Environment, Photosynthesis, and Adventitious Rooting of Manchurian Lilac Cuttings Propagated in Overhead Mist, Submist, and Combination Systems

Olivia Sanchez, Stephanie E. Burnett, and Bryan J. Peterson

School of Food and Agriculture, University of Maine, Orono, ME 04469

Additional index words. aeroponic, humidity, temperature, *Syringa pubescens* subsp. *patula*, vapor pressure deficit

Abstract. We propagated manchurian lilac (*Syringa pubescens* subsp. *patula* ‘Miss Kim’) vegetatively from stem cuttings using overhead mist, submist, and combination propagation systems. Cuttings were collected when terminal buds were already set, after the period of tender growth that is optimal for lilac propagation. Net photosynthesis (P_n) was recorded to assess whether differences in rooting could be attributed to differences in photosynthetic activity of cuttings within each system. The propagation environment differed significantly among systems, with vapor pressure deficit (VPD) substantially greater for submist systems than for overhead mist or combination systems, and root zones warmer in submist and combination systems than in overhead mist. P_n of cuttings did not differ among systems and was initially low, but increased about when the first root primordia were visible. Rooting percentages were 90% among cuttings in the combination system, with cuttings in overhead mist and submist rooting at lower, but similar, percentages (68% and 62%, respectively). Cuttings in the combination and submist systems produced significantly more and longer roots than those in the overhead mist system, and retained nearly all of their leaves. Overall, the use of systems that provide intermittent mist to the basal end of each cutting was effective for propagating manchurian lilac. Our results demonstrate that cuttings in submist alone experience a much greater VPD than those in overhead mist, but may nonetheless root at comparable percentages and produce superior measures of root system quality. Combination systems show promise for rooting of species like manchurian lilac, because cuttings rooted at high percentages and with consistent root system quality, despite having been collected after the optimal spring period for lilac propagation.

During the past century, horticulturists have investigated various systems to propagate plants from stem cuttings. Initially,

cutting propagation involved inserting cuttings into soil within a high-humidity enclosure (Bailey, 1896). However, high humidity and poor air circulation in enclosed cases often resulted in cutting decay and disease symptoms, likely caused by gray mold [*Botrytis cinerea* (Preece, 2003)]. The first published research using overhead mist systems for propagation dates back to about 1940, followed by numerous studies through the 1950s that established the effectiveness of overhead mist systems (Preece, 2003), a technique that continues to be used for propagation today.

Although overhead misting of cuttings inserted into a soilless substrate is standard, research has demonstrated that leafy stem cuttings of woody plants can be propagated in environments that provide mist to the basal ends of the cuttings, with or without overhead mist. At the 14th annual meeting of the International Plant Propagator’s Society, Eastern Region, Nitsch (1964) speculated that supplying cuttings with moisture only to the basal end of the stem, instead of to the leaves, might improve propagation results, particularly for slow-to-root species. In response, Briggs (1964) constructed a mist chamber into which he inserted stem cuttings

of several species from above, both with and without overhead mist or heated water. Although no statistical data were provided, Briggs (1964) found that cuttings rooted in his system transplanted well, after producing roots that were “tougher and more hardened” than those produced during conventional propagation. Coston et al. (1983) obtained 97% rooting of semihardwood peach (*Prunus persica*) cuttings collected in early August and provided with overhead mist while inserted into a submist chamber, with roots on many cuttings evident within the first week. Among cuttings collected in September, Coston et al. (1983) reported rooting of 65% and 27% when cuttings received overhead mist or no overhead mist, respectively. Recently, we reported that stem cuttings of manchurian lilac and inkberry (*Ilex glabra*) inserted into submist systems with no overhead mist produced more root growth than cuttings in a soilless substrate with overhead mist (Peterson et al., 2018).

