Flooding Tolerance of Six Native Landscape Plants for Use in Southeastern Rain Gardens

by:

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Abstract

Rain gardens are an effective, attractive, and sustainable stormwater management solution for residential areas and urban green spaces. Although design considerations such as size, susbstrate depth, substrate type, and stormwater holding time have been rigorously tested, little research has been conducted on the living portion of rain gardens. This study subjected six landscape plant species native to southeastern United States to repeated short term flooding to evaluate tolerance to the type of flooding seen in rain gardens. Flooding lasted for 2 d followed by 5 d of no inputs for 7 – 8 wks. Plants were evaluated based on initial and final size index, shoot dry weight, leaf: stem dry weight ratio, stomatal conductance, and leaf chlorophyll content. A diverse set of plants with assorted seasonal benefits was chosen and included two evergreen shrubs (*Morella cerifera* and *Illicium floridanum*), two herbaceous perennials (*Lobelia cardinalis* and *Chasmanthium latifolium*), and two ferns (*Polystichum acrostichoides* and *Osmunda cinnamomea*). Assessment of *L. cardinalis* was not possible due to extensive damage by herbivorous insects. Damage was not related to flooding and undamaged plants demonstrated the ability to withstand repeated short term flooding. Based on the findings in this research, *M. cerifera, C. latifolium,* and *O. cinnamomea* are recommended for use in southeastern rain gardens. *P. acrostichoides* is not recommended for southeastern rain gardens that do not receive supplemental

irrigation during dry periods due to suspected drought intolerance. Future studies are needed to determine if *I. floridanum* and confirm that *L. cardinalis* would tolerate short term flooding followed by intermittent dry periods.

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Introduction

Water management, particularly fresh water management, is a global concern. Fresh water is not limitless, a realization that becomes more obvious as the world's population increases and humankind's footprint on the globe expands. Urbanization causes extensive changes to land surfaces that exceed the geographic borders of developed areas (Pickett et al., 2008). Urbanization can cause the degradation of fresh water resources above and below the ground (Burns et al., 2005). The negative impacts of urbanization on associated watersheds result in changes to hydrology, elevated concentrations of nutrients and contaminants, altered channel morphology, and reduced biodiversity (Walsh et al., 2005). Urbanization also decreases groundwater recharge, which often leads to diminished groundwater supply (Erickson and Stefan, 2009). Contributors to altered watersheds and reduced groundwater reserves are numerous, but the primary driver is stormwater runoff.

Stormwater

Stormwater plays an extensive role in the degradation of water bodies (Paul and Meyer, 2001). Stormwater is created when water associated with rain or snow flows over land before collecting in natural channels or man-made hydrologic systems. Urbanization is correlated with an increase in impervious area that leads to decreased precipitation infiltration and increased surface runoff (Dunne and Leopold, 1978). Impervious areas include rooftops, road surfaces, parking areas, sidewalks, and concrete drainage basins. When the amount of impervious area exceeds 10 to 20% of total surface area, watershed degradation occurs, and runoff increases twofold (Arnold and Gibbons, 1996). When impervious area exceeds 30%, severe degradation of water and habitat quality occurs. Loss of vegetative cover is synonymous with increased impervious surface coverage and also contributes to increased runoff (Clayden and Dunnett, 2007). When vegetative cover is lost, evapotranspiration rates decrease. Evapotranspiration rates of urban areas compared to non-urban areas may decrease total hydrologic flow from 40 to 30% (Arnold and Gibbons, 1996).

Beyond large-scale changes to natural hydrology, stormwater is also associated with pollution (Paul and Meyer, 2001). Stormwater carries pollutants and discharges them to surface waters. Pollutants include: heavy metals (such as lead, zinc, copper, and cadmium), polycyclic aromatic hydrocarbons, soluble salts, pesticides, nitrogen, phosphorus, solids, pathogens, and pharmaceuticals (Paul and Meyer, 2001; Göbel et al., 2007; Rodriguez-Hernandez et al., 2013). In 1998, the United States Environmental Protection Agency (EPA) studied the effectiveness of best management practices (BMPs) in addressing stormwater runoff problems (EPA, 1999). Several BMPs were identified that included structural and non-structural practices. Another term used in conjunction with stormwater BMPs is low impact development (LID). LID approaches focus on stormwater management alternatives that mimic nature more than traditional stormwater design (Dietz, 2007). The EPA has published guidelines for LID practices and implementation (EPA, 2015) and recently, Alabama-specific guidelines have been published in the Alabama Low Impact Development Handbook (ADEM, 2015).

Rain gardens

A particularly beneficial BMP/LID practice is a filtration system. Filtration systems such as large-scale bioretention systems and small-scale rain gardens have the ability to reduce runoff quantity and pollutant content of contaminated water (EPA, 1999). Filtration systems reduce stormwater flow discharge, remove pollutants through natural processes, remove sedimentation by allowing more time for particles to settle, increase filtration of particulates, increase infiltration of contaminants through adsorption, increase biological uptake of nutrients, increase biological conversion of contaminants, and degrade harmful organic compounds. Most importantly, runoff and pollutants are retained onsite (Dietz, 2007). Rain gardens are effective stormwater filtration systems that also serve as attractive additions to landscapes (Russell, 2000; Cramer, 2006). In addition to the aforementioned benefits, rain gardens contribute to recharging aquifers, cycling plant nutrients, sequestering carbon, and reducing the urban heat island effect (Bortolini and Semenzato, 2010). Rain gardens also help to reduce the need for irrigated water in the landscape (Seymour, 2005).

A rain garden is a shallow depression in the landscape that receives the first inch of runoff during a storm event. Trees, shrubs, and herbaceous landscape plants are often planted along with a groundcover or mulch layer (Dietz and Clausen, 2005). Rain gardens are watered naturally and therefore may experience very dry conditions as well as temporary flooded conditions (Clayden and Dunnett, 2007; Steiner and Domm, 2012; Kraus, 2013). One important design consideration of a rain garden is ponding time. A maximum of 48 h is recommended to prevent mosquitoes from breeding (Seymour, 2005) and prolonged exposure of plant roots to anaerobic conditions (Dussaillant et al., 2005). While studies have been conducted regarding the design and substrate composition of rain gardens to maximize capture potential and pollutant retention (Akan, 2013; Turk et al., 2014), rain garden plant selection has not been as thoroughly investigated.

Rain Garden Plant Research in the Southeast

In light of limited rain garden plant research, initial studies focused on plant selection. Subsequently, several southeastern U.S. native landscape plants have been evaluated for tolerance to rain garden conditions (Jernigan and Wright, 2011; Christian et al., 2012; Dylewski et al., 2012; Meder, 2013). Species tolerant of cyclic flooding included: *Viburnum nudum* L. 'Winterthur' ('Winterthur' possumhaw), *Viburnum nudum* L. 'Brandywine'™ (possumhaw), *Ilex verticillata* (L.) A Gray 'Winter Red' (winterberry), *Ilex* vomitoria Aiton 'Schillings dwarf' (yaupon holly), *Coreopsis verticillata* L. 'Zegreb' (whorled tickseed coreopsis), *Andropogon tenarius* Michx. (broomsedge), and *Muhlenbergia capillaris* (Lam.) Trin. (gulf muhly grass). Alternatively, two species recommended for rain gardens, *Fothergilla x intermedia* L. 'Mt. Airy' (dwarf witchalder) and *Echinacea purpurea* L. Moench. 'Magnus Superior', did not perform well under repeated cyclic flooding. Failure to thrive suggests that these plants are not good candidates for use in rain gardens and reiterates the need for more research for sound plant recommendations. Interestingly, *Clethra alnifolia* L. 'Ruby Spice" ('Ruby Spice' summersweet) did poorly in one study (Dylewski et al., 2012), but thrived in another study (Jernigan and Wright, 2011). The difference in performance was attributed to plant size. Larger plants seemed more tolerant of flooding possibly due to more robust root systems. This suggests that initial plant size should be considered when installing a rain garden. Furthermore, plant-related rain garden research is not limited to plant selection.

