Propagation of Herbaceous and Woody Perennials in Submist and Overhead Mist Systems[1]

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Abstract

Submist aeroponic propagation systems apply mist to the bases of cuttings, rather than from overhead. These systems improve rooting in some plant species and reduce water usage compared to overhead mist systems. Submist systems, which were supplemented with infrequent overhead mist, were used to propagate stem cuttings collected from six plant species: bluestar (*Amsonia tabernaemontana* Walter), purple smoke false indigo (*Baptisia australis* x *B. alba* 'Purple Smoke'), threadleaf coreopsis (*Coreopsis verticillata* L.), panicle hydrangea (*Hydrangea paniculata* Siebold), sweetgale (*Myrica gale* L.), and ninebark (*Physocarpus opulifolius* (L.) Maxim 'Diablo'). The submist systems were compared to traditional overhead mist with a solid propagation medium. Rooting was better in overhead mist for cuttings of threadleaf coreopsis and purple smoke false indigo. Rooting was comparable between systems for all other species (bluestar, panicle hydrangea, sweetgale, and ninebark). In the submist systems, water usage was reduced by 67% compared to overhead. This is probably due to reduced evaporative water loss and more targeted application of water directly to cuttings, resulting in less water lost on the ground or benchtops.

Species used in this study: *Amsonia tabernaemontana* Walter, *Baptisia australis* x *B. alba* 'Purple Smoke', *Coreopsis verticillata* L., *Hydrangea paniculata* Siebold, *Myrica gale* L., *Physocarpus opulifolius* (L.) Maxim 'Diablo'.

Chemicals used in this study: KIBA (potassium salt of indole-3-butyric acid).

Index words: Aeroponic, root formation, water use.

Significance to the Horticulture Industry

Submist systems, which apply water to the bases of cuttings, show promise as an alternative to overhead mist that reduces the amount of water used in propagation. Four plants, bluestar, panicle hydrangea, sweetgale, and ninebark, may be propagated in either overhead mist or submist. Threadleaf coreopsis and purple smoke false indigo did not form high quality roots unless they were propagated in overhead mist. The submist system used in this study used 67% less water compared to overhead mist; it may be an option to reduce water use in propagation for plants that form roots readily in this system.

Introduction

Overhead mist has been the traditional system to propagate stem cuttings of woody and herbaceous perennials for decades. Although overhead mist is successful for many species, some aspects of these systems could be improved. One drawback to overhead mist is that it uses more water than other systems (Burnett et al. 2021). Reducing applied irrigation would improve water conservation, but it can also help with production challenges such as algae forming on benches or walkways. Another challenge with overhead mist is that some species are difficult or slow to propagate in these systems. Of these, some may require specific environmental conditions such as light or water that replicates conditions in their natural habitats (Foster et al. 2017). Alternative propagation systems may be useful to propagate plants unsuited to overhead mist production, including many plants for which effective propagation protocols have not been identified.

Propagation by submist, a relatively new system, has been explored as an alternative to overhead mist. In this system, mist is applied to the bases of stem cuttings, rather than from overhead. Several plant species, including *Coleus scutellarioides* (L.) Benth (coleus), *Ilex glabra* (L.) A. Gray (inkberry), and *Syringa pubescens* Turcz. subsp. *patula* (Palib.) M.C. Chang and X.L. Chen 'Miss Kim' (manchurian lilac), have superior rooting quality when they are propagated in submist instead of overhead mist (Peterson et al. 2018a, Peterson et al. 2018b). A commercial scale submist propagation system was recently developed that consisted of a custom-built lid for cuttings suspended over an ebb and flow tray housing a manifold with mist nozzles (Burnett et al. 2021). In this work, cuttings of bluestar and *Nepeta x faassenii* Stern 'Six Hills Giant' (faasen nepeta) had superior rooting in submist compared to overhead mist, and the submist system applied less water than a traditional overhead mist system occupying a similar bench space (Burnett et al. 2021).

One potential challenge with submist, particularly in hot or dry environments, is that the leaves may desiccate before stem cuttings have time to form roots. A combination system that provides mist both to the bases of cuttings and from overhead (at a reduced misting frequency) may be a better option than submist alone.

