD was used for means separation among systems.

3.4. Results

Rooting outcomes of cuttings differed among the three systems, with OM systems consistently producing lower measures of rooting in all variables. HY systems produced cuttings with the greatest number of roots, which were significantly longer than roots produced by cuttings in both SM and OM systems, but root dry weights were not significantly different than for SM. The cuttings rooted in SM systems usually retained all four leaves, while cuttings that received mist from above typically lost leaves (Table 3.1).

Table 3.1. Leaf number, root rating, root length, root number, and root dry weight of manchurian lilac cuttings after 69 d in hybrid, overhead mist, and submist propagation systems

System	% Survival	% Rooted	Leaves (no.)	Root rating (0-5 scale) [a]	Root length (cm)[b]	Roots (no.)	Root dry wt (mg)[c]
Hybrid (HY)	90 a	90 a	2.6 b	1.9 a	13.5 a	16.9 a	76 a
Overhead mist (OM)	68 b	68 b	0.9 c	0.7 b	1.1 c	3.2 c	2 b
Submist (SM)	98 a	62 b	3.8 a	1.5 a	7.4 b	10.1 b	58 a

[a] 0 (no roots) to 5 (robust rooting).
[b] Length of the longest root present on the cutting; 1 cm = 0.3937 inch.
[c] 1 mg = $3.5274 \cdot 10^{-5}$ oz

There were few differences in the measured A_n between systems, with cuttings from all systems responding similarly as roots emerged, beginning at the initial observations of root primordia around July 27th and a second noticeable increase with root emergence August 22nd. Stomatal conductance in cuttings in the HY and OM systems were similar overall, with an increasing trend over time as roots

emerged. Cuttings propagated in the SM systems remained near zero, with only periodic increases that appeared to correspond with changes in vpd (Table 3.2). VPD difference between OM and HY were non-significant, however there was a significant difference between SM and overhead mist utilizing systems (Figure 3.4). These differences also appear to correspond to differences seen in g (Figures 3.5 – 3.7).

Table 3.2. Stomatal conductance, net photosynthesis, and leaf vapor pressure deficit for manchurian lilac measured over 69 d root period in a leaf photosynthesis system.

Day	Stomatal conductance (mmol H_2O•m^{-2}·s^{-1})			Net photosynthesis (µmol CO_2•m^{-2}·s^{-1})			Vapor pressure deficit (kPa)		
	HY	OM	SM	HY	OM	SM	HY	OM	SM
7/12/2018	911 a	238 b	296 b	1.2 a	0.8 b	0.8 b	-1.6 a	-1.7 a	-1.8 a
7/13/2018	1605 a	659 a	322 a	1.3 a	1.3 a	1.5 a	-1.5 a	-1.5 a	-2.1 a
7/16/2018	710 a	1769 a	268 a	2.0 a	2.0 a	2.1 a	-0.3 a	0.1 a	-0.9 a
7/18/2018	1042 a	2004 a	304 a	2.4 a	3.6 a	2.7 a	-0.2 ab	0.2 a	-0.8 b
7/20/2018	817 a	787 a	159 b	2.7 a	2.5 a	2.4 a	-1.4 a	-1.5 a	-2.2 a
7/23/2018	883 b	1297 a	142 c	2.5 a	3.0 a	2.7 a	0.2 a	-0.1 ab	-0.5 b
7/25/2018	1451 a	772 a	170 a	3.4 a	3.2 a	2.8 a	0.9 a	0.6 ab	0.2 b
7/27/2018	495 ab	838 a	96 b	5.4 a	6.4 a	6.0 a	0.4 a	0.2 a	-0.3 a
7/30/2018	688 a	494 a	0.0 b	10.0 ab	10.1 b	10.5 a	-0.1 a	-0.4 ab	-0.8 b
8/01/2018	18.9 a	13.6 a	1.6 b	10.5 a	10.5 a	10.8 a	-0.7 a	-1.0 a	-1.5 a
8/03/2018	19.2 a	23.6 a	2.0 b	10.6 a	10.8 a	10.8 a	-1.1 a	-1.2 a	-1.8 b
8/06/2018	19.3 ab	33.9 a	0.0 b	10.2 a	10.8 a	10.8 a	0.2 a	-0.2 ab	-0.7 b
8/08/2018	32.4 a	28.7 a	3.0 b	10.6 a	10.8 a	10.9 a	0.2 a	-0.2 a	-0.7 a
8/10/2018	29.6 ab	43.7 a	1.2 b	11.4 a	10.9 a	10.9 a	-0.8 a	-1.0 ab	-1.6 b
8/13/2018	46.0 a	59.8 a	0.4 b	10.4 a	10.7 a	10.8 a	0.4 a	0.2 a	-0.4 b
8/15/2018	48.1 a	61.0 a	3.1 a	10.7 a	11.4 a	11.1 a	0.6 a	0.5 a	0.0 a
8/17/2018	55.4 a	51.4 ab	3.4 b	10.7 a	11.2 a	10.8 a	0.0 a	-0.2 a	-0.7 b
8/20/2018	57.1 ab	67.0 a	2.4 b	10.8 a	11.2 a	11.4 a	0.8 a	0.5 ab	-0.1 b
8/22/2018	67.1 a	87.1 a	0.0 b	14.3 a	15.7 a	16.3 a	0.7 a	0.6 a	-0.3 b
8/24/2018	72.4 a	69.1 a	2.7 b	12.2 a	12.2 a	12.2 a	0.3 a	0.0 a	-0.7 b
8/27/2018	95.6 a	73.4 a	4.0 a	12.5 a	12.5 a	13.6 a	0.4 a	0.3 ab	-0.6 b
8/29/2018	106.6 a	71.8 a	3.5 a	14.2 a	13.9 a	15.8 a	0.1 a	-0.5 ab	-1.2 b
9/01/2018	52.2 a	40.3 ab	2.4 b	13.0 a	12.9 a	13.9 a	-0.9 a	-1.2 ab	-2.0 b
9/04/2018	79.9 a	60.3 ab	11.1 b	13.5 a	14.1 a	15.3 a	-0.8 a	-1.2 ab	-2.0 b
9/06/2018	99.2 ab	113.3 a	22.1 b	13.6 a	15.0 b	16.0 ab	0.7 a	0.7 a	-0.1 b
9/08/2018	122.5 a	86.1 a	1.8 b	14.4 a	13.6 ab	15.5 b	0.0 a	-0.5 ab	-1.3 b
9/12/2018	134.3 a	108.0 a	5.6 b	14.0 a	14.0 b	16.3 b	1.4 a	1.0 a	0.3 b
9/14/2018	132.8 a	85.1 ab	6.3 b	13.9 a	14.0 a	16.2 a	0.6 a	0.2 ab	-0.4 b

