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

by

MELVIN ENNIS DODD, JR.

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The University of Kansas, 1971

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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, physio-lobical 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

Overhead Mist

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°C to 22°C, whereas, highly lignified cuttings rooted better at 27°C to 30°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°C to 25°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 $4^{\circ}C$ 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.

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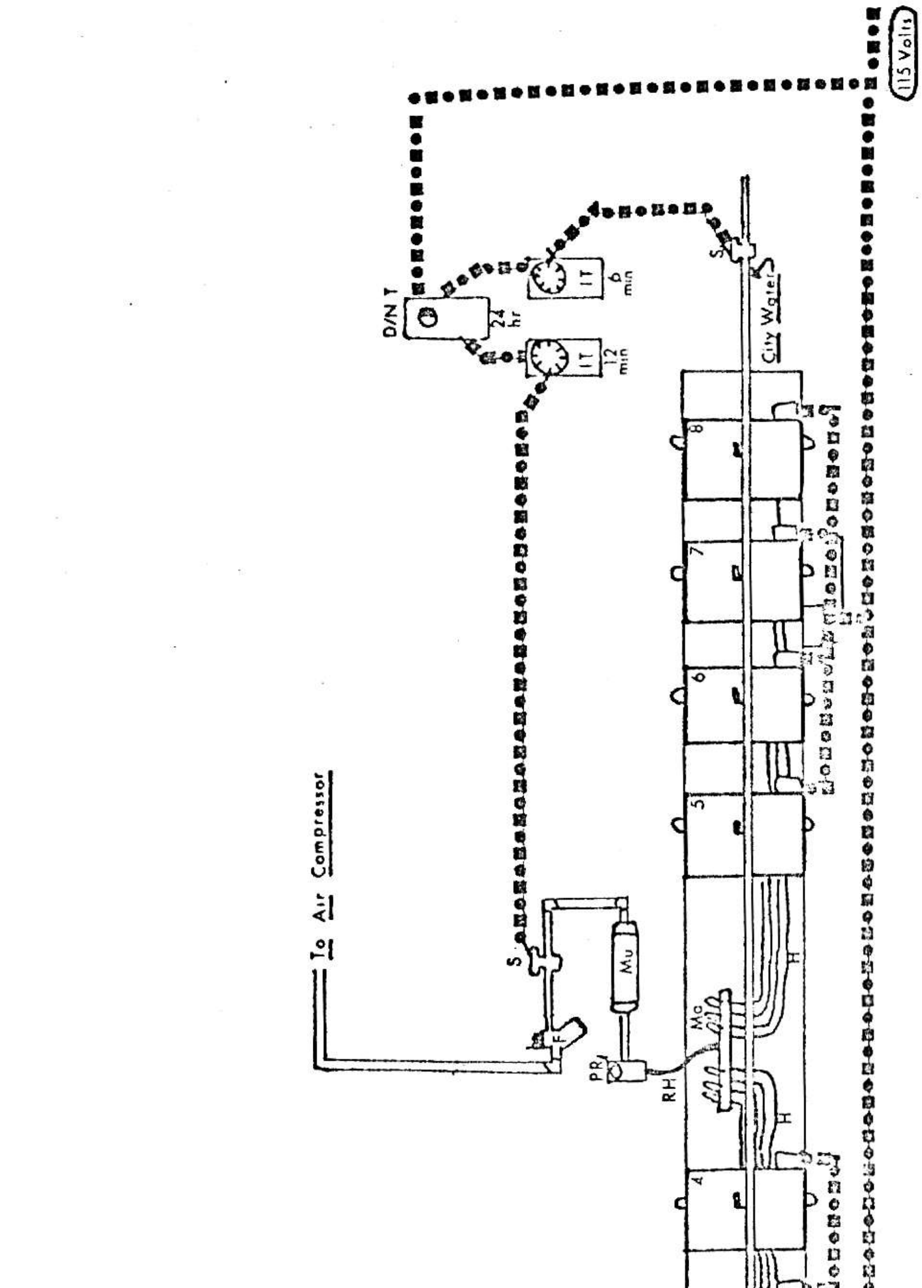

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a fresh cut was made on the proximal end of the cuttings. The buds from the lower two nodes were removed from the cuttings to facilitate a clean, water-tight chamber.

Treatments

(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.

Data Collected

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.

Statistical Analysis

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

Comments

The purpose of this experiment was to determine the best