Although P_n has been measured in several species during propagation from cuttings, the results are mixed. Svenson et al. (1995) reported that P_n of poinsettia (*Euphorbia pulcherrima*) cuttings rooted in a growth chamber was low initially, but increased with the emergence of root primordia. Smalley et al. (1991) showed that P_n of red maple (*Acer rubrum*) cuttings collected in May was low until root emergence after 42 d. In contrast, P_n remained high among cuttings collected in September, all of which rooted within 2 weeks. Although P_n often is low in unrooted cuttings, the accumulation of photosynthates before roots have formed can be substantial and may impact rooting. For example, Klopotek et al. (2012) reported that dry mass of petunia (*Petunia ×hybrida* ‘Mitchell’) cuttings increased by ≈60% before the emergence of roots, indicative of prerooting carbon assimilation. Moreover, P_n of cuttings without visible primordia doubled from ≈3 to 6 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ when CO_2 concentrations were increased from 300 to 1200 ppm, and increased from <1 to 7.8 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ when irradiance was 150 instead of 80 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Klopotek et al., 2012). Tombesi et al. (2015) demonstrated that both the accumulation of carbohydrates and rooting percentages in terminal stem cuttings of hazelnut (*Corylus avellana*) increased when irradiance was increased from <100 to 200–300 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Although these studies provide insight into the physiology of cuttings during propagation, they only investigated cuttings under mist or in humid enclosures, not in aeroponic propagation systems within a greenhouse environment.

In our previous trials, we observed that leaves may wilt soon after cuttings are inserted into submist systems, indicating that water stress may be greater among cuttings in submist systems than among those in overhead mist systems. However, this wilting is not necessarily followed by leaf abscission, and the progression of wilting may halt or reverse with no obvious detrimental effects on rooting. Nonetheless, a propagation system that combines the features of

Received for publication 9 Aug. 2019. Accepted for publication 28 Oct. 2019.

Published online 12 December 2019.

Maine Agricultural and Forest Experiment Station Publication Number 3713. This research was supported by a grant from the Horticultural Research Institute (HRI). Its contents are solely the responsibility of the authors and do not necessarily represent the views of HRI. This project was supported by the U.S. Department of Agriculture (USDA) National Institute of Food and Agriculture, Hatch project no. ME021614 and Multistate Project no. ME031901 through the Maine Agricultural & Forest Experiment Station. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the views of the National Institute of Food and Agriculture or the USDA.

We thank Lia MacLellan and Bradly Libby for providing technical assistance, and Matthew Wallhead for providing editorial comments on an early draft of the manuscript.

B.J.P. is the corresponding author. E-mail: bryan.j.peterson@maine.edu.

This is an open access article distributed under the CC BY-NC-ND license (<https://creativecommons.org/licenses/by-nc-nd/4.0/>).

overhead mist and submist could be compared with each alone to identify the separate influences, and potential synergies, of overhead mist and submist on adventitious rooting. Likewise, the rates of P_n among cuttings in each system, compared with rooting outcomes, may suggest potential hypotheses to account for measured rooting differences among systems.

According to Dirr and Heuser (2006), manchurian lilac can be propagated from softwood stem cuttings treated with 8000 mg·L⁻¹ indole-3-butyric acid (IBA). Hartmann et al. (2011) stress the importance of collecting lilac cuttings before bud set, while cuttings are still somewhat tender, because cuttings collected after bud set are difficult to root. Fordham (1959) describes the need to collect cuttings at a precise stage at which stems are just sufficiently lignified to snap when bent, but seasonal primary growth has not ceased. Interestingly, the advent of intermittent mist now permits more tender lilac cuttings to be collected and rooted, but more lignified cuttings still tend to root poorly even under mist (Fordham, 1959). Methods of propagation that could extend the collection and propagation window for lilac and other taxa that are sensitive to seasonal cutting stage might make better use of nursery growing facilities and labor hours by permitting cutting collection times to be staggered across longer propagation seasons.

This study had three objectives. Our first objective was to validate the results of Peterson et al. (2018) during a second propagation season, using difficult-to-root cuttings of manchurian lilac collected from shoots with terminal buds already set. Our second objective was to determine whether a combination system, which applied overhead mist to cuttings placed in a submist system, would improve rooting further compared with overhead mist. Our third objective was to evaluate environmental conditions in each of the three systems and investigate the possible role of cutting photosynthesis in differential rooting responses.