An Auburn University study explored previous flooding exposure to flooding tolerance, growth and physiology (Dylewski et al., 2012). Results indicated that previous flood exposure did not affect growth as much as plant maturity. *Itea virginica* L. 'Henry's Garnet' ('Henry's Garnet' sweetspire) demonstrated decreased sensitivity to flooding with greater plant maturity. However, the opposite was true for *Vibrurnum nudum* L. 'Winterthur' ('Winterthur' possumhaw). *Ilex glabra* 'Shamrock' (L.) A. Gray ('Shamrock' inkberry holly) was tolerant at any growth stage.

Recent studies found that plant diversity benefits rain gardens (Christian et al., 2012; Meder, 2013). Christian et al. (2012) concluded that nutrients were released during cool season perennial die back, even when evergreens were present. However, evergreens likely helped increase nutrient uptake during the cool season. The same study also noted high tissue concentrations of metals in ferns. Ferns may in turn have potential for greater metal uptake and removal from rain garden systems. Meder (2013) evaluated two plant growth substrates and planting diversity (i.e. monoculture vs. polyculture) for phosphorus removal. Results showed greater plant growth in substrates containing organic matter. This suggests that substrate can influence plant health and growth and thus nutrient removal. In addition, polyculture plantings were more effective in removing phosphorus from the leachate. Because some plant species had more growth in the fall while others had more growth in the spring, the author concluded that seasonal vegetative gaps may have been avoided in the polyculture plantings making them better able to remove phosphorus. Additionally, polyculture plantings have greater potential to remove niche nutrients and thereby increase overall nutrient removal (Karathanasis et al., 2003; Liang et al., 2011; Calheiros et al., 2015). Together, these studies demonstrate that using a variety of plant species should be considered when designing rain gardens. Due to seasonal, climatic, and environmental differences, area specific rain garden plant research is needed. Research-based rain garden plant selections for the southeast are listed in Appendix A.

Plant Diversity

Plant diversity in rain gardens is beneficial for reasons beyond those demonstrated in the rain garden studies by Christian et al. (2012) and Meder (2013). Plant diversity promotes overall ecosystem biodiversity. Therefore, incorporating a variety of plants in urban settings such as rain gardens should be an important consideration. Urban gardens, specifically residential gardens, collectively make a sizable contribution to urban green space (Doody et al., 2010). Urbanization fragments large natural habitats, which has negative consequences for native flora and fauna. Native plant species, especially rare species, suffer tremendously from urbanization, while nonnative plant species flourish (Kuhn and Klotz, 2006). Rain gardens may be the ideal environment for many native plant species due to their low maintenance characteristics. Thus, residential rain gardens may provide a mechanism for native plant and animal populations to move into urbanized areas (Doody et al., 2010; Russo et al., 2013).

Inclusion of native plants is one way to maintain a diverse urban ecosystem. Predictably, native flora are known to attract native fauna (Pardee and Philpott, 2014). Pollinator species are particularly important fauna that play a key role in the long-term survivability of low maintenance gardens such as raingardens. Pollinators can be supported by diverse floral resources that provide assets throughout their active season (Kearns et al., 1998; Smith et al., 2005). Additionally, urban environments that support native pollinator communities benefit surrounding ecosystems, including nearby agricultural sites that rely on pollinators for production (Russo et al., 2013). Rain gardens have the potential to mitigate the effects of increased urbanization on hydrology, as well as increase and preserve native biodiversity.

Although limited studies have focused on which plants are best suited for rain garden survival and function (Pfeiffenberger and Dougher, 2008; Werneth, 2009; Jernigan, 2010; Meder, 2013), native plants are traditionally considered to be good rain garden candidates. Native plants are adapted to meet the challenges of local climate variations, diseases and pests (Clayden and Dunnett, 2007; Steiner and Domm, 2012; Kraus, 2013). From a survivability standpoint, the best rain garden plants can tolerate short-term flooding and intermittent dry conditions. A high degree of phenotypic plasticity is required for plants that live in environments with fluctuating water tables (Crawford, 1996). Adaptations often reduce general fitness, and it cannot be assumed that every plant that thrives in an area with cyclic wet and dry soil conditions will be a good rain garden candidate. For example, drought tolerant plants have been shown to suffer if drought stress fails to occur (Crawford, 1996; Vivian et al., 2014). Additionally, rain gardens support different plant zones ranging from wet to moist/dry to dry (Indiana Lake Michigan Coastal Program, 2015). Wet zones would typically be found in the center of the rain garden and dry zones along the berm. Understanding the degree to which each plant can withstand wet or dry conditions can help to determine proper rain garden placement. Testing individual plant selections is important in determining rain garden plant effectiveness.

Flooding

Flooding, sometimes referred to as soil water logging or inundation, imposes a major abiotic stress on plants that often affects growth, distribution, and productivity (Jackson and Colmer, 2005). The major stress on flooded plants is an inadequate supply of oxygen to submerged tissues (Blom and Voesenek, 1996). Gas diffusion is severely inhibited in flooded soils. Within 24 to 48 h of flooding, plant roots deplete soil oxygen and exhibit root stress (Crawford and Braendle, 1996). Eventually, toxic products of anaerobic metabolism accumulate, causing harm to plant cells. Plants unable to withstand flooding stress eventually succumb to depleted carbohydrate reserves, accumulation of toxic metabolites, hormonal dysfunction, or some combination of the above (Crawford, 1996). Even after flooding subsides, a plant is susceptible to post-anoxic injury as it is reintroduced to oxygen. It is also susceptible to biotic stresses, such as pests and abiotic stresses such as wind.

Flooding duration can be permanent or last anywhere from a few hours to multiple seasons (Ernst, 1990). Survival strategies depend on the specific environment of each plant (Blom and Voesenek, 1996). The impact of changes in the water regime depends on the predictability, duration, intensity, and frequency of flooding (Ernst, 1990). Flood tolerant plants overcome flooding stress through a suite of morphological and physiological adaptations (Blom and Voesenek, 1996; Jackson and Colmer, 2005). At the onset of inundation, short-term metabolic adaptations may ensue. For example, glycolysis can generate ATP when aerobic respiration is inhibited. Plants may also decrease their respiratory rate, especially when totally submerged due to limited CO_2 and light (Jackson and Colmer, 2005). Long-term adaptations often develop in the roots, shoots, and life cycles of plants. Usually, the first signs of flooding response occur in the roots. Adventitious roots may form near the upper, more aerated soil. Submerged roots may even grow upward to access oxygen (Blom and Voesenek, 1996).

Long-term survival strategies in the shoot can be contradictory. Some plants cease growth to conserve energy to "ride out the storm." Other plants increase shoot elongation to restore contact with air. Subsequently, growth may be restored, and flowering and seed production may be stimulated. The epitome of plant adaptation to flooding is the formation of aerenchyma in the roots and shoots (Ernst, 1990). Aerenchyma provide internal ventilation channels between submerged tissue and tissue in contact with air. Finally, adaptations to flooding include life history adaptations such as seed dispersal and seedbank characteristics (Jackson and Colmer, 2005). Flooding may be a means to spread seed or assist seedling germination (Crawford, 1996; Blom and Voesenek, 1996). Nevertheless, some plants will succumb to flooding.

