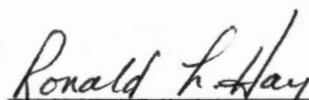


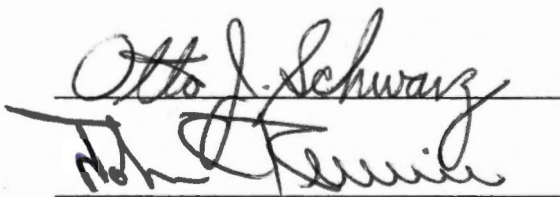
To the Graduate Council:

I am submitting herewith a thesis written by Tanya Annette Minton entitled "Growth Characteristics and Soluble Carbohydrate Concentrations of Containerized Eastern White Pine Seedlings." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Forestry.



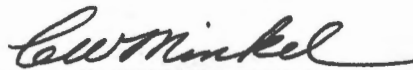
Ronald L. Hay, Major Professor

We have read this thesis
and recommend its acceptance:





Accepted for the Council:



The Graduate School

GROWTH CHARACTERISTICS AND SOLUBLE CARBOHYDRATE
CONCENTRATIONS OF CONTAINERIZED EASTERN
WHITE PINE SEEDLINGS

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Tanya Annette Minton

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ABSTRACT

This study investigated relationships between growth characteristics and soluble carbohydrate concentrations of 10-month old eastern white pine (Pinus strobus L.) seedlings grown in four different greenhouse environments: (1) augmented photoperiod and ambient carbon dioxide, (2) augmented photoperiod and supplemental carbon dioxide, (3) continuous photoperiod and ambient carbon dioxide, and (4) continuous photoperiod and supplemental carbon dioxide. Within each of these environments, four different media were used in two rootrainer sizes with each rootrainer tray further divided such that 50 percent received foliar mist fertilization. Root collar diameters, heights, root fresh weights, root dry weights, top fresh weights, top dry weights, leaf dry weights, stem dry weights, total fresh weights, total dry weights, and root/shoot ratio were recorded. Total soluble sugars, ketose sugars, and reducing sugars were determined for leaf, stem, and root tissues and for the total seedlings.

Continuous photoperiod increased all growth characteristics, except height, by 20-25 percent. Ketose and reducing sugar concentrations in stem tissue were least in seedlings grown in continuous light. Enriched carbon dioxide environments produced larger seedlings (20-25 percent) than those grown under ambient environments and also resulted in significantly higher total sugar and ketose sugar concentrations within root tissue. Pine bark-vermiculite and Redi-Earth media were superior for promoting diameter, height, and

biomass growth. Soluble sugar concentrations were approximately 25 percent greater within seedlings grown in Redi-Earth medium.

Seedlings in Hillson rootrainers had larger diameter, height, and biomass (5 percent, 25 percent, and 25 percent, respectively). Foliar mist fertilization showed very little effect on seedling growth and no effect on sugar concentrations.

Leaf soluble carbohydrate concentrations were positively correlated with height, root collar diameter, and root growth; whereas, root soluble sugar concentrations were positively correlated with top growth and root collar diameter growth. Soluble carbohydrate concentrations within stem tissue were negatively correlated with biomass growth.

Greenhouse environments can be manipulated to produce desired seedling growth characteristics and seedling soluble carbohydrate concentrations by extending the photoperiod, increasing the carbon dioxide atmospheric concentration, and using Redi-Earth growing medium in Hillson rootrainers.

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CHAPTER I

INTRODUCTION

Germination through seedling establishment is a critical period in successful reforestation (Daniel et al., 1979). Productive forests can be maintained only to the extent that new trees survive and flourish. Outplanting of seedlings has become the most common method of regeneration in most areas. Seedlings which are cultured under favorable growth conditions before outplanting have greater potential to cope with natural environmental stresses (Stein, 1975).

Greenhouse culture of containerized seedlings is becoming increasingly important in reforestation because the greenhouse environment can easily be manipulated to favor vigorous seedling growth. Accelerated growth is desirable for species like eastern white pine (Pinus strobus L.) which usually do not form secondary leaves during the first growing season; therefore, outplanting is usually done with at least 2-0 bareroot seedlings. Using containerized culture methods in a greenhouse, eastern white pine seedlings have been successfully outplanted at ten months of age (Hay and Keegan, 1982; Keegan, 1981). Another advantage of container grown seedlings is that their root systems remain intact and undisturbed, thereby facilitating survival after outplanting (Tinus, 1974).

Most research that has been done with containerized seedling culture has concerned seedling growth characteristics plus growing media and container types. Seedling height, stem diameter, and biomass at time of outplanting have been considered to be an

indication of future field survival and growth performance. Barnett (1980) found that stem dry weights of loblolly pine (Pinus taeda L.) seedlings were positively correlated with growth in the field, while seedling height and diameter exhibited little relationship. Seedling height in another study was the best indicator of outplanting success, i.e. the taller the seedling, the greater the likelihood that it will become established (McGilvray and Barnett, 1982). In contrast to both of these studies, Hay and Keegan (1982) analyzed field survival of eastern white pine containerized seedlings grown under varying greenhouse conditions and found that the seedlings with the greatest survival in the field had the smallest height and root collar diameter at the time of outplanting.

Since seedling size has not shown a consistent relationship to outplanting success, investigations on physiological conditions within seedlings warrant consideration. Height, diameter, and/or biomass of a seedling at outplanting age may neither totally nor currently reflect nutrient or carbohydrate status within the seedling (Kramer and Kozlowski, 1979; Tinus, 1974).

Carbohydrates are the most important constituents of woody plants and comprise 75 percent of their dry weight (Kramer and Kozlowski, 1979). They are used in growth, construction of cell walls, and protection from harsh environmental conditions. Carbohydrates are also the precursors to most other organic compounds within the plant (Kramer and Kozlowski, 1979; Mooney, 1972). When supplied with an abundance of carbohydrates, seedlings can readily

carry out metabolic processes producing a quality seedling for outplanting.

Conditions that promote carbohydrate synthesis and translocation can be provided during greenhouse culture by supplying supplemental light, carbon dioxide, and fertilizer. For example, terminal bud set can be inhibited by lengthening the photoperiod, thereby providing a longer period of vegetative growth and accumulation of carbohydrate reserves within the seedlings (Downs, 1958). Carbon dioxide concentrations exceeding that normally found in the atmosphere have been shown, in many instances, to enhance photosynthesis and thereby increase carbohydrate production (Holley, 1970; Larson, 1974; Tinus, 1972). A plentiful supply of essential minerals plus a quality growing medium result in seedlings with the necessary materials to carry out photosynthetic and translocation processes (Salisbury and Ross, 1978). An understanding of various greenhouse environmental effects on seedling carbohydrate concentrations and seedling size variables is necessary before an optimum culture technique for growing containerized seedlings can be developed.

Objective

The major objective of this study was to determine the best greenhouse regime for producing quality eastern white pine seedlings, based upon growth characteristics and soluble carbohydrate concentrations.

CHAPTER II

LITERATURE REVIEW

Although many containerized seedling studies have suggested a need to understand the physiological condition of the seedlings (Hanover, 1976; Hay and Keegan, 1982; McGilvray and Barnett, 1982; Tinus, 1974), very little research has directly addressed this study area. Soluble carbohydrate concentrations are of primary interest because sugars and their derivatives are directly used in seedling growth and respiration, and they are stored as reserves. Carbohydrates are the direct products of photosynthesis; therefore, environmental factors that influence photosynthesis ultimately affect carbohydrate synthesis (Kramer and Kozlowski, 1979).

Carbohydrates

Woody plants contain numerous sugars including monosaccharides, oligosaccharides, and polysaccharides. However, glucose, fructose, and sucrose were the most common sugars found to any great extent by Shallenberger and Birch (1975) and Shiroya et al. (1962). The aldose sugar, glucose, is the most widely distributed sugar in nature, existing in its free state as well as in combination with other sugars and with other glucose molecules--most commonly forming starch and cellulose. Fructose, another abundant monosaccharide, together with glucose, comprises sucrose which is a major photosynthetic product, existing in high concentration in plant cells and representing over 95 percent of the dry weight of

translocated material. Sucrose and starch, a non-soluble carbohydrate reserve, are the principle products stored within plants (Kramer and Kozlowski, 1979; Miller, 1973; Shallenberger and Birch, 1975).

Carbohydrates can be classified either as reducing sugars or as nonreducing sugars. Reducing sugars have either an exposed aldehyde group (glucose) or an exposed ketone group (fructose) that will reduce Cu^{+2} to Cu^{+} in Benedict's or Fehling's solution. Nonreducing sugars lack this reaction because the aldehyde or ketone group has been combined with another molecule to form a polysaccharide or has been changed to nonreducing alcohol structure (Salisbury and Ross, 1978).

Carbohydrate synthesis is accomplished through the photosynthetic reduction of carbon dioxide into sugar phosphates. These simple sugars form all carbohydrate and carbohydrate derivatives used in plant metabolism (Mooney, 1972). A large portion of carbohydrates are used in plant growth and respiration, providing the plant with energy and forming its support system (Miller, 1973). Other carbohydrates are diverted to symbionts such as mycorrhizae and nitrogen-fixing bacteria, and others are lost by leaching and volatilization from leaves and by exudation from roots (Kramer and Kozlowski, 1979). Carbohydrate transformations such as phosphorylation and conversion to sugar derivatives are continually occurring within the plant in order to provide necessary compounds for use in growth, respiration, and synthesis of other organic compounds (Kramer and Kozlowski, 1979).

During seed germination, carbohydrate stores are converted to sucrose which is transferred to the developing shoot and root (Goodwin and Mercer, 1972). With further seedling development, the site of carbohydrate synthesis shifted from the cotyledons to the primary needles to the secondary needles (Kozłowski, 1979). The amount of carbohydrates and the way they were used, however, differed greatly among species and under different environmental conditions, (Kramer and Kozłowski, 1979). During favorable conditions, excess carbohydrates are stored for later utilization, e.g. in hardwoods after the winter dormant period. Likewise, seedlings that are lifted from nursery or greenhouse conditions and outplanted have limited immediate ability to produce adequate photosynthate. Therefore, they must rely on carbohydrate reserves until establishment (Ronco, 1973; Tinus, 1974).

Light

Light is the principle environmental factor influencing photosynthesis and it is the most powerful single force regulating plant growth (Kramer and Kozłowski, 1979; Leopold and Kriedemann, 1975). Two major processes involving light stimulated reactions are chlorophyll synthesis and ATP (adenosine triphosphate) and NADPH (reduced nicotinamide adenine dinucleotide phosphate) production. Light also acts as a regulator of several chloroplast enzymes including ribulose biphosphate carboxylase, 3-phosphoglyceraldehyde dehydrogenase, fructose-1,6-bisphosphate phosphatase, and ribulose-5-phosphate kinase (Salisbury and Ross, 1978). Carbohydrate

production is minimized in darkness because of ATP and NADPH deficiencies and enzyme inactivity, along with closure of the stomata.

Light affects tree growth by its quality, intensity and duration (Daniel et al., 1979). Plant response indicated that red light (650-700nm) was the most efficient for photosynthesis and blue light (430-470nm) was more effective in promoting stomatal opening (Leopold and Kriedemann, 1975). Along with enhancing photosynthesis, McCree (1971) found that red light favored carbohydrate accumulation, accounting for 68 percent of leaf dry matter compared with 42 percent in blue light. Red light converts the chromoprotein, phytochrome, to its active form which is responsible for many photomorphogenic responses and prevention of dormancy (Pratt, 1979; Tinus and McDonald, 1979). Under natural conditions, light quality is usually not limiting; however, it can be very important when growing plants with artificial illumination. A few incandescent lamps added to banks of flourescent tubes provides a satisfactory combination of the red light needed for photosynthesis and the far-red wavelengths (730nm) necessary to prevent excessive stem elongation (Kramer and Kozlowski, 1979).

Photosynthetic response to different light intensities varied greatly among species (Kozlowski and Keller, 1966). Generally, however, the higher the light intensity, the higher the rate of net photosynthesis until saturation is reached (Daniel et al., 1979). A difference in saturation points between selected species of Pinus and some angiosperms was noted by Kozlowski and Keller (1966). With increasing light intensity up to full sunlight, Pinus continued to

photosynthesize at increasing rates; whereas, angiosperms achieved maximum photosynthesis at much lower light intensities. Bright light induced stomatal opening, thereby allowing greater uptake of carbon dioxide (Leopold and Kriedemann, 1975). Although light intensity affected photosynthetic rates, Shiroya et al. (1962) found no difference in the ethanol-soluble sugar concentration of eastern white pine needles exposed to light intensities of either 2,690 lux or 26,900 lux. Wardlaw (1966) and Shiroya et al. (1966) both reported greater translocation to the roots with increasing light intensities.