Materials and Methods

Plant material. On 12 July 2018, 150 semihardwood terminal stem cuttings were collected from a ‘Miss Kim’ manchurian lilac hedge at the University of Maine campus in Orono, ME. The stem cuttings consisted of three nodes, with the lowest node stripped of leaves and four leaves retained. 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·L⁻¹ K-IBA (Sigma Chemical Co., St. Louis, MO) by dipping the basal 2.5 cm of each cutting in the solution for 10 s and allowing them to air-dry for up to 10 min before placing them in one of three propagation systems: overhead mist, submist, and a combination system.

Propagation systems. Each overhead 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). Ten cells of a 50-cell propagation flat (Dillen-ITML, Middlefield, OH) were filled with 1:1 (by vol.) 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.

Each submist system (Fig. 1) consisted of 16 mist nozzles (Botanicare 330 Micro Sprayer; American Agritech, Chandler, AZ) tapped into to a 3/4-inch, 33 × 56-cm PVC manifold that was placed in a 74 × 52 × 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) 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 32 L through the duration of the experiment. For each submist system, a shallow 1020 germination tray (T.O. Plastics, Clearwater, MN) was used as a lid, with 10 5-cm-diameter holes drilled into the tray to match the layout of cells used for cuttings in the propagation flat within the overhead mist system. Cuttings were inserted into a 5-cm-diameter neoprene puck (Black Clone Collar, xGarden, Amazon.com) in each hole. A 1.25-cm layer of perlite was placed in the tray to reduce solar heat gain in the black plastic tray and to reduce light transmission to the rooting chamber through the drainage holes in the tray.

Each combination system (Fig. 1) consisted of the submist system described earlier with the addition of a single mist nozzle mounted on a PVC riser, identical to those used in the overhead mist systems. Cuttings were placed as described for the submist system. A 0.3-cm-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 32 L. The application of overhead mist and submist in the combination system was controlled by separate controllers, as described for the submist and overhead mist systems.

Environmental conditions. The experiment was conducted in a triple-layer polycarbonate glazed Quonset-style greenhouse, where temperature, relative humidity (RH), and photosynthetically active radiation (PAR) were measured using a weather station (EM50 datalogger; Meter Group, Pullman, WA) with a quantum light sensor (SQ120; Apogee Instruments, Logan, UT), and a combined temperature and RH sensor (VP-4; Meter Group). The average daily temperature during the experiment was 27.3 °C, with a maximum temperature of 39.6 °C and maximum instantaneous PAR reading of 1157 μmol·m⁻²·s⁻¹. The daily light integral (DLI) was computed by multiplying daily PAR readings by 0.0864. The average DLI during the experiment was 4.2 mol·m⁻²·d⁻¹. A portable temperature and RH data logger (Omega OM-92; Omega Engineering, Norwalk, CT) was also placed at canopy level over each experimental unit to record environmental conditions at the system level. The VPD was calculated from air temperature (T) and RH using the formula $VPD = (1 - RH/100) \times 0.611 \times e^{(17.27 \times T / (T + 237.3))}$. On 4 to 7 Aug. 2019, the same data loggers were also used to compare relative temperatures of the rooting zone over four consecutive days, in one replicate of each system. These measurements were recorded in the summer of 2019 because we did not have enough data loggers to measure aerial temperatures and root-zone temperatures concurrently in 2018. The loggers, which are not waterproof, were sealed in plastic bags and suspended in the rooting chambers of the submist and combination systems, or buried in the substrate of the overhead mist system. Systems were rerandomized between days to account for potential spatial effects, and days were treated as blocks for the analysis of root-zone temperature.

Net photosynthesis. Using a portable open-flow photosynthetic system (LI-COR

Fig. 1. Schematic of overhead mist, submist, and combination systems used to propagate ‘Miss Kim’ manchurian lilac (*Syringa pubescens* subsp. *patula*) by stem cuttings. Each of five randomized complete blocks consisted of one replicate system of each type. In overhead mist systems, cuttings were stuck into 50:50 (by vol.) peat:perlite in a propagation sheet that was inserted into a 1020 flat and placed under mist. In submist systems, cuttings were inserted into a chamber in which a submersible pump (black rectangle) in a water reservoir supplied mist through a manifold to the basal ends of cuttings. In combination systems, cuttings received both submist and overhead mist.

