CONK, CARLIN ELIZABETH. Assessing the Reliability of Hydrologic Characterization of Four Wetland Plant Communities. (Under the direction of Drs. Michael J. Vepraskas and Stephen W. Broome).

Previous field work characterized the wetness requirements of four plant communities by modeling their hydrologic regimes in three reference wetlands and correlating the results. The objectives of this work were to: 1) test the response of four tree species that represented different plant communities to controlled hydrologies in the greenhouse that simulated those predicted by the model, and 2) determine how different tree species acclimate to saturated soil conditions. Bald cypress (*Taxodium distichum*), sweet bay (*Magnolia virginiana*), pond pine (*Pinus serotina*), and swamp chestnut oak (*Quercus michauxii*) were grown in greenhouse experiments under three hydrologic regimes: i) ponded for 100 d, ii) ponded for 15 d, and iii) unsaturated. Loamy sand and sapric materials from a restored Carolina Bay were the substrate materials used to represent mineral and organic soils. Bald cypress (representing Non-riverine Swamp Forest) acclimated well to 100 d of ponding by producing lateral roots near the surface, aerenchyma tissue in roots and stem, and increasing P uptake. In organic soils ponded for 100 d, bald cypress had significantly greater height, diameter, and total biomass than all other tree species. Sweet bay (representing Bay Forest) acclimated well to 100 d of ponding by producing adventitious roots on the submerged portion of the stem. Pond pine (representing Pond Pine Woodland) was intolerant to 100 d of ponded conditions and 75% of the seedlings died in the ponded experiments. Swamp chestnut oak (representing Non-riverine Wet Hardwood Forest) was intolerant to ponding and all seedlings died in ponded treatments. A rhizotron study was conducted to further observe and quantify biological, morphological, and physiological changes in two wetland tree species with differing tolerances to saturated conditions. Bald cypress and
swamp chestnut oak seedlings were grown under ponded and unponded conditions for 106 d in rhizotrons containing the same soils used in the container study. Within 2 wk of ponding, bald cypress seedlings in both organic and mineral soils began to produce lateral roots within the top 36 cm of soil. The swamp chestnut seedlings subjected to ponding did not produce many new roots, and root death was observed within 4 wk of flooding. Scanning electron microscopy images of basal stems and roots of bald cypress subjected to saturation showed substantial development of aerenchyma, which may have aided in the overall tolerance of bald cypress to ponded conditions. This study showed bald cypress seedlings developed numerous adaptations to saturated conditions, which allowed them to grow in wet organic soils of the Coastal Plain. Results from the container and rhizotron experiments showed that modeling of the long-term hydrology of natural communities was accurate in predicting the preferred wetness requirements of representative trees in three of the four plant communities evaluated.
Assessing the Reliability of Hydrologic Characterizations of Four Wetland Plant Communities

by
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A thesis submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the Degree of Master of Science

Soil Science
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DEDICATION

I would like to dedicate this work to my family. They have provided me with unwavering support throughout my life, with every decision that I have made, even if they secretly felt my choices were unwise. I would also like to thank my husband, Mike, who was always willing to put life into perspective for me, when I needed it most.
BIOGRAPHY

Carlin Elizabeth Conk was born in Richmond, Virginia in 1980. She was raised by her mother, Joyce Ann Conk, and her older sister, Courtney Lynn Conk, until the age of 15. She graduated high school early, at the age of 16, and went to work full-time. After saving money, she traveled the country for several months, while hiking and biking most of the national parks in the United States. In the following four years, she traveled to Europe, becoming an au pair in Switzerland, traveled extensively in East Africa, and volunteered for a non-profit conservation group in Ecuador. She met her future husband in 2000, while working for a wholesale nursery and enrolled in a local community college. Eventually, she graduated Summa Cum Laude from Virginia Polytechnic Institute and State University with an Environmental Science bachelor’s degree. During her time at Virginia Tech, she was recruited for the award-winning Soil Judging Team and discovered her interest in Soil Science. In the fall of 2006, she moved with her husband, Mike, and her dog, Bourbon, to Raleigh, North Carolina, to enroll in the Soil Science Master’s program at North Carolina State University under the direction of Dr. Mike Vepraskas and Dr. Stephen Broome.
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Chapter 1
Literature Review.

INTRODUCTION

Wetland restoration is a complex process that requires extensive knowledge about the interaction of soil, hydrology, and plant communities. It is imperative to understand these relationships before beginning a wetland restoration project, because the plant community types found in wetlands are determined, in part, by the wetland’s hydroperiod. There have been numerous studies conducted on how hydroperiod, or the length of saturation, affect the growth of wetland tree species. In this chapter, the importance of wetland restoration, the role of hydrology and vegetation, as well as biological and physiological responses will be discussed.

WETLAND RESTORATION

Importance of Wetland Restoration

Wetlands provide integral functions to our environment, including: providing wildlife habitat, filtering water, acting as storage basins during flooding events, protecting the shorelines, producing valuable products, and serving as recreational areas (Brady and Weil, 2002). However, wetlands have not always been recognized as economically or aesthetically valuable. In fact, government policies encouraged the destruction of wetlands for agricultural, commercial, or residential purposes before the 1970’s. It is estimated that approximately 53% of the wetlands in the lower 48 states were lost in the past two centuries (Mitsch and Gosselink, 2000). Legislation passed during the late 1970’s gave several governmental agencies the authority to begin...
protecting wetlands, followed by a policy of “no net loss” of wetlands in the late 1980’s. These policies raised public awareness and created interest in restoring and creating wetlands. The knowledge required for “successful” wetland restoration/creation continues to be developed.

**Restoration Success**

Regulators, consultants, and wetland researchers use a variety of guidelines to assess wetland restoration “success”. Success is thought to be the viable establishment of a biologically and ecologically sound wetland ecosystem (Mitsch and Wilson, 1996). Consultants and regulators typically use indicators that rely on vegetation parameters to gauge success. These parameters, such as plant cover, target species, and mortality, are easy to measure and require little equipment. However, studies have shown that wetland restoration projects have been less than 100% successful in the past (Maguire, 1985; Reimold and Cobler, 1985). Erwin (1991) determined that problems with hydrology caused a 60% failure rate in 40 restoration projects in south Florida. According to Mitsch and Wilson (1996), a lack of understanding of general principles of wetland functions can lead to the “failures” of restoration wetlands.

**Conversion of Wetlands to Agriculture**

The conversion of wetlands to agricultural cultivation has bearing on all components of the ecosystem. The construction of drainage ditches alters the hydrology of wetlands by lowering the water table, allowing rapid drainage during precipitation events, and promoting continuous surface water drainage (Bruland et al., 2003). It is often thought that establishing wetland hydrology is the most important step in wetland restoration projects (Kusler and Kentula, 1990) due to the many chemical and physical
properties that hydrology influences. Steven and Toner (2004) found that of all of the factors researched, hydrologic regime most significantly influenced vegetation type when compared to soil texture, disturbance history, and depression size.

Wetland soils typically occur under reduced conditions where low redox potentials result in low rates of decomposition and increased accumulation of organic matter in wetland soils. The establishment of drainage ditches leads to higher rates of decomposition, which causes the loss of organic material and subsidence (Lilly, 1981). When the land is put into agricultural production, tillage increases the bulk density of soils (Brady and Weil, 2002) and liming increases the soil pH, base saturation, and can lead to higher than optimal levels of nitrate and phosphates (Bruland et al., 2003). Braekke (1999) found that concentrations of N and P were significantly raised to a depth of 20 cm, lower concentrations of Ca, K, and Mg occurred, and higher bulk density occurred to a depth of 40 cm in ombrogenous soils which had been drained, fertilized, and/or limed. Ombrogenous soils are soils that are low in fertility and derive most nutrients from precipitation, rather than from groundwater. Like hydrology, the restoration of integral soil properties, such as pH, bulk density, and fertility, is imperative in the reestablishment of wetlands and their original vegetative communities.

**CAROLINA BAYS**

**Introduction**

Carolina bays are elliptical depressions, which occur throughout the southeastern United States. These depressions are most abundant in the Coastal Plain of southeastern North Carolina and mid-coastal South Carolina (Sharitz, 2003). Although early estimates of the number of bays ranged from 100,000 to 500,000 (Nifong, 1998; Prouty, 1952), it is
most likely that as many as 20,000 exist (Richardson and Gibbons, 1993). However, many of these bays have been drained and altered due to agricultural and commercial development pressures. It is estimated that approximately 50% of Carolina bays in Bladen County, NC were drained by 1982 (Weakley and Scott, 1982). Carolina bays are oriented in a unique way; the major axis is oriented in a northwest-southeast direction in North Carolina (Prouty, 1952). These depressions can be small, with the major axis only 90m, or large, with the major axis as long as 11km (Prouty, 1952). Carolina bays typically have a sandy rim (Sharitz and Gibbons, 1982; Prouty, 1952) and poorly drained, darker mineral or organic soils in the central portion of the bay. The hydrologies of these depressions are dominated by precipitation and evapotranspiration but some may be influenced by shallow ground water inputs (Schalles and Shure, 1989; Lide et al., 1995; Chmielewski, 1996).

**Juniper Bay (JB)**

Juniper Bay is a 256 ha Carolina bay, located in Robeson county, NC, whose hydrology is dominated by rainfall and evapotranspiration. This depression wetland was selected by NCDOT in 1999 as a wetland restoration project. The entire site, except for the central portion of the bay, was cleared for lumber in the mid-1960’s (Ewing, 2003). By the mid 1970’s, a drainage ditch system had been constructed and consisted of a perimeter ditch at the northwestern end of the bay, five lateral ditches on the north side of the bay, and one ditch that ran the width of the bay (Ewing, 2003). However, this was only the initial stage of drainage ditch development. In the summer of 1979, a crew of 15 people was hired to begin the construction of many more lateral, perimeter, and main ditches to drain the Carolina bay (Ewing, 2003). After the bay was sufficiently drained,
the agricultural production of soybeans, oats, cotton, corn, tobacco, wheat, millet, and other vegetable crops, began in 1981 (Ewing, 2003). Consequently, major physical and chemical changes to the soils of Juniper Bay occurred, as well as major alterations to the hydrology of the depression wetland.

**Characterization of JB Soils**

Ewing (2003) characterized the chemical and physical properties of soils in Juniper Bay (JB) and three reference bays (RB) to determine the impacts of agricultural production on the soils of Juniper Bay. The three reference bays were selected based on the relative similarity in soil properties to JB soils. Ewing (2003) used four classes to describe the soils based on the presence and depth of organic material. Mineral soils lacked a histic epipedon (20 cm of organic material), histic soils had a histic epipedon 20 to 40 cm thick, shallow organic soils had a histic epipedon 40 to 60 cm thick, and deep organic soils had a histic epipedon greater than 60 cm thick. Ewing (2003) found significant differences between the JB organic soils and RB organic soils, including: higher organic carbon, N, CEC, and surprisingly higher levels of K throughout the RB soil profiles but higher pH and base saturation in JB soils, and higher Ca, Mn, P, and Mg in surface horizons of JB soils. Similar results were found in the mineral soils of JB compared to the mineral soils of the RB, including: higher organic carbon, N, CEC, K, and Mg in RB surface horizons and higher base saturation, pH, Ca, P, and Zn levels in the upper 30 cm of the JB soils. The higher levels in base saturation and nutrient levels in the JB soils were due to fertilizer and lime applications. Fertilizer records from 1976 showed that between 2240-6720 kg ha\(^{-1}\) of dolomitic lime, 56-226 kg P\(_2\)O\(_5\) ha\(^{-1}\), and 226-336 kg K ha\(^{-1}\) were applied in that year alone (Ewing, 2003). However, the higher K
levels in RB soils compared to JB soils may be due to plant uptake of K and leaching in JB soils. The decreased levels of organic carbon, N, and CEC in JB soils is due to the increased rate of decomposition and loss of the litter layer in JB mineral and organic soils. Bruland et al. (2003) found that Ca, Mg, and pH were the three parameters most affected by agricultural cultivation in wetland soils. Differences in chemical soil properties were found to be greatest in the upper 20 cm of the soil profile (Bruland et al., 2003), which corroborates the findings of Ewing (2003). Braekke (1999) found that N and P concentrations were significantly higher in ombrogenous organic soils that had been drained, limed, and fertilized. The soils used in Braekke (1999) were from a wetland in Norway, which had been drained, limed, and fertilized in 1953-1956 and then sampled almost 40 years later. However, Ewing (2003) found that only P was higher in organic and mineral soils, which had been drained, limed, and fertilized. Ewing (2003) found that the total amount of N is highly correlated with the amount of organic material in the soils and therefore, was lower in the soils that had been cultivated for crop production.

Characterization of JB Hydrology and Plant Communities

Two other factors to be considered in wetland restoration projects are hydrology and vegetation. Caldwell (2005) characterized the hydrologic regime of four plant communities that were associated with three reference bays, including: Tatum Millpond Bay, Charlie Long Bay, and Causeway Bay. The four plant communities found in these bays were Pond Pine Woodland (PPW), High Pocosin (HP), Bay Forest (BF), and Non-riverine Swamp Forest (NRSF). Using water table depths and rainfall data, DRAINMOD, a hydrologic model, was calibrated, for each well sampled, and a 40-year
simulation of water table levels was associated to the respective plant communities. PPW was found to be the least tolerant to ponding depth and duration, whereas NRSF was found to be the most tolerant to ponding depth and duration (Caldwell, 2005). PPW averaged 89 total days of ponding per year and <15 consecutive days of ponding with an average of 0.8 cm of water on the soil surface. BF averaged 299 total days of ponding per year and 104 consecutive days of ponding at an average depth of 8.3 cm. NRSF averaged 307 total days of ponding per year and 123 consecutive days of ponding at a depth of 9.5 cm. The longest consecutive ponding events occurred during the winter months when evapotranspiration rates were the lowest. NRSF and BF exhibited similar hydrological characteristics when compared to PPW. These two communities had water levels above the soil surface, whereas PPW had a water table depth of 15 cm below the soil surface for the 40-year simulation average. The NRSF and BF were ponded for 82-84% of the time compared to PPW, which was ponded only 24% of the time. The PPW plant community had water table depths between 0 and 30 cm below the soil surface for 60% of the time, and 8% of the time the water table was below 45 cm. The NRSF and BF exhibited water table depths below 45 cm only 1% of the time. There was very little difference between the NRSF and BF communities. However, the water table in NRSF communities would occasionally reach mineral soils and higher levels of soil available P were identified. These small differences in soil fertility could explain differences in dominant vegetation types. Overall, strong differences between the NRSF/BF communities and PPW communities are apparent with respect to hydrology.
PLANT COMMUNITIES

Nonriverine Swamp Forest (NRSF)

NRSF was described by Schafale and Weakley (1990) as occurring in wet, very poorly drained peat deposits of the outer Coastal Plain. The canopy is dominated by bald cypress (\textit{Taxodium distichum}), pond cypress (\textit{Taxodium ascendens}), swamp tupelo (\textit{Nyssa biflora}), loblolly pine (\textit{Pinus taeda}), Atlantic white cedar (\textit{Chamaecyparis thyoides}), pond pine (\textit{Pinus serotina}), tulip tree (\textit{Liriodendron tulipfera}), and red maple (\textit{Acer rubrum}). NRSF can be easily distinguished from other peatland communities due to the dominance of bald cypress and swamp tupelo. The hydrology is described as seasonally or frequently saturated by a high water table. However, Schafale and Weakley (1990) admitted that this community is “poorly understood”.

Bay Forest (BF)

Like NRSF, BF is also a poorly understood community type. BF usually occurs on the outer parts of peat-filled Carolina bays in shallow organic or deep peat deposits (Schafale and Weakley, 1990). The canopy is dominated by many trees including: sweet bay (\textit{Magnolia virginiana}), loblolly bay (\textit{Gordonia lasianthus}), swamp bay (\textit{Persea palustris}), pond pine (\textit{Pinus serotina}), red maple (\textit{Acer rubrum}), and Atlantic white cedar (\textit{Chamaecyparis thyoides}). BF is easily distinguishable from other plant communities by the dominance of the three bay species: loblolly bay, sweet bay, and swamp bay. This soils of this community type are usually seasonally saturated and nutrient poor.
**Pond Pine Woodland (PPW)**

PPW was described by Schafale and Weakley (1990) as occurring in shallow Histosols or mineral soils with organic surface horizons in peat-filled Carolina bays of the Coastal Plain. The canopy is dominated by pond pine (*Pinus serotina*), loblolly bay (*Gordonia lasianthus*), and sometimes red maple (*Acer rubrum*), loblolly pine (*Pinus taeda*), and sweet bay (*Magnolia virginiana*). PPW is easily discernable from other plant communities due to the dominance of pond pine. PPW occurs on temporarily flooded or saturated, nutrient poor soils. PPW is a relatively common plant community type, especially in North Carolina.

**Nonriverine Wet Hardwood Forest (NRHWF)**

The Nonriverine Wet Hardwood Forest (NRWHF) community occurs on the margins of peatland areas in the outer parts of the Coastal Plain (Schafale and Weakley, 1990). NRWHF contains many bottomland tree species including: swamp chestnut oak (*Quercus michauxii*), diamond leaf oak (*Quercus laurifolia*), cherrybark oak (*Quercus pagoda*), sweetgum (*Liquidambar styraciflua*), and red maple (*Acer rubrum*). It is discernable from other communities by the dominance of bottomland oaks and sometimes mixed hardwood trees. This community typically occurs on the edges of large peatlands and grades to a PPW community. It is seasonally saturated but is less tolerant of wet conditions when compared to NRSF.
HYDROLOGY

Depth, duration, and frequency

Water regime is a major factor of plant community development. The depth, duration, frequency, and the timing of alternating periods of saturated and unsaturated conditions, assist in describing the water regime. Plant responses to flooding or saturation will differ with the type of plant, the duration of saturation, the depth of saturation, and the timing of the ponded conditions (Kozlowski, 1984b). As a soil saturates, water replaces air in soil pores and this drastically slows gas exchange between the soil and the atmosphere. Respiration of microorganisms and plants depletes the oxygen that remains in the water, and the soil becomes anaerobic. Anaerobic conditions affect flood-tolerant and flood-intolerant species differently. In flood-intolerant species, anaerobic conditions reduce plant growth and increases plant mortality, due to changes in metabolic functions (Kozlowski, 1984a). Plants tolerant of saturated conditions are able to switch from aerobic to anaerobic pathways, which results in less efficient metabolism (Pezeshki, 1994). Depending on the type of plant, photosynthesis and translocation of carbohydrates can be reduced (Kramer and Kozlowski, 1979), mineral uptake of specific minerals can be reduced, growth hormones altered, and toxic compounds can be accumulated (Kozlowski, 1984b) under anaerobic conditions. The timing of the ponding event may also impact the health and survival of a plant. Some studies have shown that ponding events during the growing season are more detrimental to flood-sensitive species than ponding events that occur during a plant’s dormancy stage (Kozlowski, 1997). Ultimately, however, the flood sensitivity of a species is a more important factor for survival in saturated soils.
Research on Bald Cypress (BC)

There have been numerous studies on how ponding depth, duration, and frequency affect the growth of bald cypress in greenhouse experiments. Dickson and Broyer (1972) found that plants in unsaturated soils produced larger roots and smaller shoots but saturated conditions caused plants to produce larger shoots and smaller roots. This outcome was also found by the large, three-year experiment completed by Megonigal and Day (1992), which concluded that root to shoot ratios were lower in continuously ponded treatments compared to periodically ponded treatments after three years. Typically, root growth is decreased, causing a decrease in root to shoot ratios. They determined that 28% of total dry mass was allocated to root production in continuously ponded soils, whereas in partially ponded treatments, trees allocated 48% of their dry mass to roots. Roots in periodically ponded soils may have had to explore deeper and more vigorously into the soil for much needed water and nutrients or roots in continuously ponded soils were inhibited by the anaerobic conditions. Although after one growing season, root and shoot biomass was greatest in periodically ponded soils, this difference between periodically and continuously ponded soils decreased after three growing seasons (Megonigal and Day 1992). After 14 weeks, a reduction in leaf and root dry weight, under continuously ponded conditions, was found by Shanklin and Kozlowski (1985); however, only differences in stem dry weight were found by Pezeshki and Anderson (1997). Pezeshki and Anderson (1997) also noted the appearance of adventitious roots and hypertrophied lenticels on the submerged areas of the bald cypress stems. These morphological responses of bald cypress seedlings may explain their ability to survive longer periods of ponding when compared to other tree species.
Differences in height and diameter have also been determined between continuously ponded and periodically ponded regimes. A decrease of 27-46% in the diameter growth of bald cypress seedlings, under continuously ponded conditions, occurred when compared to seasonally ponded conditions (Dicke and Toliver, 1990). However, it has been determined that diameter was greatest in continuously ponded conditions (Pezeshki and Anderson, 1997). In flood-tolerant plants, stem diameter may actually increase due to an increase in the efficiency of nutrient uptake (McKevlin et al., 1995) or possibly an increase in the size of xylem cells (Yamamoto et al., 1995). Shanklin and Kozlowski (1985) measured the lowest heights in continuously ponded conditions but Dickson and Broyer (1972) found the lowest heights in unsaturated conditions when compared to saturated conditions. There is a great amount of research on bald cypress, but much of the research is contradicting, possibly because durations of saturation being compared differ.

**Research on Pond Pine (PP)**

Fewer greenhouse experiments utilizing pond pine seedlings have been reported than those researching hydroperiod effects on bald cypress seedlings. Hunt (1950) found reduced shoot growth in pond pine seedlings that were continuously saturated with stagnant water for 3 months. Saturation can negatively impact the shoot growth of trees by suppressing leaf formation, expansion of leaves, and premature senescence and abscission of leaves (Kozlowski, 1997). Topa and McLeod (1986) found that pond pine seedlings subjected to 8 weeks of anaerobic conditions had a 33-36% reduction in shoot fresh weight. Hunt (1950) found decreased dry weights of the roots of continuously ponded pond pine seedlings. The roots were in such poor health that the authors believed
that the pond pine seedlings may not have survived if the experiment had continued any longer. Standing water proved to be more detrimental to root growth than flowing water due to the lower supply of oxygen in the standing water (Hunt, 1950) and there were no significant differences in root growth in the pond pine seedlings subjected to ponding and drying treatments. Topa and McLeod (1986) found a 56% reduction in the root fresh weight under anaerobic conditions when compared to aerobic conditions. Although pond pine is considered to be moderately tolerant to saturated conditions (Hook, 1984), pond pine seedlings produce less shoot and root biomass under ponded, or anaerobic, conditions.

**Research on Swamp Chestnut Oak (SCO)**

There is also limited greenhouse research on swamp chestnut oak responses to saturated conditions. Angelov et al. (1996) found that after one year of continuously ponded conditions, 100% of the swamp chestnut oak seedlings had died. After two years of intermittently ponded conditions, the survival rate for swamp chestnut oak was between 90-100% (Angelov et al., 1996). Anderson and Pezeshki (1999) found decreased height growth in swamp chestnut oak under intermittently ponded conditions but found no significant differences in the diameter growth. However, swamp chestnut oak did have significantly lower leaf mass, stem dry mass, and total mass in intermittently ponded conditions when compared to the control (Anderson and Pezeshki, 1999). Swamp chestnut oak is considered to be weakly tolerant to saturated conditions (Hook, 1984), which is evident in the decreased survival rate and biomass of saturated seedlings.
Morphological Adaptations

Some species are able to acclimate to saturated conditions by producing certain physical features such as: aerenchyma tissue, adventitious roots, and hypertrophied lenticels (Hook, 1984).

