Effects of Short Interval Cyclic Flooding on Growth and Physiology of Selected Native Shrubs

by

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A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
May 14, 2010

Keywords: rain garden, pre-conditioning, flood tolerance, landscape

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Abstract

Clethra alnifolia L. 'Ruby Spice' ('Ruby Spice' summersweet), *Ilex glabra* 'Shamrock' (L.) A. Gray. ('Shamrock' inkberry holly), *Itea virginica* L. 'Henry's Garnet' ('Henry's Garnet' sweetspire), and *Viburnum nudum* L. 'Winterthur' ('Winterthur' possumhaw) were flooded in 2.5 L (trade gal) pots in a greenhouse. Plants were flooded for 0 (non-flooded), 3, or 7 days and during draining plants received no irrigation. All taxa except *C. alnifolia* 'Ruby Spice' seemed tolerant of flooding in spite of growth differences. Flooded plants for all taxa except *V. nudum* 'Winterthur' showed decreased root dry weight (RDW), shoot dry weight (SDW), and final growth index (GI) when compared to non-flooded plants. All taxa except *C. alnifolia* 'Ruby Spice' were potted in trade gal pots and were overwintered. Plants were moved to a greenhouse and subjected to 0, 3, or 6 days of flooding and to impose drought, during draining plants received no irrigation. During greenhouse flooding, flooded substrates with decreased root growth dried down more slowly during draining. *I. virginica* 'Henry's Garnet' had the largest relative growth index (RGI) followed by *V. nudum* 'Winterthur' and *I. glabra* 'Shamrock'. *I. virginica* 'Henry's Garnet' and *I. glabra* 'Shamrock' had the largest differences among treatments for final GI, RDW, and SDW, but *V. nudum* 'Winterthur' had no differences among treatments. Plants from greenhouse flooding were planted in 170.6 L (45 gal) tubs. Each tub contained 3 plants (same taxon) with one each that had been flooded for 0, 3, or 6 days during greenhouse flooding. Tubs were randomly assigned a flooding treatment and were flooded for 0, 3, or 6 days. Treatments were in a factorial of flooding treatments from greenhouse flooding x flooding treatments from outdoor flooding. Results for RGI were similar for greenhouse flooding and outdoor flooding. *I. virginica* 'Henry's Garnet' seemed to recover during outdoor flooding which was evident by smaller treatment differences that occurred during outdoor flooding. *V. nudum* 'Winterthur' appeared more affected by outdoor flooding than by greenhouse flooding, which was evident by increased treatment differences that occurred during outdoor flooding. RGI of *I. glabra* 'Shamrock' was not different among treatment combinations and did not appear to be affected by flooding treatments applied in the greenhouse or outdoors. However, RDW, SDW, and root to shoot ratio (RDW : SDW) of *I. glabra* 'Shamrock' appeared more affected by flooding treatments applied in the greenhouse than by flooding treatments applied outdoors. RGI was lowest in *I. glabra* 'Shamrock' which was likely due to the slow rate of growth associated with *I. glabra*. Photosynthesis (Ps) and stomatal conductance (SC) rates were highest in *I. glabra* 'Shamrock' followed by *V. nudum* 'Winterthur' and *I. virginica* 'Henry's Garnet'. *V. nudum* 'Winterthur' maintained intermediate rates of growth, Ps, and SC making flooding treatment differences from greenhouse flooding and outdoor flooding easier to evaluate. Results indicate that *I. virginica* 'Henry's Garnet' may become less sensitive to flooding with maturity, but that *V. nudum* 'Winterthur' may become more sensitive to flooding with maturity, and *I. glabra* 'Shamrock' seems to be tolerant of flooding at any stage of growth.

Acknowledgments

The author would first like to thank Dr. Amy Noelle Wright for believing in her from the beginning and convincing her to apply for graduate school. Dr. Amy Noelle Wright was and is the person whom the author gained the majority of her educational insight. Her friendship over the last two years and the years to come will always be special to the author. The author would also like to thank Dr. Ken Tilt and Charlene Lebleu for their direction and contributions to her research. To all graduate students who helped the author even when they didn't have time or didn't want to, the author would like to express her most sincere gratitude. Special thanks to Bryan Wilkens and Greg Creech, whose help will not soon be forgotten. Most of all, the author would like to express her deepest thanks to her family for their constant support and love. Their confidence in her and what she had the ability to accomplish far exceeded her own ideas. Last but certainly not least, the author would like to thank Brian Dylewski for his continuous support and unyielding love. Without him, the author would not have been able to do this, and to him she offers her most genuine thanks.

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Literature Review

Introduction

Water conservation practices have become a focal point for many communities due to drought conditions and water restrictions. Changing climates due to global warming have increased circumstances of drought, while increasing the likelihood of strong storms, subsequent flooding (Dunnet and Clayden, 2007; Bailey-Serres and Voesenek, 2008), and rising sea levels (Kozlowski, 1997). Groundwater recharge has decreased with urbanization (Dietz and Clausen, 2005) resulting in increased areas of compacted soil and expanses of impervious surfaces (Dunnet and Clayden, 2007; Shuster et al., 2007; Dietz and Clausen, 2008). Moreover, urbanization has led to additional pumping of groundwater supplies resulting in a need for stormwater management practices that focus on maximizing groundwater recharge (Dussaillant et al., 2005).

Environmental impacts have already directly affected the landscape industry in regard to the design and construction of landscapes (Cabrera, 2005). Non-point source pollution has become a critical issue for both homeowners and agricultural entities regarding ground water supplies and water quality (Dietz and Clausen, 2008) with over half of the U.S. population depending on underground sources for potable water (Cabrera, 2005). Furthermore, concerns have been raised on the use of potable water for irrigation practices. Non-porous surfaces such as concrete increase storm water runoff taking fertilizers, chemicals, and fecal matter with it resulting in negative environmental consequences (Dunnet and Clayton, 2007). Stormwater runoff containing excess nitrogen (Dietz and Clausen, 2005) is routed into water ways causing algal blooms, fish kills (Cabrera, 2005; Dietz and Clausen, 2006), and decreased water quality (Dietz and Clausen, 2008). Nitrate is the main form of N found in U.S. streams due to it being highly soluble and easily leached from soils (Craig et al., 2008). The EPA reported in 2001 that at least half of the U.S. waters cannot sustain aquatic life due to the presence of excess nutrients (Cabrera, 2005). The maximum contaminant level (MCL) for nitrate in public drinking water is set for 1 mg·L and is regulated by the federal government (Weyer, 2001). Nitrates alone are not considered harmful to humans, but once consumed, bacteria in the human body convert nitrates to nitrite, which can negatively impact human health. Problems such as methemoglobinemia (blue baby syndrome) and possible cancer risks are associated with increased levels of nitrates in humans (Cabrera, 2005; Weyer, 2001). Rain gardens and constructed wetlands are considered a best management practice in the collection of storm water, allowing it to seep into the ground while filtering out harmful pollutants (Shuster et al., 2007; U.S. EPA, 1983; Prince George's County, 1993; Dietz and Clausen, 2006).

Rain Gardens

Rain gardens are attractive additions to landscapes that serve as an appropriate application for stormwater management and infiltration. A rain garden is a shallow depression that collects stormwater from a roof, parking lot, or any other impervious surface (Dussaillant et. al, 2005). Plant roots support a biologically active rhizosphere by maintaining soil porosity. Rain gardens are self sustaining environments that only receive irrigation during a rain event and usually contain plants that are indicative of low lying areas (Dunnet and Clayden, 2007). Rain gardens depend on seasonal precipitation for the source of their irrigation and therefore, may remain dry for a period of weeks until the next rain event. Furthermore, plant evapotranspiration between rain events provides increased soil water storage capacity for the next stormwater event (Dussaillant et. al, 2005). During precipitation events, rain gardens may flood and remain saturated above the substrate level until water permeates the ground. Plants that are adapted to moist conditions are more likely to withstand periodic dry conditions (Dunnet and Clayden, 2007). Native plants adapted to low wetland areas are desirable for rain gardens because they are low maintenance, not invasive, relatively pest free (N.C. Coop. Ext., 2009), and are usually able to persist during periods of low rainfall or drought (Dunnet and Clayden, 2007).

There is a lack of research to determine specific plants for rain gardens. A variety of plant lists are available, but they are based on observation rather than rigorous scientific evaluation (Cabrera, 2005; U.S. Dept. Agr., 2008; Ranney et al., 1998; U.S. Fish and Wildlife Service, 1996). Such lists include but are not limited to: Qualifiers for Quagmires: Landscape Plants for Wet Sites (Ranney et al., 1998), National list of vascular plant species that occur in wetlands (U.S. Fish and Wildlife Service, 1996), Plants for Rain Gardens (Glen, 2009), Wetland Indicator Status List (USDA, 2008), and Shrubs for Rain Gardens (Dunnet and Clayton, 2007).

Dussaillant et al. (2005) developed a model to evaluate rain garden dimensions with respect to the amount of groundwater recharge and duration of soil saturation plant roots would experience. Simulations were made for three cities based on their climate and precipitation data. Results showed that soil would likely remain saturated for 1-2 days and plant roots would need to be able to withstand at least 2 days of standing water in the root zone for optimal recharge to occur. The addition of a subsurface drain would eliminate extended soil anaerobisis in the root zone but would likely decrease recharge, so plants for rain gardens need to be able to endure at least 2 days of flooding in order to maximize groundwater recharge. However, approximate length of flooding in a rain garden would depend on soil type, rain garden size and depth, and precipitation received (Hunt, 2001).

Research concerning rain gardens and constructed wetlands is specifically geared toward the removal of nitrates, phosphates, and pesticides (Headly et al., 2001; Lu et al., 2006; Stearman et al., 2003; Runes et al., 2003). Dietz and Clausen (2006) found that concentrations of nitrate, nitrite, ammonia, and total-N from roof runoff were reduced in a rain garden. The removal of N relies heavily on the microbial process of denitrification, or the reduction of nitrate to gaseous forms; denitrification usually involves the oxidation of organic matter under anoxic conditions (Craig et al., 2008). Dietz and Clausen (2006) also found that phosphorous (P) concentrations leaving a rain garden increased which was thought to have been due to high P content of a native soil used. Hunt et al. (2006) suggested that bioretention areas designed to remove P should utilize soils that have a low P-index to increase P adsorption and minimize total P leaving a system.

Flooding

Flooding elicits environmental responses such as waterlogging, soil oxygen deficiency, and even anoxia resulting in the accumulation of toxic compounds (Vartapetian, 2006). Excess water in the root zone prevents transfer of oxygen and other gases between the soil and atmosphere (Drew, 1997). Soil waterlogging fills soil pores with water, displacing soil oxygen, resulting in oxygen deficiency and anaerobic conditions due to the slow diffusion of oxygen in water (Kramer and Boyer, 1995). Oxygen deficiency occurs in flooded soils due to the diffusion of oxygen being 10^4 fold slower in water than in air (Jackson and Colmer 2005, Mitsch and Gosselink, 2007). The rate at which soils become oxygen deficient is dependent on temperature and the presence of organic substrates for microbial consumption (Mitsch and Gosselink, 2007).

Oxygen deficiency is elevated by the consumption of oxygen by microorganisms and plant roots (Visser et al. 2003). Under elevated temperatures, microbial respiration can deplete oxygen in soil in less than 24 hrs (Drew, 1997).

Reduced Soil

As anaerobic soil conditions persist, chemical compounds present are transformed into reduced forms making elements more or less available to plants and in some cases toxic. Under anoxic conditions, elements such as nitrogen, manganese, iron, and carbon, become reduced ions (Blom and Voesenek, 1996) and are more soluble under these conditions (Mitsch and Gosselink, 2007). Under waterlogged conditions, Mn and Fe are reduced and may become toxic to flood sensitive plants (Huang, 2000). Flood tolerant species avoid uptake of toxic compounds with well developed aerenchyma root tissue which aerates the rhizosphere and oxidizes toxic forms of Mn and Fe rendering those insoluble (Nilsen and Orcutt, 1996). Soil anaerobisis inhibits nitrification and other oxygen requiring microbial processes as well as provides a favorable environment for toxic by-products resulting from anaerobic metabolism of plants or bacteria (Blom and Voesenek, 1996). Plants adapted to wetland conditions are usually able to withstand periods of little or no soil oxygen, but may not be able to endure severe soil reducing conditions (Pezeshki, 2001). Moreover, as reduction of soil components increases, oxygen diffusion from roots into the rhizosphere of flooded plants increases.

Soil waterlogging may cause both nutrient accumulation and limited nutrient availability. Under flooded conditions, soil nitrate is less abundant, and soil N exists in the form of ammonium (NH_4^+) (Nilson and Orcutt, 1996; Huang, 2000). NH_4^+ ions can be immobilized and bound to negatively charged soil particles where they could potentially build up to high levels were it not for the uppermost oxidized soil layer occurring in most flooded soils (Mitsch and

Gosselink, 2007). Phosphate generally becomes more available during soil flooding (Mengel and Kirkby, 1987). Usually soil pH of waterlogged soils increases; however, in calcareous or sodic soils pH decreases under flooded conditions.

Adaptations to Flooding

As oxygen is depleted in the root zone, plants respond and adapt to flooding stress morphologically with the formation of adventitious roots, aerenchyma tissue, stem hypertrophy, and lenticels (Mitsch and Gosselink 2007). Aerenchyma tissue forms when air spaces develop in the roots and shoots allowing for diffusion of oxygen from above ground portions of plants to the roots (Mitsch and Gosselink 2007, Vartapetian, 2006). Roots possessing aerenychma tissue are able to penetrate lower anaerobic soil layers (Nilsen and Orcutt, 1996). Diffused oxygen in the root zone aids in the detoxification of soil nutrients by oxidizing reduced chemical compounds (Pezeshki, 2001). The aerenchyma tissue itself acts as a pathway for the removal of unstable compounds such as methane and ethylene (Serres and Voesenek, 2008) from the root zone (Vartapetian, 2006). Adventitious roots may develop at the shoot base in aerated soil layers (Blom and Voesenek, 1996) as a result of flooding and signal severe damage to a plant's original root system (Robbani et al., 2006; Mitsch and Gosselink, 2007). Furthermore, plants having shallow root systems are less likely to experience severe anoxic conditions associated with lower soil layers due to root growth occurring in the oxidized rhizosphere (Blom and Voesenek, 1996; Nilson and Orcutt, 1996). Surface rooting is common in flood tolerant plants and allows roots to function in a hypoxic state where they can undergo partial aerobic respiration (Nilsen and Orcutt, 1996). Under completely submerged situations or when roots must carry out anaerobic respiration, the risk for carbohydrate starvation is great because of the inefficiency associated with anaerobic respiration. *Pyrus betulaefolia* Bunge. (birch-leaved pear) formed callus tissue at the trunk and produced adventitious roots in response to 3 months of flooding (Robbani et al., 2006), while *Alnus japonica* (Thunb.) Steud. (Japanese alder) developed adventitious roots on submerged stems following 3 weeks of flooding (Iwanaga and Yamamoto, 2008). Flooded *Mangifera indica* L. (mango) trees developed hypertrophied stem lenticels and when these were covered, plants died within three days (Larson et al., 1993).

Ethylene Production

Ethylene production is promoted under anaerobic conditions associated with flooding. Flooding stress induces ethylene production causing leaf epinasty, senescence, aerenchyma tissue development, and the formation of adventitious roots (Bradford and Yang, 1981). Moreover, ethylene is less soluble in water and is therefore, retained in plants under flooded conditions (Nilsen and Orcutt, 1996). Several experiments using *Zea mays* L. (maize) roots indicate that in the absence of ethylene synthesis or functioning, aerenchyma formation stopped (Vartapetian, 2006; Brailsford et al., 1993; He et al., 1996). Decreased soil oxygen due to flooding promotes an increase in 1-aminocyclopropane- 1-carboxylic acid (ACC), the precursor to ethylene, and an increase in the enzymes required for ACC synthesis in the conversion to ethylene (Bradford and Yang, 1981; Evans, 2003; Kozlowski and Pallardy, 2002; Kramer and Boyer, 1995). Bakhtenko et al. (2007) found that ethylene was biosynthesized in *Avena sativa* L. (oat) and *Triticum aestivum* L. (wheat) after 24 h of flooding; ethylene biosynthesis was higher in flooded plants compared to control plants in both cereal crops. Ethylene has been found to advance synthesis of abscisic acid (ABA); however, high accumulations of ABA can hinder ACC synthase and ACC oxidase thereby decreasing ethylene synthesis. Bakhtenko et al. (2007) showed that the rate of ethylene biosynthesis decreased when high ABA levels were reached by the second day of flooding.

Reduced Shoot Elongation

Plants respond physiologically to flooding stress by leaf abscission and a reduction in shoot growth. Larson et al. (1993) reported that flooded *Mangifera indica* L. mango trees showed a reduction in root growth and reduced shoot : root ratios compared to control plants. Similarly, shoot elongation rates of *Vitis vinifera* L. (sultana grape vine) were reduced by 40% when plants were flooded for 5 days; shoot dry weights decreased linearly as flood periods increased (Stevens and Prior, 1994). Robbani et al. (2006) found that grafted scions of *Pyrus pyrifolia* (Burm.) Nak. 'Kosui' showed reduced shoot elongation and increased defoliation in response to increased flooding stress on rootstocks of *P. betulaefolia* and *Pyrus calleryana* Decne. (bradford pear). Moreover, flooded *Betula papyrifera* Marsh. (paper birch) and *Prunus virginiana* L. 'Canada Red' ('Canada Red' chokecherry) were severely defoliated after 44 and 57 days of flooding, but stem tissue under the bark was still green indicating the presence of living tissue (Ranney and Bir, 1994; Ranney, 1994). Plants termed flood-tolerant however, may show increases in shoot elongation which has been observed in semi-aquatic plants (Blom and Voesenek, 1996).

Net Photosynthesis and Stomatal Conductance

Reduced stomatal activity and net photosynthesis has also been noted in flooded plants. Flood tolerant species however, may not exhibit a reduction in photosynthetic activity, and some researchers associate flooding tolerance with maintaining adequate rates of photosynthesis (Arbona et al., 2009). Moreover, carbohydrate metabolism via photosynthesis and respiration is essential in the tolerance of anaerobic conditions (Huang, 2000). Reduction in net photosynthesis may be due to limited stomatal aperture during anoxic conditions (Li et al., 2007). Stomatal closure is a response of plants to depleted soil oxygen in the root zone; flooding cripples leaf gas exchange and impacts transport of soil water and solutes (Sojka, 1992). *Quercus nuttallii* Palmer. (nuttall oak) exhibited reduced stomatal conductance and net photosynthesis during flooding, but increased to non-flooded plant levels during draining and remained high through subsequent flood cycles indicating its ability to resume photosynthetic activity when flooding ceased (Anderson and Pezeshki, 1999). Flood tolerant species acclimate to anaerobic soil conditions and recover net photosynthesis levels during draining more quickly compared to non-flood tolerant species (Anella and Whitlow, 2000). However, Stevens and Prior (1994) reported that flooded *Vitis vinifera* showed reduced stomatal conductance and net photosynthesis during both flooding and draining periods as a result of speculated physiological or anatomical damage. Stomata of flood tolerant species reopen following 1-2 weeks of flooding; however, *Mangifera indica* trees flooded for 2 weeks showed a reduction in stomatal conductance and took 2 months for rates to recover to pre-flood levels (Larson et al., 1993).

Transpiration

Under flooded conditions transpiration is reduced due to closure of stomata and higher levels of atmospheric humidity associated with flooding (Nilsen and Orcutt, 1996). When transpiration is decreased, water absorption by roots is also decreased since plant water loss is decreased making plant water balance easier to maintain. Yordanova et al. (2005) observed reduced stomatal activity and reduced rates of transpiration in *Hordeum vulagare* L. (barley) after 2- 24 hours of flooding. Glaz et al. (2004) studied the effects of periodic flooding on *Saccharum officinarum* L. (sugarcane); *S. officinarum* completed 4 cycles of 21 day cycles consisting of 7 days of flooding and 14 days of draining. Transpiration rates of *S. officinarum* decreased during the first flood cycle, but were maintained at an optimum level for all subsequent flood cycles. Authors suggested that formation of stalk aerenchyma on flooded plants explained why a decline in transpiration was not experienced during flooding.