6400; LI-COR, Inc., Lincoln, NE), we measured P_n on one of the two distalmost leaves on one representative cutting per system per block ($n = 5$, $N = 15$). A 2×3 -cm leaf chamber was clamped on the leaf, ensuring the entire 6-cm² chamber area was covered by the leaf. The readings were taken 3 d per week between 11:00 AM and 1:00 PM. To obtain a single value for P_n for each experimental unit per measurement date, the logged values were averaged over a 30-s period after the LI-COR readings stabilized. 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 recorded. CO_2 concentrations ranged from 250 to 300 $\mu\text{mol}\cdot\text{mol}^{-1}$ and PAR ranged from 250 to 1150 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ during measurements of P_n .

Cutting harvest. We harvested cuttings on 18 Sept. 2018 and assessed the percentage of cuttings that rooted. Subjective ratings were assigned for root system quality on a scale from 0 to 5, with 0 for no roots, 1 for one to several roots (not transplantable), 2 for a lopsided and/or weakly developed root system, 3 for a moderately developed root system, 4 for a well developed root system, and 5 for an extensive root system distributed symmetrically around each cutting. For each cutting, the length of the longest root was measured, the number of roots and leaves was counted, and roots were removed, placed in unbleached cone coffee filters (Melitta USA, Clearwater, FL), dried in a room heated to 69 °C for 2 weeks, and weighed.

Data analysis. The experimental design consisted of five blocks, each with one overhead mist system, one submist system, and one combination system serving as experimental units. Ten cuttings, serving as subsamples, were placed in each system. Rooting data were analyzed using RStudio Version 1.0.136 with the agricolae package (RStudio, Inc., Boston, MA). We checked equality of variances with Levene's test and normality of residuals with the Shapiro-Wilk test, after which we used analysis of variance (ANOVA) with an alpha of 0.05 to test for an effect of systems, and Tukey's honestly significant difference (HSD) test for means separation among systems. To assess differences in air temperature, RH, VPD, and root-zone temperature among systems, and in P_n of cuttings within systems, we conducted a separate ANOVA for each date, followed by means separation using Tukey's HSD with an alpha of 0.05.

Results and Discussion

Cutting survival and rooting. Cuttings of manchurian lilac in the combination systems rooted at greater percentages than cuttings receiving overhead mist or submist alone (Table 1). Combination systems produced a rooting percentage of 90%, whereas overhead mist and submist produced similar percentages of 68% and 62%, respectively. However, cuttings in submist alone retained

the most leaves, with more than four times the leaf retention of cuttings that received overhead mist alone (Table 1). Despite similar rooting percentages between the two systems, cuttings in overhead mist that did not root always lost their leaves, whereas unrooted cuttings in submist retained turgid leaves and produced callus on the basal ends of stems.

Measures of root system quality also differed among cuttings in the three systems, with overhead mist alone consistently producing the lowest values for all root system measurements. For example, root ratings of cuttings in submist and combination systems, which did not differ significantly from one another, averaged 2.4 times those of cuttings in overhead mist (Table 1). Cuttings in combination systems produced 1.7 times the number of roots as those in submist systems, and more than five times the number of roots as those in overhead mist systems. Longest root lengths in combination systems were also 1.8 and 12.3 times those produced in submist and overhead mist, respectively (Table 1). Finally, root dry weights of cuttings in submist and combination systems did not differ significantly, but averaged more than 30 times the dry weight of cuttings in overhead mist alone.

The positive results from this study are consistent with previous studies in which propagation of woody plants in aeroponic systems was evaluated. In systems identical to the submist systems in this study, Peterson et al. (2018) showed that manchurian lilac cuttings that were rooted in submist and overhead mist had equal rooting percentages, but 96% of inkberry cuttings produced roots in submist compared with only 54% in overhead mist. Sharma et al. (2018) reported that an aeroponic system was successful for the propagation of sexually mature athel tamarisk (*Tamarix aphylla*) from stem cuttings, with aeroponic cuttings rooting at greater percentages and producing more and longer roots than those inserted into polybags containing a mix of soil and soilrite. Coston et al. (1983) showed that semihardwood stem cuttings of peach could be rooted at high percentages using an aeroponic system, but that reliable rooting required misting both the basal and distal ends of cuttings, an approach that is similar to our combination system. In contrast to the success of aeroponic systems in our study and others, Oakes et al. (2012) reported that rooting of american elm (*Ulmus americana*) cuttings under overhead mist and in an aeroponic system varied by genotype; responses were either comparable between systems or cuttings were entirely unrooted in aeroponics. However, the authors used a humidity dome over shoots, and a nutrient solution instead of water, which was prone to excessive algal growth.