Flooding injuries to roots, shoots, and leaves are measurable indications of plant fitness during and after a flooding event. Original roots may dieback and be replaced by adventitious roots (Vartapetian and Jackson, 1997). The ratio of dead to living root tissue may be compared to other root systems. Leaf yellowing and death is another common injury caused by flooding (Kramer, 1951). Leaf yellowing, or chlorosis, usually begins in the lower portion of the plant and progresses upward. Chlorosis due to flooding resembles nitrogen deficiency; however, it often appears 4-6 days after flooding occurs. Leaf pigment composition can be measure with a SPAD meter to detect changes in pigment content (Mielke et al., 2010; Verma et al., 2014). Flooding may also cause a decrease in the capacity of plants to absorb and conduct water (Kramer, 1951). Stomatal conductance can be measured to determine how much water vapor is being emitted via the stomata (Mielke et al., 2003; Yordanova et al., 2005; Jing et al., 2009). Decreases in stomatal conductance during flooding are common. Finally, flooding often limits plant size; therefore, plant dry weight, total leaf area, and other measures of growth are also good indications of a plant's tolerance to flooding (Pociecha et al., 2008).

Objective

Characterization of plant health in response to short-term cyclic flooding is critical for selection of plants for inclusion in rain gardens. Measurements, such as those described above, can provide quantifiable characteristics to differentiate tolerance to flooding by plant species. The objective of this research is to evaluate six diverse native landscape plant species for tolerance to repeated short-term flooding. Plant selection will be based on published recommendations (Clayden and Dunnett, 2007; Kraus, 2013; ACES, 2014; NCCE, 2014) and will include trees, shrubs, herbaceous perennials, and ferns. To date, published research has identified two grasses, two perennials, six deciduous shrubs and two evergreen shrubs as being tolerant of repeated short-term flooding. We will add two ferns, two perennials, and two evergreen shrubs to the list of plants evaluated. All plants are shade tolerant. Results will expand the list of rain garden plant recommendations. Ultimately, this research will identify plants that can be used to treat stormwater runoff, increase groundwater infiltration, preserve native plant species, and beautify southeastern landscapes.

Materials and Methods

Six shade tolerant plant species, including two evergreen shrubs, two ferns, and two herbaceous perennials were selected for these experiments. All are commonly recommended for use in rain gardens (ACES, 2014; NCCE, 2014; Kraus, 2013b). Shrubs included 11.3 L *Illicium floridanum* Ellis (Florida anise) and *Morella cerifera* L. (wax myrtle) [Dodd and Dodd Nursery Inc. (Semmes, AL)]. Shrubs were obtained in Jan. 2014. Ferns included 3.8 L *Osmunda cinnamomea* L. (cinnamon fern) and *Polystichum acrostichoides* Michx (Christmas fern) [Buck Jones Nursery (Grayson, GA)]. Ferns were obtained in Aug. 2014. Perennials included *Chasmanthium latifolium* Michx. (river oats) and *Lobelia cardinalis* L. (cardinal flower). For the first experimental run of perennials, 3.8 L *C. latifolium* [Buck Jones Nursery] and 3.4 L *L. cardinalis* [Dodd and Dodd Nursery Inc.] were purchased in Jan. 2014. For the second and third experimental run of perennials, liners were purchased in Aug. 2014 [Emerald Coast Growers (Pensacola, FL)] and replanted in 3.8 L containers in Sept. 2014. All plants were held outdoors at the Paterson Horticulture Greenhouse Complex in Auburn, AL, until use in an experiment. With the exception of *C. latifolium*, each plant was subjected to testing during two experimental runs. *Chasmanthium latifolium* was subjected to three experimental runs (Table 1).

Plants were repotted in large, 97 L plastic nursery containers [Classic 10,000, Nursery Supplies, Inc. (Chambersburg, PA)] (Figure 1). Containers with normal drainage holes were used for non-flooded treatments. Containers without drainage holes were modified with a drainage valve to allow flooding for flooded treatments. For the drainage valves, a hole was cut 8 cm from the bottom of the container. A 2.54 cm PVC socket ball valve with a male and female coupling was screwed into the hole. Wire mesh was fastened over the inlet of the drain to prevent debris from clogging the drain. A 2.54 cm washer was placed onto each of the couplings (male and female). A ribbon of plumbers putty was placed on the inside coupling (male) between the washer and the tub wall to prevent leaks. The couplings were arranged so that the valve resided on the outside of the tub.

Containers were filled with an 8:1 pinebark:sand substrate amended with 1.2 kg/m^3 of dolomitic limestone and 8.0 kg/m^3 of 15N-3.9P-10K Osmocote Plus (with micronutrients, Marysville, OH). A slow-release fertilizer was used to avoid mass leaching during flooding inundation. Each container was filled with approximately 0.08 m^3 of substrate. Shrubs were planted one plant per container. One of each perennial was planted per container; likewise, one of each fern was planted per container (total of two plants per container for perennials and ferns). Containers were placed under a shade structure at the Paterson Horticulture Greenhouse Complex. The shade structure was constructed with an overhead sloped frame to support a layer of clear poly plastic to exclude rainfall and a 40% shade cloth. The sloped frame allowed the water to drain off of the structure. All water (flooded and non-flooded) was applied to containers by hand. Containers were arranged in a completely randomized design with each species as separate experiments. There were six single container experimental units per treatment per species.

Plants received one of two treatments: flooded or non-flooded. Flooded plants were flooded for 48 hours followed by five days of draining (no additional water added). During flooding, water level was maintained approximately 2 cm above the substrate to ensure complete inundation. Plants were flooded weekly for 8 wks. Non-flooded plants were hand watered every other day with approximately 11 L of water. Weekly water totals were nearly equal for flooded and non-flooded treatments. All plants were harvested after 8 wks. Each experiment was repeated once (two runs).

Three plants of each species (not used in the experiment) were harvested for initial size index (SI) [(height + widest width +width perpendicular the widest width) / 3], leaf area (LA), shoot dry weight (SDW) [leaf + stem dry weight]. Leaf area was measured using a LI-3100 leaf area machine (LI-COR, Inc. Lincoln, NE). For SDW, plant tissue was placed in a 77° C drying oven for 3 d and weighed immediately upon removal.

Initial and final SI, LA, and SDW were collected for all plants. Final LA was measured for all ferns and perennials at experiment termination. For the shrubs, final LA was measured for three plants per species per treatment due to time restraints. Leaf chlorophyll content (LCC) and stomatal conductance (SC) was measured from newly matured leaves at the end of draining and flooding periods. A Konica Minolta Chlorophyll Meter SPAD-502Plus was used for LCC, and a Decagon Devices, Inc. Leaf Porometer (Pullman, WA) was used for SC. For shrubs and the first experimental run of perennials, LCC and SC was measured beginning midway through an experimental run and continuing for the last 3 wks. For the ferns and subsequent experimental runs of perennials, LCC and SC was measured at 2, 4, 6, and 8 wks. Stomatal conductance was measured between 8:00 AM and 11:00 AM for morning measurements and between 1:00 PM and 3:00 PM for afternoon measurements. Lastly, soil moisture was measured after the end of several flooding and draining periods with a AT Delta-T Devices ML3 – ThetaProbe Soil Moisture Sensor (Cambridge, UK).