Adventitious Roots

Adventitious roots develop on the original root system or on the inundated portion of the plant stem, as the roots of the original root system (produced under aerobic conditions) begin to die. Adventitious roots are thought to increase the absorption of water, minerals, and hormones (Vartapetian and Jackson, 1997) but may also oxidize the rhizosphere and detoxify harmful compounds (Hook et al., 1970; Hook and Brown, 1973). These specialized roots can form in both flood-tolerant and flood-sensitive species, but are more commonly found in flood-tolerant species (Kozlowki, 1984; Kozlowski, 1997). Adventitious roots can form within 2 weeks of flooding in some species (Mielke, 2003) or longer (Parolin, 2001). Adventitious roots have been found to initiate from either beneath or within the cork cambium (phellogen) and then erupt through the center of the lenticel, in the cork cambium within a lenticel, or originate deep within the vascular cambium independent of lenticels (Hook et al., 1970). However, it is thought that adventitious roots typically form in conjunction with hypertrophied lenticels.

Hypertrophied Lenticels

Hypertrophied lenticels are abnormally enlarged pores on submerged portions of the stem. The time needed to form these hypertrophied lenticels depends on the species.
and the length of inundation. Some bald cypress seedlings developed hypertrophied lenticels within 28 days of saturation (Pezeshki and Anderson, 1997). Lenticels are the major pathway through which gases, especially oxygen, travel to the living cells of the bark. Therefore, hypertrophied lenticels provide a greater surface area for gas exchange, especially the uptake of dissolved oxygen in flood waters, and enhance internal aeration. Hypertrophied lenticels also provide an exit path for potentially toxic compounds (such as ethanol, acetaldehyde, and ethylene) in some species (Chirkova and Gutman, 1972).

**Aerenchyma Tissue**

Another important morphological adaptation is the formation of aerenchyma tissue. Aerenchyma tissue is the term for an airy plant tissue that contains enlarged gas-filled cavities or lacunae. Aerenchyma tissue forms in the above-ground and below-ground portions of many wetland species, as well as some dryland species during periods of stress (Evans, 2003). In roots, aerenchyma tissue typically forms in the cortex of roots (Armstrong, 1979) but can form either in the cortex or pith cavity of stems (Armstrong and Armstrong, 1988). Aerenchyma tissue can form through the differentiation and separation of cells (schizogenous) or through the death of some cells (lysigenous), leading to a less ordered cell pattern (Kawase, 1981). Aerenchyma tissue provides the plant with an alternative strategy for obtaining oxygen from the atmosphere and transporting it to parts of the plant that are stressed due to saturated conditions (Drew et al., 2000). Root rhizospheres, exposed to saturated conditions, quickly become anaerobic due to the consumption of low levels of dissolved oxygen by microorganisms. The interconnected, enlarged pore spaces provide the plant an extensive, low-resistance
internal aeration system, which can transfer oxygen, via diffusion and convection, to anaerobic rhizospheres (Evans, 2003). The degree of longitudinal oxygen diffusion to roots is enhanced by large aerenchyma tissues, a barrier impermeable to radial oxygen loss in the basal zones (Colmer, 2003), thicker roots (Armstrong, 1979), a small number of lateral roots (Armstrong et al, 1983), or short lateral roots that occur close to the root base (Armstrong et al, 1990). Furthermore, toxic gases, such as carbon dioxide, ethylene, and methane, are capable of moving through aerenchyma tissues to the shoots and then released to the atmosphere (Colmer, 2003). Evidence for the lack of fluid in aerenchyma tissue has become evident through the measurement of gas-filled porosity and microscopic observation (Evans, 2003). The morphological adaptation of gas-filled aerenchyma tissue enhances the flood tolerance of wetland tree species and can be observed using scanning electron microscopy (SEM).

**REDOX POTENTIAL**

A soil solution can be determined to be anaerobic through redox potential measurements. The reduced state of saturated soils, or redox potential (Eh), is the measure of the electron availability due to the occurrence of oxidation-reductions reactions in the soil. Redox potential is typically measured using an inert platinum electrode and a reference electrode, which are attached to a voltmeter, to determine the electron flow between the two electrodes. A correction factor must be applied to the redox potential measurement, depending on which reference electrode is used. Actual redox potentials are measured using a standard hydrogen reference electrode, which is impractical for field use (Vepraskas and Faulkner, 2000). The actual redox potential is equivalent to the redox potential measured in the field plus the correction factor for the
reference electrode used. The Eh of a soil solution may provide a gauge as to the oxidation-reduction status of the soil. Soil Eh measurements give an indication of the oxygen availability and presence of reduced substances (Yamasaki, 1952). A decrease in redox potential indicates an increase in the abundance of the reduced component and an increase in H+ (Patrick et al., 1996). Typically, reduced forms of nitrogen, manganese, iron, and sulfur are abundant around the soil rhizosphere in reduced soils with low redox potential (Patrick and DeLaune, 1977). An Eh range of +400 to +700 mV is characteristic of aerobic soils and saturated soils may have Eh levels as low as -250 to -300 mV (Pezeshki, 1991; Patrick and DeLaune, 1977). However, the pH of the soil solution must be determined before determining threshold Eh values since Eh is pH dependent. According to the National Technical Committee for Hydric Soils (http://soils.usda.gov/use/hydric/ntchs/), the Eh required for anaerobic conditions, to identify hydric soils, can be calculated using the following equation:

\[
\text{Eh (mV)} = 595 - 60 \times \text{(pH)} \quad [1]
\]

Redox potential is an important component of the soil solution, which must be quantified to determine if anaerobic conditions exist under saturated conditions.

**NITROGEN AND PHOSPHORUS IN THE SOIL SYSTEM**

**N and P in Anaerobic Environments**

Reduced conditions influence the chemical composition of the root environment but they also control the availability of certain macronutrients, such as N and P, to plants (Patrick and DeLaune, 1977). Nitrogen is available to plants when organic N is mineralized to the \( \text{NH}_4^+ \) form. Under aerobic conditions, when oxygen is present in the soil system, \( \text{NH}_4^+ \) is converted by nitrification to the \( \text{NO}_3^- \) form, which is also available
to plants. However, under anaerobic and saturated conditions, denitrification can occur, which removes NO$_3$ from the soil system as N$_2$ gas. Alternating periods of drying and wetting in a soil system can cause alternating periods of nitrification and denitrification in the soil (Patrick and DeLaune, 1977). This can cause large losses of soil N in the soil-plant system, which may or may not be balanced by atmospheric deposition of N. Unlike N, the plant availability of P is increased when reduced conditions occur in the soil system. Under aerobic soil conditions, P forms insoluble ferric phosphate complexes with Fe$^{3+}$. However, as Fe$^{3+}$ is reduced to the ferrous form of Fe$^{2+}$ under anaerobic conditions, P is then released into the soil solution. Phosphorus and Fe-oxides are typically co-precipitated on soil particle surfaces, but as reduction processes occur, the Fe is released into the soil solution along with the P that has co-precipitated with it (Patrick and DeLaune, 1977). Patrick (1964) found that increased amounts of extractable P occurred when redox potential fell below +200 mV. The availability of N and P, two essential macronutrients, are imperative to the continued growth and health of plants under aerobic and anaerobic conditions. Therefore, the availability of these nutrients to plants must be researched.

**P in High OC Environments**

Brownfield (2007) utilized a continuously stirred reactor and static incubation experiment to determine the potential of P being dissolved from organic soils under reduced conditions. In the continuously stirred reactor experiment, four soil series were used from Juniper Bay, including: Ponzer (Terric Haplosaprist), Leon (Aeric Alaquod), Pantego (Umbric Paleaquult), and Rutledge (Typic Humaquept). Samples from the soil slurry were taken and dissolved reactive phosphorus (DRP) and Fe$^{2+}$ were measured
colorimetrically. The only soil sample to show a net increase in P dissolution was from the Ponzer soil series, which doubled to 1.2 mg L$^{-1}$ (Brownfield, 2007). Likewise, Fe$^{2+}$ and dissolved organic carbon (DOC) increased. Both DOC and Fe$^{2+}$ were correlated to the dissolved P, however, the reduction of Fe$^{2+}$ could not fully account for all of the P that was dissolved. The static incubation experiment utilized the Leon, Rutledge, and Ponzer soil series to determine the amount of DRP and Fe$^{2+}$ at the beginning and end of the experiment. After 60 days of incubation, all four soil series showed an increase in P dissolution. However, the largest increase and highest final concentration was measured in the Ponzer muck. Dissolved organic carbon and Fe$^{2+}$ also increased. Brownfield (2007) concluded that dissolved organic C played a vital role in the increase in DRP in these soils. It is thought that dissolved organic matter (DOM) can increase during soil reduction due to a lower rate of microbial activity, which decreases C mineralization, and therefore, decreases the conversion of OM to CO$_2$. Dissolved organic matter is then capable of competing with DRP for exchange sites on Fe and Al oxides. As the DOM preferentially binds to Fe and Al surfaces, P is released from these complexes. This would result in an increase of DRP in a soil solution under reduced conditions.

**N and P Uptake**

N and P in soil systems are transported to the root surface of a plant by diffusion and mass flow. The rate of nutrient uptake by the root depends on nutrient supply and the ability of the root to absorb important nutrients (Chapin, 1980). Nitrogen is thought to have the greatest effect on growth due to the influence of N on cell development, both the number and size of cells (Chapin, 1980). Phosphorus has similar impact on cell growth but the influence is much less than N. Plant species from habitats that have adequate
supplies of N and P are characterized by high relative growth rates, high root absorption capacities, high photosynthetic rates, low root: shoot ratios, lower rates of luxury consumption, and greater response to applied nutrients (Chapin, 1980). On the other hand, plant species from infertile habitats tend to have low relative growth rate, low root absorption rates, low photosynthetic rates, high root: shoot ratios, and higher rates of luxury consumption (Chapin, 1980). Luxury consumption was described by Chapin (1980) as occurring when plants increase the uptake of a limiting nutrient during a period when the limiting nutrient is readily available. However, luxury consumption may not occur in all plants that lack one or more nutrients (McJannet et al., 1995). Luxury consumption and nutrient uptake are important topics to be considered due to the high availability of P but low total N content in the soils of JB, in comparison to reference wetlands.

Nutrient limitation may be a factor in JB soils due to the low N content and high P content in the soils of this Carolina bay. Liebig’s Law of the Minimum states that growth is not controlled by the total of resources but by the most limiting nutrient available. Nutrient limitation is determined by observing an increase in growth when a limiting nutrient is made available to the plant (Chapin et al., 1986). Nutrient limitation is easily observed in individual plants or crops but may be more difficult to relate to a natural plant community due to three major factors: communities differ in nutrient availability; communities occurring on less fertile soils will have a greater limitation, and nutrient demands change with plant age (Chapin et al., 1986). However, the nature of nutrient limitation can have an important effect on plant community growth, composition, and diversity.
**N: P Ratio**

One method for determining nutrient limitation, at the community level, is the use of the N: P ratio in plant tissue. Koerselman and Meuleman (1996) hypothesized that plant species have a “critical” N: P ratio which would indicate whether growth was N-limited or P-limited. In reviewing numerous fertilization studies from European wetlands, Koerselman and Meuleman (1996) concluded the following: N-limitation occurred when N: P<14, P-limitation occurred when N: P>16, and co-limitation of N and P occurred with N: P ratios between 14 and 16. Although this study was significant, there are differences between European and North American wetlands which must be considered including: differences in management practices, histories, hydroperiods, water chemistry, watershed characteristics, and atmospheric N deposition rates (Bedford et al., 1999). Gusewell et al. (2003) found that N and P limitation, at a community and species level, was consistent with critical ratios given by Koerselman and Meuleman (1996), although the co-limitation of N and P were more variable than the critical ratios presented in 1996. Gusewell et al. (2003) determined, based on the findings of their study and the study of Koerselman and Meulman (1996), that co-limitation could not be precisely separated from N and P limitation, using vegetation N: P ratios exclusively. In a review of over 50 studies from North American wetlands, Bedford et al. (1999) found that mean N:P ratios in plant tissue were greater in peatlands when compared to tissue from mineral soils. Although the utilization of N:P ratios may be a significant tool in identifying nutrient limitation at the community and species levels in Europe, it seems that nutrient limitation may have more variability in North American wetlands (Bridgham et al., 1996).
PHYSIOLOGICAL RESPONSES

Chlorophyll Fluorescence

Photosynthesis is a physiological process that occurs when solar energy is converted to the chemical energy of organic compounds. The first step in photosynthesis is for solar energy, or light, to be captured by chlorophylls and carotenoids. As chlorophyll absorb light energy, the energy is carried to chlorophyll reaction centers where an electron is released. Upon de-excitation of the electron from the excited state to the ground state, one of three outcomes may occur including: photochemistry, heat dissipation, or the re-emission of light in the form of chlorophyll fluorescence (Maxwell and Johnson, 2000). A small proportion of this energy (1-2% of total light absorbed) is emitted as red fluorescence (Maxwell and Johnson, 2000). In general, chlorophyll fluorescence is highest when heat dissipation and photochemistry are the lowest due to the competition among these processes. Measurement of chlorophyll fluorescence is relatively easy due to the difference between the spectrums of fluorescence and absorbed light. A leaf is exposed to a specific light and the fluorescence is determined by measuring the amount of light re-emitted at the longer wavelengths, typically above 680nm (Maxwell and Johnson, 2000).

Among the first studies, Kautsky and Hirsch (1931) found that when dark-adapted leaves were illuminated with continuous light, changes in chlorophyll fluorescence occurred over a short time period. This phenomenon was named the Kautsky effect. Typically, a leaf is adapted to the dark for an adequate time period prior to measurement. The initial measure of fluorescence, $F_0$, represents the minimal fluorescence in dark-adapted tissue. This occurs when $Q_A$ is oxidized and the PSII centers are fully open. The
Q_A is the primary stable electron acceptor of PSII. Photosystem II (PSII) is a protein complex that absorbs light at the 680 nm wavelength to produce energy-producing reactions and is the only photosystem fluorescence that varies with fluctuation in photochemistry. In the initial state, F_0, the efficiency of photochemistry is at its’ maximum. A saturating flash is emitted and the PSII absorbs the light which reduces Q_A. When Q_A is fully reduced and the PSII centers have closed, chlorophyll fluorescence is maximized, F_m. This sequence is given by the equation (F_m - F_0) / F_m = F_V / F_m, which represents the maximum quantum yield, or maximum efficiency, of PSII. In healthy leaves, the ratio value of F_V / F_m should be between 0.75 and 0.85 (Bolhar-Nordenkampf and Oquist, 1993) or above 0.78 (Adams et al., 1990). If a lower value is calculated, some photoinhibition may be occurring due to damaged PSII reaction centers. Photoinhibition occurs when the capacity for photosynthesis is reduced due to an environmental stress or when leaves absorb more light energy than the system is able to process (Osmond, 1981). It is important to determine increases in F_0 from decreases in F_m when changes in the ratio of F_V / F_m occur (Bolhar-Nordenkampf and Oquist, 1989). A decline in F_m may be due to nonphotochemical quenching but an increase in F_0 may be due to a damage sustained by PSII reaction centers (Bjorkman, 1986; Baker and Horton, 1988). With the introduction of hand-held fluorometers, it has become easier to use chlorophyll fluorescence as a tool to understand plants’ tolerances to environmental stresses and furthermore, the extent to which photosynthetic systems may be damaged (Maxwell and Johnson, 2000).

Although measuring the maximum efficiency of PSII has been useful in understanding environmental stresses in trees such as drought, temperature extremes,
salinity, and air pollution, the use of $F_V / F_m$ to determine stress due to flooding has proven less exact. There were no significant differences in the ratio of $F_V / F_m$, after 56 days of treatment, in black willow (*Salix nigra*) cuttings that were grown under four soil moisture treatments, including a continuously ponded treatment (Li et al., 2004). In a 2003 study of Genipa (*Genipa americana*) seedlings, which were continuously ponded for 63 days, there were no differences in $F_V / F_m$ ratio when compared to the control seedlings (Mielke et al., 2003). One 2-year study of an evergreen trees species, *Symmeria paniculata*, examined the fluorescence differences between submerged, older, and younger leaves, as well as with changing water levels (Waldhoff et al., 2002). In this study, they found no differences in the $F_V / F_m$ ratio with rising or falling waters or with trees that had been in pooled water for the entire experiment. Furthermore, they determined that younger leaves had greater $F_V / F_m$ than more mature leaves, which had larger $F_V / F_m$ values than submerged leaves. In a study conducted in 2005, five tropical flood-tolerant tree species were studied to determine how seasonal water level changes affected fluorescence and other physiological properties (Rengifo et al., 2005). Although all five species showed decreases in $F_V / F_m$ during rising waters, the mean value never decreased below 0.76, which is still in the healthy range reported by Bolhar-Nordenkampf and Oquist (1993). Little change in $F_V / F_m$ is to be expected, however, in flood-tolerant tree species. A study by Wagner and Dreyer (1997) used three oak species, which represented a gradient of flood tolerance, to test for physical and physiological differences in response to periods of ponding over a 1-year period. *Quercus rubra*, the most intolerant to soil hypoxia of the three species, experienced a decline of $F_V / F_m$ to a value of 0.6 within the first week of ponding. This value is outside the range of healthy
plants and indicates that some chronic photoinhibition may have occurred. However, they found that the decreases in $F_V / F_m$ were due to a decline in $F_m$, instead of an increase in $F_o$. A decline in $F_o$ indicates photoinhibition but a decline in $F_m$ indicates a change in the efficiency of heat dissipation, or non-photochemical quenching (Maxwell and Johnson, 2000). $F_m$ is representative of the point where photochemical efficiency is at its highest and heat dissipation is at its lowest (Maxwell and Johnson, 2000). Using five levels of soil moistures in 2-year old Pinus sylvestris seedlings, Otronen and Rosenlund (2001) compared chlorophyll fluorescence readings to detect differences in stress levels. Although chlorophyll fluorescence differences were found to be non-significant across the five moisture levels, there was an interaction between the moisture level and the different parts of the seedlings sampled, indicating that stress was unevenly distributed within the plant. Furthermore, $F_V / F_m$ was found to increase with increasing nutrient levels, which coincided with previous research on Scots pine (Linder and Troeng, 1980).

**Stomatal Conductance**

Stomata are microscopic pores on the epidermis of plants that are surrounded by a pair of specialized epidermal cells, called guard cells that open and close the pores in response to specific environmental conditions such as light, temperature, and humidity. Stomata are typically open in the light and closed in the dark. Stomata are critical to plants because of their role in gas exchange, specifically carbon dioxide and oxygen gases, and water movement from the roots to the atmosphere. The movement of water from the roots to the shoots via the stomata supplies the water and minerals necessary for the entire plant system. When stomata are not functioning properly, the efficiency of photosynthesis and respiration are significantly reduced and the health of the plant may
become compromised. Stomatal conductance, which is the measure of leaf exchange rates of water vapor and carbon dioxide, measured in \( \mu \text{mmol m}^{-2} \text{s}^{-1} \), is an effective way to measure the rate of stomatal aperture.

There are many theories as to why stomata close after saturation occurs. A decrease in root hydraulic conductivity has been linked to stomatal closure in several studies (Anderson et al., 1984). Excess hormones, such as abscisic acid, sent from the root to the shoot may also trigger stomatal closure (Kozlowski, 1997). Abscisic acid may simply be transported from the chloroplast to the apoplast or it may be produced at a higher-than-normal rate to accumulate in the apoplast (Taiz and Zeiger, 2002).

With the onset of flooding, stomatal aperture, transpiration rates, and the absorption of water by roots all decrease, but the rate at which these changes occur depends on the plant species and also the characteristics of the flood water (Kozlowski, 1984b). Flood-sensitive plants are severely damaged within 24 hours of inundation but flood-tolerant plants can withstand inundation and oxygen depletion for a few days. Other plants, such as those adapted to wetlands, can survive prolonged periods of anoxia for several months due to morphological or physiological adaptations. One of these adaptations of wetland plants is to reopen stomata very quickly, after inundation of the soil, to resume transpiration and respiration. However, species that are not well-adapted to saturating cycles may maintain stomatal closure for long periods of time or until the flood waters recede.

There has been a substantial amount of research on the changes in leaf gas exchange of bald cypress (\textit{Taxodium distichum}). Bald cypress is a highly flood-tolerant species that can be found in sites that experience prolonged periods of saturation. In a
1996 rhizotron study, Pezeshki et al. (1996), found that leaf conductance of bald cypress seedlings recovered quickly after the ponding treatments were initiated. However, the stomatal conductance of *Quercus falcata*, a flood-sensitive species, and *Quercus lyrata*, a flood-tolerant species were found to be significantly decreased throughout the 22-day experiment. In a study on the affects of four different ponding regimes on bald cypress, nuttall oak (*Q. nuttalli*), and cherrybark oak (*Q. falcata*), it was found that the stomatal conductance of all species was reduced in the continuously ponded treatment (Pezeshki and Anderson, 1997). Although bald cypress was significantly affected by the continuously ponded treatment, it was not affected by the partially ponded or intermittently ponded treatments. Eventual recovery was noted in bald cypress. Both oak species had significantly reduced stomatal conductance throughout the 68-day experiment. It was determined that bald cypress had the highest stomatal conductance, followed by the nuttall oak, and then the cherrybark oak. In this study, adventitious roots and hypertrophied lenticels were observed in the bald cypress and nuttall oak species, which may have contributed to the higher overall leaf gas exchange rates when compared to the cherrybark oak. In a companion study, bald cypress, nuttall oak (*Q. nuttalli*), and swamp chestnut oak (*Q. michauxii*) seedlings were subjected to intermittent ponding periods of 5 days for a total of 30 days (Anderson and Pezeshki, 1999). Bald cypress represented the most flood-tolerant species and swamp chestnut oak was considered the most flood-sensitive of the three species. Bald cypress experienced a decrease in stomatal conductance within the first week of ponded conditions but quickly recovered and remained high for the rest of the experiment. Nuttall oak experienced a decline in gas exchange measurements during the final ponding period and remained low
throughout the rest of the experiment. Swamp chestnut oak experienced a 35% decrease in stomatal conductance after the first day of ponding and remained low throughout the study. The changes in stomatal conductance measurements followed the gradient of flood-tolerant to flood-sensitive species in this experiment. In a 1999 study of four bottomland oak species, responses to ponding with fresh water and salt water were examined (McLeod et al., 1999). The flood tolerance of the four oak species decreased from overcup oak (Q. lyrata) to willowcup oak (Q. phellos) to swamp chestnut oak (Q. michauxii) to water oak (Q. nigra). Stomatal conductance of swamp chestnut oak was quickly reduced after the first day of ponding. After approximately 23 days of ponding, the remaining 3 oak species had reduced stomatal conductance readings. Overall, the ranking of flood tolerances was similar to the outcome of the experiment.
REFERENCES


Chapter 2
Dynamic responses of bald cypress and swamp chestnut oak seedlings to saturated conditions

ABSTRACT

Successful establishment of wetland vegetation in restoration projects requires matching the hydrologic and soil preferences of the target vegetation. The objective of this study was to observe and quantify biological, morphological, and physiological changes in two wetland tree species, with differing tolerances to saturated conditions. Bald cypress (Taxodium distichum) and swamp chestnut oak (Quercus michauxii) seedlings were grown under ponded and drained conditions for 106 d in rhizotrons containing either mineral or organic soils collected from a Carolina Bay in Robeson County, NC. Within 2 wk of ponding, bald cypress seedlings in both organic and mineral soil began to produce lateral roots within the top 36 cm of soil. The swamp chestnut oak seedlings subjected to ponding produced a few new roots, and root death was observed within 4 wk of flooding. Overall, bald cypress seedlings grown in organic soils and continuously ponded conditions exhibited the best growth in diameter, height, and shoot biomass. Scanning electron microscopy images of basal stems and roots of bald cypress subjected to saturation showed substantial development of aerenchyma, an airy plant tissue, which may have aided in the overall tolerance of bald cypress to ponded conditions. Results from this study show significant biological adaptations of bald cypress seedlings to saturated conditions, which allow them to grow in wet organic soils of the Coastal Plain. These results indicate that swamp chestnut oak seedlings may be better adapted to wetlands, where soils are inundated only briefly.
INTRODUCTION

Successful wetland restoration requires matching the hydrologic needs of the planted vegetation to the restored hydrology. The depth, duration, frequency, and the timing of alternating periods of saturated and unsaturated conditions, assist in describing the water regime. Plant responses to flooding or saturation differ with the type of plant, the duration of saturation, the depth of saturation, and with the timing of the flooded conditions (Kozlowski, 1984b).

