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AIR AS A ROOTING MEDIUM: AN EXPERIMENTAL AIR-MIST CHAMBER SYSTEM FOR ROOTING CUTTINGS USING *FORSYTHIA* X *INTERMEDIA*

by

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I. INTRODUCTION

It was a search for an alternative system of vegetative plant propagation by stem cuttings whereby the rooting process could be more easily observed which lead to the air-mist chamber system (or the air-rooting system) for my thesis project.

In typical systems, cuttings are stuck in sand, peat, perlite, vermiculite, hadite, water, etc., or mixtures thereof. According to Marston (20) the two main requirements of a propagation medium are good aeration and the proper moisture retention. Other requirements important for certain plant taxa include temperature, pH, and darkness of the medium. Each plant taxon has its own peculiar requirements for optimum rooting response. To adjust to these differences in requirements, the air-mist chamber system can be altered readily for each plant taxon; eg., the temperature, the mist schedules, and the composition of the water solutions in the propagating chambers. A properly designed air-mist chamber system offers the exciting possibility of a good medium for vegetative propagation of cuttings in which the rooting process can be observed and controlled.

The object of this thesis was to develop a workable air-mist chamber system for experimental use. The first three experiments of this study were essentially engineering experiments whereby changes were made to the system to test conditions favorable for rooting. The final experiment was to determine whether various IBA (indole butyric acid) treatments on different cuttings in the same chamber could be tested without a treatment "dilution" effect due to misting i.e., the leaching and remisting of the IBA in the leachate to nontreated cuttings.

II. REVIEW OF LITERATURE

A. Air-Mist Chamber Systems

The first reported use of an air-mist chamber was by Pearse (22) in 1937 at the East Malling research station. The chamber was designed for the observation of root growth for entire apple (Malus domestica Borkh.) plants rather than for vegetative propagation studies. Two year old M.2 (Malling 2) and M.5 apple trees were grown in a nutrient mist in 12 inch pots coated with paraffin wax. The roots of the trees were suspended in the pots from a paraffin-coated wood cover. The trees were fitted through the holes and held in place by loose fitting corks. The nozzle was simply a constricted glass tube. Air pressure forced water through the nozzle to create a fine mist. The nutrient solution in this recycled system was reused continuously for two weeks, then replaced.

As a propagation medium, the nutrient misted-air was superior to nutrient water culture media, one of which was a water solution bubbled with air; the other was a circulating water medium. With the nutrient water cultures, M.2 and M.5 roots became covered with a "slimy fungus." Trees were decidedly unhealthy and defoliation occured earlier than nutrient misted air.

Other similar air-mist systems for whole plants were reported by Rains (23) in 1940, Carter (6) in 1942, Klotz (14) in 1944, Vyvyan and Trowell (30) in 1952, and Went (32) in 1957. The systems were similar in function if not design. All systems, except for Carter's (and

Rains') collected and recycled the nutrient solution; all systems except Klotz's were designed to keep out the light when the plants were not under observation; and all systems except those of Rains and Went had a constant mist. Clogging of the nozzles (14, 30, 32) and regulation of the amount of misting (23, 32) appeared to be problems.

The first reported use of air-mist chambers for the rooting of cuttings was by Rains (23) in 1940. In two brief reports (23, 24) he described his system as a moist-chamber in which the cuttings were given an interrupted mist. There was an "abundant regeneration of roots on relatively large leafy cuttings of poplar and other plants". The main advantage of the system was that soluble carbohydrates could be fed to the plants without deleterious growth of bacteria and fungi on the root surfaces. An admitted disadvantage was the prolonged "washing" effect which was apparently harmful to the plants.

Briggs (2, 3, 4) described a "mist chamber" system for vegetative propagation of cuttings constructed like a grafting case with a 1 X 12 board on each side. Black 1 1/2 MIL polyethylene covered the top of the chamber. The cuttings were stuck halfway through the plastic. A single layer of plastic was used during the summer months, and a double layer was used during the winter to provide additional insulation against the cold. Hot water was piped to the chambers and misted with fogger nozzles. In first trials the cuttings were misted for 5 sec/6 min interval for 24 hrs a day. Later, the mist was gradually tapered to afternoons only. After the weather had warmed sufficiently in the spring, overhead mist was applied through no. 300 brass Flora-Mist nozzles for 5 sec/12 min interval during daylight hours.

Indole butyric acid (IBA) and naphthalene acetic acid (NAA) were applied to the cuttings by three different methods, all with some success - the concentrated dip, in the overhead mist, in the underneath or chamber mist. Dipped cuttings were dried for a day to insure uptake of the growth regulator.

Important observations Briggs (3) made about the air-mist chamber system were the following: (a) some cuttings were able to root under clear plastic, but with most cuttings, black plastic was necessary so that there was darkness at the base of the cutting; (b) with the application of IBA and NAA to stimulate rooting, higher chamber misting rates required higher growth regulator concentrations; (c) too much misting slowed down or inhibited rooting; (d) air rooted cuttings were easily transplanted and proceeded towards normal growth almost immediately; (e) cuttings were successfully held in air-mist chambers for 3 months without nutrients.

B. Environmental Effects of Moist-Air Chambers

Oxygen Concentration

No information was found on the amount of oxygen available to cuttings in air-mist chambers. There is literature which describes the oxygen needs for root growth and root initiation. Root growth and root initiation are separate processes, but "rooting" may include both.

It is now known that a combination of relatively high oxygen and low carbon dioxide levels in the soil air enhances root respiration and water uptake as well as greater root growth (19).

In a soil medium, the water-saturated walls of root hairs are intimately connected to the water-films of the soil with a cell wall-water interface; likewise, the water-film has a water-gas interface. In a normal soil medium which has gas filled pores, both molar gaseous oxygen movement and diffusion take place but, in a location where there are no gas pores, the oxygen movement is limited to diffusion (5).

Oxygen is essential for rooting cuttings, but the amount of oxygen required (oxygen demand) varies with different plant taxa. Willow cuttings root readily in water with 1 ppm oxygen whereas English ivy requires 20 ppm for adequate rooting (34). Others (5, 9) have reported variations of root growth responses to oxygen concentration in whole plants.

Cannon (5) described the situation thusly: There is a characteristic "lower critical" concentration or partial pressure as well as an "upper critical" partial pressure for oxygen below and above which there is reduced root growth. This non-critical range of normal plant growth fluctuates according to the temperature of the root medium.