Statistical Analysis

An analysis of variance was performed on all responses using PROC GLIMMIX in SAS version 9.3 (SAS Institute, Cary, NC). Plant height and size index were analyzed by plant species as split plots with flooding treatment in the main plot and initial and final date in the subplot. Leaf chlorophyll was analyzed by plant species and condition as two-way, completely randomized designs with sample date as repeated measures and leaves sampled per plant as subsamples. Stomatal conductance was analyzed by plant species and condition as two-way, completely randomized designs with sample date as repeated measures. Where only main effects were significant in two-way designs, the difference in treatments with only two levels were based on the AVOVA F-tests, and linear and quadratic orthogonal contrasts were examined over sampling dates. Where the interaction was significant, differences in flooding treatments at each sample date were determined using Tukey's test, and linear, quadratic, and cubic orthogonal contrasts were examined over sampling dates for each flooding treatment. Leaf area and dry weight were analyzed by plant species as a one-way, completely randomized design. Leaf and stem dry weight as a percent of total dry weight were analyzed as a one-way, completely randomized design using the beta probability distribution. Differences among treatments were determined using Tukey's test. Where residual plots and a significant COVTEST with the HOMOGENEITY option indicated heterogeneous variance among treatments, a RANDOM statement with the GROUP option was used to correct heterogeneity. All significances were at $\alpha = 0.05$.

Results

Shrubs

Except for SI in the summer run for *M. cerifera*, there were no effects of flooding on SI, LA or SDW for either shrub species (Figures 2 and 3 and Table 2). Size index of *M. cerifera* in summer was higher for non-flooded plants (82.9 cm) than flooded plants (75 cm). Although there was no effect of flooding on SDW for either shrub species, leaf: stem dry weight ratios did differ (Table 2). The leaf: stem ratio for flooded *I. floridanum* in the summer was higher than in non-flooded plants. The leaf: stem ratio for non-flooded *M. cerifera* was higher than those of non-flooded plants in the fall.

Leaf chlorophyll content was affected by flooding for both species in the fall but not the summer. For the fall experimental run, LCC was higher in non-flooded *I. floridanum* plants than flooded plants and was higher in flooded *M. cerifera* plants than non-flooded plants (Table 3). Although not compared statistically, stomatal conductance trends were similar between *M. cerifera* and *I. floridanum* (Figure 4). Time of day (morning or afternoon) did not affect SC for either species (data not shown). In general, SC was higher for non-flooded plants than flooded plants (Figure 4). For both species, SC also changed over time (Figure 4). A time of year by treatment interaction for SC for both shrub species occurred during summer (Figure 4). For both species, non-flooded SC was higher than that in flooded SC in the summer, and SC of flooded plants decreased over time during the summer months (Figure 4). There were no differences in SC between treatments in the fall for *I. floridanum*. Stomatal conductance was lower for the flooded plants in the fall for *M. cerifera* (Table 4).

Herbaceous Perennials

The first experimental run of *C. latifolium* and *L. cardinalis* was summer 2014. A few weeks after treatments began, *L. cardinalis* was infested with an unidentified herbivorous insect (possibly *Lepidoptera*), and damage to the plants (leaves and stems) was severe. *C. latifolium* and *L. cardinalis* were repeated in spring 2014 and summer 2015, and *L. cardinalis* suffered similar infestation each time. As result of this damage, data sufficient for statistical analysis could not be collected from this species. In the following paragraphs, only results for *C. latifolium* are presented.

Size index and SDW of *C. latifolium* were not affected by flooding in summer 2014 or spring 2015 (data not shown). Although SDW did not differ between flooded and non-flooded plants in summer 2014, size index and leaf: stem SDW ratios was higher for flooded plants than non-flooded plants in summer 2015 (Table 2, Figure 2).

Leaf area was higher for flooded plants than non-flooded plants in both summer runs (Figure 3). Leaf area was 3398 cm^2 for flooded plants and 2146 cm^2 for non-flooded plants in the summer of 2014. In the summer of 2015, LA was 6678.3 cm^2 for flooded plants and 4630.7 cm^2 for non-flooded plants. Size index was affected by flooding in the summer of 2015 only (Figure 2). There was no difference in LCC for either summer runs (data not shown). Conversely, in the spring LCC was higher for non-flooded plants (38) than flooded plants (30.6); however, differences in color were not apparent (personal observation). Stomatal conductance was affected by flooding in the summer of 2014 only. Stomatal conductance was higher in flooded plants (325.1 $mmol \bullet m^{-2} \bullet s^{-1}$) than non-flooded plants (226.9 $mmol \bullet m^{-2} \bullet s^{-1}$).

Ferns

Osmunda cinnamomea was not affected by flooding for any of the measurements recorded in spring 2015 and summer 2015. *Polystichum acrostichoides* was also not significantly affected by flooding in spring 2015, with the exception of SC. Stomatal conductance was higher in the flooded plants (177.7 mmol•m^{-2}•s^{-1}) than non-flooded plants (131.7 mmol•m^{-2}•s^{-1}). In summer 2015, two of the six flooded *P. acrostichoides* plants died, and SI was lower for flooded plants (Figure 2). Additionally, a date by treatment interaction occurred for LCC and SC for flooded plants (Table 5). Stomatal conductance for flooded plants decreased linearly over time, but there was no trend for non-flooded plants. Non-flooded plants had a higher SC than flooded plants at 4, 6, and 8 wks, but no difference was found at 2 wks. Flooded plant LCC followed a quadratic trend over time, increasing up to 4 wks and then decreasing, but there was no trend for non-flooded plants. Non-flooded plants had a higher LCC than flooded plants at 6 and 8 wks, but no differences were found at 2 and 4 wks.

Substrate Moisture and Whole-Plant Stomatal Conductance Estimates

Although not analyzed statistically, substrate moisture content in the flooded (at the end of the draining period) and the non-flooded treatments appeared similar within each species (Table 6). Stomatal conductance was multiplied by leaf area to estimate the potential total amount of water that could be transpired by each species per second (Table 7). There were no differences between flooding treatments for *M. cerifera* and *O. cinnamomea*. *Illicium floridanum* and *P. acrostichoides* showed differences only during the summer when total transpiration was higher in non-flooded. Conversely, *C. latifolium* showed differences during both summer runs when total transpiration was higher with flooding.

Discussion

Shrubs

Morella cerifera tolerated repeated short term flooding for the duration of each run. Although SI in the summer run was smaller for the flooded than non-flooded, this species grew over the course of that run. Additionally, there was no difference in visual appearance between *M. cerifera* plants in different treatments (personal observation) suggesting that the difference in LCC between treatments was likely not biologically significant. Based on the results of the experiment, *M. cerifera* is recommended for southeastern rain gardens receiving partial to full shade.

Results for *I. floridanum* were less clear. Although growth measurements (SI, LA, and SDW) suggested tolerance of short-term cyclic flooding, physiological measurements (LCC, SC) and personal visual observations did not. Visual appearance of *I. floridanum* in the fall was consistent with some results but not others. For example, LCC measurements supported personal observations. Leaves appeared greener in non-flooded plants than flooded plants, and flooding resulted in lower LCC. Also, SC decreased over time in the summer run. Meanwhile, leaf yellowing, wilting, and senescence increased as the experiment progressed although LA and DW measurements did not support these personal observations. A likely explanation for the disparity relates to sample size. Destructive harvests were used for LA and DW. Due to time restrictions, only three plants of each shrub species per treatment were recorded. With such a small sample number, confidence intervals for detecting significant differences were large. A larger sample size could have decreased confidence intervals and increased precision (Whitlock and Schluter, 2009). Although leaf senescence and chlorosis are common symptoms in flooded plants, no previous research on flooding tolerance or drought tolerance was found for this species. Based on these results, it is difficult at this point to classify *I. floridanum* as suitable or unsuitable for use in rain gardens.