Caldwell (2005) characterized the hydrologic regime of four plant communities, from three reference wetlands, using the simulation model DRAINMOD. The four plant communities evaluated were: Non-riverine Swamp Forest (NRSF), Bay Forest (BF), High Pocosin (HP), and Pond Pine Woodland (PPW). Using measured water table levels and rainfall data, DRAINMOD was calibrated for each well sampled, and a 40-year simulation of water table levels was determined for the soils in each plant community. Mean water table levels were defined for the respective plant communities. Non-riverine Swamp Forest (NRSF) was found to be the most flood-tolerant of the plant communities with an average of 307 total days of ponding per year and 123 days of consecutive ponding (Caldwell, 2005). Bay Forest (BF) had a similar hydrologic regime to NRSF with 299 total days of ponding per year (Caldwell, 2005). A major difference between BF and NRSF was the estimated level of available phosphorus (P) in the soils from these two plant communities. Higher available P occurred in the NRSF plant communities. Pond Pine Woodland (PPW) was found to be the least flood tolerant of the plant communities characterized with an average of 89 total days of ponding and <15 days of consecutive ponding (Caldwell, 2005).
Conk et al. (2008) conducted a container study to determine to test Caldwell’s (2005) characterization of the hydrologic regime of plant communities. The container study utilized the hydrologic regimes and the dominant tree species from three of four of the plant communities described. High Pocosin (HP) plant community was not used in the container study because it is considered to have similar dominant species and hydrologic regime as the PPW, BF, and NRSF plant communities (Caldwell, 2005; Schafale and Weakley, 1990). The species used to represent each plant community were: bald cypress (*Taxodium distichum* - NRSF), sweet bay (*Magnolia virginiana* - BF), pond pine (*Pinus serotina* - PPW), and swamp chestnut oak (*Quercus michauxii*). Swamp chestnut oak was included in the container experiment in order to study a range of wetland tree species with differing tolerances to saturated conditions; swamp chestnut oak is considered to be weakly tolerant to saturated and ponded conditions (Hook, 1984).

In conducting the container study, it was observed within several weeks that there were significant differences in the responses between the most flood-tolerant species, bald cypress, and the least flood-tolerant species, swamp chestnut oak. It was hypothesized that the differences in flood tolerance of these two species was due to differences in root growth and anatomy.

As a soil becomes saturated, water replaces air in soil pores, which prevents gas exchange between the soil and the atmosphere. Microorganisms and plants quickly use the oxygen that remains in the water and causes anaerobic conditions to occur in the soil. Anaerobic conditions affect flood-tolerant and flood-intolerant species differently. Ponding inhibits the formation of new roots, growth of existing roots, and lessens root branching in most species (Kozlowski, 1984a; Kozlowski, 1984b). Megonigal and Day
(1992) determined that 28% of total dry mass of bald cypress seedlings was allocated to root production in continuously ponded soils, whereas, partially saturated treatments allocated 48% of their dry mass to roots. Anaerobic and reduced soil conditions can lead to development of root rot in some susceptible, flood-sensitive tree species because of increasing soil fungi activity. However, some flood-tolerant species are able to adapt to saturated conditions by producing aerenchyma tissue. Aerenchyma tissue is the term for an exceptionally porous plant tissue that contains enlarged gas-filled cavities or lacunae. Aerenchyma tissue forms in the above-ground and below-ground portions of many wetland species, as well as some dryland species during periods of stress (Evans, 2003).

Root systems can be studied by growing plants in a controlled environment. The use of containers allows researchers to study the interaction of specific environmental factors and root growth. Root boxes, or rhizotrons, are a common container approach to studying root systems. These root boxes are glass- (or Plexiglass) walled to make observation of root growth and development easier. The root boxes are kept at a 3-45° angle from the vertical, to force roots to make contact with the glass plate. The use of a glass-faced root box allows direct observation of root morphology.

Quantifying roots can be very difficult and time consuming. Measuring root biomass is practical but root length is the characteristic responsible for rates of water and nutrient uptake. Böhm (1979) reviewed most of the systematic procedures for studying roots systems in the field. In the trench-profile method, roots are mapped or counted using a square grid, which is placed against the soil profile. The counting method works well for quantifying the root distribution in a soil profile. Using root counts, root data are expressed as numbers of roots per unit area. Many researchers prefer to use the core
method, which quantifies roots based on root length density. However, this practice is much more labor intensive and time consuming. Vepraskas and Hoyt (1988) compared the trench-profile method (root counts) to the core method (root length density) and determined that the trench-profile method produced similar results to the core method, which required five times as much labor to quantify root distributions.

In addition to observing morphological changes in roots of continuously ponded seedlings, differences in physiological responses also may occur during ponding. The first signs of most plant stresses are likely to be found in the photosynthetic electron transport system. Chlorophyll fluorescence has the potential of providing an important diagnostic test for the efficiency of photosynthesis (Bjorkman and Demmig, 1987). Chlorophyll fluorescence has often been used as an indicator of physiological stress in plants (Lichtenthaler and Rinderle, 1988). The ratio of variable fluorescence (Fv) to the maximum fluorescence measured (Fm) indicates the maximum efficiency of the photosystem center (PSII) and should be between 0.75 and 0.85 for healthy leaves (Bolhar-Nordenkamp and Oquist, 1993).

Another physiological measure of stress is stomatal conductance. When stomata are not functioning properly, the efficiency of photosynthesis and respiration are significantly reduced and the health of the plant declines. Stomatal conductance, which is a measure of leaf exchange rates of water vapor, measured in mmol m$^{-2}$ s$^{-1}$, is an effective way to measure the rate of stomatal function. A decrease in stomatal conductance is a common response to ponded conditions in both flood-tolerant and flood-intolerant tree species (Kozlowski, 1984b; Kozlowski, 1997; Pezeshki and Anderson, 1997).
The purpose of this study was to determine, in a controlled environment using rhizotrons, the effects of saturated conditions and soil type on: 1) morphological responses (e.g. formation of aerenchyma tissue, lateral root growth near surface) 2) physiological responses (e.g. chlorophyll fluorescence, stomatal conductance), and 3) growth responses (e.g. changes in height, diameter, biomass partitioning) in seedlings of bald cypress, considered a flood-tolerant species, and swamp chestnut oak, considered a weakly flood-tolerant species (McKnight et al., 1981).

MATERIALS AND METHODS

Soil Collection

Soil was collected from Juniper Bay in December 2006, a 256 ha Carolina bay, located in Robeson county, near Lumberton, NC (34°30’30”N 79°01’30”E). Juniper Bay was drained in 1972 and used for agricultural production until 2006 when it was restored back to a wetland. During the years in agriculture, the soils were fertilized and limed annually to soil test recommendations for the production of soybeans (Glycine max), oats (Avena sativa), cotton (Gossypium hisutum), corn (Zea mays), tobacco (Nicotiana tabacum), wheat (Triticum aestivum), and other vegetable crops (Ewing, 2003).

Two sites were located to collect soil samples based on ease of access and soil type. Mineral soil was collected from the Ap horizon of a Leon sand (sandy, siliceous, thermic Aeric Haplaquod). Organic soil material was collected from the Oa horizon of a Ponzer muck (loamy, mixed, dysic, thermic Terric Haplosaprist). Small pits were dug by hand in each soil area to a depth of 60 cm. Undisturbed cores were collected in triplicate from the 15 to 25 cm depth using metal cylinders (10 cm in diameter by 10 cm in height)
for bulk density determination. Soil materials were collected from the upper 25 cm of the wall of each pit, placed in buckets and transported to the greenhouse. Soil materials were air-dried for 3 days and then passed through a 1.25 cm mesh sieve. Particle size distribution was determined by the hydrometer method after passing the material through a 2-mm mesh sieve (Gee and Bauder, 1986). In the laboratory, soil cores were weighed, oven-dried at 105°C for 24 hours and weighed again. Before potting, sub-samples of organic and mineral soils were taken to determine gravimetric water content, which was used to calculate for bulk densities similar to bulk densities measured in the field.

The samples were analyzed at North Carolina Department of Agriculture, Soil Testing Services, Raleigh, North Carolina for extractable P, K, Ca, Mg, Mn, Cu, Zn, and S using the Mehlich-3 extractant (Mehlich, 1984) and an inductively coupled plasma emission spectograph (ICP). Buffer acidity, cation exchange capacity, and the sum of base cations was also determined (Mehlich, 1976). The pH was determined using a 1:1 soil to water ratio. Two separate bulk samples were air-dried and ground to pass through a 2 mm sieve for the determination of organic carbon and total nitrogen with a Perkin-Elmer PE2400 CHN Elemental Analyzer (Culmo, 1988) in the North Carolina State University Soil Science Analytical Lab, Raleigh, North Carolina.

**Rhizotron Construction**

Sixteen rhizotrons were constructed from two, 61.5 × 35.5 × 1 cm sheets of transparent polycarbonate, held apart by a 2.5 × 2.5 × 0.3 cm steel channel that was welded together and spray-painted with Rust-oleum (Rust-oleum, Vernon Hills, IL) to prevent oxidation. Two 2.5 × 0.3 cm thick rubber gaskets (Raleigh-Durham Rubber & Gasket Co., Inc, Raleigh, NC) were used between the polycarbonate and steel frame to
provide an airtight seal. Silicone sealant (Dow Corning, Midland, MI) was used between the rubber gasket and the steel channel and vacuum grease was used between the gasket and polycarbonate sheet to further improve the seal. Three small drainage ports were added, to insure proper drainage, at the bottom of each rhizotron by drilling 3 small holes through one sheet of polycarbonate and installing a brass elbow, threaded on one end, and barbed on the other. The rhizotrons were supported at a 30° angle, from vertical, by two wooden legs that were 49 × 3.3 × 3.3 cm. These wooden legs were secured to the steel frame by a bolt and wing nut that could be loosened or tightened to ease movement.

When the rhizotrons were in the vertical position, binder clips were used to hold the rhizotron securely against a rack to minimize the possibility of the rhizotron falling off the bench. To minimize the effects of light on root growth, each polycarbonate plate was covered with a removable piece of 24-guage galvanized metal sheeting that was held on with binders. The edges of the polycarbonate were covered in aluminum foil tape (Intertape Polymer Group, Bradenton, FL, model 0802) to further reduce the infiltration of light.

Each rhizotron was equipped with three platinum electrodes to record redox potential. Electrodes were placed 10 cm, 20 cm, and 30 cm below the soil surface. The redox platinum electrodes were constructed and tested similar to the techniques of Wafer et al. (2004). Accurate redox probes were gently inserted by hand into the soil of the rhizotrons. A small amount of soil was mixed with water to create a soil slurry and any gaps around the redox probe were filled with the slurry.

The rhizotrons were also equipped with a tensiometer to determine the soil water pressure head for watering purposes. A 2.86-cm long and 0.95-cm wide porous ceramic
cup (Soilmoisture Equipment, Santa Barbara, CA, model 0652X07), with an air entry valve of 1 bar, was attached to a 15-cm long section of 1-cm diameter polycarbonate tubing with marine epoxy (ITW Philadelphia Resins, Montgomeryville, PA). A separate piece of 7.5-cm long and 1.25-cm diameter polycarbonate tubing was cemented to the other end of the 15-cm long polycarbonate tubing with marine epoxy. The tensiometers were inserted to a depth of 7.5 cm below the soil surface in all plant containers before the treatments were begun. The septum stoppers were periodically replaced to maintain pressure inside the tensiometer. The tensiometers were read using a tensimeter (Soil Measurement Systems, Tucson, AZ).

Tree Seedlings

Bald cypress (Taxodium distichum) and swamp chestnut oak (Quercus michauxii) seedlings were purchased from North Carolina Division of Forest Resources, Goldsboro, North Carolina. The seedlings were kept in a cold room for several weeks at 7°C with adequate moisture until planted. Fifteen seedlings from each species were potted in temporary 2.83 liter containers (Stuewe & Sons, Inc., Corvallis, OR, model Tall One), containing a potting soil medium, for approximately 14 days before being transferred to the rhizotrons.

The tree seedlings were carefully removed from their containers, at the time of planting, and their roots washed with tap water. Eight tree seedlings from each species were selected based on similar root lengths, height, and overall health. The seedlings were planted on April 13 with one seedling per rhizotron. Four seedlings, from each species, were planted in rhizotrons containing mineral soil and the remaining four seedlings were planted in rhizotrons containing organic soil. The seedlings were then
transplanted to the rhizotrons with the root collars of the seedlings at the soil surface, which was at a similar level in all rhizotrons. The seedlings were maintained under well-watered and well-drained conditions for 78 days until the treatments were initiated on June 29. During the 78-day acclimation period, the rhizotrons were kept at a 30° angle to allow for gravitropic response of the roots.

Two hydroperiod treatments were imposed: 1) control (C) where water content was maintained at -300 cm or close to field capacity and 2) continuously ponded (CP) where seedlings were saturated and water was ponded on the surface to a depth of 6 cm for 106 consecutive days. Eight rhizotrons were ponded and included four rhizotrons from the mineral soil and four rhizotrons from the organic soil. A total of two replications per treatment combination (2 reps x 2 hydroperiods x 2 soils x 2 tree species) were achieved by using 16 total rhizotrons in the experiment. The root boxes were placed vertically, after hydroperiod treatments were implemented, in a completely randomized design along a rack that was constructed to hold the rhizotrons securely in the vertical position. The rhizotrons were randomly rearranged every month to account for variable conditions in the greenhouse.

**Measurements**

Redox measurements were conducted on a weekly basis using an auto-range digital multimeter (Radio Shack, Fort Worth, TX, model 22-163) and a calomel reference electrode (Thermo Fisher Scientific, Waltham, MA, model 13-620-259). The pH of the organic and mineral soils were measured every other month using a portable pH/mV meter (Thermo Fisher Scientific, Waltham, MA, model 13-636-62A) in order to correctly interpret the redox measurements. Height and diameter were measured on a monthly
basis using a meter stick and caliper. At the end of the study, the total growth of the 106-day experiment was determined by subtracting the initial height and diameter from the final height and diameter. Roots were counted every two weeks for all rhizotrons using a uniform grid that was created by measuring and drawing two hundred and fifty-two 2.5 x 2.5 cm squares on a 2-mm wide sheet of transparent Plexiglass. The number of live roots were counted and recorded for each 2.5 x 2.5 cm square. The increases in the number of roots were determined by subtracting the initial root counts for each grid from the final root counts for each grid. The root boxes were divided into 3 depth increments: 1) top depth increment (0-18 cm below the soil surface), 2) middle depth increment (18-36 cm below the soil surface), and 3) bottom depth increment (36-54 cm below the soil surface). Each depth increment had an area of 542 cm$^2$. The increases in root counts reported for each depth increment represent the number of roots proliferated in the study in each 542 cm$^2$ area. Photographs were also taken bi-weekly.

Chlorophyll fluorescence measurements were conducted using a hand-held OS-30p fluorometer (Opti-sciences, Hudson, NH, model OS-30p) using the ‘Fv/Fm Kinetics’ option. Two measurements were taken on each plant including: the second leaf from the bottom and the second fully developed leaf from the tallest shoot apex. However, leaves with obvious signs of senescence or poor overall health were not measured. Measurements were conducted in the pre-dawn hours on leaves, which had been adapted to the dark for at least 6 hours. The relative quantum yield, or quantum efficiency, of Photosystem II was calculated by the hand-held fluorometer as $\Phi_{PS2} = (F_m - F_o)/F_m$.

Stomatal conductance measurements were measured once in week 12 with a Delta-T Porometer (Delta-T Devices Ltd, Burwell, Cambridge, England, model AP4-
Stomatal conductance was measured between the hours of 11:00 and 1:00 on a sunny day. One leaf per plant was measured per measurement day. The second fully developed leaf from the tallest shoot apex was measured and recorded. Relative humidity (RH) of the air entering the chamber was fixed. RH in the greenhouse was also measured during this time and ranged from 45 to 50%. Photon flux density (PPFD) was also measured with the Delta-T Porometer and reached a maximum of 680 µmol m$^{-2}$ s$^{-1}$.

Final measurements were made on October 15$^{th}$ and 16$^{th}$, 2007. The rhizotrons were dismantled 2 days later. The leaves and stems were separated and oven-dried for 48 hours at 70ºC, or until a constant weight was reached. Leaves were ground and analyzed by the North Carolina State University Soil Science Analytical Lab, Raleigh, North Carolina. Nitrogen was measured with a CHN analyzer and P, Ca, Mg, Mn, Fe, and Zn were measured with ICP-emission spectrometer. The root zone was divided into three depth increments (0-18 cm, 18-36 cm, and 36-54 cm). Soil samples from each depth increment were collected and air-dried for 7 days. On October 18, root and stem tissue samples were taken for SEM analysis. The root tissue was sampled between 0-18 cm and 36-54 cm and fixed in a 3% glutaraldehyde in a 0.05M sodium phosphate buffer at pH 7.2. The stem tissue samples were sampled in approximately 2 cm diameters and fixed in a 3% glutaraldehyde in a 0.05M sodium phosphate buffer at pH 7.2. These tissue samples were sent to Center for Electron Microscopy at North Carolina State University for SEM analysis.

**Data Analysis**

Statistical analyses were performed using SAS version 9.1 software (SAS Institute, Cary, NC, 2005). The PROC MIXED function was used to test for significance
at the $p \leq 0.05$ level for measurements including: changes in height, diameter, root counts, biomass, root/shoot ratio, and stomatal conductance data. The Tukey procedure was used to determine all pair-wise differences between treatment combinations. The data was transformed, as needed, to fulfill the ANOVA assumptions, especially the assumption of equal variances. Repeated measures analysis of variance was used to test for differences in means of fluorescence and redox potential data.

**RESULTS**

**Survival Rates**

Survival rates were 100% for all bald cypress seedlings at the conclusion of the experiment. Survival rates were 100% for swamp chestnut oak seedlings that were maintained at field capacity (control) in both mineral and organic soils. Some swamp chestnut oak seedlings, maintained under ponded conditions and grown in organic soils died during week six of the experiment. By week eight, 100% of swamp chestnut oak seedlings grown in organic soils under ponded conditions appeared to be dead due to brittle stems and senesced leaves. However, the roots of these swamp chestnut oak seedlings grown in ponded organic soils maintained a healthy appearance until the conclusion of the experiment.

Swamp chestnut oak seedlings grown in mineral soils under ponded conditions had a 100% survival rate, at the conclusion of the experiment. However, if the experiment had lasted longer, it is likely that these seedlings would have died due to the senescence of leaves. The onset of root death was noted in swamp chestnut oak seedlings in this treatment in week 10 and gradually continued until the conclusion of the
experiment. Hypertrophied lenticel development was observed for swamp chestnut oak seedlings, grown in mineral soils and saturated conditions, by the end of the experiment.

**Redox Potential**

Redox potential, measured 10 cm below the soil surface, averaged +300 mV for rhizotrons in mineral soil and above +400 mV for seedlings in organic soils in the control treatment (Fig. 1). Redox potential was +350 mV at 20 cm below the soil surface, but decreased to an average of +275 mV, when measured 30 cm below the soil surface, for seedlings in the controlled moisture treatment (Fig. 1). An Eh range of +400 to +700 mV is characteristic of aerobic soils (Pezeshki, 1991). The root boxes, which were maintained at field capacity, were close to aerobic conditions for the duration of the experiment. Soil redox potential in mineral soils quickly dropped within the first week of saturation. However, the redox potential in organic soils steadily declined, before leveling off. Redox potential, measured 10 cm below the soil surface, stabilized at -100 mV after 12 weeks of saturation for both mineral and organic soils. Redox potential, measured 20 cm below the soil surface, stabilized after 9 weeks of saturation and after 8 weeks for redox potential measured 30 cm below the soil surface. Anaerobic soils typically have redox potentials below +350 mV (Patrick and DeLaune, 1977). The root boxes, under continuously ponded conditions, were anaerobic for the duration of the experiment. There was no significant species effect on measured redox potential, indicating that redox potential did not vary significantly between the two species at the 3 measured depths. However, there was a significant effect due to hydropertiod ($p=0.0011$) and also an interaction between hydropertiod and the timing of the measurements ($p<0.0001$) for the three depths measured.
**Chlorophyll Fluorescence**

Values of Fv/Fm, measured in the top leaves of bald cypress seedlings remained above 0.80 for all treatment combinations (Fig. 2). In week 11 there was a substantial increase in Fv/Fm measured in bald cypress seedlings, especially those seedlings under ponded conditions, which peaked close to 0.90. Swamp chestnut oak seedlings, maintained under controlled moisture conditions, maintained an Fv/Fm value at or above 0.80. A plant is considered healthy when the Fv/Fm ratio is between 0.75 and 0.85 (Bolhar-Nordenkampf and Oquist, 1993) or above 0.78 (Adams et al., 1990). Therefore, the seedlings of bald cypress and swamp chestnut oak, grown in moist soil conditions close to field capacity, were not stressed for the entire experiment. However, swamp chestnut oak seedlings under ponded conditions and grown in organic soils maintained an Fv/Fm value of 0.80 before abruptly dying in week 8. A decrease in Fv/Fm values were observed in swamp chestnut oak seedlings, grown under ponded conditions and mineral soils, during week 12. From week 12 until the end of the experiment, values of Fv/Fm steadily decreased by 14% to 0.716 (Fig. 2). The decrease in fluorescence to an unhealthy fluorescence level of 0.716, by the end of the experiment, suggests that the swamp chestnut oak seedlings, maintained under ponded conditions in mineral soils, were declining in health. Chlorophyll fluorescence measured using the bottom leaves of seedlings followed the same general trend and are not reported here. Overall, there was a significant main effect of species ($p<0.0001$) and hydroperiod ($p=0.0036$), which was used to interpret the results.
**Stomatal Conductance**

Stomatal conductance was measured once during the experiment in week 12, after 78 days of ponding (Fig. 3). Bald cypress seedlings had a significantly ($p=0.015$) higher stomatal conductance compared to swamp chestnut oak seedlings, when all treatment combinations were averaged together for each species. Bald cypress seedlings, which were subjected to ponded conditions, had the highest rate of stomatal conductance, close to 425 mmol (H$_2$O) m$^{-2}$s$^{-1}$ (Fig. 3). Swamp chestnut oak seedlings, which were subjected to continuously ponded conditions and mineral soils, had the lowest stomatal conductance. Saturated swamp chestnut oak seedlings, in organic soils, were not included in the measure of stomatal conductance because 100% of the seedlings had died, by week 12.