As for the "lower critical" partial pressure of oxygen for <u>root growth</u>, Greenwood (11) found that the rate of oxygen uptake by the roots in mustard plant was unimpeded by dropping the oxygen concentration in the pore space to a very low level, less than 0.02 atm. Greenwood cited several root elongation studies which gave similar results with many other species.

From the above it is obvious that <u>root growth</u> is unimpeded by relatively low levels of oxygen. Yet, this does not indicate whether root initiation is likewise affected. Mahlstede and Haber (19) state that cuttings which have performed primordia generally have a lower oxygen requirement for rooting than cuttings which must initiate roots. This hints that root initiation requires more oxygen than root growth. Zimmerman (34) found that aerating water culture with 16-20 ppm oxygen improved the number of roots produced in several species of cuttings as xompared with 4-6 ppm. These data do not have the range to give the lower critical oxygen concentration for rooting, however.

Leaching

One of the most important factors in the mist propagation of cuttings is leaching (10). Minerals and metabolites are leached similarly from the "above ground part" of the plant and the roots. Tukey and Morgan (29) found that above ground plant tissue leachates include amino acids, amino acid derivatives, carbohydrates, growth regulators (including phenolics), and mineral nutrients. Rovira (25) listed compounds which were exuded (or leached) from the roots. These included amino acids, peptides, sugars, enzymes, vitamins, organic acids, nucleotides, inhibitors (growth regulators), mineral nutrients, etc.

The "above ground" and the root tissue leachates vary in amounts and relative proportions according to the plant taxon, physiolobical age, and the environmental conditions. More leaching occurs in hardwood cuttings than softwood or even semihardwood cuttings; plants in bloom are leached more readily than vegetative plants; and old leaves leach more than young leaves. More leaching occurs with high light intensity, and more carbohydrates are leached with high temperatures. Leaching is increased when the plant is injured. The duration of the misting is more important than the volume of the misting. A light mist that continuously baths the plant tissue leaches considerably more from the plant than a short voluminous sprinkling (28).

In general calcium, magnesium, manganese, potassium, sodium, and strontium are leached more readily than clorine, iron, phospherous, and zinc. Losses by leaching may be as much as 80 to 90 percent of the potassium content, and 50 to 60 percent of the calcium content in mature leaves. Plants can be literally starved by excessive leaching (29).

Leaching has been found to cause a chemical interaction among plants (or cuttings). These interactions may affect nutrition, growth promotion or inhibition (eg. promoting or inhibiting root initiation, etc.), or allelopathic death (by the leaching of toxins or toxin precursors) (28).

C. Effects of Experimental Treatments

<u>Overhead Mist</u>

Hess and Snyder (13) found that cuttings (stuck in a conventional medium) with overhead mist rooted better than those with no overhead mist but under double glass and that intermittent mist was superior to constant mist. Theoretically, overhead mist was superior to double glass chambers because the tissues of the cuttings were maintained 10 - 15^{o}F cooler. Cuttings with mist had lower respiration rates. Due to the lower temperatures this lowered respiration rate allowing the cutting to retain more food reserves for the process of rooting. Likewise, due to lower tissue temperatures, leaves under mist maintained their turgor better than those under double glass. Tugor was maintained better because the temperature-dependent vapor pressure gradients from the leaf tissues to the surrounding air would, in theory, have been much less.

Additional evidence by Lee and Tukey (17) indicated that intermittent overhead mist (constant overhead mist was not tested) stimulated a build up of root-inducing substances in leaf tissues and delayed dormancy and senescence of Euonymus alatus 'Compactus' cuttings.

Interrupted mist was superior to constant overhead mist because (a) the temperature of the rooting medium was maintained closer to the optimum i.e. warmer (this advantage would not apply to heated air-mist chambers), (b) nutrients were not leached from the cuttings to such an extent, (c) there was better aeration of the cuttings, and (d) the cuttings were more easily hardened for transplanting (13).

Temperature

The temperature of the rooting medium affects the rate of root initiation, the amount of root growth, and the structure of the roots produced. Komissarov (15) concluded from reports that cuttings from different plant taxa responded differently to temperature treatments. Optimal rooting response varied from 16°C to 37°C. Cuttings of tropical and subtropical plants tended to require higher temperatures for optimum rooting than plants from temperate origins. All cuttings seemed to root better when the basal medium was maintained 3°C to 5°C higher than the ambient air temperature. The night temperature should be lower than the day temperature so that the oxygen solubility would be enhanced for respiratory gas exchange. Usually cuttings with little lignification in the stem tissue rooted better at $20^{o}C$ to $22^{o}C$, whereas, highly lignified cuttings rooted better at $27^{o}C$ to $30^{o}C$

Root growth is limited or stopped by both low and high temperature extremes. The optimum temperature for root growth varies with the species, stage of development, and the oxygen supply, but it tends to be from $20^{o}C$ to $25^{o}C$ for most plant species (18).

The root zone temperature also affects both the morphology and the distribution of roots. At the optimum root temperature, cells divide more rapidly, and for a greater duration than at lower temperatures. At cool temperatures, the roots usually are whiter, thicker in diameter, and less branched. Maturity is delayed by the cooler temperatures, thus cell elongation is favored. At higher temperatures the roots are filamentous, short, and highly branched (21).

Indole Butyric Acid

Indole butyric acid (IBA) is one of the best and most commonly used growth regulators for the stimulation of root initiation. It is nontoxic compared to other auxins, a stable compound which resists breakdown by enzyme peroxidases and, because it translocates poorly, remains at the site of application (33).

Information about the application of growth regulators to *Forsythia* spp. cuttings for the stimulation of rooting is limited. Some (12, 19) do not use growth regulators for rooting *Forsythia* spp. cuttings. Wells (28) found with the use of Hormodin ^{R} rooting powders that softwood cuttings of *Forsythia x intermedia* rooted quickest with Hormodin ^{R} powder no. 2 (4mg IBA/g talc), whereas hardwood cuttings of the same rooted the fastest with Hormodin® powder no. 3 (8 mg IBA/g talc). No data could be found about the use of 5 sec dips of IBA solutions.