Although the rooting percentages of cuttings in overhead mist and submist were lower for this study than in that of Peterson et al. (2018), the results for root count, length, and dry weight were comparable between

studies. In this study, the submist systems produced cuttings with root counts more than three times those of cuttings in the overhead mist systems, with longest root lengths 6.7 times, and dry weights 29 times, those of cuttings in overhead mist (Table 1). In comparison, Peterson et al. (2018) reported that cuttings rooted in submist had 2.4 times the roots, which were 2.7 times the length with 3.2 times the dry weight of cuttings rooted in overhead mist. Our addition of a combination system produced more favorable results in this study (Table 1) than the most favorable results for overhead mist or submist in either study. Dirr and Heuser (2006) stated that lilacs are difficult to root from stem cuttings, with timing a critical factor, and recommended that softwood cuttings be taken before the youngest leaves mature and rooted in an overhead mist system. Our study shows that manchurian lilac collected after terminal buds have set can root at high percentages (90%) and produce adequate measures of rooting when inserted into combination systems, even when cuttings propagated using overhead mist or submist alone do not. Taken together, the results suggest that combination systems might buffer, to some extent, the year-to-year variation in cutting condition and physiology to produce more robust rooting outside the optimal window for propagation of manchurian lilac.

Environmental conditions. Submist systems differed significantly from the overhead mist or combination systems in the propagation environment they provided. The air temperature at cutting height in submist systems was almost always greater, and the RH lower, than in the overhead mist or combination systems (Fig. 2A and B). Because of these differences, the calculated VPD was greater in submist systems on nearly every date (Fig. 2C), which suggests a greater risk of cutting desiccation. Previous researchers exploring the use of VPD for dynamic control of mist in propagation have considered a VPD greater than 2.0 kPa to be injurious to plants (Gates et al., 1998). LeBude et al. (2005) demonstrated that rooting of loblolly pine (*Pinus taeda*) cuttings under mist was maximized when misting rates produced a mean daily VPD between 0.6 to 0.85 kPa, and rooting percentages decreased sharply when the mean daily VPD reached 1 kPa. However, a VPD of 0.9 to 1.7 kPa is evidently not injurious to cuttings of manchurian lilac when a consistent supply of water is available as mist applied to the basal ends of cuttings. In the submist systems, mist is applied for 10 s every 10 min, 24 h per day. Following the application of mist, each cutting retains a bead of water on its basal end, making water consistently available for uptake. In other environments with higher temperatures, it is possible that the VPD may exceed 2.0 kPa, which could cause wilting. In our case, although root system quality in submist systems was superior to that in overhead mist, the reduced VPD in combination

Table 1. Rooting percentage, leaf number, root rating, longest root length, root number, and root dry weight of ‘Miss Kim’ manchurian lilac (*Syringa pubescens* subsp. *patula*) cuttings rooted in overhead mist, submist, or combination propagation systems. Cuttings were collected on 12 July and harvested on 18 Sept. 2018.

System	Rooted (%)	Leaves retained (no.)	Root rating (0–5 scale) ^z	Longest root (cm) ^y	Roots (no.)	Root dry wt (mg) ^x
Overhead mist	68 b ^w	0.9 c	0.7 b	1.1 c	3.2 c	2 b
Submist	62 b	3.8 a	1.5 a	7.4 b	10.1 b	58 a
Combination	90 a	2.6 b	1.9 a	13.5 a	16.9 a	76 a

^zRating scale with 0 for no roots, 1 for one to several roots (not transplantable), 2 for a lopsided and/or weakly developed root system, 3 for a moderately developed root system, 4 for a well-developed root system, and 5 for an extensive root system distributed symmetrically around each cutting.

^yLength of the single longest root found on the cutting; 1 cm = 0.3937 inch.

