LEBUDE, ANTHONY VINCENT. Adventitious Rooting And Physiology Of Stem Cuttings Of Loblolly Pine. (Under the direction of Drs. Frank A. Blazich and Barry Goldfarb)

Vegetative propagation by stem cuttings can be used to multiply improved seedlings of timber species before deployment for reforestation. Before full scale deployment of rooted stem cuttings can be accomplished on an operational level, however, various obstacles need to be overcome. Among these obstacles are development of rooted cutting production systems and effective control of the rooting environment to stimulate adventitious root formation. Therefore, two separate series of experiments were conducted to develop protocols for clonal propagation of loblolly pine (*Pinus taeda* L.) by stem cuttings. The first series of experiments were conducted to test the efficacy of two containerized production systems on rooting percentage, root system morphology, and first year field growth of rooted stem cuttings of loblolly pine. The second series of experiments focused on the effect of the rooting environment on adventitious root formation to define a propagation protocol with broad application that stimulates rooting among several rooting environments.

In the first series of experiments, three studies were conducted to test adventitious rooting of juvenile hardwood (dormant) or softwood (succulent) stem cuttings of six unrelated full-sib families of loblolly pine in various sizes of Jiffy® Forestry Peat Pellets or Ray Leach Conetainers™ and the subsequent effect on first year field growth after outplanting. Controls in all experiments were Ray Leach Super Cells filled with a medium of 2 peat : 3 perlite (v/v). After adventitious rooting in the greenhouse and 12 months of field growth, all plants, with the exception of plants produced in one Jiffy pellet size, were shorter than the controls and had less root dry weight (DW) and shoot DW. Results suggest that preplant root DW is an important predictor of first year field performance. Therefore, cuttings rooted in pellets may need to be transplanted to a nursery bed for further root growth and development prior to field planting. Rigid plastic containers remain a viable production system because cuttings rooted consistently, had large root mass, and plants were of commercial size after 1 year of field growth.

In the second series of experiments, six studies were conducted. Four studies investigated the influence of cutting water potential (Ψ_{cut}) on rooting of juvenile dormant and succulent stem cuttings of loblolly pine propagated under varying substrate water potentials (Ψ_{sub}) and volumes of mist application (mist levels). In the first two studies, mist level and Ψ_{sub} contributed to the Ψ_{cut} of nonrooted stem cuttings. In the second two studies, when Ψ_{sub} was held constant across various mist treatments, mist level contributed strongly to Ψ_{cut}. In the first two studies, Ψ_{sub} affected rooting percentage when mist was suboptimal or excessive; otherwise, mist had a stronger effect than Ψ_{sub} on rooting percentage. For all four studies, cuttings rooted best when experiencing moderate water deficits (- 0.5 to - 1.2 MPa) during the period of adventitious root formation. Results demonstrate that monitoring the physiological status of stem cuttings during adventitious rooting can provide important information for controlling the rooting environment.

The final two studies of the second series of experiments investigated the relationships between mist application, vapor pressure deficit (VPD), Ψ_{cut}, and rooting percentage of dormant or succulent stem cuttings of loblolly pine. In addition, net photosynthesis at ambient conditions ($A_{ambient}$) and stomatal conductance (g_s) of succulent stem cuttings were measured during adventitious root formation to determine their relationship to rooting percentage. Dormant stem cuttings rooted ≥ 80% when mean daily VPD between 1000 and 1800 HR ranged from 0.60 to 0.85 kPa. Although rooting percentage was related to Ψ_{cut} and g_s, and $A_{ambient}$ was related to Ψcut and g_s, rooting percentage of succulent stem cuttings was not related to $A_{ambient}$. Using VPD as a control mechanism for mist application during adventitious rooting of stem cuttings of loblolly pine might increase rooting percentages across a variety of rooting environments.

ADVENTITIOUS ROOTING AND PHYSIOLOGY OF STEM CUTTINGS OF LOBLOLLY PINE

by

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A dissertation submitted to the Graduate Faculty of
North Carolina State University
In partial fulfillment of the
Requirements for the Degree of
Doctor of Philosophy
In

HORTICULTURAL SCIENCE

Raleigh, NC

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Personal Biography

Anthony V. LeBude was born in Camden, New Jersey and raised in Florida and Virginia Beach, Virginia. His parents still reside in Suffolk, Virginia, his older sister in Chesapeake, Virginia with her husband, Jimi, and three boys, Mark, Matthew, and Brent, and his older brother lives in Burlington, New Jersey with his wife Geannine. Anthony graduated from Bayside High School in Virginia Beach in 1986 and then attended Virginia Tech. After receiving a B.S. in Horticultural Science, he worked as a nursery manager for White's Old Mill Garden Center in Chesapeake, Virginia. It was there that he formalized his idea of teaching and research as an alternative career path.

Although not entirely embraced by the graduate school administration, due in part to previous grades and a lackluster performance on standardized tests, Anthony was determined to gain admission to graduate school and continue on. He continued by using the words of his older brother Neil as a mantra, "[Screw] them, they don't know what they're talking about, do it anyway". He was finally admitted and received an MS in Agricultural and Extension Education in 1998. In 1999, Anthony began his PhD degree in the Department of Horticultural Science.

Throughout his graduate career, Anthony worked as a Research Assistant for the NCSU Loblolly and Slash Pine Rooted Cutting Program in the Department of Forestry to help defray the cost of graduate education. During his tenure as Research Assistant, Anthony gained valuable experience managing people, resources and data effectively. He attributes much of his success to the hard work, diligence, and perseverance embodied in his maternal grandfather John Neill, a brick layer and sportsman, his paternal grandfather Jean LeBude, a carpenter, his father Ron LeBude, a sailor, and his older brother Neil who is just himself.

Anthony will be living and working in Western North Carolina after graduation. He begins employment, June 1, 2005, as an Assistant Professor in the Department of Horticultural Science at NC State University stationed at the Mountain Horticultural Crops Research and Extension Center in Fletcher, NC.

Acknowledgements

This research would not have been completed without the help of the Employees and Cooperators of the NC State University Loblolly and Slash Pine Rooted Program. Their assistance with ideas, funding, and willingness to work, even at odd hours of data collection, is a testament to their faith in research-based knowledge and information. Their assistance is gratefully acknowledged.

Frank Blazich and Barry Goldfarb served as co-chairs, and John Frampton and Farrell Wise completed the rest of my advisory committee. They all could not have been more trusting, empowering, enlightening, controlling, supportive and nurturing. To some, what might appear as a hands-off, laissez-faire approach looks to me like the perfect opportunity to create a new idea out of whole cloth. At times, when the outcome looked doubtful, they still gave me the latitude to make decisions that ultimately led to success. Their approach of guided-discovery coupled appropriate parts trial with an inordinate amount of error on my part. Together we managed to complete a project worthy of all our efforts.

Mollie E. Bowles, my lovely girlfriend, thank you for your guidance, assistance, friendship, fellowship, devotion, and love. Thank you also for formatting the entire thing perfectly, I would still be doing that now. I look forward to life together, beside you.

Neil LeBude is best described by the phrase “to live and to work”. His love for work and his love for his wife, Geannine, underscore his love for life. I have no doubt he will continue to be happy. To my parents, Ron and Johan LeBude, and my sister, Gigi, I thank you for all your hard work, devotion, and support of my efforts. I could not have done it without you, and I wish you all great success.

Maria Wirth Wilkes, we need to go to the PR. I’ll miss you in the mountains, but the memory of you and your family will always be with me.

There are friends too numerous to mention. These include fellow graduate students, old high school and college chums, as well as current fellow co-workers and extended families. They aided by providing such encouraging comments as “when are you going to be finished with that book report”, or “when does the university fit you for a tweed jacket and pipe”, or “you must love school, I bet you’re one of those professional students I’ve heard about", or "it must be nice having summers off your whole life" or "by the time you figure out the solution, it won't be a problem no more". I will continue to help others as you have helped me during this process.

Please know that I am sincerely grateful for all the help and assistance you have all graciously bestowed upon me in these many years. All of your prayers, well wishing, energy, thoughts, and luck are manifested in this document and my career. Thank you.

TABLE OF CONTENTS

LIST OF TABLES

Chapter 1. Container Type Influences Adventitious Rooting and Subsequent Field Growth of Stem Cuttings of Loblolly Pine

Chapter 2. Mist, Substrate Water Potential, and Cutting Water Potential Influence Rooting of Stem Cuttings of Loblolly Pine

Chapter 3. Mist Level Influences Vapor Pressure Deficit and Gas Exchange During Rooting of Juvenile Stem Cuttings of Loblolly Pine

LIST OF FIGURES

Chapter 1. Container Type Influences Adventitious Rooting and Subsequent Field Growth of Stem Cuttings of Loblolly Pine

Chapter 2. Mist, Substrate Water Potential, and Cutting Water Potential Influence Rooting of Stem Cuttings of Loblolly Pine

Chapter 3. Mist Level Influences Vapor Pressure Deficit and Gas Exchange During Rooting of Juvenile Stem Cuttings of Loblolly Pine

General Introduction

Tree improvement efforts in the southeastern United States have increased growth, wood volume, and disease resistance of seedlings of loblolly pine (*Pinus taeda* L.) deployed for reforestation. Current estimates of seedling deployment exceed a billion seedlings per year in the Southeast. Bare-root seedlings from open-pollinated parents, known as half-siblings or half-sibs, are planted almost exclusively and they are inexpensive to produce. Seedlings from controlled-pollinated crosses of two superior parents, known as full-siblings or full-sibs, are generally more expensive to produce but offer some advantages over deploying half-sibs. Generally, when full-sib seedlings are deployed, trees are taller and have more volume at rotation age, which also may be shortened compared to deploying less superior populations. To capitalize on these genetic gains from full-sib seedlings, vegetative propagation by stem cuttings can be used to multiply improved seedlings before deployment. This is particularly true if genetically improved seed is limited. Furthermore, elite individuals (clones) within these superior full-sib families can be selected, multiplied separately to increase uniformity in planting stock, and deployed to capitalize completely on all breeding efforts invested in specific, controlled-pollinated crosses.

The benefits of vegetative propagation and its use in clonal forestry have been reported for some time (Grigsby, 1961; Isik et al., 2003; Libby, 1974; Zobel and Talbert, 1984). Current deployment of rooted stem cuttings of loblolly pine on an operational level, however, is still a small fraction of the total number of plants deployed. Despite some challenges (Hare, 1979; Kleinschmit, 1977; Libby, 1974; McAlpine and Jackson, 1959), rooted cutting technology has been steadily increasing and the future of vegetative propagation as a means to produce deployment stock appears encouraging (Goldfarb et al., 1997). Researchers have investigated stock plant (hedge) management (Cooney and Goldfarb, 1999; Rowe and Blazich, 1999; Rowe et al., 2002), care and handling of stem cuttings (Grigsby, 1971; Murthy and Goldfarb, 2001), various production systems (Dougherty et al., 2004; Frampton et al., 1999, 2002; Gocke, 2001; Goldfarb et al., 1998), and field performance of rooted cuttings (Foster et al., 2000). Continued progress to refine protocols, which produce consistently uniform, high quality rooted stem cuttings efficiently, is necessary to move toward operational deployment.

Efficient production systems are needed to combine high rooting percentages and root system quality with excellent field performance. Rooting stem cuttings in containers is one option to achieve this goal. Because container volume and design vary so widely, and numerous rooting substrates may be employed, many combinations of containers and substrates may achieve successful results. Ray Leach Super cells are long, conical plastic tubes and have been used with good success to produce containerized seedlings of loblolly pine for deployment on droughty sites (Anderson et al., 1984) and for numerous research trials. Jiffy® Forestry Peat pellets are compressed peat pellets enclosed by a soft mesh covering. When soaked in water, pellets rehydrate to form a planting container and substrate ready for use. Both container systems have been used successfully to root stem cuttings of various forest species (Gocke, et al., 2000; Wright et al., 1999). However, there are few reports of these systems having been used for rooting stem cuttings of loblolly pine.

Several kinds of production systems can be used to produce rooted stem cuttings of particular timber species efficiently; however, all production systems will require a uniform rooting environment for success. Rooting environments will need to be large, especially if the quantity of rooted stem cuttings deployed approaches that of bare-root seedlings. This creates a challenge regarding maintenance of a uniform rooting environment. Moreover, rooting environments vary because of the inherent, individual components, for example substrates, containers, as well as the regional climate. Also, sufficient mist application needed to maintain favorable water relations of cuttings during adventitious rooting depends on the time of year, the type of propagation system used, the species being rooted, and tissue maturity of the cuttings (Loach, 1988). Thus, specific protocols of mist application will not be applicable to all rooting environments and time will not permit testing every combination of the individual components.

An alternative approach would be to investigate environmental factors affecting stem cutting physiology during adventitious root formation. By designing rooting environments to elicit physiological responses associated with successful adventitious root formation, propagators may be able to achieve success with many production systems in a variety of rooting environments, regardless of the regional climate. However, this hypothesis has not been tested and reported for stem cuttings of loblolly pine.

Therefore, two separate series of experiments were conducted to develop protocols for propagation of loblolly pine by stem cuttings. In the first series of experiments, three studies were conducted to test adventitious rooting of juvenile hardwood (dormant) or softwood (succulent) stem cuttings of six unrelated full-sib families of loblolly pine in various sizes of Jiffy® Forestry Peat pellets or Ray Leach Conetainers™ and the subsequent effect on first year field growth after outplanting. Controls in all experiments were Ray Leach Super cells filled with a medium of 2 peat : 3 perlite (v/v). In the second series of experiments, six studies were conducted. Four studies investigated the influence of cutting water potential (Ψ_{cut}) on rooting of juvenile dormant and succulent stem cuttings of loblolly pine propagated under varying substrate water potentials (Ψ_{sub}) and volumes of mist application (mist levels). The final two studies of the second series of experiments investigated the relationships between mist application, vapor pressure deficit (VPD), Ψ_{cut}, and rooting percentage of dormant or succulent stem cuttings of loblolly pine. In addition, net photosynthesis at ambient conditions ($A_{ambient}$) and stomatal conductance (g_s) of succulent stem cuttings were measured during adventitious root formation to determine their relationship to rooting percentage.

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Chapter 1

Container Type Influences Adventitious Rooting and Subsequent Field Growth of Stem Cuttings of Loblolly Pine

(In the format appropriate for submission to the Southern Journal of Applied Forestry)

Container Type Influences Adventitious Rooting and Subsequent Field Growth of Stem Cuttings of Loblolly Pine

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Container Type Influences Adventitious Rooting and Subsequent Field Growth of Stem Cuttings of Loblolly Pine

ABSTRACT*:* *Three experiments were conducted to study rooting of juvenile stem cuttings of six unrelated full-sibling families of loblolly pine (Pinus taeda L.) in various sizes of Jiffy® Forestry Peat Pellets or Ray Leach Conetainers™ and the subsequent effect on first-year field growth. In Expts. 1 and 2, respectively, juvenile, dormant (hardwood) and succulent (softwood) stem cuttings of three full-sibling families were inserted (set) in pellet sizes 25-65 mm, 30-65 mm, 36-65 mm, 36-75 mm (Expt. 1 only), 42-65 mm, 42-80 mm, or 50-95 mm (designated by dry diameters - expanded heights in millimeters) and a Super cell (164* cm^3*) containing a substrate of 2 peat : 3 perlite (v/v), which served as the control. In Expt. 3, juvenile, softwood stem cuttings of three other families were set in either Super cells, Stubby cells (115* cm^3*), or Pine cells (66* cm^3*). Overall, rooting percentages, 12 weeks after setting, were 72%, 62%, and 55% in Expts. 1, 2, and 3, respectively. In Expt. 1, cuttings set in 30-65 mm (93%) or 36-75 mm pellets (84%) rooted greater than or equal to the control (83%). Rooting was less frequent than the control for cuttings rooted in 42-80 mm (36%) or 50-95 mm pellets (57%). Performance of stem cuttings in other pellet sizes depended on family. In Expt. 2, stem cuttings in all pellet sizes rooted greater than or equal to the control (64%) with the exception of 42-80 mm (45%) or 50-95 mm pellets (58%). In Expt. 3, the rooting container did not affect the variables measured. In Expt. 1, cuttings from all families in all pellet sizes generally had lower root dry weights (dws) than the control. In Expt. 2, cuttings rooted in 42-80 mm and 50-95 mm pellets had similar root dw as the control, whereas, cuttings in all other pellets sizes had lower root dw than in the control. Excluding data for the control, root dw increased linearly with pellet volume. After 12 months of field growth, plants produced from 42-80 mm (38 cm) and 50-95 (41 cm) pellets were as tall as plants of the control (42 cm), whereas all other plants were shorter. Excluding data for the control, stem height, root collar diameter, root dw, and shoot dw increased linearly with volume of the rooting pellet. Although cuttings in pellet sizes 42-80 mm and 50-95 mm produced large root dw, rooting percentage was low and inconsistent between experiments. Thus, the large* *size coupled with low rooting percentage renders their use unsatisfactory for rooting stem cuttings of loblolly pine. Alternatively, cuttings could be rooted in the smaller diameter pellets, i.e., 25-65 mm, 30-65 mm, 36-65 mm, and then transplanted into a nursery bed for further root growth and development prior to field planting. Rigid plastic containers are a viable option for rooting stem cuttings of loblolly pine, due primarily to consistent rooting percentages and development of large root systems. South. J. Appl. For. _________.*

Key Words: Vegetative propagation, clonal forestry, root development, field growth, *Pinus taeda*.

Loblolly pine is the most important timber species in the southeast United States. Tree improvement efforts within the last 50 years have increased growth, volume, and disease resistance in seedlings of loblolly pine deployed for reforestation (Li et al. 1999). Although seedlings are planted almost exclusively, vegetative propagation of loblolly pine by stem cuttings is closer to being realized on an operational scale due to recent advances in control of the rooting environment (LeBude et al. 2004), production systems (Frampton et al. 1999, Rowe and Blazich 1999, Rowe et al. 2002a, 2002b), and research reporting field performance of rooted cuttings (Goldfarb et al. 1998, Foster et al. 2000, Frampton et al. 2002).

Vegetative propagation by stem cuttings can be used to multiply genetically improved seedlings of full-sibling families (controlled pollination between known parents) or generate populations of elite individuals (clones) within families of forest species (Zobel and Talbert 1984). Rooted stem cuttings can then be planted in field tests to determine superior traits of a family or clone, or, if these characteristics are known, rooted cuttings can be deployed for reforestation (McKeand et al. 2003). Production of rooted stem cuttings, however, must be accomplished in a low-cost, efficient manner, yet produce high-quality planting stock that realizes its potential during deployment (Goldfarb et al. 1997).

Stem cuttings of woody species can be rooted in innumerable combinations of containers and substrates or even aeroponically (Beyl et al. 1995, Dirr and Heuser 1987, Dougherty et al. 2004, Hinesley and Snelling 1997, Rieckermann et al. 1999). Currently, various production systems for rooting stem cuttings of loblolly pine are being considered (Gocke 2001). Some systems consist of rooting stem cuttings in raised greenhouse benches and then transplanting the rooted cuttings to nursery beds to improve root system quality (Goldfarb et al. 1998), while other systems root stem cuttings directly outdoors in nursery beds (Frampton et al. 1999). One production system has not been determined to be superior because high quality rooted cuttings can be produced equivalently from several production systems.

Containerized production of seedlings of loblolly pine using various containers and substrates has been tested previously (Anderson et al. 1984, Goodwin 1981). A current standard container used for research purposes for both seedling and stem cutting production is the Ray Leach Super cell filled with a substrate of peat and perlite. Various factors determine the use of rooting containers. Central to the decision are the effects of the container on rooting percentage, root system quality, and subsequent field growth.

Jiffy® Forestry Peat Pellets are “quasi-containers” of compressed peat enclosed by a soft mesh covering. Pellets are pathogen free and ready to use after soaking in water to expand the overall size of the pellet. After pellets have rehydrated completely, stem cuttings or seeds may be placed into a pre-formed cavity at the top of the pellet. Because pellets are placed into trays designed to improve air circulation surrounding the surface area of the pellet, roots extending past growing surface regions are air pruned to induce potential lateral root regeneration. This design allows root tips to extend horizontally from the entire vertical region of the pellet into the surrounding substrate after transplanting. By colonizing the substrate horizontally as well as vertically, seedling root systems create an architecture resembling natural stand establishment for some species (Baliskey et al. 1995). Stem cuttings of subtropical pine species [*Pinus patula* (Schiede ex Schltdl. & Cham.), *P. tecunumanii* (Eguiluz & J.P. Perry), and *P. maximinoi* (H.E. Moore)] as well as rose gum (*Eucalyptus grandis* Hill ex Maiden) (Wright et al. 1999) and American sweetgum (*Liquidamber styraciflua* L.) (Gocke et al. 2000), have been rooted successfully in Jiffy Forestry Peat Pellets.

Whether rooted cuttings are produced in containers, nursery beds, or a combination of both, plants intended for deployment will be graded for quality. Various morphological traits are used to assess seedling stock quality. Root collar diameter (rcd), the diameter of the stem 1 cm above the highest root, has been a central, effective predictor of seedling field growth, and grading standards have been based on such data (South 2000, Wakeley 1954). Because adventitious root systems of stem cuttings differ from seedling root systems by having roots arranged centrally around the stem instead of a main taproot, quality standards may need to be amended for rooted cuttings. Frampton et al. (1999), for example, used the presence of a symmetrical root system and at least one vertically oriented root per rooted cutting to supplement the original seedling grading standard based on rcd. With this method, fewer rooted cuttings were classified as grade 1 or acceptable, and more were classified as culls. Rooted cuttings then were further subdivided into various classes, including “cull”, based on rcd, and a visual integration of root system fibrosity, symmetry and total root mass. They were field planted along with seedling controls and data were recorded after 5 years. The authors concluded that rooted stem cuttings do not need separate culling criteria prior to field planting to have similar survival rates and stem volumes as seedlings (Frampton et al. 2002). Further evidence has supported the absence of a meaningful relationship between either the number of roots or root system symmetry of rooted cuttings of loblolly pine prior to field planting, and subsequent shoot height or rcd after several years of field growth (Foster et al. 2000, Goldfarb et al. 1998). Other investigators have found either weak or no correlations between various morphological traits of cutting root systems of white pine (*Pinus strobus* L.) and their subsequent field performance (Struve and McKeand 1990, Struve et al. 1984). Alternately, some researchers suggest that root mass [root dry weight (dw)], volume, and absorbing area of the cutting root system are more important predictors of field growth, but simultaneously caution that the effects of root system morphology on long-term field performance are yet to be determined (Foster et al. 2000, Goldfarb et al. 1998).

Many production systems utilizing stem cuttings of loblolly pine have inserted (set) cuttings directly into substrate in greenhouse benches, or in loamy sand in an outdoor nursery bed, or some combination of both systems. Few reports have characterized influences of propagation containers on rooting and subsequent growth of stem cuttings of loblolly pine, whether containers are rigid plastic similar to those used for seedling production, or prefabricated units that contain a distinct rooting substrate such as Jiffy Forestry Peat Pellets. Therefore, three experiments were conducted to determine the effect of container type on adventitious rooting and root system morphology of stem cuttings of loblolly pine. After rooting, samples of cuttings were field planted to determine the effect of container type on subsequent field performance. The objectives of this investigation were to compare rooting and subsequent first-year field growth of stem cuttings of three full-sibling families of loblolly pine rooted in different sizes of Jiffy® Forestry Peat Pellets and Ray Leach Conetainers™.

Methods

Plant Material

Seeds for production of hedged stock plants for all experiments were from six unrelated full-sibling families of a Florida provenance of loblolly pine selected from the North Carolina State University-Industry Cooperative Tree Improvement Program on the basis of diallel-mated progeny tests of second-cycle and plantation-selected parents. Families in Expts. 1 and 2 were designated B, C, and D and seeds were germinated December 1995, whereas, in Expt. 3, families were designated G, H, and K and seeds were germinated October 1996. Seedlings were grown continuously through the winter in a heated, polyethylene-covered greenhouse at the North Carolina State University Horticultural Field Laboratory (HFL) (lat. 35°47’ N, long. 78°39’ W). In March 1996, two hundred seedlings per family were potted individually in 12-l containers filled with a substrate of 4 composted, shredded pine bark : 1 sand (v/v) for Expts. 1 and 2. In May 1997, this was repeated for Expt. 3. All plants were then grown outdoors on a gravel pad with overhead irrigation.

At the beginning of March and August of each year, seedlings were sheared to a height of approximately 15 cm (5.9 in), and maintained as hedged stock plants (hedges) by removing all remaining visible terminal buds from lateral stems. Approximately 51 g of 18N-6P_2O_5–12K_2O Osmocote, 8-9 month controlled-release fertilizer (Grace-Sierra, Milpitas, Calif.), was applied as a top-dressing to each hedge after shearing. Other macro- and micronutrients were applied as indicated by periodic foliage analyses following recommendations for seedlings of loblolly pine (C.B. Davey and J.B. Jett, Dept. of Forestry, NC State University, Raleigh, personal communication). Hand weeding was employed as necessary and pesticides were applied throughout the growing season to control tip moth (*Rhyacionia frustrana* (Comst.) and fusiform rust [*Cronartium quercuum* (Berk.) Miyabe *ex* Shirai f. sp. f*usiforme*]. The container pad was covered with white polyethylene and left unheated to over-winter the hedges from December to April.

For all rooting experiments, terminal stem cuttings were severed from hedges before 1200 HR, wrapped in moist paper towels, and stored in insulated coolers. Coolers were placed into a cold room maintained at 4° C for 7 days for dormant cuttings in Expt. 1, or under the greenhouse bench overnight at ambient greenhouse temperatures for softwood cuttings in Expts. 2 and 3. Prior to inserting (setting) cuttings into the rooting substrate to a depth of 2 cm, cuttings were recut at the proximal ends to a final length of 9 cm, and the basal 1 cm was dipped for 3 s in either 10 mM 1-naphthaleneacetic acid [NAA; 1.86 g l^{-1} 30% ethanol (v/v)] for Expt. 1, or 2.5 mM NAA [0.46 g l^{-1} in 20% ethanol (v/v)] for Expts. 2 and 3. Needles were not removed from the basal portions of the cuttings that were inserted into the rooting substrate.