**Changes in Height and Diameter**

Growth in height was significantly affected by species ($p<0.0001$) and soil ($p=0.0001$) (Table 1). Overall, bald cypress had the greatest change in height with a mean of 20 cm, compared to an average growth of 1.6 cm in swamp chestnut oak, when all treatment combinations were averaged for each species. Bald cypress and swamp chestnut oak seedlings grown in organic soils had a higher total growth with a mean of 17 cm, compared to seedlings grown in mineral soils with an average of 4 cm. There were many significant interactions between species and soil ($p=0.0004$), species and hydroperiod ($p=0.03$), and soil and hydroperiod ($p=0.0002$) (Table 1). Bald cypress seedlings grown in organic soils had the greatest increase in height for all species and soil combinations, which was four times greater than bald cypress seedlings grown in mineral soils.
soils (Fig. 4). Bald cypress seedlings grown in continuously ponded conditions had the best overall growth for all species and hydroperiod combinations, which was almost three times as much growth as the bald cypress seedlings grown under controlled moisture conditions (Fig. 4). There were no significant differences within swamp chestnut oak seedlings grown under differing soil or hydroperiod conditions (Fig. 4).

Growth in diameter was affected by species ($p=0.0001$), soil ($p=0.035$), and hydroperiod ($p=0.0025$) (Table 1). Similar to the change in height, bald cypress growth in diameter was greater than swamp chestnut oak seedlings, when examined over all treatments. Bald cypress had a mean growth in diameter of 6 mm, compared to a 1.3 cm growth in swamp chestnut oak seedlings. Seedlings grown in organic soils had a greater growth in diameter, compared to seedlings grown in mineral soils, while seedlings grown under continuously ponded conditions also had the greatest growth during the experiment, compared to the control moisture treatment. There were significant interactions between species and soil ($p=0.030$) and between species and hydroperiod ($p<0.0001$) (Table 1). Bald cypress seedlings subjected to continuously ponded conditions experienced a five-fold increase in diameter, compared to seedlings in the control (Fig. 4). Differences within swamp chestnut oak seedlings subjected to different hydroperiods were not significant (Fig. 4).

**Biomass**

Biomass data was gathered at the end of the study and was divided into shoot and root biomass (Table 2 & 3). Total shoot biomass was significantly ($p=0.014$) higher for seedlings subjected to continuously ponded conditions. On average, seedlings grown under continuously ponded conditions had a shoot biomass of 36 grams, compared to 25
grams of shoot biomass, for the controlled moisture treatment. There were significant interactions between species and soil \( (p=0.010) \), as well as species and hydroperiod \( (p=0.018) \) (Table 1). Bald cypress seedlings grown in organic soils were significantly \( (p=0.0099) \) different in the amount of shoot biomass accumulated from those grown in mineral soils; biomass of bald cypress seedlings grown in organic soils averaged 37 grams compared to an average of 20 grams for those grown in mineral soils.

Bald cypress seedlings, grown under continuously ponded conditions had the greatest shoot biomass with an average of 39 grams, which was significantly \( (p=0.0029) \) different from the shoot biomass accumulated by bald cypress seedlings grown in the control moisture treatment (Fig.5). Furthermore, bald cypress seedlings grown under controlled moisture conditions accumulated the least amount of shoot biomass out of all species and hydroperiod combinations (Fig. 5).

Root biomass was calculated at three depth increments: 0-18 cm, 18-36 cm, and 36-54 cm (Table 2 & 3). Root biomass measured in the first depth increment, closest to the surface, differed significantly between species \( (p=0.019) \). Differences in root biomass at this depth, between species, were most likely due to the differences in the tap root of these two species. Swamp chestnut oak seedlings had a higher root biomass when compared to bald cypress seedlings due to the presence of a woodier tap root. Significant interactions between species and soil \( (p=0.013) \), as well as species and hydroperiod \( (p=0.0006) \) were present (Table 1). Swamp chestnut oak seedlings grown in mineral soils had the greatest root biomass, at the 0-18 cm depth increment, with 36 grams, which was significantly \( (p<0.05) \) different from all other species and soil treatment combinations.
Likewise, swamp chestnut oak seedlings grown under control moisture conditions had the greatest root biomass at this depth with 34 grams.

Root biomass measured at the second depth increment, between 18 and 36 cm, had a significant ($p=0.010$) main effect from species. However, at this depth increment, bald cypress seedlings had a greater root biomass with an average of 28 grams. There was also a significant interaction between species and hydroperiod ($p=0.019$). Bald cypress seedlings grown under continuously ponded conditions had the greatest root biomass in this depth increment.

In the lowest depth increment, which was from 36 to 54 cm, there was a significant ($p=0.044$) main effect from hydroperiod. Seedlings grown under control moisture conditions had the greatest root biomass at this depth with an average of 11 grams of roots, compared to 1.3 grams from seedlings grown under saturated conditions.

Total belowground biomass, summed from the three depth increments, had a significant interaction between species and hydroperiod ($p=0.016$) (Table 1). Bald cypress seedlings grown under ponded conditions had the greatest total root biomass with an average of 64 grams (Fig. 5). Swamp chestnut oak seedlings grown under ponded conditions had the lowest total root biomass with an average of 28 grams of root mass (Fig. 5).

Root: shoot ratios were calculated to determine the partitioning of biomass between the root and shoots (Table 2 & 3). There was a main effect from both species ($p=0.0098$) and hydroperiod ($p=0.0041$) (Table 1). Bald cypress seedlings allocated more biomass into root production compared to swamp chestnut oak seedlings, when all treatment combinations were averaged together for each species. An average root: shoot
ratio of 2.24 was calculated for bald cypress seedlings, whereas a root: shoot ratio of 1.39 was determined for swamp chestnut oak seedlings. Seedlings subjected to continuously ponded conditions allocated less biomass to their roots with an average root: shoot ratio of 1.36, compared to seedlings under controlled moisture conditions, which had a root: shoot ratio of 2.27 (Fig. 5).

Bald cypress seedlings grown in continuously ponded organic soils had the best growth in height and diameter, as well as the most shoot and total biomass accumulation, when compared to all other species and treatment combinations. Soil solutions from the continuously ponded containers of bald cypress and swamp chestnut oak seedlings were sampled and measured for PO$_4$-P concentrations (Table 4). There were significant main effects of soil type ($p=0.0005$) and species ($p=0.0058$) on the PO$_4$-P concentrations in the soil solutions sampled. Organic soils had the greatest concentrations of PO$_4$-P in the soil solution; organic soils contained an average of 0.32 mg/L compared to only 0.068 mg/L PO$_4$-P in mineral soils. Bald cypress seedlings, grown in organic soils, had an average soil solution concentration of 0.19 mg PO$_4$-P/L, which was lower than the average soil solution concentration for swamp chestnut oak seedlings grown in organic soils. This is most likely due to the higher biomass accumulation for bald cypress seedlings, which created a greater need for macronutrients, but may also be due to an increased efficiency of phosphorus uptake in bald cypress seedlings. Soil water analysis was also conducted for NH$_4$-N but no significant differences were observed between soil types or species.

**Leaf Tissue Analysis**

Leaf tissue samples were analyzed for percentage of N, P, and Ca, as well as the concentrations of Mg, Fe, Mn, and Zn (Table 5 & 6). There were significant main effects
from species \( p=0.0092 \) and soil \( p=0.017 \) for the percentage of N in leaf tissue. On average, swamp chestnut oak seedlings had 2.03\% N in leaf tissue, which was significantly more than bald cypress seedlings, which had an average of 1.69\% N. Overall, seedlings grown in organic soils had a greater percentage of N than seedlings grown in mineral soils. For percentage of P accumulated in leaf tissue, there was only a significant \( p=0.0013 \) main effect from soil and no significant interactions between factors. Seedlings grown in organic soils, contained an average of 0.11\% P, in leaf tissue, compared to 0.08\% P for seedlings grown in mineral soils (Table 4). The same was true for the concentrations of Mg accumulated in leaf tissue as there was only a significant \( p=0.014 \) main effect from soil. Seedlings grown in organic soils accumulated more Mg than seedlings grown in mineral soils. Interestingly, seedlings grown in organic soils had a significantly greater amount of N, P, and Mg in leaf tissue samples, when compared to leaf tissue samples from those grown in mineral soils. The soils used in this study were analyzed for nutrient concentrations prior to the start of the experiment. Organic soils contained 10 times more total N and 4 times as much Mg than mineral soils. These higher soil concentrations of these nutrients most likely led to the increased uptake of N and Mg by seedlings grown in organic soils.

For Mn, there was a significant \( p<0.0001 \) effect from species. Bald cypress seedlings accumulated an average of 111 ppm Mn, which was 20\% of the Mn accumulated by swamp chestnut oak seedlings. There was a significant \( p=0.040 \) interaction between species and soil for accumulation of Mn in plant leaf tissue. Swamp chestnut oak seedlings that were grown in organic soils accumulated approximately 690 ppm Mn, compared to 426 ppm Mn for swamp chestnut oak seedlings grown in mineral
soils. However, bald cypress seedlings, grown in mineral soils accumulated more Mn than bald cypress seedlings that were grown in organic soils. Mineral soils used in this study contained nearly twice as much Mn as organic soils. There were no significant effects or differences to report for % Ca or for concentrations of Fe and Zn.

Root Counts

Roots were counted every two weeks for each rhizotron and the changes in root counts were calculated for three depth increments: 0-18 cm, 18-36 cm, and 36-54 cm (Fig. 6). Each depth increment area represented 540 cm$^2$ (18 cm x 30 cm) of soil area. Increases in roots over the 106-day experimental period are expressed as increases in the number of roots per 540 cm$^2$. The greatest differences were observed in the top 18 cm of the soil. Significant main effects included species ($p<0.0001$) and hydroperiod ($p=0.0033$), as well as an interaction between species and hydroperiod ($p=0.0003$), for the first depth increment of soil. Bald cypress seedlings, overall, had the greatest proliferation of roots near the soil surface, compared to swamp chestnut oak seedlings. Seedlings grown in continuously ponded conditions increased their root production near the surface of the soil compared to seedlings grown under the controlled moisture treatment. While bald cypress seedlings grown in continuously ponded conditions had the most production of roots in the top depth increment, swamp chestnut oak seedlings subjected to continuously ponded conditions produced the least amount of roots near the surface (Fig. 6). The average root increase was 950 roots per 540 cm$^2$ for bald cypress seedlings subjected to ponded conditions, which was significantly ($p<0.05$) different from all other species and hydroperiod combinations. A photograph illustrating the
proliferation of roots in a bald cypress seedling, grown under ponded conditions, near the soil surface is included (Fig. 7).

Increases in root growth, at a depth increment of 18-36 cm, included a significant main effect from hydroperiod ($p=0.0056$) as well as an interaction between soil and hydroperiod ($p=0.020$). Like root proliferation in the top 18 cm of the soil surface, seedlings grown in continuously ponded soils produced more roots in the middle 18 cm of the soil for both species. Seedlings grown in continuously ponded organic soils produced an average 10-fold increase of roots between 18 and 36 cm of the soil, compared to seedlings grown in organic soils of the control moisture treatment. There were no significant effects or differences between treatment combinations in the root proliferation in the bottom third of the rhizotrons to report.

**Aerenchyma Tissue Development**

Bald cypress seedlings, which were subjected to continuously ponded conditions, developed a significant amount of aerenchyma tissue in the roots of the seedlings (Fig. 8 A-D). In the mineral soil, aerenchyma tissue development was more pronounced at the shallower depth, 0-18 cm, but aerenchyma tissue development was more visible in the deeper depth, 36-54 cm, for bald cypress seedlings grown in organic soils. It was difficult to sample bald cypress roots in the organic soil, at both depths, because the root tissue was extremely “spongy” and prone to fall apart (Fig. 8 C-D). Overall, bald cypress seedlings, grown in organic and continuously ponded conditions, had a greater amount of aerenchyma tissue development than bald cypress seedlings grown in ponded, mineral soils. In swamp chestnut oak seedlings grown in continuously ponded conditions there was minimal aerenchyma tissue development in the roots (Fig. 8 E-F). Flood-sensitive
species are less able to develop extensive, enlarged air spaces in their roots and shoots, in response to saturation, and eventually, root and plant death is observed. Aerenchyma tissue was also observed in the basal stems of bald cypress seedlings, in the pith cavity, which were subjected to ponded conditions in both organic and mineral soils (Fig. 9). The development of aerenchyma tissues is a significant morphological adaptation for seedlings subjected to saturated and anaerobic soil conditions.

**DISCUSSION**

Of the two wetland tree species studied, bald cypress was the more flood-tolerant of the two species, as was expected according to the flood tolerance rankings of Hook (1984). One hundred percent survival was observed for bald cypress seedlings that were saturated and ponded for 106 days consecutively. This survival rate is in accordance with several other studies (Anderson and Pezeshki, 1999; Pezeshki and Anderson, 1997; Megonigal and Day, 1992; Shanklin and Kozlowski, 1985). Swamp chestnut oaks are considered to be weakly tolerant to saturated conditions (Hook, 1984) and it was expected that 100% survival would not be observed for this species. By the end of the study (15 weeks), 50% of continuously ponded swamp chestnut oak seedlings had died. Conner et al. (1998) observed 10% death for swamp chestnut oaks saturated for 17 weeks.

Two physiological responses were measured to further understand the health of the plants being observed. Chlorophyll fluorescence has often been used as an indicator of physiological stress in plants (Lichtenthaler and Rinderle, 1988). Woody seedlings that are experiencing greater physiological stress emit greater red fluorescence, which leads to decreased chlorophyll fluorescence (Fv/Fm) ratios. A chlorophyll fluorescence
ratio of between 0.75 and 0.85 is considered to represent healthy plants (Bolhar-Nordenkampf and Oquist, 1993). Bald cypress seedlings maintained chlorophyll fluorescence ratios greater than 0.800 for the entire experiment, which indicates that bald cypress seedlings of all treatment combinations were unstressed (Fig. 2). However, swamp chestnut oak seedlings grown in continuously ponded mineral soils had a declining chlorophyll fluorescence ratio, indicating that plant stress was occurring (Fig. 2). Swamp chestnut oak seedlings grown in continuously ponded mineral soils had chlorophyll fluorescence ratios within this “healthy” zone before abruptly dying within 50 days (Fig. 2). Chlorophyll fluorescence measurements were taken bi-weekly, so plant stress increased significantly within a two-week period before the plants died.

Bald cypress seedlings grown in continuously ponded organic and mineral soils had the greatest stomatal conductance measured in this experiment, after 12 weeks of ponding (Fig. 3). However, stomatal conductance for swamp chestnut oak seedlings of continuously ponded soils had the lowest measured stomatal conductance of all species and treatment combinations in the current study (Fig. 3). Pezeshki and Anderson (1997) found similar results in that stomatal conductance of continuously saturated bald cypress seedlings initially decreased but remained significantly higher than the two oak species (e.g. nuttall oak and cherrybark oak) studied, which were also ponded continuously. Pezeshki et al. (1996) found similar results as described previously. Southern red oak (Q. falcata) and overcup oak (Q. lyrata) had significantly reduced stomatal conductance, when compared to bald cypress seedlings. Studies have also shown no significant reduction in stomatal conductance for bald cypress seedlings during periods of ponding (Anderson and Pezeshki, 1999). It has been shown that swamp chestnut oak seedlings
experienced significantly reduced stomatal conductance on the first day of saturation and continued throughout the experiment (Anderson and Pezeshki, 1999; McLeod et al., 1999). The decrease in stomatal conductance indicates decreased photosynthetic and respiration rates occurring in swamp chestnut oak seedlings subjected to continuously ponded organic soils.

Overall, bald cypress seedlings grown in continuously ponded organic soils had the best growth in height and diameter (Fig. 4), as well as the most accumulation in the shoots and total biomass. This was most likely due to the increased uptake of P in the soil solution of continuously ponded organic soils. In this study, we found higher PO$_4$-P concentrations in continuously ponded organic soils for both bald cypress and swamp chestnut oak seedlings (Table 4). Brownfield (2007) showed that increased dissolved phosphorus levels were evident in organic soils from Juniper Bay in Robeson County, N.C. This was due to the action of dissolved organic carbon (DOC) displacing phosphorus from the soil colloids in organic soils similar to the organic soils used here. We observed the highest percentages of N and P, as well as the highest accumulation of Fe in plant tissues of bald cypress seedlings grown in continuously ponded organic soils (Table 5). The increased uptake of these nutrients may have aided in the increased growth in height and diameter, as well as the overall biomass.

It is surprising, however, that there was no significant effect on plant tissue nutrient concentrations due to hydroperiod. Plant nutrient uptake in flooded soils can be inhibited by the physiological consequences of oxygen depletion near the roots. However, some nutrients, such as Fe and Mn, can become more soluble in saturated conditions and therefore, become more available to plants. Generally, the macronutrients, especially N and P, are adversely affected by saturated and anaerobic conditions.
conditions. In *Quercus* species, leaf N, S, and K concentrations decreased when seedlings were subjected to flooded or saturated conditions (Dreyer et al., 1991).

Typically, differences in nutrient uptake are apparent between flood-tolerant and flood-sensitive species. Flood-tolerant species, such as bald cypress, are able to absorb more nutrients due to adaptations than flood-intolerant species, which are unable to translocate oxygen to areas that require aerobic conditions. In Dickson et al. (1972), bald cypress seedlings grown in saturated soils had greater P, K, Ca, and Mg concentrations when compared to seedlings grown in aerobic soils. Therefore, we expected to observe some differences in nutrient uptake between bald cypress seedlings that were continuously ponded and swamp chestnut oak seedlings that were continuously ponded.

In addition to the increased uptake of P from continuously ponded organic soils, bald cypress seedlings grown in organic soils adapted well to ponded conditions by producing the most lateral roots near the soil surface when compared to bald cypress and swamp chestnut oak seedlings grown in other treatment combinations (Fig. 7). Soil inundation typically results in the decay of roots due to the increased activity of root-rotting fungi. Woody tree species that are intolerant to ponding will lose part of their original root system and fail to regenerate new roots. However, flood-tolerant species will lose part of that original root system and subsequently regenerate new roots on the original root system, on the submerged stem, or both (Kozlowski, 1984). Root death was observed with a few weeks of soil inundation in bald cypress seedlings grown in organic soils. However, new roots, which appeared to be more succulent than original roots, quickly emerged near the soil surface. It is thought that adventitious roots compensate for the loss of portions of the original root system through increasing the water absorption
by roots, as well as oxidizing the soil rhizosphere and transforming toxic compounds into less harmful compounds (Kozlowski, 1997). Megonigal and Day (1992) found similar results in continuously flooded bald cypress seedlings. Once new roots were produced near the soil surface, Megonigal and Day (1992) saw improved growth of continuously ponded bald cypress seedlings.

In addition to the presence of lateral surface roots, bald cypress seedlings subjected to continuously ponded conditions produced aerenchyma tissue in the roots and stems after 106 days of flooding (Fig. 8 & 9). There was minimal development of enlarged cavities in continuously ponded swamp chestnut oak seedlings. Flood-tolerant species, such as bald cypress, are known to develop aerenchyma tissue in response to saturated conditions. Aerenchyma tissue provides the plant an extensive, low-resistance internal aeration system, which can transfer oxygen, via diffusion and convection, to anaerobic rhizospheres (Evans, 2003). The development of aerenchyma tissue is typically induced by soil saturation or reduced soil conditions. Plants with interconnected gas spaces are able to transport oxygen from stems and leave to the roots, while toxic gases, such as carbon dioxide, ethylene, and methane, are capable of moving through these spaces, or tissues, to the shoots and then released to the atmosphere (Colmer, 2003). Bald cypress seedlings have produced aerenchyma tissue in saturated conditions, and to a lesser extent, in aerated conditions (Kludze et al., 1994). However, Pezeshki (1991) found that roots of bald cypress seedlings subjected to low soil Eh conditions had increased amounts of aerenchyma tissue due to cell separation in newly generated cortical cells. Bald cypress seedlings from the control had formed no aerenchyma tissue during the 14-day experiment (Pezeshki, 1991). The formation of
aerenchyma is an invaluable asset to flood-tolerant tree species surviving prolonged periods of soil inundation.

**CONCLUSION**

Bald cypress seedlings easily adapted to saturated and anaerobic conditions by producing aerenchyma tissue and lateral roots near the surface of the soil, as roots in lower depths began senescing. Bald cypress seedlings, grown in continuously ponded, organic soils had the greatest overall growth as measured by increase in height, diameter, and shoot biomass. This increase in diameter was most likely due to the increase in aerenchyma tissue development in the basal portion of the stem, which was more developed than in the stems of continuously ponded bald cypress seedlings in mineral soils. Aerenchyma tissue development was also greater in the roots of bald cypress seedlings that were grown in continuously ponded organic soils. The results of this study showed that bald cypress seedlings acclimated well to continuously ponded conditions for 106 days, and grew better than the control moisture treatment. Bald cypress seedlings grown in the controlled moisture treatment showed less growth in height, diameter, as well as root and shoot biomass. In fact, bald cypress seedlings grown in the control moisture treatment allotted more biomass to roots, especially in the lower portions of the soil, which was most likely due to roots seeking moisture and nutrients. Stomatal conductance was lower in the control moisture treatment compared to continuously ponded bald cypress seedlings, indicating greater photosynthetic efficiency in continuously ponded seedlings. Furthermore, chlorophyll fluorescence was slightly elevated in bald cypress seedlings subjected to continuously ponded, organic soils compared to the control moisture treatment. Based on the information gathered in this
experiment, bald cypress seedlings would grow best in very wet, organic soils, especially organic soils which were once in agricultural production that received higher inputs of N and P. The ability of dissolved organic carbon to displace P on Fe- and Al-complexes aids in the superior growth of bald cypress seedlings in continuously ponded, organic soils.