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Manchurian lilac stem cuttings had greater survival in a combination system than cuttings propagated in either submist or overhead mist. Cuttings in both submist and combination systems had more and longer roots than those in overhead mist systems (Sanchez et al. 2020). However, this combination system was tested in small plastic tubs, rather than on a scale practical for commercial propagation. No plants other than manchurian lilac were propagated.

Some species that are either slow or difficult to root may be more suited to a combination system than either submist or overhead mist alone. Although bluestar formed roots more quickly in submist than in overhead mist (Burnett et al. 2021), results with manchurian lilac suggest that a combination system may provide even greater improvement of rooting for bluestar. Threadleaf coreopsis, purple smoke false indigo, and ninebark have not been propagated in either submist or a combination system. One of these species, baptisia, is challenging to propagate in overhead mist (Cullina 2000). Sweetgale and panicle hydrangea have been propagated in a submist system, and there was no improvement in rooting (Burnett et al. 2021). However, a combination system may be superior to submist for these plants.

Previous research indicates that a combination propagation system may be a promising alternative to overhead mist that provides benefits similar to submist without the danger of losing cuttings. However, this combination system has not been tested on a commercial scale or with a variety of plants. Therefore, our primary objective was to test a combined overhead and submist propagation system at a commercial scale using a wide variety of herbaceous perennials and woody plants. We also determined how much water was used in a commercial scale submist system.

Materials and Methods

Cuttings of bluestar, purple smoke false indigo, threadleaf coreopsis, panicle hydrangea, sweetgale, and ninebark were propagated in overhead mist or submist with supplemental overhead mist (submist). Five independently controlled overhead mist systems were installed so that three nozzles (Misty Mist Red/White nozzles MM-RW; Dramm Corporation, Manitowoc, WI) on 1.27 cm (½ in) PVC risers provided mist to a 3′ by 8′ bench. The cuttings received mist for 10 sec every 10 min from 5:00 a.m. until 8:30 p.m. EST. Five independently controlled submist systems were built within 1.8 m (6′) x .9 m (3′) x 18 cm (7 in) ebb and flow tables (Active Aqua Premium High Rise Flood Table AAHR36W; Hydrofarm; Petaluma, CA). In an effort to improve lid design from Burnett et al. (2021), thicker lids were constructed to provide more support and consistently hold cuttings upright. For each lid, a frame containing three layers of white vinyl privacy lattice with 2.54 cm (1 in) grid holes was installed on the ebb and flow table. The middle layer of lattice was offset from the top and bottom layers to create holes just large enough to insert cuttings through the lid (Fig. 1). A manifold with twelve mist nozzles (T-Spray #7 Lime nozzle; Senninger, Clermont, FL) installed in 1.27 cm (1/2”) pex tubing provided mist to the chamber of the ebb and flow bench with lid. The benches were larger than needed, so half of the bench was covered with black and white polyethylene film with the white side facing out (Hydrofarm; Petaluma, CA). Other than the lid design and nozzle count, the system was similar to that described by Burnett et al. (2021). Mist was applied to the bases of cuttings for 10 s every 10 min. Supplemental overhead mist was provided to the submist systems by three nozzles mounted on 1.27 cm (½ in) PVC risers placed on top of the lid for the submist system. The frequency of overhead mist, which was applied daily from 5:00 am until 8:30 pm EST starting on 10 July 2020, was tapered from 10 s every 15 min for the first two days (d 1 and 2), to 10 s every 30 min on d 3 and 4, 10 s every 45 min on d 5, and 10 s every 60 min on d 6 and for the remainder of the experiment.