3.5 Discussion

Rooting performance, leaf retention, and overall survival of cuttings in systems utilizing submist were greater than for cuttings in the OM system. However, cuttings propagated in HY had a greater rooting percentage than cuttings in the OM and SM systems alone. The rate of photosynthesis was initially low, regardless of system, but increased over time as roots emerged. Stomatal conductance followed a similar trend for HY and OM systems, but this trend was less pronounced in cuttings propagated in SM systems.

3.5.1. Propagation

Cuttings rooted in the HY system rooted at a greater percentage compared to cuttings receiving overhead mist or submist alone. Manchurian lilac propagated in the HY system had a rooting percentage of 90%; there were lower rooting rates in the submist and overhead mist (68% and 62%, respectively). Results from other studies comparing alternative methods of vegetative propagation to overhead mist vary for woody plants. Rooting percentage of ficus cuttings propagated in an aero-hydroponic system were significantly lower with overhead mist than without mist (60% and 95%, respectively). Both misted and unmisted cuttings in an aero-hydroponic system rooted at significantly higher percentages than cuttings propagated in solid media (Soffer and Burger, 1988). Peterson et al. (2018a) reported that manchurian lilac cuttings rooted in submist and overhead mist had equal rooting percentages, but 96% of inkberry cuttings produced roots in submist compared to 54% in overhead mist. By contrast, Oakes et al. (2012) propagated *Ulmus americana* (American elm) using overhead mist, aeroponics, and subirrigation systems. Cuttings of American elm from the subirrigation system had a higher rooting percentage than those from the aeroponic system (44% and 10%, respectively).

Cuttings in systems utilizing submist (HY and SM systems) retained more leaves and produced more and longer roots than cuttings in overhead mist alone (Table 3.1). In our study, cuttings in the two systems utilizing submist survived at higher rates than cuttings in overhead mist alone. Survival was

over 90% for cuttings in the HY and SM systems and 68% for those in the OM system. Survival of cuttings in the HY and OM corresponded the rooting rate, because cuttings that did not root were dead. In the case of the cuttings in the SM system, 98% of the cuttings survived, but only 62% of the cuttings rooted. The remaining cuttings had green, turgid leaves with callus formation on the basal end. Zhang and Graves (1995) found similar results when propagating Japanese tree lilac by overhead mist and subirrigation, stating that cuttings placed in the mist system had a 0% root rating and no surviving leaves, whereas the subirrigation cuttings had a 21% root rating but 28% had living leaves. Aiello and Graves (1998) propagated six different woody species in mist and subirrigation systems and found similar results. For example, 100% of *Syringa vulgaris* 'Charles Joly' (common lilac) cuttings taken on June 18, 1996 retained their leaves, while only 75% rooted. Among cuttings of the same plant taken on July 25, 88% retained their leaves but only 8% rooted (Aiello and Graves, 1998). Dirr (2009) stated that lilacs are difficult to root from stem cuttings, with timing a critical factor, and recommended that softwood cuttings be taken before youngest leaves mature and rooted in an overhead mist system. This study, in which cuttings of manchurian lilac were taken on July 12, 2017, shows that cuttings taken after leaf maturation and propagated in systems utilizing submist can root at high percentages.

Cuttings of manchurian lilac in OM and HY systems began to show signs of senescence after 2-3 d in the systems. After 12 d, leaves on cuttings in the OM systems began to abscise. Leaves on cuttings in on the HY system also began to abscise but extent varied by block. Only cuttings that did not survive in the submist system had signs of leaf senescence, with all leaves on surviving cuttings remaining green. While the survival and rooting rates differed from the results seen for Peterson et al. (2018a), the results for root lengths and root dry weights are similar between studies. In our study, the systems utilizing submist (SM and HY) produced cuttings with over 65% more roots than those in the OM system, with roots over 85% longer and with 97% more dry mass. In comparison, Peterson et al. (2018a) reported

that cuttings rooted in submist had 59% more roots that were 62.5% longer, with a 69% greater dry weight than cuttings rooted in overhead mist