Swamp chestnut oak seedlings grew far less, compared to bald cypress seedlings, in this 106-day experiment. Although swamp chestnut oak is considered to be one of the more flood-tolerant Quercus species (McKnight et al., 1981), plants died within 40 days for swamp chestnut oak seedlings growing in organic soils that were continuously ponded, while no swamp chestnut oak seedlings died in mineral soils continuously ponded for 106 days. The poor conditions of the trees indicated that if the experiment had continued, they most likely would have died. The rapidly declining chlorophyll fluorescence observed in these seedlings was evidence of their decline. There were no significant differences in growth of height, diameter, or shoot biomass between hydroperiod or soil treatments within swamp chestnut oak seedlings. The only significant difference between hydroperiod treatments was that swamp chestnut oak seedlings, which were subjected to continuously ponded conditions, experienced greater root growth in the middle depth increment, 18-36 cm, especially in organic soils. Based on all of the data gathered for swamp chestnut oak seedlings, in this experiment, it would be wise to plant swamp chestnut oak seedlings on the outer fringes of wetlands where prolonged periods of saturation rarely occur. Swamp chestnut oak seedlings were unable to quickly acclimate to saturated and anaerobic conditions, unlike bald cypress seedlings, which were able to acclimate through the formation of aerenchyma tissue, growth of
lateral roots near the surface, and increased uptake of P. This research reinforces the necessity of understanding the interaction between soil, hydroperiod, and vegetation type before selecting the target plant community during wetland restoration projects.

ACKNOWLEDGEMENTS

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REFERENCES


Patrick, W.H., and R.D. DeLaune. 1977. Chemical and biological redox systems...


Figure 1. Redox potential (mV) conditions for two wetland tree species, baldcypress and swamp chestnut oak, measured 20 cm below the soil surface. Redox potentials for organic soils are shown on the left and mineral soils are shown on the right. Black line indicates the boundary between aerobic (>400 mV) and anaerobic (<400 mV) conditions.
Figure 2. Daily mean fluorescence measured at the top of baldcypress and swamp chestnut oak seedlings. Fluorescence for organic soils are shown on the left and mineral soils are shown on the right.
Figure 3. Stomatal conductance means based on hydroperiod and species. No significance is calculated for swamp chestnut oak seedlings subjected to continuously ponded conditions due to only one seedling being measured at that time. Mean values for bars followed by the same lower case letter are not significantly different at the p<0.05 significance level. Standard error bars are shown.
Figure 4. Growth in height and diameter of baldcypress and swamp chestnut oak seedlings under two soil treatments (O-organic, M-mineral) and two hydroperiods (C-control, P-continuously ponded). Mean values for bars followed by the same lower case letter are not significantly different at the p<0.05 significance level. Standard error bars are shown.
Figure 5. Averages of root and shoot biomass and root: shoot for control (C) and continuously ponded (CP) treatments of baldcypress and swamp chestnut oak seedlings. Mean values for bars followed by the same lower case letter are not significantly different at the p<0.05 significance level. Standard error bars are shown.
Figure 6. Average increases in the number of roots counted for two wetland tree species, baldcypress and swamp chestnut oak, at three root depths (0-18, 18-36, 36-54 cm). Measured as the increase in the number of roots per 540 cm². Mean values for bars followed by the same lower case letter are not significantly different at the p<0.05 significance level. Standard error bars are shown.
Figure 7. Root growth of baldcypress seedlings before (left) and after (right) continuously ponded conditions for 106 days. Root proliferation near the surface of the soil was observed for continuously ponded bald cypress seedlings in both organic and mineral soils.
Figure 8. SEM micrographs of cross-sections of roots from two wetland tree species. Figs. A-B of baldcypress roots from continuously ponded, mineral soils at a depth < 18 cm (Fig. A) and > 36 cm (Fig. B). Figs. C-D of baldcypress roots from continuously ponded, organic soils at a depth of < 18 cm (Fig. C) and > 36 cm (Fig. D). Figs. E-F of swamp chestnut oak roots from continuously ponded, mineral soils (Fig. E) and continuously ponded, organic soils (Fig. F) at a depth > 36 cm.
Figure 9. SEM micrographs of cross-sections of stems from baldcypress seedlings. Fig. A is a stem cross-section from a seedling grown in organic soil with no ponding. Fig. B is a stem cross-section from an organic soil and ponded conditions. Fig. C is a stem cross-section from a seedling grown in a mineral soil with no ponding. Fig. D is a stem cross-section from a seedling grown in mineral soil with ponded conditions.
Table 1. Significances for selected measurements. S=significant. NS=not significant. Significances are reported at the $p \leq 0.05$ level.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Growth</th>
<th>Biomass</th>
<th>Root Counts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\Delta$ Height</td>
<td>$\Delta$ Diameter</td>
<td>Shoot</td>
</tr>
<tr>
<td>Species</td>
<td>S</td>
<td>S</td>
<td>NS</td>
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<tr>
<td>Soil</td>
<td>S</td>
<td>S</td>
<td>NS</td>
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<tr>
<td>Hydroperiod</td>
<td>NS</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Species*Soil</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Species*Hydroperiod</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Soil*Hydro</td>
<td>S</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Species<em>Soil</em>Hydroperiod</td>
<td>S</td>
<td>NS</td>
<td>NS</td>
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</table>
Table 2. Change in height, diameter, and final biomass (roots and shoots) accumulation of bald cypress seedlings after 106 days. Standard errors are shown. Values followed by the same lowercase letters within each row are not significantly different at p<0.05 level.

<table>
<thead>
<tr>
<th>Bald cypress</th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Organic</td>
<td></td>
<td>Mineral</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Ponded</td>
<td>Control</td>
<td>Ponded</td>
</tr>
<tr>
<td>∆ Height</td>
<td>12.6±3.1 a</td>
<td>53.2±3.3 b</td>
<td>10.8±2.8 a</td>
<td>2.1±0.5 c</td>
</tr>
<tr>
<td>∆ Diameter</td>
<td>2.7±0.6 a</td>
<td>12.9±2.2 b</td>
<td>0.7±0.3 ac</td>
<td>7.7±1.3 ad</td>
</tr>
<tr>
<td>Shoot Biomass</td>
<td>23.6±1.2 ab</td>
<td>50.9±11.7 a</td>
<td>13.9±1.7 b</td>
<td>27.5±0.3 ab</td>
</tr>
<tr>
<td>Root Biomass:</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-18 cm</td>
<td>12.0±0.6 ab</td>
<td>31.0±6.3 a</td>
<td>11.7±3.9 b</td>
<td>25.1±2.7 ab</td>
</tr>
<tr>
<td>18-36 cm</td>
<td>19.9±1.6 a</td>
<td>24.3±5.2 a</td>
<td>21.3±4.5 a</td>
<td>43.8±17.2 a</td>
</tr>
<tr>
<td>36-54 cm</td>
<td>21.9±0.8 a</td>
<td>3.1±0.9 a</td>
<td>8.6±4.0 a</td>
<td>1.6±1.3 a</td>
</tr>
<tr>
<td>Total Root Biomass</td>
<td>53.7±3.0 a</td>
<td>58.4±10.7 a</td>
<td>41.5±8.4 a</td>
<td>70.4±21.4 a</td>
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<tr>
<td>Total Biomass</td>
<td>77.3±4.2 a</td>
<td>109.3±22.3 a</td>
<td>55.5±10.1 a</td>
<td>97.9±21.1 a</td>
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<tr>
<td>Root:shoot</td>
<td>2.28±0.01 a</td>
<td>1.16±0.06 a</td>
<td>2.95±0.25 a</td>
<td>2.57±0.80 a</td>
</tr>
</tbody>
</table>
Table 3. Change in height, diameter, and final biomass (roots and shoots) accumulation of swamp chestnut oak seedlings after 106 days. Standard errors are shown. Values followed by the same lower case letters within each row are not significantly different at p<0.05 level.

<table>
<thead>
<tr>
<th>Swamp chestnut oak</th>
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<th></th>
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<tr>
<td></td>
<td>Organic</td>
<td>Mineral</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Ponded</td>
<td>Control</td>
<td>Ponded</td>
</tr>
<tr>
<td>∆ Height</td>
<td>1.8±0.0 a</td>
<td>2.1±1.0 a</td>
<td>1.7±0.1 a</td>
<td>0.8±0.5 a</td>
</tr>
<tr>
<td>∆ Diameter</td>
<td>2.7±1.2 a</td>
<td>0.4±0.4 a</td>
<td>1.0±0.2 a</td>
<td>1.2±0.1 a</td>
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<tr>
<td>Shoot Biomass</td>
<td>28.9±2.1 a</td>
<td>29.8±8.6 a</td>
<td>35.4±3.3 a</td>
<td>36.9±2.7 a</td>
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<tr>
<td>Root Biomass:</td>
<td></td>
<td></td>
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<tr>
<td>0-18 cm</td>
<td>25.8±0.2 ab</td>
<td>13.5±5.4 a</td>
<td>42.3±2.6 b</td>
<td>29.8±6.1 ab</td>
</tr>
<tr>
<td>18-36 cm</td>
<td>19.9±10.7 a</td>
<td>8.0±2.1 a</td>
<td>22.3±10.1 a</td>
<td>4.4±0.2 a</td>
</tr>
<tr>
<td>36-54 cm</td>
<td>8.2±4.0 a</td>
<td>0.3±0.3 a</td>
<td>5.6±1.1 a</td>
<td>0.3±0.1 a</td>
</tr>
<tr>
<td>Total Root Biomass</td>
<td>53.8±18.5 a</td>
<td>21.8±5.1 a</td>
<td>70.1±8.6 a</td>
<td>34.5±6.2 a</td>
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<tr>
<td>Total Biomass</td>
<td>82.7±20.6 a</td>
<td>51.6±13.7 a</td>
<td>105.5±5.3 a</td>
<td>71.4±3.5 a</td>
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<td>Root:shoot</td>
<td>1.82±0.51 a</td>
<td>0.75±0.05 a</td>
<td>2.02±0.43 a</td>
<td>0.95±0.23 a</td>
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</tbody>
</table>
Table 4. Phosphorus concentrations in soil solution (mg/L) and in dry leaf tissue samples (%) of continuously ponded tree seedlings. Standard errors are shown. Values followed by the same lower case letters within each row are not significantly different at p<0.05 level.

<table>
<thead>
<tr>
<th></th>
<th>Bald cypress</th>
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<tr>
<td></td>
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<td>Mineral</td>
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<td>Soil Solution PO4-P (mg/L)</td>
<td>0.19±0.019 a</td>
<td>0.0033±0.03 a</td>
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<td>P in Dry Leaf Tissue (%)</td>
<td>0.11±0.0057 a</td>
<td>0.080±0.0080 b</td>
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<td>Average Dry Weight of Leaves (g)</td>
<td>14.08±3.28</td>
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<td>P Content of Leaves (g/plant)</td>
<td>1.5</td>
<td>0.58</td>
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Table 5. Leaf tissue nutrient analysis for bald cypress seedlings after 106 days. Standard errors are shown. Values followed by the same lower case letters within each row are not significantly different at p<0.05 level.

<table>
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<th>Mineral Control</th>
<th>Ponded</th>
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<td></td>
<td>N (%) 1.80±0.04 a</td>
<td>1.82±0.11 a</td>
<td>1.74±0.22 a</td>
<td>1.41±0.06 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P (%) 0.11±0.01 a</td>
<td>0.12±0.00 a</td>
<td>0.09±0.01 ab</td>
<td>0.07±0.00 b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ca (%) 0.85±0.19 a</td>
<td>0.94±0.11 a</td>
<td>1.18±0.22 a</td>
<td>0.86±0.02 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mg (%) 0.42±0.02 a</td>
<td>0.44±0.014 a</td>
<td>0.39±0.00 a</td>
<td>0.28±0.03 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fe (ppm) 97.0±3.2 a</td>
<td>106.5±13.9 a</td>
<td>95.2±7.5 a</td>
<td>73.2±0.7 a</td>
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<tr>
<td></td>
<td></td>
<td>Mn (ppm) 132.6±6.8 a</td>
<td>62.8±7.0 a</td>
<td>154.4±18.0 a</td>
<td>93.7±0.4 a</td>
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<td></td>
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<td>Zn (ppm) 64.6±18.20 a</td>
<td>25.8±0.5 a</td>
<td>32.8±4.6 a</td>
<td>27.7±5.1 a</td>
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</tbody>
</table>
Table 6. Leaf tissue nutrient analysis for swamp chestnut seedlings after 106 days. Standard errors are shown. Values followed by the same lower case letters within each row are not significantly different at p<0.05 level.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Swamp chestnut oak</th>
<th></th>
<th></th>
<th></th>
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<tr>
<td></td>
<td>Organic</td>
<td>Mineral</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Ponded</td>
<td>Control</td>
<td>Ponded</td>
</tr>
<tr>
<td>N (%)</td>
<td>2.28±0.27 a</td>
<td>2.14±0.01 a</td>
<td>1.90±0.08 a</td>
<td>1.80±0.11 a</td>
</tr>
<tr>
<td>P (%)</td>
<td>0.11±0.01 a</td>
<td>0.08±0.01 a</td>
<td>0.08±0.00 a</td>
<td>0.09±0.00 a</td>
</tr>
<tr>
<td>Ca (%)</td>
<td>0.91±0.10 a</td>
<td>0.93±0.02 a</td>
<td>0.89±0.14 a</td>
<td>0.76±0.04 a</td>
</tr>
<tr>
<td>Mg (%)</td>
<td>0.34±0.03 a</td>
<td>0.40±0.01 a</td>
<td>0.35±0.06 a</td>
<td>0.31±0.02 a</td>
</tr>
<tr>
<td>Fe (ppm)</td>
<td>92.9±16.7 a</td>
<td>80.8±8.6 a</td>
<td>88.4±3.4 a</td>
<td>183.9±74.7 a</td>
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<tr>
<td>Mn (ppm)</td>
<td>722.3±101.5 a</td>
<td>659.8±210.0 a</td>
<td>385.5±45.8 a</td>
<td>466.0±1.1 a</td>
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<td>Zn (ppm)</td>
<td>28.7±5.4 a</td>
<td>29.0±3.8 a</td>
<td>25.1±2.8 a</td>
<td>27.5±5.6 a</td>
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Chapter 3
Assessing the reliability of hydrologic characterizations of four wetland plant communities.

ABSTRACT

The species composition of wetland plant communities is influenced by hydrology. This greenhouse container study was conducted to determine whether previous work correctly characterized the wetness requirements of four plant communities by utilizing four tree species that represented different plant communities. Bald cypress (*Taxodium distichum*), sweet bay (*Magnolia virginiana*), pond pine (*Pinus serotina*), and swamp chestnut oak (*Quercus michauxii*) were grown in greenhouse experiments under three hydrologic regimes: i) ponded for 100 d, ii) ponded for 15 d, and iii) unsaturated. Loamy sand and sapric materials from a restored Carolina Bay were the substrate materials used to represent mineral and organic soils. Bald cypress (representing Non-riverine Swamp Forest) acclimated well to 100 d of ponding by producing lateral roots near the surface, aerenchyma tissue in roots and stem, and increasing P uptake. In organic soils ponded for 100 d, bald cypress had significantly greater height, diameter, and total biomass than all other tree species. Sweet bay (representing Bay Forest) acclimated well to 100 d of ponding by producing adventitious roots on the submerged portion of the stem. Pond pine (representing Pond Pine Woodland) was intolerant to 100 d of ponded conditions and 75% of the seedlings died in the ponded experiments. Swamp chestnut oak (representing Non-riverine Wet Hardwood Forest) was intolerant to ponding and all seedlings died in ponded treatments. Results from this study show that modeling of the long-term hydrology of natural communities
was accurate in predicting the preferred wetness requirements of representative trees in three of the four plant communities evaluated.
INTRODUCTION

Wetlands provide integral functions to our environment, including wildlife habitat, filtering water, acting as storage basins during flooding events, protecting the shorelines, and serving as recreational areas (Mitsch and Gosselink, 2000). However, wetlands have not always been recognized as economically or aesthetically valuable. Legislation passed during the late 1970’s gave several governmental agencies the authority to begin protecting wetlands, followed by a policy of “no net loss” of wetlands in the late 1980’s. These policies raised public awareness and created interest in restoring wetlands.

Typical wetland restoration projects will include: choosing a reference wetland as a project target, establishing hydrology, establishing vegetation or target plant communities, and monitoring for 3 to 5 years to gauge the project’s “success”. Regulators, consultants, and wetland researchers use a variety of guidelines to assess wetland restoration “success”. However, success is thought to be the viable establishment of a biologically and ecologically sound wetland ecosystem (Mitsch and Wilson, 1996).

Wetland restoration is a complex process that requires extensive knowledge about the interaction of soil, hydrology, and plant communities. Through the use of hydrologic models, such as DRAINMOD (Skaggs, 1978), those working on wetland restoration projects may be able to better understand the hydrology of reference wetlands as well as predict the hydrology of restored wetlands. Although DRAINMOD was originally designed to predict the effects of drainage and water management practices on water table depths, it has been used to determine the hydrology of natural wetlands (He et al.,
2002) as well as to determine whether the wetland hydrologic standard could be met at specific sites (Wright, 2006).

Caldwell (2005) characterized the hydrologic regime of four plant communities, from three reference wetlands using DRAINMOD. The three undrained and naturally vegetated wetlands, from Bladen County, NC, were chosen as reference wetlands for a 256-ha Carolina Bay wetland restoration project. DRAINMOD was calibrated using measured rainfall, as well as water table depths and soil data from three wetlands. A 40-year simulation of water table levels was completed, using historic rainfall data, and correlated to the following plant communities: Non-riverine Swamp Forest (NRSF), Bay Forest (BF), High Pocosin (HP), and Pond Pine Woodland (PPW). Over the 40-year simulation period, Pond Pine Woodland was ponded an average of 89 days per year, with consecutive days of ponding lasting < 15 days. The average ponding depth was 0.8 cm. Bay Forest averaged 299 total days of ponding per year and 104 consecutive days of ponding at an average ponding depth of 8 cm. Non-riverine Swamp Forest, the most flood tolerant of the communities, averaged 307 total days of ponding per year and 123 consecutive days of ponding at a ponding depth of 10 cm. The longest consecutive ponding events occurred during the winter months when evapotranspiration rates were the lowest. Non-riverine Swamp Forest and Bay Forest exhibited similar hydrological characteristics. These two communities had water levels above the soil surface, whereas Pond Pine Woodland had a water table depth of 15 cm below the soil surface for the 40-year simulation average. Overall, strong differences between the Non-riverine Swamp Forest/Bay Forest communities and Pond Pine Woodland communities were apparent with respect to hydrology.
While the hydrology of Non-riverine Swamp Forest community and Bay Forest plant community were similar; the Non-riverine Swamp Forest grew in soils with higher levels of available phosphorus. These small differences in soil fertility could explain differences in dominant vegetation types. The vegetation of NRSF is dominated by bald cypress (*Taxodium distichum*), pond cypress (*Taxodium ascendens*), swamp tupelo (*Nyssa biflora*), loblolly pine (*Pinus taeda*), Atlantic white cedar (*Chamaecyparis thyoides*), pond pine (*Pinus serotina*), and tulip tree (*Liriodendron tulintermittently pondedfera*) (Schafale and Weakley, 1990). Non-riverine Swamp Forest can be easily distinguished from other peatland communities by the dominance of bald cypress and swamp tupelo. Bay Forest, however, is dominated by sweet bay (*Magnolia virginiana*), loblolly bay (*Gordonia lasianthus*), red bay (*Persea borbonia*), pond pine (*Pinus serotina*), and Atlantic white cedar (*Chamaecyparis thyoides*) (Schafale and Weakley, 1990). Bay Forest is easily discerned from other plant communities by the dominance of the three bay species: loblolly bay, sweet bay, and red bay. Pond Pine Woodland is also easily distinguished from other plant communities due to the dominance of pond pine.

The objective of this study was to determine if growth of plant species from three wetland communities responded to hydrologic regimes as predicted by calibrating and simulating 40 years of hydrologic data using the DRAINMOD model (Caldwell, 2005). High Pocosin (HP) was not used in this study because it is considered to have similar dominant species as the Pond Pine Woodland and Bay Forest plant communities (Schafale and Weakley, 1990) and a similar hydrologic regime as the Non-riverine Swamp Forest and Bay Forest plant communities (Caldwell, 2005). Swamp chestnut oak, which is the dominant species of the Non-riverine Wet Hardwood Forest was
included in order to study a range of flood tolerant tree species. If hydrologic regimes can be correctly predicted for target plant communities using modeling approaches, before wetland restorations begin, then higher success rates for these projects could most likely be achieved.

**MATERIALS AND METHODS**

**Soil Collection**

This study was conducted in a greenhouse using organic and mineral soils collected from Juniper Bay, a 256 ha Carolina bay wetland, located in Robeson County, near Lumberton, NC (34°30’30”N 79°01’30”E). Juniper Bay was drained in 1972 and used for agricultural production until 2006 when it was restored back to a wetland. During the years in agriculture, the soils were fertilized and limed annually to soil test recommendations for the production of soybeans (*Glycine max*), oats (*Avena sativa*), cotton (*Gossypium hisutum*), corn (*Zea mays*), tobacco (*Nicotiana tabacum*), wheat (*Triticum aestivum*), and other vegetable crops (Ewing, 2003).

Two sites were located to collect soil samples based on ease of access and soil type. Mineral soil was collected from the Ap horizon of a Leon sand (sandy, siliceous, thermic Aeric Haplaquod). Organic soil material was collected from the Oa horizon of a Ponzer muck (loamy, mixed, dysic, thermic Terric Haplosaprist). Small pits were dug by hand in each soil area to a depth of 60 cm. Undisturbed cores were collected in triplicate from the 15 to 25 cm depth using metal cylinders (10 cm in diameter by 10 cm in height) for bulk density determination. Soil was collected from the upper 25 cm of the wall of each pit, placed in buckets and transported to the greenhouse. Soil materials were air-dried for 3 days and then passed through a 1.25 cm mesh sieve. Particle size distribution
was determined by the hydrometer method after passing the material through a 2-mm mesh sieve (Gee and Bauder, 1986). In the laboratory, soil cores were weighed, oven-dried at 105°C for 24 hours and weighed again. Before potting, sub-samples of organic and mineral soils were taken to determine gravimetric water content, which was used to calculate for bulk densities similar to bulk densities measured in the field.

Soil samples were analyzed at North Carolina Department of Agriculture, Soil Testing Services, Raleigh, North Carolina for extractable P, K, Ca, Mg, Mn, Cu, Zn, and S using the Mehlich-3 extractant (Mehlich, 1984) and an inductively coupled plasma emission spectograph (ICP). Buffer acidity, cation exchange capacity, and the sum of base cations were also determined (Mehlich, 1976). The pH was determined using a 1:1 soil to water ratio. Two separate bulk samples were air-dried and ground to pass through a 2 mm sieve for the determination of organic carbon and total nitrogen with a Perkin Elmer PE2400 CHN Elemental Analyzer (Culmo, 1988) in the North Carolina State University Soil Science Analytical Lab, Raleigh, North Carolina.