Daylength has a major impact on photosynthate production and vegetative growth. Short photoperiods induce dormancy and long photoperiods prolong growth (Downs, 1958; Larson, 1974). For example, eastern white pine and Japanese black pine (Pinus thunbergii Parl.) grown under sixteen or twenty hour photoperiods showed continuous shoot elongation; whereas, photoperiods of eight or twelve hours caused a terminal resting bud to form (Struve and Blazich, 1980). Hay and Keegan (1982) found an increase in biomass of both tops and roots of eastern white pine seedlings grown in extended photoperiods. Other investigations (Wheeler, 1979; Fowler 1961) also emphasized the positive effect that extended photoperiod had on seedling root growth. Some pines responded to extended photoperiods by expanding in a series of flushes instead of a single growth flush (Kramer and Kozlowski, 1979).

The accumulated time that a plant was exposed to light above the compensation point largely determined the amount of carbohydrates that were produced (Daniel et al., 1979). Seedlings grown under long

days had higher rates of photosynthesis than those grown under short days. In addition to rate increases, a lengthened photoperiod allowed a longer period in which photosynthetic processes occurred, thereby increasing potential growth (Downs, 1958). Photoperiodism also regulates other plant processes including seed germination, leaf abscission, and frost hardiness (Kramer and Kozłowski, 1979). A continuous photoperiod, however, cannot eliminate seasonal rhythms completely. For example, production of new seedling primordia required temporary rest, i.e. apical growth cessation, regardless of the length of the photoperiod (Larson, 1974).

Carbon Dioxide

To maximize the utilization of solar energy, relative levels of water, carbon dioxide, and mineral fertility must not be limiting (Brun and Cooper, 1967). Atmospheric concentration of carbon dioxide (300ppm) may not be sufficient to maximize photosynthetic processes (Funch et al., 1970; Leopold and Kriedemann, 1975; Salisbury and Ross, 1978; Tinus, 1972). This phenomenon is especially true for greenhouse crops during the winter months when the greenhouse must be closed for heating purposes (Salisbury and Ross, 1978). In a limited air volume, carbon dioxide is depleted through photosynthesis faster than it can be replenished by plant respiration.

Carbon dioxide is necessary for the dark phase of photosynthesis during which it acts with the enzyme ribulose biphosphate carboxylase to form 3-phosphoglyceric acid from ribulose biphosphate, which ultimately leads to carbohydrate formation

(Salisbury and Ross, 1978). High concentrations of carbon dioxide decreased photorespiration (the process by which ribulose biphosphate carboxylase combines with oxygen in the presence of light, resulting in carbon dioxide evolution) by increasing the ratio of carbon dioxide to oxygen (Pallas, 1979).

Enriched carbon dioxide atmospheres of 1000ppm resulted in twice as much height and stem diameter growth of eastern white pine seedlings over that which occurred at 300ppm (Funsch et al., 1970). Ponderosa pine (Pinus ponderosa Laws.) seedlings grown at high carbon dioxide concentrations were 48 percent heavier by dry weight than those grown at ambient carbon dioxide (Tinus, 1972). Likewise, Hay and Keegan (1982) observed that the best developed seedlings in the greenhouse were those grown in atmospheres containing supplemental carbon dioxide supplied by a propane fueled burner. Kramer (1981) reported a larger leaf area on some plants subjected to high levels of carbon dioxide.

Increased weight when plants were grown in enriched carbon dioxide was attributed to an increase in photosynthesis (Frydriych, 1976; Kramer, 1958; Pallas, 1979). By raising the carbon dioxide concentration from 350ppm to 4000ppm with corresponding increases in light intensity, Larson (1974) observed a fourfold increase in photosynthetic rate. Helms (1976) found higher rates of photosynthesis after increasing the carbon dioxide concentration where it had previously been limiting. Increased plant growth responses were related to the increased carbohydrate supply resulting from higher photosynthetic activity (Holley, 1970).

Some disagreement has arisen over the long range benefits of carbon dioxide enrichment. Kramer (1981) discovered that exposure to high concentrations of carbon dioxide yielded only a temporary increase in the rate of photosynthesis. Over an extended period of time, the rate often fell below that of plants grown at ambient levels. Similar trends with cucumber were described by Aoki and Yabuki (1977).

Decreases in photosynthetic rates following prolonged exposure to high concentrations of carbon dioxide was thought to be due to increased accumulation of starch in the leaves or changes in the leaf tissue itself such as increased thickness of palisade and spongy parenchyma cells, stomatal closure, or reduced number of stomata (Aoki and Yabuki, 1977; Kramer, 1981). Starch normally accumulates in the chloroplast during periods when photosynthate production exceeds translocation (Salisbury and Ross, 1978). Mauney and co-workers (1979) found high starch concentrations in several agricultural crops which were grown in enriched carbon dioxide atmospheres. However, no significant changes occurred in soluble sugar concentration. Tinus and McDonald (1979) recommended carbon dioxide concentrations of 800-1500ppm in greenhouse production, thereby providing a good balance between the advantages and disadvantages of supplemental carbon dioxide effects on plant growth.

Fertilizer

Fertilizer regimes in containerized seedling culture have been designed to promote rapid growth, ultimately yielding superior quality

seedlings (Brix and van den Driessche, 1974). In addition to producing seedlings of maximum size, fertilizer programs must benefit the physiological condition of the plants (Kozlowski, 1979).

Three macronutrients essential for plant growth are nitrogen, phosphorus, and potassium. Deficiencies of these elements inhibit seedling growth in a variety of ways. For example, low nitrogen levels created leaf chlorosis due to a lack of chlorophyll synthesis. Phosphorus deficiencies impeded photosynthesis by disturbing the energy transfer of ADP (adenosine diphosphate) to ATP, causing stunting and sparsely developed root systems. Potassium deficiencies hindered translocation of carbohydrates and nitrogen metabolism (Kramer and Kozlowski, 1979). Studies by Natr (1975) showed that deficiencies of any of these elements inhibited carbon dioxide uptake and increased stomatal and mesophyll resistance to carbon dioxide transfer. Of the macronutrients, nitrogen had the greatest impact on photosynthesis and was correlated to carbohydrate production and consumption (Kozlowski and Keller, 1966). Under high nitrogen conditions, more chlorophyll was produced and, therefore, more available carbohydrates were synthesized (Meyer and Splittstoesser, 1971).

Research has shown that medium levels of fertilizer were most effective at enhancing seedling growth. Lister et al. (1968) observed superior growth, mycorrhizae development, photosynthesis and translocation in three year old eastern white pines grown at intermediate levels of nitrogen and phosphorus. Trujillo (1976)

showed that best growth occurred in those ponderosa pine seedlings grown in 25 percent strength Hoagland's nutrient solution.

Nutritional deficiencies should not limit growth of containerized seedlings because fertilizers and growing media that will not restrict root growth and nutrient uptake can easily be managed. Although slow release fertilizers such as Osmocote are often used in containerized seedling culture, Tinus and McDonald (1979) did not recommend them because the amount and kind of nutrient solution needed varies with seedling growth and development stage, and it is difficult to match pellet dissolution with plant growth needs. Soluble fertilizer applications incorporated with regular waterings were often disadvantageous because they sometimes promoted top growth at the expense of root growth (Hay, 1981) and inhibited mycorrhizae formation (Marx and Barnett, 1974; Pritchett, 1979). These problems can be minimized by foliar mist fertilization which alleviates deficiencies yet minimizes dripping of nutrients into the rooting medium (Dixon et al., 1979).

Mycorrhizae

Mycorrhizae inoculation is extremely important in containerized seedling culture. Advantages to the plant from mycorrhizal infection are increased root surface area, increased tolerance to drought and temperature extremes, provision of a barrier (fungal mantel) that protects the plant from pathogenic infection, and provision of growth-regulating substances such as minerals, vitamins, and hormones (Langlois and Fortin, 1981; Marx and Barnett, 1974;

Ruehle, 1981). Because of these improved conditions, containerized seedlings grown with mycorrhizae have shown an increase in size and dry weight over those grown without mycorrhizae, as was the case in Walker's et al. (1983) study of sweet birch (Betula lenta L.) inoculated with Pisolithus tinctorius (Pers.). Fungal infection increased the rate of net photosynthesis of eight month old containerized ponderosa pine and lodgepole pine (Pinus contorta Loud.) seedlings (Kidd and Reid, 1979).

Excessive fertilization inhibits mycorrhizal development. Marx and co-workers (1977) found negative correlations between high levels of nitrogen and phosphorus and susceptibility of loblolly pine short roots to Pisolithus tinctorius infection. Similarly, Langlois and Fortin (1981) noted that excessive fertilization decreased infection of jack pine roots (Pinus banksiana Lamb.). Seedlings grown at high fertility levels rapidly use photosynthate for growth, thereby minimizing root carbohydrate concentrations necessary to maintain the mycobionts (Marx et al., 1982; Pritchett, 1979). Mycorrhizae parasitize roots for soluble sugars and will not infect those which do not contain a surplus of soluble carbohydrates (Hacskeylo, 1973). In their study of mycorrhizae and loblolly pine, Marx et al. (1977) found increased root susceptibility to mycorrhizal infection as the concentration of soluble sugars, especially sucrose, increased. Hacskeylo (1973) concluded that glucose was the sugar that mycorrhizae absorbed most rapidly. Handley and Sanders (1962) reported that the concentration of soluble reducing sugars within the roots resulted from mycorrhizal infection, rather than caused the infection. Fungi

absorb carbohydrates from root tissues, thereby increasing the carbohydrate flow gradient from leaves to roots (Hacskeylo, 1973).

Growing Medium

Growing medium supplies mechanical support, heat, air, water, and nutrients that influence plant growth (Brady, 1974). Therefore, the growing medium for containerized seedlings must be carefully selected. Two major media types have frequently been used in containerized seedling production. The most common is a peat moss and vermiculite (50:50) mixture. It has uniform composition, light weight, high water holding capacity, high cation exchange capacity (CEC), and is slightly acidic, which is favorable for conifer growth (Tinus and McDonald, 1979). Sometimes perlite is used instead of vermiculite because it does not compress as easily; however, the root plugs are more difficult to extract from the containers (Tinus and McDonald, 1979). Experiments by Phipps (1974a) using growing media showed the largest seedlings were produced in peat-vermiculite or peat-vermiculite-arcellite mixtures. Increases in peat were positively correlated with average stem length. Incorporating sand into the medium inhibited growth due to its low CEC (Bosley, 1967; Phipps, 1974b). Peat also encouraged lateral root formation, producing a more favorable root/shoot relationship (Edgren, 1973).

Because peat is relatively expensive, milled pine bark is often preferred as a growing medium. Like peat-vermiculite mixtures, bark is light in weight and has a high CEC (Gartner et al., 1973). In addition, bark provides a well-drained, well-aerated medium that is

difficult to overwater (Bosley, 1967). Bark and sawdust mixtures produced white fir (Abies concolor Lindl.) of equal quality to peat-vermiculite mixtures (Montano et al. 1977). Forsythia (Forsythia intermedia Zabel.) and pfitzer juniper (Juniperus chinensis var. Pfitzeriana Spaeth.) plants also exhibited best growth in media containing bark fragments (Klett et al., 1972). Problems which may arise from using milled pine bark as a growing medium are plant nitrogen deficiencies and high soil acidity. However, both of these problems can be overcome by addition of nitrogen fertilizers and limestone (Lunt and Clark, 1959; Tinus and McDonald, 1979).

Container Size

For economic reasons, small containers at close spacings are usually utilized in tree seedling production (Van Eerden, 1981). This type of arrangement, however, confines seedlings to an environment which is characterized by narrow limits of reserves and tolerances, and may lead to small seedlings. For instance, water and nutrients are rapidly depleted from the small quantity of medium in the containers (Van Eerden, 1981). Endean and Carlson (1975) found increased root dry weight, shoot dry weight, total dry weight, and shoot length of loblolly pine seedlings in large containers. Likewise, southern red oak (Quercus falcata Michx.) grew taller in larger containers and also had a greater leaf surface area (Elam et al., 1981). Barnett and McGilvray (1981) recommended container diameter for growing southern pines to be no less than 2.5cm to 3.2cm.

More critical than container volumes is the effect that roottrainer sizes have on seedling density (Barnett and McGilvray, 1981). Experiments on Douglas-fir (Pseudotsuga menziesii Franco) grown at densities ranging from 270 to 1080 plants per square meter showed that those seedlings grown at the greatest density were 50 percent taller and 13 percent smaller in root collar diameter than seedlings grown at the least density (Timmis and Tanaka, 1976). van den Driesshe (1981) also found heavier Douglas-fir and Sitka spruce (Picea sitchensis Carr.) seedlings at low stocking densities.