Abscisic Acid

Abscisic acid (ABA) is considered to be responsible for decreased stomatal aperture during flooding. ABA concentrations in leaves accumulate due to the inability of ABA to be translocated from the leaves in response to root zone flooding (Lui and Dickmann, 1992). In a study using *Populus tristis* Fisch. x *P. balsamifera* L. 'Tristis No. 1' (Tristis genotype) and *Populus* x *euramericana* (Dode) Guinier. 'Eugenei' (Eugenei genotype) Lui and Dickmann (1992) found that after 5 days of flooding, ABA concentrations increased 6-fold in Tristis genotype, while ABA increased 10-fold gradually *over* 10 days of flooding for Eugenei genotype. However, escalations in ABA were not significant during the entire period of flooding and it was reported that ABA accumulation did not influence the noted decrease in photosynthesis and stomatal conductance.

Methodologies

Research pertaining to flooding is available, but the majority deals with longterm flooding of agronomic crops and wetland plants, not landscape horticulture crops. Flooding tolerance is based on plant age, survival, and on the depth and duration of flooding (Kozlowski, 1994). Abbot and Gough (1987) flooded highbush blueberries continuously for 4 months and evaluated the effects of flooding on fruit yield and quality. When compared to non-flooded *Vaccinium corymbosum* L. 'Bluecrop' ('Bluecrop' blueberries), flooded *V. corymbosum* 'Bluecrop' blueberries showed a 45% decrease in fruit yield, exhibited delayed anthesis, and had reduced fruit weight and size. In a flooding tolerance experiment, rootstocks of *Betula papyrifera, Betula pedula* Roth. (European birch), *Betula nigra* L. (river birch). and *Betula* *platyphylla* var. *japonica* ‘Whitespire’ (‘Whitespire’ Japanese birch) were flooded for 44 days and the water level was maintained at 3.5 cm above the root crown in containers (Ranney and Bir, 1994). It was determined that *B. nigra* rootstock was the most flood tolerant having higher rates of photosynthesis and stomatal conductance compared to other birch rootstocks. Ranney (1994) incrementally flooded 10 *Prunus spp.* (cherry, plum, and apricot) for 57 days to assess flooding tolerance; containers were submerged in buckets of water in two week stages until the entire root zone was completely submerged. This research showed that ((*P. salicina* Lindl. x (*P. americana* Marsh. x *P. nigra* Ait.)) x *P. cerasifera* J.F. Ehrh.) ‘Newport’ (‘Newport’ plum) had the highest survival rate with the least amount of defoliation and was the most tolerant of root zone flooding. In other research, clones of *Pyrus calleryana* and *Pyrus betulaefolia* rootstocks were flooded for 3 months with the water level maintained at 5 cm above the soil surface to determine flooding tolerance (Robbani et al., 2006). *P. calleryana* rootstock was considered more tolerant than *P. betulaefolia* despite varied responses within each species.

Short term flooding research is minimal and deals for the most part with fruit. Bradford and Hsiao (1982) flooded *Lycopersicon esculentum* Mill. (tomato) for 24 hrs by placing pots inside of larger containers with the water level maintained at the cotyledonary node. Results indicated that stomatal conductance and transpiration rates of tomato plants were decreased by 30-40% following 24 hours of flooding. Ortuno et al. (2007) flooded *Citris limon* (L.) Burm. ‘Verna’ (‘Verna’ lemon) trees for 3 days similarly by placing containers in larger containers and maintaining water level at 3-4 cm above the soil surface; recovery was studied over the next 15 day period. When compared to non-flooded *C. limon*, flooded *C. limon* had soil O_2 levels that were reduced by 0.18 mmol dm $^{-3}$. Furthermore, soil O_2 levels in flooded plants took 11 days to recover from when flooding ceased. Nicolas et al. (2005) flooded *Prunus armeniaca* L. (apricot) trees for 50 hrs with the water level at 4 cm above the soil surface; after draining, flooded plants were re-watered when soil matric potential reached -40kPa. Flooded apricot trees had significantly reduced photosynthesis rates and stomatal conductance and took 14 days to recover to control plant levels. Larson et al. (1993) flooded *Mangifera indica* trees for 0, 14, or 28 days to investigate growth responses to flooding; flooding was simulated by submerging plants in tubs filled with tap water to maintain the water table at 10 cm above the soil surface. Results showed that survival of flooded *M. indica* trees was dependent on the development of hypertrophied stem lenticels.

Cyclic flooding has been used to evaluate agricultural crops and wetland species. Cyclic flooding consists of a cycle of flooding and draining that is repeated over time. *Vitis vinifera* grapevines completed 4 cycles of flooding for 0, 3, 5, or 7 days and drained for 7 – 14 days over a two week cycle with plants irrigated 5 times a day with 0.17 L of water (Stevens and Prior, 1994). Flooded *V. vinifera* showed reduced shoot elongation and stomatal conductance during flooding and draining periods. Anderson and Pezeschki (1999) subjected seedling of *Taxodium distichum* (L.) Rich. (baldcypress), *Quercus nuttallii*, and *Quercus michauxii* Nutt. (swamp chestnut oak) to 3 cycles of flooding for 5 days each followed by draining for 5 days to evaluate trees used in bottomland forest restoration. Rates of photosynthesis and stomatal conductance of *T. distichum* did not show significant differences between flooded and non-flooded plants indicating their ability to maintain rates of photosynthesis during flooding. *Q. nuttallii*, however, showed decreased rates of photosynthesis during flooding, but rates rapidly improved during draining.

Summary

Global climate change and drought have led to an increased awareness of the importance of water conservation practices. Rain gardens can improve aquifer recharge while providing an esthetically pleasing addition to the landscape. A lack of research concerning the evaluation of landscape horticultural plants for use in rain gardens has led to this research. Plants for use in rain gardens should be able to withstand alternating periods of wet and dry conditions since rain gardens on receive irrigation during a rain event. Rain gardens may remain flooded for up to 2 days, so plants should also be able to survive and grow under periods of anaerobisis. Therefore, the objective of this study is to determine the effects of short interval cyclic flooding on selected native shrubs selected for use in rain gardens and to determine whether plants exposed to short interval cyclic flooding can be pre-conditioned to better tolerate subsequent cyclic flooded conditions.

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Chapter II

Effects of Short Interval Cyclic Flooding on Growth and Survival of Four Native Shrubs

Index Words: Rain garden, flood tolerance, landscape

Abstract: The effect of short interval cyclic flooding on root and shoot growth of *Clethra alnifolia* L. 'Ruby Spice' ('Ruby Spice' summersweet), *Ilex glabra* 'Shamrock' (L.) A. Gray. ('Shamrock' inkberry holly), *Itea virginica* L. 'Henry's Garnet' ('Henry's Garnet' sweetspire), and *Viburnum nudum* L. 'Winterthur' ('Winterthur' possumhaw) was studied in a greenhouse in Auburn, Ala. Thirty 0.25 L (2.25 in) liners of each taxa were planted into 2.5 L (trade gal) pots in 1:1 pinebark:peat (PB : P) or fine textured calcined clay (CC). *C. alnifolia* 'Ruby Spice', *I. glabra* 'Shamrock', and *I. virginica* 'Henry's Garnet' were planted 18 April 2008; the experiment was repeated with the addition of *V. nudum* 'Winterthur' on 16 Jun. 2008. Plants were flooded to substrate level for 0 (non-flooded), 3, or 7 days. Flooding cycles were repeated at least 5 times with 7 days of draining between each flood cycle. During draining plants received no irrigation. Non-flooded plants were watered as needed. Flooded plants for all taxa except *V. nudum* 'Winterthur' showed decreased RDW, SDW, and final GI when compared to non-flooded plants. Survival was higher in CC than PB : P for both experiments. Generally, growth was similar in non-flooded plants and plants flooded for 3 days in Experiment I, but in Experiment II growth was similar in plants flooded for 3 and 7 days. Mortality rates were higher in Experiment I than in Experiment II. Pot in pot flooding methodology was determined to be an

effective method for evaluating plants ability to withstand alternating wet and dry periods. Overall, plants seemed tolerant of flooding in spite of differences in growth.

Introduction

Water conservation practices have become a focal point for many communities due to drought conditions and water restrictions. Groundwater recharge has decreased with urbanization (Dietz and Clausen, 2005) which has led to additional pumping of groundwater supplies resulting in a need for stormwater management practices that focus on maximizing groundwater recharge (Dussaillant et al., 2005). When the amount of groundwater pumped exceeds the amount of recharge, aquifers become deficient (Seo et al., 2008).

Non-point source pollution has become a critical issue for both homeowners and agricultural entities regarding ground water supplies and water quality (Dietz and Clausen, 2008) with over half of the U.S. population depending on underground sources for potable water (Cabrera, 2005). Non-porous surfaces such as concrete increase storm water runoff taking fertilizers, chemicals, and fecal matter with it resulting in negative environmental consequences (Dunnet and Clayton, 2007). The EPA reported in 2001 that at least half of the U.S. waters cannot sustain aquatic life due to the presence of excess nutrients (Cabrera, 2005). Rain gardens and constructed wetlands are considered a best management practice in the collection of storm water, allowing it to seep into the ground while filtering out harmful pollutants (Shuster et al., 2007; U.S. Environ. Protection Agency, 1983; Prince George’s County, 1993; Dietz and Clausen, 2006).

Rain gardens are attractive additions to landscapes that serve as an appropriate application for stormwater management and infiltration. A rain garden is a shallow depression that collects stormwater from a roof, parking lot, or any other impervious surface (Dussaillant et.

al, 2005). Rain gardens depend on seasonal precipitation for the source of their irrigation, therefore, during precipitation events, rain gardens may flood and remain saturated above the substrate level until water permeates the ground. Dussaillant et al. (2005) found that soil in a rain garden would likely remain saturated for 1-2 days and plant roots would need to be able to withstand at least 2 days of standing water in the root zone for optimal recharge to occur. Plants adapted to moist conditions are more likely to withstand periodic dry conditions (Dunnet and Clayden, 2007).

Cyclic flooding has been used to evaluate agricultural crops and wetland species. Cyclic flooding consists of a cycle of flooding and draining that is repeated over time which is similar to what a rain garden might experience in the landscape. Rain gardens are self sustaining environments that only receive irrigation during a rain event and may remain dry for a period of weeks until the next rain event. Native plants adapted to low wetland areas are desirable for rain gardens because they are low maintenance, not invasive, relatively pest free (N.C. Coop. Ext., 2009), and are usually able to persist during periods of low rainfall or drought (Dunnet and Clayden, 2007).

There is a lack of research to determine specific plants for rain gardens. A variety of plant lists are available, but they are based on observation rather than rigorous scientific evaluation (Cabrera, 2005; USDA, 2008; Ranney et al., 2008; U.S. Fish and Wildlife Service, 1996). Such lists include but are not limited to: Qualifiers for Quagmires: Landscape Plants for Wet Sites (Ranney et al., 1998), National list of vascular plant species that occur in wetlands (U.S. Fish and Wildlife Service, 1996), Plants for Rain Gardens (Glen, 2009), Wetland Indicator Status List (U.S. Dept. Agr., 2008), and Shrubs for Rain Gardens (Dunnet and Clayton, 2007).

Increased circumstance of drought and water restrictions has led to an increased awareness of the importance of water conservation practices. Rain gardens can improve aquifer recharge while providing an esthetically pleasing addition to the landscape. A lack of research concerning the evaluation of landscape horticultural plants for use in rain gardens has led to this research. Plants for use in rain gardens should be able to withstand alternating periods of wet and dry conditions since rain gardens only receive irrigation during a rain event. Rain gardens may remain flooded for up to 2 days, so plants should also be able to survive and grow under periods of anaerobisis. Therefore, the objective of this study is to determine the effects of short interval cyclic flooding on selected native shrubs selected for use in rain gardens.

Materials and Methods

Experiment I

On 18 Apr. 2008, thirty 11.2 cm (4.4 in) rooted stem cuttings [0.25 L (2.25 in liners)] of *Ilex glabra* ‘Shamrock’ (ink berry holly), *Itea virginica* ‘Henry’s Garnet’ (sweetspire), and *Clethra alnifolia* ‘Ruby Spice’ (summer sweet) (Spring Meadow Nursery, Grand Haven, Mich.) were planted into 2.5 L (trade gal) pots with drainage holes. Liners were approximately 6 months old at planting. At planting, root balls were submersed in a solution of 0.5% (w/v) methylene blue for 10s to facilitate identification of new root growth outside of the original root ball. Substrates used were 1 pine bark : 1 peat (by volume) referred to hereafter as PB : P or fine textured calcined clay (CC) (Profile Products, Buffalo Grove, Ill.); both substrates were amended with dolomitic limestone [1.2 kg·m^{3} (2 lbs·yd^{3})] and controlled release fertilizer [8 kg·m^{-3} (13.8 lbs·yd^{3})(8 month 15N-2.64P-9.96K; Polyon®, Pursell Industries, Sylacauga, Ala.)]. Plants were arranged on benches in an 8mm polycarbonate greenhouse in on campus of Auburn University in

Auburn, Ala. The greenhouse was ventilated and maintained at a minimum night temperature of 18˚C (65˚F) and a maximum day temperature of 30˚C (80˚F) with a natural photoperiod.

Treatments were initiated on 12 May 2008 (24 DAP). Plants were flooded for 0 (non-flooded), 3, or 7 days. Flooded conditions were created by nesting a container with drainage holes containing a plant inside a 2.5 L (trade gal) container without drainage holes. Plants were flooded to the level of the substrate by adding approximately 1 L (0.26 gal) tap water on the first day of flooding and adding approximately 50 mL (0.013 gal) tap water on each additional flood day to maintain water table at substrate level. At the end of each flood cycle, exterior container was removed and plants were allowed to drain for one week, and no water was added until the next flood cycle began. Percent moisture was measured daily for control plants using a Theta moisture probe (Delta- T Devices Ltd., Cambridge, England). Non-flooded plants were irrigated with 500 mL (0.13 gal) tap water when substrate percent moisture reached 25% (Bailey, 2009; Wilkin, 2007). Substrate percent moisture was measured in 3 and 7 flood day treatments every other day during the draining period. At experiment termination roots and shoots were harvested. Shoots were harvested for shoot dry weight (SDW) [shoots removed from the root ball and dried at 68.3˚C (155˚F) for 48 hrs]. New roots were harvested from the original root ball and root dry weight (RDW) [roots removed from the root ball and dried at 68.3˚C (155˚F) for 48 hrs] was recorded when all shoots had been removed for SDW. During the experiment, if a plant displayed necrotic tissue, SDW and RDW were determined at that point. The experiment was terminated on 27 Aug. 2008 (131 DAP), and plants in 3 and 7 day flood treatments experienced a total of 9 and 7 flood cycles, respectively. Plant growth indices [(height + widest width + width perpendicular to widest width)/3] were recorded at planting and at experiment termination. Each species was treated as a separate experiment and the experimental design was a split plot design with substrate as the main plot and treatments randomized within. Data were analyzed using mixed models procedures with least square means ($P<0.05$) (SAS Institute, Inc., 2004).

Experiment II

On 16 Jun. 2008, thirty 11.2 cm (4.4 in) rooted stem cuttings of all previously listed taxa with the addition of *Viburnum nudum* ‘Winterthur’ (possumhaw) (Spring Meadow Nursery, Grand Haven, Mich.) were planted into 2.5 L (trade gal) pots. Materials and methods were the same as Experiment I with the following exceptions. For this experiment, the entire root ball was removed and washed for RDW when SDW was recorded. Substrate percent moisture was measured every six hours using ECH_2O soil moisture sensors (model EC-5) (Decagon Devices, Inc., Pullman, WA). Treatments were initiated on 21 July 2008 (35 DAP). The experiment was terminated on 10 Oct. 2008 (117 DAP). Plants in the 3 and 7 day flood treatments experienced 7 and 5 flood cycles, respectively.

Results

***Clethra alnifolia* ‘Ruby Spice’**

Experiment I

RDW was higher for plants in PB : P (4.4 g) than in CC (2.4 g). RDW and SDW were higher in non-flooded plants and than in flooded plants (Table 1). PB : P dried down more slowly than CC, and substrate percent moisture of the end of a draining period for plants flooded for 3 and 7 days was higher in PB : P (23.0 and 31.2, respectively) than CC (17.9 and 23.5, respectively). Plants flooded for 7 days dried more slowly and had higher substrate percent moisture than plants flooded for 3 days. Mortality was higher in flooded plants (40%) than control plants (0%), and was higher in PB : P (47%) than CC (13%). By 84 DAP, 40% of plants flooded for 3 days in PB : P were dead, and at 95 DAP, 100% of plants in flooded for 7 days in PB : P were dead.

Experiment II

RDW was higher for plants in CC (3.3 g) and than in PB : P (2.3 g). There was an interaction between substrate and treatment for SDW. SDW was higher for plants in CC than in PB : P for non-flooded plants but was not different between substrates in flooded plants (Table 1). Final GI was higher for plants in CC (21.7 cm) than in PB : P (16.7 cm). There were no differences among flood treatments for RDW and final GI (data not shown). PB : P dried down more slowly than CC and substrate percent moisture of the end of a draining period for plants flooded for 3 and 7 days was higher in PB : P (24.8 and 31.4, respectively) than CC (16.4 and 19.2, respectively). On average substrate percent moisture reached approximately 50.7% moisture during flooding. Survival rate was higher for plants in CC (100%) than in PB : P (93%). All plants across the 3 and 7 day flood treatments in PB :P and CC had 100% survival rates, however 6.7% of non-flooded plants died.

Ilex glabra **'Shamrock'**

Experiment I

RDW and SDW were higher for plants in CC (0.9 g and 6.5 g, respectively) than in PB : P (0.5 g and 4.7 g, respectively). RDW, SDW, and final GI were lower in flooded plants than in non-flooded plants (Table 2). PB : P dried down more slowly than CC and substrate percent moisture of the end of a draining period for plants flooded for 3 and 7 days was higher for plants in PB : P (30.3 and 34.8, respectively) than in CC (20.9 and 25.8, respectively). All plants in CC had 100% survival rate. At 86 DAP, 40% of plants flooded for 7 days in PB: P were dead and at 95 DAP 20% of plants flooded for 3 days in PB: P were dead.

Experiment II

There was an interaction between substrate and treatment for RDW and SDW. RDW was higher in CC than in PB : P for non-flooded plants and plants flooded for 3 days (Table 3), and SDW was higher in CC than PB :P for non-flooded plants (Table 3). RDW and SDW decreased linearly with increased flood days (Table 3); however, SDW in PB : P responded quadratically to flood treatments (Table 3). During flooding and draining, substrate percent moisture was similar among substrates but not different among flood treatments (not statistically analyzed, n=2). On average substrate percent moisture during flooding was higher in PB : P (52.2) than CC (49.0). PB : P dried down more slowly than CC and substrate percent moisture of the end of a draining period for flooded plants was higher in PB : P (29.6) than in CC (20.1). Mortality occurred only in plants flooded for 7 days in both substrates and mortality rates was higher in PB : P (40%) than CC (20%).

***Itea virginica* 'Henry's Garnet'**

Experiment I

There was a significant interaction between substrate and treatment for RDW and SDW. RDW and SDW were higher for plants in CC than in PB: P for non-flooded plants (Table 4). However, RDW and SDW were higher in PB: P for plants flooded for 3 days, but similar in plants flooded for 7 days (Table 4). RDW and SDW were lower in flooded plants in CC and PB: P (Table 4). Mortality rates of plants flooded for 7 days were lower in CC (20%) than in PB : P (40%), but mortality occurred sooner in CC (57 DAP) than PB: P (113 DAP). Non-flooded plants and plants flooded for 3 days in PB : P had similar mortality rates of 40% by 113 DAP; however mortality was more vigorous in plants flooded for 3 days with all mortalities occurring by 75 DAP.