**Tree Seedlings**

Pond pine (*Pinus serotina*), bald cypress (*Taxodium distichum*), and swamp chestnut oak (*Quercus michauxii*) seedlings were purchased from North Carolina Division of Forest Resources, Goldsboro, North Carolina. Sweet bay (*Magnolia virginiana*) seedlings were purchased from SuperTree Nursery, Blenheim, South Carolina. Prior to planting, the seedlings were kept in the cold room at 7°C with adequate moisture.

Thirty seedlings of each species were chosen for the study based on their similar heights and overall health. Fifteen seedlings were potted in each soil type using 7.65-liter
tree containers (Stuewe & Sons, Inc., Corvallis, OR, model TPOT3). Drain holes in the pots were covered with fiberglass mesh to minimize the soil loss from the bottom of the pots. The pots were filled with an appropriate amount of soil and the soil was packed to the desired field bulk density. The final bulk density for containers filled with mineral soil was 1.28 g/cm$^3$ and 0.47 g/cm$^3$ for organic soils. The tree seedlings were acclimated to greenhouse conditions by allowing them to grow under unsaturated conditions for 79 days. Twenty-four of the original 30 potted seedlings from each species were then selected for the study based on similar heights and overall health.

Three hydroperiods were utilized and included: control (C), intermittently ponded (IP), and continuously ponded (CP). Control treatments were watered to maintain a soil water pressure potential of -300 cm which was considered near field (or container) capacity. The intermittently ponded treatment was imposed by saturating pots from the base upward and maintaining a water level above the surface. After 14 days, the soil was drained and kept unsaturated for 42 days. This saturation and drainage cycle was repeated 6 times, totaling 84 days of ponding and 282 days of unsaturated conditions. The intermittently ponded hydroperiod treatment was intended to represent the 40-year average hydrologic characterization of the Pond Pine Woodland (PPW) plant community (Caldwell, 2005). The continuously ponded treatment was saturated and kept ponded for a total of 300 days and allowed to drain for 65 total days over the 49-week experiment. The continuously ponded treatment was initially inundated for 110 days and then two subsequent 95-day periods of saturation were utilized. The continuously ponded hydroperiod treatment was intended to represent the 40-year average hydrologic characterization of the Non-riverine Swamp Forest (NRSF) and Bay Forest (BF) plant
communities (Caldwell, 2005). The water level was maintained 10 to 12 cm above the soil surface for the continuously ponded and intermittently ponded treatments, during their ponded phases. During the unponded cycles, soil moisture content was maintained close to field capacity. Tap water was used for all treatments. Experiments conformed to a split-plot design, containing four replications per treatment combination, after 3 months of treatment initiation.

Each plant container was equipped with two platinum electrodes: one placed at 10 cm below the soil surface and a probe placed at 20 cm below the soil surface. The two redox probes were placed approximately 13 cm apart, on either side of the tree seedling, in the containers. The redox platinum electrodes were constructed and tested similar to the techniques of Wafer et al. (2004). Accurate redox probes were gently inserted by hand into the soil of the rhizotrons. A small amount of soil was mixed with water to create a soil slurry and any gaps around the redox probe were filled with the slurry.

The containers were equipped with a tensiometer to determine the soil water pressure head for watering purposes. A 2.86-cm long and 0.95-cm wide porous ceramic cup (Soilmoisture Equipment, Santa Barbara, CA, model 0652X07), with an air entry valve of 1 bar, was attached to a 15-cm long section of 1-cm diameter polycarbonate tubing with marine epoxy (ITW Philadelphia Resins, Montgomeryville, PA). A separate piece of 7.5-cm long and 1.25-cm diameter polycarbonate tubing was cemented to the other end of the 15-cm long polycarbonate tubing with marine epoxy. The tensiometers were inserted to a depth of 7.5 cm below the soil surface in all plant containers before the treatments were begun. The septum stoppers were periodically replaced to maintain
pressure inside the tensiometer. The tensiometers were read using a tensimeter (Soil Measurement Systems, Tucson, AZ).

**Measurements**

Redox potential measurements were made weekly using an auto-range digital multimeter (Radio Shack, Fort Worth, TX, model 22-163) and a calomel reference electrode (Thermo Fisher Scientific, Waltham, MA, model 13-620-259). The pH measurements of the organic and mineral soils were made bi-monthly using a portable pH/mV meter (Thermo Fisher Scientific, Waltham, MA, model 13-636-62A) in order to correctly interpret the redox measurements. Height and diameter were measured on a monthly basis using a meter stick and caliper. At the end of the study, the total growth of the 49-week experiment was determined by subtracting the initial height/diameter from the final height/diameter.

Chlorophyll fluorescence measurements were conducted using a hand-held OS-30p fluorometer (Opti-sciences, Hudson, NH, model OS-30p) using the ‘Fv/Fm Kinetics’ option. Two measurements were taken on each plant including: the second leaf from the bottom and the second fully developed leaf from the tallest shoot apex. However, leaves with obvious signs of senescence or poor overall health were not measured. Measurements were conducted in the pre-dawn hours on leaves, which had been adapted to the dark for at least 6 hours. The relative quantum yield, or quantum efficiency, of Photosystem II was calculated by the hand-held fluorometer as:

\[
\Phi_{PS2} = \frac{(F_m-F_o)}{F_m} \tag{1}
\]

Stomatal conductance measurements were conducted in week 16 and again in week 29 with a Delta-T Porometer (Delta-T Devices Ltd, Burwell, Cambridge, England,
model AP4-UM-3). Stomatal conductance was measured between the hours of 11:00 a.m. and 1:00 p.m. on a sunny day. One leaf per plant was measured per measurement day. The second fully developed leaf from the tallest shoot apex was measured and recorded. Relative humidity (RH) of the air entering the chamber was fixed, while RH in the greenhouse ranged from 45 to 50%. Photon flux density (PPFD) was also measured with the Delta-T Porometer and reached a maximum of 680 µmol m\(^{-2}\) s\(^{-1}\).

Water samples were taken in week 22 to test for N and P concentrations in the soil solution of the continuously ponded seedlings. Phosphate and ammonium were determined using the colorimetric method with a lachat flow injection analyzer. Soil samples were taken at the end of the study. Soil samples were analyzed for extractable P using the Mehlich-3 extractant (Mehlich, 1984) and an inductively coupled plasma emission spectrograph (ICP). Soil samples were also analyzed for phosphate (PO\(_4\)) using the colorimetric method with a lachat flow injection analyzer. Final measurements were conducted on February 8\(^{th}\), 2008. The containers were dismantled 3 days later. Biomass was divided into shoot (stems and leaves) and roots. Stems and roots were washed, oven-dried for 72 hours at 70°C, and weighed to calculate shoot, root, and total biomass.

**Data Analysis**

Statistical analyses were performed using SAS version 9.1 software (SAS Institute, Cary, NC, 2005). The PROC MIXED function was used to test for significance at the \(p \leq 0.05\) level for measurements including: changes in height, diameter, biomass, and root/shoot ratio. The Tukey procedure was used to determine all pair-wise differences between treatment combinations. The data were transformed, as needed, to fulfill the ANOVA assumptions, especially the assumption of equal variances. Repeated
measures analysis of variance was used to test for differences in means of fluorescence, stomatal conductance, and redox potential data.

**RESULTS**

**Redox Potential**

Redox potential measured at 10 and 20 cm below the soil surface did not vary significantly among species and data were combined for all species for each depth (Fig. 1). The redox potential measured in the control and intermittently ponded organic soils tended to be slightly higher when compared to redox potential measured in mineral soils. The average redox potential for seedlings in the control moisture treatment was +425 mV for seedlings grown in mineral soils, and +500 mV for seedlings grown in organic soils. Well-drained soils typically have a redox potential range of +400 to +700 mV but redox potentials measured below +350 mV are thought to be characteristic of anaerobic soils (Patrick and DeLaune, 1977).

Seedlings subjected to the intermittently ponded hydroperiod treatment experienced 6 cycles of saturation. After the 4th period of inundation, redox potentials stabilized near -25 mV during the saturated cycle (Fig. 1). During the drained cycles for seedlings subjected to intermittently ponded conditions, the redox potentials closely followed the redox potentials observed for seedlings subjected to the control moisture treatment. The first saturated cycle for continuously ponded seedlings lasted 110 days. During this first inundation cycle, redox potentials fell to -100 mV for both mineral and organic soils. The second two cycles consisted of 95 days of consecutive ponding and the redox potentials were slightly elevated at approximately 0 mV during saturation for both soils. Redox potential measured 20 cm below the soil surface followed the same
general trend as redox potential measured 10 cm below the soil surface (Fig. 1). More variability in redox potential was observed at the lower depth, especially for seedlings subjected to control and intermittently ponded hydroperiod treatments. The differences in redox potentials measured in both mineral and organic soils from the control and intermittently ponded treatments disappeared for the lower depth measured.

**Survival Rates**

Survival was 100% for bald cypress seedlings in all treatment combinations. Survival was also 100% for sweet bay seedlings grown in the control and intermittently ponded hydroperiod treatments. However, survival was 75% for sweet bay seedlings grown in organic soils under continuously ponded conditions but was 100% for sweet bay seedlings grown in mineral soils under continuously ponded conditions. One sweet bay seedling grown in continuously ponded organic soils died after 50 days of ponding. This seedling was the smallest of all the sweet bay seedlings used in the study. After a month of continuously ponded conditions, one sweet bay seedling grown in mineral soils dropped all of its leaves but regenerated them within several weeks and survived a total of 300 days of ponding.

Pond pine seedlings grown in the control and intermittently ponded hydroperiod treatments had 100% survival. After 110 days of consecutively ponded conditions, 75% of pond pine seedlings in organic soils had died. The last surviving pond pine seedling grown in continuously ponded organic soils died after 150 days of saturation. Survival was 50% for pond pine seedlings grown in continuously ponded mineral soils. The first pond pine seedling grown in continuously ponded mineral soils died after 191 days of ponding and the other pond pine seedling died after 205 days of ponding. The remaining
two pond pine seedlings grown in continuously ponded mineral soils maintained healthy chlorophyll fluorescence levels until the end of the 338-day experiment.

Like sweet bay and pond pine, swamp chestnut oak seedlings had 100% survival in the control and intermittently ponded hydroperiod treatments. All of the swamp chestnut oak seedlings grown in continuously ponded organic soils had died within 99 consecutive days of ponding. The death of swamp chestnut oak seedlings grown in continuously ponded mineral soils was more sporadic than the death observed in continuously ponded organic soils. The first swamp chestnut oak seedling grown in continuously ponded mineral soils died within 29 days of consecutive ponding, the second seedling within 50 days of consecutive ponding, and the final seedling died within 110 days of consecutive ponding. However, the remaining swamp chestnut oak seedling in this treatment combination survived the 300 total days of ponding, possibly due to the formation of hypertrophied lenticels. Overall, swamp chestnut oak seedlings in continuously ponded mineral soils had a 75% survival rate.

**Chlorophyll Fluorescence**

A plant is considered healthy, or unstressed, when the Fv/Fm ratio is between 0.75 and 0.85 (Bolhar-Nordenkampf and Oquist, 1993) or above 0.78 (Adams et al., 1990). Bald cypress seedlings subjected to the three hydropersiods maintained an Fv/Fm above 0.78 throughout the experiment indicating that bald cypress seedlings subjected to continuously ponded, intermittently ponded, and the control moisture treatment were healthy throughout the 49-week experiment (Fig. 2). The three remaining species maintained Fv/Fm of 0.78 or higher for most of the experiment in the controlled and intermittently ponded treatments. Marked declines in Fv/Fm were observed in the
continuously ponded treatment with the lowest Fv/Fm found for swamp chestnut oak seedlings (Fig. 2). The steep declines in Fv/Fm indicate seedling death.

During week 29, there was a significant peak in Fv/Fm for all of the seedlings subjected to intermittently ponded and control moisture conditions (Fig. 2), which was most likely due to the declining temperatures in the greenhouses. Temperatures in the greenhouse, during July and August, frequently exceeded 46ºC. When Fv/Fm was analyzed together, there were no significant main effects or interactions (Table 1). However, when Fv/Fm was analyzed separately, by species, there was a significant interaction between hydroperiod and the time of measurements for all species, except swamp chestnut oak (Table 1). There was also a main effect from hydroperiod in all species, except bald cypress, which showed similar Fv/Fm for all hydroperiod treatments (Table 1).

**Stomatal Conductance**

Stomatal conductance was measured during week 16, after the intermittently ponded seedlings had undergone two periods of saturation and drainage, and in week 29, after the continuously ponded seedlings had been ponded for 110 days, followed by a short drainage period and another 11 weeks of ponding (Fig. 3). There were significant main effects for species and soil (Table 1). Bald cypress and sweet bay seedlings typically had higher stomatal conductance when compared to the less flood-tolerant species (i.e. pond pine, swamp chestnut oak). All species, except pond pine, had higher stomatal conductance in seedlings grown in organic soils. Bald cypress seedlings grown in continuously ponded conditions had the greatest stomatal conductance compared to the two other hydroperiod treatments and stomatal conductance increased from week 16 to
week 29 (Fig. 3). Sweet bay seedlings grown in continuously ponded and intermittently ponded hydroperiod treatments showed increased stomatal conductance from week 16 to week 19 (Fig. 3). The increase in stomatal conductance indicates respirative recovery after several weeks of inundation and drainage.

**Changes in Height and Diameter**

Bald cypress had the greatest overall growth in height and diameter for all hydrologic treatments (Fig. 5). The change in height was four times larger than pond pine and sweet bay seedlings and 34 times greater than swamp chestnut oak seedlings. When the growth in height and diameter was averaged across species, seedlings grown in organic soils had greater growth in height and diameter than seedlings grown in mineral soils. Continuously ponded seedlings had the greatest growth in height and diameter, followed by seedlings subjected to intermittently ponded conditions, and finally, seedlings subjected to the control moisture treatment had the least amount of growth in height and diameter. Most of the significant effect caused by hydroperiod was due to the tremendous growth exhibited by bald cypress seedlings subjected to continuously ponded conditions.

Bald cypress seedlings grown in continuously ponded soils had twice as much growth in height as intermittently ponded bald cypress seedlings and nearly 2.5 times the growth of bald cypress seedlings grown in the well-drained conditions (Fig. 4). Bald cypress subjected to continuously ponded conditions had two times the growth in diameter of intermittently ponded bald cypress seedlings and five times the growth of bald cypress seedlings under well-drained conditions. Likewise, sweet bay seedlings subjected to continuously ponded conditions exhibited the best growth in height when
compared to sweet bay seedlings grown in intermittently ponded and the control moisture treatments (Fig. 4). Sweet bay seedlings subjected to continuously ponded conditions also experienced the greatest growth in diameter when compared to the sweet bay seedlings subjected to the remaining hydroperiod treatments due to the growth of adventitious roots at the base of the stem. However, there was only a significant difference between sweet bay seedlings grown in continuously ponded and intermittently ponded hydroperiod treatments.

Hydroperiod treatments did not significantly affect height in pond pine and swamp chestnut oak seedlings. However, pond pine seedlings grown in intermittently ponded conditions had the best growth in diameter when compared to pond pine seedlings grown in the control and intermittently ponded treatments. The growth in height of swamp chestnut oak seedlings, subjected to continuously ponded conditions, was the least of any species and hydroperiod combination. Unlike growth in height, there was also a significant ($p=0.0063$) interaction between species and soil (Table 2). Bald cypress and sweet bay seedlings grown in organic soils exhibited more than 1.5 times more growth in diameter than bald cypress and sweet bay seedlings grown in mineral soils (Fig. 5).

**Biomass**

Biomass was partitioned into shoots and roots and compared among species (Fig. 6). There was a significant main effect from species ($p=0.0008$) and soil ($p<0.0001$), as well as an interaction between species and hydroperiod ($p<0.0001$) (Table 2). Overall, swamp chestnut oak had the greatest shoot biomass and sweet bay had the least accumulation of shoot biomass compared to all other species. Seedlings grown in
organic soils had greater shoot biomass than seedlings grown in mineral soils. Bald
cypress seedlings grown in continuously ponded soils had greater shoot biomass
accumulation than bald cypress seedlings grown in intermittently ponded or soils
maintained in the control moisture treatment (Fig. 6). However, pond pine seedlings
subjected to continuously ponded conditions produced less shoot biomass than pond pine
seedlings of intermittently ponded or control moisture treatments. There were no
significant differences within sweet bay or swamp chestnut oak seedlings.

Root biomass was significantly \( p<0.0001 \) affected by species, soil, and
hydroperiod (Table 2). Bald cypress seedlings accumulated the most root biomass,
followed by swamp chestnut oak, pond pine, and sweet bay seedlings. Seedlings grown
in organic soils accumulated 1.5 times more root biomass when compared to seedlings
grown in mineral soils. Seedlings grown in continuously ponded conditions accumulated
the least amount of root biomass, whereas there was no significant difference in root
biomass of seedlings grown in intermittently ponded and control moisture treatments.

There were two significant interactions between species and soil \( p=0.0005 \) as
well as, species and hydroperiod \( p<0.0001 \). Bald cypress seedlings grown in
continuously ponded conditions accumulated the most root biomass of the three moisture
treatments (Fig. 6). Sweet bay seedlings grown in continuously ponded conditions
exhibited tremendous adventitious root growth above the soil surface but there were no
significant differences due to the hydroperiod treatments in root biomass accumulation
within sweet bay seedlings. Ponded conditions significantly reduced the amount of root
biomass for both pond pine and swamp chestnut oak seedlings. Bald cypress and sweet
bay seedlings grown in organic soils had greater root biomass accumulation than
seedlings grown in mineral soils. There were no significant differences within pond pine and swamp chestnut oak seedlings grown in the two soil types.

Bald cypress had the largest root: shoot ratios (2.95), whereas the other three species had root: shoot ratios between 1.64 and 1.89. Seedlings grown in continuously ponded soils allocated less of their biomass to root production whereas seedlings grown in the control moisture treatment allocated the most biomass to root production. There were two significant interactions between species and soil \( (p=0.0010) \), as well as species and hydroperiod \( (p=0.0005) \) (Table 2). Bald cypress seedlings grown in the control moisture treatment allocated 3.5 times more biomass into root production than shoot production, whereas bald cypress seedlings of the continuously ponded moisture treatment only allocated 2.5 times more biomass into root production (Fig. 6). Pond pine and swamp chestnut oak seedlings subjected to continuously ponded conditions allocated significantly less biomass into root production when compared to the control and intermittently ponded treatments (Fig. 6). Bald cypress and sweet bay seedlings grown in organic soils allocated significantly more biomass to the roots than seedlings of the same species grown in mineral soils. There were no significant differences in biomass allocation for pond pine and swamp chestnut oak seedlings grown in the two soil types.

There were significant main effects from species \( (p=0.0001) \), soil \( (p<0.0001) \), and hydroperiod \( (p<0.0001) \) for total biomass accumulation (Table 2). Bald cypress seedlings accumulated the most total biomass, followed by swamp chestnut oak, pond pine, and finally, sweet bay seedlings. Seedlings grown in organic soils accumulated nearly 1.5 times the total biomass of seedlings grown in mineral soils. However, seedlings of all four species grown in continuously ponded conditions accumulated the
least amount of total biomass when compared to seedlings grown in intermittently ponded or the control moisture treatments. There were also two interactions from species and soil ($p=0.0042$) as well as species and hydroperiod ($p<0.0001$) (Table 2). Bald cypress seedlings grown in continuously ponded conditions accumulated more total biomass than bald cypress seedlings grown in the control moisture treatment (Fig. 7). On the other hand, pond pine and swamp chestnut oak seedlings grown in continuously ponded conditions accumulated significantly less total biomass than seedlings grown in the other two hydroperiod treatments. Three out of four species in this study accumulated more total biomass when grown in organic soils of any hydroperiod treatment than seedlings of the same species grown in mineral soils; although pond pine accumulated more total biomass in organic soils, the difference was not significant when compared to pond pine seedlings grown in mineral soils (Fig. 7).

**DISCUSSION**

Bald cypress is one of the dominant species of the Non-riverine Swamp Forest (NRSF) plant community that is adapted to wet, very poorly drained peat deposits of the outer Coastal Plain, according to Schafale and Weakley (1990). Caldwell (2005) predicted that NRSF communities averaged 307 total days of ponding per year and 123 consecutive days of ponding over the 40-year hydrologic simulation. This prediction was verified in this study as all bald cypress seedlings survived in the continuously ponded treatment where ponding was maintained for 110 days initially with two subsequent 95-day periods of saturation. The 100% survival rates found in this study corroborated the findings of other studies (Anderson and Pezeshki, 1999; Pezeshki and Anderson, 1997; Megonigal and Day, 1992; Shanklin and Kozlowski, 1985). It was also shown that bald
cypress seedlings subjected to 300 total days of ponding in a 49-week period experienced a significantly greater growth in height and diameter, as well as accumulated significantly more shoot, root, and total biomass when compared to bald cypress seedlings grown in the control moisture treatment (Figs. 4 and 6). Likewise, the growth in height and diameter and shoot biomass accumulation were significantly greater in continuously ponded bald cypress seedlings when compared to bald cypress seedlings subjected to intermittently ponded conditions (Figs. 4 and 6). Although bald cypress is considered to be a very flood-tolerant woody species (Hook, 1984), previous studies have shown that prolonged saturation has caused decreased shoot biomass (Shanklin and Kozlowski, 1985; McLeod et al., 1986; Conner et al., 1998; Anderson and Pezeshki, 1999), decreased root biomass (Dickson and Broyer, 1972; Shanklin and Kozlowski, 1985; McLeod et al., 1986; Conner et al., 1998; Anderson and Pezeshki, 1999), as well as decreased growth in height (Shanklin and Kozlowski, 1985; Conner, 1994). However, increases in stem diameter of continuously ponded bald cypress seedlings have been found to occur (Conner, 1994; Pezeshki and Anderson, 1997). The growth differences between bald cypress seedlings grown in the control moisture treatment and the intermittently ponded seedlings were not significant except for the change in diameter. Growth in diameter was twice as much for bald cypress seedlings grown in continuously ponded than in intermittently ponded conditions, which was, in turn, twice as much when compared to bald cypress seedlings grown in the control moisture treatment.

Previous research has shown that bald cypress seedlings grown in anaerobic, saturated soils have had initial decreases in stomatal conductance but regained stomatal function within a few weeks (Pezeshki, 1991; Anderson and Pezeshki, 1999). Although
we measured stomatal conductance in week 16, the stomatal conductance for bald cypress seedlings grown in continuously ponded conditions was higher when compared to the other hydroperiod treatments (Fig. 3). Thirteen weeks later, stomatal conductance doubled for bald cypress seedlings under continuously ponded conditions, as well as for bald cypress seedlings in the intermittently ponded hydroperiod (Fig. 3). Furthermore, chlorophyll fluorescence was not reduced due to the various hydroperiod and soil treatments used in the current study (Fig. 2). This indicates that photosynthesis and respiration were not reduced in bald cypress seedlings due to the saturated and ponded conditions.