CHAPTER III

MATERIALS AND METHODS

Eastern white pine seeds, Morgan and Scott County source, obtained from the Tennessee Division of Forestry at Pinson were used in this study. The first phase was conducted in a glass-paneled greenhouse, where the seedlings were grown for ten months under varying experimental conditions. Following the greenhouse phase, some seedlings were harvested, measured, and prepared for laboratory investigations of soluble carbohydrate concentrations. Other seedlings were outplanted in a separate study.

Greenhouse Study

Experimental design. A randomized complete block design containing a split plot at the lowest level was selected for the study. The greenhouse was divided into four locations for implementation of the following:

1. augmented photoperiod and ambient carbon dioxide,
2. augmented photoperiod and supplemental carbon dioxide,
3. continuous photoperiod and ambient carbon dioxide,
4. continuous photoperiod and supplemental carbon dioxide.

Within each of these locations, seedlings were grown in two different sizes of roottrainers and in four different kinds of media.

Furthermore, each tray of roottrainers was split, 1/2 receiving weekly foliar fertilization and 1/2 receiving no additional fertilizer.

Eight replications of each combination were used, making a total of 512 experimental units.

Experimental establishment. The eastern white pine seeds were stratified for 60 plus days; seeding began in July. Spencer-Lemaire book rootrainers were chosen for their corrugated walls which promote downward root growth and for their convenience during seedling production. The Hillson rootrainer contains four cavities per book with cavity dimensions of 3.81cm X 3.81cm X 12.70cm deep and eight books per tray. Healthy eastern white pine seedlings had been produced in the Hillson rootrainers previously (Keegan, 1981). The Fives rootrainer is much smaller, containing five cavities per book with cavity dimensions of 2.54cm X 2.54cm X 10.80cm deep. Each tray holds 14 books. Cavity volumes within this range (170cc and 62cc respectively) have usually been satisfactory for production of a quality seedling stock (Van Eerden, 1981).

Four different growing media were used in this study (Table 1). Ruehle et al. (1981) successfully used a pine bark medium in their research on containerized seedlings. A peat moss vermiculite medium has been shown to promote good seedling growth (Tinus and McDonald, 1979).

Each of the two kinds of rootrainers was filled with one of the various media and located at random in each location of the greenhouse. Prior to seeding, the seeds were surface sterilized in a 5 percent sodium hypochlorite solution for 20 seconds and thoroughly rinsed in running tap water. The seeds were then sowed in the

Table 1
Media Selected for Growing Eastern White Pine Seedlings

Medium	Source	Composition	Additional Treatments
pine bark-vermiculite	Fern Nursery Washington, Ga	pine bark (80%) fine vermiculite (20%)	methyl bromide fumigated, 1.00 cm screened, addition of Tween 20, Osmocote 18-6-12 plus phosphorus
Redi-Earth	W. R. Grace	peat moss, vermiculite	Osmocote 18-6-12 plus phosphorus
Metro-Mix 220	W. R. Grace	peat moss, vermiculite, perlite	Osmocote 18-6-12 plus phosphorus
Metro-Mix 500	W. R. Grace	peat moss, vermiculite, sand, pine bark	Osmocote 18-6-12 plus phosphorus

roottrainers with two or three seeds per cavity. In addition, several seeds were planted in seed flats to use for transplanting into empty cavities. Within two weeks of germination, the seedlings were thinned and transplanted such that each cavity contained one seedling.

Application of treatments. Two 40 watt Gro-Lux fluorescent lights placed 40cm above each bench of seedlings were used to extend the photoperiod. The Gro-Lux lamps were desirable because they emit light mainly in the red portion of the visible spectrum which is the peak wavelength for photosynthesis (Salisbury and Ross, 1978). During the first four months of growth, the photoperiod was lengthened to 18 hours for those seedlings receiving the extra light treatments. In mid-November, the photoperiod was further extended to 24 hours and remained as such until the following April when a modified hardening off period began. The other group of seedlings, that were not treated with extended photoperiod, were subjected to an augmented photoperiod to prevent terminal bud set. The augmented photoperiod was accomplished by interrupting the dark period using 75 watt incandescent light bulbs that were placed above the benches. The lights initially came on for one hour at 5:30p.m. and for 15 minutes every hour thereafter until 4:00a.m.

Supplemental carbon dioxide was supplied to the experimental seedlings by a propane fueled burner which enriched the atmospheric concentration to approximately 1000ppm. The propane burner was activated in mid-November and initially generated carbon dioxide from 6:00p.m. to 10:00p.m. every evening. After two months, the propane

burner was synchronized with the incandescent lights timer for convenience. When night ventilation of the greenhouse was necessary in April, carbon dioxide enrichment was discontinued.

The fertilizer regimes used in this study included slow release fertilization, phosphate fertilization, and foliar mist fertilization. Osmocote 18-6-12 was incorporated into all growing media at a rate of 5kg/m³. This N-P-K formulation promotes good seedling growth plus good mycorrhizal infection. However, without mycobiont inoculation, triple super phosphate was added at Osmocote's recommended rate of 0.89g/l. High phosphate concentration aids newly planted crops with limited root structure and restricted ability to absorb phosphorus from the soil (Salisbury and Ross, 1978). Beginning in mid-November and extending to April, foliar mist of 25-10-10 (concentration of 3.96ml/l) was applied weekly to one-half of each tray. The fertilized roottrainer books were randomly selected.

A few chemicals were used on the seedlings to combat disease and insect problems. The most serious problem encountered was excessive damping-off. Seedlings were sprayed with a mixture of Dexon and Terrachlor as per label instructions. After a few months, some seedlings became infested with mealy bugs for which malathion was used.

Harvesting and measurements. At the conclusion of the greenhouse phase, a randomly selected sample of seedlings from each experimental unit was harvested and measured. Seedlings were removed from the roottrainers and the media was gently shaken from the roots.

Root collar diameter, height, root fresh weight, and top fresh weight were measured before drying the seedlings in an oven at 70 C. Root dry weight, stem dry weight, and leaf dry weight were then recorded. From these data, total fresh weights, total dry weights, and root/shoot ratios were calculated.

Data analysis. Data were analyzed with The University of Tennessee's IBM 370/3031 computer using SAS 82 (SAS Institute Inc., 1982) General Linear Models (GLM) and t-Test procedures. The GLM procedure generated analyses of variance and Duncan's multiple range tests for analyzing photoperiod, carbon dioxide, growing media and roottrainer size effects on the measured growth variables. Significant interactions between the treatment variables were also determined. The t-Test procedure was used for analysis of fertilizer effects and fertilizer interactions with other greenhouse treatments.

Laboratory Study

Experimental establishment. Three replications from among the eight original replications were randomly selected for soluble carbohydrate analyses. Within these three replications, further selections were made for Fives roottrainers and pine bark-vermiculite or Redi-Earth media. Large roottrainers are impractical from a production standpoint because of space limitations (Tinus and McDonald, 1979; Van Eerden, 1981). Therefore, restricting the soluble carbohydrate analyses to seedlings grown in the smaller cavities seemed appropriate for this study. Survival varied greatly among the

four different media but it was especially poor in Metro-Mix 220 and Metro-Mix 500, so seedlings grown in these two media were also excluded from soluble carbohydrate analyses.

The same randomly selected seedlings from each experimental unit that were measured at the conclusion of the greenhouse phase were used for carbohydrate analyses. The dry leaf, root, and stem tissues from each group of seedlings were compiled according to tissue type, ground to powder in a Wiley mill, and stored in airtight plastic vials at -20 C until carbohydrate analyses.

Analytical techniques were based upon those of Hay (1968) and Rowe (1980) (Figure I). Numerous preliminary tests were made to determine the best procedures and materials for this particular study. The available prepared tissue limited the sample size to approximately 100mg, but smaller samples were used when necessary.

To derive the final extract volume of 15-20ml needed for the individual sugar tests, the tissue samples were mixed with 15ml aliquots of 80 percent ethanol and placed in a 85 C water bath for 10 minutes. This treatment was repeated once to insure that all soluble carbohydrates were extracted. An 80 percent ethanol blank was included with every group of extractions to provide a control.

Following centrifugation of the extracts, the supernatants required further mixing and filtering with activated charcoal to remove coloring substances. A charcoal aliquot of 1.0g added to the supernatants and stirred periodically over a 20 minute time period was sufficient for the root and stem tissue samples. For the leaf tissue

SOLUBLE CARBOHYDRATE ANALYSES

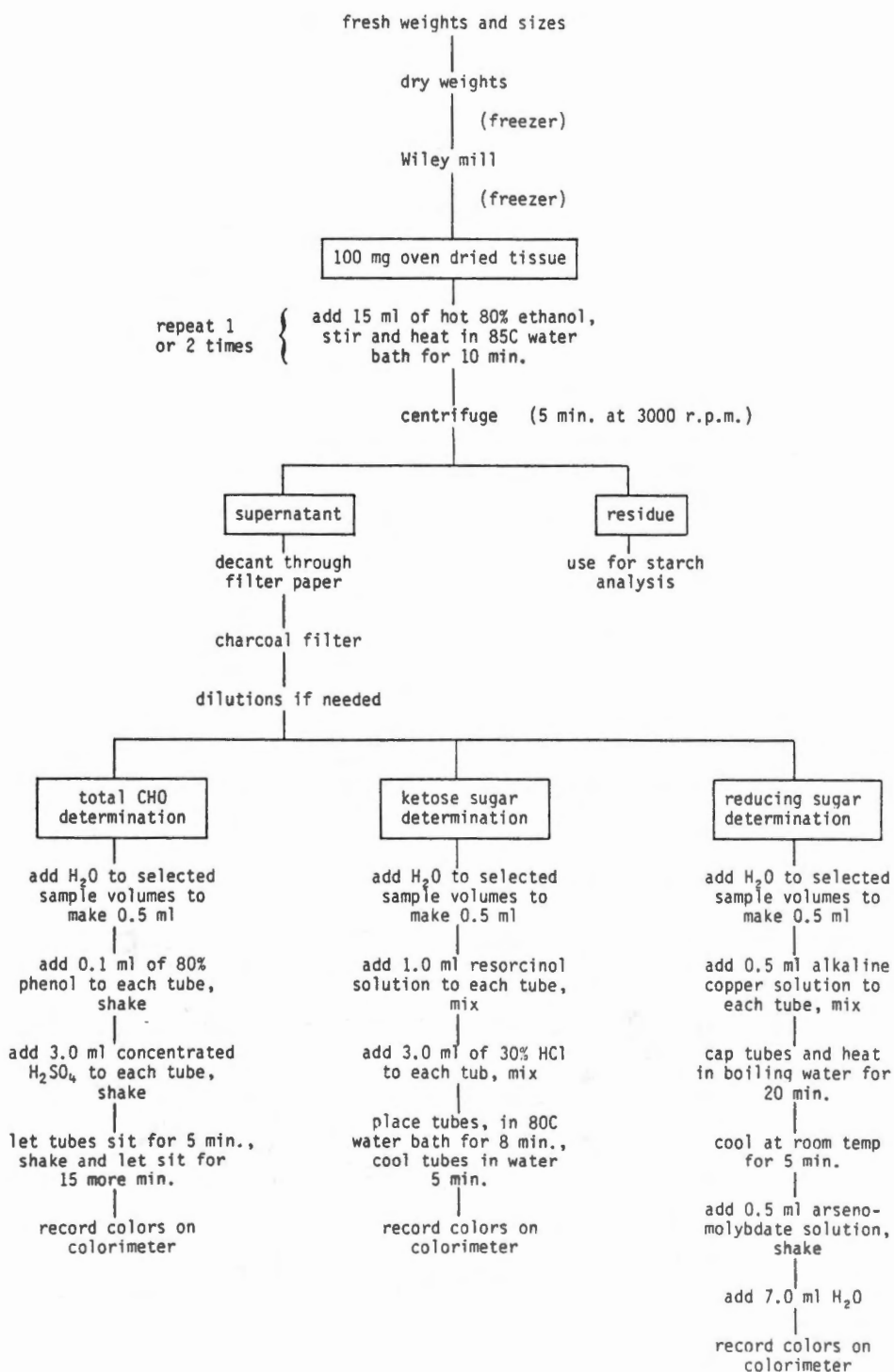


FIGURE I

PROCEDURE FOR SOLUBLE CARBOHYDRATE DETERMINATIONS
(HAY, 1968)

samples, however, the charcoal filtering procedure was repeated to obtain a colorless solution. After the charcoal treatment, the supernatants were filtered through Whatman No. 1 paper into graduated cylinders for final volume determinations. Samples were then stored in test tubes at 5 C.