III. EARLY TRIALS

The air-mist chamber system was still in a crude state when experiments with grape cuttings *Vitis labrusca* L. 'Concord' were conducted. Experimental technique was not strictly adhered to since it was necessary to make adjustments in the system while the experiment was in progress.

The original chamber was a 55 gallon barrel cut in half lengthwise, open side placed up, with pressed styrofoam (1/4 inch) as a cover. The chamber system consisted of 8 chambers on a 33 ft bench in a (glass) greenhouse. Aluminum foil was glued to the styrofoam boards with an excess lapping over the sides to exclude light from the inside of the chamber. It did not exclude all light, but it did hold the styrofoam board in place over the chamber.

On opposite ends of the chamber were single holes into which glass atomizers (custom built at Kansas State University by F.W. Schwenk and Mitsugi Ohno) were placed. The atomizer was two glass tubes lined together at right angles, each tube tapered to a small hollow point. One tube extended through the chamber hole and was connected to a compressed air source by vinyl tubing. The other tube was immersed in a reservoir pool of distilled water at the chamber bottom.

The chambers were painted inside by a Rustoleum® primer and a Rustoleum® enamel paint, and the seams were filled with an "epoxy glue repair kit." Approximately 5 liters of distilled water were placed in each chamber as a reservoir for misting. Tap water proved to be undesirable since it clogged nozzles.

The dormant hardwood grape cuttings were stuck through nail-sized holes in the styrofoam boards. The proximal ends of the cuttings were cut diagonally to a point so the cuttings could be plunged through the smaller holes and held loosely by the styrofoam. The cuttings uniformly had three nodes with two nodes stuck into the air-mist chamber. The fog or mist was continuous within the chamber 24 hours a day; air pressure to the atomizers was held at 12 psi by a Binks® air pressure regulator.

This air-mist chamber system was located in the research (glass) greenhouse of the Department of Horticulture and Forestry, Kansas State University, Manhattan.

The greenhouse was steam heated with perimeter pipe and vertical discharge unit heaters. It was cooled with ridge ventilators, or during the warmer months by a CEL-DEK® fan and pad cooling system. The glass roof was covered with a glazing compound during the summer months to mitigate solar radiation heat.

Foremost among the problems with this system was that the cuttings were over-watered and leached considerably. A fungal slime built up around the cuttings which was especially thick at the base. The abundance of water caused guttation of the new leaves of the cuttings. A pungent odor was noticed when the cover was lifted from the chamber.

Some light entered the chamber through the gaps between the cuttings and the board. The aluminum foil draped over the top did not adhere tightly to the sides of the cutting. Briggs (3) reported that light was detrimental to the rooting process of many plant taxa.

There seemed to be excessive heat on the tops of the cuttings since no shade or overhead mist was provided. The solar radiation heat was compounded by reflection from the aluminum foil. Komissarov (15) reported that the tops of cuttings should be 3-5^{o}C cooler than the basal medium, and that excessive heat reduces rooting.

The paint coating the inside of the chamber bubbled and peeled under the resevoir pool. There was a faint paint odor in the chamber.

Lastly, some grape cuttings were too long for the chambers. The proximal ends were immersed in the reservoir pool of water which defeated the purpose of the air-mist chambers.

Few cuttings rooted under these conditions. Those that did transplanted to pots easily. Since no overhead mist was provided, it was not necessary to harden the cuttings by gradually weaning them from the chamber mist.

An attempt was made to rectify the leaching and fungal problems by coating the cuttings with paraffin wax. This seemed to prevent aeration of the cuttings and the rooting performance dropped to zero.

The air pressure to the atomizers was dropped to 4 psi and benomyl fungicide was placed in the reservoir pools of the chambers. Each of the four chambers received a different concentration of benomyl (concentrations were not accurate since benomyl was nearly insoluable in the distilled water). In each of the four remaining chambers four different concentrations of IBA were applied to the reservoir pools, 0 ppm, 0.1 ppm, 1 ppm, 10 ppm to speed the rooting process before the cuttings were leached. Few cuttings rooted with the fungicide treatments or the IBA treatments. Leaching or some other unknown factor was involved.

A new approach was established. *Forsythia* x *intermedia*, a hybrid of *F. suspensa* x *F. viridissima*, cuttings were smaller which allowed more cuttings per barrel. A new kind of cover was constructed to reduce light intensity within the chambers. A black polyethylene film was stretched over a wooden frame and stapled to it. The frame was constructed to be larger than the open top of the chamber so that the polyethylene film sealed against the top edges of the chamber with the weight of the wooden frame. The black polyethylene film sealed against the cuttings better than the styrofoam boards yet some light still was admitted into the chamber.

It was with these changes that I began the first experiment.

IV. MATERIALS AND METHODS

A. IBA Dilute Soak Experiment No. 1

Comments

The experiment ran from January 8, 1975 to March 4, 1975. There was no overhead mist or shade provided to the cuttings. Data were not collected as very few cuttings rooted.

Description of the System

The system was the same as that most previously mentioned. It had a constant mist with 4 psi air pressure to the atomizers. The chambers were covered with a black polyethylene film (4 MIL). Forty-eight were cuttings stuck into each chamber (two nodes beneath the plastic, one or two nodes above the plastic). There were 8 rows of cuttings with 6 in each row (spaced 50 mm apart in the row).

Type of Cuttings

The cuttings were dormant 5 inch straight cuttings of *Forsythia x intermedia* 'Spring Glory' collected from Skinner's Nursery, Topeka, Ks. on January 3, 1975.

Treatments

Some cuttings received a dilute soak for 24 hours of 20 ppm IBA others were left untreated. Three chambers contained all treated cuttings, three chambers were stuck with one-half treated and one-half nontreated cuttings (randomized within the chamber) and two chambers were stuck with untreated cuttings.

B. Description of Revised System Used in Remaining Experiments

The cover for the chambers was altered for the overhead mist. A piece of no. 3 galvanized wire was bent and secured over the wooden frame such that when the black polyethylene film was draped over the wire and the frame a "hill" was created in the center of the cover as a high point for drainage. Two layers of end-roll unprinted newspaper were stapled over the plastic film which gave a 3 layered cover. This insulated the chambers from solar heat and provided additional humidity to the cuttings with the overhead mist.

The cuttings were punched through the cover such that at least two nodes of a cutting were inside the chamber. There were 48 cuttings per barrel, 8 rows of 6 cuttings. Trowbridges Grafting Wax [R] (manufactured by W.E. Clark and Son, Orange, Conn.) was used to juxtapose both the cutting and the end-role paper thus sealing the chamber from water and light.