Experiment II

SDW and GI were higher in non-flooded plants than in flooded plants (Table 5). RDW was similar in non-flooded plants and plants flooded for 3 days and RDW for flooded plants was not different (Table 5). Substrate percent moisture for plants flooded for 3 and 7 days was similar among PB : P (19.6 and 22.3, respectively) and CC (21.2 and 20.9, respectively) during dry down (not statistically analyzed, n=2). All plants in both substrates maintained 100% survival rate throughout this experiment.

***Viburnum nudum* ‘Winterthur’**

Experiment II

There was an interaction between substrate and treatment for RDW and SDW. RDW and SDW were higher in CC than PB : P for non-flooded plants (Table 6). SDW in PB : P decreased linearly over all treatments (Table 6). RDW was similar for plants flooded for 3 and 7 days in both substrates. SDW was higher in CC than in PB : P for plants flooded for 3 days (Table 6). Final GI was higher in CC (20.2 cm) than in PB : P (18.2 cm). There were no differences among treatments for final GI (data not shown). PB : P dried down more slowly than CC and substrate percent moisture of the end of a draining period for plants flooded for 3 and 7 days was higher in PB : P (24.4 and 23.5, respectively) than CC (16.7 and 21.3, respectively). PB : P mortality rates were higher in non-flooded plants (30%) than in plants flooded for 3 days (10%). CC mortality was higher in plants flooded for 7 days (20%) than in plants flooded for 3 days (10%).

Discussion

All taxa with the exception of *Clethra alnifolia* ‘Ruby Spice’ appear tolerant of cyclic flooding, which suggests they would be appropriate selections for rain gardens. During Experiment I, severe defoliation occurred during the beginning of the experiment which may have been due to prematurity of liners used (personal observation). Liners used in Experiment I were shipped at the earliest possible date and seemed more fragile than the liners used in Experiment II that were shipped later in the season. Final GI was not measured for *Clethra alnifolia* 'Ruby Spice' for Experiment I due to severe infestation of broad mite (*Polyphagotarsonemus latus* Banks). Damage associated with broad mite was first noted on 25 July 2008 and subsequent applications of Abamectin (Avid® 0.15 EC, Syngenta, Greensboro, N.C.) were made in an attempt to control them. However, broad mite damage was significant and treatment causal differences were no longer discernable by experiment termination.

Results from Experiments I and II indicated that flooded plants in all taxa except *V. nudum* 'Winterthur' showed decreased RDW, SDW, and final GI when compared to non-flooded plants. Similarly, shoot elongation rates of *Vitis vinifera* L. (sultana grape vine) were reduced by 40% when plants were flooded for 5 days and shoot dry weights decreased linearly as flood periods increased (Stevens and Prior, 1994). Larson et al. (1993) reported that flooded *Mangifera indica* L. (mango) trees showed a reduction in root growth and reduced shoot: root ratios compared to control plants.

In Experiment I, non-flooded plants across all taxa grew more than flooded plants, but plants flooded for 3 days were visually similar to non-flooded plants. Plants flooded for 7 days showed extremely reduced canopies. Flooded plants showed visual symptoms of flooding stress and were generally more defoliated and less vigorous compared to non-flooded plants. Plants flooded for 3 days in Experiment I appeared able to adapt to flooding stress and continue growth after initial shock of flooded conditions. By the end of Experiment I, *Ilex glabra* 'Shamrock' plants had visible new growth across both flood treatments (personal observation, not quantified). In Experiment II, plants flooded for 3 days were visually similar to plants flooded for 7 days. Flooded plants in Experiment II showed more dense canopies and decreased defoliation in comparison to flooded plants in Experiment I. This was likely due to the reduction in number of flood cycles from 9 and 7 flood cycles in Experiment I to 7 and 5 flood cycles in Experiment II. However, other factors such as maturity of liners and seasonal climate differences may have also contributed to overall higher quality plants observed at termination of Experiment II.

Mortality rates were generally higher in Experiment I than in Experiment II. Elevated mortality rates of flooded plants in Experiment I may have been due to prematurity of liners and decreased acclimation of plants before flood treatments were initiated. Plants had more time to acclimate to their environment (ambient temperature and light) before treatments were initiated in Experiment II (35 DAP) than in Experiment I (24 DAP). Higher mortality rates were associated with flooded plants across all taxa with the exception of *C. alnifolia* 'Ruby Spice' which had higher mortality rates in non-flooded plants than in flooded plants. Mortality of non-flooded *C. alnifolia* 'Ruby Spice' did not seem to be treatment related, but rather an effect of severe broad mite damage.

Methylene blue was not useful in identifying new roots due to considerable amounts of dead roots in flooded treatments. In the past, methylene blue has been used to help identify new root growth and was found to have no effect on root or shoot growth up to 90 days after application (Arnold and Young, 1990, Wright et al., 2004). However, in our study, because plants were subjected to flooding, roots were more likely to rot and turn black. Therefore, harvesting only new roots was abandoned after Experiment I due to similarity in color between new and old roots.

Pot in pot flooding methodology was determined to be an effective method for creating flooded conditions. CC drained more quickly than PB : P, and plants in CC substrate experienced lower substrate percent moisture during dry down which could better evaluate tolerance to short term drought episodes. Although PB : P did dry down during draining, a longer draining period may be necessary to impose more severe drought circumstances.

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Table 1. Effect of length of flooding cycle on root dry weight (RDW) and shoot dry weight (SDW) of *Clethra alnifolia* 'Ruby Spice' grown in a greenhouse in Auburn, Ala. For May 2008, plants in the 3 and 7 day flood treatments experienced a total of 9 and 7 flood cycles, respectively. For July 2008, plants in the 3 and 7 day flood treatments experienced a total of 7 and 5 flood cycles, respectively.

May 2008		
Treatment[z]	RDW (g)	SDW (g)
0	6.4a[w]	33.1a
3	3.2b	13.2b
7	0.7c	5.8c
	L***[y]	L***

July 2008			
	SDW (g)		
	Treatment[z]		
Substrate[x]	0	3	7
CC	7.3a	5.3	5.3
PB : P	2.7b	4.0	3.8

[z] Treatments were based on days of flooding. Plants were flooded for 0 (control), 3, or 7 days.

[y] Linear trend at alpha = <0.0001 (***) among treatments.

[x] Substrates used were CC = calcined clay and PB : P = 1:1 pine bark to peat.

[w] Lowercase letters denote mean separation using Tukey at $p<0.05$ (Sas Institute, 2004); if no differences then letters are omitted.

Table 2. Effect of length of flooding cycle on root dry weight (RDW), shoot dry weight (SDW), and final growth index (GI) of *Ilex glabra* ‘Shamrock' grown in a greenhouse in Auburn, Ala. For May 2008, plants in the 3 and 7 day flood treatments experienced a total of 9 and 7 flood cycles, respectively. For July 2008, plants in the 3 and 7 day flood treatments experienced a total of 7 and 5 flood cycles, respectively.

	May 2008			July 2008
Treatment[z]	RDW (g)	SDW (g)	GI (cm)	GI (cm)
0	1.7a[w]	9.8a	25.3a	20.5a
3	0.3b	4.2b	14.5b	16.9b
7	0.02b	2.8b	13.0b	15.5b
	L***[y]	L***	L***	L***

[z] Treatments were based on days of flooding. Plants were flooded for 0 (control), 3, or 7 days.

[y] Linear trend at alpha = <0.0001 (***), 0.001 (**), and 0.01(*) among treatments.

[x] Substrates used were CC = calcined clay and PB : P = 1:1 pine bark to peat.

[w] Lowercase letters denote mean separation using Tukey p<0.05; if no differences then letters are omitted.

Table 3. Effect of length of flooding cycle on root dry weight (RDW), shoot dry weight (SDW), and final growth index (GI) of *Ilex glabra* ‘Shamrock' grown in a greenhouse in Auburn, Ala. For July 2008, plants in the 3 and 7 day flood treatments experienced a total of 7 and 5 flood cycles, respectively.

	July 2008							
	RDW (g)				SDW (g)			
	Treatment[z]							
Substrate[x]	0	3	7		0	3	7	
CC	8.6a[w]	5.7a	3.0	L***[y]	10.1a	7.8	6.2	L**
PB : P	3.6b	3.6b	2.1		4.7b	7.1	4.2	Q*

[z] Treatments were based on days of flooding. Plants were flooded for 0 (control), 3, or 7 days.

[y] Linear or quadratic trend at alpha = <0.0001 (***), 0.001 (**), and 0.01(*) among treatments.

[x] Substrates used were CC = calcined clay and PB : P = 1:1 pine bark to peat.

[w] Lowercase letters denote mean separation using contrast statements in Proc Glimmix p<0.05; if no differences then letters are omitted.

Table 4. Effect of length of flooding cycle on root dry weight (RDW), shoot dry weight (SDW), and final growth index (GI) of *Itea virginica* ‘Henry’s Garnet’ grown in a greenhouse in Auburn, Ala. For May 2008, plants in the 3 and 7 day flood treatments experienced a total of 9 and 7 flood cycles, respectively.

	May 2008							
	RDW (g)				SDW (g)			
	Treatment[z]							
Substrate[x]	0	3	7		0	3	7	
CC	8.9a[w]	0.5b	0.1	L***[y]	21.6a	4.1	1.3	L***
PB : P	3.0b	3.3a	0.1	L*	6.7b	5.4	1.1	L**

[z] Treatments were based on days of flooding. Plants were flooded for 0 (control), 3, or 7 days.

[y] Linear trend at alpha = <0.0001 (***), 0.001 (**), and 0.01(*) among treatments.

[x] Substrates used were CC = calcined clay and PB : P = 1:1 pine bark to peat.

[w] Lowercase letters denote mean separation using contrast statements in Proc Glimmix p<0.05; if no differences then letters are omitted.

Table 5. Effect of length of flooding cycle on root dry weight (RDW), shoot dry weight (SDW), and final growth index (GI) of *Itea virginica* 'Henry's Garnet' grown in a greenhouse in Auburn, Ala. For July 2008, plants in the 3 and 7 day flood treatments experienced a total of 7 and 5 flood cycles, respectively.

	July 2008		
Treatment[z]	RDW (g)	SDW (g)	GI (cm)
0	8.5a[w]	12.6a	30.7a
3	6.5ab	8.5b	25.3b
7	5.3b	6.5b	22.9b
	L*[y]	L***	L**

[z] Treatments were based on days of flooding. Plants were flooded for 0 (control), 3, or 7 days.

[y] Linear trend at alpha = <0.0001 (***), 0.001 (**), and 0.01(*) among treatments.

[w] Lowercase letters denote mean separation using Tukey at p<0.05 (Sas Institute, 2004); if no differences then letters are omitted.

Table 6. Effect of length of flooding cycle on root dry weight (RDW), shoot dry weight (SDW), and final growth index (GI) of *Viburnum nudum* 'Winterthur' grown in a greenhouse in Auburn, Ala. Plants in the 3 and 7 day flood treatments experienced a total of 7 and 5 flood cycles, respectively.

	May 2008							
	RDW (g)				SDW (g)			
	Treatment[z]							
Substrate[x]	0	3	7		0	3	7	
CC	10.8a[w]	6.5	5.8	L**[y]	8.56a	7.7a	6.0	
PB : P	3.4b	5.0	5.8		3.4b	5.2b	6.8	L*

[z] Treatments were based on days of flooding. Plants were flooded for 0 (control), 3, or 7 days.

[y] Linear trend at alpha = 0.001 (**) and 0.01(*) among treatments.

[x] Substrates used were CC = calcined clay and PB : P = 1:1 pine bark to peat.

[w] Lowercase letters denote mean separation using contrast statements in Proc Glimmix p<0.05; if no differences then letters are omitted.

Chapter III

Effects of Previous Flood Exposure on Flood Tolerance, Growth, and Physiology of Selected Native Shrubs

Index Words: Rain garden, landscape

Abstract: *Ilex glabra* 'Shamrock' (L.) A. Gray. ('Shamrock' inkberry holly), *Itea virginica* L. 'Henry's Garnet' ('Henry's Garnet' sweetspire), and *Viburnum nudum* L. 'Winterthur' ('Winterthur' possumhaw) were subjected to 0 (non-flooded), 3, or 6 days of flooding in a greenhouse and then outdoors. Plants were potted in 2.5 L (trade gal) pots in 5 : 3 : 1 pine bark : peat : perlite (PB) or sterilized field soil and placed in a greenhouse in Feb. 2009. All taxa were subjected to 0 (non-flooded), 3, or 6 days of flooding. During draining, plants received no irrigation. Non-flooded plants were watered as needed. Plants in the 3 and 6 day flood treatments experienced a total of 7 and 5 flood cycles, respectively. All taxa maintained 100% survival during greenhouse flooding. All taxa were then planted outdoors in 170.6 L (45 gal) tubs in PB or calcined clay (CC). There were three plants (same taxon) per tub with one each that had been flooded for 0 (non-flooded), 3, or 6 days in the greenhouse. Outdoor flooding treatments were the same as greenhouse flooding treatments and were randomly assigned to each tub. Overall flooding treatments were in a factorial combination of greenhouse flooding treatment x outdoor flooding treatment. Following greenhouse and outdoor flooding, *I. virginica* 'Henry's Garnet' had the largest relative growth index (RGI) followed by *V. nudum* 'Winterthur' and *I. glabra* 'Shamrock'. *I. virginica* 'Henry's Garnet' and *I. glabra* 'Shamrock' had the largest differences among treatments for final growth index (GI), but *V. nudum* 'Winterthur'had no differences among treatments. During outdoor flooding, photosynthesis (Ps) and stomatal conductance (SC) rates were highest in *I. glabra* 'Shamrock' followed by *V. nudum* 'Winterthur' and *I. virginica* 'Henry's Garnet'. Results indicate that *I. virginica* 'Henry's Garnet' may become less sensitive to flooding with maturity, but that *V. nudum* 'Winterthur' may become more sensitive to flooding with maturity. *I. glabra* 'Shamrock' seems to be tolerant of flooding at any stage of growth.

Introduction

Rain gardens are attractive additions to landscapes that facilitate stormwater management and infiltration. A rain garden is a shallow depression that collects stormwater from a roof, parking lot, or any other impervious surface (Dussaillant et. al, 2005). Rain gardens depend on seasonal precipitation for the source of their irrigation, therefore, during precipitation events, rain gardens may flood and remain saturated above the substrate level until water permeates the ground. Dussaillant et al. (2005) found that soil in a rain garden would likely remain saturated for 1-2 days and plant roots would need to be able to withstand at least 2 days of standing water in the root zone for optimal recharge to occur. Plants adapted to moist conditions are more likely to withstand periodic dry conditions (Dunnet and Clayden, 2007).

Cyclic flooding has been used to evaluate agricultural crops and wetland species. Cyclic flooding consists of a cycle of flooding and draining that is repeated over time which is similar to what a rain garden might experience in the landscape. Rain gardens are self sustaining environments that only receive irrigation during a rain event and may remain dry for a period of weeks until the next rain event. Native plants are desirable for rain gardens because they are low maintenance, not invasive, relatively pest free, and are usually able to persist during periods of low rainfall or drought. Plants for use in rain gardens should be able to withstand alternating periods of wet and dry conditions since rain gardens only receive irrigation during a rain event. Rain gardens may remain flooded for up to 2 days, so plants should also be able to survive and grow under periods of anaerobisis.

Reduced stomatal activity and net photosynthesis has also been noted in flooded plants. Flood tolerant species however, may not exhibit a reduction in photosynthetic activity, and some researchers associate flooding tolerance with maintaining adequate rates of photosynthesis (Arbona et al., 2009). Moreover, carbohydrate metabolism via photosynthesis and respiration is essential in the tolerance of anaerobic conditions (Huang, 2000). Reduction in net photosynthesis may be due to limited stomatal aperture during anoxic conditions (Li et al., 2007). Stomatal closure is a response of plants to depleted soil oxygen in the root zone; flooding cripples leaf gas exchange and impacts transport of soil water and solutes (Sojka, 1992).

Rain gardens can improve aquifer recharge while providing an esthetically pleasing addition to the landscape. Previously flooded plants may be able to adapt to flooded conditions and better survive future flood events. Anderson and Pezeshki (2001b) exposed *Taxodium distichum* (L.) Rich., *Quercus nuttallii* Palmer, and *Quercus michauxii* Nutt. to short-term flood events in an attempt to pre-condition seedlings to subsequent flood tolerance. Results indicated that after repeated exposure to flooding, seedling response did not improve during subsequent flood events, but that additional research using more mature plants exposed to other frequencies and durations of flooding was needed. Pre-conditioning plants for greater flood tolerance during subsequent flooding episodes could also be utilized for better establishment of plants in rain gardens in the designed landscape. Therefore, the objective of this study is to determine if plants exposed to short interval cyclic flooding could be pre-conditioned to better tolerate subsequent cyclic flooded conditions.

Materials and Methods

Greenhouse Flooding

In Oct. 2008, 11.2 cm (4.4 in.) rooted stem cuttings [0.25 L (2.25 in) liners] (Spring Meadow Nursery, Grand Haven, Mich.) of *Ilex glabra* ‘Shamrock’ (inkberry), *Itea virginica* ‘Henry’s Garnet’ (sweetspire), and *Viburnum nudum* ‘Winterthur’ (possumhaw) were planted into 2.5 L (trade gal) pots with drainage holes. Liners were approximately 6 months old at planting. Container substrates used were 5 pine bark : 3 peat : 1 perlite (by volume) hereafter referred to as PB or soil [(Marvyn sandy loam) collected from research field plots on Auburn University campus in Auburn, Ala.]. Prior to use, soil was sterilized in an autoclave at 129˚ C (265˚ F) for 3 hrs at the Plant Sciences Research Center on campus in Auburn, Ala. PB was amended with dolomitic limestone [1.2 $kg{\cdot}m^{-3}$ (2 $lbs{\cdot}yd^{-3}$)], controlled release fertilizer [8 $kg{\cdot}m^{-3}$ (13.8 $lbs{\cdot}yd^{-3}$)(8 month 18N-2.64P-9.96K; Polyon®, Pursell Industries, Sylacauga, Ala.)], and micronutrient fertilizer [0.7 $kg{\cdot}m^{-3}$ (1.5 $lbs{\cdot}yd^{-3}$) (Micromax®, Scott’s Company, Marysville, Ohio)]. Plants grown in soil were top dressed with controlled release fertilizer at a rate of 14 g (0.5 oz) per container. Plants were overwintered in a shade house on campus in Auburn, Ala. from 16 Oct. 2008 to 23 Feb. 2009. During the overwintering period, plants received daily overhead irrigation for 10 min at 10:00 AM. If temperatures decreased to approximately -4˚ C (25˚ F) or lower, plants were covered with white polyethylene plastic with ventilation holes.

Plants were arranged on raised benches on 23 Feb. 2009 [(131 days after planting (DAP)] in an 8 mm (0.3 in) polycarbonate greenhouse on campus in Auburn, Ala. The greenhouse was ventilated and maintained at a minimum night temperature of 18˚C (65˚F) and a maximum day temperature of 29.7˚C (80˚F) with a natural photoperiod. There were 21 single pot replications per treatment per substrate per taxon. Due to lack of available plant material, *V. nudum* 'Winterthur' grown in soil contained only 18 single pot replications per treatment.

Plants were flooded for 3 days, 6 days, or non-flooded. Flooded conditions were created by nesting a plant's container with drainage holes inside a 2.5 L (trade gal) container without drainage holes. Plants were flooded to the level of the substrate by adding approximately 1 L (0.26 gal) tap water to the substrate on the first day of flooding and adding approximately 50-100 mL (1.7-3.4 oz) tap water on each additional flood day to maintain water table at substrate level. At the end of each flood cycle, the exterior container without holes was removed, plants were allowed to drain for 6 days, and no water was added to the substrate until the next flood cycle began. Substrate percent moisture was measured daily for non-flooded plants using a Theta moisture probe (Delta- T Devices Ltd., Cambridge, England). Non-flooded plants were irrigated with 500 mL (0.13 gal) tap water when substrate percent moisture reached 25% (Bailey, 2009; Wilkin, 2007). Substrate percent moisture was measured in 3 and 6 flood day treatments every other day during the draining period.