3.5.2. Physiology

The rate of photosynthesis (A_n) was initially low, regardless of system, but increased over time (Table 3.2). A trend of slowly increasing A_n was evident through the first 2 weeks, followed by a steep increase in A_n during the third week, during the period in which the first visible root primordia became evident in the systems utilizing submist. Over the next three weeks, cuttings in SM and HY systems continued to show increasing development of visible root primordia and callus. By the end of week six, some roots were longer than 5 mm, after which roots continued to elongate daily. These visual observations in the systems with submist correspond to the increasing trend of A_n. The cuttings in the overhead mist system were not inspected for roots to prevent damage to any roots that may have been growing. Although there was generally not a significant difference in photosynthetic activity among systems, the overall trend appears to be consistent with results of other studies. For example, Humphries and Thorne (1964) measured A_n on detached leaves from *Phaseolus vulgaris* (dwarf bean) that were rooted in compost, and reported low rates of A_n until roots appeared, after which there was a noticeable increase in A_n over time. Machida et al. (1977) investigated the rate of photosynthesis in cuttings of *Camellia japonica* (Japanese camellia), *Camellia sasanqua* (sasanqua camellia), *Viburnum awabuki* (Awabuki sweet viburnum), *Forsythia suspensa* (weeping forsythia), and garden mum. Cuttings of weeping forsythia, garden mum, and Japanese camellia showed a sharp increase in A_n at root emergence. Likewise, among cuttings of *Acer rubrum* 'Red Sunset' (red maple) collected in May 1987, Smalley et al. (1991) found an increase in A_n 42 d after sticking, which corresponded to root emergence. This trend was not evident in cuttings of the same species taken in September 1987. Moreover, cuttings of two cultivars of *Euphorbia pulcherrima* (poinsettia) rooted in a growth chamber did not differ in A_n during the first 10 d, but at 13 d, root primordia were visible and A_n increased by approximately 35% for

each cultivar, followed by a 70-78% increase from 13 to 15 d when roots emerged (Svenson et al., 1995). While these studies reported an overall trend for A_n during rooting, LeBude et al. (2005) reported no significant relationship between rooting percentage and A_n. The results of this study are consistent with the results of LeBude et al. (2005), as A_n of manchurian lilac cuttings increased over time, but was similar for each system and did not correspond to differences in rooting percentage.

Although A_n did not differ between systems, g_s of cuttings in the SM system was lower than that of cuttings in the systems using overhead mist (HY and OM; Figure 3.2). During the first week that cuttings were in each system, there were no significant differences in g_s among systems. Readings for cuttings in the OM and HY systems ranged from 283 to 2000 mmol $H_2O \bullet m^{-2} \cdot s^{-1}$, while those of cuttings in the SM systems ranged between 250-300 mmol $H_2O \bullet m^{-2} \cdot s^{-1}$, Cuttings in all three systems showed a decrease in g_s during the first two weeks. By the third week, g_s of cuttings in all three systems dropped to their lowest point (Figure 3.2). As was seen for A_n, the g_s of cuttings in the OM and HY systems steady increased over time, corresponding to root growth. However, g_s in the SM system remained near zero, with a slower increase over the last two weeks. Carvalho et al. (2016) reported significantly lower g_s in whole plants of 'Touriga National' and 'Trincadeira' grape when subject to heat stress, water stress, and a combination of heat plus water stress. Although timing of rooting of cuttings in all systems was similar, cuttings propagated in systems utilizing overhead mist had increases in both A_n and g_s, while g_s in cuttings propagated in SM systems remained low, indicating they may also be undergoing heat stress. However, the overall trend in A_n and g_s observed in this study corresponds to other researchers' observations. Gas exchange data from Svenson et al. (1995) on two cultivars of poinsettia rooted in a growth chamber showed inconsistent readings of g_s during the first 8 d, with alternating increasing and decreasing readings during those days. After 13 d, when root primordia were reported present, g_s began to increase, with a final reported value of 70.0 mmol $H_2O \bullet m^{-2} \cdot s^{-1}$ after 23 d. Svenson et al. (1995) also found a corresponding increase in A_n at the same time, with readings similar to those from cuttings

of manchurian lilac rooted in the systems utilizing overhead mist. Stomatal conductance measurements of *Corylus maxima* (purple filbert) cuttings rooted in a fog environment by Fordham et al. (2001) showed that g_s varied with leaf age, and older, more fully expanded leaves showed fluctuating readings over a 21 d study, in contrast with increasing readings for younger, newly unfolded leaves. A study on loblolly pine varying the rate of overhead mist application from 45 – 310 mL · m^2 showed at lower mist levels, g_s remained significantly lower than in cuttings receiving higher mist levels (LeBude, et. al., 2005). While previous reports of g_s in cuttings is restricted to environments that include methods to reduce moisture stress (i.e., fog, high relative humidity, or mist), this seems to be the first study to report g_s of cuttings in rooting environments with and without overhead mist.

3.6 Conclusion

Systems utilizing submist are effective for propagation of manchurian lilac. Morphological differences among cuttings rooted in OM, SM, and HY systems showed that systems with submist produce cuttings with a greater number of roots, which are also significantly longer. Rooting percentage in cuttings propagated in the HY systems were significantly greater than the OM and SM systems. While the rooting percentage between cuttings in OM systems and the SM systems did not differ significantly, the survival of cuttings in OM systems was significantly lower than that of cuttings in SM and HY systems.

In contrast to rooting results, physiological differences were not pronounced among systems. Photosynthetic rate did not differ significantly among systems but increased over time in all systems as roots emerged. Stomatal conductance of cuttings in systems utilizing overhead mist appeared to increase once root emergence was apparent but remained relatively constant for the cuttings rooted in submist. Overall, physiological measurements indicate that differences in photosynthetic rates and stomatal conductance are not evidently responsible for differences in rooting among cuttings in the three systems.