Propagation Environment

Rooting was conducted in a clear, two-layer, polyethylene-covered greenhouse with natural photoperiod and irradiance. For Expts. 2 and 3, irradiance was decreased 60% by placing shade cloth on the greenhouse exterior. Heating and cooling systems were set to maintain the daily air temperature between 23 and 26˚ C and the night temperature between 20 and 23˚ C. Environmental management software (Q-Com Corp., Irvine, Calif.) calculated mist frequency and triggered a traveling gantry (boom) system (Solaris, McConkey Co., Mt. Puyallup, Wash.) to apply mist. Cuttings were misted intermittently at a variable frequency related inversely to the relative humidity (rh) within the greenhouse. Variable frequencies were defined by designating minimum (60% rh) and maximum (99% rh) off-times between mist applications. Off-times for intermediate humidity values were calculated using a linear function. The minimum and maximum off-times varied according to the time of day. For the period from 0600 HR to 0900 HR, the minimum and maximum off times were 10 min and 35 min, respectively. For the periods from 0900 HR to 1800 HR, 1800 HR to 2100 HR, and 2100 HR to 0600 HR, minimum and maximum off times were 8 min and 24 min, 10 min and 40 min, and 60 and 240 min, respectively.

Experimental Design

Rooting in Jiffy® Forestry Peat Pellets (Expts. 1 and 2)

These experiments were conducted in January 1998 (Expt. 1) and June 1998 (Expt. 2) to evaluate rooting of stem cuttings of three unrelated full-sibling families of loblolly pine in varying sizes of Jiffy® Forestry Peat Pellets (Jiffy Products, Shippagan, New Brunswick, Canada). The peat pellets differed in dry diameters-expanded heights and are designated as such in Table 1. Both studies generally used the same pellets although Expt. 2 excluded the 36-75 mm pellet. Ray Leach Super cells (vol. = 164 ml) (Stuewe and Sons, Inc., Corvallis, Ore.) containing a substrate of 2 peat: 3 perlite (v/v) served as the control for both experiments. The experimental design was a split-plot with eight (or seven in Expt. 2) container sizes as the main plots and three full-sibling families as the sub-plots. Treatments were replicated in eight blocks with 10 cuttings per family per treatment in each block. In Expt. 1, 1920 dormant hardwood cuttings were collected and set, and in Expt. 2, 1680 softwood cuttings were collected and set. One row of border cuttings surrounded all pellet treatments within blocks.

Rooting percentage, based on a cutting having at least one root > 1 mm long, number of primary roots > 1 mm long, root system symmetry (at least two primary roots $\geq 130^{o}$ apart using the stem as a vertex), root collar diameter (rcd, measured 1 cm above the highest root), stem height (measured from the base of the terminal or resting bud at the tip of the stem to the base of the cutting), root dry weight (dw), and shoot (stem plus needles) dw were recorded in both experiments 12 weeks after setting. Secondary roots were severed from the primary root system and both secondary and primary roots, as well as the shoots, were dried at 70° C for 72 h and then weighed separately. All rooting data presented, except rooting percentage, are based only on cuttings that rooted.

In Expt. 1, all cuttings were destructively scored for all root and shoot variables. In Expt. 2, five cuttings per plot were chosen randomly (irrespective of rooting) and rooted cuttings were destructively scored for root and shoot variables. The remaining five cuttings were scored for rooting percentage only. Rooted cuttings remaining from Expt. 2 were grown without fertilization or mist for 2 months in their respective rooting containers in the same greenhouse, and then hardened off outside for 1 week until field planting.

Rooting in Ray Leach Conetainers™ (Expt. 3)

This experiment was initiated in June 1998 to investigate the effect of three container volumes on rooting percentage and root quality of juvenile, softwood stem cuttings of three unrelated full-sibling families of loblolly pine. The containers were Ray Leach Pine cells (vol. = 66 ml), Ray Leach Stubby cells (vol. = 115 ml), and Ray Leach Super cells (vol. = 164 ml) (Stuewe and Sons, Inc., Corvallis, Ore.) filled with a substrate of 2 peat : 3 perlite (v/v) (Table 2). Ray Leach Super cells constituted the control since we have used them consistently for producing seedlings and rooted cuttings for field trials, and since Super cells have been the primary container type used for our rooted cutting research. The experimental design was a split-plot with eight blocks. Container type was the main plot and family was the sub-plot. Seven stem cuttings were set per family per treatment in each block for a total of 504 stem cuttings. Test cuttings were surrounded by one row of border cuttings.

After 12 weeks, four cuttings per plot were chosen randomly and scored destructively for root and shoot variables while the remaining three cuttings were scored for rooting percentage only. Remaining rooted cuttings were grown in their respective containers without fertilization or mist for 2 months in the same greenhouse, and then hardened off outside for 1 week prior to field planting.

Field Phase (Expts. 2 and 3)

This phase was designed to investigate the influence of various rooting containers on first-year field growth. The experimental design was a randomized complete block with single-tree plots in eight (Expt. 2) or four (Expt. 3) blocks. In Expt. 2, plants were maintained in the original eight blocks from the greenhouse phase. Actual numbers of rooted cuttings planted per family and container in Expt. 2 are in Table 3. For Expt. 3, all rooted cuttings were randomly assigned evenly among four blocks (See Table 4 for actual numbers planted in Expt. 3). Since numbers of cuttings from each rooting container were unequal, the numbers of treatment plots were unequal within blocks. A planting bar was used to plant rooted cuttings approximately 5 cm deep, measured from the highest root, on December 20, 1998 in Glenville, Georgia, in a portion of the Rayonier, Inc., seedling nursery. Plant spacing was 0.3 m x 0.3 m. Root systems of cuttings in all Ray Leach Conetainers™ in both experiments were severed approximately 14 cm below the highest root to increase root branching and inhibit narrow, girdled root systems. Two rows of commercial bare-root seedlings were planted as borders around the experiments, but none were planted between blocks.

The field site consisted of a sandy loam nursery soil normally used to germinate and grow bare-root seedlings of loblolly pine for reforestation. The site was chosen to provide uniform soil conditions for short-term growth and was not intended as a transplanting step prior to deployment. The soil was relatively well drained, but was disked prior to planting to break a shallow hardpan. Plants received Oust (sulfometuron; 2-[[[[(4,6-dimethyl-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]benzoic acid) at 73.2 ml ha^{-1} twice during the growing season (1999) to inhibit weed competition, and were fertilized through overhead irrigation periodically.

All plants were lifted approximately 1 year later by digging a 0.3 m x 0.3 m square area around each individual plant. Most vertical roots were excavated. Root system symmetry, rcd, stem height, shoot dw, and root dw were recorded for each plant. Primary and secondary roots were not separated, but were dried as described previously. Root system symmetry was measured by focusing on both specific roots and the overall distribution of the root system. Root systems were considered symmetrical if two or more roots were $\geq 130^{o}$ apart, using the stem as the vertex, and root systems were distributed horizontally in a similar manner. Root systems were considered asymmetrical if two roots were $\geq 130^{o}$ apart, but if little or no horizontal root branching had occurred during the growth period. This method of measurement emphasizes horizontal root distribution arising from lateral branching and is different from the method used to evaluate symmetry after greenhouse rooting.

During the growing season an outbreak of phytophthora root rot (*Phytophthora* sp.) occurred in both field experiments. In Expt. 2, 38 plants appeared yellowish, stunted, and possessed short, stringy, root systems with very little secondary branching. In Expt. 3, only three plants appeared infected. Data from infected plants were included for percent survival but not for analysis of root and shoot variables.

Statistical Analyses

Data were tested for normality and homogeneity of variances by univariate procedures, using SAS v. 8.2 (SAS Institute, Cary, NC), prior to analysis of variance (ANOVA) procedures. Data for rooting percentage were transformed using the arcsine square root prior to ANOVA procedures. Shoot dw:root dw data were log transformed.

Primary, secondary, and total root dw data were transformed using the square root of the data to improve normality. There were no differences between analyses of raw data and transformed data in the ANOVA for any variable. Therefore, data presented are based on nontransformed values. When main effects were significant, treatment means were compared to control means using Fisher's protected least significant difference (LSD). When the family x container interaction was significant, a Dunnett's test was used to compare the response of one family in a pellet to its response in the control. Multiple comparisons among families, pellet sizes, and the control were not performed. For example, the response of cuttings from family C, rooted in 25-65 mm pellets, was not compared to the response of cuttings of family B rooted in the control. When appropriate and significant, regression analyses determined the relationships between cuttings rooted in the Jiffy pellets and the dimensions of the pellets, excluding data for the control.

In Expt. 2, all field phase data were not normally distributed. The log transformation significantly improved distribution and these data were used in all analyses. For percent field survival after 1 year, data were transformed using the arcsine square root. Data for the control were excluded and regression analysis determined the relationship between growth data and volumes of the rooting pellets. In Expt. 3, data for root dw and shoot dw were transformed using the square root and log functions, respectively, prior to all analyses. Distribution was improved significantly; however, no differences were found between analyses using transformed or nontransformed data in either experiment. Therefore, means presented in tables and figures for both field growth phases of the experiments are those of nontransformed data. Unfortunately, in Expt. 3, all dw data in block 3 and some in block 4 were lost, so data for dws were analyzed with only three blocks. All other field data recorded in Expt. 3 remained intact. To account for unequal numbers of observations among treatments and blocks in both experiments, least squares means were used to compare pellet with control responses when the probability of a greater F-value for the main effect was < 0.05.

Results and Discussion

Rooting Percentage

Overall, rooting was 72% and 62% for Expts. 1 and 2, respectively. In Expt. 1, rooting percentage was affected by family, container, and family x container (Table 5). With the exception of cuttings of family B rooted in 36-65 mm and 42-65 mm pellets, cuttings from all families set in 25-65 mm, 30-65 mm, 36-65 mm, 36-75 mm, and 42-65 mm pellets rooted equal to those set in the control (Fig. 1I). All families rooted less than or equal to the control in 42-80 mm and 50-95 mm pellets. When data for the control were excluded and rooting percentage by family was expressed as a function of pellets per square meter (density), rooting percentage exhibited a linear and quadratic relationship with density (Fig. 2). Families B and D responded equally at all pellet densities as indicated by similar regression equations (Fig. 2). Stem cuttings from family C had higher rooting percentages at all pellet densities, especially at lower densities, whereas families B and D rooted poorly; this was also indicated by the family x container interaction for rooting percentage in Table 5. Increased density of pellets creates a dense canopy of foliage that intercepts more mist, increases rh surrounding the stem cuttings, and prevents excessive mist from falling to the substrate of the pellets (peat). Thus, the low rooting percentages observed for pellet sizes 42-80 mm and 50-95 mm could have been a combination of low rh surrounding the cuttings and low oxygen concentrations near the stem bases in the rooting substrate.

In Expt. 2, rooting percentage was affected by family and container (Table 6). Similar to Expt. 1, cuttings of family C rooted higher (68%) than those of families B (60%) or D (59%), which rooted equally. Stem cuttings rooted higher in 36-65 mm pellets than in the control (77% vs. 64%), whereas cuttings in 42-80 mm (49%) and 50-95 mm (52%) pellets rooted less than cuttings in the control (Fig. 3). Rooting percentages of cuttings in pellets, however, excluding the control, did not exhibit a linear or quadratic relationship with pellet density, or any other dimensions of the Jiffy pellets when tested using regression analyses.

In Expts. 1 and 2, rooting was generally higher for cuttings set in the smaller pellets (i.e., 25-65, 30-65, 36-65, and 42-65 mm) than in larger pellets (i.e., 42-80 and 50-95 mm).

Cuttings of subtropical pine species (*Pinus* L. sp.) also rooted at high percentages in the smaller pellets but were not tested in the larger pellets (Wright et al. 1999). Cuttings of American sweetgum clones rooted well in all sizes of Jiffy Forestry Peat pellets, including the larger pellets (Gocke et al. 2000). Because optimal mist application for stem cuttings can differ due to pellet density or size, an industrial supporter of the research conducted a separate study to test mist application rates between some smaller-sized pellets and these same larger-sized pellets. Although specific results are proprietary, cuttings of loblolly pine rooted poorly in the larger pellet sizes regardless of the amount of mist applied (personal communication, Ben Cazell, Rayonier, Inc. Yulee, FL).

Mean rooting for Expt. 3 was 56%. Family significantly affected rooting percentage and rcd ($P < 0.05$), but family and rooting container did not affect any other root or shoot variables measured (ANOVA table not presented). Rooting percentages for families G (75%) and I (61%) were similar, but greater than that of family K (31%). The low overall rooting percentage in Expt. 3, as compared to Expts. 1 and 2, could have been due to reduced rooting ability of families chosen for Expt. 3, particularly family K. In other species, Keever and Cobb (1988) also reported no effect of container on rooting percentage, however, we are not suggesting this is true for every species and/or container. Even though the dimensions of the rooting containers in Expt. 3 did not affect rooting percentage or root system morphology, the merits of each container might be more evident in investigations of substrate moisture availability and various substrates.

Root And Shoot Morphology of Rooted Cuttings

In Expt. 1, number of primary roots, root system symmetry, and shoot height were all affected by family and container (Table 5). Number of primary roots and root system symmetry were also affected by the family x container interaction, and rcd was influenced by family (Table 5). With the exception of cuttings of family B rooted in 36-65 mm, 42-80 mm, or 50-95 mm pellets, and cuttings of family D rooted in 42-80 mm pellets, cuttings from all families set in all pellets sizes produced greater (family C) or equal numbers of roots per cutting and had similar root system symmetry as those set in the control (Fig. 1II and III). The family x container interactions for root number and symmetry (Table 5) were due to magnitude changes among families in each rooting container and not to rank changes among families (Fig. 1II and III). Generally, cuttings of family C produced more roots per cutting (6.0) than families B (3.6) or D (4.2), which produced statistically similar numbers of roots per cutting. Cuttings of family C produced more symmetrical root systems (77%) than cuttings of families B (51%) or D (58%), which were similar. Rooted cuttings of families C and D had equal but greater rcds (2.4 mm) than cuttings from family B (2.2 mm), although a 0.2 mm difference between families may not be biologically significant. Mean stem height per rooted cutting was lowest for cuttings rooted in the larger 42-80 mm (12.9 cm) and 50-95 mm (11.9 cm) pellets compared to the control (14.1 cm). Mean stem height of all other cuttings in all other pellets (13.7 cm) was equal to the control.

In Expt. 2, number of new roots, root system symmetry, and rcd were affected by family, while stem height was unaffected (Table 6). Similar to Expt. 1, cuttings from family C produced more roots per cutting (3.7) than families B (2.4) or D (2.5), which were equal. Moreover, cuttings from family C had a higher percentage of symmetrical root systems (57%) in all rooting containers than families B (30%) or D (35%), which were equal. Rcd of rooted cuttings was equal for families C and D (2.23 mm), but greater than family B (2.07mm). Stem height of all rooted cuttings in all rooting containers was 10.6 cm. In Expt. 3, rcd of stem cuttings of family I (2.6 mm) was larger than those of families K (2.4 mm) and G (2.3 mm), which were equal. Stem height was 10 cm for all cuttings.

Collectively, root and shoot system morphology was determined largely by genetic control of the root system and to a lesser extent by the type of rooting container. As is common with many studies utilizing several unrelated families (or clones), either one family surpasses the others entirely, or each family is equal to one family but different from the remaining families when comparing several variables (Anderson et al. 1999, Frampton et al. 1999, Gocke et al. 2000, Wright et al. 1999,). In some cases, the family with the highest rooting percentage also has a high-quality root system (Frampton et al. 1999). Moreover, genotype may affect the initial rcd of the stem cutting and subsequently the rcd measured after rooting. The effect of the rooting container on root and shoot morphology may not have been fully realized because data were recorded only 12 weeks after setting. Had data been recorded later, more differences due to container type might have been evident. Depending on the species, some containers may severely limit root system quality regardless of when data are recorded (e.g., stem cuttings of loblolly pine set in 42-80 mm pellets and to a lesser extent 50-95 mm pellets). Overall root evaluation of three sub-tropical pine species was better in smaller Jiffy pellets than in a traditional plastic container filled with 1 sifted coal ash : 1 mineral soil; however, the effect was reversed with clones of rose gum (Wright et al. 1999). In the present investigation, most cuttings set in Jiffy pellets performed similarly to the Super cell control, therefore, container choice might depend on the interaction between species, rooting substrates, and when the resulting propagules are field planted.

Dry Weight of Rooted Cuttings After Rooting

In Expt. 1, primary and total root dw was affected by family, container, and family x container, while secondary root dw was affected by family and container (Table 5). Primary and total root dw of cuttings of most family by pellet combinations, with few exceptions for cuttings of family C, was less than those in the control (Fig. 4I and III). Mean secondary root dw per rooted cutting in 30-65 mm (0.07 g) or 50-95 mm (0.07 g) pellets was equal to the control (0.09 g), whereas, secondary root dw of rooted cuttings in all other pellet sizes was less than in the control (Fig. 4II). Shoot dw was affected by family while shoot dw:root dw was affected by container (Table 5). Shoot dw per cutting was greater for family C (1.2 g) than for families B (1.0 g) or D (1.1 g), which were equal. Shoot dw:root dw for all pellet sizes (25-65 mm = 8.06, 30-65 mm = 5.3, 36-65 mm = 7.7, 36-75 mm = 6.8, 42-65 mm = 7.9, 42-80 mm = 15.7, and 50-95 mm = 7.3) were significantly greater than the control (3.5). Dry weight variables were not related linearly or quadratically to pellet volume or any other dimension of Jiffy pellets when data for the control were excluded and the data were subjected to regression analyses.

In Expt. 2, primary, secondary, and total root dw per rooted cutting was affected by container (Table 6). Primary (0.07 g) and total root dw (0.10 g) for cuttings in the control was higher than those rooted in 25-65 mm (0.04 g and 0.05 g), 30-65 mm (0.04 g and 0.06 g), 36-65 mm (0.04 g and 0.05 g), or 42-65 mm (0.05 g and 0.07 g) pellets. Secondary root dw for cuttings rooted in the control (0.03 g) was higher than those for cuttings rooted in 25-65 mm (0.01 g) or 36-65 mm (0.01 g) pellets. Excluding data for the control, primary, secondary, and total root dws per rooted cutting increased linearly as volume of the rooting pellet increased (Fig. 5). In Expts. 1 and 2, however, root growth in smaller pellets extended into adjacent pellets and resulted in loss of primary and secondary root mass during destructive harvests. The relationship with volume could reflect the greater percentage of root mass harvested from the larger pellets, rather than larger pellets directly inducing growth of greater root mass. Widespread co-mingling of roots into adjacent pellets can be prevented by regulating the humidity in the propagation greenhouse after rooting, or transplanting rooted cuttings prior to extensive root growth (Baliskey et al 1995, Wright et al. 1999). Shoot dw was affected by family and container, whereas shoot dw:root dw ratio was not affected by any factor (Table 6). Shoot dw per rooted cutting in 25-65 mm (0.65 g) pellets was lower than the control (0.72 g), whereas shoot dw in all other pellet sizes was equal to the control. Shoot dw was not related linearly or quadratically to the dimensions of the Jiffy pellets using regression analyses. Shoot dw:root dw for all rooted cuttings in all containers was 9.5. All rooted cuttings in Expt. 3 produced a mean of 2.4 roots per rooted cutting and the root system weighed 0.18 g regardless of family or rooting container.

In Expts. 1 and 2, cuttings rooted in the larger Jiffy pellets (42-80 and 50-95 mm) had root systems equal to, or very similar in either dw or quality, to root systems produced in the control. Rooting percentage was very poor across families in these pellets, however, which would ultimately require setting more cuttings to achieve the same number of rooted cuttings as the smaller pellets or control. As a result, the additional area required to accommodate the larger pellets, and the additional cuttings and pellets required to compensate for reduced rooting would make use of these pellet sizes unsatisfactory. Use of smaller pellets on an operational scale would have to allow for roots growing into adjacent pellets, especially when pellet density per unit area was high.

After rooting in the greenhouse for 12 weeks, all of these rooted stem cuttings would have been considered “culls” because rcds were < 3 mm (South 2000, Wakeley 1954). Generally, succulent stem cuttings collected and set for rooting in Raleigh, N.C., are unlikely to grow as well as those in more southerly locations due to earlier onset of cold temperatures in late fall. During the 2-month growth period following rooting in Expts. 2 and 3, but prior to field planting, roots had ample time to reach and exceed the capacities of their original rooting containers. The effect of container volume was probably greatest at this stage, causing the initial measured differences in root systems to increase further before field planting. Although data were not collected at this time, some rooted cuttings might have been classified as "plantable" or "acceptable" (slightly larger than a cull, rcd > 3 mm) (South 2000, Wakeley 1954). This number could have been greater had fertilizer been applied during or after the rooting period and continued until field planting. Moreover, if dormant cuttings rooted in Expt. 1 had been grown and field planted instead of softwood cuttings in Expts. 2 or 3, plants presumably would have had greater root dws after a longer growing period and would have been much larger prior to field planting.

Field Phase (Expts. 2 and 3)

Mean field survival after 1 year was 96% in Expt. 2, and was affected significantly by family (Table 7). Plants from family B had only 92% survival, whereas, 98% of families C and D survived.

Container type significantly affected shoot height, rcd, root system symmetry, root dw and shoot dw (Table 7). Shoot height of cuttings rooted originally in 42-80 mm (38.2 cm) or 50-95 mm pellets (40.7 cm) was equal to the control (41.6 cm), while heights for plants in all other pellet sizes were shorter than the control (Table 8). Rcd of plants in 50-95 mm pellets (9.9 mm) was equal to the control (10.4 mm), while rcds of plants in all other pellet sizes were thinner than the control (Table 8). The percentages of symmetrical root systems of plants in all pellet sizes were greater than those of the control (Table 8). Cuttings rooted in Jiffy pellets had more horizontal root growth into the surrounding substrate, perhaps due either to the air pruning design of the pellets, or indirectly, by the researchers pruning the roots to separate the plants prior to planting. We observed, as did Baliskey et al. (1995), that root systems produced in plastic containers tended to grow vertically rather than horizontally. Differences in root symmetry between the rooting phase and field growth phase have been reported previously for loblolly pine, especially if lateral root growth is also evaluated during field performance (Goldfarb et al. 1998). Nevertheless, plants in the control produced greater root mass (20.7 g) than all plants with the exception of those produced in 50-95 mm pellets (20.7 g). Shoot mass was greater in the control (29.0 g) than for plants in all other pellet sizes (Table 8). Shoot dw: root dw averaged 1.5 for all plants in all container types.

Shoot height, rcd, root dw, and shoot dw in Expt. 2 all increased linearly with increasing pellet volume when data for the control were excluded (Fig. 6). No relationship was noted between number of primary roots, root system symmetry, rcd, shoot height, or shoot dw and pellet volume after the initial greenhouse rooting phase. Root dw was related linearly to pellet volume prior to field planting, and this relationship persisted in the field after 1 year. Root mass is thought to be a more important predictor of shoot height during field growth than other morphological factors such as number of roots, root orientation, or root system symmetry (Foster et al. 2000, Frampton et al. 2002, Goldfarb et al. 1998). These results support total root mass of cuttings prior to planting as a good indicator of shoot height after 1 year of field growth.

In Expt. 3, field survival was 89% and was not affected significantly by family or container (ANOVA table not presented). Rcd (10.2 mm), percent symmetrical root systems (48%), or shoot dw: root dw (1.22) were not significantly affected by family or container type. Shoot height, shoot dw, and root dw were influenced by container, but not by any other factors (ANOVA table not presented). Plants from stem cuttings of all families rooted in the Super cell controls were 51 cm tall, whereas, cuttings were 40 cm or 42 cm tall from the Pine or Stubby cells, respectively. Similarly, shoot dw and root dw were greater for cuttings in Super cells (shoot dw = 35.4 g, root dw = 27.3 g) than for cuttings in either the Pine cells (shoot dw = 21.1 g, root dw = 18.5 g) or Stubby cells (shoot dw = 18.2 g, root dw = 16.6 g). Similar to Expt. 2, the larger volume of the Super cell probably accounted for pre-plant differences in root mass among containers that persisted in the field after 1 year.

Rooting succulent stem cuttings in Ray Leach Super cells and then field planting for 1 year can produce plants similar in height to plants produced from other production systems after 1 year of field growth. Mean heights of plants in Ray Leach Super cells in Expt. 2 and 3 (47 cm) were slightly shorter than those reported in Goldfarb et al. (1998) (53 cm), Foster et al. (2000) (55 cm), or Frampton et al. (2000) (56 cm). Plants in those reports, however, may have been larger because plants were transplanted to nursery beds (Goldfarb et al. 1998) or to larger containers (Foster et al. 2000, Frampton et al. 2000) and grown for a longer period of time prior to field planting. Height growth increment of plants produced from the Ray Leach Super cell control in Expts. 2 and 3 (32 cm) were greater than those of plants produced in Jiffy pellets (22 cm) in Expt. 2, or bare-root (24 cm) in Goldfarb et al. (1998). Moreover, in Expts 2 and 3, plants might have been larger if fertilizer been applied to cuttings after rooting and grown outdoors rather than in the greenhouse before field planting.

Conclusions These results suggest two systems for producing rooted stem cuttings of loblolly pine. One would set and root stem cuttings in rigid plastic containers. Depending on the time interval between rooting and deployment, plant size at field planting would be influenced by the dimensions of the container, i.e., overall volume and depth. Rooting in individual containers (direct rooting), rather than flats, raised beds, or nursery soil facilitates successful transplanting, because root systems are less disturbed (Frampton et al. 1999). Plastic containers are also reusable and can establish a deeper root system which can improve survival in nonirrigated fields. For example, loblolly pine seedlings produced in Ray Leach containers prior to field planting had higher survival rates than bare-root seedlings or seedlings produced in open trays (Anderson et al. 1984). Container storage, cost, usable life, and the sanitation required after storage, however, are notable considerations when choosing a rigid container production system.