Sweet bay is one of the three dominant bay species of the Bay Forest plant community, which was estimated by Caldwell (2005) to average 299 total days of ponding per year and 104 consecutive days of ponding over the 40-year simulation period. Based on these findings, we hypothesized that 100% survival would also be observed for sweet bay subjected to an initial saturation of 110 days, followed by another 2 cycles of 95 days of ponding. However, survival rate was slightly greater than 87% due to one seedling dying within 50 days of the experiment initiation. This seedling was the smallest of the sweet bay seedlings used in the study and may have been naturally weaker than the rest of the continuously ponded seedlings. However, sweet bay seedlings in the continuously ponded hydroperiod treatment exhibited the greatest growth in height, which was significantly greater than the intermittently ponded treatment as well as the greatest growth in diameter, which was significantly greater from the two other hydroperiod treatments within the sweet bay species (Fig. 4). Sweet bay seedlings subjected to continuously ponded conditions produced the most new shoots; however,
total shoot biomass in continuously ponded conditions was not significantly different from the other hydroperiod treatments. There were also no significant differences in total biomass between sweet bay seedlings subjected to continuously ponded conditions and the other two hydroperiod treatments. It was expected that the greatest overall growth would be observed in continuously ponded sweet bay seedlings due to the findings of Caldwell (2005). This was not the case, although sweet bay seedlings were able to survive continuously ponded conditions while maintaining a comparable amount of growth to the other two hydroperiod treatments.

Furthermore, the physiological measurements conducted did not indicate that sweet bay seedlings were significantly harmed due to the intermittently ponded or continuously ponded hydroperiod treatments. Although chlorophyll fluorescence decreased initially in sweet bay seedlings subjected to intermittently ponded and continuously ponded conditions, the plants recovered well and only one death was observed (Fig. 2). The fluorescence remained close to the “healthy” fluorescence level of 0.78 for the remainder of the experiment. Stomatal conductance measured in sweet bay seedlings was unique from the other species (Fig. 3). Stomatal conductance of seedlings subjected to intermittently ponded and continuously ponded conditions increased by 100-140 mmol/m²/s, while stomatal conductance of seedlings subjected to the control moisture conditions decreased from week 16 to week 29. Sweet bay seedlings, like bald cypress, recovered well after 13 weeks to the continuously ponded and intermittently ponded conditions.

Caldwell (2005) hypothesized that the main difference between Bay Forest and Non-riverine Swamp Forest plant communities was the higher plant available phosphorus
in the soils of Non-riverine Swamp Forest compared to Bay Forest soils. Total P (organic and inorganic P) and phosphate (PO$_4$-P) were extracted from the soils at the end of the current study. Organic soils contained significantly ($p<0.0004$) more PO$_4$-P (1.55 mg/L) than mineral soils (1.28 mg/L) after the 49-week experiment. Likewise, continuously ponded soils contained significantly ($p<0.0001$) higher concentrations of PO$_4$-P (1.88 mg/L) when compared to soils of the control (1.16 mg/L) and intermittently ponded (1.21 mg/L) hydroperiod treatments.

There was an interaction between soil and hydroperiod in that organic soils that were subjected to continuously ponded conditions contained the greatest concentrations of PO$_4$-P (2.2 mg/L). The increase in PO$_4$-P in continuously ponded organic soils may be due to the action of dissolved organic carbon (DOC) displacing phosphorus from the soil mineral surfaces (Hutchison and Hesterberg, 2004; Brownfield, 2007). Dissolved organic carbon is organic material that has been broken down into smaller molecules, whose chemical structure may or may not be recognizable. Many of the recognizable molecules contain carboxylic acid functional groups, which compete with PO$_4$-P for mineral surface binding sites (Bolan et al., 1994; Violante et al., 1991). Organic soils from containers where sweet bay seedlings were grown contained significantly more PO$_4$-P at the end of the study when compared to the organic soils from where bald cypress seedlings were grown. Overall, the soils of bald cypress seedlings contained the least amount of PO$_4$-P and total P by the end of the study which suggests that bald cypress, one of the dominant tree species of Non-riverine Swamp Forest, is efficient in taking up available P from the soil, especially in continuously ponded organic soils. Caldwell (2005) may have been correct in suggesting that the differences in species of the
Bay Forest and Non-riverine Swamp Forest communities are due to differences in available P in the soils and not due to difference in flood-tolerance.

Pond pine is the dominant species of the Pond Pine Woodland plant community, which Caldwell (2005) characterized as typically occurring in soils that were ponded for <15 days consecutively over the 40-year simulation period. Seventy-five percent of pond pine seedlings subjected to three periods of >95 days of consecutive ponding had died by the end of the study. If the study had continued longer, the remaining two seedlings most likely would have died as the needles were turning brown and senescing. Pond pine seedlings subjected to continuously ponded conditions had the least amount of shoot, root, and total biomass accumulation compared to the other two hydroperiod treatments. Hunt (1950) found reduced shoot growth in pond pine seedlings that were continuously saturated with stagnant water for 3 months. Saturation can negatively impact the shoot growth of trees by suppressing leaf formation, expansion of leaves, and premature senescence and abscission of leaves (Kozlowski, 1997). Topa and McLeod (1986) found that pond pine seedlings subjected to 8 weeks of anaerobic conditions had a 33 to 36% reduction in shoot fresh weight. Furthermore, Hunt (1950) found decreased dry weights of the roots of continuously ponded pond pine seedlings. The roots were in such poor health that the pond pine seedlings may not have survived if the experiment had continued any longer. Topa and McLeod (1986) found a 56% reduction in the root fresh weight under anaerobic conditions when compared to aerobic conditions. Although pond pine is considered a moderately tolerant species to saturated conditions (Hook, 1984), pond pine seedlings produce less shoot and root biomass under prolonged ponded, or anaerobic, conditions.
The total shoot, root, and overall total biomass accumulation were comparable in intermittently ponded pond pine seedlings and pond pine seedlings grown in the control moisture treatment. Pond pine seedlings grown in intermittently ponded soils accumulated more total shoot biomass than pond pine seedlings grown in the control moisture treatment, although the differences were not significant. Growth in height was not significantly different in the three hydroperiod treatments. Hunt (1950) found the greatest increase in height in pond pine seedlings subjected to 14 days of ponding and 14 days of drainage. Survival was 100% for pond pine seedlings grown in the 14-day intermittently ponded hydroperiod treatment. Pond pine seedlings grown in intermittently ponded conditions had greater stomatal conductance than seedlings in the control moisture treatment and continuously ponded conditions, although the difference between intermittently ponded and the control were not significant (Fig. 3). The intermittently ponded treatment in the current study was intended to mimic the hydrology of the Pond Pine Woodland plant community characterized by Caldwell (2005). Caldwell (2005) determined that the Pond Pine Woodland plant community averaged 89 total days of ponding per year and <15 consecutive days of ponding. Based on the results of this study, we would agree with the hydrologic characterization of the dominant species of the Pond Pine Woodland plant community.

The Non-riverine Wet Hardwood Forest community occurs on the margins of peatland areas in the outer parts of the Coastal Plain and grades into the Pond Pine Woodland (Schafale and Weakley, 1990). Swamp chestnut oak is one of the three dominant bottomland oak species of the Non-riverine Wet Hardwood Forest. Although Caldwell (2005) did not describe the hydrologic profile of this community, we included
this species to determine if swamp chestnut oak is, in fact, weakly tolerant to ponding as described by Hook (1984). Most (87%) swamp chestnut oak seedlings subjected to 300 total days of ponding died, but all lived that were subjected to 84 total days of ponding. Angelov et al. (1996) found that after one year of continuously ponded conditions, 100% of the swamp chestnut oak seedlings had died. After two years of intermittently ponded conditions, the survival rate for swamp chestnut oak was between 90-100% (Angelov et al., 1996). Furthermore, continuously ponded swamp chestnut oak seedlings had a significantly lower root and total biomass accumulation when compared to swamp chestnut oak seedlings subjected to the intermittently ponded and control moisture treatments in this study. Although intermittently ponded swamp chestnut oak seedlings had the greatest shoot, root, and total biomass accumulation, the differences were not significantly different from the swamp chestnut oak seedlings in the control moisture treatment. The differences in growth in height were not significantly different between the three hydroperiods used in this study. Anderson and Pezeshki (1999) found decreased height growth in swamp chestnut oak under intermittently ponded conditions but found no significant differences in the diameter growth. However, swamp chestnut oak did have significantly lower leaf mass, stem dry mass, and total mass in intermittently ponded conditions when compared to the control (Anderson and Pezeshki, 1999). Based on the findings of this experiment, we confirmed the assessment of Hook (1984) that swamp chestnut oak, one of the dominant species of the Non-riverine Wet Hardwood Forest, is weakly tolerant to ponded conditions.
The objective of this study was to determine whether hydrologic simulations can be used to correctly assess the hydrologic profile of wetland plant communities. Caldwell (2005) characterized the hydrologic regime of Non-riverine Swamp Forest, Bay Forest, and Pond Pine Woodland. Using similar durations and frequency of ponding, the accuracy of the hydrologic profiles was assessed by utilizing the dominant tree species of the respective plant community.

Caldwell (2005) determined that Non-riverine Swamp Forest could withstand the longest durations of ponding and total days of ponding per year over the 40-year simulation period of the four plant communities described. Bald cypress, the dominant tree species of the Non-riverine Swamp Forest plant community, was also found to be the most tolerant of continuously ponded conditions of the four tree species studied in this 49-week experiment. Bald cypress seedlings had 100% survival in the three hydroperiods studied, including the continuously ponded hydroperiod treatment that was intended to mimic the hydrologic regime of the most tolerant plant communities. Bald cypress seedlings subjected to 300 total days of ponding experienced the greatest growth in height and diameter, as well as accumulated the most total biomass when compared to the control moisture treatment. Sweet bay, one of the dominant trees of the Bay Forest community, had close to 100% survival in the continuously ponded treatment, but growth was not significantly different when compared to the other hydroperiods studied. Bay Forest was characterized as averaging 299 total days of ponding per year and 104 consecutive days of ponding. Due to the ability of sweet bay to withstand 300 total days of ponding in >95-day increments, Caldwell’s (2005) characterization of Bay Forest was
correct. The main difference between the dominant species between Bay Forest and Non-riverine Swamp Forest appears to be the utilization of increased available phosphorus in continuously ponded soils.

Pond Pine Woodland was characterized as experiencing 89 total days of ponding per year, on average, as well as <15 days of consecutive ponding. Pond pine seedlings subjected to 300 total days of ponding had a 75% death rate. However, pond pine seedlings subjected to 84 total days of ponding had a 100% survival rate. The biomass accumulation of pond pine subjected to 84 total days of ponding was similar to pond pine seedlings maintained close to field capacity. Therefore, the hydrologic characterization of the Pond Pine Woodland was correct and the dominant species of Pond Pine Woodland can withstand 84 total days of ponding in <15 days increments of consecutive ponding.

It is common for success rates of wetland restoration projects to be less than 100%. Utilizing a hydrologic model, such as DRAINMOD, to more fully understand the target wetland hydrology and the hydrologic regime of the target plant community, higher success rates for these projects could most likely be achieved.

ACKNOWLEDGEMENTS

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REFERENCES


Figure 1. Redox potential measured 20 cm below the soil surface for all seedlings averaged together. The line indicates the boundary between aerobic (>400 mV) and anaerobic (<400 mV) conditions.
Figure 2. Fluorescence ($F_v/F_m$) measured on the top leaves of four wetland tree species over the 49-week experiment. Fluorescence ratios greater than 0.78 represent unstressed, or healthy, plants.
Figure 3. Stomatal conductance measured in week 16 and week 29 for four wetland tree species. Standard errors bars are shown.
Figure 4. Effect of species and hydroperiod on change (final-initial) in height (cm) and diameter (mm). Mean values for bars followed by the same lower case letter are not significantly different at the p<0.05 significance level. Standard error bars are shown.
Figure 5. Effect of soil and species on change (final-initial) in height (cm) and diameter (mm). Mean values for bars followed by the same lower case letter are not significantly different at the p<0.05 significance level. Standard error bars are shown.
Figure 6. Effect of species and hydroperiod on shoot and root biomass (g) and root: shoot ratio (g/g). Mean values for bars followed by the same lower case letter are not significantly different at the p<0.05 significance level. Standard error bars are shown.
Figure 7. Effect of species and hydroperiod and species and soil type on total biomass (g) accumulation. Mean values for bars followed by the same lower case letter are not significantly different at the p<0.05 significance level. Standard error bars are shown.
Table 1. Significances for repeated measures of stomatal conductance and fluorescence. S=significant. NS=not significant. Significances reported at the $p<0.05$ level.

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<th>Pond pine</th>
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Table 2. Significances of change in height and diameter and biomass accumulation. Significances reported at the p<0.05 level. S=significant. NS=not significant.

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Chapter 4
Morphological responses of four wetland tree species to saturated and anaerobic soils.

ABSTRACT

Morphological adaptations aid in the survival of woody species that experience prolonged periods of saturation, and might be used to as hydrologic indicators by wetland delineators. The objective of this study was to determine the type and order of development of morphological adaptations in trees that were inundated for periods up to 110 consecutive days. Four wetland tree species were evaluated: bald cypress (T. distichum), sweet bay (M. virginiana), pond pine (P. serotina), and swamp chestnut oak (Q. michauxii). Tree seedlings were grown in a greenhouse in pots containing either mineral or organic soils obtained from the upper 30 cm of a drained Carolina Bay wetland that had been used for agriculture for 30 years. Three moisture treatments were evaluated during the 49-wk study: i) control (unsaturated), ii) alternately ponded for 14 d followed by drainage for 30 d, and iii) continuously ponded for ≤110 d consecutively. Bald cypress seedlings grown in continuously ponded conditions expressed the greatest degree of morphological acclimation by forming adventitious roots near the soil surface, development of air-filled cavities in the stem, and stem hypertrophy. Sweet bay seedlings also formed an extensive network of adventitious roots and hypertrophied lenticels to survive continuously ponded conditions. Hypertrophied lenticel development was the only morphological acclimation found in both pond pine and swamp chestnut oak seedlings. Morphological acclimations probably will not provide information on ponding duration beyond indicating that trees have experienced ponded conditions for periods
between 5 to 31 weeks. Soil factors will also confound the using of adaptations for hydrologic information on ponding duration.
INTRODUCTION

When soils become saturated, water displaces air from soil pores. Through the activity of microorganisms and plants, any remaining oxygen in the soil system is depleted and the soil becomes anaerobic. Anaerobic conditions affect flood-tolerant and flood-intolerant species differently. In flood-intolerant plant species, prolonged anaerobic conditions reduce growth and increase mortality, due to changes in metabolic functions (Kozlowski, 1984). Some plants, which are tolerant to saturated conditions are able to switch from aerobic to anaerobic pathways, although these metabolic pathways are less efficient (Pezeshki, 1994).

Morphological adaptations are another type of mechanism that are utilized by flood-tolerant woody species to adapt to anaerobic soils. These include the emergence of hypertrophied lenticels, adventitious root production, the formation of aerenchyma tissue, and stem hypertrophy (Hook, 1984). Lenticels are openings located on stems and roots that act as the major pathway through which gases, especially oxygen, travel to the living cells of the cambium. Oxygen enters herbaceous species through the leaf but it has been found that lenticels at the base of the stem are more important for oxygen entering woody plants (Coutts and Armstrong, 1976). Lenticels are connected through intercellular spaces in the cortex and phloem (Hook, 1984). Hypertrophied lenticels are enlarged lenticels that provide a greater surface area for gas exchange, especially the uptake of dissolved oxygen in flood waters, and enhance internal aeration. Gill (1970) used a vacuum on the submerged portion of a stem to show that air in the stem could be continuously pulled out of submerged lenticels if lenticels were present above the submerged portion to allow air into the stem. Hypertrophied lenticels also provide an
exit path for potentially toxic compounds (such as ethanol, acetaldehyde, and ethylene) in some species (Chirkova and Gutman, 1972). Kozlowski (1982) found that the enlargement of lenticels was due to ethylene formation in response to flooding. The time needed for the formation of hypertrophied lenticels, in the submerged portion of the stem, depends on the flood-sensitivity of the species being inundated with water.

Although many flood-sensitive species will lose part of the original root system, during submergence due to death, flood-tolerant species will respond by generating new roots, either on the original root system or on the submerged portions of the stem, or both. Adventitious roots grow during saturation and are thought to increase the absorption of water and minerals (Vartapetian and Jackson, 1997) but may also oxidize the rhizosphere and detoxify harmful compounds (Hook et al., 1970; Hook and Brown, 1973). Megonigal and Day (1992) determined that adventitious roots of bald cypress seedlings replaced the primary root system after years of saturation. Adventitious roots are often more succulent and permeable than roots of the original root system (Hook et al., 1970). Furthermore, they also tend to be larger in diameter and less branched than original roots (Megonigal and Day, 1992). Adventitious roots have been found to initiate from either beneath or within the cork cambium (phellogen) and then erupt through the center of the lenticel, in the cork cambium within a lenticel, or originate deep within the vascular cambium independent of lenticels (Hook et al., 1970). However, it is thought that adventitious roots typically form in conjunction with hypertrophied lenticels.

Some flood-tolerant woody species will increase the proportion of aerenchymatous (i.e. air-filled) tissue in the xylem and phloem of their stems in response to prolonged saturated conditions (Kozlowski, 1997). Aerenchyma tissues are a
specialized form of parenchyma tissue. Aerenchyma contain enlarged gas-filled cavities, or lacunae, that form through the differentiation and separation of cells (schizogenous) or through the death of some cells (lysigenous), leading to a less ordered cell pattern (Kawase, 1981). Aerenchyma provide the plant with an alternative strategy for obtaining oxygen. The interconnected, enlarged pore spaces provide the plant an extensive, low-resistance internal aeration system, which can transfer oxygen, via diffusion and convection, to anaerobic rhizospheres (Evans, 2003). Furthermore, toxic gases, such as carbon dioxide, ethylene, and methane, move through aerenchyma to the shoots and are then released to the atmosphere (Colmer, 2003). In flood-tolerant plants that produce these specialized intercellular spaces for greater aeration, stem diameters are sometimes increased in response to aerenchyma formation. The term for the increase in diameter is stem hypertrophy or stem buttressing. Stem hypertrophy has been found to occur in bald cypress, water tupelo, and black willow (McKevlin et al., 1998), all very flood-tolerant tree species.

Although much is known about the types of morphological adaptations that occur due to saturated conditions, very little is known about the specific hydrology and soil conditions that are needed to produce these adaptations in woody species. With present knowledge, field observation of morphological adaptations is not used for estimating the duration and frequency of inundations of the soil. A better understanding of the hydrologic and soil combinations required for morphological adaptations to occur could possibly be developed into a useful tool for field assessment of the hydrologic regime in several important wetland tree species. For example, if hypertrophied lenticels were found to develop within 2 weeks of ponding, while adventitious roots developed after 10
weeks, it would be possible to estimate relative durations of ponding in a wetland by looking for these adaptations on a particular species of tree.

The objectives of this study were to experimentally determine the effects of periodic and prolonged ponding on: 1) hypertrophied lenticel development, 2) adventitious root growth, 3) aerenchyma tissue formation, and 4) stem hypertrophy in four wetland tree species. The four wetland tree species used in this study are listed from the most flood-tolerant to the least flood-tolerant (Hook, 1984): bald cypress (*T. distichum*), sweet bay (*M. virginiana*), pond pine (*P. serotina*), and swamp chestnut oak (*Q. michauxii*).

**MATERIALS AND METHODS**

This study was conducted in a greenhouse using organic and mineral soils collected from Juniper Bay, a 256 ha Carolina bay, located in Robeson County, near Lumberton, NC (34°30'30"N 79°01’30”E). Juniper Bay was drained in 1972 and used for agricultural production until 2006 when it was restored back to a wetland. During the years in agriculture, the soils were fertilized and limed annually to soil test recommendations for the production of soybeans (*Glycine max*), oats (*Avena sativa*), cotton (*Gossypium hisutum*), corn (*Zea mays*), tobacco (*Nicotiana tabacum*), wheat (*Triticum aestivum*), and other vegetable crops (Ewing, 2003). Two sites were located to collect soil samples based on ease of access and soil type. Mineral soil was collected from the Ap horizon of a Leon sand (sandy, siliceous, thermic Aeric Haplaquod). Organic soil material was collected from the Oa horizon of a Ponzer muck (loamy, mixed, dysic, thermic Terric Haplosaprist). Small pits were dug by hand in each soil area to a depth of 60 cm. Undisturbed cores were collected in triplicate from the 15 to 25 cm
depth using metal cylinders (10 cm in diameter by 10 cm in height) for bulk density
determination. Soil materials were collected from the upper 25 cm of the wall of each
pit, placed in buckets and transported to the greenhouse. Soil materials were air-dried for
3 days and then passed through a 1.25 cm mesh sieve. Particle size distribution was
determined by the hydrometer method after passing the material through a 2-mm mesh
sieve (Gee and Bauder, 1986). In the laboratory, soil cores were weighed, oven-dried at
105°C for 24 hours and weighed again. Before potting, sub-samples of organic and
mineral soils were taken to determine gravimetric water content, which was used to
estimate the amount of soil needed to put in the containers to achieve the bulk densities
measured in the field.

Pond pine (*Pinus serotina*), bald cypress (*Taxodium distichum*), and swamp
chestnut oak (*Quercus michauxii*) seedlings were purchased from North Carolina
Division of Forest Resources, Goldsboro, North Carolina. Sweet bay (*Magnolia
virginiana*) seedlings were purchased from SuperTree Nursery, Blenheim, South
Carolina. Prior to planting, the seedlings were kept in a cold room at 7°C with adequate
moisture. Roots were rinsed with tap water before planting to remove any excess soil.

Thirty seedlings from each species were chosen for the study based on similar
heights and overall health. Fifteen seedlings were potted in each soil type using 7.65-liter
tree containers (Stuewe & Sons, Inc., Corvallis, OR, model TPOT3). Drain holes in the
pots were covered with fiberglass mesh to minimize the soil loss from the bottom of the
pots. The pots were filled with an appropriate amount of soil and the soil was packed to
the desired field bulk density. The final bulk density for containers filled with mineral
soil was 1.28 g/cm³ and 0.47 g/cm³ for organic soils. Tree seedlings were planted in
containers and acclimated to greenhouse conditions for 79 days, while being maintained under unsaturated conditions. Twenty-four of the original 30 potted seedlings from each species were then selected for the study based on similar heights and overall health.

Three hydroperiods were imposed that included: control (C), intermittently ponded (IP), and continuously ponded (CP). Control treatments were watered to maintain a soil water pressure potential of -300 cm which was considered to be near field (or container) capacity. The intermittently ponded treatment was saturated from the base upward and a water level maintained above the surface. After 14 days, the soil was drained and kept unsaturated for 42 days. This saturation and drainage cycle was repeated 6 times, totaling 84 days of ponding and 282 days of unsaturated conditions. The continuously ponded treatment was saturated and kept ponded for a total of 300 days and allowed to drain for 65 total days over the 49-week experiment. The continuously ponded treatment was initially inundated for 110 days and then 2 subsequent 95-day periods of saturation were utilized. When ponded, the water level was maintained 10-12 cm above the soil surface for the continuously ponded and intermittently ponded treatments, during their ponded phases. During the unponded cycles, soil moisture content was maintained close to field capacity. Tap water was used for all treatments. Experiments conformed to a split-plot design, containing four replications per treatment combination.