An overhead mist system was installed over barrels with Flora Mist [R] "fogger nozzles" (Model 300A). One was spaced over each chamber. A 4 MIL black polyethylene film was placed into the chamber to cover and protect the chamber from water corrosion and leakage.

To each of the eight chambers was added a 250 watt copper immersion heater, Van Waters and Rogers Cat. No. 33890-049, Brisbane, Calif. Two holes were drilled into the front end of the chamber to hold the immersion heaters. The holes were sealed against leakage with DAP [R] white Kwik Seal [R] and tile caulk (DAP Inc., Dayton, Ohio 45401). Eight General Electric HSC-6 thermostats (range -1^{o} to 49^{o}C were installed with the sensor immersed in the reservoir pool of each chamber.

The chamber mist was made intermittent (20 sec per 12 min from 7 AM to 6 PM). Each mist system had its own solenoid valve and short-interval programmer. Both solenoid values were 3/4 inch Asco® gasses and liquids electric solenoid valves. Between the solenoid valve for the compressed air and the pressure regulator was a 1 1/2 inch muffler pipe to weaken the explosion of air when the solenoid valve opened. The programmer for the chamber mist was a Model 12 M 8001 Tork® "time switch", the timer for the overhead mist was a Model 6M8001 Tork® "time switch". There was a common day/night programmer, an Intermatic® "DPST switch" Model T 103. The thermostat controls and all electrical outlets were covered with polyethylene film to prevent water damage. Please note Figure 1.

C. Overhead Mist Experiment No. 2

Comments

This experiment determined the effects of overhead mist on cuttings in air-mist chambers. The duration of the experiment was from March 15, 1975 to May 16, 1975 (63 days). Additional data were taken from the misted chambers for Experiment 3.

Type of Cuttings

Straight 4 inch dormant hardwood cuttings were collected from Skinner Nursery, of Forsythia x intermedia 'Spring Glory', on March 6, 1975. The cuttings were stored for 8 days at $4^{o}C$ in sterilized moist peat and wrapped in 4 MIL polyethylene film. The day of the experiment

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Figure 1: Diagram of the air-mist chamber system.

F	Screen filter, 100 mesh.
S	Solenoid valve, 3/4 inch Asco gasses and liquids, electric.
Mu	Muffler pipe.
PR	Air pressure regulator with a rag filter, Binks Model 86-130.
RH	Rubberhose, from pressure regulator to manifold.
Ma	Manifold (8 holes).
H	Vinyl hose, from manifold to the two chamber atomizers.
1-8	Air-mist chambers no. 1 through no. 8 (55 gal. barrels cut lengthwise in half).
A	Exterior location of chamber atomizers.
N	(8) Flora-Mist Fogger Nozzles Model 300A, for overhead mist.
T	(8) GE HSC-6 thermostats, range -1°C to 49°C.
D/NT	Intermatic DPST Switch, 24 hr timer Model 103.
IT (12 min)	Model 12M8001 Tork Time Switch.
IT (6 min)	Model 6M8001 Tork Time Switch.

Note that the 250 watt immersion heaters and the thermostat sensors 1 each in all 8 chambers are not shown in this figure, as they are inside the chambers.

THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

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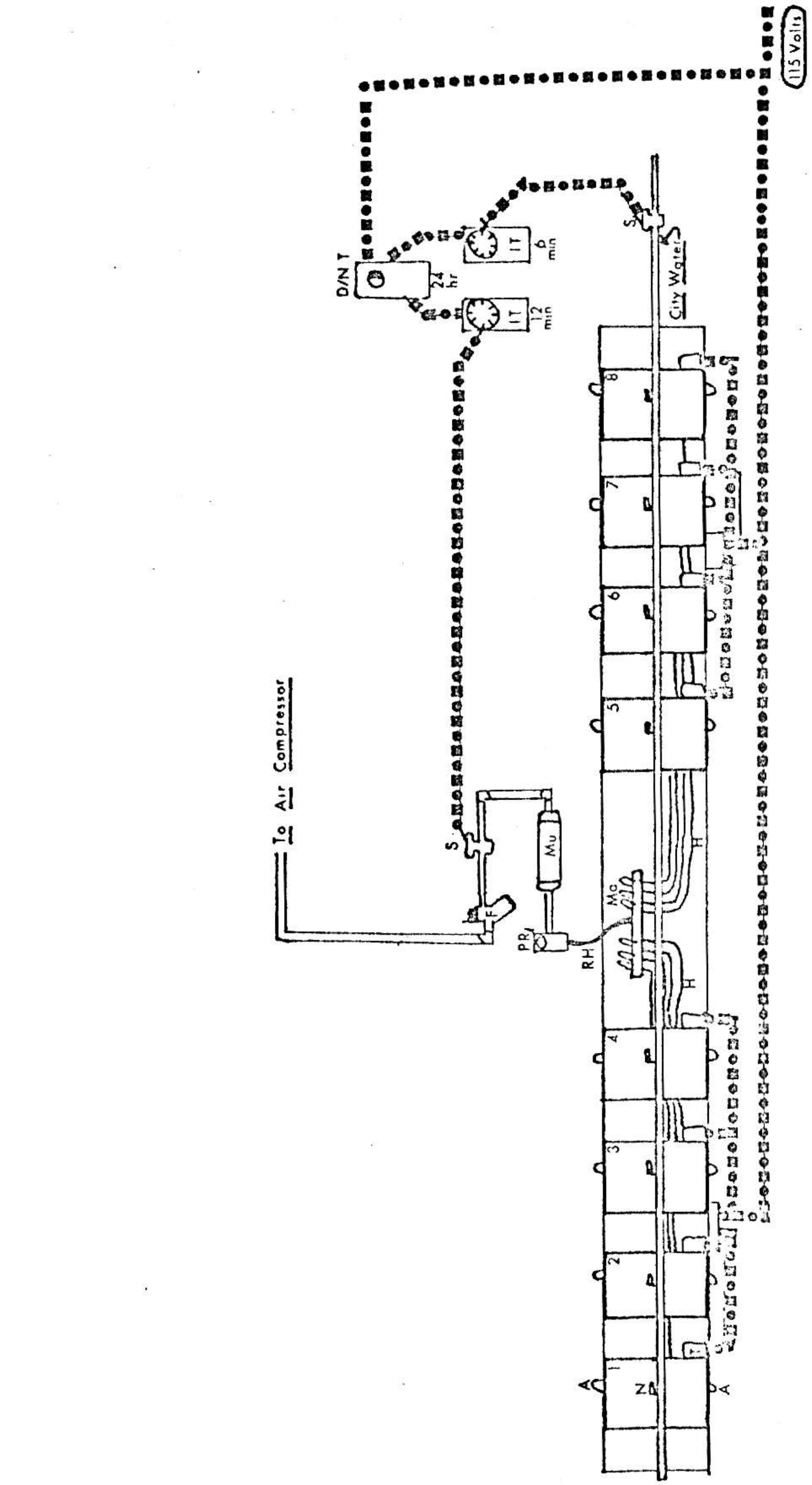
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a fresh cut was made on the proximal end of the cuttings. The buds from the lower two nodes were removed from the cuttings to facilitate a clean, water-tight chamber.