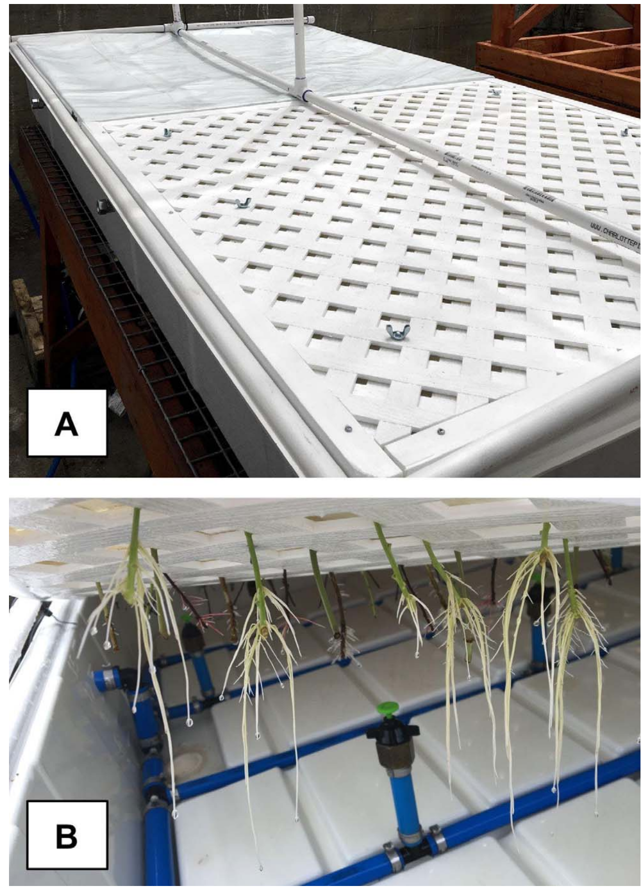

Fig. 1. Photos of a submist system constructed in a white ebb and flow table. A. The lid consisted of three stacked lattices with the middle layer offset to create small holes for cuttings. Half of the submist chamber was covered with plastic because the space was not needed for cuttings. An overhead mist system was placed on top of the lid to provide a small amount of supplemental mist to cutting shoots. B. Cuttings in submist are shown from inside the ebb and flow bench.

One hundred terminal cuttings of bluestar, ninebark, sweetgale, purple smoke false indigo, and panicle hydrangea were taken from the Lyle E. Littlefield Ornamentals Trial Garden in Orono, ME on 9 and 10 July, 2020. Seventy cuttings of threadleaf coreopsis were taken from a private residence in Orono, ME on 10 July. Bluestar cuttings had 10-12 nodes and were approximately 7.6 cm

(3 in) long. Ninebark cuttings were softwood, had six-nodes, and were approximately 8.9 cm (3.5 in) long. Sweetgale cuttings were semi-hardwood and approximately 7.6 cm (3 in) long with about 12 nodes. Purple smoke false indigo cuttings had approximately 3-4 nodes. Panicle hydrangea cuttings were softwood, had 3-4 nodes, and were approximately 8.9 cm (3.5 in) long. Threadleaf coreopsis cuttings with 3-4 nodes had flowers, which were removed before propagation. Lower leaves were removed from all cuttings. Cuttings of all species except sweetgale were dipped for 5 s in 1,000 ppm KIBA (potassium salt of indole-3-butyric acid) in water, and then air-dried. Sweetgale received no rooting hormone (Peterson et al. 2019). Cuttings in the submist were stuck directly into the systems. Those propagated under overhead mist were stuck into a substrate 1:1 peat:perlite in 50-cell vacuum propagation sheets (Dillen-ITML, Middlefield, OH) inserted into 1020 trays.

Total water applied was measured for each of the 10 independent overhead mist systems by installing a flow valve (½ in flow meter; model DLJSJ50; Daniel L. Jerman Company, Hackensack, NJ) between each mist controller and nozzle manifold. Water that was added to the recirculating pump basin of each submist system was measured initially, after which no additional water was added. Some water from the overhead mist nozzles located above each submist system entered the submist chamber through the lid, which kept the pump basins full for the remainder of the experiment. For submist systems, total water use was accounted for by totalling the water initially added to the basin of each system plus water measured by the flow valve.

Cuttings were propagated in a glass greenhouse, where the environment was monitored 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 relative humidity sensor (VP-4, Meter Group, Pullman, WA). The daily light integral (DLI = average *PAR* x 0.0864) was 13.9 $mol{\cdot}m^{-2}{\cdot}d^{-1}$, the average daily temperature was 28.5 C (83.3 F), and the relative humidity was 77%.