Flooding treatments were initiated when all plants within a taxon had produced approximately four new nodes. Due to differences in growth rates among taxa, treatment initiation dates were different among taxa. For *I. virginica* 'Henry's Garnet' treatments were initiated 18 Mar. 2009 (155 DAP) and ended 19 May 2009 (217 DAP). For *I. glabra* 'Shamrock' treatments were initiated 2 Apr. 2009 (170 DAP) and ended 4 Jun. 2009 (233 DAP). For *I. virginica* 'Henry's Garnet' and *I. glabra* 'Shamrock', treatments were initiated on the same day for both substrates. Due to growth rate differences between the two substrates in *V. nudum* 'Winterthur', treatments were initiated in PB 24 Mar. 2009 (161 DAP) and ended 26 May

2009 (224 DAP); for plants in soil, treatments were initiated 8 Apr. 2009 (176 DAP) and ended 10 June 2009 (239 DAP).

Plants in the 3 and 6 day flood treatments experienced a total of 7 and 5 flood cycles, respectively. Three plants per treatment per substrate were harvested when treatments were discontinued for shoot dry weight (SDW) [shoots removed from the root ball and dried at 68.3˚C (155˚F) for 48 hrs]. The root ball was rinsed to remove substrate and dried at 68.3˚C (155˚F) for 48 hrs, and root dry weight (RDW) was determined. Due to lack of plant material, RDW and SDW were not recorded for plants in soil. Plant growth indices [(GI) (height + widest width + width perpendicular to widest width)/3] were recorded at planting and at experiment termination. Relative growth index [(RGI) (Final GI – Initial GI)/Initial GI)] was calculated for each plant. Each taxon was treated as a separate experiment and the experimental design was a split plot design with substrate as the main plot and treatments randomized within. Data were analyzed using contrast statements in the Glimmix procedure with least square means ($P<0.05$) (SAS Institute, Inc., 2004).

Outdoor Flooding

Plants used in the above greenhouse flooding experiment were held in the greenhouse for 40 days after flooding treatments ended. During this period, plants were irrigated with 300 to 500 mL (0.08 to 0.13 gal) tap water when substrate percent moisture reached 25%. Plants were pruned within each taxon x substrate x treatment combination 40 days after greenhouse flooding treatments ended. The purpose of pruning was to create a uniform size within each taxon x substrate x treatment combination while preserving growth differences that occurred during greenhouse flooding. *I. virginca* ‘Henry’s Garnet’ and *I. glabra* ‘Shamrock’ plants were pruned (if needed) to a height of approximately 30 cm (11.8 in). *V. nudum* ‘Winterthur’ plants growing in PB were pruned to a height of approximately 40 cm (15.75 in). *V. nudum* ‘Winterthur’ plants growing in soil were not pruned because very little new growth occurred during greenhouse flooding. GI was recorded for all plants after pruning before Experiment II treatments were initiated.

Plants from the greenhouse flooding experiment were planted outdoors into [170.6 L (45 gal) 93 cm x 53 cm x 50 cm (36.5 in x 21 in x 19.5 in)] tubs (Sterilite Corporation, Townsend, Mass.) in a 5 : 3 : 1 pinebark : peat: perlite (PB) or fine textured calcined clay (CC) (Profile Products, Buffalo Grove, Ill.). This outdoor experiment required too large of a volume of soil to be collected and sterilized, as a result, CC was used instead of soil. CC is a mineral substrate that is similar in texture to soil used in the greenhouse flooding experiment. Plants grown in PB in the greenhouse were planted in PB outdoors, and plants grown in soil in the greenhouse were planted in CC outdoors. Tubs were modified using basic PVC plumbing parts to include a drain. A 3.8 cm (1.5 in.) hole was centered 5 cm (2 in.) from tub bottom on one short side of each tub (Fig. 1A). The slip ends of a 3.8 cm (1.5 in.) female coupling and a 3.8 cm (1.5 in.) male trap adapter were cemented together using PVC primer and cement (Oatey, Cleveland, Ohio) (Fig. 1B). A 4.5 cm (1.75 in.) flat rubber washer was placed around the hole on each side of the tub using a bead of pressure formed gasket maker (Permatex®, Hartford, Conn.) (Fig. 1C). Another bead of gasket maker was placed on top of the flat washer to insure a press seal was formed as the male end of the trap adapter was screwed into hole. A 3.8 cm (1.5 in.) female bushing was modified to include a filter to prevent substrate loss through drain by using a plastic zip tie to adhere a small piece of window screening (Fig. 1D). Threads of the female bushing were painted with teflon tape alternative (Rectorseal, Houston, Texas), and the bushing was screwed onto the male end the male trap adapter on the inside of the tub. A 3.8 cm (1.5 in.) screw plug on the outside of the tub (Fig. 1E) was tightened and loosened using a pair of channel lock pliers to enable tub to be flooded or drained. Leaks were minimal, but those that occurred were patched with silicone.

Tubs were filled with 0.12 m^{-3} (4.2 ft^{-3}) substrate to a height of approximately 30 cm (11.8 in.). Each tub contained three plants (same taxon), one each that had been non-flooded, flooded for 3 days, and flooded for 6 days in the greenhouse. Plants were planted in each tub in a row approximately 10.2 cm (4 in) from the long edge of the tub and 17.8 cm (7 in) on center from each other and the short end of the tub. Outdoor flooding treatments were randomly assigned to each tub. Tubs were non-flooded, flooded for 3 days, or flooded for 6 days. Flooding treatments were thus in a factorial combination of flooding treatment in the greenhouse x flooding treatment outdoors for a total of nine flooding treatments outdoors in the tubs. There were a total of 18 tubs per substrate per taxon with the exception of *V. nudum* ‘Winterthur’ in CC which only had 15 tubs. Within each taxon and substrate combination there were 6 tubs per treatment (except *V. nudum* ‘Winterthur’ in CC which only had 5 tubs). Treatments were initiated for *I. glabra* ‘Shamrock’ on 23 July 2009 (282 DAP) and ended on 23 Sept. 2009 (344 DAP). Treatments were initiated for *I. virginica* ‘Henry’s Garnet’ on 7 July 2009 (266 DAP) and ended on 7 Sept. 2009 (328 DAP). Treatments were initiated for *V. nudum* ‘Winterthur’ in PB on 14 July 2009 (273 DAP) and ended on 14 Sept. 2009 (335 DAP). Treatments were initiated for *V. nudum* ‘Winterthur’ in CC on 29 July 2009 (288 DAP) and ended on 28 Sept. 2009 (349 DAP).

Tubs were placed under a 7.9 m x 4.6 m (26 ft x 48 ft) shade house. The top of the structure was pitched [(3.4 m (10 ft) tall sloping to 1.8 m (6 ft) tall along the short side)] and covered in a double layer of 6 mil white polyethylene plastic to keep any rainfall from entering and a 60% woven shade cloth. The long sides of the shade house were covered in a 60% knitted shade cloth. Photosynthetic photon flux (PPF) was measured using a quantum meter (Model LQM50-3, Spectrum Technologies, Inc., East-Plainfield, Ill.), and PPF was approximately 275 μmol·m^2·sec during full sun. Short sides were oriented north to south and left open to ensure adequate air movement and ventilation. Within each taxon x substrate combination, tubs were randomly arranged in two rows of nine tubs placed side by side with no space between them.

Substrate percent moisture was measured every 6 hours using ECH$_2$O soil moisture sensors (model EC-5) (Decagon Devices, Inc., Pullman, WA). Two soil moisture sensors were installed per tub in two tubs per treatment within a species and substrate. Sensors were installed in the substrate on opposite sides of a tub approximately 12 cm (4.7 in) deep and centered between the end and middle plant approximately 10 cm (4 in) from the side of the tub. On 22 Aug. 2009, soil moisture sensors used to measure dry down of flooded tubs were changed from measuring every 6 hours to every 15 min due to speed of initial draining of substrate and to better assess initial dry down. Irrigation (tap water) was applied by hand using a standard hose and watering wand with a flow rate of 26.5 L·min^{-1} (7 gal·min^{-1}). Each irrigation application was timed and volume of water applied was calculated. Non-flooded tubs were irrigated with 20 L (5.3 gal) tap water when substrate percent moisture reached 20%. At 25% percent moisture, substrates remained saturated and were still considered wet which was likely due to the large volume of substrate within each tub. Tubs were flooded to the substrate level by adding 26.5 L (7 gal) initially and by adding 1.5 L (0.4 gal) water on each additional flood day to maintain the water table at substrate level. Leachate was collected during the first 15 min of draining during the last draining cycle for three tubs in each taxon x substrate x treatment combination, and electrical conductivity (EC) and pH were measured using a handheld pH meter (Model EP11·pH, Myron L Company, Carlsbad, Calif.)

Net photosynthesis (Ps) and stomatal conductance (SC) were measured using the LI-COR 6400 (Model 1000, LI-COR Biosciences, Inc., Lincoln, Nebr.) on the last day of flooding and draining within a flood cycle during an intermediate and the final flood cycle of both flooding treatments. Ps and SC were measured on all plants within a tub in three tubs of each taxon x substrate x treatment combination. Ps and SC of control plants were measured concurrently with flooded plants. Leaves of *I. glabra* 'Shamrock' had to be removed from the plant and immediately placed in the cuvette for measuring due to short length of petiole. Following each Ps and SC measurement for *I. glabra* 'Shamrock', the leaf was sealed in a ziploc bag and transported to a lab where leaf area was measured using a LI-COR 3100 leaf area meter (LI-COR Biosciences, Inc., Lincoln, Nebr.). Ps and SC values for *I. glabra* 'Shamrock' were reconfigured based on exact leaf areas using LI-COR Simulator software (version 5.3.2, LI-COR Biosciences, Inc., Lincoln, Nebr.) in file exchange mode.

Plants in the 3 and 6 day flood treatments experienced a total of 7 and 5 flood cycles, respectively. SDW and RDW were recorded at experiment termination and root : shoot ratio (RDW : SDW = RDW/SDW) was calculated for each plant. All replications within a taxon x substrate x treatment combination were harvested except for *I. virginica* 'Henry's Garnet' in PB which had only 3 replications per treatment combination harvested due to difficulty separating roots from substrate. Final GI was recorded at experiment termination. RGI was calculated for each plant to indicate the change in growth that occurred outdoors in tubs. Each taxon was treated as a separate experiment. The experimental design was a split – split plot design with substrate as the main plot and outdoor flooding treatments as the subplot with greenhouse flooding treatments randomized within each tub. Data were analyzed using the Glimmix procedure with least square means (P<0.05) (SAS Institute, Inc., 2004).

Results

Greenhouse Flooding

***Ilex glabra* 'Shamrock'**

In PB, RDW and SDW were higher in non-flooded plants than in flooded plants (Table 1). RDW and SDW were not different among flood treatments for plants in soil (Table 1). In PB, RGI was similar between non-flooded plants and plants flooded for 3 days for plants (Table1), and RGI was higher in non-flooded plants than in flooded plants for plants in soil (Table 1). In PB, Final GI was higher in non-flooded plants than in flooded plants for plants (Table 1). However, in soil final GI in non-flooded plants was higher than in plants flooded for 3 days (Table 1). In both flood treatments PB dried down more slowly than soil (Fig. 2). In general, substrate percent moisture of PB and soil showed a similar rate of drying, but overall values were different (Fig. 2). Substrates flooded for 3 days dried down more slowly during the fourth and sixth flood cycle with substrate percent moisture drying to 43% and 44% in PB and 22% and 26% in soil. Substrate percent moisture during draining was highest in PB and soil (42.5% and 21.2%) during the fourth flood cycle in the 6 day flood treatments (Fig. 2). Substrates flooded for 6 days dried down the most during the second flood cycle with percent moisture reaching 36% and 15% in PB and soil, respectively (Fig. 2). All plants survived.

***Itea virginica* 'Henry's Garnet'**

In PB, RDW and SDW were lowest for plants flooded for 6 days (Table 2). In soil, RDW was higher in non-flooded plants than in flooded plants (Table 2). In soil, SDW was lowest in plants flooded for 6 days (Table 2). In PB, RGI and final GI were highest in non-flooded plants and lowest in plants flooded for 6 days (Table 2). In PB and soil, RGI and final GI were higher in non-flooded plants than in flooded plants (Table 2). Substrate percent moisture decreased more slowly in PB than in soil (Fig. 3). Substrate percent moisture in PB flooded for 3 days was lowest during dry down of the fifth cycle reaching 11% (Fig. 3). Soil flooded for 3 days dried down the most during the fourth cycle reaching 7% (Fig. 3). In general, substrate percent moisture of PB and soil showed a similar rate of drying, but overall values were different (Fig. 3). Substrate percent moisture of had a quantitative response between substrates and dried down more slowly in PB than in soil when flooded for 6 days (Fig. 3). Percent moisture was lowest during dry down of the third cycle reaching 33.6% and 9.4% in PB and soil, respectively in the 6 day flood treatment. All plants survived.

Viburnum nudum 'Winterthur'

In PB and soil, final GI and RGI were not different among treatments (Table 3). Similarly, in PB, RDW and SDW were not different among treatments (Table 3). PB dried down more slowly than soil (Fig. 4). In general, substrate percent moisture of PB and soil showed a similar rate of drying, but overall values were different (Fig. 4). Substrate percent moisture in PB was lowest during dry down of the fourth flood cycle in the 3 day flood treatment and reached 23% (Fig. 4). Substrate percent moisture in soil was lowest during dry down of the second flood cycle in the 3 day treatment and reached 13% (Fig. 4). Substrate percent moisture in PB was highest during dry down of the first flood cycle in the 6 day flood treatment (37.5%) and lowest during dry down of the last flood cycle (20%) (Fig 4). Substrate percent moisture in soil was lowest during dry down of the first flood cycle (11.8%) of the 6 day flood treatment and highest during dry down of the fourth flood cycle (25.3%) (Fig. 4). All plants survived.

Outdoor Flooding

***Ilex glabra* 'Shamrock'**

In PB and CC, relative growth index (RGI) was not affected by flooding treatments in the greenhouse or outdoors (Table 4). For both substrates, SDW and RDW were generally more affected by greenhouse flooding than by outdoor flooding, and were highest for plants that were non-flooded in the greenhouse followed by plants that were flooded for 3 and 6 days in the greenhouse (Table 4). For both substrates, RDW : SDW was generally more affected by greenhouse flooding than by outdoor flooding, and overall, RDW : SDW was lowest in plants that were flooded for 6 days in the greenhouse (Table 5). Ps of plants in PB was not significantly affected by greenhouse flooding or outdoor flooding or the by the combination of greenhouse flooding and outdoor flooding. There was a significant interaction between greenhouse flooding and outdoor flooding for SC of plants in PB and SC was lowest when plants were flooded for 6 days in the greenhouse and outdoors (Table 6). Ps and SC of plants in CC were more affected by greenhouse flooding than outdoor flooding. For plants in CC, Ps and SC were higher in plants that were non-flooded in the greenhouse (13.9 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 0.24 $mmol \cdot m^{-2} \cdot s^{-1}$) than in plants that were flooded for 3 (11.9 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 0.2 $mmol \cdot m^{-2} \cdot s^{-1}$) or 6 days in the greenhouse (11.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 0.18 $mmol \cdot m^{-2} \cdot s^{-1}$). Beginning with draining of the 4th flood cycle for both flooding treatments, PB began to dry down more slowly than in the beginning of the experiment (Fig. 5). Substrate percent moisture of CC showed similar trends during dry down for all draining cycles (Fig. 6). EC and pH of leachates were higher in PB than in CC and were similar in the 3 and 6 day flood treatments (Table 7).

Itea virginica **'Henry's Garnet'**

For plants in PB, RGI was generally more affected by greenhouse flooding than by flooding outdoors (Table 8) and was higher in plants that were flooded for 3 or 6 days in the greenhouse (Table 8). For plants in CC, RGI was not affected by flooding treatments in the greenhouse or outdoors and was similar overall among flooding treatments applied in the greenhouse and outdoors (Table 8). For both substrates, SDW and RDW were generally more affected by greenhouse flooding than by outdoor flooding and were lower overall in plants that were flooded for 6 days in the greenhouse (Table 8). For plants in PB, RDW : SDW was generally more affected by greenhouse flooding than by flooding outdoors and was generally lower in plants that were flooded for 6 days in the greenhouse (Table 5). For plants in CC, RDW : SDW was not affected by flooding treatments in the greenhouse or outdoors and was similar overall among flooding treatments applied in the greenhouse and outdoors (Table 5). Ps and SC of plants in both substrates were not affected by greenhouse flooding or outdoor flooding, and data were therefore pooled across all treatment combinations. For both substrates, Ps and SC was generally higher during flooding and draining at the end of the experiment than midway through the experiment (Table 9). Ps of plants in PB was more affected by flooding treatments applied outdoors and was generally higher in plants that were flooded for 3 days outdoors (10.2 $\mu mol \cdot m^{-2} \cdot s^{-1}$) than in plants that were flooded for 6 days outdoors (9.0 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Beginning with draining of the 5th flood cycle of plants flooded for 3 days and the 4th flood cycle of plants flooded for 6 days, PB began to dry down more slowly than in the beginning of the experiment (Fig. 7). Substrate percent moisture of CC showed similar trends during dry down for all draining cycles (Fig. 8). EC and pH of leachates were similar in the 3 and 6 day flood treatments

(Table 7). PB and CC pH of leachates were the similar, but EC was higher in PB than in CC (Table 7).

***Viburnum nudum* ‘Winterthur’**

RGI, SDW, and RDW of plants in PB were generally more affected by outdoor flooding than by greenhouse flooding (Table 10) and were higher overall for plants that were non-flooded outdoors, followed by plants that were flooded for 3 and 6 days outdoors (Table 10). RGI of plants in CC was more affected by outdoor flooding than by greenhouse flooding and was higher overall in plants that were flooded for 6 days outdoors than in plants were non-flooded or flooded for 3 days outdoors (Table 10). SDW and RDW for plants in CC were generally more affected by outdoor flooding than by greenhouse flooding and were generally higher in plants that were flooded for 3 days outdoors than in plants that were flooded for 3 or 6 days outdoors (Table 10). RDW : SDW of plants in PB was generally more affected by greenhouse flooding than by outdoor flooding and was higher in plants that were flooded for 3 or 6 days in the greenhouse and lower in plants that were non-flooded in the greenhouse (Table 5). RDW : SDW in CC did not appear to be affected by greenhouse flooding or outdoor flooding (Table 5). There was a significant interaction for Ps and SC of plants in PB between flooding treatments applied outdoors and stage within a flood cycle which Ps and SC were measured. Ps of plants in PB was generally higher during flooding and draining at the end of the experiment than midway through the experiment (Table 11). Within outdoor flooding treatments for plants in PB, Ps was lower during draining of the final cycle for plants flooded for 3 days outdoors (Table 11). SC of plants in PB was higher during final flooding across all outdoor flooding treatments (Table 11). Within outdoor flooding treatments for plants in PB, SC was higher during draining of the final cycle for plants flooded for 6 days outdoors (Table 11). Ps of plants in CC was not significantly affected by greenhouse flooding or outdoor flooding, and data were therefore pooled across all treatment combinations. Ps of plants in CC was highest during draining of both cycles measured and lowest during flooding midway through the experiment (Table 12). There was an interaction for SC of plants in CC between flooding treatments applied outdoors and stage within a flood cycle which Ps and SC were measured. SC of plants in CC was generally lower during flooding and draining midway through the experiment for plants flooded for 6 days outdoors and generally higher during flooding and draining at the end of the experiment for plants flooded for 6 days (Table 12). Beginning with draining of the 5th flood cycle of plants flooded for 3 days and the 4th flood cycle of plants flooded for 6 days, PB began to dry down more slowly than in the beginning of the experiment (Fig. 9). Substrate percent moisture of CC showed similar trends during dry down for all draining cycles (Fig. 10). For plants in PB, leachates of plants flooded for 6 days had a higher pH than plants flooded for 3 days and EC was higher in plants flooded for 3 days than in plants flooded for 6 days (Table 7). For plants in CC, leachates of plants flooded for 3 days had a higher pH than in plants flooded for 6 days and EC was similar between plants flooded for 3 and 6 days (Table 7).