A second production system would involve stem cuttings rooted in smaller Jiffy pellets. Pellet size would depend on total propagation area and production system objectives. Extended growth periods after rooting in smaller pellets, however, would increase root growth into adjacent pellets, which eventually would result in root loss when separating pellets for field planting. An alternative solution would be transplanting the cuttings after a limited number of weeks in the propagation structure into an outdoor nursery bed and growing them similarly to seedlings produced for bare-root field planting. Early transplanting of cuttings of other woody species rooted in containers has enhanced subsequent root and shoot growth (Knight et al. 1993). This would allow rooted cutting and seedling production systems to be interfaced. Seeds are currently sown directly into nursery soil and then seedlings are lifted after a period of growth for cold storage and/or deployment. Direct setting of stem cuttings or transplanting rooted cuttings into nursery beds would supplement this process. Moreover, machinery and labor used for cultural practices and lifting bare-root seedlings could be used for rooted cuttings. Post-harvest procedures would be similar for both stock types. Notable considerations for Jiffy pellets include the cost of pellets for each use, an additional transplanting step, storage of pellets prior to use, and root growth into adjacent pellets.

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Table 1. Dimensions of Jiffy® Forestry Peat pellets and a control [Ray Leach Super cell (vol. = 164 ml) containing a substrate of 2 peat : 3 perlite (v/v)] used in Expts. 1 and 2 for rooting juvenile stem cuttings of loblolly pine.

Peat pellet	Dry diameter (mm)	Expanded diameter (mm)	Expanded height (mm)	Expanded volume (ml)	Pellets/unit area (m^2)
25-65	25	28	65	35	1130
30-65	30	33	65	60	732
36-65	36	39	65	80	592
36-75	36	39	75	90	592
42-65	42	46	65	110	355
42-80	42	46	80	140	355
50-95	50	55	95	250	226
Control	n/a	38	210	164	527

Table 2. Dimensions of Ray Leach Conetainers™ used in Expt. 3 to root succulent, juvenile stem cuttings of loblolly pine. All containers were filled with a substrate of 2 peat : 3 perlite (v/v).

Conetainer Type	Diameter (mm)	Height (mm)	Volume (ml)	Containers/ unit area (m^2)
Pine cell	25	160	66	1076
Super cell “Stubby”	38	140	115	528
Super cell (control)	38	210	164	528

Table 3. Number of rooted cuttings in Expt. 2 produced from three families of loblolly pine rooted originally in six sizes of Jiffy® Forestry Peat pellets and a control [Ray Leach Super cell (vol. = 164 ml) containing a substrate of 2 peat : 3 perlite (v/v)] and then field planted in December 1998.

	Jiffy® Forestry Peat Pellets							
Family	25-65 mm	30-65 mm	36-65 mm	42-65 mm	42-80 mm	50-95 mm	Control	Total
B	21	29	33	26	20	16	30	175
C	19	32	34	28	17	28	24	182
D	14	25	31	22	17	16	24	149
Total	54	86	98	76	54	60	78	506

Table 4. Number of rooted cuttings in Expt. 3 produced from three families of loblolly pine rooted originally in three sizes of Ray Leach Conetainers™ and then field planted in December 1998. All containers were filled with a substrate of 2 peat : 3 perlite (v/v) and the Super cell served as the control.

	Ray Leach Conetainers™			
Family	Pine cell	Stubby cell	Super cell (control)	Total
G	16	15	16	47
I	11	12	13	36
K	6	4	9	19
Total	33	31	38	102

Table 5. ANOVA for Expt. 1 on the effects of family and rooting container on rooting percentage and growth responses of juvenile dormant stem cuttings of loblolly pine rooted in seven sizes of Jiffy® Forestry Peat pellets and a control [Ray Leach Super cell, vol. = 164 ml containing a substrate of 2 peat : 3 perlite (v/v)]. Values in bold type are significant at $P < 0.05$.

Source	df	Rooting (%)	No. of primary roots per cutting	Root system symmetry	Root collar diameter	Shoot height
Rep	7	**0.02**	**0.04**	0.14	**0.01**	**0.01**
Family (F)	2	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**
Container (C)	7	**0.01**	**0.01**	**0.01**	0.25	**0.01**
F x C	14	**0.01**	**0.01**	**0.01**	0.86	0.18

Source	df	Shoot dw	Primary root dw	Secondary root dw	Total root dw	Shoot dw: total root dw
Rep	7	**0.01**	**0.01**	0.11	**0.01**	0.17
Family (F)	2	**0.01**	**0.01**	**0.01**	**0.01**	0.12
Container (C)	7	0.31	**0.01**	**0.01**	**0.01**	**0.01**
F x C	14	0.54	**0.01**	0.18	**0.01**	0.59

Table 6. ANOVA for Expt. 2 on the effects of family and rooting container on growth response variables of succulent juvenile stem cuttings of loblolly pine rooted in six sizes of Jiffy® Forestry Peat pellets and a control [Ray Leach Super cell (vol. = 164 ml) containing a substrate of 2 peat : 3 perlite (v/v)]. Values in bold type are significant at *P* < 0.05.

Source	df	Rooting (%)	No. of primary roots per cutting	Root system symmetry	Root collar diameter	Shoot height
Rep	7	**0.01**	0.87	0.87	**0.01**	**0.01**
Family (F)	2	**0.04**	**0.01**	**0.01**	**0.01**	0.34
Container (C)	6	**0.01**	0.35	0.75	0.62	0.33
F x C	12	0.80	0.73	0.24	0.74	0.30

Source	df	Shoot dw	Primary root dw	Secondary root dw	Total root dw	Shoot dw: total root dw
Rep	7	0.22	**0.01**	0.19	**0.01**	0.33
Family (F)	2	**0.01**	0.06	0.66	0.14	0.12
Container (C)	6	**0.04**	**0.01**	**0.01**	**0.01**	0.50
F x C	12	0.60	0.53	0.11	0.37	0.06

Table 7. ANOVA for Expt. 2 on the effects of family and rooting container on growth characteristics of rooted cuttings of three full-sibling families of loblolly pine rooted initially in six sizes of Jiffy® Forestry Peat pellets and a control [Ray Leach Super cell (vol. = 164 ml) containing a substrate of 2 peat : 3 perlite (v/v)] and then field planted for 1 year. Values in bold type are significant at $P < 0.05$.

Source	df	Survival	Height	Root collar diameter	Root system symmetry	Root dw	Shoot dw	Shoot dw: root dw
Rep	7	0.75	**0.03**	**0.01**	**0.02**	**0.01**	**0.02**	**0.01**
Family (F)	2	**0.03**	0.54	0.10	0.07	0.07	0.15	0.12
Container (C)	6	0.10	**0.01**	**0.01**	**0.03**	**0.01**	**0.01**	0.49
F x C	12	0.13	0.50	0.26	0.15	0.51	0.46	0.83

Table 8. Growth variables in Expt. 2 after 1 year of field growth for juvenile, succulent stem cuttings rooted initially in Jiffy® Forestry Peat pellets and a control [Ray Leach Super cell (vol. = 164 ml) containing a substrate of 2 peat : 3 perlite (v/v)] and then field planted in December 1998. Means are averaged over eight blocks and three full-sibling families for each container. An asterisk within a column denotes a significant difference from the control at *P* < 0.05 using LSMEANS.

Rooting container (n)	Expanded vol. (ml)	Survival (%)	Stem height (cm)	Root collar diameter (mm)	Root system symmetry (%)	Root dw (g)	Shoot dw (g)
25-65 mm (38)	35	89	35.8*	8.4*	40.6*	11.9*	16.5*
30-65 mm (74)	60	96	33.9*	8.2*	52.4*	10.9*	15.3*
36-65 mm (86)	80	96	36.5*	8.7*	58.5*	12.6*	18.0*
42-65 mm (66)	110	95	37.9*	8.7*	42.8*	12.2*	17.8*
42-80 mm (50)	140	96	38.2	9.1*	59.8*	14.1*	20.9*
50-95 mm (59)	250	99	40.7	9.9	60.4*	17.7	25.2*
Control (73)	164	99	41.6	10.4	21.1	20.7	29.0

Fig. 1. (I) Rooting percentage, (II) number of new roots per cutting, and (III) root system symmetry for juvenile, hardwood stem cuttings of three families (B,C, D) of loblolly pine rooted in seven sizes of Jiffy Forestry Peat Pellets, designated by dry diameters - expanded heights, in Expt. 1. The control was a Ray Leach Super cell (vol. = 164 ml) containing a substrate of 2 peat : 3 perlite (v/v). For each graph, the hatched, unfilled, cross hatched, and filled bars are families B, C, D, and the mean, respectively. In all graphs, the response of family B rooted in the control is represented by the broken line, the solid line is family C, and the dotted line represents family D. An asterisk denotes a significant difference between the response of a family in the pellet and its response in the control, as determined by a Dunnett's test ($P < 0.05$). Multiple comparisons among all family-by-pellet combinations and the control were not performed.

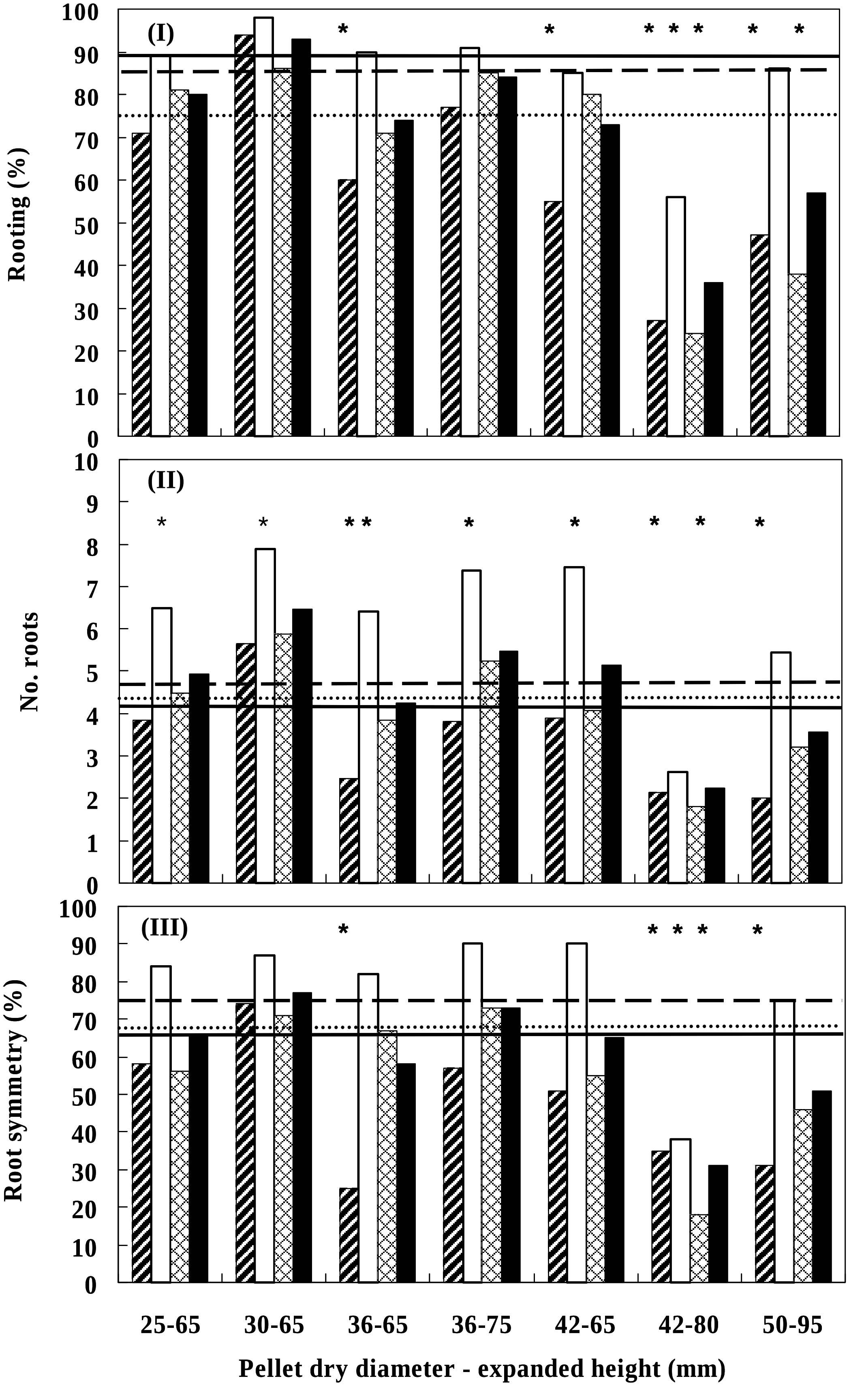

100
90
80
70
60
50
40
30
20
10
0
Rooting (%)
(I)
*
*
* * *
*
*
10
9
8
7
6
5
4
3
2
1
0
No. roots
(II)
*
*
* *
*
*
*
*
*
100
90
80
70
60
50
40
30
20
10
0
Root symmetry (%)
(III)
*
* * *
*
25-65
30-65
36-65
36-75
42-65
42-80
50-95
Pellet dry diameter - expanded height (mm)

Fig. 2. Rooting percentage in Expt. 1 as a function of Jiffy® Forestry Peat pellet density per square meter for three families of juvenile hardwood stem cuttings of loblolly pine. Regression equations are: Rooting in family B (bottom solid line) = - 0.049 + 0.002(density) – 0.000001(density^2), P = 0.01, r^2 = 0.42; Rooting in family C (top solid line) = 0.60 + 0.0007(density) - 0.0000004(density^2), P = 0.05, r^2 = 0.11; and rooting in Family D (middle broken line) = - 0.005 + 0.002(density) – 0.000001(density^2), P = 0.01, r^2 = 0.40.

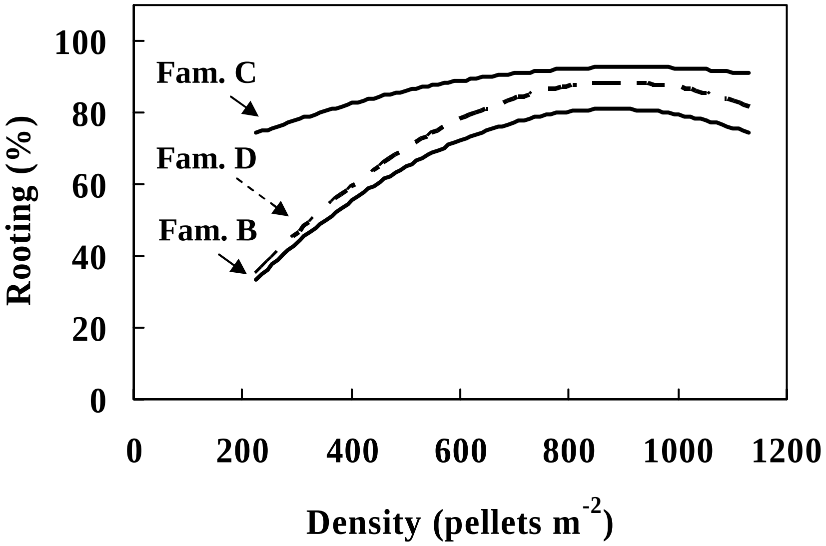

Fam. C
Fam. D
Fam. B
Rooting (%)
0
20
40
60
80
100
0
200
400
600
800
1000
1200
Density (pellets m-2)

Fig. 3. Mean rooting percentage in Expt. 2 of juvenile softwood stem cuttings of three families of loblolly pine rooted in six sizes of Jiffy® Forestry Peat pellets, designated by their dry diameters - expanded heights, and a control [Ray Leach Super cell (vol. = 164 ml) containing a substrate of 2 peat : 3 perlite (v/v)] (solid line). Each bar and control are means of three families and eight blocks. Asterisks denote significant differences from the control at $P = 0.05$ using Fisher's protected LSD.

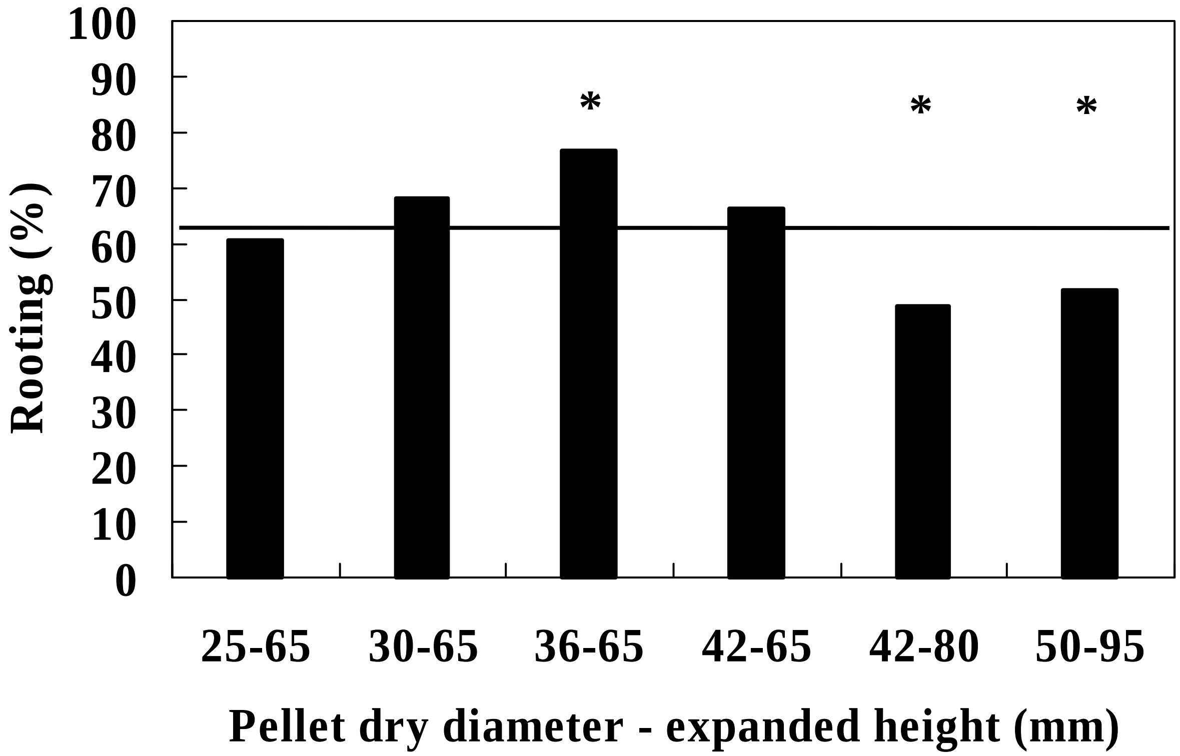

100
90
80
70
60
50
40
30
20
10
0
Rooting (%)
*
*
*
25-65
30-65
36-65
42-65
42-80
50-95
Pellet dry diameter - expanded height (mm)

Fig. 4. (I) Primary, (II) secondary, and (III) total root dry weight of juvenile hardwood stem cuttings of three families (B, C, and D) of loblolly pine rooted in seven Jiffy Forestry Peat Pellets, designated by dry diameters - expanded heights, and a control [Ray Leach Super cell (vol. = 164 ml) containing a substrate of 2 peat : 3 perlite (v/v)] in Expt. 1. For each graph, the hatched, unfilled, cross hatched, and filled bars are families B, C, D, and the mean, respectively. In (I) and (III), the response of family B in the control is represented by the broken line, the solid line is family C, and the dotted line represents family D. In graph (II) the solid line is the mean of all three families in the control. An asterisk denotes a significant difference between the response of a family in the pellet and its response in the control, as determined by a Dunnett's test (graphs I and III) or F-protected LSD (graph II) at $P = 0.05$.

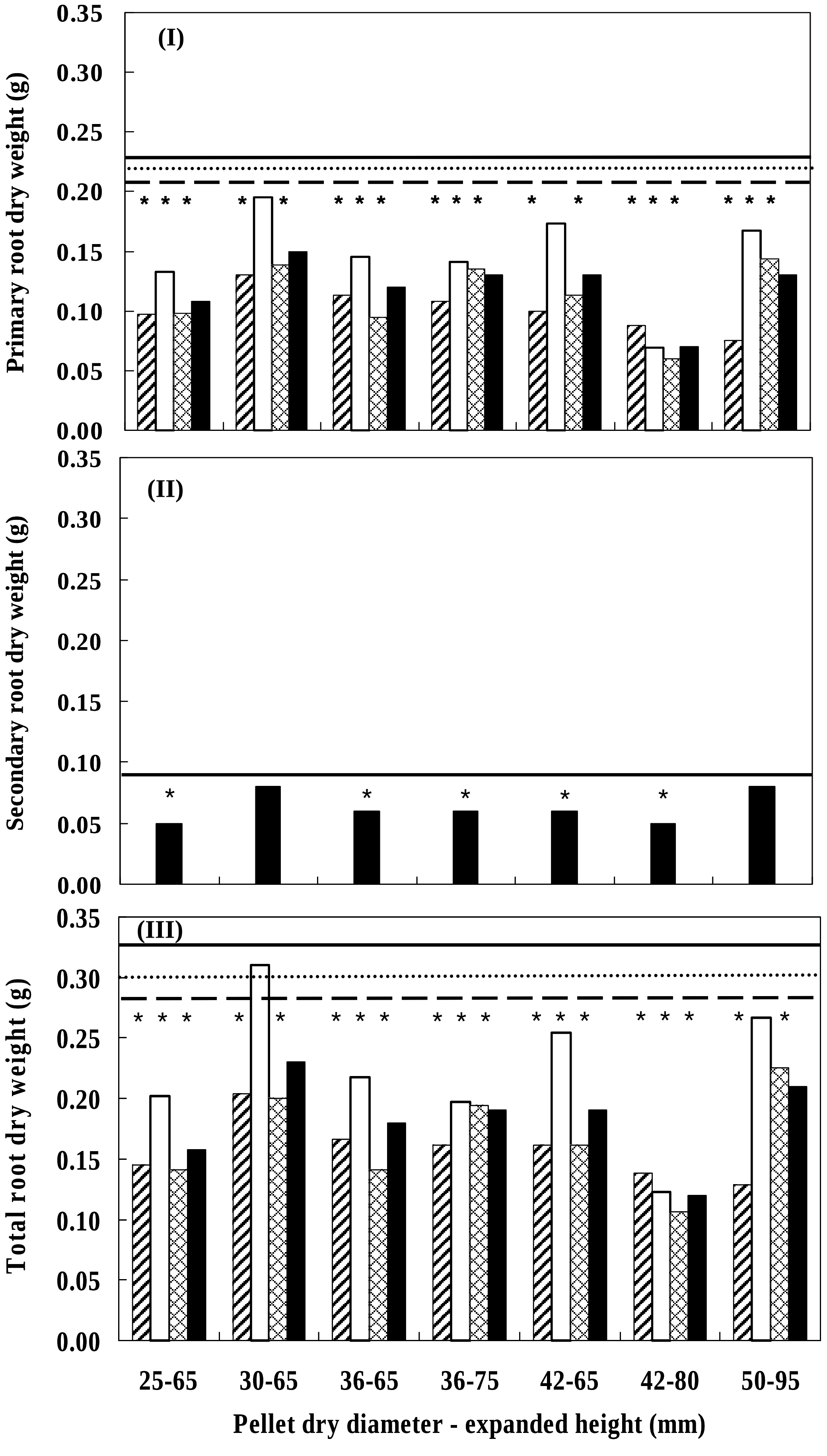

(I)
Primary root dry weight (g)
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
(II)
Secondary root dry weight (g)
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
(III)
Total root dry weight (g)
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
25-65
30-65
36-65
36-75
42-65
42-80
50-95
Pellet dry diameter - expanded height (mm)

Fig. 5. Primary, secondary, and total root dw in Expt. 2 of juvenile succulent stem cuttings of loblolly pine rooted in Jiffy® Forestry Peat pellets (designated by volume). Regression equations are primary root dw = 0.03 + 0.0001(vol.), $P = 0.01$ $r^2 = 0.72$; secondary root dw = 0.01 + 0.00009(vol.), $P = 0.01$, $r^2 = 0.76$; and total root dw = 0.04 + 0.0002(vol.), $P = 0.01$, $r^2 = 0.80$.