The systems were arranged in a randomized block design with five blocks, each containing one submist and one overhead mist system. Each replicate system (n=5) held 7 cuttings of threadleaf coreopsis and 10 cuttings each of the remaining species, with individual cuttings serving as subsamples. Cuttings were harvested from each block once they seemed to be well established in at least one of the two systems. This was on July 29th for bluestar, and between August 10th -13th for ninebark, purple smoke false indigo, sweetgale, threadleaf coreopsis, and panicle hydrangea. Data collected included a root rating on a scale of 0-5, with 0 for no roots, 1 for one to several roots (not transplantable), 2 for a weakly developed root system that may have roots only on one side, 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. The length of the longest root, the number of roots, and the root dry weight were also recorded. Roots were dried in a soil drying room (temperature = 68 C (154 F)) for at least a week before measuring their dry weight. Root rating was determined visually and root length was measured on all subsamples in each system. For all species except threadleaf coreopsis, root number and root dry weight were measured on 7 of the 10 subsamples in each system. For threadleaf coreopsis, all seven subsamples were measured for root number, and root dry weight was not measured due to poor rooting in the submist system. Subsamples for each species in each system were averaged for each block prior to data analysis. Data were analyzed using Anova and Tukey's HSD test in JMP (JMP Pro 14, Statistical Analysis Systems, Cary, NC).

Results and Discussion

Root rating, length, dry weight and number varied among the species propagated in submist and overhead mist systems (Table 1). Rooting of purple smoke false indigo and threadleaf coreopsis was generally better in overhead mist. Results were mixed for bluestar, sweetgale, and ninebark; some measures of rooting were better in the submist system, while others were better in overhead mist. Cuttings of panicle hydrangea rooted similarly in both propagation systems.

Some species, such as purple smoke false indigo and threadleaf coreopsis, rooted better in overhead mist than the combination system. Purple smoke false indigo root rating and number were significantly greater when cuttings were propagated in overhead mist (Table 1). The root rating was 35% greater, and cuttings in the overhead mist system produced an average of 11 more roots than those in submist. Root dry weight was 38% higher for purple smoke false indigo cuttings propagated in overhead mist (Table 1). Root length was not significantly different for purple smoke false indigo grown in either system. Most baptisia are propagated from seed because stem cuttings do not readily form adventitious roots (Cullina 2000). However, as purple smoke false indigo is a hybrid that does not form seed readily, an alternative method of propagation would be desirable. The submist system does not appear to be a good option for propagating hybrid baptisia, such as purple smoke false indigo.

Similarly, root rating, length, and number were greater for threadleaf coreopsis grown in overhead mist. Cuttings in overhead mist formed 7 more roots, roots were 72% longer, and root ratings averaged 3.0 in overhead mist, compared to 0.48 in submist (Table 1). Many cuttings of threadleaf coreopsis did not form roots in the combination system, so dry weights were not analyzed (data not shown). Since a root rating of 1 corresponds to cuttings with only a few roots that would not be transplantable, a combination system is not recommended for propagating threadleaf coreopsis. Threadleaf coreopsis is considered easy to propagate by stem cuttings in overhead mist, although bottom heat is recommended (Cullina 2000, Hartmann et al. 2002, Kessler and Keever 2007). Neither threadleaf coreopsis nor purple smoke false indigo were propagated previously in submist systems.

It is unclear why some species did not root as well in a submist system as compared with previous studies. This is a relatively new propagation system, and many parameters

Table 1. **Rooting response of *Amsonia tabernaemontana* (bluestar), *Hydrangea paniculata* (panicle hydrangea), *Baptisia* 'Purple Smoke' (purple smoke false indigo), *Physocarpus opulifolius* 'Diablo' (ninebark), *Myrica gale* (sweetgale), *Coreopsis verticillata* (threadleaf coreopsis) cuttings in response to propagation in either submist or overhead mist systems.**