^x1 mg = 3.5274 × 10⁻⁵ oz.

^wAnalysis of variance with an alpha of 0.05 was used to test for an effect of system. Values for each system are the mean of five replicated systems, with 10 cuttings as subsamples within each system. Means separation by Tukey’s honestly significant difference was used to test for pairwise differences among systems; means followed by the same letter within columns are not significantly different.

Fig. 2. Average daily air temperature (A), relative humidity (B), and vapor pressure deficit (C) recorded at the height of ‘Miss Kim’ manchurian lilac (*Syringa pubescens* subsp. *patula*) cuttings inserted into overhead mist, submist, and combination systems on 12 July. The combination systems provided both overhead mist and submist to cuttings. Circles represent daily means for each system, averaged across five blocks. Means separation by Tukey’s honestly significant difference with an alpha of 0.05 showed that the temperature in submist systems was significantly higher, and relative humidity was significantly lower, compared with overhead mist or combination systems on every date except the final date, when all three systems were equivalent statistically. The vapor pressure deficit in submist systems was significantly greater than in overhead mist or combination systems on every date except the final date, when values for submist systems were only significantly greater than those for the combination systems. Overhead mist and combination systems did not differ significantly on any date for any measurement.

systems seems to improve rooting responses compared with submist alone.

Very little research has explored the effects of air temperature on root emergence and elongation, and we are not aware of suggestions regarding the optimum temperature during propagation for manchurian lilac. Cuttings that were rooted in the combination systems were grown under temperatures similar to those rooted in overhead mist during this study (Fig. 2A), so it is unlikely that their greater rooting percentages are the result of differences in air temperature. Systems also differed in the temperatures of the root zones measured across four consecutive days (Table 2). Root-zone temperatures of the overhead mist system averaged 2.5 and 3.9 °C less than those of the combination system and submist system, respectively. The combination system also produced a root zone averaging 1.4 °C cooler than that of the submist system (Table 2), likely because the combination system was cooled by tap water draining into the chamber from intermittent overhead mist. Wilkerson et al. (2005) suggested that the poinsettia rooting percentage is greatest when root zones are 27 to 29 °C, and declines at higher or lower temperatures. Owen and Lopez (2018) showed that both root-zone temperature and light impact rooting percentage of purple fountain grass (*Pennisetum xadvena*). At a low DLI (4–10 mol·m⁻²·d⁻¹), increasing temperature increased rooting percentage, whereas at a higher DLI (8–16 mol·m⁻²·d⁻¹), rooting percentage increased up to an optimum temperature of 23 °C and then declined (Owen and Lopez, 2018). Greater root-zone temperatures in submist and combination systems might, in part, account for their increased quality of root systems relative to overhead mist, but lower aerial temperatures in combination systems might, in turn, account for the increased rooting percentage in combination systems relative to submist alone. Alternatively, because root-zone temperatures in systems using submist were greater than others have reported to be optimal for propagation, it is conceivable that decreasing root-zone temperatures in these systems could improve rooting success further. Ultimately, it is unclear which environmental and physiological factors account for the relative performance of systems in this study.

Photosynthesis. P_n was low initially, regardless of system, but increased over time (Fig. 3). From 12 July to 25 July, P_n increased

slowly from ≈1 to 3 μmol·m⁻²·s⁻¹ CO₂. Because rooting chambers allowed nondestructive assessment of root formation, we observed visible root primordia in systems using submist on 24 July. This observation was followed by a rapid increase in P_n among cuttings in all three systems, from ≈3 to 10 μmol·m⁻²·s⁻¹ CO₂ from 25 July to 30 July (Fig. 3). During the next 3 weeks, cuttings in submist and combination systems continued to show increasing development of visible root primordia and callus, with roots elongating on some cuttings. P_n remained fairly stable until 22 Aug., when it increased slightly and remained between 12 and 16 μmol·m⁻²·s⁻¹ CO₂ until the end of the study. During this time, roots continued to elongate on many cuttings in the submist and combination systems. P_n was similar for cuttings in all three systems despite final differences in rooting percentage and rooting quality among systems. One explanation for this similarity in P_n is that cuttings selected for photosynthesis readings needed green leaves, which necessarily excluded the least successful cuttings in each system.