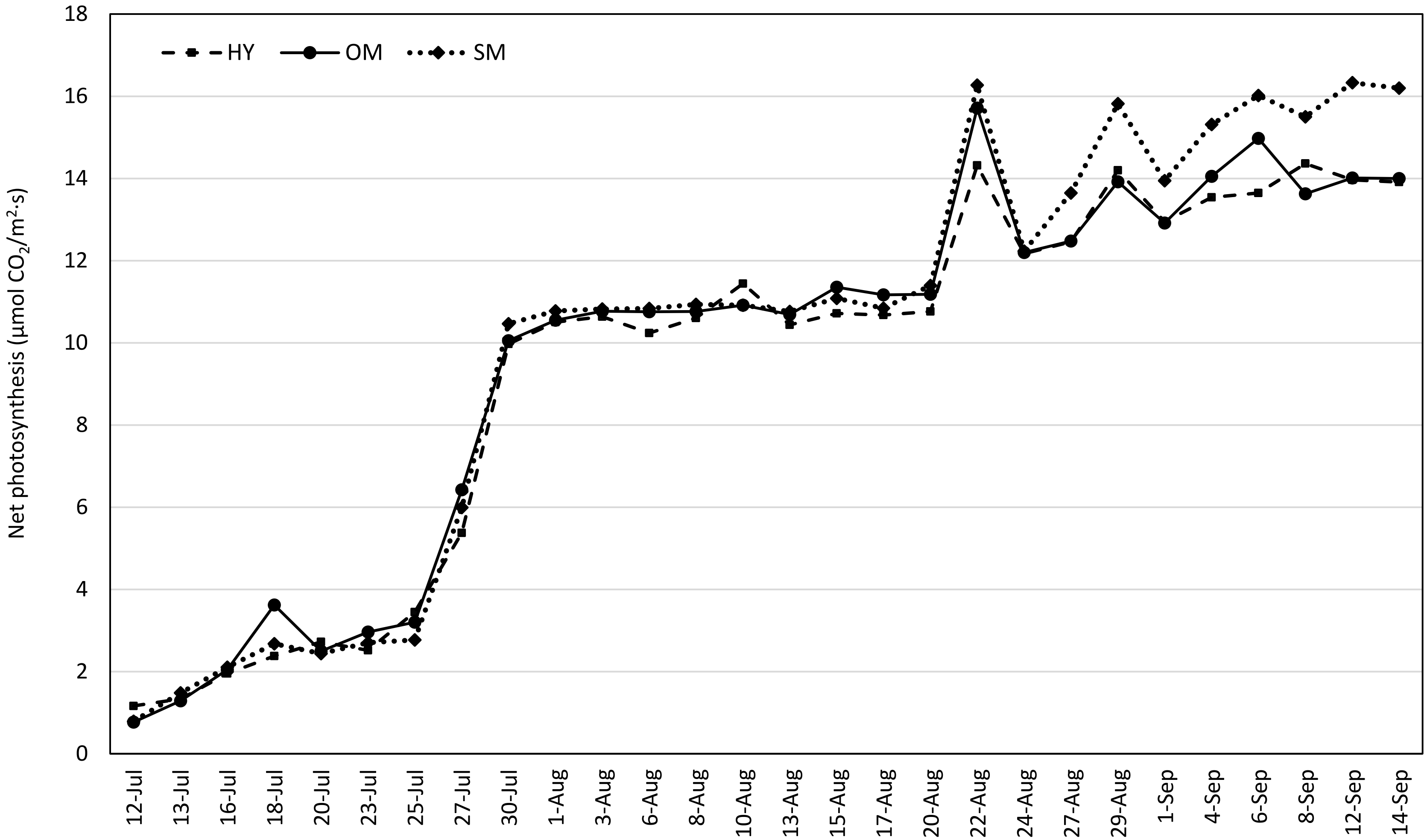

Figure 3.1. Net photosynthesis (A_n) of manchurian lilac stem cuttings rooted in submist, overhead mist, and hybrid propagation systems. A_n was measured using a Li-Cor Li-6400.