<u>Treatments</u>

(a) Overhead mist - chamber no. 5 through no. 8 received overhead mist; chambers no. 1 through no. 4 received none.

(b) Chamber heat - the chamber liquid in one overhead misted and one non-overhead misted chamber was heated to 20°C, 25°C, 30°C, or 35°C (thermostat settings for heaters). Random order of chamber selection relative to heat treatments was established.

(c) Wax capping - in each of the 8 rows of (6) cuttings, a random 3 cuttings were capped with grafting wax on the distal cut surface of the cutting.

<u>Data Collected</u>

The data taken were the number of cuttings which rooted per chamber, the number of root-tips per cutting in each chamber, and the number of cuttings with leaves per chamber.

<u>Statistical Analysis</u>

No statistical analysis was necessary as the difference between the overhead mist treatment and the nonmisted (overhead) treatment was obvious.

D. Chamber Heating and Wax Capping Experiment No. 3

<u>Comments</u>

The purpose of this experiment was to determine the best immersion heat treatment for air-mist chambers in combination with or without a wax capping treatment and the rooting percentage at three time periods.

There were three trial periods in this experiment: 1) 63 days from March 15, 1975 to May 16, 1975 (temperature replication no. 1); 2) 57 days, from June 4, 1975 to July 31, 1975 (temperature replications nos. 2 and 3); 3) 54 days, from August 20, 1975 to October 13, 1975 (temperature replications nos. 4 and 5). Trials were ended when no new roots formed within a 2 day period in any of the eight chambers.

Since it was necessary to discard replication no. 2 after the immersion heaters malfunctioned (with faulty electrical wiring), the original 5 temperature replications were reduced to 4.

Description of the System

The system (Figure 1) was the same as in Experiment No. 2 except the overhead mist extended to all 8 chambers. Some leakage of overhead mist into the chambers occurred but this was minimal and did not impair the performance of the chamber.

Type of Cuttings

There were three types of cuttings in this experiment. The heat treatment replication no. 1 had straight, dormant, hardwood cuttings of *F.* x *intermedia* 'Spring Glory' (from Skinner's Nursery, Topeka, Ks.). The heat treatment replications nos. 2 and 3 had straight, dormant, hardwood cuttings of *F.* x *intermedia* 'Spring Glory' (from Skinner's Nursery, Topeka, Ks.) which were removed from 4°C storage after 90 days, March 6, 1975 to June 4, 1975). Heat treatment replications nos. 4 and 5 had straight, semi-hardwood, leafy cuttings of *F.* x *intermedia* (cultivar unknown) collected at the Kansas State University campus on the first day of the experiment, August 20, 1975. All three types of cuttings were 4 inches in length.

Treatments

There were two treatments - (a) chamber heat and (b) wax capping.

(a) Chamber heat - immersion heat treatment of 20°C, 25°C, 30°C, and 35°C were given randomly to the liquid reservoir of different chambers.

(b) The wax capping treatment - in each of the 8 rows of cuttings, distal cut surface of a random 3 cuttings were covered with grafting wax.

Data Collected

Data taken at the end of the experiment were collected in samples of 24 cuttings indicated the number of cuttings rooted, the number of root-tips per cutting, and the number of cuttings with leaves. "Leaves" refer to both the original leaves in the semi-hardwood cuttings and the newly emerged leaves after the cuttings (dormant hardwood and semi-hardwood) were stuck.

Data taken while the experiment was in progress indicated the number of cuttings rooted during the designated periods of time (0 to 40 days, 41 to 47 days, and 48 to 54 days) for each temperature treatment (one chamber, 48 cuttings).

Statistical Analysis

The experiment was a split-plot design (each plot is split with waxed and unwaxed treatments). The data was analyzed by a two-way analysis of variance. The means were separated by Fisher's least significant difference (LSD) test at the 5% level of significance.

E. IBA and Nozzle Experiment No. 4

Comments

The purpose of this experiment was to test (a) the effect of IBA on the performance of the cuttings, (b) whether there is a "dilution" of differences between treated and untreated cuttings in the same chamber (leaching with leachate mists), and (c) the effect of the location of the cutting relative to the nozzle (or to test the evenness of the chamber mist).

The experiment was divided into two trials. Trial 1 began October 24, 1975 and ended Dec. 23, 1975 (60 days). Trial 2 began January 23, 1976 and ended April 2, 1976. The trials were terminated by the criterion of Experiment No. 3.

The overhead mist during trial 2 was accidently left off for 4 days at 52 to 56 days after sticking the cuttings. This killed some cuttings but delayed the rooting process for the remaining cuttings.

Description of the System

The system was the same as Experiment 3, except that all immersion heater thermostats were set at 25°C.

Type of Cuttings

The cuttings were all - four inch, straight, dormant, hardwood cuttings of F. x intermedia (cultivar unknown) taken from Rocky Ford Experiment Station (4 miles north of Manhattan, Ks.). The cuttings were taken the day before the trials had begun. The dates of collection were October 23, 1975 (Trial 1) and January 22, 1976 (Trial 2).

IBA Treatments

Two chambers received no treatment. In 3 chambers one-half the cuttings were dipped for 5 sec in a solution of 500 ppm IBA in 50% ethyl alcohol and water. These treatments were randomized along each row of 6 cuttings such that 3 were dipped in IBA and 3 were not dipped. Lastly, in 3 more chambers all of the cuttings were given the same IBA dip treatment (500 ppm IBA, 5 sec).