Prior to extraction of the experimental tissue samples, the entire carbohydrate analysis procedure was carried out on a known quantity of sucrose to test efficiency of the procedure. The results showed no interference with charcoal filtering, indicating that solute-clearing with charcoal would have little effect on soluble carbohydrates recovered from test tissues.

Application of treatments. The sample extracts were used in three separate tests to determine total soluble sugar concentrations, ketose sugar concentrations, and reducing sugar concentrations within leaf, root, and stem tissues (Figure 1). Light absorbancy for all three tests was performed in a Bausch and Lomb Spectronic 20 colorimeter.

Total soluble carbohydrate determinations were accomplished by the modified version of Nalewaja and Smith's (1963) 80 percent phenol and sulfuric acid method described by Hay (1968). A wavelength of 490nm was used for absorbancy readings and a sucrose standard provided the control. Ketose sugar tests followed the procedure outlined by Roe (1934) using alcoholic resorcinol, 30 percent hydrochloric acid, and heating. Resultant colors, after cooling, were read at 490nm against a fructose standard. Reducing sugar analyses were conducted

using Nelson's (1944) procedure which involved an alkaline copper reagent, a boiling water bath, and an arsenomolybdate color reagent. The final dark blue color was read at 630nm. Glucose was the standard.

For each sugar determination, the extracted samples were tested at two different concentrations in triplicate. Sample volumes of 0.5ml and 0.3ml (brought to a final volume of 0.5ml with 80 percent ethanol) proved to be satisfactory quantities, requiring no further dilutions and easily readable with the colorimeter for every test.

Data analysis. The SAS 82 GLM procedure was also used for data analysis of the laboratory phase of this study. This procedure provided analyses of variance and Duncan's multiple range tests to determine significant relationships between greenhouse treatments and total sugar, ketose sugar, and reducing sugar concentrations within the seedlings. Analyses were made on individual leaf, root, and stem tissues and root/shoot concentrations, as well as on total seedling concentrations. Treatment interaction effects were also examined. Further analyses of the greenhouse data and laboratory data were accomplished by the SAS Correlation procedure. The measured growth variables were correlated with the sugar concentrations within the leaf, root, and stem tissues and analyzed for significance and percentage of variation.

CHAPTER IV

RESULTS

Greenhouse Study

Following ten months of seedling growth in the greenhouse, a sample of seedlings was selected and root collar diameter, height, root fresh weight, root dry weight, top fresh weight, top dry weight, total fresh weight, and total dry weight were measured to determine relationships between varying greenhouse treatments and seedling sizes. Statistically significant seedling growth differences were attributed to the individual effects of (1) photoperiod, (2) carbon dioxide concentration, (3) growing medium, and (4) roottrainer size (Table 2). Growing medium/roottrainer size interaction and photoperiod/roottrainer size interaction were significant at the .05 level for several of the growth characteristics (Table 2). The fertilizer treatments did not appear to have any effect on seedling size.

Photoperiod. Photoperiod had a statistically significant effect on root collar diameter, root fresh weight, root dry weight, top fresh weight, top dry weight, leaf dry weight, stem dry weight, total fresh weight, total dry weight, and root/shoot ratio (Table 2). Seedlings grown in continuous photoperiod were approximately 25 percent larger in all biomass measurements and 20 percent larger in root collar diameter (1.70mm compared to 1.49mm) than those seedlings grown under augmented

Table 2
Mean Square Values for Growth Variables of 10-Month Old Eastern White Pine
Seedlings Grown under Various Greenhouse Treatments

Treatment	Root Collar Diameter	Height	Root Biomass		Top Biomass				Seedling Biomass		Root/Shoot Dry Weight
			Root Fresh Weight	Root Dry Weight	Top Fresh Weight	Top Dry Weight	Leaf Dry Weight	Stem Dry Weight	Total Fresh Weight	Total Dry Weight	
Photoperiod	2.88*	1.69	2.71*	0.23*	11.92*	1.31*	0.88*	0.04*	26.02*	2.62*	0.10*
CO ₂	3.16*	6.73*	1.27*	0.10*	7.09*	0.68*	0.54*	0.01	14.36*	1.29*	0.05*
Photoperiod x CO ₂	1.47*	0.95	1.05*	0.02	0.34	0.12	0.06	0.01	2.58	0.25	0.03
Rep (Photoperiod x CO ₂)	0.25	0.92	0.23	0.01	0.59	0.06	0.04	0.00	1.48	0.12	0.01
Medium	0.55*	1.95*	0.52*	0.05*	2.65*	0.27*	0.19*	0.01*	5.49*	0.52*	0.05*
Medium x Photoperiod	0.07	0.45	0.11	0.00	0.21	0.02	0.01	0.00	0.55	0.02	0.02*
Medium x CO ₂	0.06	0.23	0.07	0.00	0.48*	0.03	0.02	0.01*	0.91	0.05	0.01
Roottrainer Size	0.76*	0.82	2.87*	0.09*	6.97*	0.72*	0.55*	0.01*	18.79*	1.33	0.00
Photoperiod x Roottrainer Size	0.24	0.41	0.63*	0.02	1.35*	0.14*	0.11*	0.01*	3.83*	0.28*	0.01
CO ₂ x Roottrainer Size	0.05	0.04	0.03	0.00	0.06	0.00	0.00	0.00	0.16	0.00	0.00
Medium x Roottrainer Size	0.44*	1.54*	0.25*	0.02*	1.26*	0.13*	0.09*	0.01*	2.60*	0.24*	0.00
Error	0.07	0.28	0.05	0.00	0.17	0.02	0.01	0.00	0.38	0.03	0.00

*Indicates significance at the .05 level according to analyses of variance.

photoperiod (Table 3). Similar differences were noted in the root/shoot ratio, i.e. 33 percent in continuous photoperiod and 29 percent in augmented photoperiod. Although continuous photoperiod produced slightly taller seedlings, no statistically significant height differences occurred between seedlings grown under 24-hour photoperiod as opposed to augmented photoperiod (Table 3).

Carbon dioxide. Carbon dioxide concentration had a significant influence on all growth variables except stem dry weight in the analysis of variance (Table 2). Root fresh weight and root/shoot ratio, however, were not significant in the Duncan's multiple range test. All other variables apparently increased due to supplemental carbon dioxide (Table 4). The root collar diameter and biomass increases were again approximately 20 percent and 25 percent respectively. For instance, total dry weight increased from 0.55g to 0.68g. Height differences were not as pronounced, showing only a 5 percent increase (5.76cm compared to 6.07cm).

Photoperiod and carbon dioxide. Root collar diameter and root fresh weight were the only growth variables which showed a statistically significant difference due to treatment interaction between photoperiod and carbon dioxide (Table 2). Under augmented photoperiod and ambient carbon dioxide, seedling root collar diameter and root fresh weight grew the least (Table 5). However, with either a continuous photoperiod or an enriched carbon dioxide atmosphere or both, root collar diameter and root fresh weight were both larger (Table 5).

Table 3
Morphological Growth Means of 10-Month Old Eastern White Pine Seedlings
Grown under Continuous and Augmented Photoperiod

Photoperiod	Root Collar Diameter	Height	Root Biomass		Top Biomass				Seedling Biomass		Root/Shoot Dry Weight
			Root Fresh Weight	Root Dry Weight	Top Fresh Weight	Top Dry Weight	Leaf Dry Weight	Stem Dry Weight	Total Fresh Weight	Total Dry Weight	
	<u>mm</u>	<u>cm</u>	<u>g</u>	<u>g</u>	<u>g</u>	<u>g</u>	<u>g</u>	<u>g</u>	<u>g</u>	<u>g</u>	<u>%</u>
Continuous	1.70 ^{a*}	5.98 ^a	0.68 ^a	0.18 ^a	1.64 ^a	0.53 ^a	0.44 ^a	0.09 ^a	2.32 ^a	0.71 ^a	33.03 ^a
Augmented	1.49 ^b	5.86 ^a	0.48 ^b	0.12 ^b	1.22 ^b	0.39 ^b	0.33 ^b	0.06 ^b	1.70 ^b	0.51 ^b	29.21 ^b

*Lower case letters indicate significance at the .05 level according to Duncan's multiple range test.

Table 4
Morphological Growth Means of 10-Month Old Eastern White Pine Seedlings
Grown under Supplemental and Ambient Carbon Dioxide Environments

CO ₂ Level	Root Collar Diameter	Height	Root Biomass		Top Biomass				Seedling Biomass		Root/Shoot Dry Weight
			Root Fresh Weight	Root Dry Weight	Top Fresh Weight	Top Dry Weight	Leaf Dry Weight	Stem Dry Weight	Total Fresh Weight	Total Dry Weight	
	<u>mm</u>	<u>cm</u>	<u>g</u>	<u>g</u>	<u>g</u>	<u>g</u>	<u>g</u>	<u>g</u>	<u>g</u>	<u>g</u>	<u>%</u>
Supplemental	1.70 ^{a*}	6.07 ^a	0.65 ^a	0.17 ^a	1.59 ^a	0.51 ^a	0.43 ^a	0.08 ^a	2.23 ^a	0.68 ^a	32.39 ^a
Ambient	1.49 ^b	5.76 ^b	0.52 ^a	0.13 ^b	1.28 ^b	0.42 ^b	0.35 ^b	0.07 ^a	1.80 ^b	0.55 ^b	29.95 ^a

*Lower case letters indicate significance at the .05 level according to Duncan's multiple range test.

Table 5

Effects of Photoperiod and Carbon Dioxide on Mean Growth
of 10-Month Old Eastern White Pine Seedlings

Interaction		Root Collar Diameter	Root Fresh Weight
<u>Photoperiod</u> ^A	<u>CO₂</u> ^B	<u>mm</u>	<u>g</u>
+	+	1.73 ^{a*}	0.69 ^a
+	0	1.66 ^a	0.67 ^a
0	+	1.67 ^a	0.61 ^a
0	0	1.27 ^b	0.33 ^b

^ASymbols represent photoperiod treatment.

++--continuous photoperiod

0--augmented photoperiod

^BSymbols represent CO₂ treatment.

++--supplemental CO₂

0--ambient CO₂

*Lower case letters indicate significance at the .05 level
according to Duncan's multiple range test.

Growing medium. Growing medium was extremely important in influencing growth, affecting root collar diameter, height, root fresh weight, root dry weight, top fresh weight, top dry weight, leaf dry weight, stem dry weight, total fresh weight, total dry weight, and root/shoot ratio (Table 2). Of the four growing media selected for this study, pine bark-vermiculite medium and Redi-Earth medium produced the largest seedlings (Table 6). Root collar diameter was the only variable which was smaller in pine bark-vermiculite medium than in Redi-Earth medium--1.52mm and 1.68mm respectively. Metro-Mix 220 medium was favorable for root collar diameter, height, and root fresh weight. However, growth in Metro-Mix 500 medium was the poorest for all growth variables measured (Table 6).

Growing medium and photoperiod. The interaction of growing medium and photoperiod only had a statistically significant influence on seedling root/shoot ratio (Table 2). Seedlings had the highest root/shoot ratio when grown in pine bark-vermiculite medium, regardless of the photoperiod (Table 7). For all other media, however, continuous photoperiod apparently increased the root/shoot ratio about 5 percent over that of seedlings grown under augmented photoperiod.

Growing medium and carbon dioxide. Seedling top dry weight and stem dry weight were the only growth variables which were significantly affected at the .05 level by the growing medium/carbon dioxide interaction (Table 2). Metro-Mix 220 or Metro-Mix 500 media interacting with ambient carbon dioxide resulted in the lowest top

Table 6
Morphological Growth Means of 10-Month Old Eastern White Pine Seedlings
Grown in Four Different Media

Growing Medium	Root Collar Diameter	Height	Root Biomass		Top Biomass				Seedling Biomass		Root/Shoot Dry Weight
			Root Fresh Weight	Root Dry Weight	Top Fresh Weight	Top Dry Weight	Leaf Dry Weight	Stem Dry Weight	Total Fresh Weight	Total Dry Weight	
	mm	cm	g	g	g	g	g	g	g	g	%
Pine bark-vermiculite	1.52 ^{b*}	6.02 ^a	0.66 ^a	0.18 ^a	1.62 ^a	0.52 ^a	0.44 ^a	0.08 ^{ab}	2.28 ^a	0.70 ^a	35.00 ^a
Redi-Earth	1.68 ^a	6.08 ^a	0.65 ^a	0.16 ^a	1.56 ^a	0.51 ^b	0.43 ^a	0.09 ^a	2.21 ^a	0.67 ^a	23.83 ^b
Metro-Mix 220	1.69 ^a	5.89 ^{ab}	0.57 ^a	0.14 ^b	1.38 ^b	0.44 ^b	0.37 ^b	0.08 ^b	2.00 ^b	0.59 ^b	29.77 ^b
Metro-Mix 500	1.51 ^b	5.71 ^b	0.46 ^b	0.12 ^c	1.18 ^c	0.38 ^c	0.32 ^c	0.06 ^c	1.64 ^c	0.50 ^c	31.26 ^b

*Lower case letters indicate significance at the .05 level according to Duncan's multiple range test.