Although P_n did not differ significantly among systems in this study, the overall trends in photosynthesis over time were consistent with the results of others. For example, Humphries and Thorne (1964) measured P_n on detached leaves from dwarf bean (*Phaseolus vulgaris*) that were rooted in a nutrient solution, and reported low rates of P_n until roots appeared, after which there was an increase in P_n over time. Likewise, Davis and Potter (1981) reported that P_n of pea (*Pisum sativum* ‘Alaska’) cuttings in growth chambers decreased over several days after cuttings were excised from stock plants, but increased when new roots emerged. Despite diminished P_n, cutting dry weight increased ≈20 mg before root development, and the number of roots per cutting increased when atmospheric CO₂ concentrations, irradiance, and photoperiod increased, and decreased when an antitranspirant was applied to cuttings. Smalley et al. (1991) showed that P_n of red maple cuttings collected in May and rooted under intermittent mist decreased initially nearly to the compensation point, and increased again after root emergence in 42 d. In contrast, P_n remained high among cuttings collected in September, and 100% of cuttings rooted within 12 d (Smalley

Table 2. Average, minimum, and maximum daily root-zone temperatures in overhead mist, submist, and combination systems measured on 4 to 7 Aug. 2019. One replicate of each system was measured each day, systems were randomized between days to account for spatial effects, and days were treated as blocks for analysis.

System	Avg (°C)	Minimum (°C)	Maximum (°C)
Overhead mist	27.5 c ^z	19.8 c	36.7 a
Submist	31.4 a	23.8 a	38.2 a
Combination	30.0 b	22.8 b	37.2 a

^zAnalysis of variance with an alpha of 0.05 was used to test for an effect of system. Means separation by Tukey's honestly significant difference was used to test for pairwise differences among systems; means followed by the same letter within columns are not significantly different.

Fig. 3. Net photosynthesis (P_n) of 'Miss Kim' manchuian lilac (*Syringa pubescens* subsp. *patula*) cuttings inserted into overhead mist, submist, and combination systems on 12 July. The combination system provided both overhead mist and submist to cuttings. Because the systems using submist permitted us to view cuttings without disturbing them, the date of first visible root primordia is based on observations of submist and combination systems only. Each point represents the mean of five cuttings, with one from each of five blocks. Asterisks represent significant differences in photosynthesis based on Tukey's honestly significant difference with an alpha of 0.05. Means for combination systems exceeded those for overhead mist on 6 Sept., and those for submist on 8 Sept. and 12 Sept.

et al., 1991). Among cuttings of poinsettia rooted in a growth chamber, P_n averaged 0.9 to 1.5 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \text{ CO}_2$ during the first 10 d, but increased to 2.1 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \text{ CO}_2$ when root primordia were visible at 13 d, and then quadrupled with root emergence by 15 d (Svenson et al., 1995). Although these studies reported an increasing trend with time for P_n during rooting, LeBude et al. (2005) reported that P_n of loblolly pine cuttings increased with mist application volumes, but not with rooting progress over time.

Conclusions

Systems using submist can root manchuian lilac stem cuttings effectively after terminal buds have set for the season. The rooting percentage of cuttings propagated in the combination systems was significantly

greater than that of cuttings in the overhead mist and submist systems. Morphological differences among cuttings rooted in overhead mist, submist, and combination systems showed that the two systems with submist produce cuttings with a greater number of roots, which were also significantly longer and of greater mass than those produced in overhead mist alone. The aerial environment in submist systems is characterized by higher temperature, lower RH, and a greater VPD than in overhead mist or combination systems, a fact that is difficult to reconcile with the greater leaf retention of submist cuttings, and root systems of greater quality than in overhead mist. Root-zone temperatures were also greater in submist and combination systems than in overhead mist systems, but the role of these differences in rooting outcomes is unknown. Finally, P_n before or after root initial formation does not explain differences in rooting outcomes, because P_n did not differ among cuttings in each of the three systems. Opportunities for future research with these systems include the evaluation of cuttings collected at different times of the growing season, the development of scaled-up systems to assess commercial feasibility, and the evaluation of nursery and landscape performance of rooted cuttings over several seasons after propagation in systems using submist.