Discussion

Although not compared statistically, values for RGI suggest differences in growth rates among taxa. When greenhouse flooding treatments were discontinued, *I. virginica* 'Henry's Garnet' had the largest overall RGI (Table 2) followed by *V. nudum* 'Winterthur' (Table 3) and *I. glabra* 'Shamrock' (Table 1). *I. virginica* 'Henry's Garnet' and *I. glabra* 'Shamrock' had the largest differences among treatments for Final GI in the greenhouse, but *V. nudum* 'Winterthur' had no differences among treatments (Table 1 – 3). Slower rates of dry down at the end of the experiment in PB and soil containing *I. virginica* 'Henry's Garnet' indicated decreased water uptake by roots and decreased root growth which correlated with lower RDW in plants flooded for 6 days during greenhouse flooding (Fig. 3, Table 2). *I. glabra* 'Shamrock' had similar rates of dry down when flooded for 3 and 6 days which correlated with similar RDW of plants flooded for 3 and 6 days during greenhouse flooding (Fig. 2, Table 1). RDW and SDW were lower in flooded plants than in non-flooded plants of *I. virginica* 'Henry's Garnet' (PB and soil) and *I. glabra* 'Shamrock' (PB) (Table 1, 2) which was consistent with others who observed reduced RDW and SDW in flooded plants (Stewart et al., 2007; Stevens and Prior, 1994; Larson et al., 1993; Pezeshki, 2001a). Root growth is an energy dependent process that occurs in the presence of oxygen, and under flooded conditions, reduced root growth can be expected (Pezeshki, 2001a). However, *V. nudum* 'Winterthur' RDW and SDW were not different among treatments (Table 3). During greenhouse flooding, plants flooded in soil experienced increased drought conditions during draining compared to plants in PB since soil dried down much more quickly than PB (Fig. 1 – 3). It can be assumed that soil in a container does not behave in the same manner as it might in the field with respect to water holding capacity and draining.

Although not compared statistically, values for RGI at the end of outdoor flooding also suggest differences in growth rates among taxa. When outdoor flooding treatments were discontinued, *I. virginica* 'Henry's Garnet' had the largest overall RGI (Table 8), followed by *V. nudum* 'Winterthur' (Table 10), and *I. glabra* 'Shamrock' (Table 4), which was similar to RGI results observed when greenhouse flooding treatments ended (Table 1 – 3). *I. virginica* 'Henry's Garnet' appeared to be more affected by flooding in the greenhouse than by flooding outdoors which was evident by smaller treatment differences that occurred during outdoor flooding (Table 8). During outdoor flooding, *I. virginica* 'Henry's Garnet' seemed to recover from greenhouse flooding and may have been due to a fast growth rate. This is consistent with Bailey (2009) who found that the fast growth rate of *I. virginica* 'Henry's Garnet' made treatment differences in their research difficult to observe. *V. nudum* 'Winterthur' appeared to be more affected by outdoor flooding than by greenhouse flooding, which was evident by increased treatment differences that occurred during outdoor flooding (Table 10). RGI of *I. glabra* 'Shamrock' did not appear to be affected by flooding treatments applied in the greenhouse or outdoors which was evident by no differences among flood treatment combinations from greenhouse and outdoor flooding (Table 4). In contrast to RGI, RDW, SDW, and RDW : SDW of *I. glabra* 'Shamrock' appeared to be more affected by flooding treatments applied in the greenhouse than by flooding treatments applied outdoors (Table 4, 5). RGI of *I. glabra* 'Shamrock' reflected the fact that *I. glabra* is considered slow growing, and the cultivar 'Shamrock' has a slower rate of growth than other cultivars of this species (Dirr, 1998). *I. glabra* 'Shamrock' and *I. virginica* 'Henry's Garnet' that were flooded in the greenhouse had decreased root growth going into outdoor flooding compared to non-flooded plants (Table 1, 2). By the end of outdoor flooding, RDW : SDW of *I. glabra* 'Shamrock' and *I. virginica* 'Henry's Garnet' were lower in plants that were flooded for 6 days in the greenhouse. Plants that were flooded for 3 days in the greenhouse had higher RDW : SDW despite flooding treatments experienced outdoors indicating that pre-flooding plants for 3 days of flooding several times may pre-condition plants to tolerate waterlogged conditions (Table 14).

Although not compared statistically, rates of Ps and SC also suggest differences among taxa. Ps and SC rates were highest overall in *I. glabra* 'Shamrock' (Table 7) followed by *V. nudum* 'Winterthur' (Tables 10 – 12) and *I. virginica* 'Henry's Garnet' (Table 8, 9). How species compared in terms of RGI outdoors was inversely proportional to how they compared for Ps and SC rates. It is not clear why this is, but *V. nudum* 'Winterthur' was intermediate in terms of RGI and Ps and SC which seemed to make treatment differences more easily observed during outdoor flooding. Tolerance to flooded conditions can be associated with maintaining adequate rates of photosynthesis and stomatal conductance (Arbona et al., 2009; Gravatt and Kirby, 1998). Ps and SC were more affected by flooding treatments applied in the greenhouse than by flooding treatments applied outdoors in *I. glabra* 'Shamrock' and *I. virginica* 'Henry's Garnet' (Table 7 – 9). Ps and SC of *I. virginica* 'Henry's Garnet' and *V. nudum* 'Winterthur' were generally higher during final flooding and draining cycles (Table 8 – 11) which is consistent with others who found higher Ps and SC rates during later flood cycles of intermittently flooded plants that were considered to be flood tolerant (Pezeshki and Anderson, 1997, 1999).

Adventitious root growth was noted in lower stem portions at the soil line of *I. virginica* 'Henry's Garnet' during outdoor flooding. Pezeshki and Anderson (1997) suggested that formation of adventitious roots in their study may have contributed to re-opening of stomata resulting in higher rates of Ps and SC later in the experiment. Adventitious roots may also develop at the shoot base in aerated soil layers (Blom and Voesenek, 1996) as a result of flooding and signal severe damage to a plant's original root system (Robbani et al., 2006; Mitsch and Gosselink, 2007). Excess water in the root zone prevents transfer of oxygen and other gases between the soil and atmosphere (Drew, 1997). Plants having shallow root systems are less likely to experience severe anoxic conditions associated with lower soil layers due to root growth occurring in the oxidized rhizosphere (Blom and Voesenek, 1996; Nilson and Orcutt, 1996). During outdoor flooding of this study, new root growth occurred in flooded plants of all taxa and was consistently found in the upper portion of the substrate which was likely to be partially aerated. Surface rooting is common in flood tolerant plants and allows roots to function in a hypoxic state where they can undergo partial aerobic respiration (Nilsen and Orcutt, 1996).

Under completely submerged situations or when roots must carry out anaerobic respiration, the risk for carbohydrate starvation is great because of the inefficiency associated with anaerobic respiration.

Tubs were used during outdoor flooding to create conditions plants might experience in a rain garden. Studies in a greenhouse are helpful in controlling water table depths but usually suffer some limitations such as reduced volumes of soil or substrate (Megonigal and Day, 1992) associated with container sizes and space constraints. Mesocosms (tubs) have been used extensively to mimic field conditions for various wetland and aquatic studies (Ahn and Mitsch, 2002). This study may have better evaluated flooding rather than drought. However, drought research conducted on *I. virginica* 'Henry's Garnet' found that plants were still visually acceptable when surrounding soil percent moisture reached 15% (Bailey, 2009). Moreover, a rootball may experience drought conditions even when the surrounding soil is considered well-watered, which is why monitoring root ball percent moisture is more accurate in determining plant drought stress. In this study, soil moisture sensors were placed in the substrate rather than the root ball. Evaluating root ball percent moisture may have indicated that plants in CC experienced increased drought during draining than previously thought.

All taxa evaluated in this study appear to be tolerant of flooding which would be likely to occur in a rain garden. It is suggested that plants for rain gardens be able to withstand 2 days of standing water (Dussaillant et al., 2005), however, this is merely a suggestion and is dependent on soil characteristics, amount of precipitation received, and the size and depth of the rain garden (Hunt, 2001). Plants in this study experienced more flooding than would typically occur in a rain garden and this study evaluated flood tolerance more so than flood and drought tolerance. Results indicate that *I. virginica* 'Henry's Garnet' may become less sensitive to flooding with maturity, but that *V. nudum* 'Winterthur' may become more sensitive to flooding with maturity. *I. glabra* 'Shamrock' seems to maintain consistent tolerance of flooding at any stage of growth. At the end of outdoor flooding, all taxa in all treatments and substrates maintained good plant visual quality and new growth along with satisfactory rates of Ps and SC which suggests their tolerance to cyclic flooding.

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Table 1. Effect of length of flooding cycle (treatment) on relative growth index (RGI), final growth index (GI), root dry weight (RDW), and shoot dry weight (SDW) of *Ilex glabra* 'Shamrock' grown in a greenhouse in Auburn, Ala. Plants in the 3 and 6 day flood treatments experienced a total of 7 and 5 flood cycles, respectively. Treatments initiated on 2 Apr. 2009 (170 DAP) and ended on 4 Jun. 2009 (233 DAP).

	Pinebark : Peat : Perlite (PB)				Soil			
Treatment (Days)	Final GI[z] (cm)	RGI[y]	RDW (g)	SDW (g)	Final GI (cm)	RGI	RDW (g)	SDW (g)
0	16.9a[x]	0.4a	5.2a	12.6a	15.8a	0.3a	3.7a	6.6a
3	15.3b	0.4a	1.6b	5.1b	15.1ab	0.2b	4.2a	5.1a
6	13.4c	0.2b	1.6b	3.6b	14.4b	0.1b	3.3a	5.5a

[z] Final GI = (height + widest width + width perpendicular to widest width)/3.

[y] RGI = (Final GI – Initial GI)/Initial GI.

[x] Lowercase letters denote mean separation using contrast statements in the Glimmix procedure at $p<0.05$ (Sas Institute, 2004).

Table 2. Effect of length of flooding cycle (treatment) on relative growth index (RGI), final growth index (GI), root dry weight (RDW), and shoot dry weight (SDW) of *Itea virginica* 'Henry's Garnet' grown in a greenhouse in Auburn, Ala. Plants in the 3 and 6 day flood treatments experienced a total of 7 and 5 flood cycles, respectively. Treatments initiated on 18 Mar. 2009 (155 DAP) and ended on 19 May 2009 (217 DAP).

Treatment (Days)	Pinebark : Peat : Perlite (PB)				Soil			
	Final GI[z] (cm)	RGI[y]	RDW (g)	SDW (g)	Final GI (cm)	RGI	RDW (g)	SDW (g)
0	66.6a[x]	2.1a	12.9a	23.7a	52.8a	1.6a	15.8a	18.3a
3	56.5b	1.5b	13.9a	23.8a	35.2b	0.7b	8.4b	13.8ab
6	38.4c	0.8c	7.0b	13.5b	33.6b	0.6b	5.9b	10.2b

[z] Final GI = (height + widest width + width perpendicular to widest width)/3.

[y] RGI = (Final GI – Initial GI)/Initial GI.

[x] Lowercase letters denote mean separation using contrast statements in the Glimmix procedure at p<0.05 (Sas Institute, 2004).

Table 3. Effect of length of flooding cycle (treatment) on relative growth index (RGI), final growth index (GI), root dry weight (RDW), and shoot dry weight (SDW) of *Viburnum nudum* ‘Winterthur’ grown in a greenhouse in Auburn, Ala. Plants in the 3 and 6 day flood treatments experienced a total of 7 and 5 flood cycles, respectively. Treatments were initiated in PB on 24 Mar. 2009 (161 DAP) and ended on 26 May 2009 (224 DAP); treatments were initiated in soil on 8 Apr. 2009 (176 DAP) and ended on 10 June 2009 (239 DAP).

	Pinebark : Peat : Perlite (PB)				Soil	
Treatment (Days)	Final GI[z] (cm)	RGI[y]	RDW (g)	SDW (g)	Final GI (cm)	RGI
0	39.6a[x]	1.3a	11.6a	19.5a	20.4a	0.4a
3	41.0a	1.5a	18.7a	28.0a	20.6a	0.3a
6	39.3a	1.5a	18.4a	27.0a	20.5a	0.3a

[z] Final GI = (height + widest width + width perpendicular to widest width)/3.

[y] RGI = (Final GI – Initial GI)/Initial GI.

[x] Lowercase letters denote mean separation using contrast statements in the Glimmix procedure at $p<0.05$ (Sas Institute, 2004).

Table 4. Effect of combination of flooding treatments applied in the greenhouse and then outdoors on root dry weight (RDW), shoot dry weight (SDW), and relative growth index (RGI) of *Ilex glabra* ‘Shamrock’ grown in Auburn, Ala. Plants flooded in the greenhouse were held in a greenhouse for 40 days after treatments ended. Plants flooded for 3 and 6 days in the greenhouse and outdoors experienced a total of 7 and 5 flood cycles, respectively.

		Pine bark : Peat : Perlite (PB)			Calcined Clay (CC)		
Greenhouse Flooding	Outdoor Flooding	RGI[y]	SDW (g)	RDW (g)	RGI[y]	SDW (g)	RDW (g)
0	0	0.37a	22.8a	8.3ab	0.17a	9.6a	13.2b
0	3	0.27a	21.2a	8.5a	0.16a	9.1a	16.3a
0	6	0.22a	24.1a	8.8a	0.12a	8.7ab	15.1ab
3	0	0.23a	16.6b	6.5bc	0.05a	7.1bc	9.5c
3	3	0.25a	15.6b	5.7cd	0.13a	6.3bcd	9.3cd
3	6	0.23a	16.6b	5.0cde	0.13a	5.7cd	9.3cd
6	0	0.37a	10.7c	3.5def	0.15a	4.5def	8.2cd
6	3	0.32a	10.9c	3.2ef	0.05a	4.0ef	6.5d
6	6	0.27a	10.9c	3.0f	0.07a	3.7f	8.1cd

[z] Treatment combinations were based on flooding treatments applied in the greenhouse for 0 (non-flooded), 3, and 6 days of flooding in combination with flooding treatments applied outdoors for 0 (non-flooded), 3, or 6 days of flooding.

[y] RGI=(Final GI – Initial GI)/Initial GI during outdoor flooding.

[x] Lowercase letters denote mean separation within a column using contrast statements in the Glimmix procedure (p<0.05).

Table 5. RDW : SDW of *Ilex glabra* ‘Shamrock’, *Itea virginica* ‘Henry’s Garnet’, and *Viburnum nudum* ‘Winterthur’ in outdoor flooding. Treatments were in a factorial combination of flooding treatment from greenhouse flooding and flooding treatment from outdoor flooding. For *I. glabra* ‘Shamrock’ greenhouse flooding treatments initiated on 2 Apr. 2009 and ended on 4 Jun. 2009; outdoor flooding treatments initiated on 23 July 2009 and ended on 23 Sept. 2009. For *I. virginica* ‘Henry’s Garnet’ greenhouse flooding treatments initiated on 18 Mar. 2009 and ended on 19 May 2009; outdoor flooding treatments initiated on 7 July 2009 and ended on 7 Sept. 2009. For *V. nudum* ‘Winterthur’ in PB greenhouse flooding treatments initiated on 24 Mar. 2009 and ended on 26 May 2009; outdoor flooding treatments initiated on 14 July 2009 and ended on 14 Sept. 2009. For *V. nudum* ‘Winterthur’ in CC in greenhouse flooding treatments initiated on 8 Apr. 2009 and ended on 10 June 2009; outdoor flooding treatments initiated on 29 July 2009 and ended on 28 Sept. 2009.

		Ilex glabra 'Shamrock'		*Itea virginica* 'Henry's Garnet'		*Viburnum nudum* 'Winterthur'	
		PB	CC	PB	CC	PB	CC
Greenhouse Flooding[z]	Outdoor Flooding	RDW : SDW[y]	RDW : SDW	RDW : SDW	RDW : SDW	RDW : SDW	RDW : SDW
0	0	0.36abc[x]	0.73a	0.44abc	1.22a	0.45b	1.30ab
0	3	0.42a	0.58abc	0.44abc	0.78b	0.44b	1.11ab
0	6	0.37abc	0.60abc	0.44abc	0.96ab	0.45b	0.96b
3	0	0.38ab	0.75a	0.50a	0.91ab	0.55a	1.60a
3	3	0.38ab	0.68ab	0.45ab	1.11ab	0.51ab	1.29ab
3	6	0.30cd	0.62ab	0.46ab	1.10ab	0.47ab	1.23ab
6	0	0.33bcd	0.56bc	0.41bcd	0.96ab	0.49ab	1.38a
6	3	0.30d	0.61abc	0.37cd	0.94ab	0.45ab	1.21ab
6	6	0.27d	0.46c	0.35d	0.79b	0.42b	0.92b

[z] Treatment combinations were based on flooding treatments applied in the greenhouse for 0 (non-flooded), 3, and 6 days of flooding in combination with flooding treatments applied outdoors for 0 (non-flooded), 3, or 6 days of flooding.

[y] RDW : SDW = RDW/SDW.

[x] Lowercase letters denote mean separation using contrast statements in the Glimmix procedure ($p<0.05$) within a column.

Table 6. Effect of flooding treatments applied in the greenhouse and then outdoors on stomatal conductance (SC) of *Ilex glabra* 'Shamrock' grown in PB. Treatments were in a factorial combination of flooding treatment from greenhouse flooding and flooding treatment from outdoor flooding. Greenhouse flooding treatments initiated on 2 Apr. 2009 and ended on 4 Jun. 2009. Outdoor flooding treatments initiated on 23 July 2009 and ended on 23 Sept. 2009.

Greenhouse Flooding	Outdoor Flooding	SC ($mmol \cdot m^{-2} \cdot s^{-1}$)
0	0	0.24a
0	3	0.21b
0	6	0.33a
3	0	0.31a
3	3	0.25a
3	6	0.29a
6	0	0.25a
6	3	0.27a
6	6	0.22b

[z] Greenhouse flooding treatments were flooded for 0 (non-flooded), 3, or 6 days in trade gal pots.

[y] Outdoor flooding treatments were flooded for 0 (non-flooded), 3, or 6 days in 45 gal tubs under a shade structure. There were 3 plants per tub with one each that had been flooded for 0, 3, or 6 days in a greenhouse.

[x] Letters denote means separation within a column using Tukey in the Glimmix Procedure at $p<0.05$ (Sas Institute, 2004).

Table 7. Electrical conductivity (EC) and pH during draining of tubs that were flooded for 3 and 6 days during outdoor flooding. EC and pH measurements were recorded during the last draining cycle on 3 tubs per treatment within a taxon and substrate combination.

		Substrate			
		PB		CC	
	Treatment[z]	pH	EC	pH	EC
Ilex glabra 'Shamrock'	3	6.2	1.6	5.5	0.7
	6	6.4	1.3	5.6	0.8
Itea virginica 'Henry's Garnet'	3	5.1	2.6	5.1	0.6
	6	5.4	2.3	5.4	0.9
Viburnum nudum 'Winterthur'	3	4.9	2.2	6.1	0.5
	6	6.1	1.6	5.6	0.6

[z] Treatments were based on days of flooding. Plants were flooded for 3 or 6 days.

Table 8. Effect of combination of flooding treatment applied in the greenhouse and then outdoors on root dry weight (RDW), shoot dry weight (SDW), and relative growth index (RGI) of *Itea virginica* 'Henry's Garnet' grown in Auburn, Ala. Plants flooded in the greenhouse were held in a greenhouse for 40 days after treatments ended. Plants flooded for 3 and 6 days in the greenhouse and outdoors experienced a total of 7 and 5 flood cycles, respectively.