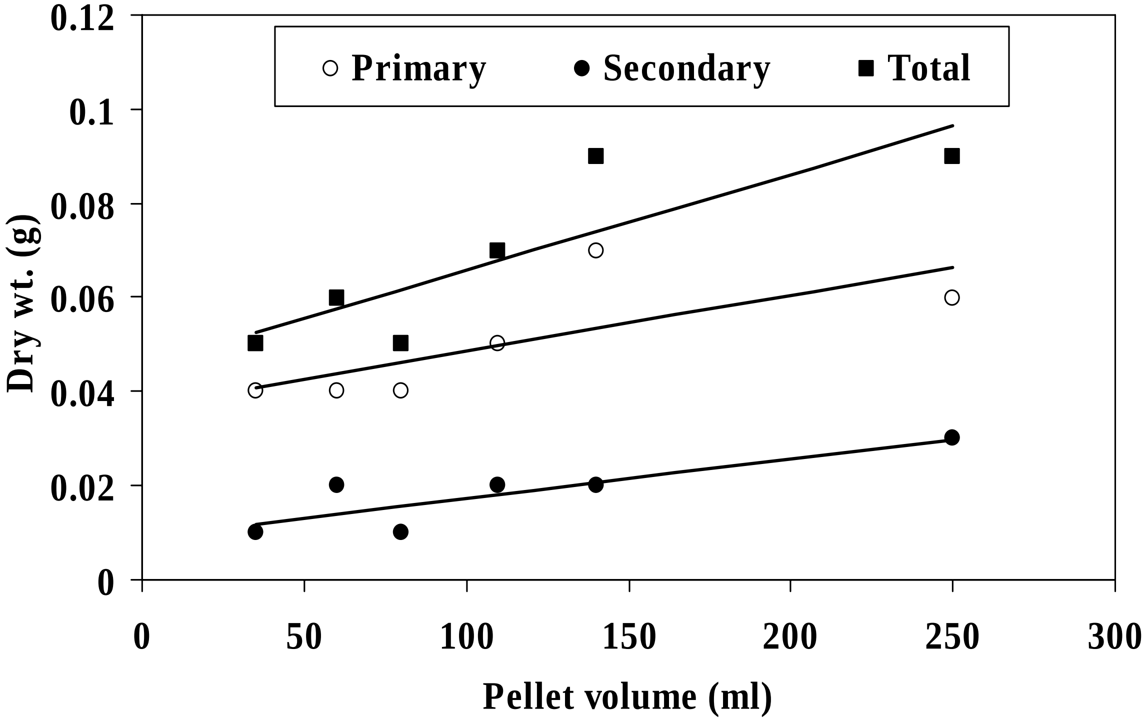
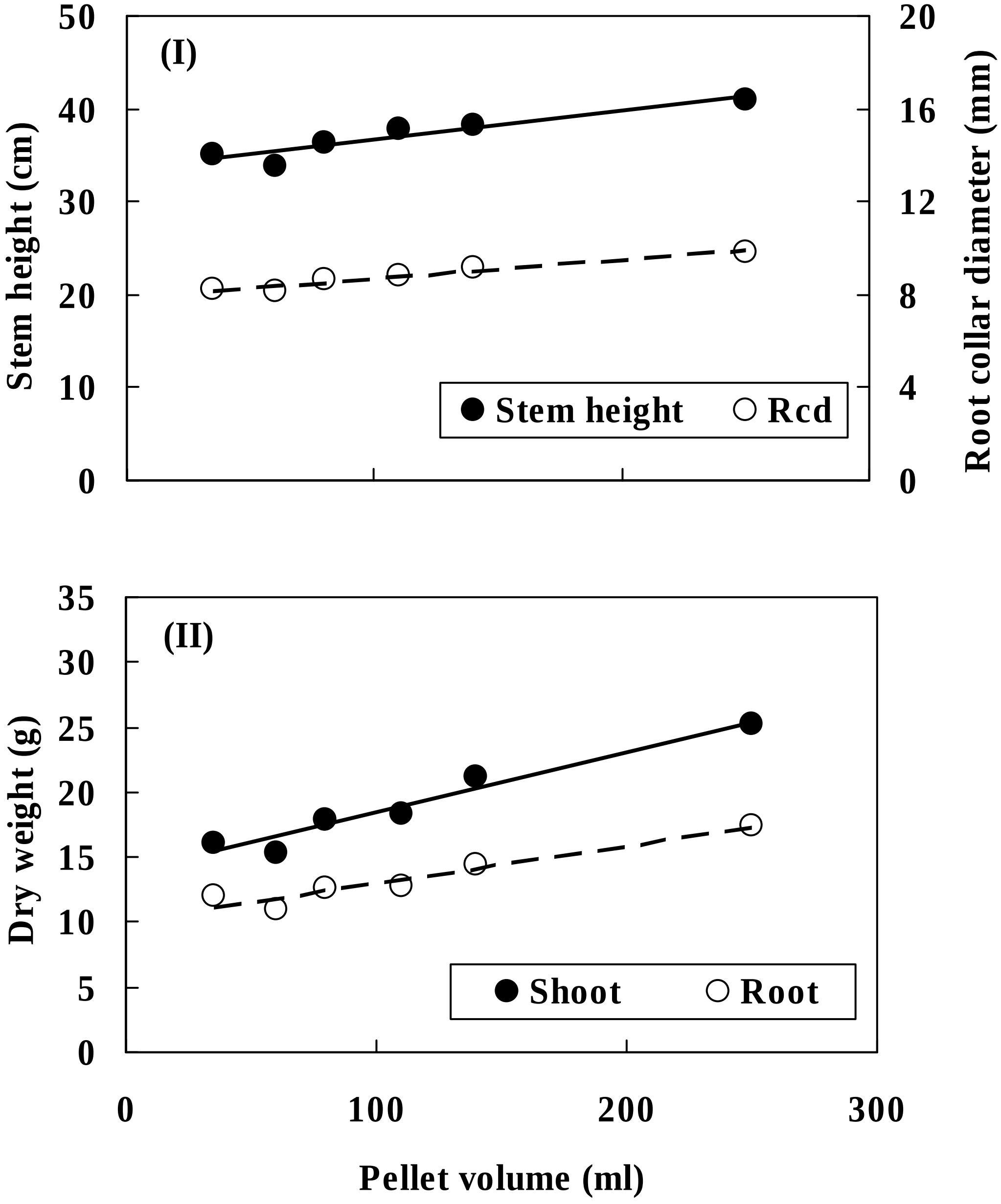

0.12
0.1
0.08
0.06
0.04
0.02
0
Dry wt. (g)
○ Primary
● Secondary
■ Total
0
50
100
150
200
250
300
Pellet volume (ml)

Fig. 6. Relationship between (I) stem height (filled symbols and solid line) or root collar diameter (rcd) (unfilled symbols and broken line) or (II) shoot dry weight (dw) (filled symbols and solid line) or root dw (unfilled symbols and broken line) of stem cuttings of three families of loblolly pine field grown for 1 year and the volume of the pellet in which the cuttings were rooted originally. Regression equations are (I) shoot height (cm) = 33.5 + 0.03(vol.), $P = 0.01$, $r^2 = 0.89$; rcd (mm) = 7.9 + 0.01(vol.), $P = 0.01$, $r^2 = 0.96$, and (II) shoot dw (g) = 13.8 + 0.05(vol.), $P = 0.01$, $r^2 = 0.95$; root dw (g) = 10.2 + 0.03(vol.), $P = 0.01$, $r^2 = 0.93$.


(I)
Stem height (cm)
Root collar diameter (mm)
0
10
20
30
40
50
0
4
8
12
16
20
● Stem height
○ Rcd
(II)
Dry weight (g)
0
5
10
15
20
25
30
35
● Shoot
○ Root
0
100
200
300
Pellet volume (ml)

Chapter 2

Mist, Substrate Water Potential, and Cutting Water Potential Influence Rooting of Stem Cuttings of Loblolly Pine

(In the format appropriate for submission to Tree Physiology)

Mist, substrate water potential, and cutting water potential influence rooting of stem cuttings of loblolly pine

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Running head: water relations during rooting of pine cuttings

Summary Four experiments were conducted over 2 years to investigate the influence of cutting water potential (Ψcut) on rooting of juvenile hardwood (dormant) and softwood (succulent) stem cuttings of loblolly pine (Pinus taeda L.) propagated under varying substrate water potentials (Ψsub) and volumes of mist application (mist levels). In year 1, mist level and Ψsub contributed to the Ψcut of nonrooted stem cuttings. In the second year, when Ψsub was held constant across various mist treatments, mist level contributed strongly to Ψcut. In year 1, Ψsub affected rooting percentage when mist was sub-optimal or excessive; otherwise, mist had a stronger effect than Ψsub on rooting percentage. For both years, cuttings rooted best when experiencing moderate water deficits (- 0.5 to - 1.2 MPa) measured weekly for 4 or 5 weeks during the beginning of the rooting period. Cuttings experiencing more severe water deficit or no water deficit rooted poorly. Results demonstrate that monitoring the physiological status of cuttings can provide important information for controlling the rooting environment such that propagators should not eliminate water deficit completely when rooting stem cuttings of loblolly pine.

Keywords: adventitious rooting, clonal forestry, Pinus taeda, vegetative propagation, water deficit, water relations.

Introduction

Propagation of loblolly pine (Pinus taeda L.) by juvenile stem cuttings, produced on recurrently sheared stock plants (hedges), can be used to multiply superior full-sib families and elite clones within superior families for reforestation (Zobel and Talbert 1984, Goldfarb et al. 1997, Frampton et al. 1999, 2000). Producing high quality rooted stem cuttings on a large scale, however, will require maintenance of a suitable rooting environment over large areas. Rooting environments can vary from polyethylene-covered greenhouses to semi-shaded structures and direct field-setting of stem cuttings. Thus, specific irrigation protocols may not be applicable to all rooting environments (Frampton and Hodges 1989, Gocke et al. 2000, Rowe et al. 1999). Therefore, a more physiological approach to understanding water relations of stem cuttings during the rooting period may aid the design and control of suitable propagation environments.

In most systems used for the rooting of stem cuttings, intermittent mist application minimizes water deficit by lowering leaf temperature and transpiration by increasing humidity surrounding the cuttings (Tukey 1978). It has been shown that the volume of water applied during intermittent mist application can affect rooting percentage (Greenwood et al. 1980, Loach 1988). Moisture in the substrate (rooting medium) is also important during rooting of woody ornamental species (Rein et al. 1991), especially when mist is not applied (Graves and Zhang 1996) or is applied infrequently to softwood cuttings (Giroux et al. 1999).

The water potential of a stem cutting (Ψ_{cut}) is a physiological indicator of the water deficit (Kramer and Kozlowski 1979) that results from the rate of transpiration exceeding water uptake (Grange and Loach 1983, Bray 1997). Although available water is not usually limiting during rooting, stem cuttings can experience highly negative values of Ψ_{cut}, presumably because of the absence of a root system for water uptake. To decrease water deficit, stem cuttings may either absorb applied mist through that portion of foliage above the rooting substrate or absorb water through the basal portion of the cutting inserted in the substrate. Although both modes of uptake have been reported to occur in stem cuttings of radiata pine (*Pinus radiata* D. Don.) (Cameron and Rook 1974, Cremer and Svensson 1979), the modes of uptake that contribute to Ψ_{cut} of loblolly pine under different environmental conditions have not been reported.

It has been suggested that rooting percentage is related linearly to Ψ_{cut} during the rooting period (Loach 1977, Loach and Whalley 1978, Hartmann et al. 2002). This implies that stem cuttings experiencing lower water deficits during the period of adventitious root formation will have a greater likelihood of root initiation and development. On the other hand, supra-optimal mist application, which decreases water deficit, also can decrease rooting (Greenwood et al. 1980, Harrison-Murray and Howard 1998). Decreased rooting under conditions of minimal water deficits, however, is usually attributed to reduced oxygen concentrations near root initials (Loach 1985, Soffer and Burger 1988) or, secondarily, to a buildup of disease-causing fungal organisms (Hartmann et al. 2002). Alternately, Harrison-Murray and Howard (1998) suggested that some degree of water stress might be required during the rooting period for adventitious root formation to occur. Moderate to severe short-term water deficits in stem cuttings of loblolly pine, prior to setting under optimal conditions, decreased percent rooting only slightly (Murthy and Goldfarb 2001). The degree of water deficit stem cuttings can experience during the rooting period that still permits adventitious rooting at high percentages is not well defined for any species.

In two studies investigating the effects of gradients in mist and substrate moisture on rooting percentage, treatments were created by positioning stem cuttings at various distances from a fixed mist nozzle placed above the rooting bed (Harrison-Murray and Howard 1998, Loach 1988). Although effective in determining the effect of overall moisture application, this method did not permit testing mist level and substrate moisture independently, because the factors were confounded. Moreover, a greater understanding of the effects of aerial mist and substrate moisture, their interaction on $\Psi_{cut,}$ and, ultimately rooting, would facilitate implementation of irrigation systems that would be applicable across rooting environments.

In the present investigation, we report on four experiments designed to study the relationship between rooting percentage and Ψ_{cut} in juvenile hardwood (dormant) and softwood (succulent) stem cuttings of loblolly pine and to determine how this relationship is affected by aerial mist and substrate water potential (Ψ_{sub}). In year 1, two experiments were conducted to determine the effects of the volume of mist delivered per application (mist level) and Ψ_{sub} on Ψ_{cut} and root formation. In the second year, two additional experiments tested the effects of various mist levels on Ψ_{cut} and root formation, with Ψ_{sub} held constant. In all four experiments, Ψ_{cut} was used as an independent variable to determine its relationship with rooting percentage.

Materials and Methods

Plant material

Two hundred seeds from two unrelated full-sib families (100 seeds per family) from a Florida provenance of loblolly pine were germinated June 1996. Individual seedlings were transplanted into 12-l containers containing a substrate of composted, shredded pine bark : sand (4:1 v/v) in May 1997. All stock plants were grown on an outdoor, gravel container pad at the North Carolina State University Horticultural Field Laboratory (lat.35°47′ N, long.78°39′ W). At the beginning of March and August of each year, seedlings were sheared to a height of approximately 15 cm, and maintained as hedged, stock plants (hedges) by removing all remaining terminal buds of lateral stems in accordance with protocols of Cooney and Goldfarb (1999). Irrigation was provided, as needed, by an overhead sprinkler system. Approximately 51g of Osmocote ($18N\text{-}6P_2O_5\text{–}12K_2O$), 8-9 month controlled-release fertilizer (Grace-Sierra, Milpitas, Calif.), was applied twice per year as a top-dress to each hedge after pruning to maintain tissue nitrogen levels according to Rowe et al. (2002). Other macro- and micronutrients were applied as indicated by periodic foliage analyses following recommendations for seedlings of loblolly pine (C.B. Davey and J.B. Jett, personal communication). Hand weeding was used as needed and several pesticides were applied throughout the growing season to control tip moth (*Rhyacionia frustrana* Comst.) and fusiform rust [*Cronartium quercuum* (Berk.) Miyabe *ex* Shirai f. sp. *fusiforme*]. The container pad was covered with white polyethylene and left unheated to overwinter hedges each year from December to April.

In February 1999, ramets were produced from the original seedling ortets by rooting stem cuttings from approximately 60 ortets per full-sib family that had rooted ≥ 80% in a previous experiment. In May 1999, rooted cuttings from these 60 ramets per family were moved to the container pad and grown for approximately 12 months in 164 ml Ray-Leach Super Cells (Steuwe and Sons, Corvallis, Ore.) before being transplanted into 12-l containers in May 2000. Hedges were pruned to a 15 cm height in July 2000. Cultural treatments were as described previously.

For all experiments, terminal stem cuttings were collected from the hedged ramets of both full-sib families before 1200 HR, wrapped in moist paper towels, and stored in insulated coolers. Coolers were placed in a cold room maintained at 4° C for Expts. 1 and 3 (winter). For Expts. 2 and 4 (spring), coolers were placed under the propagation bench overnight at ambient greenhouse temperatures. Prior to setting cuttings into the rooting medium to a depth of 1 cm, cuttings were recut from the proximal portions to a final length of 9 cm, and the basal 1 cm was dipped for 3 s in either 10 mM 1-naphthaleneacetic acid (NAA; 1.86 g l^{-1} in 30% ethanol v/v) for Expts. 1 and 3, or 2.5 mM NAA (0.46 g l^{-1} in 20% ethanol v/v) for Expts. 2 and 4. Needles were not removed from the basal portions of the cuttings that were inserted into the rooting medium.

Propagation environment

Experiments were conducted in a clear polyethylene-covered greenhouse with natural photoperiod and irradiance. For Expts. 2 and 4, irradiance was decreased 60% by placing shade cloth on the greenhouse exterior. Heating and cooling systems were set to maintain the daily air temperature between 23° and 26° C and the night temperature between 20° and 23° C. Cuttings were misted intermittently at a variable frequency related inversely to the relative humidity within the greenhouse. Variable frequencies were defined by designating minimum (60% RH) and maximum (99% RH) off-times between misting applications. Off-times for intermediate humidity values were calculated using a linear function. The minimum and maximum off-times varied according to the time of day. For the period from 0600 HR to 0900 HR, the minimum and maximum off times were 10 min and 35 min, respectively. For the periods from 0900 HR to 1800 HR, 1800 HR to 2100 HR, and 2100 HR to 0600 HR, minimum and maximum off times were 8 min and 24 min, 10 min and 40 min, and 60 and 240 min, respectively. An environmental management software package (Q-Com Corp., Irvine, Calif.) calculated mist frequency and triggered a traveling gantry (boom) system (ITS, McConkey Co., Mt. Puyallup, Wash.) to apply mist. Misting frequency (number of boom passes) was similar for all cuttings within each experiment; however, boom traveling speeds were altered to create different mist application treatments. For each boom speed, mist application was calculated by dividing the total output for all nozzles (258 ml min^{-1} per nozzle x 26 nozzles) (TeeJet nozzle #800067, Spraying Systems, Co., Neuvo, Calif.) by the area covered by the boom in 1 min, expressed as ml m^{-2}. Main plots were surrounded by either white porous cloth (Expts. 1 and 2) or clear polyethylene plastic (Expts. 3 and 4) to minimize environmental gradients within the greenhouse and to separate treatments. Individual plots were surrounded by two rows of border cuttings.

Effect of mist level and Ψsub on Ψcut and rooting percentage (Year 1)

Two experiments investigated the effect of two mist levels and four Ψsub treatments on Ψcut and rooting of stem cuttings. The experimental design for each was a split-plot with two levels of mist as the main plots and two replications of each mist level. Cuttings in the high mist treatment (HM) received approximately 40% more mist than cuttings in the low mist treatment (LM) during each boom pass. Four Ψsub treatments: wet, intermediate, dry, and a control were the sub-plots. Each Ψsub treatment in a replication of mist was represented as two separate rooting tubs (sub-samples within sub-plots). Ψsub treatments were created by using rooting tubs of various heights for each treatment (wet = 15.2 cm, intermediate = 30.5 cm, and dry = 43.2 cm) filled with fine silica sand (BX-30, Foster Dixiana Corp., Columbia, S.C.). After watering each rooting tub to container capacity, the different distances between the perched water tables at the bases of the containers and the rooting zones at the tops of the sand columns in each container created the three Ψsub treatments. To maintain Ψsub at the desired treatment threshold levels more precisely, tensiometers (Irrometer Co., Santa Monica, Calif.) monitored Ψsub and then triggered supplemental irrigation delivered through drip irrigation emitters (360° shrub-bubblers, Antelco Pty. Ltd., Sydney, Australia) placed at the level of the sand surface in the rooting containers. One tensiometer was placed in each of the wet, intermediate, and dry Ψsub treatments (six tensiometers total) in one replication of each mist level. Tensiometers were inserted so that the top of the porous ceramic tip was at the surface of the sand and the base was 3.8 cm below the surface. This was done to measure

Ψsub near the basal portion of stem cuttings. When Ψsub for each treatment decreased below the critical values set within each experiment (described below), supplemental irrigation was applied to both replications for that Ψsub treatment within a mist regime. A commonly used medium, consisting of peat : perlite (2:3 v/v), was placed in a container with a height equal to the intermediate Ψsub treatment to serve as a control and supplemental irrigation was not provided.

Twenty-one hundred cuttings, bulked from the two families, were collected for each experiment and approximately 120 stem cuttings were placed in each sub-plot (60 cuttings per sub-sample x 2 sub-samples x 4 Ψ_{sub} treatments x 2 mist levels x 2 replications = 1920 total stem cuttings set). A pressure chamber (Scholander et al. 1965) (SoilMoisture Equipment Corp., Santa Barbara, Calif.) was used to measure Ψ_{cut} destructively at 0500 and 1400 HR on one cutting per sub-sample 7, 14, 21, 28, and 35 days after setting (DAS). Cuttings selected randomly for Ψ_{cut} measurements in this and subsequent experiments were replaced to maintain canopy dynamics. Ψ_{sub} was also measured in each sub-sample at these times. Data for Ψ_{cut} and Ψ_{sub} for the two sub-samples were averaged to obtain the means for each sub-plot. These data were then averaged over the two measurement times each day and, finally, for the 35-day measurement period. The percentage of cuttings that had not been sampled and produced at least one root ≥ 1 mm was recorded for each sub-plot 70 DAS.

Experiment 1 Juvenile hardwood stem cuttings were collected January 5, 2001, and stored until setting on January 11, 2002. Chronologically, stem cutting material was 4½-years-old from seed; however, cuttings were collected from hedges that had been propagated vegetatively 18 months previously. Cuttings in the HM treatment received 121 ml m^{-2} of mist, whereas cuttings in LM received 72 ml m^{-2}, approximately 40% less during each boom pass. Mist regimes remained constant for 36 DAS then HM was reduced to 102 ml m^{-2} until 50 DAS and finally reduced to 72 ml m^{-2} for the remainder of the experiment. LM remained unchanged during the experiment.

The Ψ_{sub} treatments were calibrated to -1.6 kPa (wet), -2.8 kPa (intermediate), and -3.8 kPa (dry) corresponding to sand column heights of 10.2 cm, 25.4 cm, and 38.1 cm, respectively, within rooting containers. Ψ_{sub} treatments, including the control, remained distinct and relatively stable over the course of the 35-day measurement period (Figure 1).

Experiment 2 Softwood stem cuttings were collected June 22, 2001, stored overnight, and set the following day. Stem cutting material was 5-years-old from seed, although cuttings were collected from hedges that had been propagated vegetatively 2 years previously. Because stem cuttings in Expt. 1 under HM remained continuously wet, modifications were made to the mist and Ψsub treatments used in Expt. 2. During the first 35 DAS in the HM treatment, boom speed was varied manually to apply 68-121 ml m-2 of mist so that the foliage of the cuttings appeared dry just prior to the next boom pass. Simultaneously, mist application in LM was adjusted to apply 40% less mist than HM. Thirty-six DAS, mist applications in both HM and LM were adjusted to 45 ml m-2 for the remainder of the experiment. Ψsub treatments were calibrated to –1.8 kPa (wet), -2.5 kPa (intermediate), and –3.5 kPa (dry) by altering heights of sand columns, and then maintained as described previously. Ψsub treatments remained constant during the measurement period, except for the control treatment where Ψsub fluctuated depending on mist regime. Ψsub in the control treatment was not maintained by supplemental irrigation, so it averaged –2.6 kPa in HM, and -3.1 kPa in LM. The remaining Ψsub treatments were significantly different from one another, yet similar between mist regimes for each Ψsub treatment as in Expt. 1 (data not presented).

Effect of mist level on Ψcut and rooting percentage (Year 2)

Experiments 3 and 4 tested the effect of mist level on Ψcut and rooting percentage. Each employed a randomized complete block design with two replications of six mist treatments. The mist treatments were 45, 61, 75, 102, 147, and 310 ml m-2 of mist application per boom pass and remained constant for the duration of the experiment. Ψsub was set at –2.2 kPa for all mist treatments and maintained as described previously for Expts. 1 and 2. A Ψsub of –2.2 kPa was created by placing 12.5 cm of fine silica sand in 91 (length) x 61 (width) x 20 cm (height) black plastic super tubs (Rosti OS, Inc., Irving, Texas).

Approximately 100 stem cuttings were set in each plot. A pressure chamber was used to measure Ψcut destructively every 3 h between 0500 and 2300 HR (seven measurements) on two cuttings per plot 7, 14, 21, 28, and 35 DAS (the 35-DAS measurements were only conducted in Expt. 4). Data for Ψcut were averaged over the seven times each day and then over the 28- (Expt. 3) or 35-day (Expt. 4) measurement period. Ψsub was measured at 0500 and 1400 HR on the same DAS and remained at -2.4 ± 0.2 kPa among plots during these periods. The percentage of cuttings that had not been sampled and produced at least one root > 1 mm was recorded for each plot 70 DAS.

Experiment 3 Hardwood stem cuttings were collected February 15, 2002, stored, and then set April 5, 2002 (1200 total stem cuttings). Stem cutting material was 5½-years-old from seed, although stem cuttings were collected from hedges that had been propagated vegetatively 2½ years previously.

Experiment 4 Three thousand softwood stem cuttings were collected June 25, 2002 and set the following day. Stem cutting material was 6-years-old from seed although cuttings were collected from hedges that had been propagated vegetatively 3 years previously. One hundred stem cuttings were placed in each of two rooting tubs (sub-samples) per plot for a total of 2400 cuttings. Two rooting tubs per replication were used in this experiment to provide enough cuttings for simultaneous measurements of photosynthesis and gas exchange (LeBude 2004). Measurements for Ψcut, Ψsub, and rooting percentage were averaged first over the sub-samples (2 rooting tubs) within a plot prior to calculating plot means.

Statistical Analyses

Data were tested for normality and homogeneity of variances using univariate procedures (Steel et al. 1997) in SAS (SAS Institute, Inc., Cary, N.C.). In all four experiments, data for Ψ_{cut} were not distributed normally, but had equal variances among treatments. Despite numerous transformations, normality was not improved significantly. However, the transformation that improved distribution according to visual examination of plots was used in a parallel analysis with the nontransformed data. No differences were found between the two comparison analyses for any experiment. Data for rooting percentage were distributed normally and had homogeneous variances among treatments; however, the arcsine square root transformed values were used in a parallel analysis. The outcome was similar in both analyses. Therefore, all test statistics and means presented herein are based on the nontransformed data of Ψ_{cut} and rooting percentage.

For Expts. 1 and 2, the main effects and interactions of mist and Ψ_{sub} on Ψ_{cut} and percent rooting were determined using analysis of variance (GLM procedures of the SAS system) (Hatcher and Stepanski 1994). Regression analysis was used to determine the relationships between Ψ_{cut} and $\Psi_{sub.}$ For Expts. 3 and 4, regression analysis was used to determine the relationship between Ψ_{cut} and mist application, and then to determine the relationship between percent rooting and mist application. For all four experiments, Ψ_{cut} was used as an independent variable to determine the relationship with rooting percentage.

Results

Effects of mist treatment and Ψsub on Ψcut and rooting percentage (Experiments 1 and 2)

Ψ_{cut} was significantly affected by mist level and Ψ_{sub} treatment at $P \leq 0.10$ in Expts. 1 and 2 (Table 1). Ψ_{cut} increased (became less negative) across all Ψ_{sub} treatments when mist application increased 40% from LM to HM (Figure 2). In Expt. 1, but not Expt. 2, Ψ_{cut} was significantly affected by the mist x Ψ_{sub} treatment interaction (Table 1). Therefore, the effect of Ψ_{sub} on Ψ_{cut} is shown by mist level for Expt. 1, whereas the main effect of Ψ_{sub} is shown for Expt. 2 (Figure 2). As Ψ_{sub} increased, the rate of increase in Ψ_{cut} was similar for cuttings in the combined mist levels in Expt. 2 and cuttings in LM in Expt. 1, as indicated by similar regression slopes (b=0.20 for Expt. 2 and 0.21 for LM in Expt. 1) (Figure 2). For cuttings in HM in Expt. 1, however, the rate of increase in Ψ_{cut} with increasing Ψ_{sub} was significantly less (b=0.09).

Mean rooting was 23% and 48% for Expts. 1 and 2, respectively. In Expt. 1, Ψ_{sub} and the mist x Ψ_{sub} interaction significantly affected rooting percentage (Table 1). In HM, mean rooting was 5% and 32% for cuttings in the wet and dry treatments, respectively. In contrast, mean rooting in LM was 42% and 22% for cuttings in the wet and dry treatments, respectively. In Expt. 2, mist level significantly affected rooting percentage, but Ψ_{sub} did not (Table 1). In this experiment, rooting percentage was higher for cuttings in HM (60%) compared to those cuttings in LM (35%), regardless of Ψ_{sub} treatment.