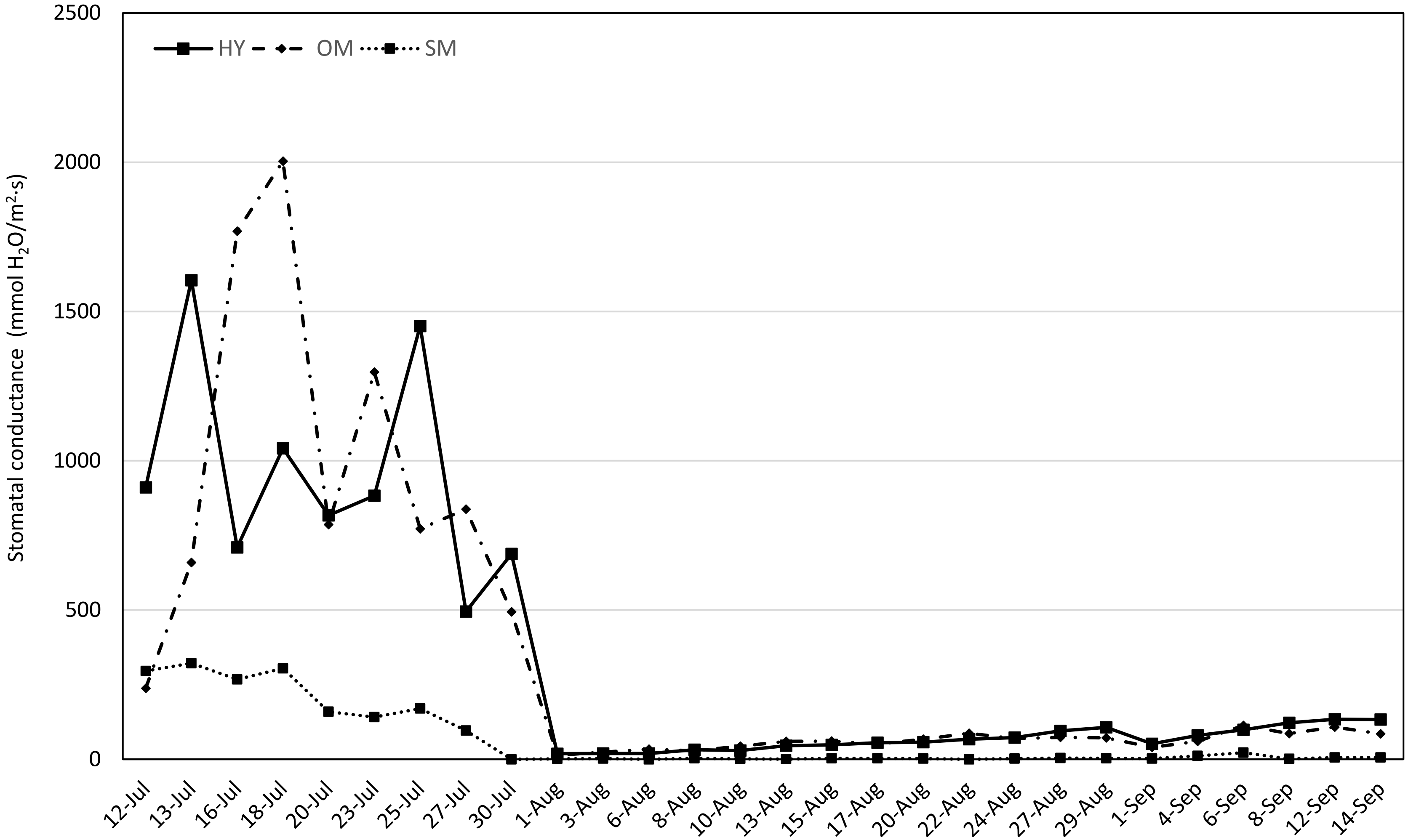

Figure 3.2. Stomatal conductance (g_s) of Manchurian lilac stem cuttings in submist, overhead mist, and hybrid systems during the entire rooting period. g_s was measured using a Li-Cor Li-6400. Note Figure 3.3 shows the last 7 weeks of rooting (Aug 1 – Sep 4) in more detail.

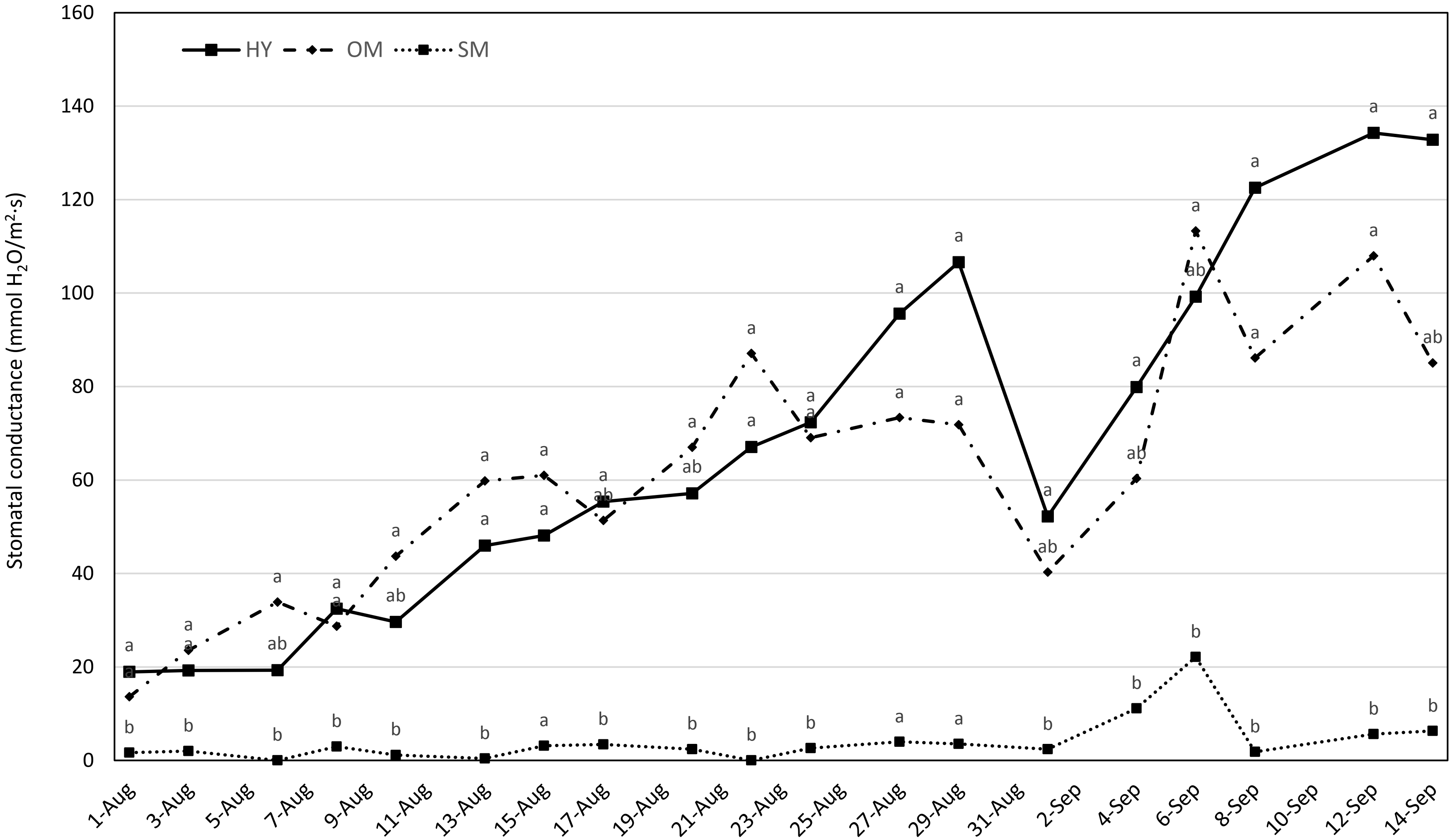

Figure 3.3. Stomatal conductance (g_s) of manchurian lilac stem cuttings in submist, overhead mist, and hybrid systems during the last 7 weeks of rooting. g_s was measured using a Li-Cor Li-6400. Letters represent results of Tukey's HSD at *p=0.05*.