Herbaceous Perennials

Chasmanthium latifolium not only tolerated repeated short-term flooding, but responded positively to flooding based on many of the data collected (Table 8). In at least one of the three runs, SI, LA, SDW, and SC were higher in flooded plants than non-flooded plants. Average SI for all plants increased during the course of each experimental run. The average difference in SI can be explained by the maturity of the plants at the beginning of the experimental runs and the fact that *C. latifolium* is known to peak in the summer (Thetford et al., 2009). Upon inspection of the plants at harvest, *C. latifolium* developed two characteristics not seen in any of the other species: robust root systems that penetrated the entirety of the 97 L containers in flooded plants and aerenchyma tissue in the stems. Aerenchyma are a well-known plant acclimation in water-logged environments that transport oxygen to depleted roots (Blom and Voesenek, 1996). Water-logged soils have been shown to be advantageous for some flood tolerant grass species, such as *C. latifolium,* because it increases the number of adventitious roots per stem and nearly doubles root porosity (Colmer, 2002). Similarly, *C. latifolium* thrived in a three year low-input performance study (Thetford et al., 2009). Although *C. latifolium* produces seeds copiously indications of an invasive tendency have not been reported. Therefore, based on the results of this experiment and others, *C. latifolium* is an excellent southeastern rain garden shade plant.

Insect pests were not discriminatory and attacked flooded and non-flooded *L. cardinalis* plants similarly. Thus, all damage appeared to be related to infestation, not flooding stress. Not all plants were damaged, and those that were undamaged maintained good visual quality regardless of flooding treatment. Additionally, vegetative growth and flowering occurred in undamaged flooded and non-flooded plants. Although data are needed to confirm, *L. cardinalis* appears to tolerate repeated short term flooding. Concerning unwanted insects, evidence suggests that drought may support outbreaks of plant-eating insects and fungi whereas flooding is associated with various types of rots and foliar diseases (Mattson and Haack, 1987). In a raingarden setting where supplemental watering is not utilized, herbivorous pests may benefit from dry conditions between rain events.

Although herbaceous perennials and ferns were potted one each per container, crowding in the 97L containers was not an issue for the time allotted for each experimental run. However, had the experiment been longer, crowding could have affected the data collected.

Ferns

This research revealed that *O. cinnamomea* was very tolerant of rain garden conditions, and *P. acrostichoides* was not. *Osmunda cinnamomea* was not affected by flooding for any of the data collected. Furthermore, this species was visually appealing under both treatments (personal observation). Under the same flooding conditions, *P. acrostichoides* performed poorly. Flooding did not affect *P. acrostichoides* in the spring run, but summer SI, LCC, and SC were negatively affected by flooding (Table 8). Two of the six summer 2015 plants died. A time by flooding treatment interaction was found for LCC and

SC (Table 5), which suggest that a longer experimental period could have resulted in more loss. Personal observations of *P. acrostichoides* were consistent with the data collected. Plants seemed to suffer from the 5 d drying period and somewhat recovered from drying when flooded. Intolerance for dry conditions may explain why this species was not negatively affected by flooding in the fall as opposed to the hot summer months. In a vegetative community study *P. acrostichoides* was identified as a strong indicator species of the presence of near surface water (defined as saturated soil or water table within 30 cm), which supports that this species was intolerant of the 5 d no watering period (Choi et al., 2012). Results from *P. acrostichoides* highlight the importance of rain garden research under various wet/dry periods as well as seasonal studies. *P. acrostichoides* would be recommended for use in rain gardens based on the spring run. However, it is not recommended in southeastern rain gardens based on the summer run. If this species were selected for use in a southeastern rain garden, it may require supplemental irrigation during periods of drought.

Stomatal conductance

Stomatal conductance was consistently higher in non-flooded plants than flooded plants in *I. floridanum, M. cerifera, and P. acrostichoides* (summer 2015 only) (Table 8). Stomata are known to close in response to flooding to prevent loss of oxygen (Kozlowski, 1984, 1997). However in plants that are adapted to waterlogged soils, such as *C. latifolium* and *P. acrostichoides*, stomatal conductance can be enhanced (as it was in this research) by continuous flooding or periodic flooding (Li et al., 2004). Understanding evapotranspiration potential is an important consideration because transpiration modifies rain garden hydrology (Rouse, 2007). Rouse (2007) reported that evapotranspiration was nearly 50% of the hydrologic budget when shrubs were planted in rain gardens, which is consistent with the findings of this research. Whole plant transpiration estimates for both shrub species were nearly double that of *C. latifolium* in the fall 2014 run. Therefore, transpiration rates may be considered when designing rain gardens. Utilizing plants with lower transpiration rates may result in longer drying times in a rain garden but may also allow more infiltration for groundwater recharge. Conversely, plants with high transpiration rates may speed drying in areas where standing water may not be acceptable. Additionally, the inclusion of evergreen plants such as the shrubs used in this research promotes year round rain garden functionality (Dougherty et al., 2007).

Rain garden plant recommendations

Plants were not evaluated long-term. Therefore, it is unknown if there would be an accumulation effect of prolonged stress, particularly for shrub species. Further, this research did not include a winter experimental run. Rain garden soil may stay waterlogged for a longer period of time and stomatal conductance rates are expected to be lower in cooler months. As such, the effects of winter intermittent flooding for the plants tested in this study are not known. However, each plant in this experiment was tested during the wettest time of year for southeastern states, May through September.

Based on the findings in this research, *M. cerifera, C. latifolium,* and *O. cinnamomea* are recommended for use in southeastern rain gardens. All three plants will tolerate the wettest zone in a rain garden because this study used the maximum recommended flooding time (48 h) (Seymour, R. M., 2005). *P. acrostichoides* is not recommended for southeastern rain gardens that do not receive supplemental irrigation during dry periods due to suspected drought intolerance. Future studies are needed to determine if *I. floridanum* and confirm that *L. cardinalis* would tolerate short-term flooding followed by intermittent dry periods. Finally, increasing the variety of plant species suitable for use in rain gardens may facilitate increased public acceptance and utilization in a range of commercial, residential, and municipal landscape situations.

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Table 1. Run dates for *Illicium floridanum, Morella cerifera, Chasmanthium latifolium, Lobelia cardinalis, Osmunda cinnamomea,* and *Polystichum acrostichoides*. The experiment was conducted in summer 2014 (SU 14), fall 2014 (FA 14), spring 2015 (SP 15) and summer 2015 (SU 15).

	Species	Run	Initiation	Termination	Duration	
					Days	Weeks
Shrubs	*I. floridanum*	SU 14	10-Jun-14	29-Jul-14	49	7
		FA 14	2-Sep-14	30-Oct-14	58	8
	M. cerifera	SU 14	10-Jun-14	29-Jul-14	49	7
		FA 14	26-Aug-14	23-Oct-14	58	8
Perennials	*C. latifolium*	SU 14	10-Jun-14	29-Jul-14	49	7
		SP 15	17-Mar-15	12-May-15	56	8
		SU 15	1-Jun-15	27-Jul-15	56	8
	L. cardinalis	SU 14	10-Jun-14	29-Jul-14	49	7
		SP 15	17-Mar-15	12-May-15	56	8
		SU 15	1-Jun-15	27-Jul-15	56	8
Ferns	*O. cinnamomea*	SP 15	24-Mar-15	19-May-15	56	8
		SU 15	25-May-15	20-Jul-15	56	8
	P. acrostichoides	SP 15	24-Mar-15	19-May-15	56	8
		SU 15	25-May-15	20-Jul-15	56	8

Table 2. Shoot dry weight (SDW) and leaf:stem DW ratio of *Illicium floridanum*, *Morella cerifera,* and *Chasmanthium latifolium* after 7-8 wks of repeated short term flooding. Plants were flooded (F) for 48 h followed by 5 d of no watering or watered every other day (NF).