In Expt. 1, rooting percentage was related moderately with both the linear and quadratic terms of Ψ_{cut} (P=0.01, R^2=0.56, for the equation containing both terms) (Figure 3A). Rooting percentage increased to approximately 35% as Ψ cutting increased from -0.8 to -0.5 MPa. However, percent rooting then decreased as Ψ_{cut} continued to increase to -0.2 MPa (Figure 3A). For cuttings in Expt. 2, rooting increased linearly as Ψ_{cut} increased (Figure 3B). Although overall rooting was higher than for Expt. 1, the highest rooting percentages were found for cuttings from several treatment combinations with mean Ψ_{cut} between -0.4 and -0.6 MPa, similar to Expt. 1.

Effects of mist treatment on Ψ_{cut} and rooting percentage (Experiments 3 and 4)

Over the 28- and 35-day measurement periods (in Expts. 3 and 4, respectively), Ψ_{cut} tended to follow a similar diurnal pattern for each mist regime (Figure 4). Ψ_{cut} decreased between 0800 and 1700 HR, and increased between 1700 and 0800 HR. Although variation occurred in both experiments, cuttings receiving 310 ml m^{-2} of mist had minimal change in Ψ_{cut} either during the day or over the measurement period (Figure 4). In both experiments, average Ψ_{cut} (across all times and measurement dates) was related strongly with the log of mist application (Expt. 3, R^2=0.97 and Expt. 4, R^2=0.96) (Figure 5). The sharpest increase in Ψ_{cut} was between 45 and 102 ml m^{-2} of mist per application, whereas above 147 ml m^{-2}, the increase was more gradual.

Mean rooting was 73% and 63% for Expts. 3 and 4, respectively. Rooting percentage was significantly affected by both the linear and quadratic terms of mist level in Expt. 3 (P=0.03, R^2=0.54, for the equation containing both terms), but neither the linear nor the quadratic terms affected rooting percentage in Expt. 4 (Figure 6). In Expt. 3, rooting increased to 94% at a mist level of 147 ml m^{-2}, then declined at the higher mist level (Figure 6).

Rooting percentage was related moderately with both the linear and quadratic terms of Ψ_{cut} and the square of Ψ_{cut} in both experiments (P=0.01, R^2=0.70, for equations containing both terms in both Expts.) (Figure 7). In Expt. 3, the greatest rooting occurred for cuttings with an average Ψ_{cut} between -0.5 and -1.0 MPa (90%). In Expt. 4, the greatest rooting occurred for cuttings with an average Ψ_{cut} between -0.7 and -1.2 MPa (80%).

Minimum (most negative) and maximum (closest to zero) daily Ψ_{cut} were selected from the weekly measurements for each plot, regardless of the time of day, and averaged over the 28- (n=4) or 35- (n=5) day measurement period. In Expt. 3, rooting percentage was moderately to highly correlated with minimum Ψ_{cut} (R^2=0.78, P=0.01) (Figure 8A). The regression equation predicted that cuttings rooted 80% or higher when mean daily minimum Ψ_{cut} was between -0.7 and -1.65 MPa (Figure 8A). In Expt. 4, rooting percentage was related moderately with minimum Ψ_{cut} (R^2=0.45, P=0.07) (Figure 8B). The regression equation did not predict 80% rooting from the observed data, so 70% rooting was used as a benchmark. At 70% rooting or higher, Ψ_{cut} ranged from –0.95 and –1.60 MPa (Figure 8B), however, variation in rooting was considerable.

The relationship between rooting percentage and maximum Ψ_{cut} was moderate for both Expts. 3 (R^2=0.61, P=0.02) and 4 (R^2=0.61, P=0.01) (Figure 8). When cuttings in Expt. 3 experienced a mean maximum Ψ_{cut} between -0.2 and -0.4 MPa, rooting was $\geq$ 80%. As cuttings in Expt. 4 experienced mean maximum Ψ_{cut} between -0.35 and -0.75 MPa, rooting was $\geq$ 70%.

Discussion

Mist and Ψ_{sub} contributed to the Ψ_{cut} of nonrooted, juvenile stem cuttings of loblolly pine. Ψ_{cut} increased as Ψ_{sub} increased for all cuttings in Expts. 1 and 2 (Figure 2). Cuttings within individual mist levels received equal mist application, so increases in Ψ_{cut} were due to uptake from the substrate. Increasing mist level also increased Ψ_{cut} for all cuttings in all experiments (Figures 2 and 5). It has been suggested that uptake of water can occur through both the aerial portion of the foliage exposed to intermittent mist application and the basal portion of the shoot inserted in the rooting substrate (Cameron and Rook 1974, Cremer and Svensson 1979, Peer and Greenwood 2001). In addition to absorption through the foliage, increased mist application could decrease transpiration, thus preventing water loss. The effect of either mist or Ψ_{sub} on Ψ_{cut} decreased for cuttings experiencing little water deficit in Expt. 1, as indicated by the significant Ψ_{sub} x mist level interaction (*P*=0.09).

Cuttings with the most negative Ψ_{cut} for each experiment had poor rooting percentages (Figures 3 and 7). This is consistent with reports of poor rooting of stem cuttings of many species that experienced substantial water deficit or severely decreased Ψ_{cut} (Loach 1977, Grange and Loach 1984, Rein et al. 1991, Murthy and Goldfarb 2001, Peer and Greenwood 2001). On the other hand, cuttings with the highest Ψ_{cut} in Expts. 1 (Figure 3A), 3 (Figure 7A), and 4 (Figure 7B) also rooted poorly. The one exception was Expt. 2 in which the revised method of mist application did not produce high values of Ψ_{cut} in stem cuttings. Greenwood et al. (1980) also reported that excessive mist application decreased rooting percentage in loblolly pine; however, those authors did not report Ψ_{cut}. Our results that juvenile stem cuttings of loblolly pine experiencing little or no water deficit root poorly have not been reported previously. In general, the deleterious effect of excessive mist on rooting may be indirect because basal portions of stem cuttings can become waterlogged and, ultimately, necrotic under saturated substrate moisture conditions. However, in our studies, this indirect effect was excluded by independently controlling mist level and $\Psi_{sub.}$ Furthermore, basal stem necrosis was not observed on cuttings for any treatments in these experiments**.**

Our results suggest that, in juvenile stem cuttings of loblolly pine, moderate water deficits imposed during the rooting period stimulated adventitious root formation and development. Cuttings with the highest rooting percentages had mean Ψ_{cut} between -0.5 and -1.2 MPa (Figures 3 and 7). These results agree with rooting percentages and Ψ_{cut} from other species. Juvenile stem cuttings of Alaskan yellow cedar [*Chamaecyparis nootkatensis* (D.Don) Spach] had a mean Ψ_{cut} of -0.8 MPa (mean at 1100 HR over 13 weeks prior to rooting) and rooted 92% (Grossnickle and Russell 1993). Two treatments of stem cuttings of mountain lilac (*Ceanothus thrysiflorus* Esch.) had mean Ψ_{cut} of –0.8 MPa and –1.6 MPa (mean of 0900 and 1400 HR) during the rooting period with rooting percentages of 96% and 56%, respectively (Loach 1977). Prior to rooting, the cuttings with mean Ψ_{cut} of -0.8 MPa had a one-time minimum stress of -1.2 MPa, whereas cuttings with mean Ψ_{cut} of -1.6 MPa had a one-time minimum stress of -3.9 MPa (Loach 1977). Cuttings of both yellow cedar and mountain lilac, which rooted in the highest percentages, also had mean Ψ_{cut} within the same intermediate range of –0.5 to –1.2 MPa that induced superior rooting in loblolly pine. However, it cannot be determined from all these reports whether rooting percentage was adversely affected by a one-time severe water deficit, repeated severe water deficits, or the mean Ψ_{cut} experienced by cuttings during the rooting period.

Stem cuttings of loblolly pine rooted ≥ 80% and ≥70%, in Expts. 3 and 4, respectively, when mean daily minimum Ψ_{cut} was > -1.6 MPa but < -0.90 MPa (Figure 8). Murthy and Goldfarb (2001) reported that rooting of juvenile hardwood and softwood stem cuttings of loblolly pine decreased below 80% and 70%, respectively, when cuttings experienced a one-time Ψ_{cut} < -3.5 MPa for hardwood and < -1.7 MPa for softwood stem cuttings. In that study, the one-time water deficit treatments were imposed in a laboratory atmosphere prior to rooting cuttings under optimal conditions. Thus, water deficits occurred one time and well in advance of adventitious root formation. By contrast, treatment differences in the present study occurred repeatedly and throughout the period leading to rooting. Moreover, our cuttings experienced environmental factors such as high irradiance and leaf temperature, which, in addition to $\Psi_{cut,}$ may induce additional deleterious effects in the cuttings. Therefore, juvenile stem cuttings of loblolly pine may experience moderate to severe water deficit during the rooting period and still produce adventitious roots at high percentages, provided these cuttings do not experience mean daily minimum Ψ_{cut} < –1.6 MPa.

The mechanism(s) by which Ψ_{cut} affects adventitious root formation in stem cuttings is unclear (Grange and Loach 1984, Sinclair and Ludlow 1985, Loach 1988). Ψ_{cut} is comprised of both the osmotic potential and turgor pressure (Kramer and Kozlowski 1979) of the cutting, and these components may differentially affect the two general stages of adventitious root formation – root initiation and root growth and development. For example, a low Ψ_{cut} may not affect root initiation, but loss of turgor at a critical point could prevent or retard growth and development, thus, decreasing the percentage of cuttings that are observed as rooted. Moreover, intact seedlings of loblolly pine exposed repeatedly to water deficit can undergo osmotic adjustment, which decreases the solute potential and maintains turgor at a lower Ψ (Seiler and Johnson 1985). Decreased percent rooting has been associated with lower osmotic potentials in response to high irradiance in stem cuttings of some woody species (Grange and Loach 1985). Whether osmotic adjustment occurs in stem cuttings and how it might affect the two stages of adventitious root formation are unknown. Further studies measuring the effects of osmotic and turgor potentials on adventitious root initiation and development are needed to better understand these mechanisms. In addition, more investigation is necessary to extend these results to other propagation environments to test whether our finding that moderate water stress is necessary to stimulate high rooting percentages in stem cuttings of loblolly pine is a general principle with broad applicability.

In conclusion, substrate moisture and mist application affected the water status of nonrooted stem cuttings of loblolly pine as cuttings absorbed moisture from the substrate, absorbed water through the foliage above the substrate, and/or experienced reduced transpirational demand. Substrate moisture affected rooting percentage to some extent when mist was sub-optimal or applied excessively. Mist level was the overriding factor determining rooting percentage, however, when adequate moisture was present in the substrate. When Ψ_{sub} was held constant across mist levels, mist level determined the Ψ_{cut} of nonrooted stem cuttings and Ψ_{cut}, in turn, strongly affected rooting percentage. Stem cuttings of loblolly pine rooted at high percentages when experiencing a moderate amount of water deficit prior to initiating adventitious roots. Thus, propagators of this species should not endeavor to eliminate all water deficit experienced by cuttings during the rooting period. These results and those of future studies determining physiological processes in cuttings and environmental measurements during rooting have the potential to guide development of automatically controlled propagation systems that function efficiently in a variety of settings.

Acknowledgements

We gratefully acknowledge funding for this research by the industrial supporters of the North Carolina State University Loblolly and Slash Pine Rooted Cutting Program and by the North Carolina Agricultural Research Service, Raleigh, NC 27695-7643. The authors also thank Drs. William H. Swallow and Cavell Brownie for statistical assistance, Bill Pogue and Michael Carnes for technical assistance, and Kelly Dougherty, Marissa K. Rodman, Maria

Wirth Wilkes, Dr. Amy Noelle Wright, Brownie Wirth and all past employees of the Rooted Cutting Program for their assistance during these experiments.

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Table 1. Analysis of variance for cutting water potential (Ψ_{cut}) and percent rooting of juvenile hardwood (Expt. 1) and softwood (Expt. 2) stem cuttings of loblolly pine rooted under two mist levels and four substrate water potential (Ψ_{sub}) treatments. Error A was used as the error term to test the main effect of replication and mist level while error B was used as the error term to test the main effect of Ψ_{sub} and the mist x Ψ_{sub} interaction. Values in bold type are significant at *P* < 0.10.

		Expt. 1		Expt. 2	
Source	df	Ψ_{cut}	Rooting (%)	Ψ_{cut}	Rooting (%)
Replication	1	0.53	0.28	0.46	0.12
Mist level	1	**0.08**	0.17	**0.02**	**0.02**
Error A = Replication x Mist level	1	0.14	0.32	0.68	0.85
Ψ_{sub}	3	**0.01**	**0.04**	**0.01**	0.40
Mist level x Ψ_{sub}	3	**0.09**	**0.01**	0.50	0.88
Error B = [Replication x Ψ_{sub} (Mist level)]	6				

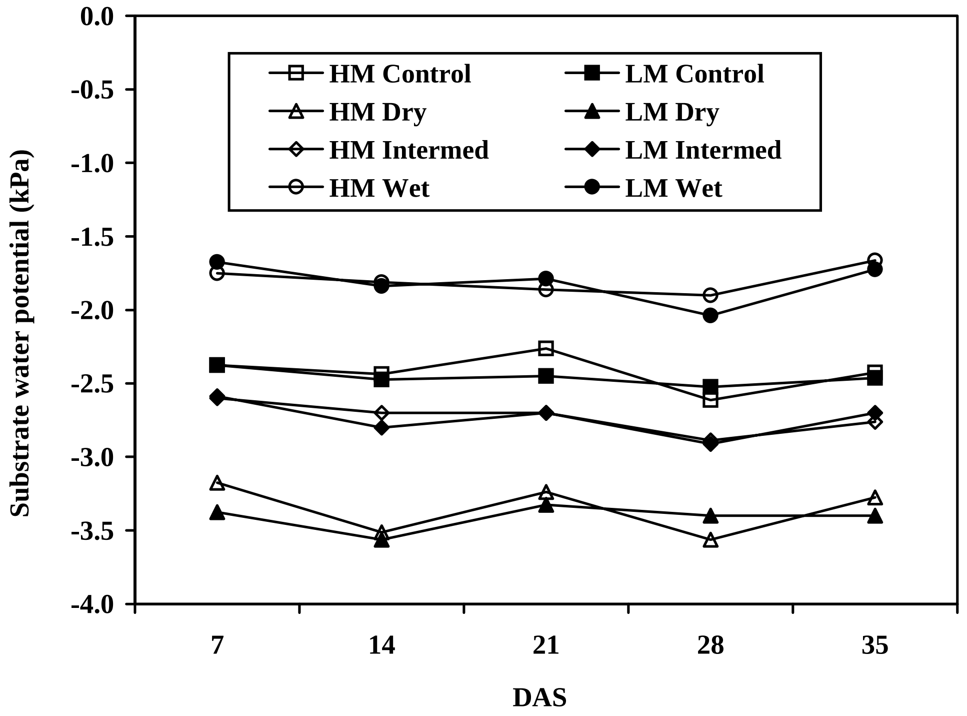


Figure 1. Mean substrate water potential (Ψ_{sub}) (averaged for measurements taken at 0500 and 1400 HR) 7, 14, 21, 28, or 35 days after setting (DAS) for juvenile hardwood stem cuttings of loblolly pine rooted under two mist levels [high mist (HM) and low mist (LM)] and four Ψ_{sub} treatments (control, dry, intermediate, or wet) in January 2001 (Expt. 1).

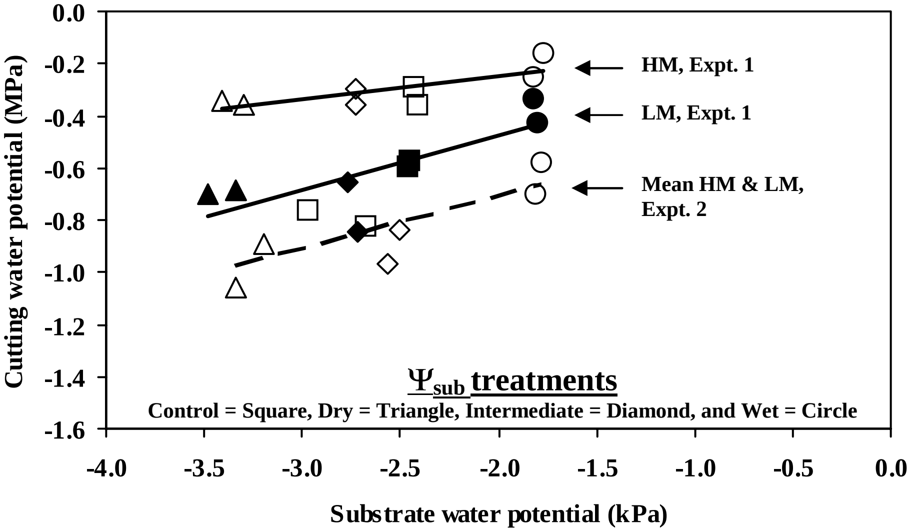


Figure 2. Effect of mist level and substrate water potential (Ψ_{sub}) on mean cutting water potential (Ψ_{cut}) of juvenile hardwood (Expt. 1, conducted in January 2001) and softwood (Expt. 2, conducted in June 2001) stem cuttings of loblolly pine. Solid regression lines represent Expt. 1. The open symbols in Expt. 1 represent the high mist (HM) and solid symbols the low mist (LM) treatments. The dashed regression line represents Expt. 2, and the open symbols represent the means of HM and LM. Symbols are means (n = 20 for Expt. 1, and n = 40 for Expt. 2) of Ψ_{cut} and Ψ_{sub} measured at 0500 and 1400 HR 7, 14, 21, 28, and 35 days after setting in each plot. Regression equations are Ψ_{cut} (HM) = - 0.07 + 0.09 (Ψ_{sub}), $P = 0.03$, $r^2 = 0.58$, and Ψ_{cut} (LM) = - 0.07 + 0.21 (Ψ_{sub}), $P = 0.02$, $r^2 = 0.61$, for Expt. 1 and Ψ_{cut} (combined HM and LM) = - 0.30 + 0.20 (Ψ_{sub}), $P = 0.04$, $r^2 = 0.61$, for Expt. 2.

Figure 3. Relationship between rooting percentage and cutting water potential (Ψ_{cut}) of juvenile (A) hardwood (Expt. 1, conducted in January 2001) and (B) softwood (Expt. 2, conducted in June 2001) stem cuttings of loblolly pine rooted under high mist (HM = open symbols) and low mist (LM = solid symbols) and four substrate water potential treatments (control = square, dry = triangle, intermediate = diamond, and wet = circle). Symbols are means (N = 20) of Ψ_{cut} measured at 0500 and 1400 HR 7, 14, 21, 28, or 35 days after setting in each plot. Values for the quadratic term, Ψ_{cut}^2, in Expt. 1 were generated for Ψ_{cut} in each plot initially, and then averaged similarly. The regression equations are (A) Rooting (%) = - 32.80 – 261.66 (Ψ_{cut}) – 257.47 $(\Psi_{cut})^2$, $P = 0.01$, $r^2 = 0.56$, and (B) Rooting (%) = 77.49 + 36.2 (Ψ_{cut}), $P = 0.01$, $r^2 = 0.67$.

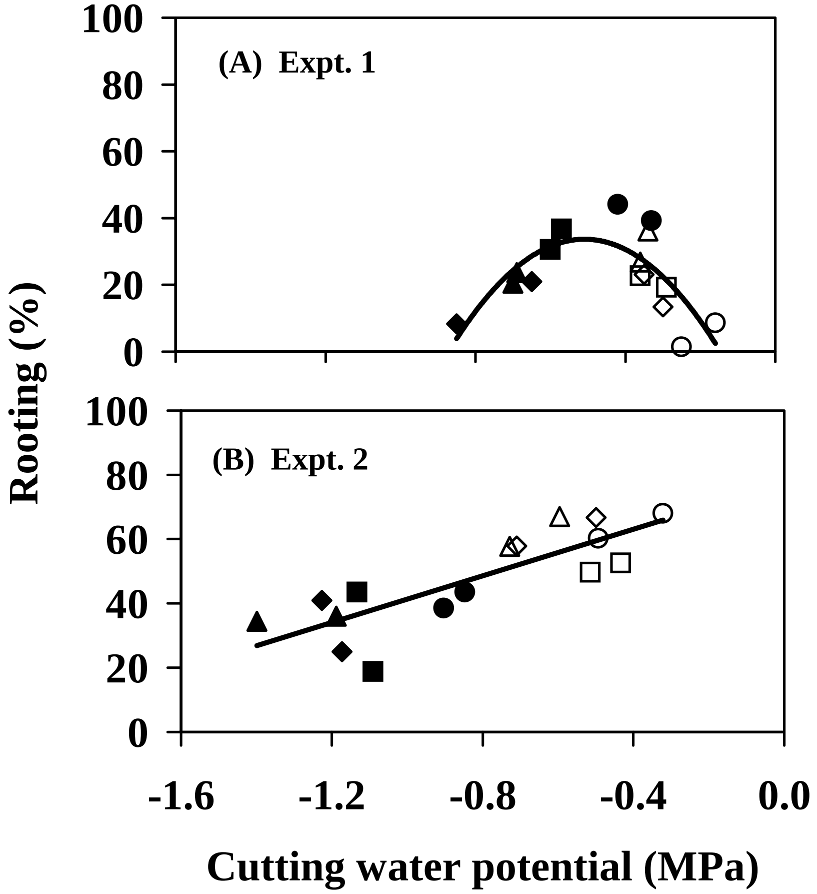

(A) Expt. 1
(B) Expt. 2
Rooting (%)
0
20
40
60
80
100
-1.6
-1.2
-0.8
-0.4
0.0
Cutting water potential (MPa)

Figure 4. Cutting water potential (Ψ_{cut}) by time of day for juvenile (A) hardwood (Expt. 3, conducted in April 2002) and (B) softwood (Expt. 4, conducted in June 2002) stem cuttings of loblolly pine receiving 45, 61, 73, 102, 147, or 310 ml m^{-2} of mist per application. Symbols are means (n = 8 for Expt. 3, and n = 10 for Expt. 4) of two replications per mist treatment averaged over 7, 14, 21, 28, or 35 (day 35 measurement for Expt. 4 only) days after setting. Vertical bars = ± 1 SE (n = 4 for Expt. 3 and n = 5 for Expt. 4).

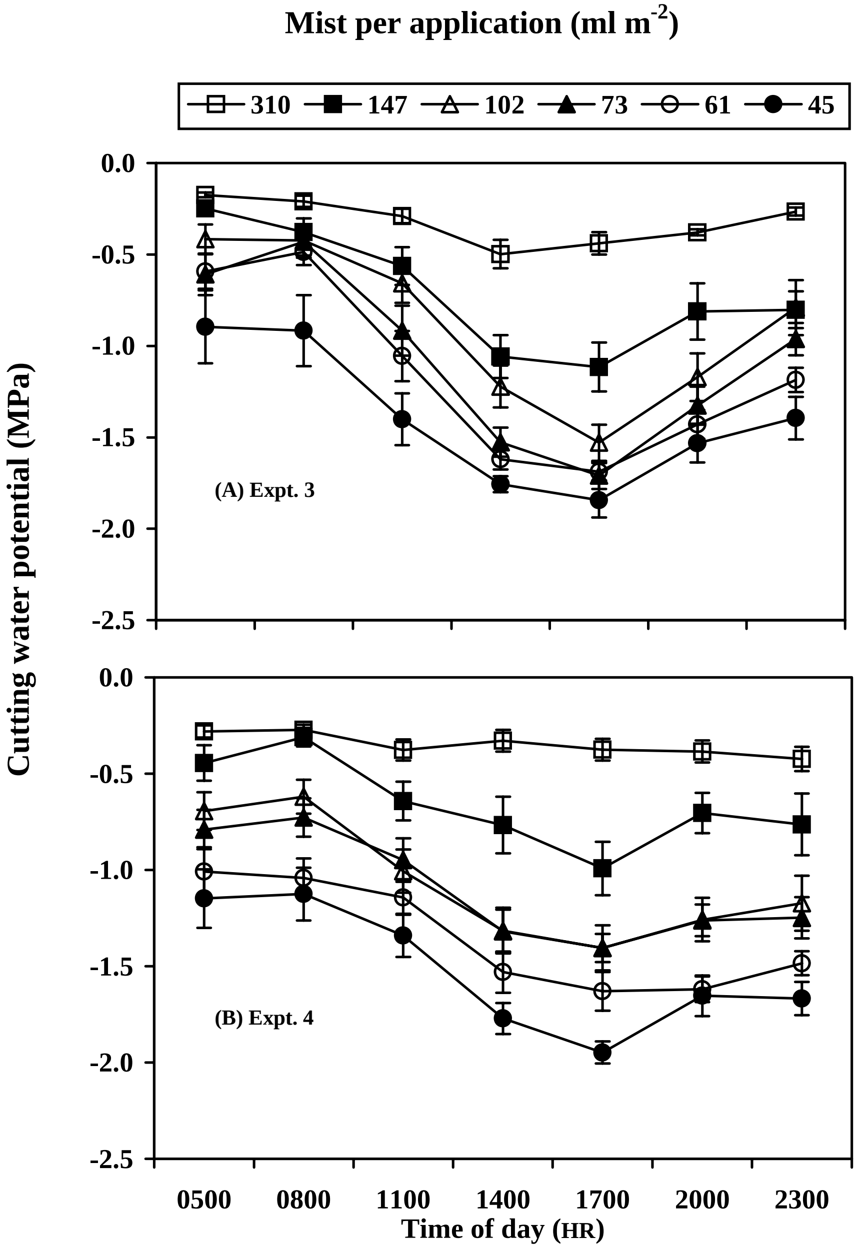
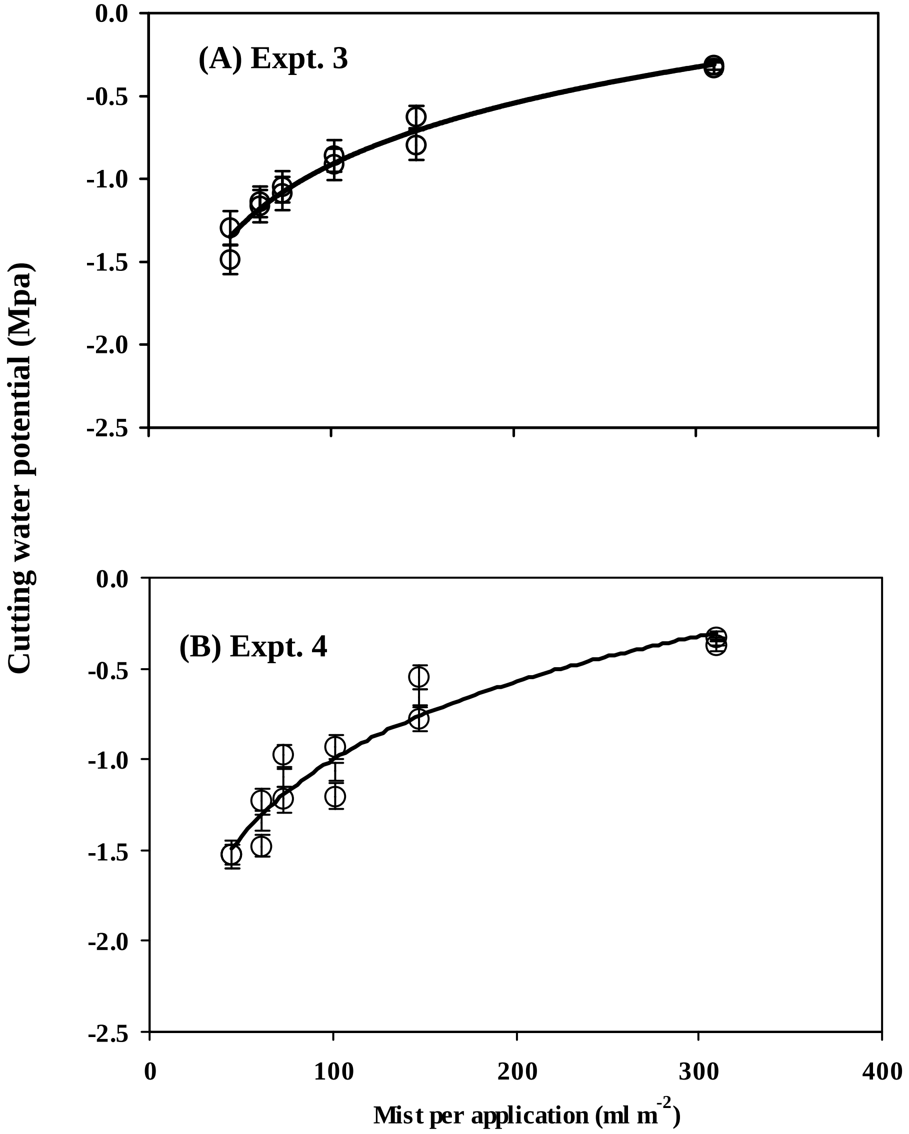

Mist per application (ml m^{-2})
310
147
102
73
61
45
Cutting water potential (MPa)
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
(A) Expt. 3
(B) Expt. 4
0500
0800
1100
1400
1700
2000
2300
Time of day (HR)

Figure 5. Relationship between cutting water potential (Ψ_{cut}) and 45, 61, 73, 102, 147, or 310 ml m^{-2} of mist per application for juvenile (A) hardwood stem cuttings in April 2002 (Expt. 3) and (B) softwood stem cuttings of loblolly pine in June 2002 (Expt. 4). Symbols are mean (n = 56 for Expt. 3, and n = 70 for Expt. 4) Ψ_{cut} for each replication recorded seven times per day 7, 14, 21, 28, or 35 (day 35 measured for Expt. 4 only) days after setting cuttings in each plot. The regression equations are (A) $\Psi_{cut} = -3.39 + 0.54\ln(\text{mist ml m}^{-2})$, $P = 0.01$, $r^2 = 0.97$, and (B) $\Psi_{cut} = -3.84 + 0.62\ln(\text{mist ml m}^{-2})$, $P = 0.01$, $r^2 = 0.96$. Vertical bars = ± 1 SE (n = 4 for Expt. 3, and n = 5 for Expt. 4).