Table 7

Effects of Growing Medium and Photoperiod on Mean Growth
of 10-Month Old Eastern White Pine Seedlings

Interaction		Root/Shoot Dry Weight
<u>Growing Medium</u> ^A	<u>Photoperiod</u> ^B	%
1	+	34.50 ^{ab*}
1	0	35.54 ^a
2	+	31.16 ^{bc}
2	0	26.34 ^d
3	+	32.82 ^{ab}
3	0	26.15 ^d
4	+	33.64 ^{ab}
4	0	28.44 ^{cd}

^ANumbers represent type of growing media.

1--pine bark-vermiculite

2--Redi-Earth

3--Metro-Mix 220

4--Metro-Mix 500

^BSymbols represent photoperiod treatment.

+-continuous photoperiod

0--augmented photoperiod

*Lower case letters indicate significance at the .05 level
according to Duncan's multiple range test.

fresh weights and stem dry weights (Table 8). In contrast, pine bark-vermiculite and Redi-Earth media showed superior top fresh weights and stem dry weights in either enriched carbon dioxide atmospheres or ambient carbon dioxide atmospheres.

Growing medium and fertilizer. Based on the statistical tests, mist foliar fertilization mainly influenced seedling growth through its interaction with growing medium. Seedlings grown in pine bark-vermiculite medium and Metro-Mix 500 medium were taller when fertilizer was added; whereas, seedlings grown in the other two media were taller without foliar mist fertilization (Table 9). This same trend also occurred for top dry weight and leaf dry weight, with the difference being most pronounced in Metro-Mix 500 medium. Except for seedlings grown in Redi-Earth medium, root/shoot ratios were greater when no foliar fertilizer was applied. The growing medium/fertilizer interaction apparently did not significantly affect the root collar diameter or other biomass variables.

Roottrainer size. Roottrainer size had a statistically significant effect on root collar diameter, root fresh weight, root dry weight, top fresh weight, top dry weight, stem dry weight, total fresh weight, and total dry weight (Table 2). Seedlings grown in the Hillson roottrainers were larger (about 25 percent) in biomass than seedlings grown in the Fives roottrainer (Table 10). However, root collar diameter was only 5 percent larger.

Table 8

Effects of Growing Medium and Carbon Dioxide on Mean Growth
of 10-Month Old Eastern White Pine Seedlings

Interaction		Top Fresh Weight	Stem Dry Weight
Growing Medium ^A	CO ₂ ^B	g	g
1	+	1.70 ^{a*}	0.08 ^{abc}
1	0	1.54 ^{ab}	0.08 ^{abc}
2	+	1.67 ^{ab}	0.09 ^a
2	0	1.46 ^{ab}	0.08 ^{ab}
3	+	1.54 ^{ab}	0.08 ^{abc}
3	0	1.19 ^c	0.07 ^c
4	+	1.44 ^b	0.07 ^{bc}
4	0	0.90 ^d	0.05 ^d

^ANumbers represent type of growing media.
 1--pine bark-vermiculite
 2--Redi-Earth
 3--Metro-Mix 220
 4--Metro-Mix 500

^BSymbols represent CO₂ treatment.
 +--supplemental CO₂
 0--ambient CO₂

*Lower case letters indicate significance at the .05 level
according to Duncan's multiple range test.

Table 9

Mean Growth Differences of 10-Month Old Eastern White Pine Seedlings
Grown without Foliar Fertilization Minus Those Grown
with Foliar Fertilization, in Four Different Media

Growing Medium	Height	Top Dry Weight	Leaf Dry Weight	Root/Shoot Dry Weight
	<u>cm</u>	<u>g</u>	<u>g</u>	<u>%</u>
pine bark-vermiculite	-0.189 ^{b*}	-0.005 ^a	-0.002 ^{ab}	0.018 ^a
Redi-Earth	0.131 ^a	0.004 ^a	0.006 ^a	-0.029 ^b
Metro-Mix 220	0.021 ^{ab}	0.004 ^a	0.003 ^a	0.022 ^a
Metro-Mix 500	-0.245 ^b	-0.061 ^b	-0.051 ^b	0.034 ^a

*Lower case letters indicate significance at this .05 level according to Duncan's multiple range test.

Table 10
Morphological Growth Means of 10-Month Old Eastern White Pine Seedlings
Grown in Hillson and Fives Roottrainers

Roottrainer size	Root Collar Diameter	Height	Root Biomass		Top Biomass				Seedling Biomass		Root/Shoot Dry Weight
			Root Fresh Weight	Root Dry Weight	Top Fresh Weight	Top Dry Weight	Leaf Dry Weight	Stem Dry Weight	Total Fresh Weight	Total Dry Weight	
	<u>mm</u>	<u>cm</u>	<u>g</u>	<u>g</u>	<u>g</u>	<u>g</u>	<u>g</u>	<u>g</u>	<u>g</u>	<u>g</u>	<u>%</u>
Hillson	1.66 ^{a*}	5.99 ^a	0.70 ^a	0.17 ^a	1.62 ^a	0.52 ^a	0.44 ^a	0.08 ^a	2.32 ^a	0.69 ^a	31.26 ^a
Fives	1.54 ^b	5.86 ^a	0.47 ^b	0.13 ^b	1.26 ^b	0.41 ^b	0.34 ^b	0.07 ^b	1.74 ^b	0.54 ^b	31.20 ^a

*Lower case letters indicate significance at the .05 level according to Duncan's multiple range test.

Photoperiod and rootrainer size. Root fresh weight, root dry weight, top fresh weight, top dry weight, leaf dry weight, stem dry weight, total fresh weight, and total dry weight measurements suggested a significant interaction between photoperiod and rootrainer size (Table 2). All biomass measurements were greatest under conditions of 24-hour photoperiod and large rootrainers (total dry weight of 0.82g) (Table 11). Small rootrainers interacting with continuous photoperiod or large rootrainers interacting with augmented photoperiod resulted in intermediate seedling weights (total dry weights of 0.60g and 0.55g respectively); whereas, small rootrainers and augmented photoperiod were least favorable for biomass growth (total dry weight of 0.47g) (Table 11).

Growing medium and rootrainer size. Analyses of variance shows root collar diameters, heights, and seedling biomass to be significantly influenced by the growing medium/rootrainer size interaction at the .05 level (Table 2). Pine bark-vermiculite medium in the Hillson rootrainers yielded the largest seedlings; whereas, those in the Fives rootrainers were some of the smallest (Table 12). Redi-Earth and Metro-Mix 220 media in Hillson rootrainers resulted in increased biomass growth over that produced in the Fives rootrainers. However, the differences were not as great as those in pine bark-vermiculite medium. Root collar diameter and height were greater in the larger rootrainers containing pine bark-vermiculite medium (Table 12). Least growth occurred in Metro-Mix 500 medium regardless of rootrainer size.

Table 11
Effects of Photoperiod and Roottrainer Size on Mean Growth
of 10-Month Old Eastern White Pine Seedlings

Interaction		Root Biomass		Top Biomass				Seedling Biomass	
		Root Fresh Weight	Root Dry Weight	Top Fresh Weight	Top Dry Weight	Leaf Dry Weight	Stem Dry Weight	Total Fresh Weight	Total Dry Weight
Photoperiod ^A	Roottrainer Size ^B	g	g	g	g	g	g	g	g
+	1	0.84 ^{a*}	0.21 ^a	1.88 ^a	0.61 ^a	0.51 ^a	0.10 ^a	2.72 ^a	0.82 ^a
+	2	0.52 ^b	0.15 ^b	1.40 ^b	0.45 ^b	0.37 ^b	0.08 ^b	1.91 ^b	0.60 ^b
0	1	0.54 ^b	0.13 ^{bc}	1.32 ^b	0.42 ^b	0.36 ^b	0.07 ^{bc}	1.86 ^b	0.55 ^b
0	2	0.42 ^c	0.12 ^c	1.12 ^c	0.36 ^c	0.30 ^c	0.06 ^c	1.54 ^c	0.47 ^c

^ANumbers represent photoperiod treatment.
+--continuous photoperiod
0--augmented photoperiod

^BNumbers represent roottrainer size.
1--Hillson
2--Fives

*Lower case letters indicate significance at the .05 level according to Duncan's multiple range test.

Table 12

Effects of Growing Medium and Roottrainer Size on Mean Growth
of 10-Month Old Eastern White Pine Seedlings

Interaction		Root Collar Diameter	Height	Root Biomass		Top Biomass				Seedling Biomass	
				Root Fresh Weight	Root Dry Weight	Top Fresh Weight	Top Dry Weight	Leaf Dry Weight	Stem Dry Weight	Total Fresh Weight	Total Dry Weight
Growing Medium ^A	Roottrainer Size ^B	mm	cm	g	g	g	g	g	g	g	g
1	1	0.70 ^{a*}	6.31 ^a	0.86 ^a	0.23 ^a	2.00 ^a	0.64 ^a	0.54 ^a	0.10 ^a	2.85 ^a	0.86 ^a
1	2	1.33 ^c	5.73 ^c	0.46 ^d	0.14 ^c	1.24 ^{de}	0.40 ^{de}	0.33 ^{de}	0.06 ^d	1.70 ^{de}	0.53 ^{de}
2	1	1.72 ^a	6.02 ^{ab}	0.76 ^{ab}	0.17 ^b	1.73 ^b	0.57 ^b	0.47 ^b	0.09 ^{ab}	2.49 ^b	0.74 ^b
2	2	1.64 ^{ab}	6.14 ^{ab}	0.54 ^{cd}	0.14 ^c	1.40 ^{cd}	0.46 ^{cd}	0.38 ^{cd}	0.08 ^{abc}	1.94 ^{cd}	0.60 ^{cd}
3	1	1.70 ^a	5.90 ^{bc}	0.66 ^{bc}	0.15 ^b	1.47 ^c	0.48 ^c	0.40 ^c	0.08 ^{bc}	2.13 ^c	0.63 ^c
3	2	1.68 ^a	5.87 ^{bc}	0.48 ^d	0.13 ^c	1.28 ^{cde}	0.41 ^{cde}	0.34 ^{cde}	0.07 ^{cd}	1.77 ^{de}	0.54 ^{cde}
4	1	1.51 ^b	5.70 ^c	0.51 ^d	0.13 ^c	1.24 ^{de}	0.40 ^{de}	0.34 ^{cde}	0.06 ^d	1.75 ^{de}	0.53 ^{de}
4	2	1.52 ^b	5.72 ^c	0.41 ^d	0.11 ^c	1.13 ^e	0.37 ^e	0.31 ^e	0.06 ^d	1.54 ^e	0.48 ^e

^ANumbers represent types of growing media.
1--pine bark-vermiculite
2--Redi-Earth
3--Metro-Mix 220
4--Metro-Mix 500

^BNumbers represent roottrainer size.
1--Hillson
2--Fives

*Lower case letters indicate significance at the .05 level according to Duncan's multiple range test.

Laboratory Study

Following seedling growth measurements, leaf, root, and stem tissues were analyzed for soluble carbohydrate concentrations. Growing medium was the only treatment effect that appeared to consistently influence sugar concentrations in all three tissues as well as in the entire seedling (Table 13). The only interaction that apparently impacted carbohydrate levels was photoperiod and carbon dioxide. Foliar mist fertilization had no statistically significant effect on seedling soluble sugar content either alone or in combination with any other treatment.

Photoperiod. All soluble carbohydrate concentrations in the leaf, stem, and root tissues and in the total seedling were slightly higher in seedlings grown under augmented photoperiod (Table 14). However, only greater ketose sugar and reducing sugar concentrations within stem tissue and reducing sugar concentration in the total seedling were statistically significant. The root/shoot ratios of sugar concentrations were a little higher for seedlings grown under continuous photoperiod, but the differences were not statistically significant.