All containers were equipped with a tensiometer to determine the soil water pressure head for watering purposes in the control and intermittently ponded hydroperiod treatments. A 2.86-cm long and 0.95-cm wide porous ceramic cup (Soilmoisture Equip., Santa Barbara, CA, model 0652X07), with an air entry valve of 1 bar, was attached to a
15-cm long section of 1-cm diameter polycarbonate tubing with marine epoxy (ITW Philadelphia Resins, Montgomeryville, PA). A separate piece of 7.5-cm long and 1.25-cm diameter polycarbonate tubing was cemented to the other end of the 15-cm long polycarbonate tubing with marine epoxy. The tensiometers were inserted to a depth of 7.5 cm below the soil surface in all plant containers before the treatments were begun. The septum stoppers were periodically replaced to maintain pressure inside the tensiometer. The tensiometers were read using a tensimeter (Soil Measurement Systems, Tucson, AZ).

Each container was equipped with two redox probes: one placed at 10 cm below the soil surface and another probe placed at 20 cm below the soil surface. The redox probes were constructed and tested according to the techniques utilized by Wafer et al. (2004). A calomel reference electrode was used and all subsequent redox potential measurements were adjusted as necessary. The redox probes were gently inserted by hand into the soil of the containers. A small amount of soil was mixed with water to create soil slurry and any gaps around the redox probe were filled with the slurry. The redox probes were approximately 40 cm to 75 cm in length and placed approximately 13 cm apart, on either side of the tree seedling, in each container.

Redox measurements were conducted on a weekly basis using an auto-range digital multimeter (Radio Shack, Fort Worth, TX, model 22-163) and a calomel reference electrode (Thermo Fisher Scientific, Waltham, MA, model 13-620-259). Soil pH was conducted bi-monthly using a portable pH/mV meter (Thermo Fisher Scientific, Waltham, MA, model 13-636-62A) in order to correctly interpret the redox measurements. Height and diameter were measured on a monthly basis using a meter.
stick and caliper. At the end of the study, the total growth during the 49-week experiment was determined by subtracting the initial height and diameter from the final height and diameter. In addition, biomass was divided into shoot (stems and leaves) and roots. Stems and roots were washed, oven-dried for 48 hours at 70°C, and weighed to calculate shoot, root, and total biomass.

Statistical analyses were performed using SAS version 9.1 software (SAS Institute, Cary, NC, 2005). The PROC MIXED function was used to test for significance at the $p \leq 0.05$ level for measurements including: changes in height, diameter, biomass, and root/shoot ratio. The Tukey procedure was used to determine all pair-wise differences between treatment combinations. The data was transformed, as needed, to fulfill the ANOVA assumptions, especially the assumption of equal variances.

**RESULTS AND DISCUSSION**

Redox potential data are shown in Fig. 1. Redox potential, measured at 10 and 20 cm below the soil surface was approximately -100 mV for the first saturated period and increased to approximately 0 mV for the final saturated period of the continuously ponded treatment. The initial cycle of saturation for the intermittently ponded hydroperiod, averaged 125 mV, when measured 10 and 20 cm below the soil surface. After the first cycle of saturation, redox potential decreased for the next three subsequent cycles to -100 mV. However, the last cycle of saturation in the intermittently ponded treatment averaged 0 mV, when measured 10 and 20 cm below the soil surface. Anaerobic soils typically have redox potentials below +350 mV (Patrick and DeLaune, 1977). Therefore, both mineral and organic soils were anaerobic during their saturated cycles. During the unsaturated phases of the continuously ponded conditions, the soils
stayed anaerobic. An Eh range of +400 to +700 mV is characteristic of aerobic soils (Pezeshki, 1991). During unsaturated phases of the intermittently ponded conditions, the soils were aerobic with their redox potential averaging +425 mV. The control treatments, which maintained close to field capacity, were aerobic during the entire experiment with an average redox potential of +450 mV.

The development of morphological adaptations observed over time for each tree species in the two soils is shown in Table 1 for the continuously ponded treatment. Major adaptations observed for continuously ponded seedlings included: stem hypertrophy, adventitious roots, hypertrophied lenticels, and aerenchyma tissue in the basal portion of the stem. The formation of morphological adaptations was less prominent in seedlings subjected to intermittently ponded conditions. Hypertrophied lenticels were observed during the first saturated period for intermittently ponded sweet bay, pond pine, and swamp chestnut oak seedlings. Slight stem hypertrophy was observed for intermittently ponded bald cypress seedlings, especially intermittently ponded bald cypress seedlings grown in organic soils. Timing of the appearance of hypertrophied lenticels, adventitious roots, and stem hypertrophy differed between mineral and organic soils for continuously ponded and intermittently ponded bald cypress and sweet bay seedlings.

Bald cypress seedlings subjected to continuously ponded conditions developed stem hypertrophy, adventitious roots below the soil surface, and aerenchyma tissue in the basal portion of the stem (Table 1). In continuously ponded organic soils, stem hypertrophy was significant (Fig. 2) with a mean increase in diameter of 19.8 mm, compared to 11.2 mm for bald cypress seedlings in continuously ponded mineral soils.
The increase in stem diameter for intermittently ponded bald cypress seedlings was less pronounced. Intermittently ponded bald cypress seedlings grown in organic soils had an average increase in diameter of 9.3 mm which was slightly higher than the growth in diameter of intermittently ponded seedlings grown in mineral soils but not significantly different. The increase in stem diameter of flood-tolerant seedlings has been attributed to aerenchyma formation in the xylem and phloem of the stems (Kozlowski, 1997). In this study, the increases in air spaces seemed to be limited to the cortex of the stem, near the bark cambium (Fig. 3). The outer surface of the bark on the lower portion of continuously ponded bald cypress seedlings split due to stem hypertrophy.

After 15 weeks of inundation, adventitious roots were visible near the soil surface on bald cypress seedlings growing in continuously ponded conditions and organic soils. Some of these adventitious roots were protruding from the soil surface and growing vertically against the container. These succulent roots were approximately 1 mm in diameter and contained very few branches. Adventitious roots were minimal for continuously ponded bald cypress seedlings grown in mineral soils, but the roots that were visible were approximately 0.5 mm in diameter (Fig. 2). Overall, root growth was noticeably greater in bald cypress seedlings subjected to continuously ponded organic soils compared to continuously ponded mineral soils (Fig. 2). The survival rate for continuously ponded and intermittently ponded bald cypress seedlings was 100% in both mineral and organic soils.

Within 3 weeks of inundation, leaves from sweet bay seedlings began to senesce. The bottom-most leaves quickly turned yellow and fell from the stem. This occurred for intermittently ponded and continuously ponded hydroperiod treatments, which were both
initially inundated on the first day of the experiment. However, seven out of eight sweet bay seedlings quickly recovered after producing hypertrophied lenticels within 2 weeks of saturation. One sweet bay seedling, grown in organic soils and continuously ponded, did not recover and died within 13 weeks of saturation.

Within 5 weeks of inundation, continuously ponded sweet bay seedlings grown in organic soils began producing adventitious roots from the submerged, hypertrophied lenticels. It appeared that the adventitious roots erupted through the center of the hypertrophied lenticels (Fig. 4), as was proposed by Hook et al. (1970). Hypertrophied lenticels were also visible on the largest adventitious roots, as well. The adventitious roots extended directly from the stems and grew long enough to reach and penetrate the soil surface. The adventitious roots of continuously ponded sweet bay seedlings that extended from the topmost, submerged portion of the stem were thick, sparsely branched roots (Fig. 5). The adventitious root diameters ranged from 1 to 6 mm. The formation of adventitious roots in continuously ponded sweet bay seedlings, grown in organic soils, was followed by a significant amount of shoot growth from the main stem but also the emergence of new side stems. Due to the increased root growth from the stem, stem diameters were significantly increased compared to the control and intermittently ponded hydroperiod treatments, for continuously ponded seedlings grown in organic soils. Sweet bay seedlings grown in continuously ponded conditions grew 9.6 cm in diameter compared to 3.8 cm in intermittently ponded conditions and 2.2 cm in sweet bay seedlings grown in control moisture conditions.

Continuously ponded sweet bay seedlings grown in mineral soils experienced some hypertrophied lenticel development, but it was much less than found for
continuously ponded seedlings grown in organic soils. Furthermore, formation of hypertrophied lenticels took longer to develop in continuously ponded sweet bay seedlings grown in mineral soils compared to continuously ponded organic soils (Fig. 6). Within 21 weeks of saturation, continuously ponded sweet bay seedlings grown in mineral soils began to produce adventitious roots from the center of lenticels. However, the adventitious roots formed in mineral soils were unlike those formed in organic soils. Adventitious roots of mineral soils were produced on the basal portion of the submerged portion of the stem and were approximately 1 to 3 mm in diameter (Fig. 7). The roots were still very succulent and contained few branches from the main adventitious root. Overall, adventitious root development was greater, although total root biomass was not significantly different, in continuously ponded organic soils when compared to sweet bay seedlings grown in continuously ponded mineral soils (Fig. 8). The sweet bay seedlings of continuously ponded, mineral soils had less shoot growth than sweet bay seedlings of continuously ponded, organic soils. The growth in diameter was also much less, due to the limited amount of adventitious root formation. Sweet bay seedlings grown in intermittently ponded hyproperiod treatments produced hypertrophied lenticels but did not produce any adventitious roots from the lenticels. At the conclusion of the study, hypertrophied lenticel development was still visible in intermittently ponded sweet bay seedlings of both soil treatments.

Pond pine seedlings, grown under continuously ponded conditions, produced hypertrophied lenticels within 9 weeks of inundation (Fig. 9). These hypertrophied lenticels were large (2 mm diameter), white, globular lenticels that became enlarged following saturation. However, during the first period of unsaturated conditions, these
hypertrophied lenticels decreased in size. Hypertrophied lenticel development was minimal on continuously ponded pond pine seedlings after the first drained cycle. This may have played a part in the increased death of pond pine seedlings that were subjected to continuously ponded conditions. After 29 weeks of saturation, six out of eight pond pine seedlings had died in both mineral and organic soils, although continuously ponded seedlings grown in organic soils died first. Hypertrophied lenticels appeared desiccated on the two surviving continuously ponded pond pine seedlings, at the conclusion of the experiment (Fig. 10). There were very few hypertrophied lenticels on pond pine seedlings that were subjected to intermittently ponded conditions, at the conclusion of the experiment, as well.

Several, but not all, swamp chestnut oak seedlings that were under continuously ponded and intermittently ponded conditions produced hypertrophied lenticels on the submerged portion of the stem (Fig. 11). The development of hypertrophied lenticels of swamp chestnut oak seedlings was less than that of the hypertrophied lenticels of sweet bay and pond pine seedlings. The size and shape of hypertrophied lenticels of swamp chestnut oak seedlings were more uniform, as well. During periods of unsaturated conditions, most of these lenticels desiccated and were not produced again. However, at the conclusion of the experiment, the one remaining continuously ponded swamp chestnut oak seedlings had significant hypertrophied lenticel development (Fig. 12). This morphological adaptation may have caused a greater ability to survive in ponded conditions, compared to the other continuously ponded swamp chestnut oak seedlings, which had died within 14 weeks of the experiment.
Bald cypress (*T. distichum*) was the most flood-tolerant of the four species studied, and produced multiple morphological adaptations. One of those adaptations was the development of air-filled cavities, or aerenchyma, which led to stem hypertrophy of continuously ponded bald cypress seedlings (Figs. 2 and 3). Similar results were found by Shanklin and Kozlowski (1985) who showed that after 8 weeks of ponding, bald cypress trees had an 18% increase in stem diameters due to swelling of the lower stems. Megonigal and Day (1992) found that bald cypress seedlings produced the greatest amount of aerenchyma in the stems and roots of continuously ponded seedlings when compared to seedlings kept in a control moisture treatment. Another important morphological feature in continuously ponded bald cypress seedlings was the production of adventitious roots, especially in continuously ponded bald cypress seedlings grown in organic soils. Megonigal and Day (1992) found that the roots of continuously ponded bald cypress seedlings were negatively geotropic, or grew upwards toward the soil surface, producing adventitious roots directly below the soil surface. Pezeshki and Anderson (1997) found that adventitious roots formed on the submerged portion of the stem within 3 weeks of saturation and also observed the formation of hypertrophied lenticels on submerged portions of bald cypress stems after 4 weeks of ponding. Hypertrophied lenticels were not observed in this experiment. Furthermore, it is thought that bald cypress seedlings are capable of oxidizing their root rhizosphere through the translocation of oxygen (Hook, 1984), but this was not determined in this experiment. However, the formation of aerenchyma tissue in the stems of continuously ponded bald
cypress seedlings leads us to believe that the translocation of oxygen, from the shoot to the root, was occurring.

Sweet bay (*M. virginiana*) seedlings were also tolerant to saturated and anaerobic conditions due to the development of an extensive network of hypertrophied lenticels and adventitious roots, especially in organic soils (Figs. 4-7). Hypertrophied lenticels were observed within 5 weeks of saturation in continuously ponded sweet bay seedlings grown in organic soils but in mineral soils, 21 weeks of saturation were required to produce hypertrophied lenticels (Table 1). Likewise, continuously ponded sweet bay seedlings grown in organic soils had a significantly higher amount of shoot growth compared to the continuously ponded seedlings grown in mineral soils. The increase in shoot growth of the continuously ponded sweet bay seedlings grown in organic soils was likely related to the superior development of adventitious roots and hypertrophied lenticels, which most likely would allow an increase in the uptake of nutrients and dissolved oxygen and purging of toxic compounds such as ethylene and ethanol. Results from this study could not be compared other studies due to the lack of research conducted with sweet bay seedlings exposed to continuously ponded conditions.

Pond pine (*P. serotina*) is considered to be moderately tolerant to saturated and ponded conditions (Hook, 1984). It is thought that mature pond pine trees are able to tolerate prolonged soil saturation (Hook, 1984). This study showed, however, that young seedlings may not be as tolerant as older trees. Pond pine seedlings initially adapted to continuously ponded conditions by producing a significant amount of hypertrophied lenticels on the submerged portion of the stem (Fig. 9). Topa and McLeod (1986) also observed hypertrophied lenticel development on the stems and root collars of pond pine
seedlings, which accounted for the majority of oxygen entry for the roots of continuously ponded seedlings. However, once saturated conditions were suspended for four to six weeks in the current study, hypertrophied lenticels essentially dried up and returned in much smaller numbers when saturated again (Fig. 10). The inability to reproduce these lenticels may have had a negative impact on continuously ponded pond pine seedlings since six out of eight continuously ponded seedlings died.

Swamp chestnut oak seedlings are considered to be weakly tolerant to saturated conditions (Hook, 1984), and in this experiment, it was the species least tolerant to saturated and ponded conditions. The only morphological adaptation observed in swamp chestnut oak was production of a few hypertrophied lenticels on the submerged portion of the stem. These hypertrophied lenticels were small and sparse when compared to the other two species that produced hypertrophied lenticels. The only continuously ponded swamp chestnut oak to survive saturated conditions for an extended amount of time had developed an extensive network of hypertrophied lenticels (Fig. 12). Anderson and Pezeshki (2001) observed the development of hypertrophied lenticels in 83% of continuously ponded swamp chestnut oak seedlings after 10 weeks of ponding. Anderson and Pezeshki (2001) further found the formation of adventitious roots in 67% of continuously ponded swamp chestnut oak seedlings, which was not observed in this experiment. The appearance of sparse hypertrophied lenticels was the only morphological adaptation produced by this species in this 49-week study.

In some species studied, there was a difference in the appearance of specific morphological adaptations between seedlings grown in mineral and organic soils (Table 1). Bald cypress seedlings grown in continuously ponded organic soils showed the
formation of adventitious roots and stem hypertrophy within 17 weeks of the start of the experiment. However, the appearance of the same features took an additional 12 weeks for continuously ponded bald cypress seedlings grown in mineral soils. Likewise, adventitious roots and hypertrophied lenticels appeared within 5 weeks for sweet bay seedlings grown in continuously ponded organic soils but appeared later in the study for sweet bay seedlings grown in continuously ponded mineral soils. It was first hypothesized that differences in redox potential between the two soils could have caused the differences in timing of the formation of morphological adaptations. However, there were no differences in redox potentials measured between organic and mineral soils (Fig. 1).

Plants that undergo stressful environmental conditions, such as soil anaerobiosis, increase the production of 1-aminocyclopropane-1-carboxylic acid (ACC), which is the precursor of ethylene, in plant tissues (Grichko and Glick, 2001). Ethylene can also be produced by anaerobic or facultative anaerobic microorganisms (Goodlass and Smith, 1978). Ethylene becomes trapped and continues to accumulate in plant tissues and in the soil, due to slow diffusion rates of the gas into water. It has been determined that elevated levels of ethylene cause distinct responses in the tissues experiencing the stress directly. The production of adventitious roots has been associated with accumulations of ethylene, as well as, the formation of aerenchyma in the roots and shoots in flood-tolerant species (Jackson, 1990). It is further thought that the accumulation of ethylene in flood-sensitive species may damage original roots and inhibit the growth of new roots. Smith and Restall (1971) determined the concentrations of several hydrocarbons for six soils under anaerobic conditions for 10 days. An organic soil used in their study, which
contained 38% organic matter (OM), accumulated 24 µg ethylene/kg soil, which was 56 times the amount of ethylene accumulated by a sandy soil with 1.4% OM. It was concluded that total evolution of ethylene was correlated with OM content of the soils - the higher the OM content, the higher the ethylene concentrations. Soils used in the current study are similar to the mineral and organic soils used in Smith and Restall (1971). It is possible that higher amounts of ethylene were produced and trapped in the organic soils used in this study when compared to the mineral soils. The flood-tolerant species, like bald cypress and sweet bay, responded to ponding by producing adventitious roots, hypertrophied lenticels, and aerenchyma tissue that may have allowed venting of the harmful gas away from plant tissues. The less flood-tolerant species, like pond pine and swamp chestnut oak, were slower in their response to the build-up of ethylene and therefore, pond pine and swamp chestnut oak seedlings grown in continuously ponded organic soils died before seedlings grown in continuously ponded mineral soils (Table 1).

The differences in responses observed within and between tree species grown in saturated organic and mineral soils may possibly be due to differences in microbial community structure. Priha et al. (1999) studied the microbial community structure in the rhizospheres of 3 tree species in mineral and organic soils, which had similar OM% and pH’s as the soils used in this study. It was concluded that in organic soils, the various tree species had a significant influence on soil microbes and microbial community structure. However, this was not the case in mineral soils. It was hypothesized that organic soils may have had a greater initial microbial diversity compared to mineral soils. Differences in exudates between tree species may affect the microbial communities differently in organic soils.
Results from this study show that the more morphological and anatomical adaptations that are utilized by a species, the greater the likelihood that the species will survive extended periods of ponding. Bald cypress seedlings adapted the best, through a variety of morphological adaptations, and therefore had 100% survival rate. Sweet bay seedlings produced an extensive network of adventitious roots and were therefore, able to survive 300 total days of ponding. The only adaptation of pond pine and swamp chestnut oak seedlings were hypertrophied lenticels on the submerged portion of the stem and therefore, had a 75% and 87% death rate, respectively. The use of morphological adaptations as indicators of ponding duration will be difficult and require additional research. This short-term greenhouse experiment indicated that the timing of the appearance of the adaptations varies among species, as well as between organic and mineral soils.

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REFERENCES


Figure 1. Redox potential (Eh) for organic (left) and mineral (right) soils for three hydroperiod treatments. Black line indicates the boundary between aerobic (>300 mV) and anaerobic (<300 mV) conditions.
Figure 2. Differences in stem hypertrophy (arrows) and root growth between bald cypress grown in continuously ponded organic (CP-O) and continuously ponded mineral soils (CP-M). Stems of bald cypress seedlings subjected to continuously ponded conditions and grown in organic soils had the greatest growth in stem diameter. Surficial and overall root growth was greatest for bald cypress seedlings in continuously ponded organic soils.
Figure 3. Scanning electron micrograph (SEM) of aerenchyma development in the basal portion of a bald cypress stem after 15 weeks of flooding.
Figure 4. Initial formation of adventitious roots (arrow) on sweet bay seedlings after four weeks of inundation in organic soils. Adventitious roots erupted through the center of hypertrophied lenticels.
Figure 5. Adventitious roots (ar), hypertrophied lenticels (hl), and stem hypertrophy (sh) of sweet bay seedlings grown in organic soils and saturated for 42 weeks. Stem diameter was greatest in sweet bay seedlings that were grown in organic soils and continuously ponded due to the prolific growth of adventitious roots. Adventitious roots of sweet bay seedlings grown in organic, continuously ponded soils occurred on the submerged portion of the stem. Hypertrophied lenticels occurred on the submerged portion of the stem and also on the largest adventitious roots.
Figure 6. Hypertrophied lenticels on sweet bay seedlings after nine weeks of saturation in mineral soils. Hypertrophied lenticels occurred on the submerged portion of the stem and were very large, white, and bulbous.
Figure 7. Adventitious roots and hypertrophied lenticel formation in sweet bays of mineral soils after 42 weeks of saturation. Adventitious roots occurred near the soil surface, at the basal portion of the stem. Hypertrophied lenticels occurred on the submerged portion of the stem, as well as on the largest adventitious roots.
Figure 8. Differences in root formation of sweet bay seedlings subjected to continuously ponded conditions in mineral (CP-M) and continuously ponded organic soils (CP-O). Adventitious roots occurred at the top of the submerged portion of the stem in continuously ponded organic soils. Adventitious roots occurred at the basal portion of the submerged stem in continuously ponded mineral soils. Overall root growth was significantly greater in continuously ponded organic soils.
Figure 9. Hypertrophied lenticel development in pond pine seedlings in continuously ponded conditions after nine weeks of saturation. Hypertrophied lenticels were apparent during the initial ponding cycle but disappeared after drainage.
Figure 10. Dessicated hypertrophied lenticels on pond pine seedlings subjected to continuously ponded mineral soils after three cycles of saturation and drainage. Hypertrophied lenticels dried up after the first cycle of saturation and drainage. Half of the pond pine seedlings subjected to continuously ponded mineral soils died. All of the pond pine seedlings subjected to continuously ponded organic soils died within 20 weeks of saturation.
Figure 11. Hypertrophied lenticel development on swamp chestnut oak seedlings in continuously ponded organic soils after 15 weeks of saturation. Hypertrophied lenticels were small and sparse in swamp chestnut oak seedlings when compared to sweet bay and pond pine seedlings. Hypertrophied lenticels of swamp chestnut oak seedlings disappeared after the first draining cycle in most swamp chestnut oak seedlings.
Figure 12. Hypertrophied lenticel development after 42 weeks of saturation in the sole surviving swamp chestnut oak in continuously ponded conditions.
Table 1. Timeline for morphological and anatomical adaptations for continuously ponded seedlings.

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<tr>
<th>Ponding Duration (weeks)</th>
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- Bald Cypress: Leaf Senescence
- Sweetbay: Hypertrophied Lenticels
- Pond Pine: Aerenchyma in stems and roots
- Swamp Chestnut Oak: 100% Death, 50% Death