		Pine bark : Peat : Perlite (PB)			Calcined Clay (CC)		
Greenhouse Flooding[z]	Outdoor Flooding[z]	RGI[y]	SDW (g)	RDW (g)	RGI[y]	SDW (g)	RDW (g)
0	0	0.48c[x]	58.0ab	27.0a	0.85a	31.7ab	40.4a
0	3	0.48c	60.8a	29.0a	0.63ab	33.3a	25.9bc
0	6	0.57bc	59.2a	24.1ab	0.62ab	30.8ab	27.9abc
3	0	0.88a	57.5ab	26.3a	0.78ab	25.8bc	23.5bc
3	3	0.80ab	58.3a	24.5ab	0.68ab	30.0ab	33.4ab
3	6	0.77ab	63.3a	25.5a	0.52b	27.5abc	30.4ab
6	0	0.75ab	41.7c	18.2bc	0.85a	30.0ab	27.8bc
6	3	1.02a	41.7c	12.0c	0.72ab	26.7bc	24.8bc
6	6	0.80ab	42.5bc	13.2c	0.65ab	22.5c	17.4c

[z] Treatment combinations were based on flooding treatments applied in the greenhouse for 0 (non-flooded), 3, and 6 days of flooding in combination with flooding treatments applied outdoors for 0 (non-flooded), 3, or 6 days of flooding.

[y] RGI=(Final GI – Initial GI)/Initial GI during outdoor flooding.

[x] Lowercase letters denote mean separation within a column using contrast statements in the Glimmix procedure ($p<0.05$).

Table 9. Effect of time on photosynthesis (Ps) and stomatal conductance (SC) of *Itea virginica* ‘Henry’s Garnet’ grown in PB and CC.

	Pine bark : Peat : Perlite (PB)		Calcined Clay (CC)	
Cycle Stage[z]	Ps ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	SC ($mmol \cdot m^{-2} \cdot s^{-1}$)	Ps ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	SC ($mmol \cdot m^{-2} \cdot s^{-1}$)
Intermediate flooding	8.7c[y]	0.11b	8.4b	0.15b
Intermediate draining	9.1bc	0.14b	8.6b	0.16b
Final flooding	10.7a	0.26a	10.2a	0.28a
Final draining	10.1ab	0.23a	10.1a	0.28a

[z] Ps and SC were measured on the last day of flooding and draining within a flood cycle during the intermediate flood cycle and final flood cycle. Ps and SC were measured in non-flooded plants concurrently with flooded plants.

[y] Letters denote means separation within a column using Tukey in the Glimmix Procedure at $p<0.05$ (Sas Institute, 2004).

Table 10. Effect of combination of flooding treatment applied in the greenhouse and then outdoors on root dry weight (RDW), shoot dry weight (SDW), and relative growth index (RGI) of *Viburnum nudum* 'Winterthur' grown in Auburn, Ala. Plants flooded in the greenhouse were held in a greenhouse for 40 days after treatments ended. Plants flooded for 3 and 6 days in the greenhouse and outdoors experienced a total of 7 and 5 flood cycles, respectively.

		Pine bark : Peat : Perlite (PB)			Calcined Clay (CC)		
Greenhouse Flooding[z]	Outdoor Flooding[z]	RGI[y]	SDW (g)	RDW (g)	RGI[y]	SDW (g)	RDW (g)
0	0	0.73a	66.7a	29.6ab	0.11bc	12.9ab	15.3a
0	3	0.43bc	50.0b	21.7b	0.06c	15.2a	15.7a
0	6	0.36c	53.3ab	23.3b	0.4a	9.1bcd	8.5b
3	0	0.52ab	57.5ab	32.1a	0.02c	6.6d	10.4b
3	3	0.47bc	55.8ab	28.0ab	0.12bc	12.0abc	15.3a
3	6	0.42bc	50.0b	22.7b	0.16bc	9.6bcd	11.5ab
6	0	0.6ab	57.5ab	27.6ab	0.08bc	7.7cd	10.6b
6	3	0.52ab	48.3b	22.9b	0.08bc	10.4bcd	12.2ab
6	6	0.37c	53.3ab	22.8b	0.24abc	13.4ab	12.0ab

[z] Treatment combinations were based on flooding treatments applied in the greenhouse for 0 (non-flooded), 3, and 6 days of flooding in combination with flooding treatments applied outdoors for 0 (non-flooded), 3, or 6 days of flooding.

[y] RGI=(Final GI – Initial GI)/Initial GI during outdoor flooding.

[x] Lowercase letters denote mean separation within a column using contrast statements in the Glimmix procedure ($p<0.05$).

Table 11. Effect of flooding treatments applied during greenhouse flooding and outdoor flooding on photosynthesis (Ps) and stomatal conductance (SC) of *Viburnum nudum* ‘Winterthur’ grown in PB. Treatments were in a factorial combination of flooding treatment from greenhouse flooding and flooding treatment from outdoor flooding. Greenhouse flooding treatments initiated on 24 Mar. 2009 and ended on 26 May 2009. Outdoor flooding treatments initiated on 14 July 2009 and ended on 14 Sept. 2009.

	Ps ($\mu mol \cdot m^{-2} \cdot s^{-1}$)			SC ($mmol \cdot m^{-2} \cdot s^{-1}$)		
	Outdoor Flooding[y]			Outdoor Flooding		
Cycle Stage[z]	0	3	6	0	3	6
Intermediate flooding	10.9b[x]	10.6b	10.3b	0.14bc	0.15b	0.14b
Intermediate draining	10.7b	10.9b	10.3b	0.10c	0.10b	0.09b
Final flooding	13.6a	13.5a	12.0ab	0.32a	0.32a	0.37a
Final draining	12.1ab A	9.9b B	13.6a A	0.20b B	0.12b B	0.36a A

[z] Ps and SC were measured on the last day of flooding and draining within a flood cycle during the intermediate flood cycle and final flood cycle.

[y] Letters denote means separation using Tukey in the Glimmix Procedure at $p<0.05$ (Sas Institute, 2004). Lowercase letters represent means separation within a column for flooding treatments applied outdoors. Uppercase letters represent means separation within a row for stage within a flood cycle measured. If no differences then letters are omitted.

Table 12. Effect of flooding treatments applied in the greenhouse and then outdoors on stomatal conductance (SC) of *Viburnum nudum* 'Winterthur' grown in CC. Treatments were in a factorial combination of flooding treatment from greenhouse flooding and flooding treatment from outdoor flooding. Greenhouse flooding treatments initiated on 8 Apr. 2009 and ended on 10 June 2009. Outdoor flooding treatments initiated on 29 July 2009 and ended on 28 Sept. 2009.

Cycle Stage[z]	Ps (µmol·m-2·s-1)
Intermediate flooding	9.8c[y]
Intermediate draining	12.9a
Final flooding	11.6b
Final draining	11.9ab

	SC (mmol·m^{-2}·s^{-1})		
	Outdoor Flooding		
Cycle Stage	0	3	6
Intermediate flooding	0.18a	0.16a	0.20b
Intermediate draining	0.22a	0.19a	0.21b
Final flooding	0.19aB	0.24aAB	0.26bA
Final draining	0.22aB	0.18aB	0.36aA

[z] Ps and SC were measured on the last day of flooding and draining within a flood cycle during the intermediate flood cycle and final flood cycle.

[y] Letters denote means separation using Tukey in the Glimmix Procedure at $p<0.05$ (Sas Institute, 2004). Lowercase letters represent means separation within a column. Uppercase letters represent means separation within a row for stage within a flood cycle measured. If no differences then letters are omitted.

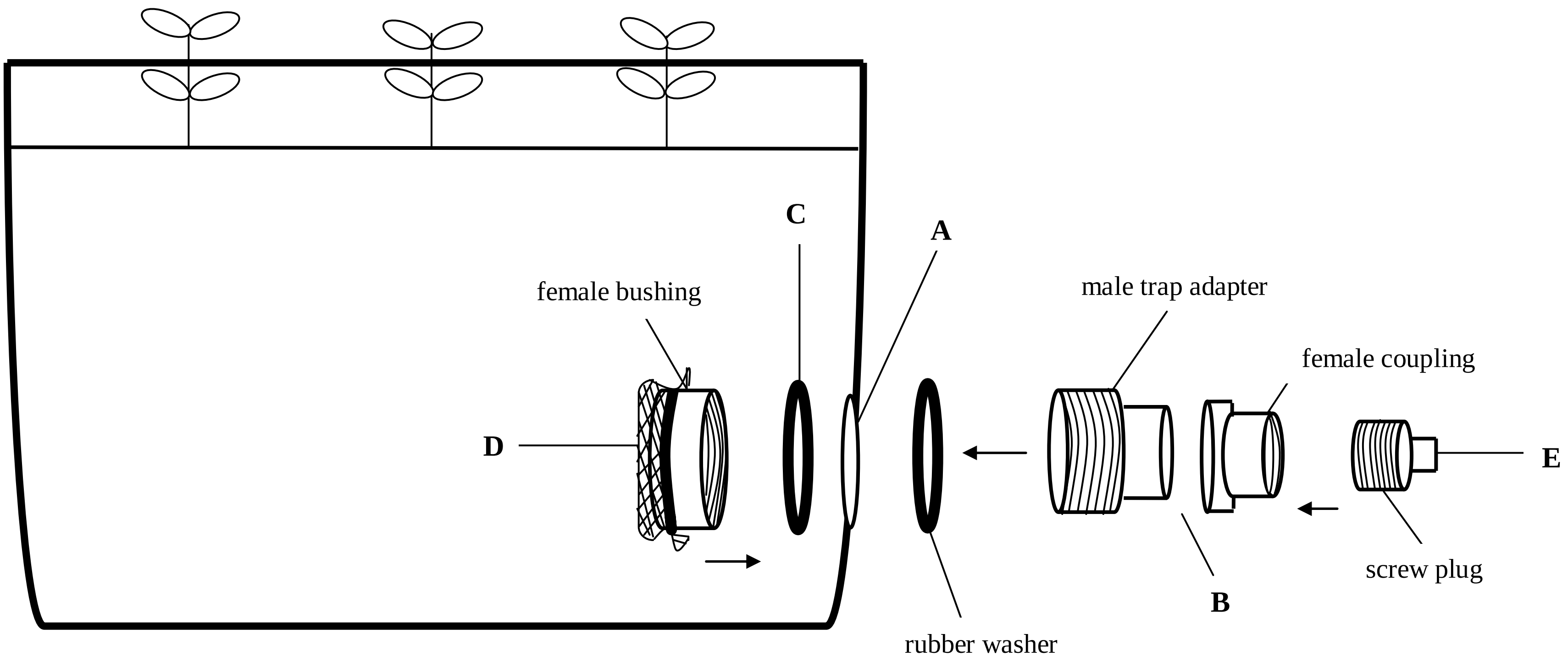
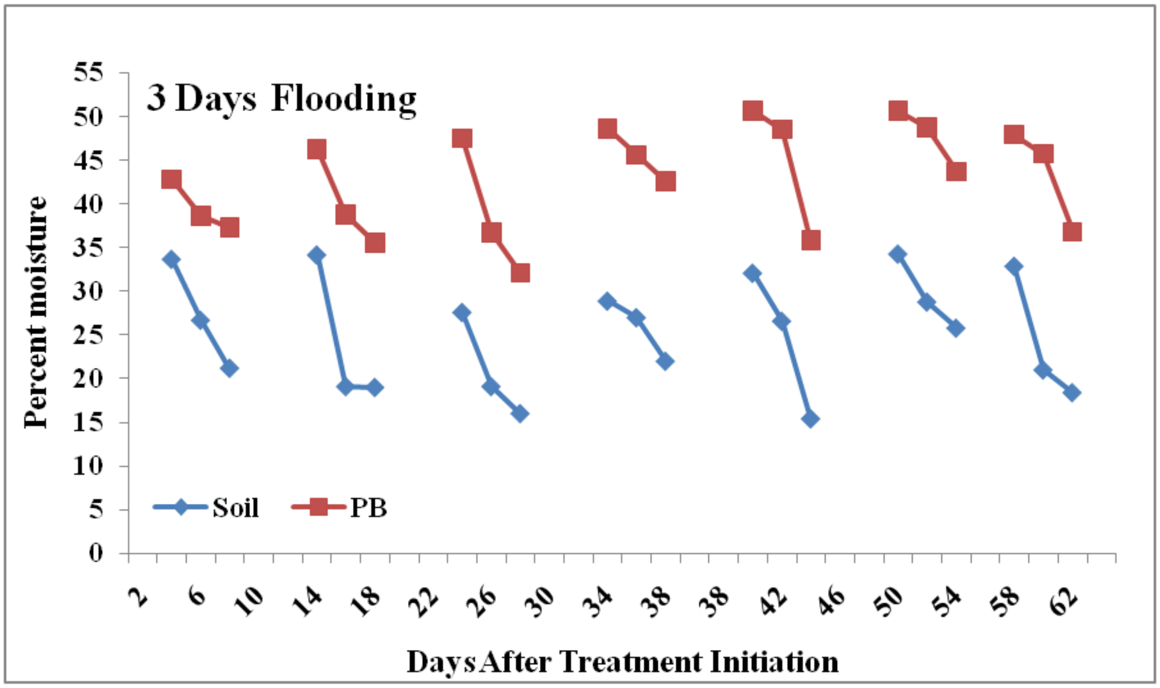


Figure 1. Tubs were modified using basic plumbing parts to include a drain. A 3.8 cm (1.5 in.) hole was centered 5 cm (2 in.) from tub bottom on one short side of each tub (A). The slip ends of a 3.8 cm (1.5 in.) female coupling and a 3.8 cm (1.5 in.) male trap adapter were cemented together using PVC cement (B). Rubber washers were placed around the hole on each side using a bead of pressure formed gasket maker (C). A 3.8 cm (1.5 in.) female bushing was modified to include a filter by using a plastic zip tie to adhere a small piece of window screening (D). A 3.8 cm (1.5 in.) screw plug (E) was tightened and loosened to enable flooding and draining.

Figure 2. Substrate percent moisture during draining for *Ilex glabra* 'Shamrock' flooded for 3 or 6 days. Flood cycles consisted of 3 or 6 days of flooding and 6 days of draining. Plants in the 3 and 6 days flood treatments experienced a total of 7 and 5 flood cycles, respectively. Plants were grown in a greenhouse in Auburn, Ala. and treatments initiated on 2 Apr. 2009 and ended on 4 Jun. 2009.



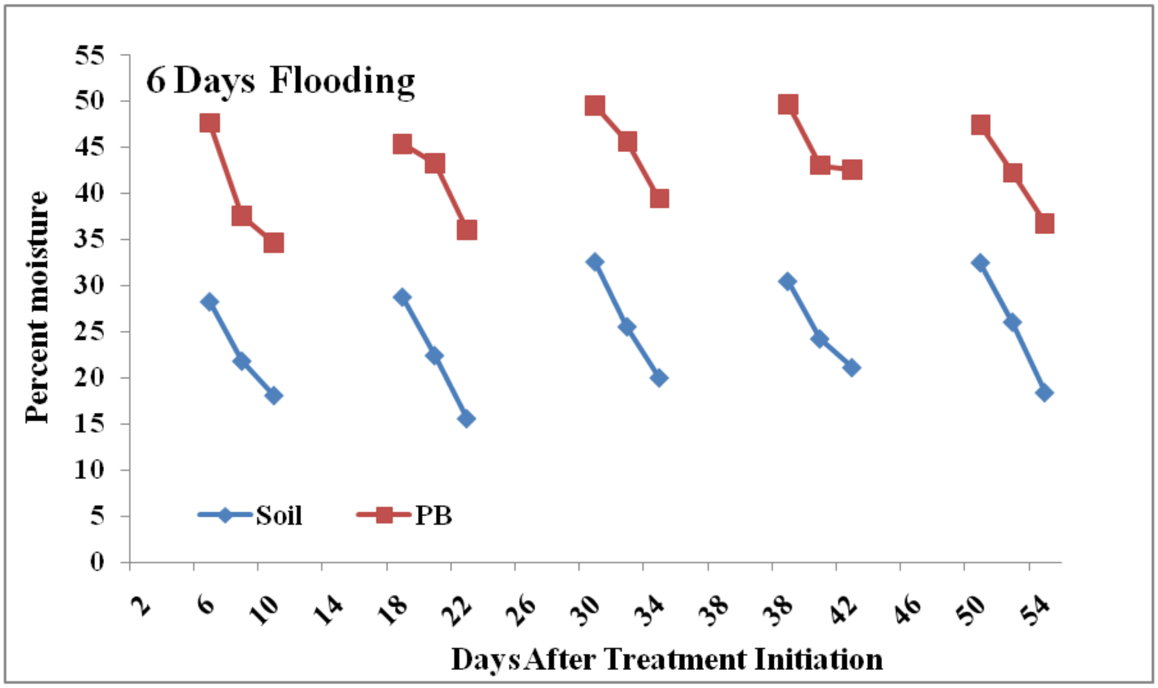


Figure 3. Substrate percent moisture during draining for *Itea virginica* ‘Henry’s Garnet’ flooded for 3 or 6 days. Flood cycles consisted of 3 or 6 days of flooding and 6 days of draining. Plants in the 3 and 6 days flood treatments experienced a total of 7 and 5 flood cycles, respectively. Plants were grown in a greenhouse in Auburn, Ala. and treatments initiated on 18 Mar. 2009 and ended 19 May 2009

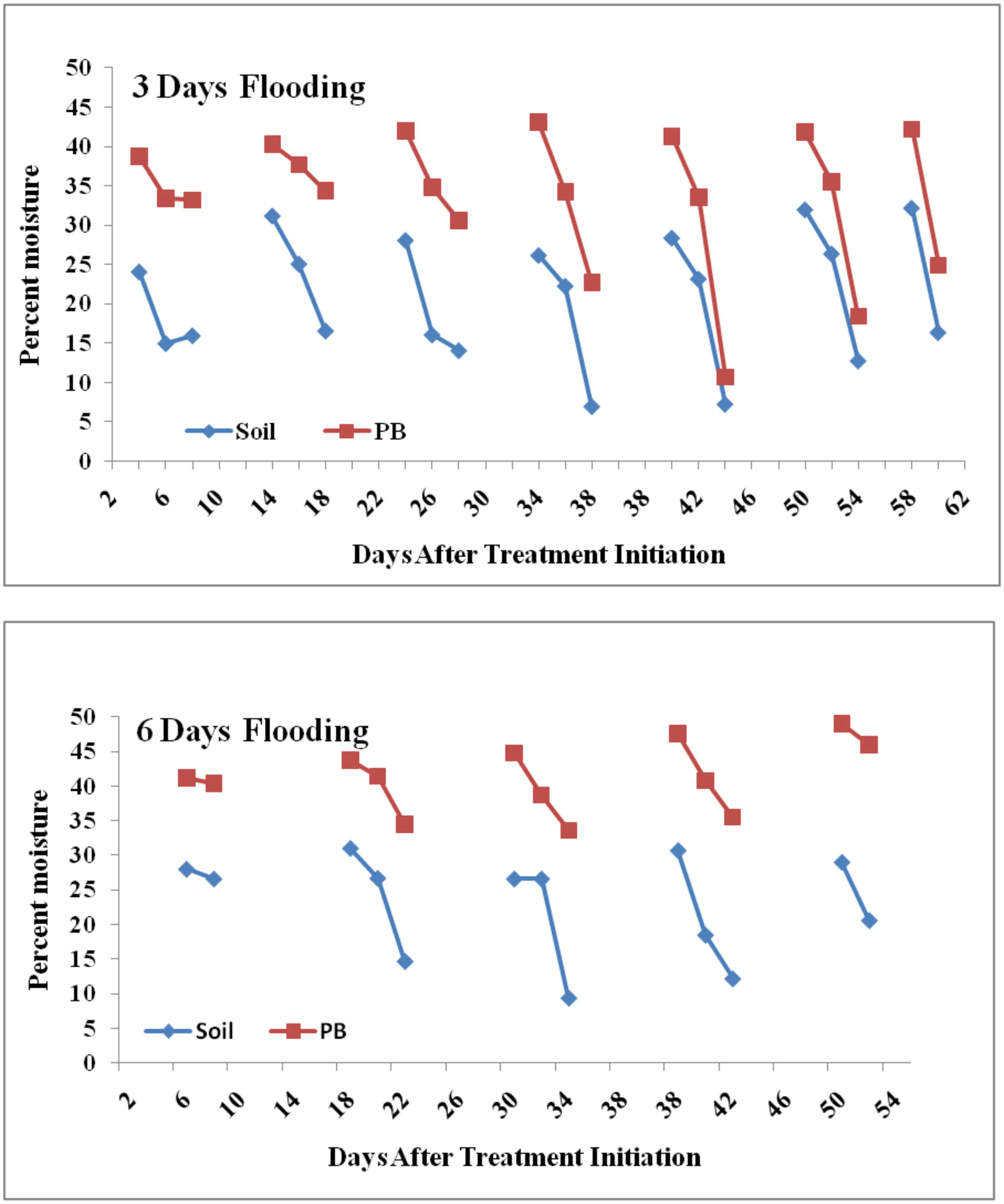


Figure 4. Substrate percent moisture during draining for *Viburnum nudum* 'Winterthur' flooded for 3 or 6 days. Flood cycles consisted of 3 or 6 days of flooding and 6 days of draining. Plants in the 3 and 6 days flood treatments experienced a total of 7 and 5 flood cycles, respectively. Plants were grown in a greenhouse in Auburn, Ala. Treatments were initiated in PB on 24 Mar. 2009 and ended on 26 May 2009; for plants in soil, treatments were initiated on 8 Apr. 2009 and ended on 10 June 2009.