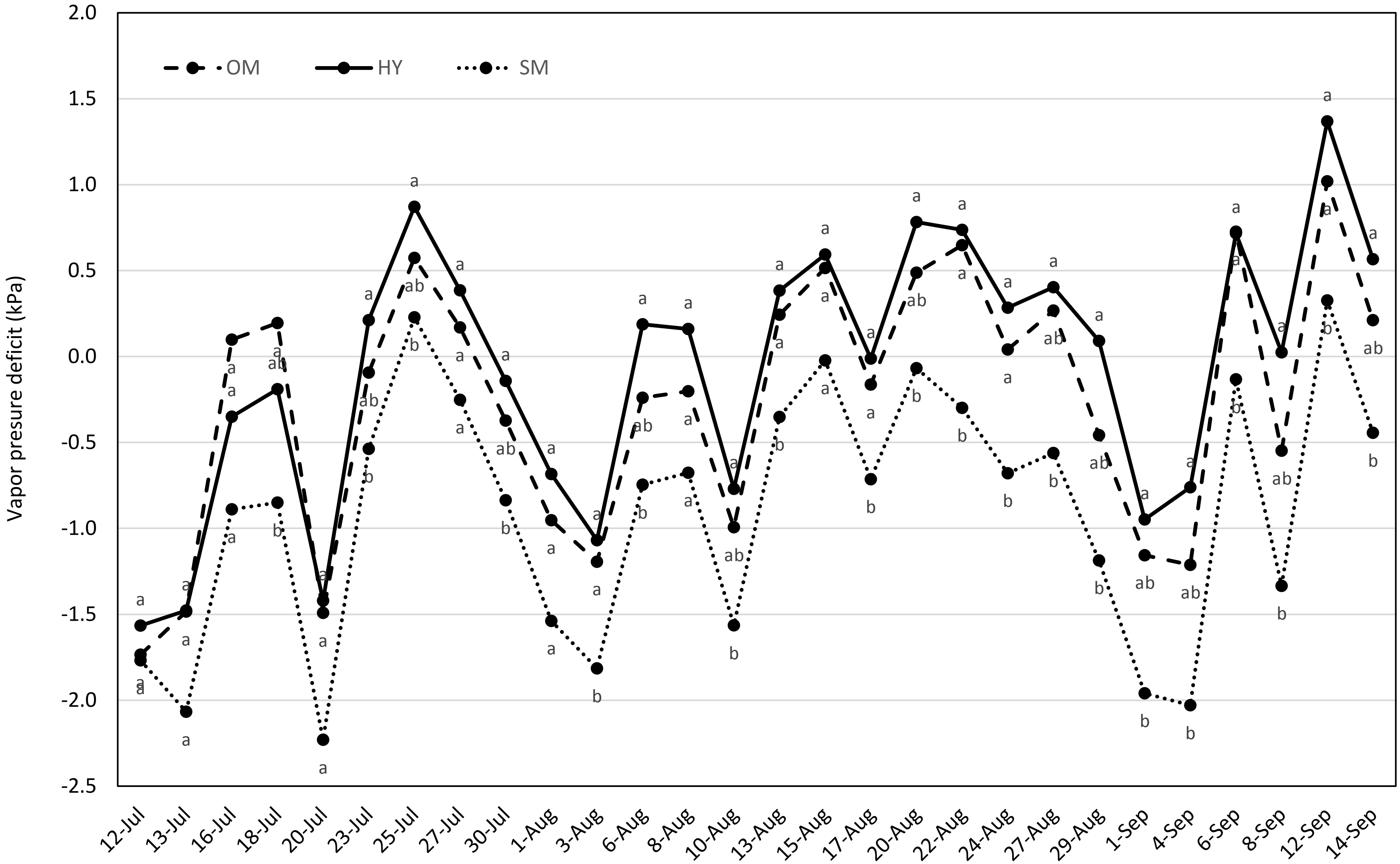

Figure 3.4. Vapor pressure deficit of lilac cuttings propagated in overhead mist, submist, and hybrid systems for 69 d. Letters represent Tukey's HSD results at p=0.05.