Data Collected

The data were collected in 2 row, 12 cutting samples. The two rows were in identical locations relative to the nozzle, closest to each (there are two nozzles at each end of a chamber). The data included the number of cuttings rooted per sample (of 12 cuttings), the no. of root-tips per cutting, and the no. of cuttings with leaves.

Statistical Analysis

The experiment was a split-plot design. An unequal subclass analysis of variance determined experimental differences in the treatments.

V. RESULTS

A. IBA Dilute Soak Experiment

No data was collected for this experiment because very few of the dormant hardwood Forsythia cuttings rooted.

B. Overhead Mist Experiment

The effect of intermittent overhead mist on the rooting performance and whether foliations occurred in the F. x intermedia 'Spring Glory' dormant, hardwood cuttings (stuck in air-mist chambers) is presented in Table 1. On March 14, 1975, 63 days after sticking the cuttings, it was found that with overhead intermittent mist there was a greater no. of cuttings rooted, and a greater no. with roots of a higher quality (more root-tips per cutting) than with those without overhead mist. Similarly more of the cuttings with overhead, intermittent mist produced green foliage than those without the overhead mist, although there was less difference between the results in this case.

All cuttings received an intermittent "underneath" mist inside the air-mist chamber.

C. Chamber Heating and Wax Capping Experiment

The results are presented in Tables 2, 3, and 4. Table 2 indicates that immersion heat treatments caused significantly different rooting performance and significantly different numbers of cuttings with foliage. Immersion heat thermostat settings of 25°C and 30°C were superior to those at 20°C and 35°C. The mean no. of cuttings rooted at

Table 1. Effect of intermittent overhead misting on the rooting performance and the presence of green foliage on *Forsythia x intermedia* Zab. 'Spring Glory' dormant hardwood cuttings which were stuck in air-mist rooting chambers.[1]

Thermostat Setting	No. of cuttings rooted		No. of root-tips		No. of cuttings with leaves	
	Unmisted	Misted	Unmisted	Misted	Unmisted	Misted
20^oC	12	24	0.83	3.40	35	29
25^oC	9	41	0.25	8.31	23	39
30^oC	0	30	0.00	4.69	0	44
35^oC	0	14	0.00	1.69	23	25
Mean	5.25	27.25	0.27	4.25	20.25	34.25

[1]Each datum represents one chamber with 48 cuttings.

Table 2. Effect of immersion heat treatments in air-mist chambers on the rooting performance and the presence of green foliage in *Forsythia x intermedia* Zab. cuttings.[1]

Thermostat Setting	No. of cuttings rooted	No. of root-tips /cutting	No. of cuttings with leaves
20°C	8.00[b][2]	2.26[b][2]	9.25[b][2]
25°C	16.63[a]	6.46[a]	14.88[a]
30°C	13.13[a]	3.92[b]	14.50[a]
35°C	8.88[b]	2.06[b]	15.75[a]
LSD .05	4.43	2.37	4.84

[1]Each datum is the mean value for 8 replications of 24 cuttings.

[2]Mean separation within each column by Fisher's least significant difference (LSD) test, 5% level.

Table 3. Effect of wax-capping the distal cut surface of *Forsythia x intermedia* Zab. cuttings on the rooting performance and foliage production in air-mist root chambers.[1]

Treatment	No. of cuttings rooted	No. of root tips / cutting	No. of cuttings with leaves
Waxed	11.56	3.65	14.00
Unwaxed	11.75	3.69	13.19

[1]All data N.S. at the 5% level by analysis of variance. Each datum is the mean value for 16 replications of 24 cuttings.

Table 4. Effect of different immersion heat treatments in air-mist rooting chambers on the rooting rates of *Forsythia* x *intermedia* Zab. cuttings.

	Number of cuttings rooted within each designated period			
	Thermostat Setting			
Rooting period	20°C	25°C	30°C	35°C
0 to 40 Days	3.33^{bc}[1]	16.33^{a}	21.00^{a}	7.67^{bc}
41 to 47 Days	2.33^{c}	9.33^{b}	2.66^{c}	2.66^{c}
48 to 54 Days	7.33^{bc}	9.33^{b}	2.00^{c}	5.00^{bc}
Total per 48 cuttings	13	35	25.66	15.33
LSD .05 = 6.38				

[1]Mean separation by Fisher's least significant difference, 5% level.

the 25°C setting was greater than that of the 30°C setting, but it was not significantly different at the LSD .05 level. Whereas, for the no. of root-tips per cutting, the 25°C temperature setting was superior to all other immersion heat treatments. Table 2 also indicates the mean no. of cuttings with green foliage. All immersion heat thermostat settings except 20°C produced new foliage or retained their original foliage (leafy semi-hardwood cuttings) equally well. At 20°C there were significantly fewer cuttings with foliage.

Table 3 shows no consistent effect of wax capping the distal cut surface of the cuttings as measured by the number of cuttings rooted, number of root-tips per cutting, or the number of cuttings with leaves.

Lastly, Table 4 demonstrates the difference in the rooting rates according to different immersion heat treatments. During the first period, 0 to 40 days, the 30°C and the 25°C thermostat settings had the greatest no. of cuttings rooted. During the second period the 25°C heat was significantly better than all other treatments. In the final period the 25°C heat was superior to the 30°C heat.

In summary, the 25°C heat gave more rooted cuttings over the longest period of 54 days whereas both 25°C and 30°C heats had the most cuttings rooted early, 40 days after sticking.

D. IBA and Nozzle Experiment

There were no significant differences between the treatments of IBA to 100%, 50% and 0% of the cuttings in a chamber as shown in Table 5. Therefore, at that concentration, for that time period of dip, IBA had no significant effect on the rooting performance, or whether foliation occurred.