Species	Run[z]	SDW (g)		Leaf:stem	
		F	NF	F	NF
I. floridanum	SU 14	247.9	236.7	1.02a[y]	0.78b
	FA 14	265.8	281.7	1.54	1.25
M. cerifera	SU 14	232.5	260.4	0.70	0.82
	FA 14	286.7	296.7	0.47b	0.63a
C. latifolium	SU 14	22.7	22.2	0.96	0.62
	SP 15	31.3	18.7	1.12	1.26
	SU 15	82.1a	49.5b	0.60	0.81

[z]Summer 2014 (SU 14), fall 2014 (FA 14), spring 2015 (SP 15) and summer 2015 (SU 15)

[y]Letters indicate significant differences within run between F and NF at $P<0.05$

Table 3. Leaf chlorophyll content (LCC, measured using SPAD meter) of *Illicium floridanum*, *Morella cerifera*, and *Chasmanthium latifolium* after 7-8 wks of repeated short term flooding. Plants were flooded for 48 h followed by 5 d of no watering (flooded, F) or watered every other day (non-flooded, NF).

Species	Run[z]	LCC	
		F	NF
I. floridanum	SU 14	59.5	63.3
	FA 14	60.4b[y]	66.4a
M. cerifera	SU 14	45.4	46.1
	FA 14	53.8a	52.0b
C. latifolium	SU 14	40.3	39.6
	SP 15	30.6b	38.0a

[z] Summer 2014 (SU 14) and fall 2014 (FA 14).

[y] Letters indicate significant differences within run between F and NF at P<0.

Table 4. Stomatal conductance for *M. cerifera* after 7-8 wks of repeated short term flooding. Plants were flooded for 48 h followed by 5 d of no watering (flooded, F) or watered every other day (non-flooded, NF).

	Stomatal conductance ($mmol \bullet m^{-2} \bullet s^{-1}$)		
Treatment	Dry[z]	Wet[y]	Overall[x]
F	107.4b	165.8b	166.1b[w]
NF	253.8a	216.4a	243.5a

[z] Stomatal conductance measured after 5 d of no watering

[y] Stomatal conductance measured after 48 h of flooding

[x] Combines all stomatal conductance measurements within each treatment

[w] Letters indicate significant differences between flooded and non-flooded at $P<0.05$.

Table 5. Stomatal conductance (SC) and leaf chlorophyll content (LCC) and for *Polystichum acrostichoides* after 8 wks of cyclic flooding (summer 2015). Plants were flooded for 48 h followed by 5 d of no watering (flooded, F) or watered every other day (non-flooded, NF).

		Weeks of flooding				
	Treatment	2	4	6	8	Sig.[y]
SC ($mmol \bullet m^{-2} \bullet s^{-1}$)	F	142.2ns	127.0b[z]	81.3b	22.8b	L**
	NF	177.7	269.8a	224.3a	222.1a	NS
LCC	F	41.8ns	45.9ns	43.5b	28.3b	Q*
	NF	48.5	50.9	59.1a	54.8a	NS

[z] Letters indicate significant differences at $P<0.05$.

[z] Significance of linear (L) or quadratic (Q) at = 0.1 (*) and 0.01 (**).

Table 6. Average volumetric substrate moisture content for all experimental runs in 97 L containers with simulated rain garden flooding. Plants were flooded for 48 h followed by 5 d of no watering (flooded, F) or watered every other day (non-flooded, NF). Substrate moisture was measured at the end of the 5 d no watering period. *Illicium floridanum* and *Morella cerifera* (11 L) were planted one per container. *Chasmanthium latifolium* and *Lobelia cardinalis* (3.8 L, herbs) were planted one each per container. *Osmunda cinnamomea, Polystichum acrostichoides* (3.8 L, ferns) were planted one each per container.

	Substrate moisture ($m^3 \cdot m^{-3}$)			
Treatment	*I. floridanum*	*M. cerifera*	Herbs	Ferns
F	28.7	25.0	18.5	18.8
NF	25.7	24.6	17	19.0

Table 7. Whole plant transpiration estimates based stomatal conductance (mmol•m^{-2}•s^{-1}) and total plant leaf area (m^2) of *Illicium floridanum* (IF), *Morella cerifera* (MC), *Osmunda cinnamomea* (OC), *Polystichum acrostichoides* (PA), and *Chasmanthium latifolium* (CL) after 7-8 weeks of flooding. Plants were flooded for 48 h followed by 5 d of no watering (flooded, F) or watered every other day (non-flooded, NF). The experiment was conducted in summer 2014 (SU 14), fall 2014 (FA 14), spring 2015 (SP 15) and summer 2015 (SU 15).

		Whole Plant Transpiration (mol•s^{-1})							
		SU 14		FA 14		SP 15		SU15	
Type	Species	F	NF	F	NF	F	NF	F	NF
Shrub	IF	18.4a[z]	0.513b	0.097	0.201	-	-	-	-
Shrub	MC	0.242	0.527	0.131	0.186	-	-	-	-
Fern	OC	-	-	-	-	0.095	0.075	0.054	0.053
Fern	PA	-	-	-	-	0.021	0.018	0.002a	0.018b
Perennial	CL	0.141a	0.047b	-	-	0.022	0.025	0.237a	0.142b

[z] Letters indicate significant differences between treatments for each species within a run at $P<0.05$.

Table 8. Summary of responses to 7-8 wks of cyclic flooding for five species: *Illicium floridanum* (IF), *Morella cerifera* (MC), *Osmunda cinnamomea* (OC), *Polystichum acrostichoides* (PA), and *Chasmanthium latifolium* (CL). Runs were summer 2014 (SU 14), fall 2014 (FA 14), spring 2015 (SP 15), and summer 2015 (SU 15). Measurements included: size index (SI), leaf area (LA), leaf chlorophyll content (LCC), shoot dry weight (SDW), leaf:stem DW ratio, and stomatal conductance (SC). Plants were flooded for 48 h followed by 5 d of no watering (flooded, F) or watered every other day (non-flooded, NF). If a difference occurred between species, the treatment with a higher value is highlighted.