(A) Expt. 3
(B) Expt. 4
Cutting water potential (Mpa)
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
0
100
200
300
400
Mist per application (ml m-2)

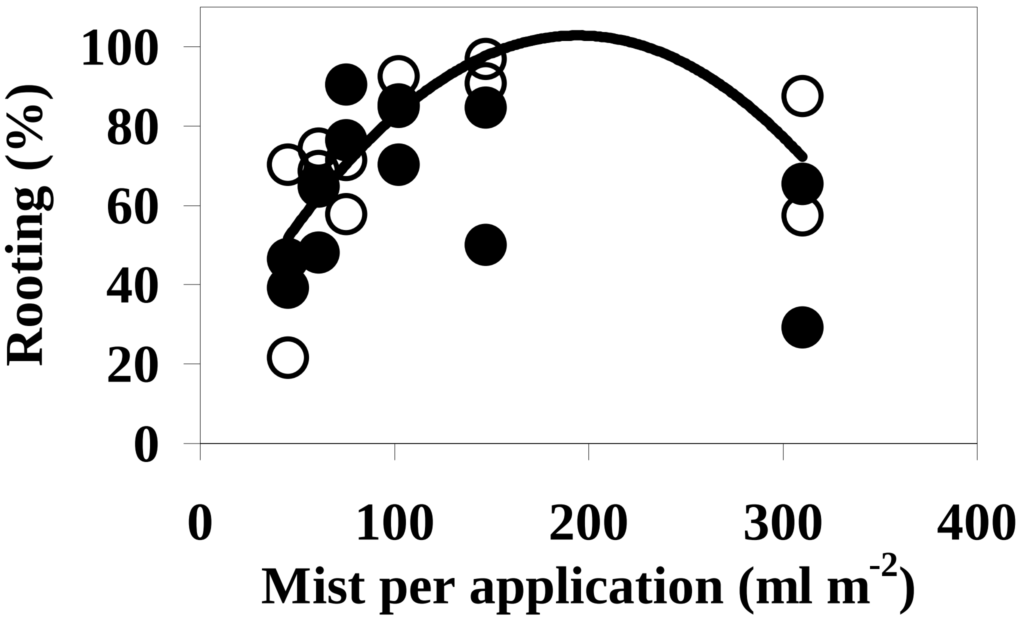
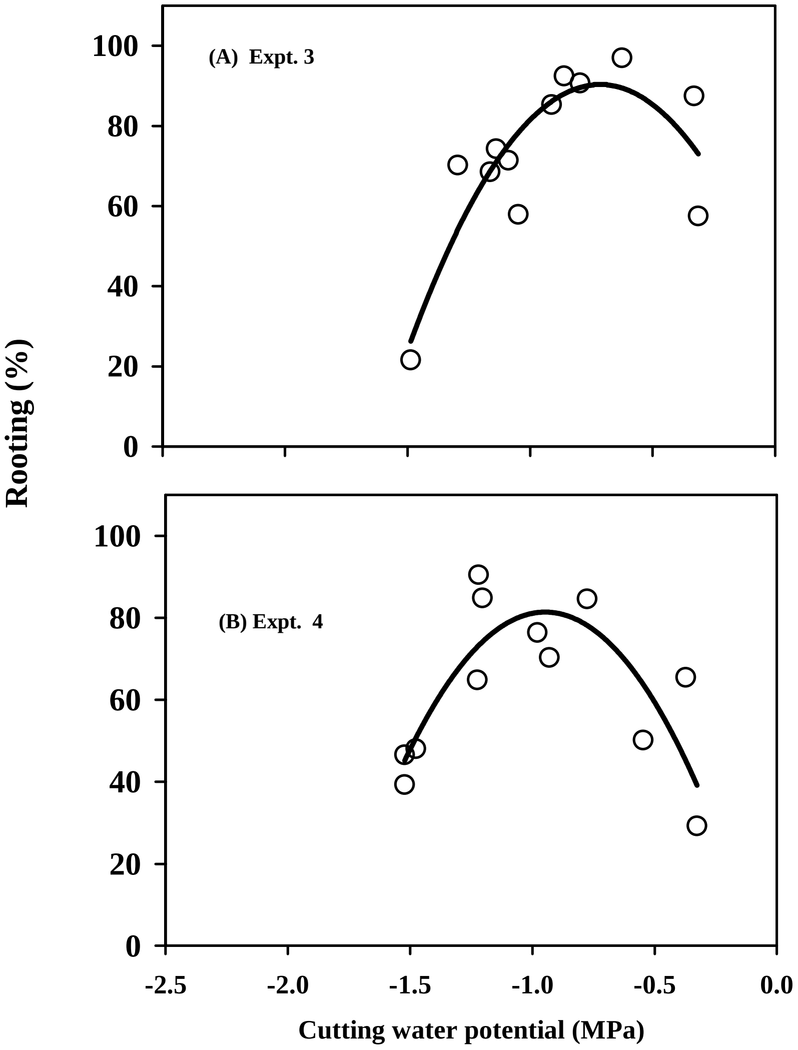


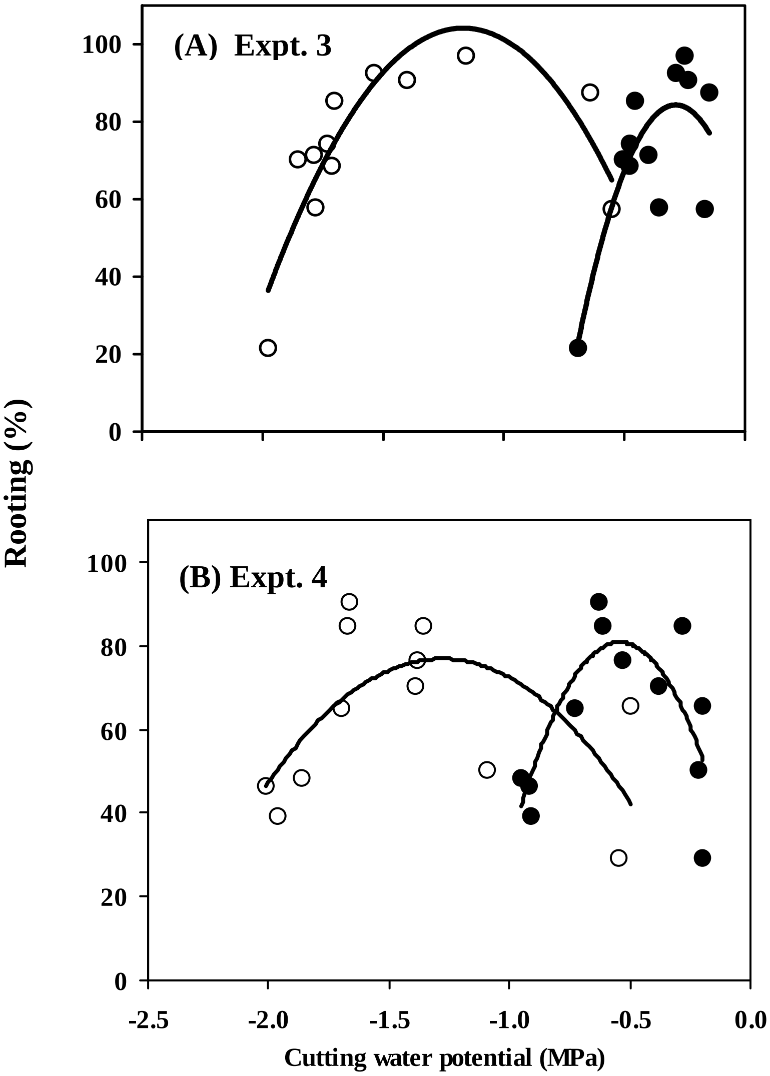
Figure 6. Effect of 45, 61, 73, 102, 147, or 310 ml m^{-2} of mist per application on percent rooting of juvenile hardwood (Expt. 3, conducted in April 2002, open symbols) and softwood (Expt. 4, conducted in June 2002, solid symbols) stem cuttings of loblolly pine. The regression equation is Rooting (%) Expt. 3 = 16.36 + 0.89 (ml m^{-2}) – 0.0023 (ml m^{-2})^2, $P = 0.03$, $r^2 = 0.54$; Mist application did not significantly effect percent rooting during Expt 4.

Figure 7. Relationship between rooting percentage and mean cutting water potential (Ψ_{cut}) of juvenile (A) hardwood (Expt. 3, April 2002) and (B) softwood (Expt. 4, June 2002) stem cuttings of loblolly pine rooted under six mist treatments. Symbols are means (n = 56 for Expt. 3, and n = 70 for Expt. 4) of Ψ_{cut} recorded seven times per day 7, 14, 21, 28, or 35 days after setting cuttings (day 35 measurement for Expt. 4 only) in each plot. Values for the quadratic term, Ψ_{cut}^2, were generated for Ψ_{cut} in each plot initially, and then averaged similarly. The regression equation for (A) is Rooting (%) = 9.26 – 261.94 (Ψ_{cut}) – 152.44 $(\Psi_{cut})^2$, $P = 0.01$, $r^2 = 0.70$, and for (B) Rooting (%) = - 31.49 - 283.57 (Ψ_{cut}) – 140.03 $(\Psi_{cut})^2$, $P = 0.01$, $r^2 = 0.70$.



(A) Expt. 3
(B) Expt. 4
Rooting (%)
0
20
40
60
80
100
-2.5
-2.0
-1.5
-1.0
-0.5
0.0
Cutting water potential (MPa)

Figure 8. Relationship between rooting percentage and mean minimum (open symbols) and maximum (solid symbols) cutting water potential (Ψ_{cut}) for juvenile (A) hardwood (Expt. 3 conducted in April 2002) and (B) softwood (Expt. 4 conducted in June 2002) stem cuttings of loblolly pine. Symbols are means of the minimum and maximum daily Ψ_{cut} values recorded 7, 14, 21, 28, or 35 days (day 35 for Expt. 4 only) after setting cuttings in each plot (n = 4 for Expt. 3 and n = 5 for Expt. 4). Values for the quadratic term, Ψ_{cut}^2, were generated for Ψ_{cut} in each plot initially, and then averaged similarly. The regression equations for (A) are Rooting (%) = - 31.24 – 237.57(Minimum Ψ_{cut}) – 102.34 (Minimum Ψ_{cut})^2, $P = 0.01$, $r^2 = 0.78$, Rooting (%) = 63.95 – 138.01(Maximum Ψ_{cut}) – 214.04 (Maximum Ψ_{cut})^2, $P = 0.02$, $r^2 = 0.61$, and for (B) Rooting (%) = - 17.42 – 154.57(Minimum Ψ_{cut}) – 60.89(Minimum Ψ_{cut})^2, $P = 0.07$, $r^2 = 0.45$, and Rooting (%) = 14.04 – 241.21(Maximum Ψ_{cut}) – 196.94(Maximum Ψ_{cut})^2, $P = 0.01$, $r^2 = 0.61$.


(A) Expt. 3
(B) Expt. 4
Rooting (%)
0
20
40
60
80
100
-2.5
-2.0
-1.5
-1.0
-0.5
0.0
Cutting water potential (MPa)

Chapter 3

Mist Level Influences Vapor Pressure Deficit and Gas Exchange During Rooting of Juvenile Stem Cuttings of Loblolly Pine

(In the format appropriate for submission to HortScience)

Subject Category: Propagation and Tissue Culture

Mist Level Influences Vapor Pressure Deficit and Gas Exchange During Rooting of Juvenile Stem Cuttings of Loblolly Pine

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Received for publication November 17, 2004. Accepted for publication ____________. This research was funded jointly by the industrial supporters of the North Carolina State University Loblolly and Slash Pine Rooted Cutting Program and by the North Carolina Agricultural Research Service (NCARS) Raleigh, NC 27695-7643. Use of trade names in this publication does not imply endorsement by the NCARS of the products named nor criticism of similar ones not mentioned. Statistical assistance of Cavell Brownie, and technical assistance of Kelly Dougherty, Jim Grissom, and Neil LeBude is greatly appreciated. From a thesis to be submitted by A.V.L. in partial fulfillment of the requirements for the PhD degree.

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Additional index words. clonal forestry, vegetative propagation, *Pinus taeda*, water relations

Abstract. Two experiments were conducted during which juvenile hardwood or softwood stem cuttings of loblolly pine (*Pinus taeda* L.) were rooted under six mist regimes in a polyethylene-covered greenhouse to investigate the effect of mist level on vapor pressure deficit (VPD) and cutting water potential (Ψ_{cut}), and to determine the relationships between these variables and rooting percentage. In addition, net photosynthesis at ambient conditions ($A_{ambient}$) and stomatal conductance (g_s) were measured in stem cuttings during adventitious root formation to determine their relationship to rooting percentage. Hardwood stem cuttings rooted ≥ 80% when mean daily VPD between 1000 and 1800 HR ranged from 0.60 to 0.85 kPa. Although rooting percentage was related to Ψ_{cut}, and $A_{ambient}$ was related to Ψ_{cut}, rooting percentage of softwood stem cuttings was not related to $A_{ambient}$ of stem cuttings. Using VPD as a control mechanism for mist application during adventitious rooting of stem cuttings of loblolly pine might increase rooting percentages across a variety of rooting environments.

Loblolly pine (*Pinus taeda*) is the most important timber species in the southeast United States. Adventitious rooting of stem cuttings of loblolly pine collected from stock plants in the juvenile growth phase can be used to multiply superior genotypes (clones) within families for progeny testing in breeding programs (Isik et al., 2004), or for use directly in reforestation (Frampton et al., 2000; 2002). Loblolly pine has been considered recalcitrant to propagate by stem cuttings (Zobel and Talbert, 1984) and somatic embryogenesis (Pullman et al., 2003), posing an obstacle to large-scale use in clonal forestry. For vegetative propagation of stem cuttings of loblolly pine to attain operational levels, successful rooting must be accomplished in low-cost environments. Several propagation environments, including greenhouses, shadehouses, and nursery beds are being tested (Gocke, 2001). Abiotic factors, such as rooting substrate, mist application, air temperature, wind, relative humidity (RH), and irradiance can vary among these environments and affect rooting percentage. Thus, irrigation schedules that are optimized for one rooting environment may not be successful in others. Understanding how environmental factors induce the physiological responses associated with increased rooting could enable propagators to reproduce those conditions utilizing a variety of production systems.

LeBude et al. (2004) determined previously that, provided substrate water potential was - 2.4 ± 0.2 kPa, mist level was the most important factor influencing rooting percentage of juvenile stem cuttings of loblolly pine. Mist level was strongly related to cutting water potential (Ψ_{cut}), a physiological indicator of water deficit, and Ψ_{cut} was related to rooting percentage. They hypothesized that stem cuttings of loblolly pine need to experience a moderate, mean daily water deficit to stimulate adventitious root formation and development. Using Ψ_{cut} as an indicator of rooting percentage, however, would be cumbersome in an operational system because measurements are labor intensive.

Vapor pressure deficit (VPD) has been used successfully to control mist frequency dynamically on stem cuttings of ‘Freedom Dark Red’ poinsettia (*Euphorbia pulcherrima* Willd. Ex Klotzsch ‘Freedom Dark Red’) during the transition period between root emergence and subsequent root growth (Zolnier et al. 2001a; 2003). Although VPD has been monitored during rooting of stem cuttings in other species, correlations between VPD, rooting percentage, and Ψ_{cut} were not intended as objectives of various studies (Aminah et al., 1997; Grossnickle and Russell, 1993; Newton et al., 1992). An initial step in utilizing an environmental factor to control mist application or predict rooting success is to define the response of stem cuttings to that factor (Zolnier et al., 2001b). Such research has not been reported for stem cuttings of loblolly pine.

Current photosynthesis (photosynthetic rate of stem cuttings during the period of adventitious root formation and development) and stomatal conductance to water vapor (g_s) are two physiological variables that may influence adventitious root formation in stem cuttings (Davis, 1988). Photosynthetic rate in stem cuttings is dependent upon a complex interaction among the level of photosynthetically active radiation (*PAR*), RH, stomatal opening, and water potential (Davis, 1988). Because Ψ_{cut} is associated with rooting percentage (Hartmann et al., 2002; LeBude et al., 2004; Loach and Whalley, 1978), its effect on current photosynthesis might explain the relationship between Ψ_{cut} and rooting percentage. Moreover, the relationship between these processes might aid design of rooting environments to induce photosynthetic responses in stem cuttings associated with increased rooting percentages.

The experiments in this report were conducted concurrently with the experiments of LeBude et al. (2004) utilizing the same stem cuttings, rooting environments, and mist treatments. The goal of this report is to first broaden the impact of LeBude et al. (2004) by developing quantitative information for controlling rooting environments, and secondly, to describe further the nature of the relationship between Ψ_{cut} and rooting percentage by studying gas exchange in stem cuttings. Therefore, the following two experiments were conducted to determine (1) the relationships between VPD and Ψ_{cut}, and VPD and rooting percentage, and to determine (2) the relationship between gas exchange and adventitious rooting of juvenile stem cuttings of loblolly pine.

Materials and Methods

Plant material. The provenance, propagation and culture of stock plants, and subsequent collection of stem cuttings for these experiments, were described previously (LeBude et al., 2004). Methods described for Expts. 3 and 4 in LeBude et al. (2004) are the same for Expts. 1 and 2 in the present study. Briefly, either juvenile hardwood (Expt. 1) or juvenile softwood (Expt. 2) terminal stem cuttings were collected and bulked from recurrently sheared (hedged) stock plants of two full-sib families of loblolly pine, and then placed in insulated coolers. For Expt. 1, the coolers were placed in a cold room and maintained at 4° C for 8 weeks until setting the cuttings (insertion into the rooting medium) on 5 Apr. 2002, whereas in Expt. 2, the coolers were placed under a greenhouse bench overnight until the cuttings were set the following day, 29 June 2002. Prior to setting cuttings to a depth of 1 cm, cuttings were recut from the proximal ends to a final length of 9 cm, and the basal 1 cm was dipped for 3 s in either 10 mM 1-naphthaleneacetic acid (NAA; 1.86 g L^{-1} in 30% ethanol v/v) for Expt. 1, or 2.5 mM NAA (0.46 g L^{-1} in 20% ethanol v/v) for Expt. 2. Needles were not removed from the basal portions of the cuttings that were inserted into the rooting medium.

Rooting environment. Both experiments were conducted under natural photoperiod and irradiance in a clear polyethylene-covered greenhouse; however, irradiance in Expt. 2 was decreased 60% by placing shade cloth on the greenhouse exterior. Heating and cooling systems were adjusted to maintain the daily air temperature between 23 and 26° C and the night temperature between 20 and 23° C. Cuttings were misted intermittently at a variable frequency related inversely to the RH (50-Y Temp/RH Probe, QCOM, Corp., Irvine, Calif.) surrounding stem cuttings being rooted on an adjacent bench within the greenhouse. The Temp/RH probe was also misted with 121 mL m^{-2} of mist at each boom pass. Variable frequencies were defined by designating minimum (60% RH) and maximum (99% RH) off-times between mist applications. Off-times for intermediate humidity values were calculated using a linear function. The minimum and maximum off-times varied according to the time of day. For the period from 0600 to 0900 HR, the minimum and maximum off times were 10 and 35 min, respectively. For the periods from 0900 to 1800 HR, 1800 to 2100 HR, and 2100 to 0600 HR, minimum and maximum off times were 8 and 24 min, 10 and 40 min, and 60 and 240 min, respectively. A greenhouse environmental management software program (GEM3, QCOM Corp., Irvine, Calif.) calculated mist frequency and triggered a traveling gantry (boom) (Solaris, McConkey Co., Mt. Puyallup, Wash.) to apply mist. Misting frequency (number of boom passes) was similar for all cuttings within each experiment; however, boom traveling speeds were altered to create different mist application treatments. For each boom speed, mist application was calculated by dividing the total output for all nozzles (258 mL min^{-1} per nozzle x 26 nozzles) (TeeJet nozzle #800067, Spraying Systems, Co., Neuvo, Calif.) by the area covered by the boom in 1 min, expressed as milliliters per square meter. The experimental design was a randomized complete block with two replications of mist. The mist treatments were 45, 61, 75, 102, 147, or 310 mL m^{-2} of mist per boom pass. Each plot was divided by clear polyethylene plastic barriers (91.4 cm tall) to minimize environmental gradients within the greenhouse and to separate treatments. Experimental stem cuttings were surrounded by two rows of border cuttings of the same genetic origin.

Dependent variables. A pressure chamber (Scholander et al., 1965) (SoilMoisture Equipment Corp., Santa Barbara, Calif.) was used to measure Ψ_{cut} destructively every 3 h beginning at 0500 until 2300 HR (seven measurements) on two cuttings per plot 7, 14, 21, 28, or 35 (Expt. 2 only) days after setting (DAS). Data for both cuttings were subsequently averaged to provide a mean for each plot per measurement time. Substrate water potential (Ψ_{sub}) was also measured in each plot using a tensiometer (Irrometer Co., Riverside, Calif.) at 0500 and 1400 HR on the same days that Ψ_{cut} was measured; however, Ψ_{sub} was not significantly different among plots [data not presented, see LeBude et al. (2004) for construction and maintenance of Ψ_{sub}]. Cuttings selected randomly for Ψ_{cut} measurements were replaced to maintain canopy dynamics, but were excluded from subsequent measurements. Adventitious roots began to emerge ≈ 28 to 42 DAS; however, the percentage of cuttings producing at least one root $\geq$ 1 mm was recorded for each plot 70 DAS.

Effect of mist level on VPD, and VPD on Ψ_{cut} and rooting percentage (Expt. 1). Leaf temperatures were recorded in all plots continuously using thermocouples (Type-T, Omega Engineering, Stamford, Conn.) connected to a micrologger (23X, Campbell Scientific, Logan, Utah). RH at the stem cutting level was recorded in each mist plot in the second replication only by six separate HOBO® data loggers (Onset Computer Corp., Pocasset, Mass.). Data for leaf temperature and RH were averaged continuously from both data loggers over 15 min intervals. VPD based on the leaf temperature and RH was calculated using equations of Buck (1981) and Prenger and Ling (2001). Leaf temperature was used as a substitute for air temperature because leaf temperature was measured in both replications and air temperature and humidity in one replication only. Because VPD was calculated for each plot using this method, data for VPD were averaged over both replications before analyses to account for this limitation. In some cases, HOBO data loggers malfunctioned while data were being recorded due to either saturated conditions or battery failure. As a result, data for the mist level of 61 mL m^{-2} were excluded from all analyses and data for the mist level of 75 mL m^{-2} include only the first 20 DAS rather than 30 DAS used for all other mist levels. Although VPD was recorded in Expt. 2, data logger malfunction prevented data from being retrieved for use. Therefore, data for VPD in Expt. 2 are not presented.