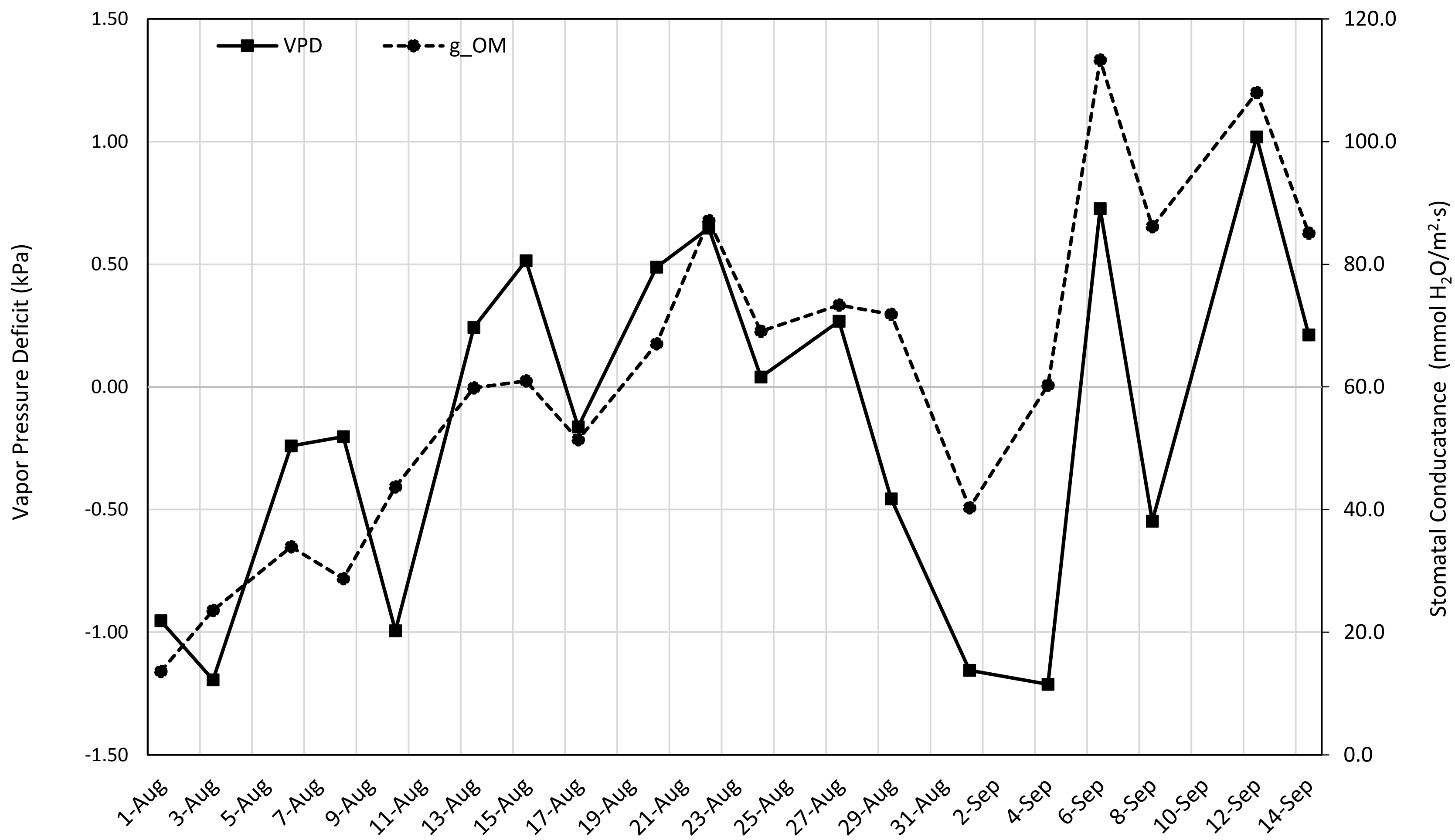

Figure 3.5. Stomatal conductance and vapor pressure deficit of cuttings in the overhead mist system from the emergence of roots through day 69.