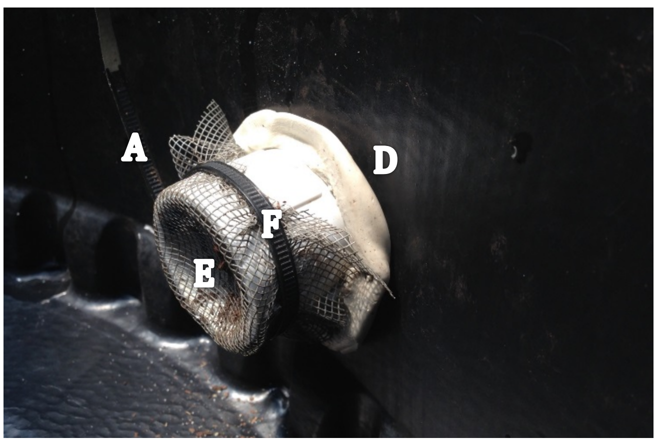
Type	Species	Run	SI[z]	LA	LCC	SDW	Leaf:stem[x]	SC
Shrub	IF	SU 14	ND[y]	ND	ND	ND	F	NF - F decreased over time
		FA 14	ND	ND	NF	ND	NF	NF when wet
	MC	SU 14	NF	ND	ND	ND	ND	NF - F decreased over time
		FA 14	ND	ND	NF	ND	NF	NF
Fern	OC	SP 15	ND	ND	ND	ND	-	ND
		SU 15	ND	ND	ND	ND	-	ND
	PA	SP 15	ND	ND	ND	ND	-	F
		SU 15	NF	ND	NF over time	ND	-	NF - F decreased over time
Perennial	CL	SU 14	ND	F	ND	ND	F	F
		SP 15	ND	ND	NF	ND	ND	ND
		SU 15	F	F	ND	F	ND	ND

[z] SI = (height + widest width + perpendicular width)/3

[y] No significant difference is denoted by ND.

[x] DW ratios were not collected for fern

Figure 1. To control flooding, custom 97L containers without drainage holes were retrofitted with a two-piece (male and female) 2.54 cm PVC socket ball valve. Holes were drilled 8 cm from the bottom of the custom containers to accommodate the drainage valves. Before adjoining the male (A) and female (B) couplings, 2.54 cm O-rings (C) were placed inside and outside of the holes. A band of plumber's putty (D) was placed between the inside (male) coupling and the O-ring to prevent leakage. Window screening (E) was cut in 7.5 cm^2 segments and affixed to the male coupling (opposite of tub) with a zip tie (F). Valves (G) were shut to maintain flooding treatments and opened to allow drainage.



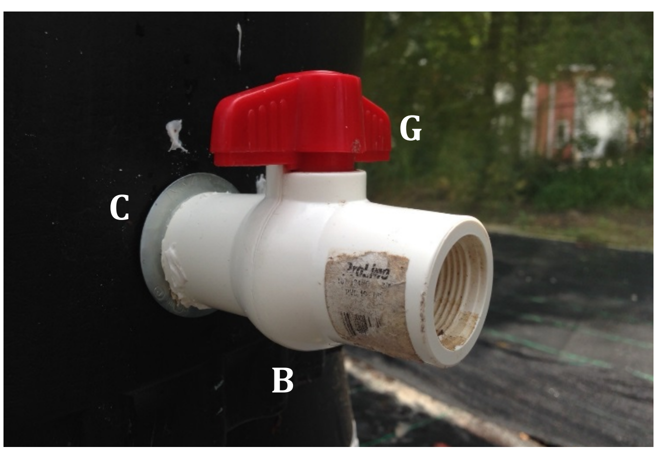


Figure 2. Size Index of shrubs: *Morella cerifera* (MC) and *Illicium floridanum* (IF), ferns: *Osmunda cinnamomea* (OC) and *Polystichum acrostichoides* (PA), and a perennial: *Chasmanthium latifolium* (CL) after 7 – 8 weeks of cyclic flooding. Plants were flooded for 48 h followed by 5 d of no watering (flooded, F) or watered every other day (non-flooded, NF). The experiment was conducted in summer 2014 (SU 14), fall 2014 (FA 14), spring 2015 (SP 15) and summer 2015 (SU 15). SI = (height + widest width + perpendicular width)/3. Significant differences between treatments for each species within a run at $P<0.05$ indicated by "*".

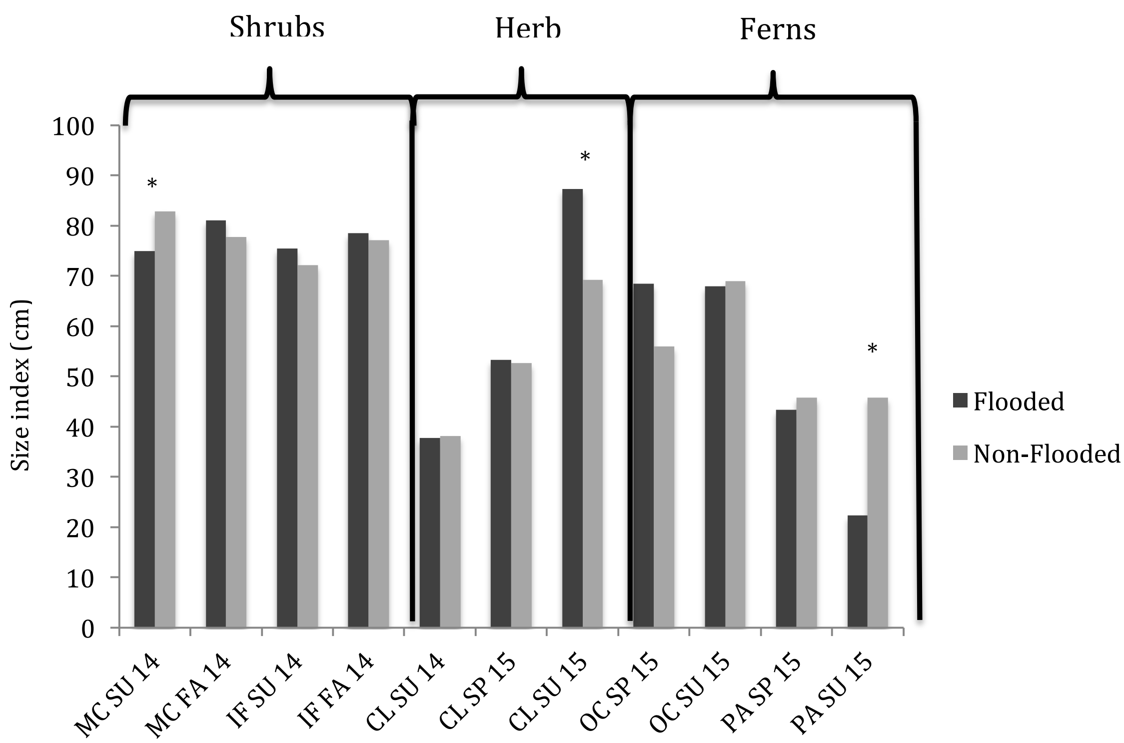


Figure 3. Leaf Area of *Morella cerifera* (MC), *Chasmanthium latifolium* (CL), *Illicium floridanum* (IF), *Osmunda cinnamomea* (OC), and *Polystichum acrostichoides* (PA) after 7 – 8 weeks of cyclic flooding. Plants were flooded for 48 h followed by 5 d of no watering (flooded, F) or watered every other day (non-flooded, NF). The experiment was conducted in summer 2014 (SU 14), fall 2014 (FA 14), spring 2015 (SP 15) and summer 2015 (SU 15). Significant differences between treatments for each species within a run at P<0.05 indicated by "*".