Mean VPD between 1000 and 1800 HR was used in analyses, because the greatest variation among mist levels occurred during this period and, subsequently, was found to contribute most meaningfully to the statistical relationships between variables. Likewise, mean Ψ_{cut} for measurements at 1100, 1400, or 1700 HR were used to coincide with this time frame. Previously, rooting percentage was found to be related to mean daily Ψ_{cut} averaged from 0500 to 2300 HR, and related to the single daily minimum Ψ_{cut} (most negative) measured (LeBude et al., 2004). The present paper differs by reporting the relationship between the environmental conditions during rooting that contribute to the mean midmorning to late afternoon Ψ_{cut}.

Effect of mist level on photosynthesis at ambient conditions ($A_{ambient}$) and stomatal conductance to water vapor (g_s) and their effect on rooting percentage (Expt. 2). $A_{ambient}$ and g_S were measured on nonrooted stem cuttings 14, 28, 42, 56, or 70 DAS using a LI-COR 6400 infrared gas analyzer (IRGA) (Software version Open 3.4, LI-COR, Lincoln, Nebr.) equipped with a 6 cm^2 cuvette and a 6400-02B Red/Blue LED light source. The same measurements were made separately on rooted stem cuttings (control plants) 28 or 70 DAS only. All measurements were recorded on a replication basis between 0750 and 1100 HR (AM) and 1300 and 1600 HR (PM) on two nonrooted stem cuttings per plot, or on two random plants per plot in the case of controls (i.e., data for replication 1 of nonrooted stem cuttings were recorded 27 DAS, while data for replication 1 of the controls were recorded 28 DAS, etc.). For AM and PM measurements, the average ambient greenhouse environmental conditions across plots (as recorded by dataloggers and QCOM for 2 weeks prior to the first measurements) were reproduced in the cuvette and used as a standard environment for measurement. During all AM measurements, *PAR* was held constant at 250 μmol m^{-2} s^{-1}, CO_2 was 400 μmol m^{-2} s^{1}, RH was 74% to 79%, and leaf temperature was 25° C. During all PM measurements, *PAR* was 450 μmol m^{-2} s^{-1}, CO_2 was 400 μmol m^{-2} s^{-1}, RH was 64% to 69%, and leaf temperature was 28° C.

Cuttings were selected randomly from plots and hand dried with paper towels and KimWipes (Kimberly Clark, Inc., Neenah, Wis.). Because we expected rates of CO_2 assimilation and g_s to be low and variable for stem cuttings, we inserted as many recently expanded needles into the cuvette as possible without overlapping. Leaf area on the IRGA was set to 2 cm^2 for all measurements, and then readjusted based on leaf area calculations described below. Prior to logging data, $A_{ambient}$ and g_s were monitored for 3 to 5 min while the total coefficient of variation was < 0.5%. Ψ_{cut} was measured immediately on each stem cutting or control plant, using a pressure chamber, after measurements of gas exchange were completed.

Controls. One hundred forty four rooted stem cuttings from Expt. 1 (originally from the same stock plants as stem cuttings used in Expt. 2) were chosen randomly from a larger group of rooted cuttings and potted in 164 mL Ray Leach Super cells (Steuwe and Sons, Corvallis, Ore.) filled with the same silica sand used for rooting the stem cuttings. The rooted cuttings were grown outdoors for 3 weeks and fertilized daily with a 20N-8.7P-16.6K water soluble fertilizer (Peters, The Scotts Co., Marysville, Ohio) providing N at 100 mg L^{-1}. Plants were then topdressed with 0.71 g 18N-2.6P-9.9K Osmocote controlled-release fertilizer (8-9 month formulation, Grace-Sierra, Milpitas, Calif) and placed beside nonrooted stem cuttings under each mist treatment. Control plants were watered twice daily over the entire experiment to maintain similar substrate water contents as rooting tubs receiving subirrigation. Substrate water potential was not measured in controls.

Fascicle surface area in the cuvette. Fascicle surface area (SA) was estimated using the equation,

$$SA=2\pi rl + 6rl ,$$

which assumes needles are the sectors of a cylinder, and r is the radius in centimeters of the fascicle (measured as the interior face of one needle in the fascicle of three needles) and l is the length of the needle in centimeters (Johnson, 1984; Svenson and Davies, 1992). During measurements of CO_2 assimilation 42 DAS, one fascicle consisting of three needles was selected independently of experimental fascicles within each replication-by-time-of-day measurement [2 cuttings per plot x 2 replications x 2 times of day (AM and PM) equals eight cuttings (eight fascicles) for each mist level]. Fascicles were cut to a length of 31 mm representing the length and portion of needle enclosed in the 2 cm x 3 cm cuvette. A thin cross section was taken from the midsection of each needle as well as each end yielding an average measure of the radius of the proposed cylinder based on nine measurements for each fascicle. The interior face of each cross section was measured to the nearest 0.1 mm using a stereo microscope (Wild HeerBrugg, Technical Instrument Co., San Francisco, Calif.). The remaining 30 mm length of needle was dried for 72 h at 70° C and weighed to the nearest 0.01 mg. SA was then regressed on the dry weight (DW) of each fascicle-by-mist treatment. The resulting regression equations were used to estimate SA based on the DW of needles actually used in measurements of gas exchange from all measurement periods. SA was estimated similarly for needles collected from control plants at 70 DAS. After SA was estimated for all measurement periods and both plant types, data for A_{ambient} and g_s were recalculated using the IRGA by re-entering the leaf area measurements.

Statistical analyses. Data were tested for normality and homogeneity of variances using univariate procedures (Steel et al., 1997) in SAS v. 8.2 (SAS Institute, Inc., 2001). Data for A_{ambient} were distributed normally and had homogeneous variances; however, data for g_s did not. Data for g_s were log transformed prior to analysis of variance (ANOVA) procedures. Nonsignificant terms for main effects and interactions were pooled into the main error term. Main effects and interactions were retested using appropriate error terms and these values are reported in Table 1. Means reported in all figures for both variables are nontransformed data.

VPD was distributed normally and had equal variances among the mist levels and time periods analyzed for this study. Data for Ψ_{cut} were not distributed normally, but had equal variances among treatments. Despite numerous transformations, normality was not improved significantly. However, the transformation that improved distribution according to visual examination of plots was used in a comparative analysis with the nontransformed data. No differences were found between the two comparison analyses. Data for rooting percentage were distributed normally and had homogeneous variances among treatments. Therefore, all test statistics and means presented herein are based on the nontransformed data of Ψ_{cut} and rooting percentage.

Regression analysis was used to determine the relationships between $A_{ambient}$, g_s, rooting percentage, VPD, and Ψ_{cut}. Each variable was used in regression either as a dependent or independent variable depending on the objective being tested. When generating the quadratic term or log function of a variable for use in regression, the transformation was made on the original datum and then averaged over measurement times, DAS, etc. For this reason, inputting variables from the X axis into the regression equations presented in the figure captions will not produce a point exactly on the lines representing the relationships within the graphs.

Results

Effect of mist level on VPD, and VPD on ψ_{cut} (Expt. 1). Mean VPD ranged from 0 to 1.2 kPa, depending on time of day and mist application (Fig. 1). For example, between 1300 and 1700 HR, VPD was highest for the mist level of 75 mL m^{-2} (1.2 kPa) and lowest for the mist level of 310 mL m^{-2} (0.6 kPa). Between 2100 and 0700 HR, VPD was near 0 kPa for all mist levels (Fig. 1). Averaged between 1000 and 1800 HR, VPD was strongly related to the log of mist application [$\text{VPD}=1.81-0.22\ln(\text{mL m}^{-2})$, $P = 0.01$, $r^2 = 0.82$]. Mean ψ_{cut}, for measurements recorded at 1100, 1400, or 1700 HR, decreased (became more negative) as the log of VPD increased (averaged between 1000 and 1800 HR) [$\psi_{cut} = -1.94 + 1.96\ln(\text{VPD})$, $P = 0.02$, $r^2 = 0.89$].

Relationship between rooting percentage, ψ_{cut}, and VPD (Expt. 1). Rooting percentage was strongly related to the linear and quadratic terms of Ψ_{cut} (Fig. 2). The regression equation predicted ≥ 80% rooting with mean ψ_{cut} between - 0.55 and -1.2 MPa (averaged over 1100, 1400, and 1700 HR). Rooting percentage was also related strongly to the linear and quadratic terms of mean daily VPD (Fig. 3A). Rooting percentages ≥ 80% occurred when mean daily VPD ranged from 0.6 to 0.85 kPa between 1000 and 1800 HR.

The maximum daily VPD value for a single 15 min interval was recorded for each mist level and averaged 20 DAS (75 mL m^{-2} only) or 30 DAS. Rooting percentage was related strongly with the linear and quadratic terms of mean maximum daily VPD (Fig. 3B). Rooting percentage was predicted ≥ 80% when daily maximum VPD ranged from 0.85 to 1.3 kPa.

Effect of mist level on $A_{ambient}$ and g_s (Expt. 2). Mist level and DAS significantly affected $A_{ambient}$ and g_s of nonrooted stem cuttings (Table 1). Greater mist volumes generally increased $A_{ambient}$ in all five measurement periods (Fig. 4A). When $A_{ambient}$ was averaged for all mist levels at each measurement period, the response declined initially between 14 and 28 DAS, and then remained relatively steady through 70 DAS (see mean Fig. 4A). Stomatal conductance of nonrooted stem cuttings responded similarly to mist level and DAS (Fig. 4B). In contrast to nonrooted stem cuttings, $A_{ambient}$ and g_s of rooted controls was affected by DAS, but not mist (Table 1.). Mean $A_{ambient}$ was 2.19 and 8.49 μmol m^{-2} s^{-1} at 28 and 70 DAS, respectively, and g_s was 59 and 328 mmol m^{-2} s^{-1} at 28 and 70 DAS, respectively, averaged across mist levels.

Photosynthesis and g_s of juvenile, nonrooted, succulent stem cuttings were related strongly to the log of mist level when data for 14, 28, or 42 DAS were averaged together to represent the period during adventitious root formation (Fig. 5A and B). Both responses increased as mist level increased. It was reported previously that daily mean Ψ_{cut} was also related strongly to mist level in juvenile stem cuttings of loblolly pine (LeBude et al., 2004). In the present study, $A_{ambient}$ increased linearly as Ψ_{cut} increased (became less negative) (Fig. 6A). Stomatal conductance, however, had a different response to Ψ_{cut}, increasing gradually (35 mmol m^{-2} s^{-1}) between - 1.3 and - 0.9 MPa, and then sharply (150 mmol m^{-2} s^{-1}) thereafter until reaching - 0.5 MPa (Fig. 6B).

Stomatal conductance affected $A_{ambient}$ in nonrooted stem cuttings similarly at 28 and 70 DAS (Fig. 7A). In rooted controls 28 DAS, the response was similar to nonrooted stem cuttings measured at the same time (compare solid symbols between Fig. 7A and B). At 70 DAS, however, overall rates of $A_{ambient}$ and g_s were greater for the controls (Fig. 7A and B, compare open symbols). When data for both 28 and 70 DAS were included in the model for the controls, there was a strong overall relationship between $A_{ambient}$ and g_s (Fig. 7B, both open and solid symbols).

Relationships between rooting percentage and $A_{ambient}$ *and* g_s *(Expt. 2).* Rooting percentage was not related to $A_{ambient}$ of nonrooted, juvenile, succulent, stem cuttings of loblolly pine (Fig. 8A). Rooting percentage was related to the linear and quadratic terms of g_s (Fig. 8B). The equation predicted ≥ 70% rooting when g_s ranged from 40 to 150 mmol m^{-2} s^{-1}.

Discussion

Effect of mist level on VPD and VPD on Ψ_{cut} *and rooting percentage (Expt. 1).* Mist level contributed to both the VPD surrounding the stem cuttings and Ψ_{cut} when data for both variables were averaged between 1000 and 1800 HR. Mist application decreases VPD by lowering leaf temperatures and increasing the RH surrounding stem cuttings (Tukey, 1978). Thus, there is less of a transpirational demand on stem cuttings and/or increased absorption of water through the foliage; both of which aid in maintaining or increasing Ψ_{cut} during the rooting period (LeBude et al., 2004).

Rooting ≥ 80% occurred for juvenile hardwood stem cuttings of loblolly pine when mean Ψ_{cut} was maintained between -0.6 and -1.2 MPa. This corresponded to a mean daily VPD between 0.60 and 0.85 kPa. Values of mean VPD associated with increased rooting are similar to those of Newton et al. (1992) when stem cuttings of terminalia (*Terminalia spinosa* Engl.) rooted at 80%. In light red meranti (*Shorea leprosula* Miq.), rooting was 60% within this range of VPD; however, higher maximum VPDs were recorded during rooting, which could have corresponded to increased water stress (Aminah et al., 1997).

Mean daily maximum VPD recorded in the present study was between 0.85 and 1.25 kPa when rooting percentage was ≥ 80%. Because individual species may respond differently to VPD, an initial step in designing suitable rooting environments would be to define the physiological response and rooting of stem cuttings to such environmental factors. After rooting percentage was 100% in stem cuttings of poinsettia, VPD was used as a dynamic control for mist application during subsequent root growth and development (Zolnier et al., 2003). Our data indicate the potential use of VPD to control mist application during the period of adventitious root formation in dormant, hardwood stem cuttings of loblolly pine to produce a range of Ψ_{cut} necessary for optimal rooting rates. Data for VPD were unavailable for succulent, softwood stem cuttings in Expt. 2 due to instrument malfunction; however, the authors believe that the results for hardwood stem cuttings would extend to softwood stem cuttings because of (1) the strong relationships between VPD and Ψ_{cut}, and VPD and rooting of dormant stem cuttings in this paper, and (2) the strong and very similar relationship between rooting and Ψ_{cut} of both dormant and succulent stem cuttings in LeBude et al. (2004).

Effect of mist on $A_{ambient}$ and g_s, and their effect on rooting percentage (Expt. 2). $A_{ambient}$ and g_s of juvenile, nonrooted, succulent, stem cuttings of loblolly pine declined as DAS progressed, which has been reported for many species (Aminah et al., 1997; Davis, 1988; Mesén et al., 1997; Newton et al., 1992; Smalley et al., 1991; Yue and Margolis, 1993), especially when compared to rooted controls in the same experiment (Gay and Loach, 1977; Grossnickle and Russell, 1993). More broadly, these processes are decreased when our results are compared to those of 1- to 2-year-old intact seedlings of loblolly pine grown in the field in other experiments (Kramer and Clark, 1947; Seiler and Johnson, 1985). Both processes increased, however, with increasing mist volume (Fig. 5A and B). This was probably due to the strong affect of mist level on Ψ_{cut} (Fig. 5, LeBude et al., 2004), and the subsequent effect of Ψ_{cut} on the relationship between $A_{ambient}$ and g_s (Figs. 6A and B and 7A and B). In stem cuttings of other species, variation among treatments or decreases in photosynthetic rate were attributed generally to water deficit (Svenson et al., 1995), to water deficit induced by high irradiance (Mesén et al., 1997), or to water deficit induced by various leaf area treatments (Newton et al., 1992). This is not surprising since the dual roles of stomata are to regulate water loss while assimilating CO_2. In some species, stomatal regulation is a response to water deficit (Sperry, 2000).

Photosynthetic rate of juvenile, nonrooted, succulent stem cuttings of loblolly pine was not related to rooting percentage. Some previous reports have also noted no relationship between photosynthetic rates during the rooting period and rooting percentage (Mesén et al., 1997; Okoro and Grace, 1976; Smalley et al., 1991; Svenson et al., 1995). Moreover, efforts to increase rooting of stem cuttings of loblolly pine by increasing levels of CO_2 artificially in polyethylene greenhouses were not successful in the 1970s [Michael Greenwood, Dept. of Forest Ecosystem Sci., Univ. of Maine, Orono (personal communication)]. In studies where photosynthetic rates among treatments were not related to percent rooting, such factors as the phase change of the stock plants (Grossnickle and Russell, 1993), clonal differences in rooting (von Schaesberg et al., 1993), or poor environmental conditions were inferred to be contributing causes (Yue and Margolis, 1993). It has been proposed that photosynthesis does not occur in nonrooted stem cuttings because of minimal leaf conductance (Gay and Loach, 1977). In contrast, other researchers have found that the photosynthetic rate in stem cuttings of tropical tree species affected rooting percentage (Aminah et al., 1997; Hoad and Leakey, 1996; Newton et al., 1992). Additional support for this hypothesis comes from Leakey and Coutts (1989) who measurements levels of carbohydrates and correlated them with rooting percentage. Rooting percentages in that study, however, also could have been explained by differences in water deficits among treatments rather than as a direct result of photosynthetic activity.

The hypothesis that current photosynthetic rate affects rooting percentage has neither been proven nor refuted, as it is supported in some cases but not in others. This might be due to the inherent obstacle of independently controlling the photosynthetic rates of stem cuttings (Davis, 1988), in addition to the difficulty of replicating experiments over time, the use of various angiosperms vs. gymnosperms, as well as the use of different genetic experimental units within species (i.e., number of different cultivars, clones, or the use of seedlings). Moreover, in the present study, gas exchange was measured on succulent rather than dormant stem cuttings because it was thought that succulent stem cuttings would be more photosynthetically active than dormant stem cuttings. We suggest that photosynthesis in juvenile, succulent, stem cuttings of loblolly pine, at least at a minimal level, might be an indication of the relative ability of cuttings to function under conditions of high stress. In this case, it is an indication of the ability of the stem cutting to assimilate carbon while experiencing repeated severe water deficit coupled with high leaf temperatures and irradiance. Above that minimal level, water deficit, photosynthesis and, therefore, carbohydrate supply are not limiting to rooting percentage.

In the present investigation, rooting percentage was more closely related to moderate rates of g_s than to $A_{ambient}$ in softwood, juvenile stem cuttings of loblolly pine. Rooting percentage and g_s could be related indirectly as indicated by the inability of the linear and quadratic equation to predict more than ≈ 50% of the variation in rooting percentage (Fig. 8B). Stomatal conductance could be related indirectly to rooting percentage by moderating Ψ_{cut}. The mechanism(s) by which Ψ_{cut} influence rooting percentage is largely unknown (LeBude et al., 2004; Sinclair and Ludlow, 1985), but this effect could be independent of any effect g_s has on rooting percentage.

Summary. This study measured various environmental variables and physiological processes to learn how to better design and control rooting environments for stem cuttings of loblolly pine. Although $A_{ambient}$, g_s, Ψ_{cut}, and VPD were affected by varying mist levels, the relationships between $A_{ambient}$ and g_s with rooting percentage were not as strong as between Ψ_{cut} and VPD with rooting percentage. Moreover, $A_{ambient}$, g_s, and Ψ_{cut} are time consuming measurements, limiting the number of measurements that can be taken and their subsequent simultaneous use in dynamic control of mist application. On the other hand, VPD was related strongly to rooting percentage, and VPD can be measured rapidly, calculated automatically, and integrated into a system to control mist application dynamically. Our results suggest that VPD could be used as a dynamic control for mist application when managing Ψ_{cut} in stem cuttings of loblolly pine. A mean daily range of VPD between 1000 and 1800 HR of 0.6 to 0.85 kPa should produce beneficial levels of Ψ_{cut} that improve rooting percentages in stem cuttings of loblolly pine. Further research is necessary to validate the VPD-based model as a control system for mist application, as well as establish appropriate levels of VPD for a wider range of species in different rooting environments.

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Table 1. Analysis of variance summary for potential effects of mist level (M), days after setting (DAS), and time of day (TOD) on photosynthetic rate ($A_{ambient}$) and stomatal conductance (g_s) of nonrooted, juvenile, softwood stem cuttings of loblolly pine in Expt. 2 (conducted June 2002) 14, 28, 42, 56, or 70 DAS, and of intact, rooted control plants evaluated 28 or 70 DAS. Values are the probability of a greater F statistic. Bold values are statistically significant at $P < 0.05$.

	Nonrooted cuttings			Rooted controls		
Source	df	$A_{ambient}$	g_s	df	$A_{ambient}$	g_s
Replication	1	**0.02**	0.23	1	**0.02**	0.10
M	5	**0.01**	**0.01**	5	0.40	0.55
DAS	4	**0.02**	**0.04**	1	**0.01**	**0.03**
TOD	1	0.54	0.31	1	0.93	0.38
DAS x M	20	0.13	0.52	5	0.43	0.51
TOD x M	5	0.92	0.71	5	0.43	0.75
DAS x TOD	4	0.42	0.45	1	0.53	0.46
DAS x TOD x M	20	0.85	0.87	5	0.50	0.37

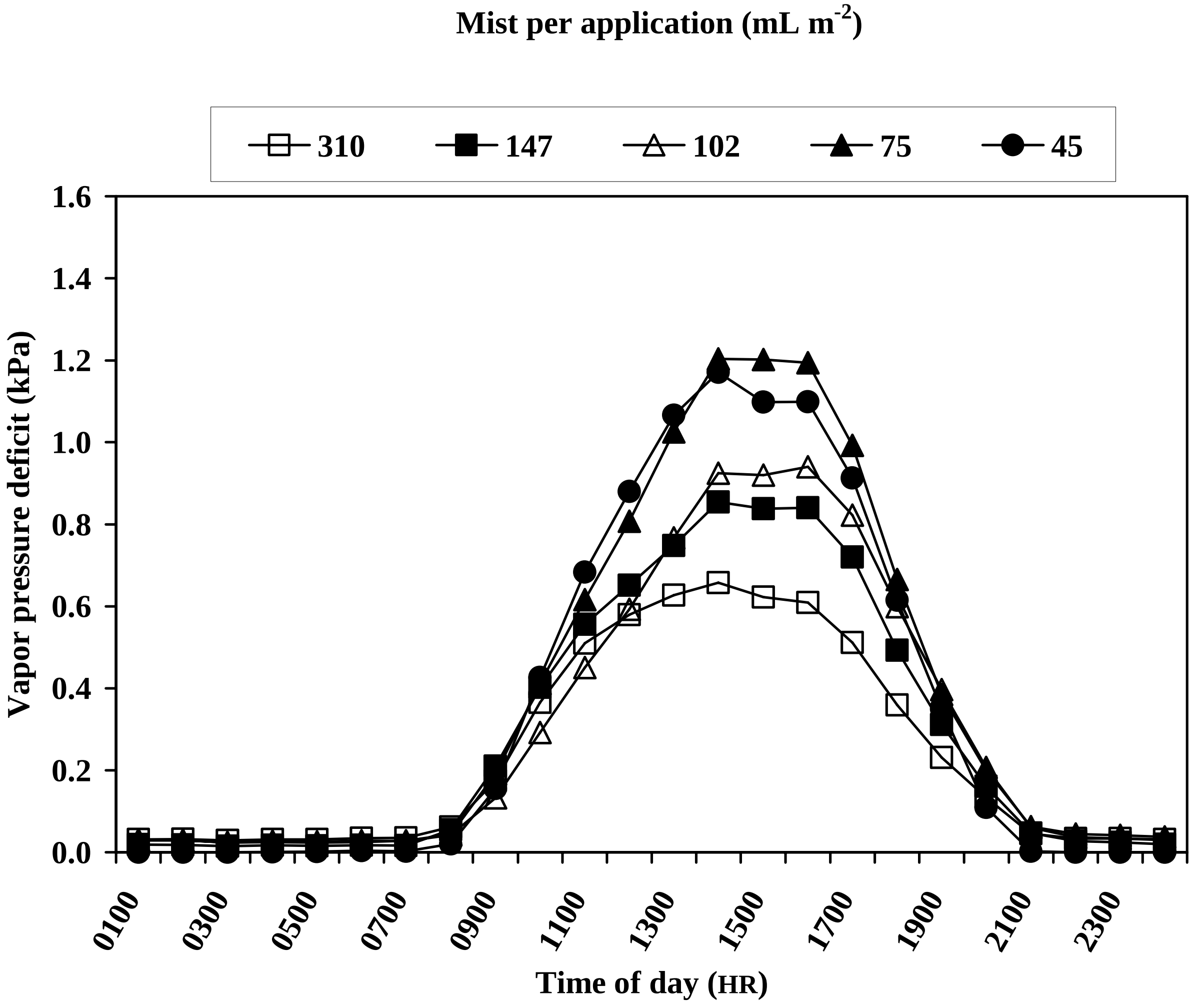


Fig. 1. Effect of mist level on mean VPD by hour over 20 (75 mL m^{-2}) or 30 DAS for Expt. 1 (Apr. 2002). (Mist level of 61 mL m^{-2} was not included due to instrument error while data were recorded). Symbols are means of two replications.

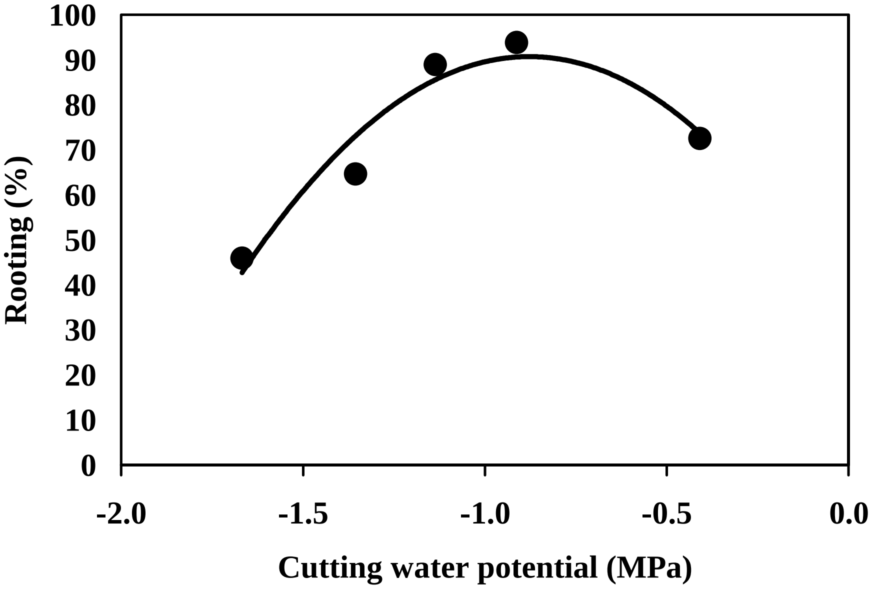


Fig. 2. Rooting percentage (70 DAS) as a function of Ψ_{cut} averaged over both replications for measurements recorded at 1100, 1400, or 1700 HR 7, 14, 20 (75 mL m^{-2} only) or 30 DAS for juvenile, hardwood stem cuttings rooted in Expt. 1 (Apr. 2002). (Mist level of 61 mL m^{-2} was not included due to instrument error while data were recorded.). The regression equation is Rooting (%) = 4.15 – 224.62(Ψ_{cut}) – 115.90(Ψ_{cut}^2), $P = 0.03$, $r^2 = 0.97$. Data for the quadratic term (Ψ_{cut}^2) were generated by squaring the linear term and then averaging over measurement times and DAS.