Literature Cited

Bailey, L.H. 1896. The nursery-book: A complete guide to the multiplication and pollination of plants. 4th ed. Macmillan, New York, NY.
 Briggs, B.A. 1964. An experiment in air rooting. Intl. Plant Prop. Soc. Proc. 14:139–141.
 Coston, D.C., G.W. Krewer, R.C. Owings, and E.G. Denny. 1983. Air rooting of peach semihardwood cuttings. HortScience 18:323–324.
 Davis, T.D. and J.R. Potter. 1981. Current photosynthate as a limiting factor in adventitious root formation on leafy pea cuttings. J. Amer. Soc. Hort. Sci. 106:278–282.
 Dirr, M.A. and C.W. Heuser, Jr. 2006. The reference manual of woody plant propagation. Timber Press, Portland, OR.
 Fordham, A.J. 1959. Propagation and care of lilacs. Arnoldia 19:36–45.

Gates, R.S., S. Zolnier, and J. Buxton. 1998. Vapor pressure deficit control strategies for plant production. Intl. Fed. Auto. Control Proc. 31:271–276.
 Hartmann, H.T., D.E. Kester, F.T. Davies, and R.L. Geneve. 2011. Hartmann and Kester's plant propagation: Principles and practices. 8th ed. Prentice Hall, Upper Saddle River, NJ.
 Humphries, E.C. and G.N. Thorne. 1964. The effect of root formation on photosynthesis of detached leaves. Ann. Bot. 28:391–400.
 Klopotek, Y., E. George, U. Druge, and H.-P. Klaering. 2012. Carbon assimilation of petunia cuttings in a non-disturbed rooting environment: Response to environmental key factors and adventitious root formation. Scientia Hort. 145:118–126.
 LeBude, A.V., B. Goldfarb, and F.A. Blazich. 2005. Mist level influences vapor pressure deficit and gas exchange during rooting of juvenile stem cuttings of loblolly pine. HortScience 40:1448–1456.
 Nitsch, J.P. 1964. Propagation in the year 2000. Intl. Plant Prop. Soc. Proc. 14:316–324.
 Oakes, A.D., N.A. Kazcmar, C.A. Maynard, and W.A. Powell. 2012. Vegetative propagation of American elm (*Ulmus americana*) varieties from softwood cuttings. J. Environ. Hort. 30:73–76.
 Owen, W.G. and R.G. Lopez. 2018. Propagation daily light integral and root-zone temperature influence rooting of single-internode *Pennisetum xadvena* culm cuttings. HortScience 53: 176–182.
 Peterson, B.J., S.E. Burnett, and O. Sanchez. 2018. Submist is effective for propagation of manchuian lilac and inkberry by stem cuttings. HortTechnology 28:378–381.
 Preece, J.E. 2003. A century of progress with vegetative plant propagation. HortScience 38: 1015–1025.
 Sharma, U., V. Kataria, and N.S. Shekhawat. 2018. Aeroponics for adventitious rhizogenesis in evergreen haloxeric tree *Tamarix aphylla* (L.) Karst.: Influence of exogenous auxins and cutting type. Physiol. Mol. Biol. Plants 24: 167–174.
 Smalley, T.J., M.A. Dirr, and A.M. Armitage. 1991. Photosynthesis and leaf water, carbohydrate, and hormone status during rooting of stem cuttings of *Acer rubrum*. J. Amer. Soc. Hort. Sci. 116:1052–1057.
 Svenson, S.E., F.T. Davies, Jr., and S.A. Duray. 1995. Gas exchange, water relations, and dry weight partitioning during root initiation and development of poinsettia cuttings. J. Amer. Soc. Hort. Sci. 120:454–459.
 Tombesi, S., A. Palliotti, S. Poni, and D. Farinelli. 2015. Influence of light and shoot development stage on leaf photosynthesis and carbohydrate status during the adventitious root formation in cuttings of *Corylus avellana* L. Front. Plant Sci. 6:973.
 Wilkerson, E.G., R.S. Gates, S. Zolnier, S.T. Kester, and R.L. Geneve. 2005. Predicting rooting stages in poinsettia cuttings using root zone temperature-based models. J. Amer. Soc. Hort. Sci. 130:302–307.