Species	System	Root rating[z]	Root length (cm)[y]	Root number	Root dry weight (mg)[y]
Bluestar	Submist	3.22 A[x]	12.3 A	29.4 B	0.037 A
	Overhead Mist	2.95 A	4.45 B	45.5 A	0.019 B
	P-value	NS	<0.0001	0.0002	0.0112
Panicle hydrangea	Submist	3.78 A	4.97 A	64.0 A	0.060 B
	Overhead Mist	3.80 A	5.09 A	82.3 A	0.096 A
	P-value	NS	NS	NS	0.006
Purple smoke false indigo	Submist	1.87 B	5.48 A	6.68 B	0.060 B
	Overhead Mist	2.86 A	7.35 A	18.09 A	0.096 A
	P-value	0.013	NS	0.0012	0.006
Ninebark	Submist	3.13 B	8.27 A	16.5 A	0.061 A
	Overhead Mist	3.76 A	6.61 B	30.8 A	0.088 A
	P-value	0.0226	0.0174	NS	NS
Threadleaf coreopsis	Submist	0.48 B	2.30 B	0.64 B	N/A[w]
	Overhead Mist	3.04 A	8.32 A	8.36 A	N/A
	P-value	<0.0001	0.0017	0.0001	N/A
Sweetgale	Submist	3.22 A	6.19 B	18.1 A	0.014 B
	Overhead Mist	3.69 A	10.7 A	13.6 B	0.081 A
	P-value	NS	0.0038	0.0422	0.0002

[z]Roots were rated on a scale of 0-5 where 0 represented a cutting with no roots and 5 represented a cutting that was well rooted.

[y]1 cm = 0.39 inches; 1 mg = $3.5274 \bullet 10^{-5}$ oz.

[x]Data were analyzed using Anova and Tukey's means separation in JMP. Means for treatments are presented; those with different letters are statistically different. Data that are not statistically significant are indicated with a *P*-value of NS.

[w]There were not enough samples of threadleaf coreopsis that grew roots in submist to include in the analysis.

must be optimized. For example, in aeroponic production systems, which are similar in concept to a submist system, the size of water droplets and time between root misting impacted shoot and root development of lettuce (*Lactuca sativa* L., Tunio et al. 2022). The submist system for this project was modified from a previously built system in which five species had either superior or comparable rooting in submist versus overhead mist (Burnett et al. 2021). One change between the two systems is that a new lid was built to provide more support for cuttings, but with a reduction in full enclosure. Consequently, the interior of the submist system could have been too dry, hot, or bright. It is possible that too much light penetrated the new lid, which had larger openings; many of these openings were not filled with cuttings, which would allow more light to penetrate the lid of the system. Previous submist systems have either been small, opaque, plastic containers or large ebb and flow benches covered with plastic that is impervious to light (Burnett et al. 2021, Peterson et al. 2018a, Peterson et al. 2018b, Sanchez et al. 2020). Moreover, the lid was thicker, which could have reduced the amount of water reaching each cutting stem from mist nozzles in the chamber. Submist propagation systems tend to have a greater vapor pressure deficit compared to overhead mist, which could impact some plants more than others (Sanchez et al. 2020). Compared to previous designs (Peterson et al. 2018a, Peterson et al. 2018b, Sanchez et al. 2020, Burnett et al. 2021), the design of the submist lid with a grid of holes for cuttings also may have reduced the amount of water vapor held in the chamber atmosphere between misting events. Unlike most previous submist propagation systems (Burnett et al. 2021, Peterson et al. 2018b), this system also included supplemental overhead mist. However, cuttings of manchurian lilac (*Syringa pubescens* Turcz. subsp. *patula* (Palib.) M.C. Chang & X.L. Chen 'Miss Kim') rooted at higher percentages in a combination system with submist and overhead mist than cuttings propagated in either overhead mist or submist alone (Sanchez et al. 2020). For this reason, it is unlikely that the addition of overhead mist reduced rooting percentages in this experiment.

For some species (bluestar, ninebark, and sweetgale), there was no clear advantage in rooting with either the combination system or overhead mist (Table 1). The root rating was not significantly different for bluestar grown in each system, but cuttings in overhead mist systems each produced an average of 16.1 more roots than those in submist. However, roots were 62% longer in submist and root dry weight was 49% greater for bluestar propagated in submist (Table 1). It is likely that cuttings with more roots that are shorter may be easier to transplant into containers and recover more quickly from transplant shock compared to cuttings with fewer, but longer roots. By comparison, Burnett et al. reported that root rating, length, number, and dry mass were greater when bluestar was propagated in submist (2021). Bluestar is generally considered easy, but slow, to root. Previous research suggested that propagating bluestar in submist without supplemental overhead mist resulted in quicker root formation (Burnett et al. 2021).