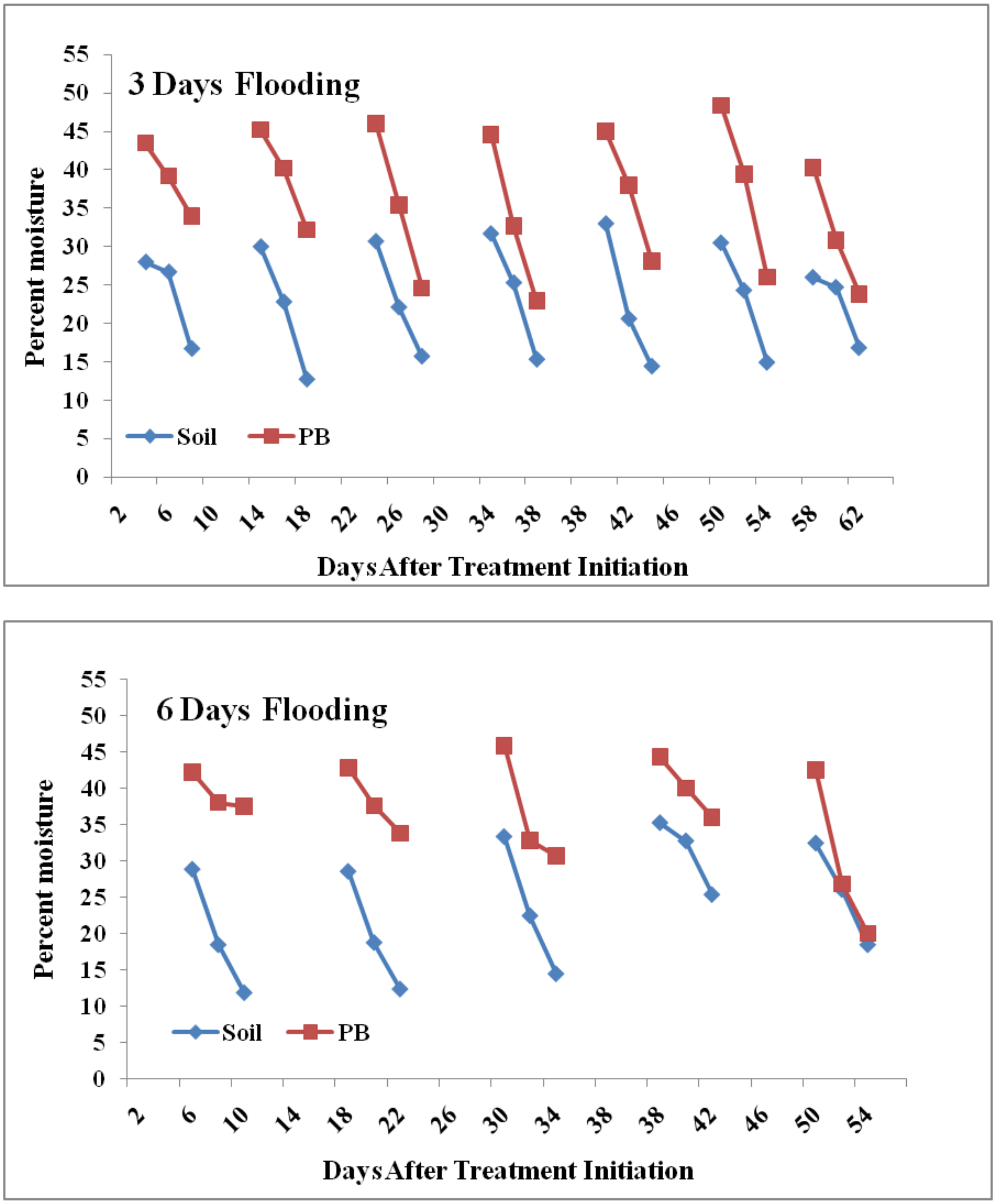


Figure 5. Dry down of substrate percent moisture of *Ilex glabra* 'Shamrock' in PB flooded for 0 (non-flooded), 3, or 6 days. Flood cycles consisted of 0, 3, or 6 days of flooding and 6 days of draining. Plants in the 3 and 6 day flood treatments experienced a total of 7 and 5 flood cycles, respectively. Plants were grown under a shade house in Auburn, Ala. and treatments initiated on 23 July 2009 (282 DAP) and ended on 23 Sept. 2009 (344 DAP).

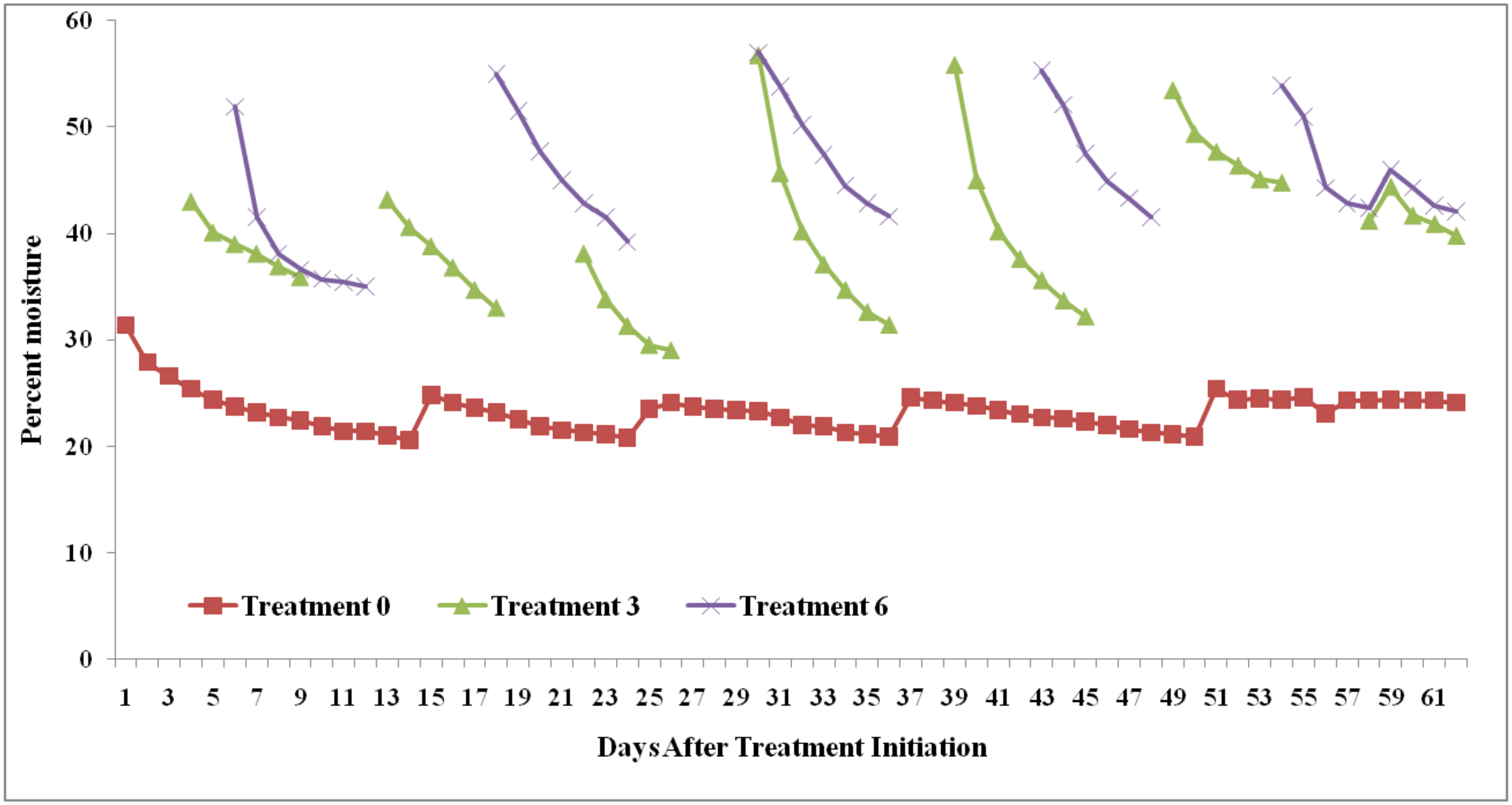


Figure 6. Dry down of substrate percent moisture of *Ilex glabra* 'Shamrock' in CC flooded for 0 (non-flooded), 3 or 6 days. Flood cycles consisted of 0, 3 or 6 days of flooding and 6 days of draining. Plants in the 3 and 6 days flood treatments experienced a total of 7 and 5 flood cycles, respectively. Plants were grown under a shade house in Auburn, Ala. and treatments initiated on 23 July 2009 (282 DAP) and ended on 23 Sept. 2009 (344 DAP).

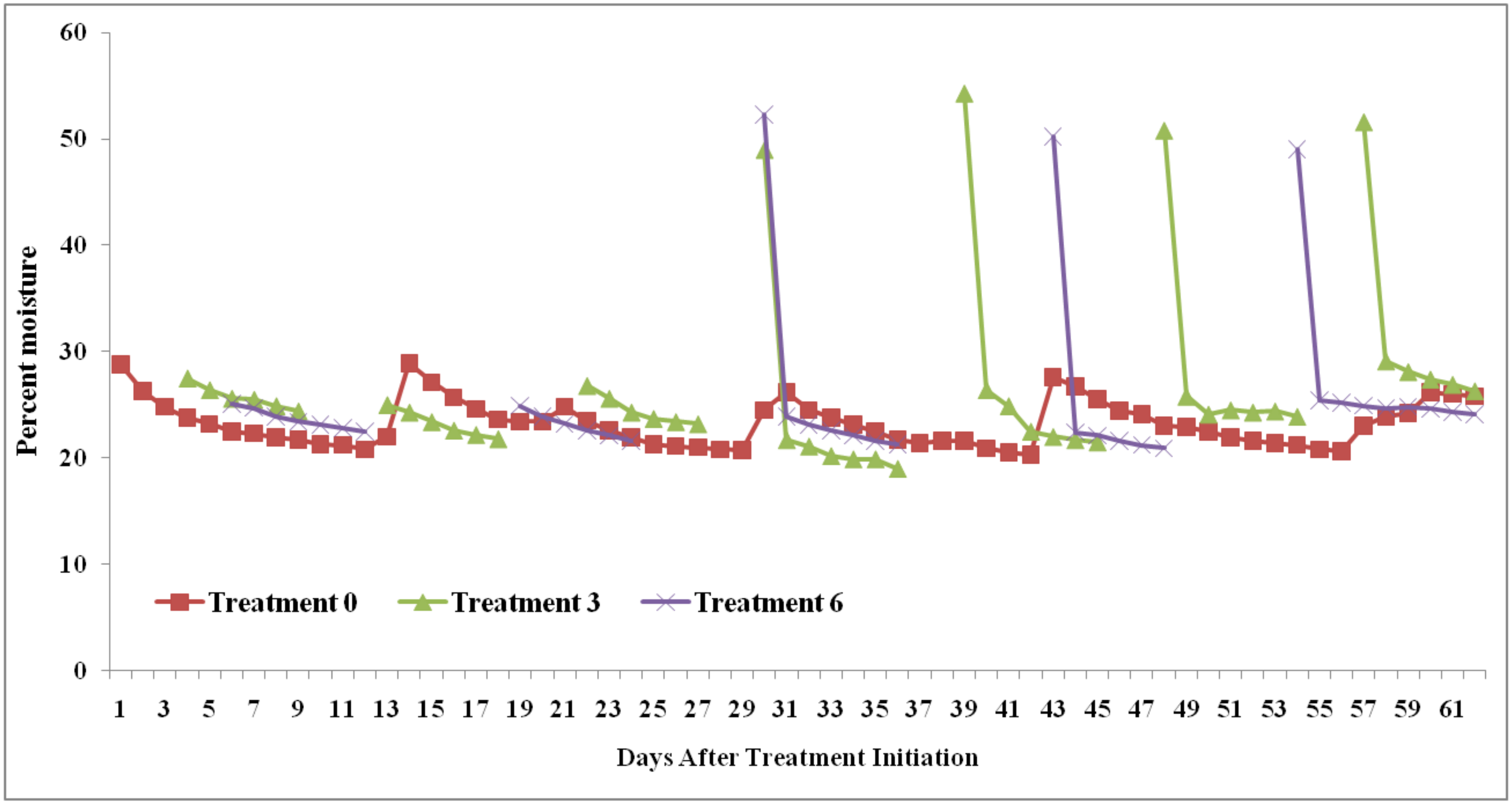


Figure 7. Dry down of substrate percent moisture of *Itea virginica* 'Henry's Garnet' in PB flooded for 0 (non-flooded), 3 or 6 days. Flood cycles consisted of 0, 3 or 6 days of flooding and 6 days of draining. Plants in the 3 and 6 days flood treatments experienced a total of 7 and 5 flood cycles, respectively. Plants were grown under a shade house in Auburn, Ala. and treatments initiated on 7 July 2009 (266 DAP) and ended on 7 Sept. 2009 (328 DAP).

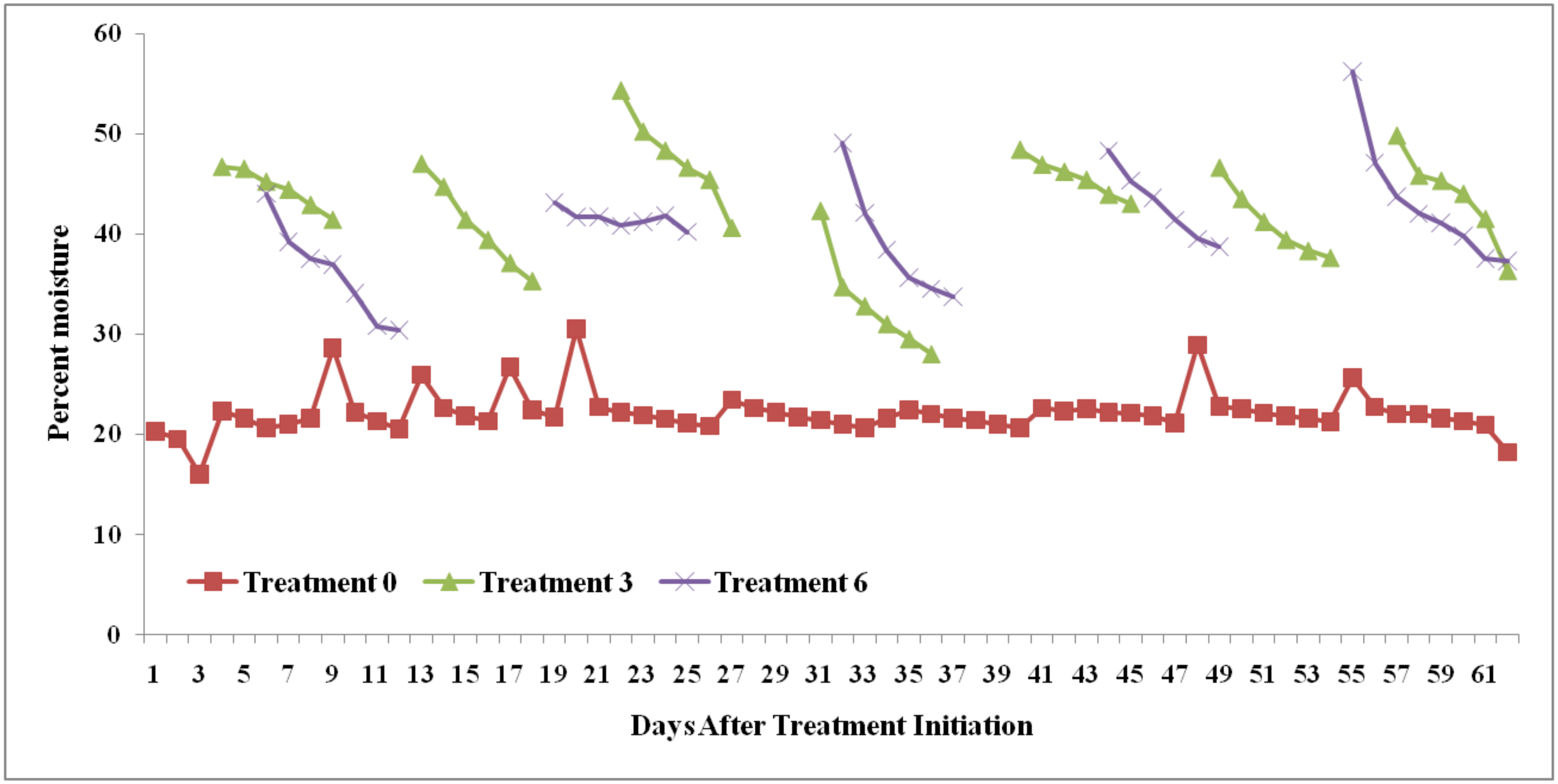


Figure 8. Dry down of substrate percent moisture of *Itea virginica* 'Henry's Garnet' in CC flooded for 0 (non-flooded), 3 or 6 days. Flood cycles consisted of 0, 3 or 6 days of flooding and 6 days of draining. Plants in the 3 and 6 days flood treatments experienced a total of 7 and 5 flood cycles, respectively. Plants were grown under a shade house in Auburn, Ala. and treatments initiated on 7 July 2009 (266 DAP) and ended on 7 Sept. 2009 (328 DAP).