Carbon dioxide. Total sugars, ketose sugars, and reducing sugars were all slightly greater in seedlings exposed to supplemental carbon dioxide; however, very few of the differences were statistically significant (Table 15). Concentrations of total sugars (41.83 μ g carbohydrates/mg tissue and 34.92 μ g carbohydrates/mg tissue) and ketose sugars (19.83 μ g carbohydrates/mg tissue and 17.17 μ g

Table 13

Mean Square Values for Soluble Carbohydrate Concentrations within 10-Month Old Eastern White Pine Seedlings
Grown under Various Greenhouse Treatments

Treatment	Leaf			Stem			Root			Total Seedling			Root/Shoot		
	Total	Ketose	Reducing	Total	Ketose	Reducing	Total	Ketose	Reducing	Total	Ketose	Reducing	Total	Ketose	Reducing
Photoperiod	11.79	5.35	11.15	211.02	102.27*	107.33*	15.95	2.49	9.16	46.72	15.35	23.22*	0.04	0.35	0.20
CO ₂	25.02	2.48	7.56	226.23	25.69	0.12	545.21*	61.52*	2.58	220.78	23.69	1.91	0.05	0.06	0.24
Photoperiod x CO ₂	347.64*	133.85*	105.45*	369.85	128.09*	64.52*	121.38	0.84	0.83	93.13	57.13*	18.60*	5.58*	5.06*	1.42*
Rep (Photoperiod x CO ₂)	48.20	8.63	17.88	85.90	12.71	2.57	48.73	7.84	6.36	43.36	7.77	2.63	0.47	0.33	0.04
Medium	181.32*	53.02*	50.77	1271.77*	338.79*	102.99*	1188.57*	182.76*	87.76*	746.84*	156.72*	45.92*	0.10	0.42	0.16*
Medium x Photoperiod	15.39	12.08	2.05	18.60	10.61	32.01*	42.23	28.82	82.98*	27.12	16.55	15.93	0.18	0.08	0.01
Medium x CO ₂	146.83*	23.27*	15.35	174.59	40.96	7.12	87.51	7.64	0.55	98.84	14.36	0.73	0.28	0.40	0.00
Photoperiod x CO ₂ x Medium	60.79	14.25	1.44	1.44	0.55	—	83.04	2.93	4.42	53.10	5.16	—	0.01	0.01	—
Rep x Medium (Photoperiod x CO ₂)	16.27	2.37	12.77	68.54	10.69	1.25	146.14	24.91	6.65	47.36	7.67	1.07	0.49	0.42	0.01
Fertilizer	13.83	5.93	2.76	2.53	0.05	0.92	12.53	3.29	1.33	7.17	1.04	0.51	0.05	0.00	0.00
Photoperiod x Fertilizer	52.31	13.74	3.91	25.68	0.74	0.18	147.57	7.86	0.68	57.24	3.65	0.07	0.09	0.03	0.04
CO ₂ x Fertilizer	35.39	7.36	3.87	57.56	4.15	0.05	64.41	1.00	0.34	41.77	1.57	0.62	0.01	0.00	0.06
Medium x Fertilizer	10.95	8.23	16.94	0.44	0.07	1.85	29.62	8.09	1.23	0.25	0.01	2.32	0.09	0.29	0.04
Error	14.18	5.85	4.28	17.50	5.66	2.98	69.75	11.28	2.81	16.95	2.52	1.05	0.12	0.11	0.04

*Indicates significance at the .05 level according to analyses of variance.

Table 14
Mean Soluble Carbohydrate Concentrations (μg carbohydrates/mg plant tissue)
within 10-Month Old Eastern White Pine Seedlings Grown
under Continuous and Augmented Photoperiod

Soluble Carbohydrates Photoperiod	Leaf	Stem	Root	Total Seedling	Root/shoot
	$\mu\text{g}/\text{mg}$	$\mu\text{g}/\text{mg}$	$\mu\text{g}/\text{mg}$	$\mu\text{g}/\text{mg}$	
Total Sugars					
Continuous	16.19 ^{a*}	27.25 ^a	37.99 ^a	27.07 ^a	1.86 ^a
Augmented	17.36 ^a	31.71 ^a	38.62 ^a	28.68 ^a	1.82 ^a
Ketose Sugars					
Continuous	7.56 ^a	12.39 ^b	18.16 ^a	12.68 ^a	1.96 ^a
Augmented	8.40 ^a	15.54 ^a	18.34 ^a	13.65 ^a	1.79 ^a
Reducing Sugars					
Continuous	8.01 ^a	10.94 ^b	9.34 ^a	9.40 ^b	1.21 ^a
Augmented	9.23 ^a	16.92 ^a	9.64 ^a	12.32 ^a	0.97 ^a

*Lower case letters indicate significance at the .05 level according to Duncan's multiple range test.

Table 15
 Mean Soluble Carbohydrate Concentrations (μg carbohydrates/mg plant tissue)
 within 10-Month Old Eastern White Pine Seedlings Grown under
 Supplemental or Ambient Carbon Dioxide Environments

Soluble Carbohydrates CO ₂ Level	Leaf	Stem	Root	Total Seedling	Root/shoot
	$\mu\text{g}/\text{mg}$	$\mu\text{g}/\text{mg}$	$\mu\text{g}/\text{mg}$	$\mu\text{g}/\text{mg}$	
Total Sugars					
Supplemental	17.59 ^{a*}	31.79 ^a	41.83 ^a	30.00 ^a	1.80 ^a
Ambient	15.95 ^a	27.17 ^a	34.92 ^b	25.81 ^a	1.79 ^a
Ketose Sugars					
Supplemental	8.29 ^a	14.74 ^a	19.83 ^a	13.79 ^a	1.93 ^a
Ambient	7.66 ^a	13.19 ^a	17.17 ^b	12.54 ^a	1.83 ^a
Reducing Sugars					
Supplemental	9.14 ^a	13.80 ^a	9.17 ^a	11.01 ^a	1.17 ^a
Ambient	8.14 ^a	14.02 ^a	9.83 ^a	10.76 ^a	1.03 ^a

*Lower case letters indicate significance at the .05 level according to Duncan's multiple range test.

carbohydrates/mg tissue) within the root tissue were the only values that were significantly greater at the .05 level in enriched carbon dioxide atmospheres than in ambient carbon dioxide atmospheres.

Photoperiod and carbon dioxide. Interaction effects of photoperiod and carbon dioxide yielded similar results as those attributed to individual treatment effects. Total sugar, ketose sugar, and reducing sugar concentrations, significant at the .05 level were the highest in augmented photoperiod and supplemental carbon dioxide (Table 16). Sugar concentrations expressed as root/shoot ratio were the largest under conditions of continuous photoperiod and supplemental carbon dioxide.

Growing medium. All individual tissues and the total seedling showed approximately a 25 percent increase in total sugar, ketose sugar, and reducing sugar concentrations when grown in Redi-Earth medium over pine bark-vermiculite medium (Table 17). The greatest difference--41.21 μ g carbohydrates/mg tissue compared to 33.12 μ g carbohydrates/mg tissue--was measured in the total sugar concentration of the root tissue. Sugar concentrations of the root/shoot ratio comparison did not appear to be significantly different between the two media.

Growing medium and photoperiod. Although soluble sugar concentrations within the root and stem tissues were consistently greater in seedlings grown in Redi-Earth medium regardless of photoperiod length, only the reducing sugar concentration was

Table 16
Effects of Photoperiod and Carbon Dioxide on Mean Soluble Carbohydrate Concentrations
(μg carbohydrates/mg plant tissue) within 10-Month Old
Eastern White Pine Seedlings

Interaction		Leaf	Stem	Total Seedling	Root/shoot
		$\mu\text{g}/\text{mg}$	$\mu\text{g}/\text{mg}$	$\mu\text{g}/\text{mg}$	
Total Sugars					
Photoperiod ^A	CO ₂ ^B				
+	+	14.33 ^{b*}	27.00 ^a	28.04 ^a	2.24 ^a
+	0	18.21 ^b	27.53 ^a	26.00 ^a	1.45 ^b
0	+	20.86 ^a	37.02 ^a	32.34 ^a	1.47 ^b
0	0	13.86 ^b	26.84 ^a	25.63 ^a	2.11 ^{ab}
Ketose Sugars					
Photoperiod	CO ₂				
+	+	6.23 ^b	11.70 ^b	12.41 ^b	2.32 ^a
+	0	9.01 ^{ab}	13.15 ^b	12.96 ^b	1.57 ^b
0	+	10.34 ^a	18.04 ^a	15.45 ^a	1.47 ^b
0	0	6.45 ^b	13.22 ^b	12.14 ^b	2.06 ^{ab}
Reducing Sugars					
Photoperiod	CO ₂				
+	+	7.02 ^b	8.96 ^c	8.59 ^c	1.67 ^a
+	0	9.10 ^b	12.07 ^b	9.87 ^{bc}	0.77 ^b
0	+	11.45 ^a	17.67 ^a	12.95 ^a	0.95 ^b
0	0	7.20 ^b	16.30 ^a	11.79 ^{ab}	1.13 ^b

^ASymbols represent photoperiod treatment.
+--continuous photoperiod
0--augmented photoperiod

^BSymbols represent carbon dioxide treatment.
+--supplemental CO₂
0--ambient CO₂

*Lower case letters indicate significance at the .05 level according to Duncan's multiple range test.

Table 17

Mean Soluble Carbohydrate Concentrations (μg carbohydrates/mg plant tissue)
within 10-Month Old Eastern White Pine Seedlings
Grown in Two Different Media

Soluble Carbohydrates Growing Medium	Leaf	Stem	Root	Total Seedling	Root/shoot
	$\mu\text{g}/\text{mg}$	$\mu\text{g}/\text{mg}$	$\mu\text{g}/\text{mg}$	$\mu\text{g}/\text{mg}$	
Total Sugars					
Pine-bark vermiculite	14.86 ^{b*}	24.43 ^b	33.12 ^b	24.03 ^b	1.85 ^a
Redi-Earth	18.80 ^a	34.98 ^a	44.21 ^a	32.34 ^a	1.82 ^a
Ketose Sugars					
Pine bark-vermiculite	6.98 ^b	11.39 ^b	16.35 ^b	11.51 ^b	1.94 ^a
Redi-Earth	9.04 ^a	16.78 ^a	20.42 ^a	15.03 ^a	1.81 ^a
Reducing Sugars					
Pine bark-vermiculite	7.58 ^b	11.00 ^b	8.17 ^b	9.26 ^b	1.12 ^a
Redi-Earth	9.76 ^a	17.45 ^a	11.24 ^a	12.79 ^a	1.05 ^a

*Lower case letters indicate significance at the .05 level according to Duncan's multiple range test.

significantly different at the 0.05 level (Table 18). Combination of Redi-Earth medium and augmented photoperiod yielded the highest reducing sugar concentrations within both root (13.34 μ g carbohydrates/mg tissue) and stem (20.43 μ g carbohydrates/mg tissue) tissues.

Growing medium and carbon dioxide. Seedlings grown in Redi-Earth medium had higher sugar concentrations within the leaf tissue than that of seedlings grown in pine bark-vermiculite medium regardless of the carbon dioxide concentration (Table 19). The lowest total sugar and ketose sugar levels were found when both the pine bark-vermiculite medium and the ambient carbon dioxide atmosphere were used.

Correlation of Greenhouse and Laboratory Studies

Several growth variables were significantly correlated with sugar concentrations within leaf, root, and stem tissue (Table 20). Root collar diameter was the most consistent, showing significant positive correlations with total sugars, ketose sugars, and reducing sugars in leaf tissue plus total sugars and ketose sugars in root tissue. Height and stem dry weight were positively correlated with sugar concentration of the leaves while leaf dry weight was positively correlated with soluble sugars in the roots. Significant correlations with reducing sugars in the stem were limited to biomass variables. Stem-reducing sugar concentrations were negatively correlated with

Table 18

Effects of Growing Medium and Photoperiod on Mean Soluble Carbohydrate Concentrations (μg carbohydrates/mg plant tissue) within 10-Month Old Eastern White Pine Seedlings

Interaction		Stem	Root
		$\mu\text{g}/\text{mg}$	$\mu\text{g}/\text{mg}$
Total Sugars			
<u>Medium</u> ^A	<u>Photoperiod</u> ^B		
1	+	22.89 ^{a*}	33.63 ^a
1	0	25.98 ^a	32.60 ^a
2	+	32.01 ^a	42.73 ^a
2	0	37.96 ^a	45.84 ^a
Ketose Sugars			
<u>Medium</u>	<u>Photoperiod</u>		
1	+	10.35 ^a	16.93 ^a
1	0	12.42 ^a	15.77 ^a
2	+	14.62 ^a	19.49 ^a
2	0	18.94 ^a	21.43 ^a
Reducing Sugars			
<u>Medium</u>	<u>Photoperiod</u>		
1	+	9.77 ^b	9.17 ^b
1	0	12.71 ^b	7.16 ^b
2	+	12.98 ^b	9.55 ^b
2	0	20.43 ^a	13.34 ^a

^ANumbers represent type of growing medium.
 1--pine bark-vermiculite
 2--Redi-Earth

^BSymbols represent photoperiod treatment.
 +--continuous photoperiod
 0--augmented photoperiod

*Lower case letters indicate significance at the .05 level according to Duncan's multiple range test.