Fig. 3. Rooting percentage of juvenile, hardwood stem cuttings of loblolly pine as a function of (A) mean daily VPD between 1000 and 1800 HR, and (B) mean daily maximum VPD for one 15 min period between 1000 and 1800 HR in Expt. 1 (Apr. 2002). Data for VPD were averaged for both replications over 20 (75 mL m^{-2} only) or 30 DAS and rooting was scored 70 DAS. (Mist level of 61 mL m^{-2} was not included due to instrument error while data were recorded.). The regression equation for (A) is Rooting (%) = -189.43 + 728.76(mean daily VPD) – 384.88(mean daily VPD^2), $P = 0.04$, $r^2 = 0.96$, and for (B) is Rooting (%) = - 198.00 + 522.78(daily max. VPD) – 211.60(daily max. VPD^2), $P = 0.05$, $r^2 = 0.95$. Data for the quadratic term (VPD^2) were generated by squaring the linear term prior to averaging over measurement times and DAS.

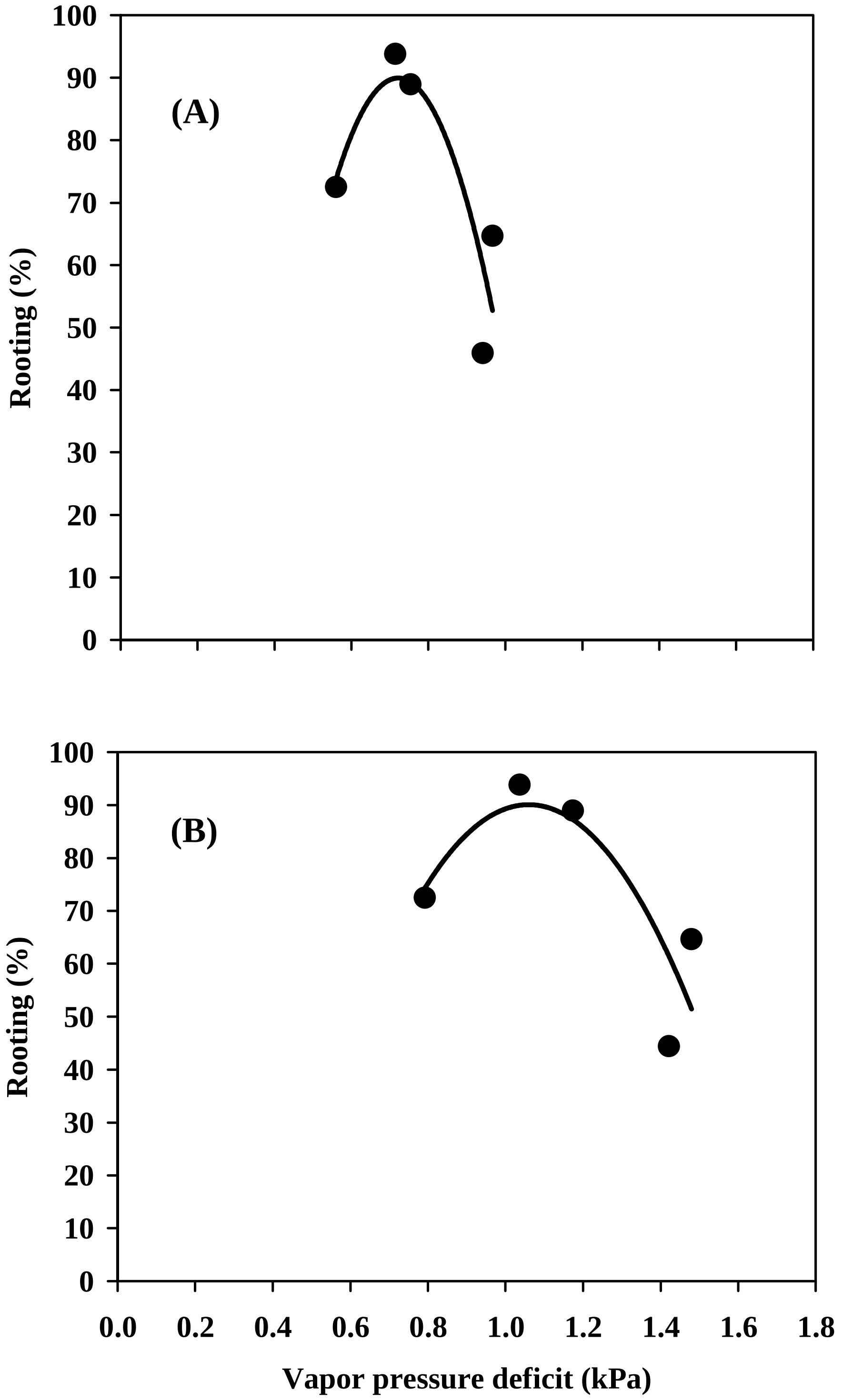
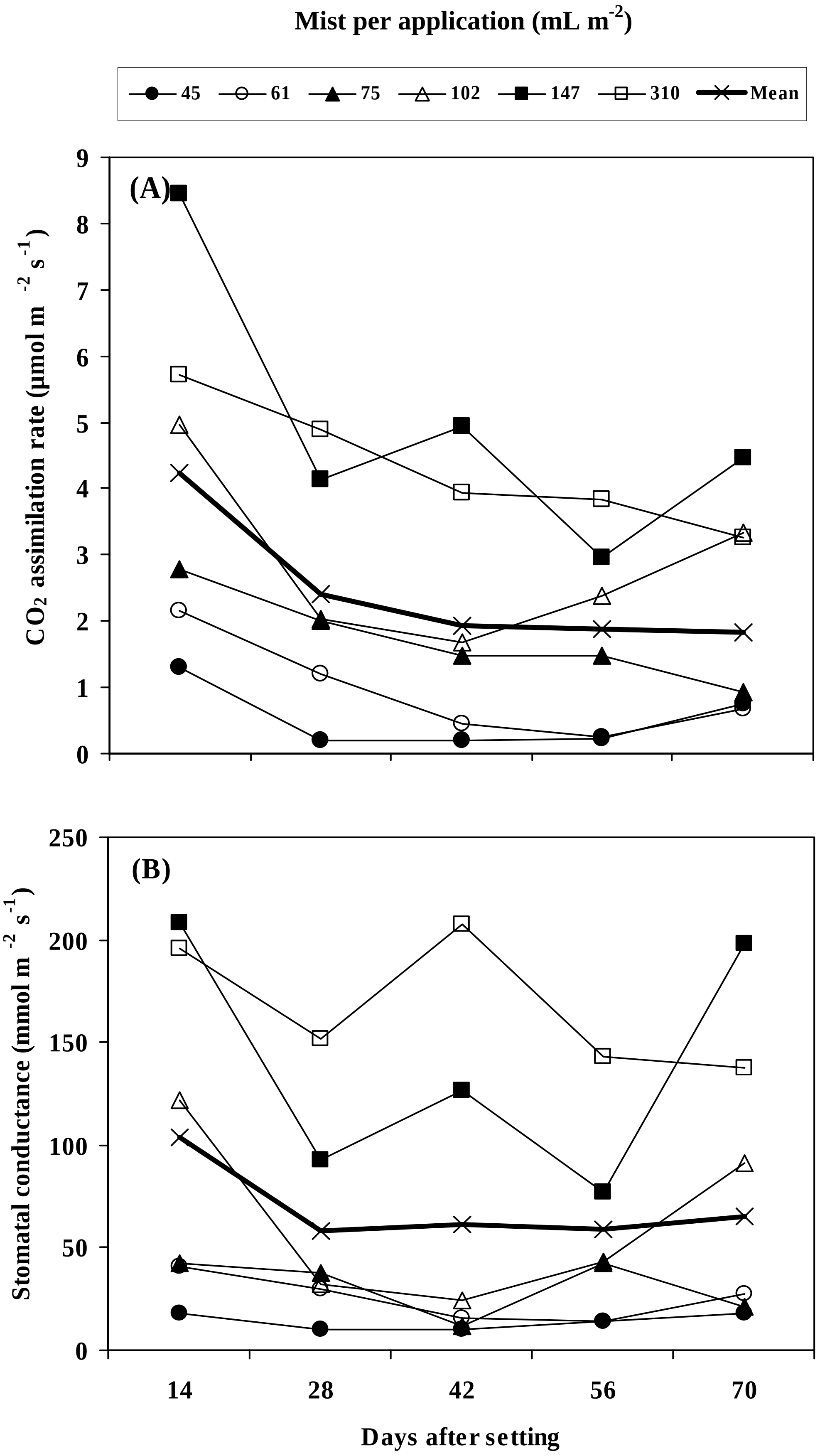

(A)
(B)
Rooting (%)
Rooting (%)
0
10
20
30
40
50
60
70
80
90
100
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
Vapor pressure deficit (kPa)

Fig. 4. (A) $A_{ambient}$ and (B) g_s of nonrooted, juvenile, softwood stem cuttings of loblolly pine in Expt. 2 (June 2002) 14, 28, 42, 56, or 70 DAS for each mist level. Symbols are means of measurements recorded in the AM or PM for two replications of each mist level.


Mist per application (mL m-2)
45
61
75
102
147
310
Mean
(A)
CO2 assimilation rate (µmol m-2 s-1)
0
1
2
3
4
5
6
7
8
9
(B)
Stomatal conductance (mmol m-2 s-1)
0
50
100
150
200
250
14
28
42
56
70
Days after setting

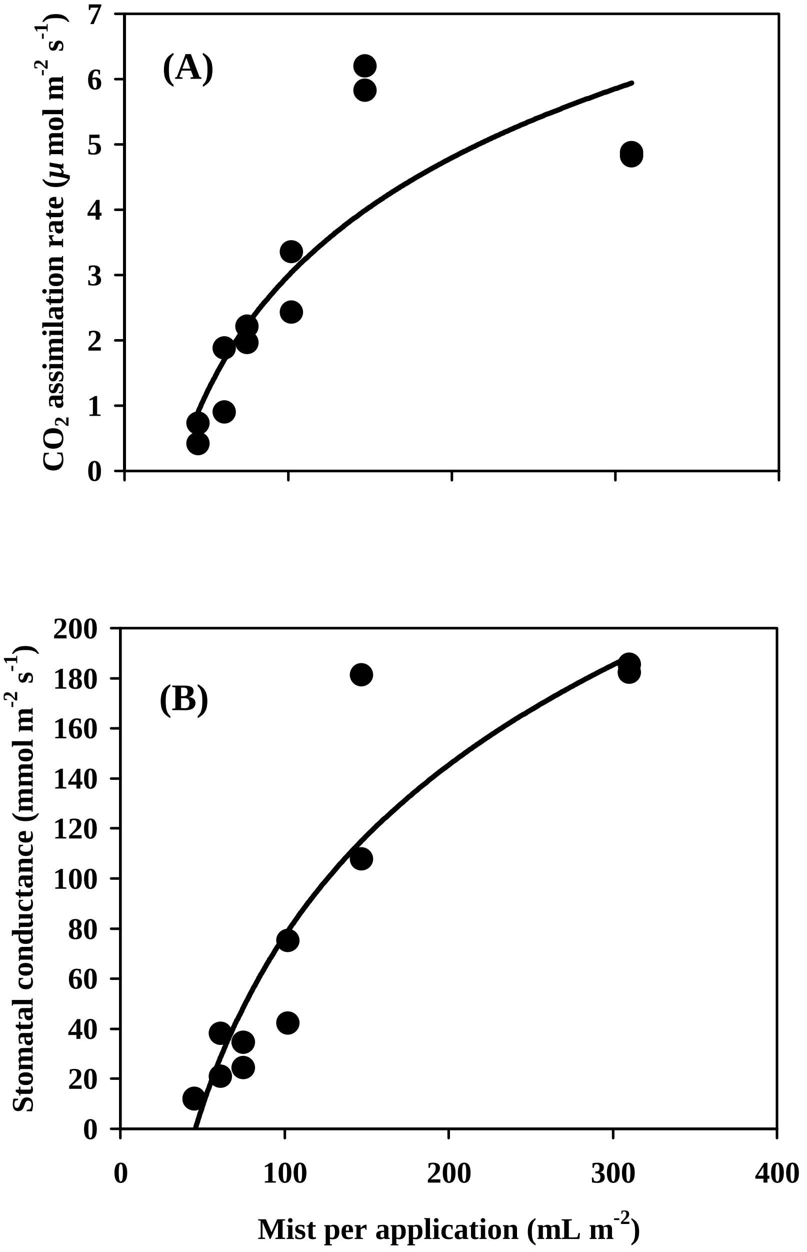


Fig. 5. Relationship between (A) $A_{ambient}$ and (B) g_s and mist level for nonrooted, juvenile, softwood stem cuttings of loblolly pine rooted under six mist regimes in Expt. 2 (June 2002). Data points are averaged over AM and PM measurements 14, 28, or 42 DAS. The regression equations are (A) $A_{ambient} = -9.03 + 2.61\ln(\text{mist mL m}^{-2})$, $P = 0.01$, $r^2 = 0.73$, and (B) $g_s = -367.55 + 98.51\ln(\text{mist mL m}^{-2})$, $P = 0.01$, $r^2 = 0.87$.

Fig. 6. Relationship between (A) $A_{ambient}$ and (B) g_s and Ψ_{cut} of nonrooted softwood stem cuttings of loblolly pine in Expt. 2 (June 2002). Ψ_{cut} was measured on each cutting immediately after $A_{ambient}$ and g_s were measured. Data points for both variables are averaged over morning and afternoon measurements 14, 28, or 42 DAS. The regression equations are (A) $A_{ambient} = 9.28 + 7.07(\Psi_{cut})$, $P = 0.01$, $r^2 = 0.73$, and (B) $g_s = 36.11 – 219.39\ln(\Psi_{cut}* -1)$, $P = 0.01$, $r^2 = 0.86$.

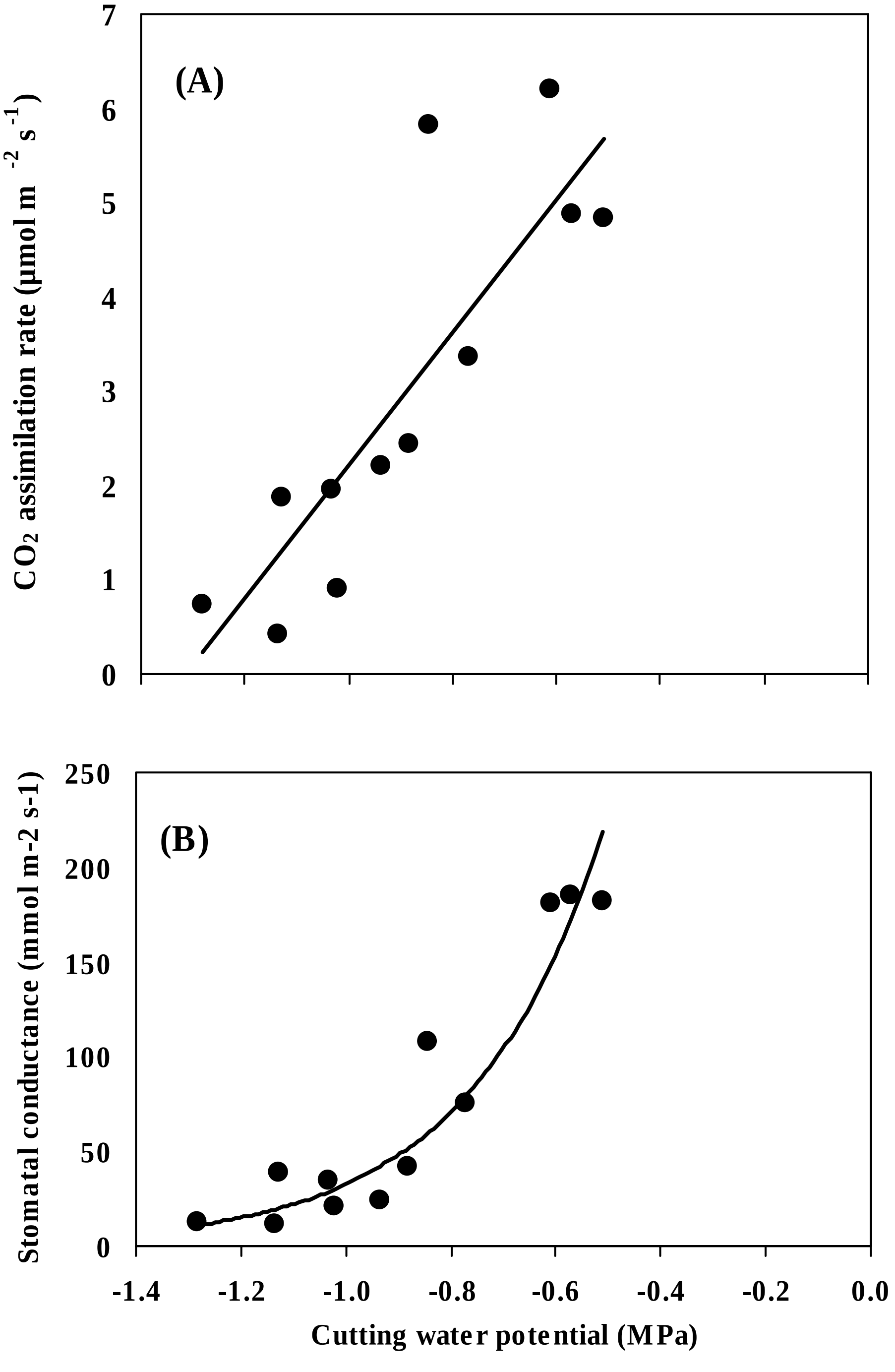

(A)
(B)
CO_2 assimilation rate (µmol m^{-2} s^{-1})
Stomatal conductance (mmol m-2 s-1)
Cutting water potential (MPa)
0
1
2
3
4
5
6
7
0
50
100
150
200
250
-1.4
-1.2
-1.0
-0.8
-0.6
-0.4
-0.2
0.0

Fig. 7. Relationship between $A_{ambient}$ and g_s for (A) nonrooted, juvenile, softwood stem cuttings of loblolly pine 28 (solid symbols) and 70 (open symbols) DAS and (B) rooted controls at 28 (solid symbols) and 70 (open symbols) DAS in Expt. 2 (June 2002). The regression equations are (A) $A_{ambient}$ (28 DAS) = - 0.27 + 0.05(g_s) – 0.0002 (g_s^2), $P = 0.01$, $r^2 = 0.92$, and $A_{ambient}$ (70 DAS) = - 0.43 + 0.08(g_s) – 0.0003 (g_s^2), $P = 0.01$, $r^2 = 0.92$ for nonrooted stem cuttings and (B) $A_{ambient}$ (28 and 70 DAS) = - 7.9 + 2.91 ln(g_s), $P = 0.01$, $r^2 = 0.80$ for rooted controls.

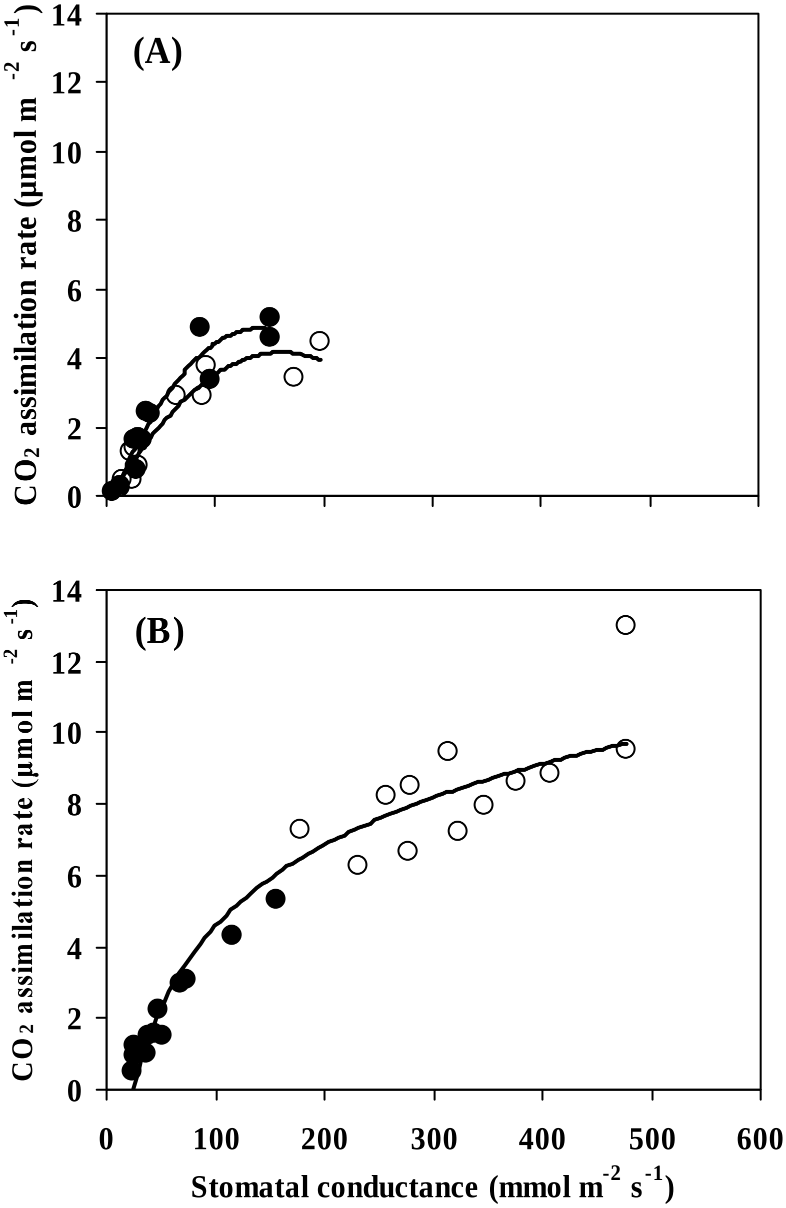
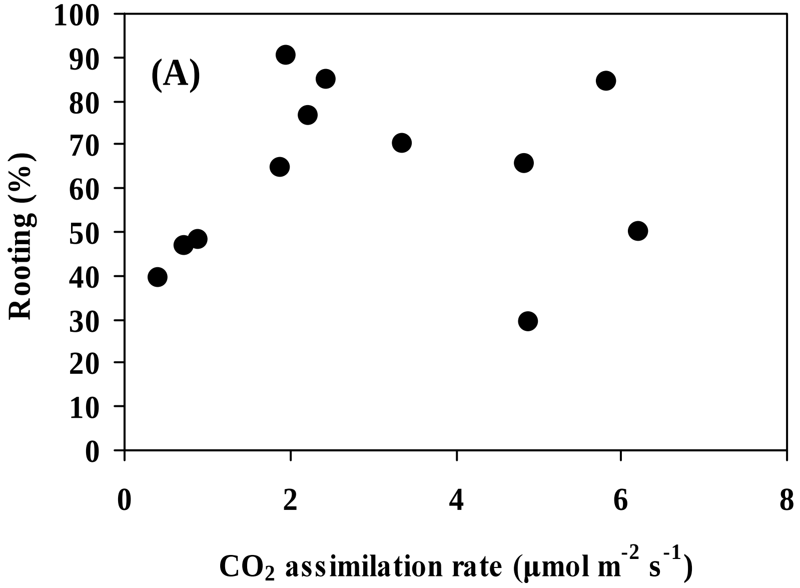
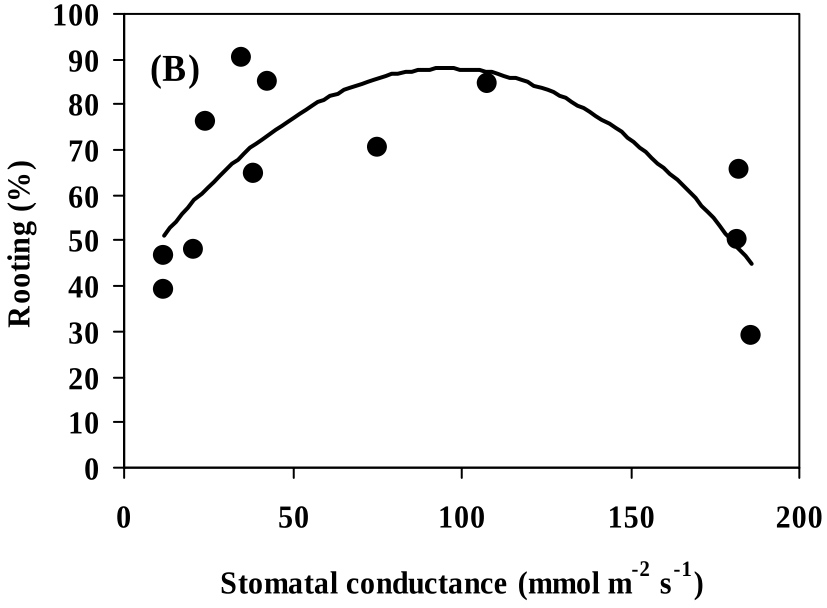

(A)
(B)
CO2 assimilation rate (µmol m-2 s-1)
Stomatal conductance (mmol m-2 s-1)
0
2
4
6
8
10
12
14
0
100
200
300
400
500
600

Fig. 8. Rooting percentage and (A) $A_{ambient}$ and (B) g_s of nonrooted, juvenile, softwood stem cuttings averaged for AM or PM afternoon measurements recorded 14, 28, or 42 DAS in Expt. 2 (June 2002). Rooting percentage (70 DAS) was not significantly related to photosynthetic rate. The regression equation in (B) is Rooting (%) = 34.48 + 1.55(g_s) – 0.007(g_s^2), $P = 0.04$, $r^2 = 0.52$.


(A)
Rooting (%)
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
CO2 assimilation rate (µmol m-2 s-1)


(B)
Rooting (%)
100
90
80
70
60
50
40
30
20
10
0
0
50
100
150
200
Stomatal conductance (mmol m-2 s-1)