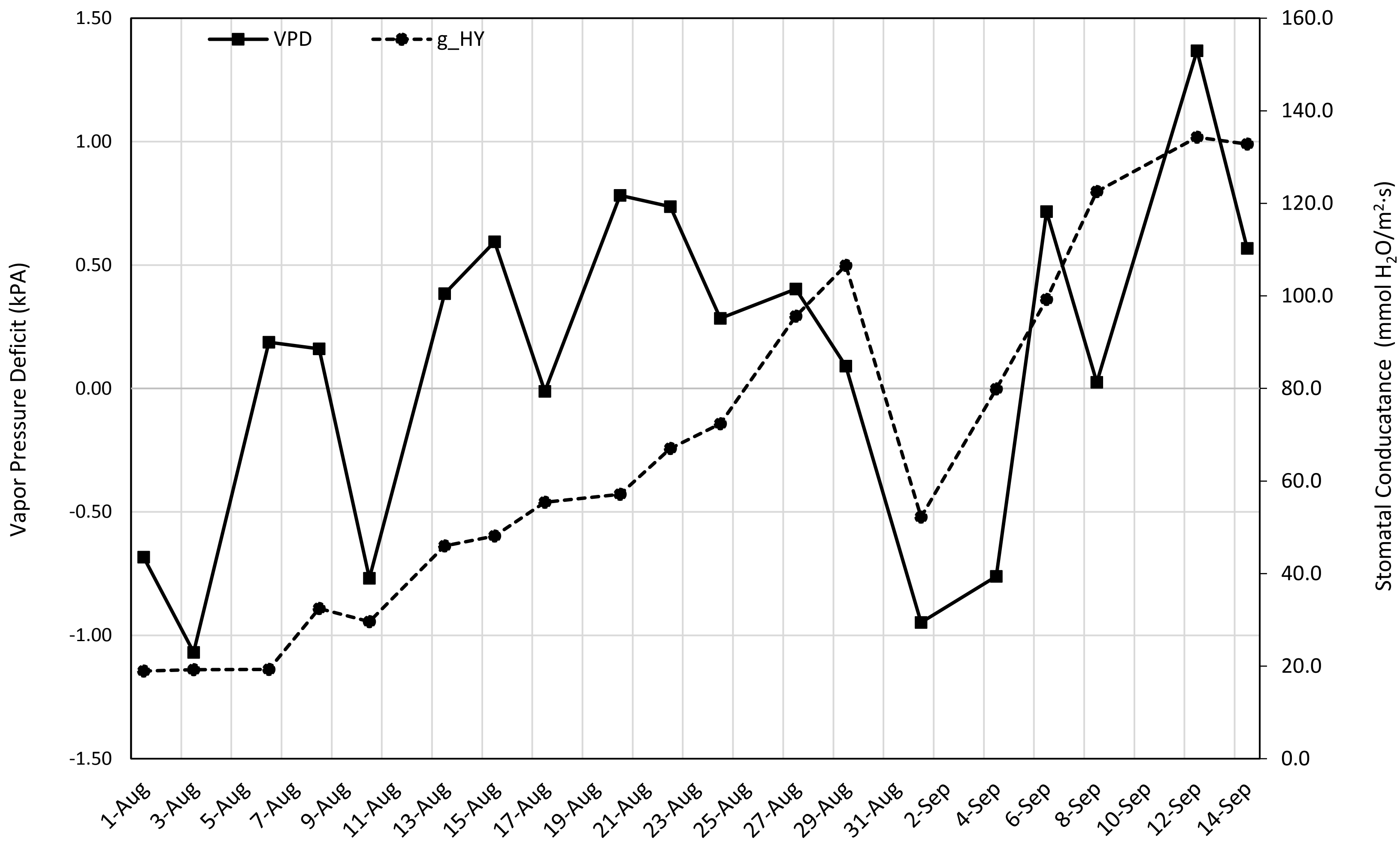

Figure 3.6. Stomatal conductance and vapor pressure deficit of cuttings in the hybrid system from the emergence of roots through day 69.

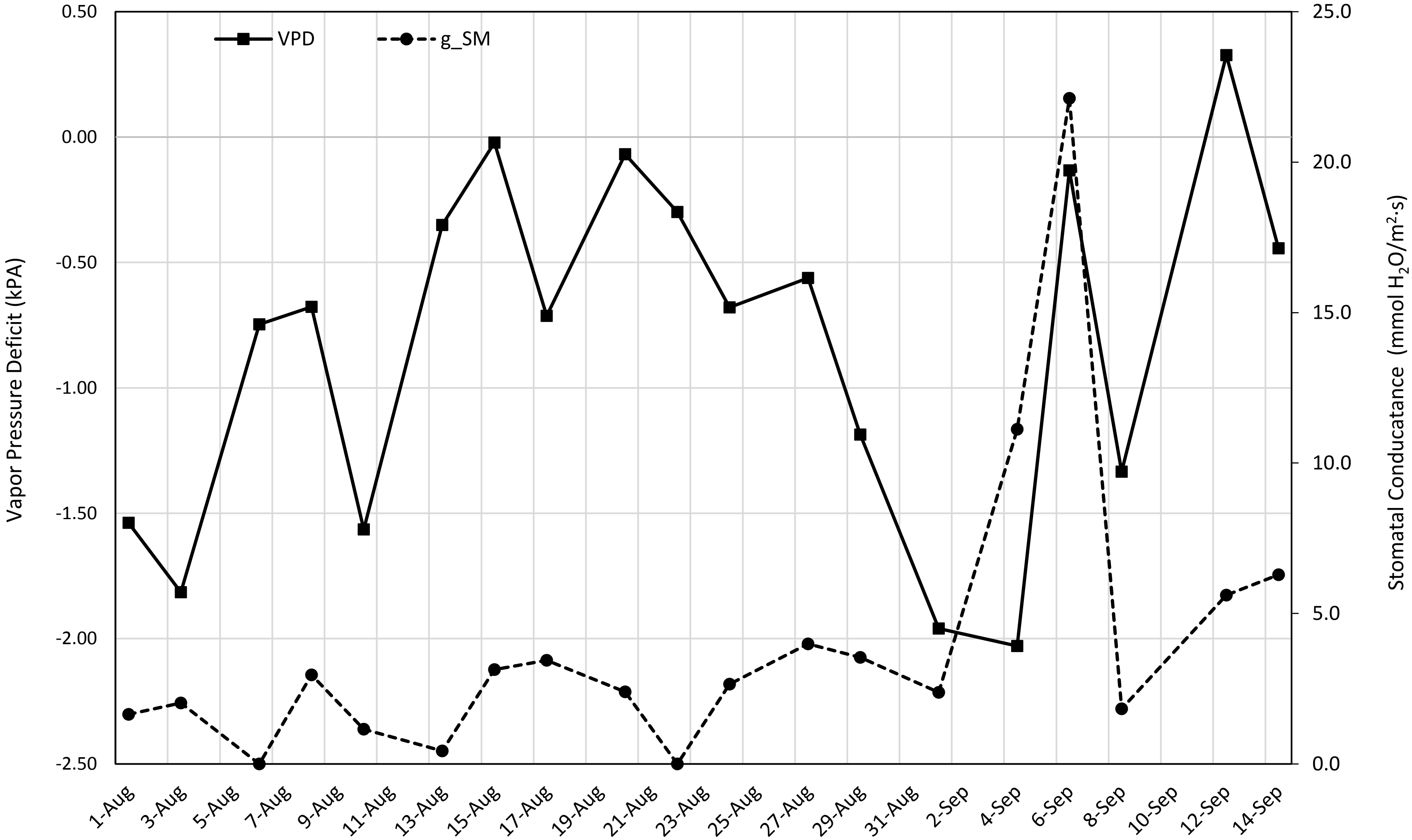

Figure 3.7. Stomatal conductance and vapor pressure deficit of cuttings in the submist system from the emergence of roots through day 69.

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BIOGRAPHY OF THE AUTHOR

Olivia Sanchez attended the University of Detroit Mercy and graduated in 1994 with a Bachelor's degree in Civil Engineering. She attended The University of Maine and graduated with a Master's degree in Civil Engineering in 2002. She worked for the Advanced Structures and Composite Center for 12 years as a Research Engineering before entering the Plant, Soil, and Environmental Science graduate program at the University of Maine. Olivia is a candidate for the Master of Science degree in Horticulture from the University of Maine in May 2019.