Table 19

Effects of Growing Medium and Carbon Dioxide on Mean Soluble
Carbohydrate Concentrations (μg carbohydrates/mg
plant tissue) within 10-Month Old
Eastern White Pine Seedlings

Interaction		Leaf
		$\mu\text{g}/\text{mg}$
Total Sugars		
<u>Medium</u> ^A	<u>CO₂</u> ^B	
1	+	17.39 ^{a*}
1	0	12.33 ^b
2	+	17.79 ^a
2	0	19.89 ^a
Ketose Sugars		
<u>Medium</u>	<u>CO₂</u>	
1	+	7.92 ^{ab}
1	0	6.03 ^c
2	+	8.65 ^{ab}
2	0	9.46 ^a
Reducing Sugars		
<u>Medium</u>	<u>CO₂</u>	
1	+	8.59 ^a
1	0	6.57 ^a
2	+	9.73 ^a
2	0	9.78 ^a

^ANumbers represent type of growing medium.
1--pine bark-vermiculite
2--Redi-Earth

^BSymbols represent carbon dioxide treatment.
+--supplemental CO₂
0--ambient CO₂

*Lower case letters indicate significance at the .05 level
according to Duncan's multiple range test.

Table 20

Significant Correlations of 10-Month Old Eastern White Pine Seedling
Morphological Variables with Seedling Soluble
Carbohydrate Concentrations

Plant Tissue : Soluble Carbohydrates	Morphological Variable	<u>r</u>	Sample Size
Leaf : Total Sugars	root collar diameter	0.348	94
	height	0.217	94
	root dry weight	0.211	94
	stem dry weight	0.257	94
Leaf : Ketose Sugars	root collar diameter	0.354	94
	height	0.232	94
	stem dry weight	0.264	94
Leaf : Reducing Sugars	root collar diameter	0.325	92
Root : Total Sugars	root collar diameter	0.285	90
	top dry weight	0.214	90
	leaf dry weight	0.211	90
Root : Ketose Sugars	root collar diameter	0.259	90
	leaf dry weight	0.209	90
Stem : Reducing Sugars	top fresh weight	-0.497	44
	top dry weight	-0.412	44
	stem dry weight	-0.322	44
	leaf dry weight	-0.417	44
	root dry weight	-0.316	44
	total fresh weight	-0.393	44
	total dry weight	-0.397	44

seedling biomass; whereas, all other correlations were positive. Although many correlations were statistically significant, the greatest r^2 -value obtained from the positive correlations was 0.125 and the highest r^2 -value obtained from the negative correlations was 0.247 (Table 20).

CHAPTER V

DISCUSSION

Greenhouse Study

Photoperiod. Seedling diameters and biomass increased with a continuous photoperiod which provided more light for photosynthesis. Light is necessary for chlorophyll synthesis, ATP and NADPH production, and chloroplast enzyme regulation (Salisbury and Ross, 1978); therefore, long duration of light allows more photosynthesis to occur. With increased photosynthesis, more carbohydrates are produced (Daniel et al., 1979) and rapidly used for energy, new growth, and storage. Increased growth and greater carbohydrate accumulation may have contributed to the larger top and root biomass of those seedlings grown under continuous photoperiod. An abundance of photosynthate allows the seedlings to grow at maximum potential and convert excess carbohydrates to storage.

Unlike the other growth variables, seedling height was not affected by a continuous photoperiod. Failure to expand in height under extended daylengths involves the light-sensing pigment, phytochrome. Photoperiod, in this experiment, was lengthened using fluorescent lights that emit light mainly in the red portion of the spectrum. Red wavelengths are efficient for photosynthesis, but do not favor stem elongation. Keegan's (1981) study of eastern white pine showed similar trends with continuous photoperiod increasing root collar diameter and biomass but not height growth.

Carbon dioxide. Supplemental carbon dioxide positively influenced seedling diameter, height, and most biomass variables. Increases were almost identical to those produced with continuous photoperiod. For those seedlings grown under continuous photoperiod, enhanced growth can be attributed to an increase in the photosynthetic rate caused by plant responses to carbon dioxide enrichment. These responses included an increase in leaf surface area, an increase in leaf temperature, an increase in dark carbon dioxide fixation, and a decrease in photorespiration (Kramer, 1981; Pallas, 1979). The other seedlings that were grown under augmented photoperiod were subject to artificial light during the dark period--initially for one hour--followed by 15 minute interruptions every hour thereafter. Since light was available intermittently, supplemental carbon dioxide may have increased photosynthesis in these seedlings resulting in increased growth. Some other mechanism may also have contributed to seedling growth, particularly since a few incandescent lights probably do not provide quality light for photosynthesis.

Stem dry weight was the only variable that was not affected by supplemental carbon dioxide--based on both analyses of variance and Duncan's multiple range tests. Instead of contributing to stem growth, it appears that the additional photosynthate from higher rates of photosynthesis was either incorporated into the leaves or translocated to the roots. Changes in the photosynthate composition and allocation have been shown to occur under enriched carbon dioxide atmospheres (Pallas, 1979). Height growth increases were not as pronounced probably because the majority of the photosynthate was

being used for biomass growth. Although Duncan's multiple range test did not show root fresh weight and root/shoot ratio to be significantly affected by atmospheric carbon dioxide concentration, the trends observed and the significance of the analyses of variance suggest that high carbon dioxide concentrations favor root growth.

Results from this study are somewhat contrasting to those found by Hay and Keegan (1982) in which biomass variables, other than top fresh weight, of 12 month old eastern white pines were not significantly influenced by an enriched carbon dioxide environment at the .05 level. Possibly the negative effects of excess carbon dioxide including increased thickness of palisade and parenchyma cells, stomatal closure, or reduced number of stomata (Aoki and Yabuki, 1977) became more pronounced with time, thereby inhibiting the positive growth effects.

Very few interactions were found between photoperiod and carbon dioxide treatments. The only statistically significant differences in the growth variables measured were lower root collar diameters and root fresh weights under augmented photoperiod and ambient carbon dioxide. As long as one treatment was favorable, i.e. 24-hour photoperiod or supplemental carbon dioxide, no further gain was attained by increasing the other treatment. Because of the expense of carbon dioxide enrichment and no apparent advantage over continuous photoperiod effects, it would be more economical to grow seedlings under 24-hour photoperiod without addition of carbon dioxide.

Growing medium. Variation in growth responses to the four growing media was very evident, with seedlings grown in pine bark-vermiculite or Redi-Earth media showing superior growth. Previous studies have reported these two media to be the preferred growing media because of their desirable characteristics that are conducive to ion uptake, water availability, and seedling growth (Gartner et al., 1973; Tinus and McDonald, 1979).

In addition to peat moss and vermiculite, present in Redi-Earth medium, Metro-Mix 220 medium contains perlite. Since peat moss and vermiculite alone favored seedling growth, perlite must either negate the positive characteristics of these materials or add some less desirable conditions. The sand portion of Metro-Mix 500 medium likely contributed to poor seedling growth due to reduced air pore space and low cation exchange capacity (Bosley, 1967).

Continuous photoperiod interacting with growing medium generally had a positive effect on root/shoot ratios. A higher root/shoot ratio in seedlings grown in Redi-Earth, Metro-Mix 220, or Metro-Mix 500 media under continuous photoperiod may have resulted from increased photosynthate translocation to the roots compared to that going to the shoots. Root/shoot ratio of seedlings grown in pine bark-vermiculite medium did not exhibit this trend, but instead showed superior ratios regardless of the photoperiod, indicating high ability of this medium to promote root growth. Superior root growth in this medium likely resulted from abundant air pore space and good drainage.

Gains in top fresh weight and stem dry weight were not attained from carbon dioxide enrichment when seedlings were grown in

pine bark-vermiculite medium or in Redi-Earth medium. However, seedling top fresh weight and stem dry weight growth in the less desirable media--Metro-Mix 220 and Metro-Mix 500--were increased with addition of supplemental carbon dioxide. Although carbon dioxide enrichment did overcome some of the negative aspects of these two media, superior growth still occurred in pine bark-vermiculite and Redi-Earth media. Growing medium seemed to exert a stronger influence on growth than did carbon dioxide enrichment.

Foliar mist fertilization had the most pronounced effect on seedlings grown in Metro-Mix 500 medium. The low cation exchange capacity of this medium limits mineral uptake which, in turn, may have inhibited seedling growth. With addition of nitrogen, phosphorus, and potassium through foliar fertilization, height growth, top dry weight, and leaf dry weight were significantly increased in the analyses of variance. In contrast, height growth, top dry weight, and leaf dry weight of seedlings grown in Redi-Earth medium apparently was inhibited by foliar fertilization. Increasing mineral content in seedlings grown in this superior medium may have raised concentrations to unfavorable levels which negatively affected shoot growth. The root/shoot ratio of seedlings grown in Redi-Earth medium showed this same trend, i.e. less of shoot growth with addition of foliar fertilizer resulting in a larger root/shoot ratio.

Overall, foliar mist fertilizer did not appear to influence seedling growth, either positively or negatively. Since fertilizer did not improve growth, seedlings gained little benefit from addition

of nitrogen, phosphorus, and potassium, which suggests that they already contain a plentiful supply of these minerals.

Roottrainer size. The Hillson roottrainers may have produced larger seedling diameters and biomass due to low seedling density and larger quantities of medium in each cavity. Less competition between seedlings occurred in low density conditions so that seedlings could utilize the available light and carbon dioxide to carry out photosynthesis to their full potential and grow without restriction from surrounding seedlings. The very small quantity of medium contained in the Fives roottrainer may be less desirable for growth because of its narrow limits of tolerances, i.e. rapid water depletion (Van Eerden, 1981).

Height growth was not affected by roottrainer size, and root collar diameter was only slightly influenced, suggesting that photosynthate may have been directed mainly toward biomass growth. Biomass increases did not favor the roots over the shoots or vice versa as reflected by the consistent root/shoot ratio regardless of the roottrainer size.

Benefits of Hillson roottrainers and continuous photoperiod are evident through their interaction effect on seedling biomass. Desirable traits of large roottrainers and long photoperiods complement each other to produce the largest fresh and dry weights (except for root fresh weight). Least biomass growth occurred when photoperiod was limited and root cavity size was small, making conditions for photosynthesis less favorable. Increasing the photoperiod to

24-hours, however, improved biomass growth of seedlings in the Fives roottrainers possibly due to greater photosynthesis. Likewise, increasing the roottrainer size under augmented photoperiod also improved biomass growth, which may resulted from lower stocking densities.

The superior characteristics of the Hillson roottrainers for promoting growth were shown in pine bark-vermiculite, Redi-Earth, and Metro-Mix 220 media. The most pronounced differences occurred within pine bark-vermiculite medium, with seedling growth in the Fives roottrainers being far inferior. A probable explanation for this large difference is the composition of the medium. Bark has a low water holding capacity and, therefore, dries out quickly in small roottrainer cavities. Vermiculite was added to alleviate this problem but may not have been added in sufficient quantities. Besides suffering from inadequate water, seedling growth in the Fives roottrainers may have been obstructed or hindered by small pieces of bark.

Laboratory Study

Photoperiod. A 24-hour photoperiod allows more photosynthesis to occur since light-demanding reactions can be carried out continuously. With additional photosynthesis, an increase in soluble carbohydrates within seedlings grown under 24-hour photoperiod would be expected; however, this was not the case. Instead, seedlings grown under augmented photoperiod contained slightly higher sugar concentrations. These results suggest that the additional carbohydrates produced from continuous photosynthesis may have been

used in respiration or rapidly converted to other forms and incorporated into the seedlings.

Following synthesis, carbohydrates are translocated throughout the plant and converted to protoplasm, cell walls, and other products of metabolism (Kramer and Kozlowski, 1979). Soluble carbohydrates are necessary to maintain accelerated growth and respiration resulting from high levels of photosynthesis; therefore, very few soluble carbohydrates are likely to accumulate in tissues of vigorously growing seedlings. In seedlings grown under continuous photoperiod, photosynthesis may to exceed translocation. When this condition occurs, excess soluble carbohydrates are converted to starch for later use rather than built up in soluble form within the seedling (Salisbury and Ross, 1978).