Ninebark root ratings were significantly higher in overhead mist (3.76 compared to 3.13; Table 1). Although root rating was higher in overhead mist, plants were likely transplantable when they were propagated in either system. Roots were about 20% longer for ninebark cuttings in submist, but root number and dry weight did not differ significantly between cuttings propagated in each system (Table 1). There is no previous work exploring propagation of ninebark in submist; however, it is easy to propagate

Table 2. Water usage in submist and overhead mist systems.

System	Water usage (L)
Submist[z]	630.4[y]
Overhead Mist[x]	1919.3
P-value	0.0002

[z]The water usage in submist systems was manually measured each time water was added to the system. Flow meters were used to monitor the amount of water added via supplemental overhead mist.

[y]Water usage is the average water from five submist or overhead mist systems during the duration of propagation. Data were analyzed using Anova in JMP. Means for treatments are presented.

[x]Overhead mist water usage was measured using flow meters.

from softwood cuttings in early spring (Dirr and Hauser 2006).

For sweetgale, the root rating was not significantly different between cuttings in each system. However, roots were 42% longer and had 87% greater dry weight when propagated in overhead mist, whereas cuttings in submist produced approximately 4 additional roots per plant (Table 1). By comparison, previous research indicated that there was no difference in rooting for sweetgale when plants were propagated in submist compared to overhead mist (Burnett et al. 2021). In overhead mist, the concentration of auxin, but not the composition of substrate, impacts rooting of sweetgale (Peterson et al. 2019). Not applying rooting hormone resulted in the greatest rooting length, percentage, and rating (Peterson et al. 2019).

There was little to no difference in rooting for cuttings of panicle hydrangea propagated in either system (Table 1). Only root dry weight differed between cuttings in each system; root dry weight was 38% greater for cuttings propagated in overhead mist. By comparison, previous work indicated that more roots formed when panicle hydrangea cuttings were propagated in submist systems, but roots were longer in overhead mist (Burnett et al. 2021). Panicle hydrangea is considered to be an easy to root species that can be propagated from softwood, semi-hardwood, or hardwood cuttings (Hartmann et al. 2002, Dirr and Heuser 2006).

While there were few differences in the rooting quality for some species, the submist systems used less water than overhead mist systems (Table 2). The submist systems which were supplemented with overhead mist, applied 67% less water than standard overhead mist during the propagation period. This compares to previous work indicating that submist alone reduces water usage by 98% compared to overhead mist (Burnett et al. 2021). Even with the addition of some overhead mist to the submist system in this study, water savings were substantial. Because Burnett et al. (2021) found that submist alone produced cuttings with more roots and a greater root rating for several species, the supplemental overhead mist in this study was limited to 10 sec every 15 min and tapered to 10 sec every 60 min within the first week to maintain water savings. Some propagators may consider water savings a reason to use a combination system, even if rooting of cuttings is comparable, but not superior. In efficient irrigation technology for crop production, rather than propagation, there are benefits to efficient water use beyond saving water. For example, growers using a moisture-sensor automated irrigation system reported labor savings that allowed for more flexibility (Wheeler et al. 2018). A similar system reduced disease incidence on gardenia (*Gardenia jasminoides* Ellis, Chappell et al. 2013). Relatively little work has addressed water-efficient propagation systems, and it is unknown whether some of these same benefits would carry over to the propagation stage. However, future work on propagation systems that reduce water use could explore potential benefits beyond saving water.

In summary, the submist propagation systems produced variable rooting success across six species compared to overhead mist, but used less water during propagation. Rooting in submist was poor for threadleaf coreopsis and purple smoke false indigo, which were better suited to overhead mist. For bluestar, ninebark, and sweetgale, submist was superior for some measures of rooting success, but not for others. Panicle hydrangea rooted equally well in both systems. Bluestar, ninebark, sweetgale, and panicle hydrangea could be propagated using either submist or overhead mist. Practical considerations, such as ease of installation, flexibility for use with multiple species, or cost may determine which system is best for a species that forms roots reliably in either system. One advantage of using a submist with supplemental overhead mist system to propagate cuttings is that water usage is reduced by 67%.

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