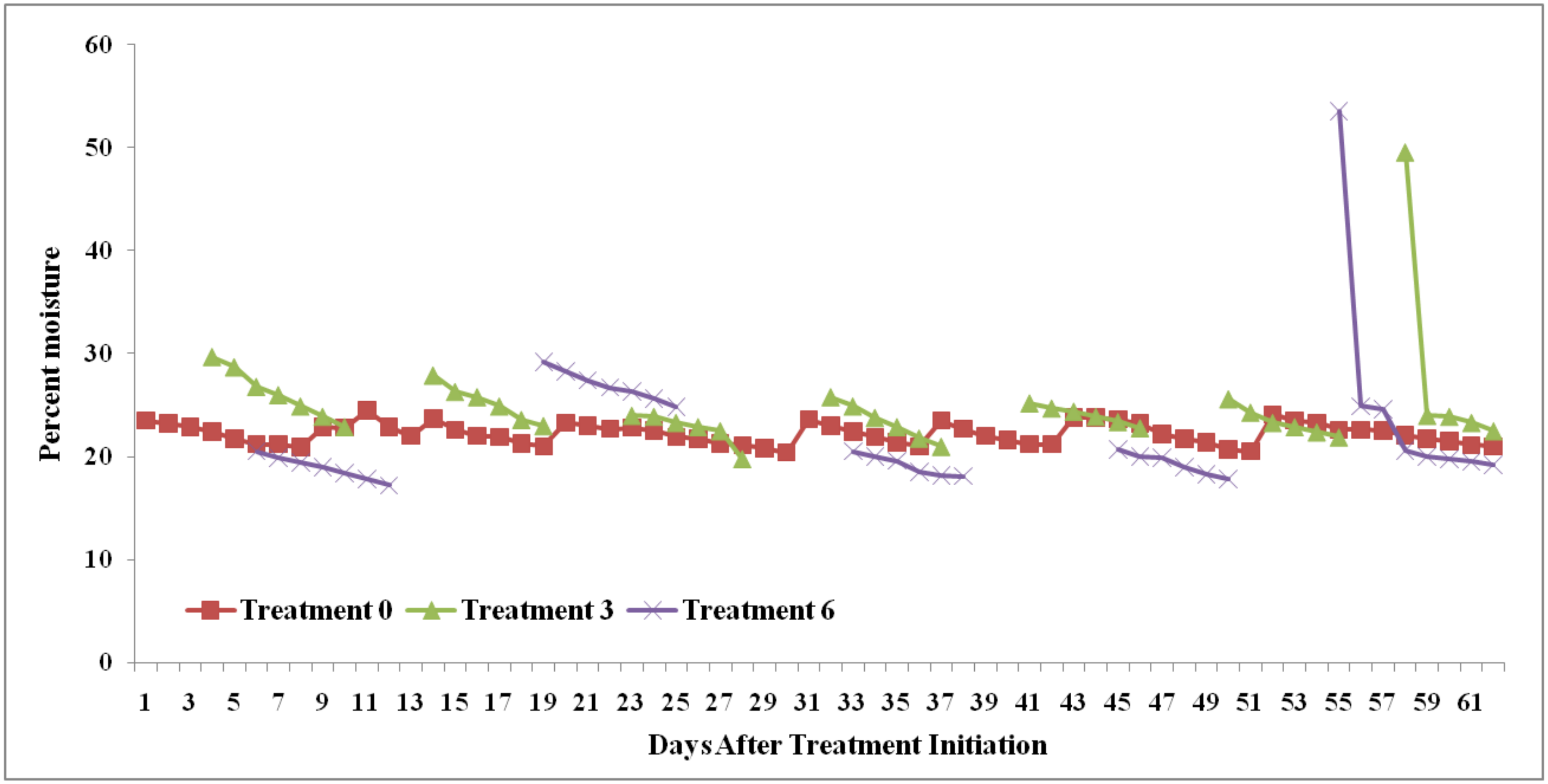


Figure 9. Dry down of substrate percent moisture of *Viburnum nudum* ‘Winterthur’ in PB flooded for 0 (non-flooded), 3 or 6 days. Flood cycles consisted of 0, 3 or 6 days of flooding and 6 days of draining. Plants in the 3 and 6 days flood treatments experienced a total of 7 and 5 flood cycles, respectively. Plants were grown under a shade house in Auburn, Ala. and treatments initiated on 29 July 2009 (288 DAP) and ended on 28 Sept. 2009 (349 DAP).

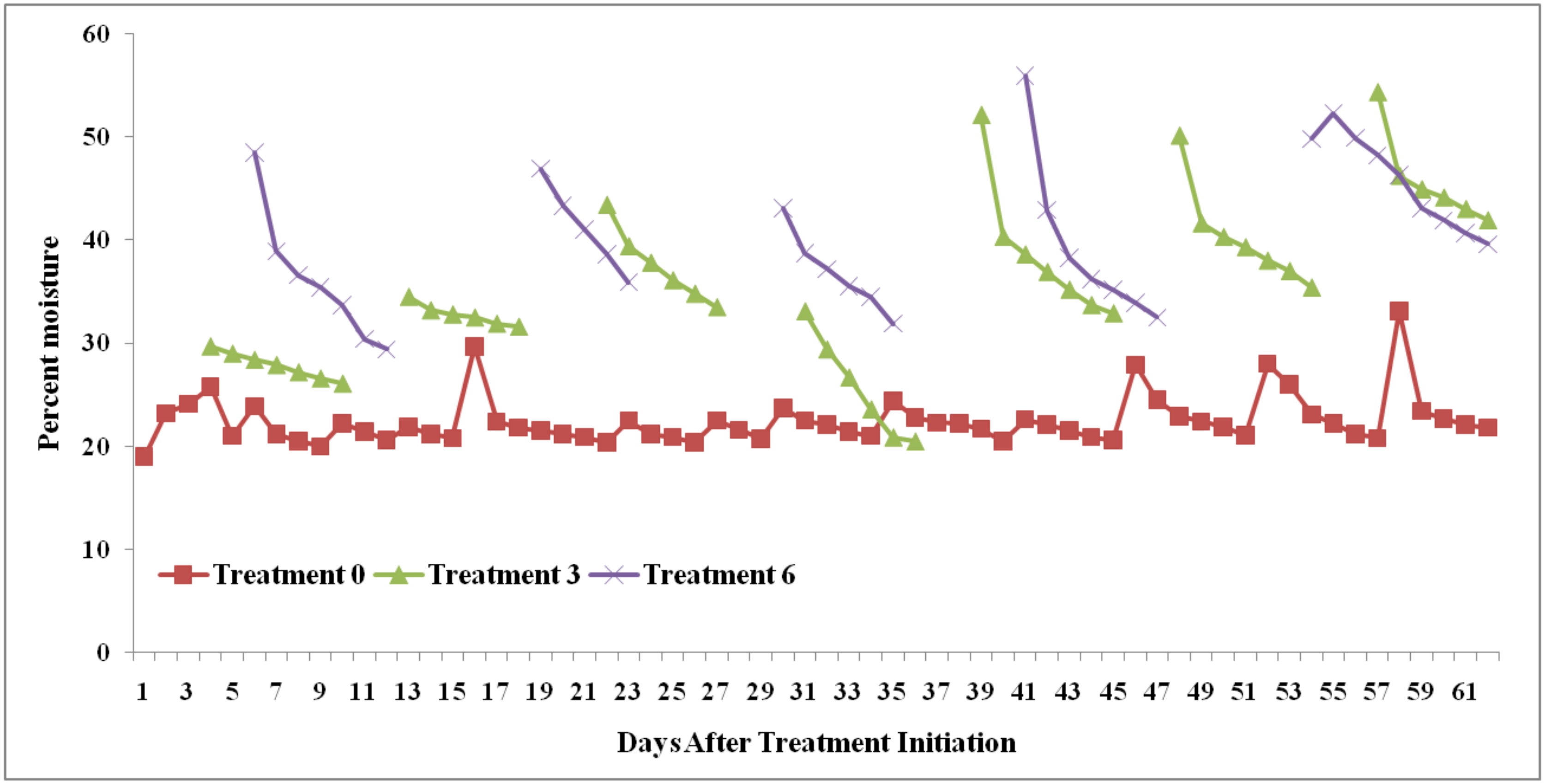
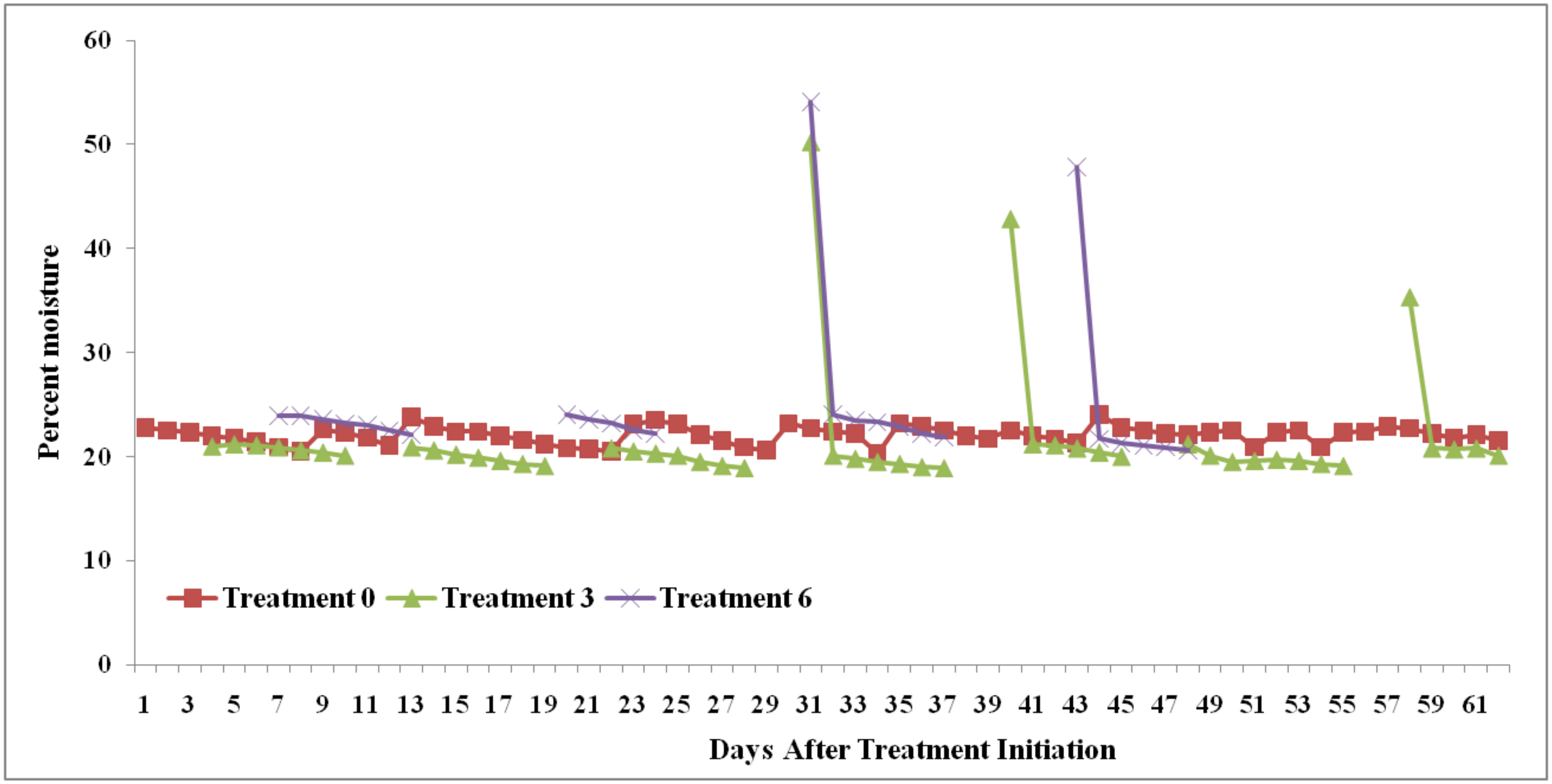


Figure 10. Dry down of substrate percent moisture of *Viburnum nudum* 'Winterthur' in CC flooded for 0 (non-flooded), 3 or 6 days. Flood cycles consisted of 0, 3 or 6 days of flooding and 6 days of draining. Plants in the 3 and 6 days flood treatments experienced a total of 7 and 5 flood cycles, respectively. Plants were grown under a shade house in Auburn, Ala. and treatments initiated on 29 July 2009 (288 DAP) and ended on 28 Sept. 2009 (349 DAP).



Chapter IV

Final Discussion

Effects of Short Interval Cyclic Flooding on Growth and Survival of Four Native Shrubs.

Clethra alnifolia L. 'Ruby Spice', *Ilex glabra* (L.) A. Gray 'Shamrock', *Itea virginica* L. 'Henry's Garnet', and *Viburnum nudum* L. 'Winterthur' are plants native to Alabama that are categorized as flood tolerant on multiple lists. However, the majority of these listings are based merely on perception rather than scientific evaluations. We proposed that because these taxa are found growing in moist conditions in their native habitats, they would be able to withstand periodic episodes of inundation and drought typical in a rain garden setting. We grew these plants in 1 pine bark : 1 peat (PB : P) and calcined clay (CC). PB : P was used as an organic bog like substrate, while CC was used as a mineral fine textured substrate that is well drained and easily rinsed from the roots for root dry weight. Using a pot in pot flooding methodology, these plants were flooded for 0 (non-flooded), 3, or 7 days. To impose drought, flooded plants did not receive any irrigation during 7 days of draining until the next flood cycle.

Chapter 2 results indicated that all taxa except *Clethra alnifolia* 'Ruby Spice' were tolerant of short interval cyclic flooding. Flooded plants generally had reduced growth index and root and shoot dry weights compared to non-flooded plants. Mortality was higher in Experiment I than in Experiment II, which we speculated was due to prematurity of liners used in Experiment I and to decreased acclimation before treatments were initiated in Experiment I. Treatments were initiated for Experiment I at 24 days after planting (DAP) and Experiment II treatments were initiated at 35 DAP. Liners used in Experiment I were shipped to us at the earliest possible shipping date and as a result, these liners appeared smaller and less vigorous than the liners that were shipped to us two months later for Experiment II. We concluded that pot in pot flooding methodology was an appropriate method to control the water table of flooded plants. PB : P dried down much more slowly than CC and was likely due to a higher water holding capacity associated with PB : P.

These plants show great promise as selections for use in rain gardens. In the event that this study was repeated, it would be beneficial to evaluate a plant that is considered intolerant of flooding. To impose greater drought conditions during draining, a growing medium with lower water holding capacity could be used or the draining period could be increased. A field study would also lend itself to a better evaluation of performance in the landscape. Repeating this experiment in larger containers would be helpful because plants would be more mature and possibly more equipped to handle such conditions as well as having larger substrate volumes similar to what may be experienced in the field. In our study, we took rooted cutting liners and planted them in trade gal containers. Plants installed in a rain garden would likely be at least one gal plants and more likely three gal plants, and future studies may be more applicable if larger plants were used. However, these experiments were beneficial in evaluating tolerance to cyclic flooding on smaller plants and also in developing methodology for imposing flooded conditions.

Effects of Previous Flood Exposure on Flood Tolerance, Growth, and Physiology of Selected Native Shrubs.

There are few studies that have evaluated the possibility of pre-conditioning plants to flood tolerance. In this study, plants overwintered in trade gal pots and developed extensive root systems before treatments were initiated. All taxa from previous experiments except *C. alnifolia* 'Ruby Spice' were used in this study. This study consisted of two phases: flooding in the greenhouse and subsequent outdoor flooding. Substrates were different for this study. 1 pine bark : 1 peat used in previous experiments evolved to 5 pine bark : 3 peat : 1 perlite (PB) for better aeration and the possibility of better drainage to impose greater drought conditions. Local native soil was used because plants would likely be growing in this or a similar soil in the landscape. Plants were flooded for 0 (non-flooded), 3, or 6 days and flooded plants drained for 6 days. During draining, flooded plants did not receive any irrigation until the next flood cycle in order to impose drought conditions. To facilitate ease of treatment scheduling, treatment days were in multiples of 3.

Following greenhouse flooding, *I. glabra* 'Shamrock' and *I. virginica* 'Henry's Garnet' had decreased growth in plants flooded for 6 days. *V. nudum* 'Winterthur' had no differences in growth across flooding treatments. *V. nudum* 'Winterthur' plants grown in soil were considerably smaller than plants grown in PB (personal observation) which may have been due to increased soil compaction as a result of overhead irrigation during the overwintering period. *V. nudum* 'Winterthur' roots are coarse textured (similar to that of a Japanese holly) and very brittle and this coupled with compaction likely resulted in decreased root growth and possible stunting of plants grown in soil. During greenhouse flooding, soil dried down more quickly during draining, which may have evaluated drought better than PB. Following greenhouse flooding, plants were held in a greenhouse for 40 days after which they were potted into 170.6 L (45 gal) tubs for outdoor flooding. This outdoor experiment required too large of a volume of soil to be collected and sterilized, as a result, CC was used instead of soil. Same flooding treatments (0, 3, and 6 days of flooding) were randomly assigned to each tub and treatments were in a factorial of flooding in the greenhouse x flooding outdoors.

Results for outdoor flooding indicated that *I. glabra* 'Shamrock' and *I. virginica* 'Henry's Garnet' were more affected by flooding treatments applied during greenhouse flooding than by flooding treatments applied during outdoor flooding. *I. glabra* 'Shamrock' grew so slowly that treatment differences were hard to distinguish during outdoor flooding. We observed flushes of new shoot growth across all treatments at the end of flooding in the greenhouse and outdoor flooding, but roots of flooded *I. glabra* 'Shamrock' were much smaller in comparison to non-flooded plants. This demonstrates the need for a long term study to determine whether this taxon thrives or only survives under these conditions. In contrast, *I. virginica* 'Henry's Garnet' grew so vigorously during outdoor flooding that treatment differences seemed to disappear and we concluded that this taxon may become more flood tolerant with maturity. *V. nudum* 'Winterthur' appeared more affected outdoor flooding which suggested that this plant may become less tolerant of flooding with maturity. During all harvests, root systems of *I. virginica* 'Henry's Garnet' and *V. nudum* 'Winterthur' were consistently larger and more robust than *I. glabra* 'Shamrock', and this was demonstrated by overall results for RDW. In all of our experiments using *I. virginica* 'Henry's Garnet' and *V. nudum* 'Winterthur', these taxa appeared to be vigorous and fast growing and as a result, all taxa except *I. glabra* 'Shamrock' were pruned before treatments initiated for outdoor flooding. Plants were pruned to create uniformity within a taxa x substrate x treatment combination while preserving growth differences that resulted from greenhouse flooding. Plants were pruned to the average GI within a taxa x substrate x treatment combination. It could be suggested that growth responses observed during outdoor flooding were directly related to pre-treatment pruning. However, *I. virginica* 'Henry's Garnet' and *V. nudum* ‘Winterthur’ both grew substantially during outdoor flooding and treatment differences either disappeared or developed in these taxa.

During outdoor flooding, tubs were held in a shade house covered with shade cloth and a double layer of polyethylene plastic. This plastic was said to be photo stable, but in the event of a weekend of an extended period of severe weather, the plastic ripped in several places. Tubs were covered and uncovered with perforated polyethylene plastic, but dry down during draining was likely affected. The shade house was also on slightly sloped terrain and if this experiment was to be repeated, it would help to conduct this research on level ground. Due to the volume of substrates in tubs, lifting or moving them was not easily accomplished, so correcting for slope was difficult. As a result, plants on low side of tub likely experienced more severe flooding. To compensate for this, plants from greenhouse flooding were randomized within each tub and previous treatments that experienced increased water levels in outdoor flooding were random.

CC drained very quickly in comparison to PB, but CC rarely drained to substrate percent moisture below that of what non-flooded plants experienced. PB drained to a higher substrate percent moisture than would be necessary to be considered water stressed. However, larger volumes of substrates in these tubs did likely resemble dry down under field conditions. Therefore, longer draining period may have been necessary to evaluate drought on these plants. This experiment may not have evaluated drought during outdoor flooding, but did evaluate tolerance of short term cyclic flooding events. Flooded tubs reached substrate percent moistures of 45% and up, so there is no question whether tolerance to periodic waterlogged conditions was observed.

Drought tolerance may not have been fully evaluated for these experiments. In our study, substrate percent moisture was monitored in surrounding soil instead of a combination of surrounding soil and rootballs of plants in 45 gal tubs. Perhaps in future experiments, sensors could be placed in root balls of plants in tubs to better assess substrate percent moisture and whether plants actually experienced drought conditions. These plants experienced more flooding than a typical rain garden would and all taxa survived, which suggests they are capable of tolerating periodic flooded conditions. Future research could use actual rainfall and drought data collected from our area and impose these conditions on plants to better simulate a rain garden.

To better understand and evaluate the effects of flooding during greenhouse and outdoor flooding, additional physiological data collection may have helped. Such data collection may have included dissolved oxygen samples to evaluate severity of anaerobic conditions experienced. We observed surface root growth that likely occurred in avoidance of deeper anaerobic soil layers. However, dissolved oxygen samples would have provided knowledge of whether roots were undergoing partial aerobic respiration. Moreover, root porosity could have been measured to determine whether plant roots developed aerenchyma tissue. Without development of aerenchyma tissue, it is unlikely that plants would have been able to maintain rates of photosynthesis and stomatal conductance during flooding. It's possible that plants that were flooded in the greenhouse were more likely to develop aerenchyma root tissue when flooded outdoors. Knowledge of length of time needed to develop aerenchyma tissue would be useful information to have regarding these taxa.

In conclusion, we flooded plants in a greenhouse setting before flooding them outdoors to simulate pre-conditioning that might occur during production. Based on results from greenhouse flooding and outdoor flooding, we concluded that a grower might be able to flood these taxa for three days periodically during the growing season to pre-condition these taxa for subsequent flood tolerance. However, further research of pre-conditioning these taxa for flood tolerance is needed to fully assess the possibility of pre-conditioned flood tolerance.