The only significant difference occurred in the stem tissue and total seedling in which sugar concentrations were greater with augmented photoperiod. Stem concentrations may have been influenced the most because the stem is neither a strong source nor a strong sink (Crafts and Crisp, 1971). It is possible that the stem sugars were rapidly depleted through translocation to the roots and uptake for maintaining growth. The ketose and reducing sugar concentrations were likely affected instead of total sugar concentration because the simple sugars are most readily used for respiration and for combination to form complex sugars. The statistically significant differences in reducing sugar concentration in the total seedling due to photoperiod effect may have resulted from the variation in the stem reducing sugars.

Carbon dioxide. Greater sugar concentrations within the roots of seedlings grown in enriched carbon dioxide environments over those in ambient carbon dioxide atmospheres may have resulted from a higher rate of translocation or lower root respiration. Carbon dioxide enrichment increases the photosynthetic rate which leads to larger production of carbohydrates and greater translocation (Salisbury and Ross, 1978). Since roots are the major carbohydrate sink, they exert the greatest pull on the photosynthate. When both roots and shoots are growing, the proportion of photosynthate retained by the shoots and translocated to the roots depends on the relative strength of these sinks (Kramer and Kozlowski, 1979). Actively growing roots imported more photosynthate than slow growing roots (Crafts and Crisp, 1971). Increases in root sugar concentrations of seedlings grown under supplemental carbon dioxide suggests that carbon dioxide enrichment favors vigorous root systems.

Soluble carbohydrate increases within the roots occurred in ketose and total sugars, but not in reducing sugars. Reducing sugars are simple sugars and, therefore, are more reactive than more complex sugars. Excess reducing sugars may have been converted to starch or other sugar derivatives necessary for respiration and growth. Since sucrose is the principle form of mobile carbon in plants, fat and starch must be formed in storage cells (leucoplasts) (Crafts and Crisp, 1971).

No significant differences occurred in the soluble sugar concentrations of leaf tissue, stem tissue, total seedling, or root/shoot ratio of seedlings grown under different atmospheric carbon

dioxide concentrations. As was the case with extended photoperiod, the seedlings may have immediately utilized or translocated excess carbohydrates that were produced from high levels of photosynthesis, so that no gain in soluble carbohydrates was noted.

The higher sugar concentrations from augmented photoperiod and enriched carbon dioxide atmospheres were also reflected in their interaction which yielded similar results for leaf tissue, stem tissue, and total seedling. Sugar concentrations expressed as root/shoot ratio, however, were greatest when both carbon dioxide concentration and photoperiod were increased. This combination of increased photosynthetic duration and increased photosynthetic rate may have produced abundant carbohydrates which were translocated to the roots faster than they could be converted to other sugar derivatives.

Growing medium. Superior growth occurred in both pine bark-vermiculite and Redi-Earth media; however, significantly greater soluble carbohydrate concentrations were only present in seedlings grown in Redi-Earth medium. Continued growth requires an adequate supply of water, mineral nutrients, organic nitrogen compounds, and certain growth regulators (Kramer and Kozlowski, 1979). Since seedlings in both media grew almost equally well, it appears that each medium supplied the seedlings with necessary materials.

Higher sugar concentrations within seedlings grown in Redi-Earth medium may have resulted from greater photosynthetic production, from building less root biomass, or from lower rates of

incorporation into storage forms and/or conversion to sugar derivatives. Conditions for photosynthesis possibly were superior in seedlings grown in Redi-Earth medium. With abundant photosynthesis, soluble carbohydrates are produced at a faster rate than they can be utilized by the seedlings. Top dry weight was larger for those seedlings grown in pine bark-vermiculite medium. Increased weight may have been due to greater storage of carbohydrates and other energy-rich compounds (Hay, 1981) which suggests that soluble carbohydrates were more readily converted to other forms in pine bark-vermiculite medium. Soluble carbohydrates expressed as root/shoot ratio were similar regardless of the growing medium indicating that soluble carbohydrates are distributed similarly throughout the seedlings grown in either medium.

Interaction between photoperiod and growing medium was only statistically significant for the reducing sugar concentration of the root and stem tissues. As would be expected, based on the individual treatment effects, the highest sugar concentrations were found in root and stem tissues of those seedlings grown in augmented photoperiod and Redi-Earth medium. Likewise, the carbon dioxide/growing medium interaction was also consistent with the individual treatment responses in which the lowest total sugar and ketose sugar concentrations were observed under conditions of ambient carbon dioxide and pine bark-vermiculite medium.

Foliar mist fertilization did not affect soluble carbohydrate concentrations within the seedlings, indicating that they did not respond to any additional minerals. Nitrogen, phosphorus, and

potassium concentrations were already present in sufficient quantities to carry on photosynthesis and sugar conversions.

Correlation of Laboratory and Greenhouse Studies

Leaf soluble carbohydrates and root soluble carbohydrates showed an inverse relationship to growth. High leaf sugar concentrations coincided with larger heights, stem dry weights, and root dry weights. Whereas, high sugar concentrations within roots led to greater top dry weights and leaf dry weights. Root collar diameter, however, was positively correlated to both leaf and root sugar concentrations. Large quantities of soluble carbohydrates within the leaf tissue indicates rapid photosynthesis. High rates of photosynthesis leads to high rates of translocation (Salisbury and Ross, 1978) of organic materials to the stem and roots (Frydriych, 1976). Supplied with abundant carbohydrates, the stem and roots can expand. Low concentrations of soluble carbohydrates in the leaves results in decreased stem and root growth because photosynthate is first incorporated into the leaves to maintain growth and production (Kramer and Kozlowski, 1979), leaving less photosynthate available for translocation.

Leaf and branch expansion occurs either at the expense of their own photosynthate or from previously produced substances that are moved into the developing shoots from the roots or from the stems (Shiroya et al. 1966). The positive correlation between root soluble sugar concentrations and leaf dry weight in this study suggests that seedling leaf growth is more strongly influenced by reserve

carbohydrates. The soluble carbohydrates within the roots are carried upward to meet the leaf growth needs. Positive correlations between root collar diameter and both leaf soluble carbohydrate concentrations and root soluble carbohydrate concentrations may be related to the positioning of the root collar, i.e. close to the roots where photosynthate is being funnelled and accumulated.

In contrast to positive correlations between seedling growth and sugar concentrations within the leaf and root tissue, biomass growth is negatively correlated to stem reducing sugar concentrations. The stem acts predominately as a channel that moves newly produced photosynthate from the leaves to the roots and carries carbohydrate reserves upward from the roots to the shoots. Small quantities of sugars in the stem might indicate that the photosynthate is being rapidly translocated to other parts of the seedling for use in biomass growth. The non-reducing sugar, sucrose, represents over 95 percent of the dry weight of translocated material (Kramer and Kozlowski, 1979; Shiroya et al., 1962). Vigorous seedling growth requires continuous translocation; therefore, most of the sugar fraction within the stems of rapidly growing seedlings would most likely be in the non-reducing form rather than in the reducing form.

Although several significant correlations exist between seedling growth variables and soluble carbohydrate concentrations, very little variation can be explained by their association, as reflected by the low r^2 values. Many other factors besides carbohydrates contribute to seedling growth including light, carbon dioxide, water, minerals, growth regulators, photosynthate

distribution mechanisms, respiration, and seasonal patterns (Evans, 1975; Kozlowski, 1979).

CHAPTER VI

SUMMARY AND RECOMMENDATIONS

Summary

Continuous photoperiod produced seedlings with greater root collar diameter, root fresh weight, root dry weight, top fresh weight, top dry weight, leaf dry weight, stem dry weight, total fresh weight, and total dry weight than seedlings grown under natural photoperiod. However, height and root/shoot ratio remained the same regardless of photoperiod. Ketose and reducing sugar concentrations within stem tissue and reducing sugar concentration in the total seedling were significantly greater under natural photoperiod.

Enriched carbon dioxide atmospheres produced seedlings with larger root collar diameter, height, root fresh weight, root dry weight, top dry weight, leaf dry weight, total fresh weight, total dry weight, and root/shoot ratio than seedlings grown in ambient carbon dioxide environments. Only stem dry weight differences were not statistically significant. Concentrations of total sugars and ketose sugars within root tissue were greater in enriched carbon dioxide atmospheres than in ambient atmospheres. Photoperiod/carbon dioxide interactions showed seedling root collar diameter and root fresh weight to be the largest when either a continuous photoperiod or an enriched carbon dioxide environment or both were present. Sugar concentrations expressed as root/shoot ratio were the largest under conditions of continuous photoperiod and supplemental carbon dioxide.

Pine bark-vermiculite and Redi-Earth media were the most conducive to seedling growth. Metro-Mix 220 medium was mediocre for promoting growth and Metro-Mix 500 was the poorest. Total sugar, ketose sugar, and reducing sugar concentrations within the leaf, root, and stem tissue and the total seedling were higher in Redi-Earth medium than in pine bark-vermiculite medium. Pine bark-vermiculite medium produced seedlings with the highest root/shoot ratio regardless of the photoperiod. Interaction of Redi-Earth medium and natural photoperiod yielded the highest reducing sugar concentrations within both root and stem tissues. Pine bark-vermiculite and Redi-Earth media showed superior top fresh weight and stem dry weight in either enriched carbon dioxide atmospheres or ambient carbon dioxide atmospheres. Least growth occurred in Metro-Mix 220 or Metro-Mix 500 media combined with ambient carbon dioxide. Greater sugar concentrations within the leaf tissue were present in seedlings grown in Redi-Earth medium regardless of the carbon dioxide concentration. Foliar mist fertilization had the greatest positive impact on height, leaf dry weight, and top dry weight of seedlings grown in Metro-Mix 500. Other than the fertilizer/growing medium interaction, foliar mist fertilization had no statistically significant effect on seedling growth or on soluble carbohydrate concentrations.

Seedlings grown in Hillson rootrainers were larger in biomass and root collar diameter than seedlings grown in Fives rootrainers. All biomass measurements except root dry weight were greatest for seedlings grown under continuous photoperiod in Hillson rootrainers. Pine bark-vermiculite medium in Hillson rootrainers produced the

largest seedlings; whereas, those in Fives roottrainers were some of the smallest. Least growth occurred in Metro-Mix 500 medium regardless of roottrainer size.

Height, stem dry weight, and root dry weight were significantly correlated with soluble carbohydrate concentrations in the leaves, while leaf dry weight was significantly correlated with soluble sugars in the roots. Root collar diameter was positively correlated with both leaf and root sugar concentrations. Stem reducing sugar concentrations were negatively correlated with seedling biomass.

Recommendations

Based upon the results of this study, the following greenhouse culture procedures are recommended to produce quality eastern white pine seedlings for outplanting:

1. Increased photoperiod. At least 18 hours of light are necessary for good seedling development.
2. Enriched carbon dioxide (1000ppm). Results indicate that soluble carbohydrates in the roots were increased by supplemental carbon dioxide. However, the expense of producing carbon dioxide is considerable, therefore enriched carbon dioxide atmospheres are marginal.
3. Container type. More than twice the number of seedlings can be produced per square meter of bench space in the Fives roottrainers compared to Hillson roottrainers.. However, seedlings grown in the Hillsons were somewhat

larger. If the greenhouse culture period is 10 months or longer, Hillsons will yield larger seedlings.

4. Growing media. Redi-Earth medium was superior to all other media and is readily available in commercial quantities from a number of suppliers.
5. Foliar mist fertilization. Assuming a good time-releasing fertilizer is in the medium, foiar fertilization does not provide additional benefits through 10 months of growth. Some fertilizer may be necessary if culture periods exceed 10 nonths.

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VITA

Tanya Annette Minton was born in Aurora, Illinois on September 4, 1959. She grew up in Oswego, Illinois; Naperville, Illinois; New Concord, Ohio; and Aurora, Illinois where she completed her high school education in June 1977. The following August, she entered Eastern Illinois University in Charleston and, in May 1981, she received a Bachelor of Science degree with high honors in Environmental Biology. As an undergraduate, she received the Walter M. Scruggs Scholarship, the G. B. Dudley Award, and the Beta Beta Beta Service Award.

In September 1981 she accepted a research assistantship in the Department of Forestry, Wildlife, and Fisheries at the University of Tennessee in Knoxville and began her graduate studies. While attending graduate school, she was awarded the Hilton A. Smith Fellowship. In March 1984, she graduated with a Master of Science degree in Forestry.

The author is a member of Phi Alpha Eta, Beta Beta Beta, Phi Sigma, Xi Sigma Pi, and the Society of American Foresters.