Table 5. Effect of a indole-3-butyric acid (IBA) 500 ppm 5 sec dip on the rooting performance and the no. of cuttings foliated for *Forsythia* x *intermedia* Zab. hardwood dormant cuttings.[1]

% cuttings in chamber dipped in IBA (%)	No. of cuttings rooted	No. of root-tips / cutting	No. of cuttings foliated
		TRIAL 1	
0	4.00 ± .68	1.43 ± .24	7.7 ± .66
50	5.83 ± .55	1.99 ± .19	8.7 ± .54
100	5.58 ± .56	1.87 ± .19	6.5 ± .54
		TRIAL 2	
0	5.12 ± .48	2.60 ± .26	2.75 ± .42
50	2.92 ± .39	0.82 ± .21	2.00 ± .34
100	3.92 ± .39	1.51 ± .21	2.08 ± .34

[1]All data N.S. at the 5% level by unequal subclass analysis of variance. Each datum is the mean value for 8 replications of 12 cuttings.

Table 6 demonstrates no apparent effect of row location or position compared to the nozzle for the rooting performance and cuttings foliated.

Table 6. The chamber nozzle misting effect according to row location on rooting performance and the no. of cuttings foliated of *Forsythia* x *intermedia* Zab. dormant hardwood cuttings.[1]

Location[2]	No. of cuttings rooted[2]	No. of root-tips /cutting[2]	No. of cuttings foliated
		TRIAL 1	
1	5.78 ± 0.69	2.22 ± 0.24	8.22 ± 0.67
2	5.00 ± 0.69	1.46 ± 0.24	7.39 ± 0.67
3	4.89 ± 0.69	1.85 ± 0.24	6.72 ± 0.67
4	4.89 ± 0.69	1.53 ± 0.24	8.33 ± 0.67
		TRIAL 2	
1	3.78 ± 0.49	1.41 ± 0.26	1.99 ± 0.42
2	3.22 ± 0.49	1.42 ± 0.26	1.77 ± 0.42
3	4.44 ± 0.49	2.08 ± 0.26	2.72 ± 0.42
4	4.50 ± 0.49	1.67 ± 0.26	2.62 ± 0.42

[1]All data N.S. at the 5% level by unequal subclass analysis of variance. Each datum is the mean value for 8 replications of 12 cuttings (± S.E.).

[2]Location numbers are designated from 1-4, 1 = rows closest to the nozzles to 4 = rows farthest from nozzles.

VI. DISCUSSION

A. IBA Dilute Soak Experiment

Although this experiment did not demonstrate the compatability of air-mist chambers to different treatments of IBA, it did show, by the poor rooting performance of the cuttings, that more adjustments to the system were necessary.

One of the inadequacies was the fungal growth on the basal portion of the cutting despite the reduced constant chamber mist. It had been reported by Briggs (2) that constant underneath mist seemed to retard root formation. Rains (24) found that there were no bacterial or fungal growth problems with interrupted mist in his air-mist chambers. This experiment confirms their reports. The air-mist chamber systems for the remaining experiments all had an intermittent chamber mist.

B. Overhead Mist Experiment

The object of this experiment was to determine whether it was advantageous to add intermittent overhead mist to the system of air-mist chambers. The results dramatically favored overhead mist. When overhead mist was used there were more cuttings that rooted and more root-tips per cutting than without overhead mist (although the improvement of foliation was less obvious). These results agree with the findings of Hess and Snyder (13) that overhead mist is superior to the non-mist treatments. Although such results would be obvious with conventional media, it would not have been so with the air-mist chamber system since the chamber (intermittent) mist provided excellent opportunity for water uptake.

It was feared that both mist systems might leach the cuttings excessively which would result in the starvation of the cutting (29). While there may have been some leaching, the new system, with overhead mist, provided much more success in the propagation of cuttings. The intermittent chamber mist apparently solved the fungal growth on the cuttings troublesome in Experiment No. 1 and Early Trials.

C. Chamber Heating and Wax Capping Experiment

This experiment was designed to further refine the air-mist chamber system to give better rooting success for *F.* x *intermedia* cuttings. Although there were problems with this experiment, it was nevertheless successful in its objective. The problems of this experiment were that (a) more than one type of cutting was used in this experiment and (b) there should have been 5 replications of the immersion heat treatments. The second replication was discarded because of faulty electrical wiring to one of the immersion heaters. Although it is known that differences exist between semi-hardwood and hardwood cuttings; eg., hardwood cuttings are more easily leached than semi-hardwood cuttings (28), this problem apparently was not critical to the experiment as there was no significant differences between replications by analysis of variance. As for the second problem, there was a loss of a more "weighted" conclusion, but the experiment still distinguished significant differences in heat treatments and heat treatments x time interactions.

The fact that the cuttings had an optimum number of cuttings rooted with the 25^{o}C and 30^{o}C heats would classify the optimum *F.* x *intermedia* cuttings as higher than the normal optimum rooting range (20°C to 25°C) designated by Kramer (16). Leaching and the nature of the rooting medium e.g. oxygen supply and the amount of water condensation on the basal portions of the cuttings (the higher heat treatments usually had more condensation of water during cool evenings) might have been factors.

What is the nature of an air-mist rooting medium? Most probably in a constant chamber mist the cuttings are covered entirely with an oxygen saturated thin layer of water but they are not directly exposed to atmosphere oxygen. In an intermittent chamber mist, air gaps in the water film exist which would allow for atmospheric oxygen exposure to plant tissues in addition to the high oxygen diffusion from the water layer. Thus oxygen is more available to the cuttings.

Since there have been no oxygen measurements with air-mist chambers, it cannot be concluded that the partial pressure for oxygen are within the "noncritical range" for the rooting of cuttings, although it is unlikely that the "upper critical" partial pressure for rooting could be reached for any plant in a mist chamber. The highest partial pressure possible for an intermittent chamber mist system would be atmospheric partial pressure. No reports have been found which indicate that the atmospheric partial pressure of oxygen reduced root growth of plants compared to lower oxygen partial pressures (1, 7, 8, 26, 27). Recall that Mahlstede and Haber (19) insinuated that root initiation requires more oxygen than root growth.

The results of the immersion heat treatments effect on the rooting rate demonstrate one of the principal advantages of this system. That advantage is the opportunity for daily observation of the rooting process. This experiment showed that some heat treatments (thermostat settings 30°C and 25°C) promote quicker rooting than others. In future experiments it might be possible to study why certain cuttings root faster than others under the same treatment. For example, suppose the experimenter wishes to investigate certain factors right at the time of first root emergence, such as the nature of the cutting or its mass, the thickness of its corky layer or what plant hormones, rooting co-factors, or what inhibitors may be present in the cutting. The advantage of this system of air-mist chambers over other types of propagation systems with conventional media is clear: it allows observation of the rooting process without disturbing the roots. It's advantage over other media which allow observation (without injury) such as water, stirred water, or water bubbled with air is that the air-mist chamber system has a higher oxygen availability. Pearse (22) found that the air-mist chamber system was indeed superior to the other water culture media mentioned above with apple trees.

Another use of the system would be for root growth studies of whole plants, e.g. what is the day to day effect on the root growth of certain "above ground" treatments of pruning, misting, temperature treatments or chemical sprays.

Observations of the anatomy of root growth may be made as well. For instance, this author observed at the lower heat treatments, the cuttings had a few white fleshy roots with little branching whereas at higher heat treatments, the roots were slender or fibrous, highly branched, with some brown color added to the white of the roots. This confirmed many of the same observations made by Nielsen (21).

D. IBA and Nozzle Experiment

It was felt that after previous experiments had refined the system to consistently get a fair percentage of rooted cuttings, it was now possible to again try an experiment to determine whether different, root-stimulating, growth regulator treatments could be made in a single growth chamber, without a deleterious "dilution effect" between treatments. As reported, however, this experiment failed to show this because there was no response to the IBA treatment.

The reason for a lack of root stimulating response by the _F. x intermedia_ cuttings could be one of three factors. The cuttings with (a) would not respond to that concentration of IBA or (b) there was not a sufficient drying period after dipping the cuttings to insure uptake of the growth regulator into the cutting without some leaching in the chamber mist or (c) there was a combination of factors.

The fact that the cuttings showed no chamber nozzle misting effect (according to row location) indicates that all cuttings apparently received effectively equal chamber mist treatments, under the situation of no IBA effect. This would not predict whether there might be a chamber nozzle misting effect concommittant with an IBA effect.

It is believed that the rooting performance was lower than expected in this final experiment due to the time of the year the trials were conducted (October 24 to December 23 and January 23 to April 2). Apparently, the amount of light available for photosynthesis in leaves of cuttings in air-mist chambers is critical when there is a large amount of leaching due to the misting. Longer days with higher light intensity would increase the photosynthesis rates and thus replenish photosynthates depleted from leaching. This concept is similar to the concept by Hess and Snyder (13) of carbohydrate economy. Probably there should have been less misting with the air-mist chambers, both overhead mist and chamber mist, during the winter months to compensate for the lower photosynthate production. With less light intensity, there would be less dehydration of the cuttings.

VII. SUMMARY AND CONCLUSIONS

It has been shown that the air-mist chamber system for rooting cuttings is potentially a workable research tool, although more adjustments must be made to improve its rooting performance.

It is probable that the observations in this study would apply to air-mist chamber systems with many plant taxa (not only F. x intermedia). This is because in this project, there were many of the same observations as that of others - Rains (23, 24) and Briggs (2, 3, 4).

The following is a summary of the findings of this study:

1. The health of the cuttings, was better with an intermittent, rather than a constant, chamber mist.

2. Cuttings in air-mist chambers with intermittent overhead mist had a better rooting performance than those in open air without overhead mist.

3. An immersion heat thermostat setting of 25°C had the best rooting performance of F. x intermedia cuttings in air-mist chambers.

4. After 54 days, the no. of cuttings rooted with 25°C immersion heat was optimal; whereas, at 40 days both 25°C and 30°C immersion heat settings were superior to the other heat treatments. Therefore, with shorter propagation periods, it is permissible to have higher immersion heat.

5. Wax capping the distal cut surface of the cuttings has no effect of rooting performance on the no. of cuttings with leaves.

6. There was no root stimulation effect from a 5 sec dip of IBA (500 ppm) on *F.* x *intermedia* cuttings in air-mist chambers with intermittent overhead mist.

7. In a situation where there was no IBA effect on the rooting performance of *F.* x *intermedia* cuttings, there was no nozzle effect (or chamber location effect) on the rooting performance of the cuttings.

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AIR AS A ROOTING MEDIUM: AN EXPERIMENTAL AIR-MIST CHAMBER SYSTEM FOR ROOTING CUTTINGS USING *FORSYTHIA X INTERMEDIA*

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Research was conducted to develop and refine an air-mist chamber system for the asexual propagation of Forsythia x intermedia from cuttings, and to establish the ability of the system to function for experimental purposes. Both dormant hardwood and leafy, semi-hardwood cuttings were used in this study.

A system built from various components, the air-mist chambers were designed for experimental control and ease of operation. The design was further adjusted by experimental results. The system had 8 air-mist chambers; each with a recycling mist system consisting of 2 atomizers immersed in a reservoir pool. The amount of mist applied to the cuttings was controlled through the regulation of the air pressure entering the chamber atomizers from the manifold and through the regulation of the duration of the intermittent mist schedule (for both the chamber mist and the overhead mist). Each chamber had its own thermostatically controlled immersion heat system. The entire chamber was lined with 4 MIL polyethylene film. This includes the cover, which consisted of two layers of paper with one layer of polyethylene film. The cuttings were stuck through the cover into the chamber so that two nodes were inside the chamber. The remainder of the cuttings were exposed to greenhouse, propagation-bench conditions.

Although the rooting performance was unsatisfactory in this study, it was demonstrated that air-mist chambers are potentially good tools for the study of asexual propagation of cuttings.

In this study, on the basis of the rooting performance of the F. x intermedia cuttings (no. of cuttings rooted and the no. of root-tips per cutting), the following was found: Air-mist chambers with intermittent overhead mist were superior to those exposed to open air with no overhead mist. An immersion heat setting of 25°C was best for air-mist chambers.

On the basis of the number of cuttings rooted, the 25°C setting was best for the long propagation period of 54 days whereas both 25°C and 30°C were superior for the shorter period of 40 days.

There were two visual observations made in this study. One was that the health of the cuttings was superior with intermittent chamber mist as compared with constant chamber mist. The other was that morphological differences in root formation exist with different chamber heats. With low chamber heat the cuttings had few fleshy roots with little branching, whereas at higher treatments cuttings had many roots that were fibrous, highly branched, with some brown color added to the white.

Wax caps on the distal cut surface of the cuttings had a 5 sec basal dip to the cuttings with 500 ppm IBA had no effect on their rooting performance.

It remains to be found whether it is possible to give different auxin treatments to the cuttings in the same air-mist chamber.