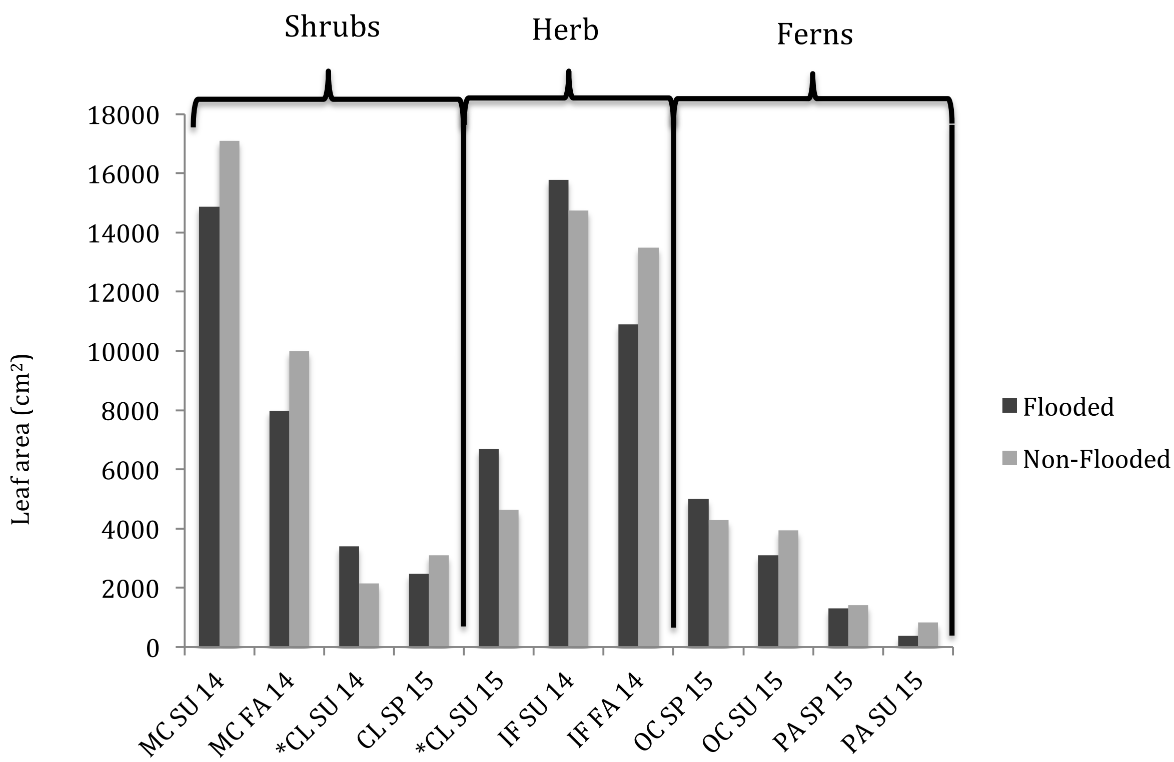
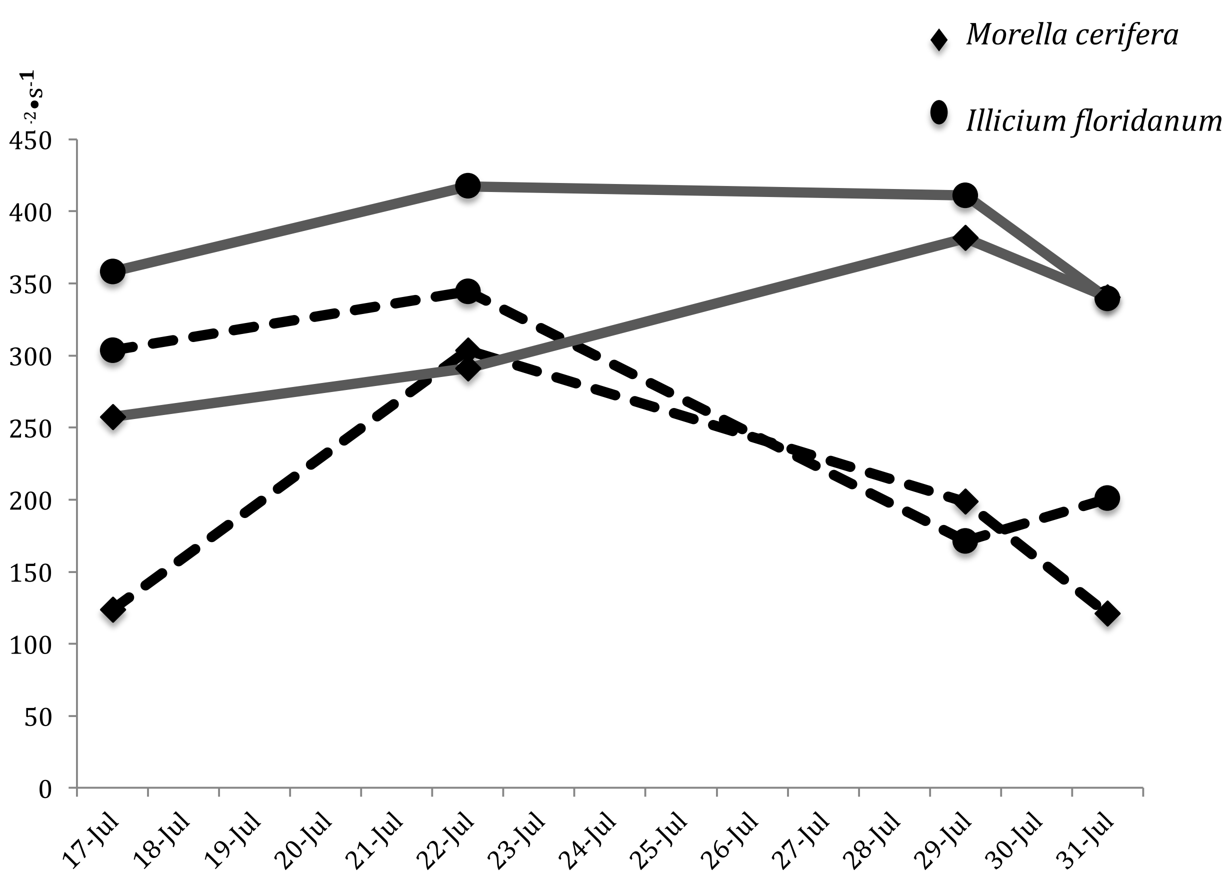


Figure 4. Stomatal conductance (SC) for *Morella cerifera* and *Illicium floridanum* after 7-8 wks of repeated short term flooding (summer 2014). Plants were flooded for 48 h followed by 5 d of no watering (dashed lines) or watered every other day (solid lines). Measurments were take after 5 d of no watering (22 Jul and 29 Jul) and after 28 h of flooding (17 Jul, 24 Jul, and 31 Jul). For both shrub species, there was a time of year by treatment interaction, and SC was higher for non-flooded plants.



Appendix A

Wetland Indicator Status by Region of Researched-based Rain Garden Native Plant Selections

		Atlantic Coastal Plains	*Eastern Mountains and Piedmont*
Evergreen Shrubs			
Ilex vomitoria 'Schillings dwarf' yaupon holly	(Christian et al., 2012; Dylewski et al., 2012)	FAC	FAC
Ilex glabra 'Shamrock' inkberry	(Dylewski et al., 2012)	FACW	FAC
Illicium floridanum Florida anise	Authors' suggestion	FACW	FACW
Morella cerifera wax myrtle	Authors' suggestion	FAC	FAC
Deciduous Shrubs			
Clethra alnifolia 'Ruby Spice' summersweet	(Jernigan and Wright, 2011)	FAC	FAC
Fothergilla x intermedia 'Mt. Airy' dwarf witch alder	(Jernigan and Wright, 2011)	FACW	FACW
Itea virginica 'Henry's Garnet' sweetspire	(Dylewski et al., 2012)	FACW	OBL
Viburnum nudum 'Winterthur' possumhaw	(Dylewski et al., 2012)	FACW	OBL
Ilex verticillata 'Winter Red' winterberry	(Jernigan and Wright, 2011)	FACW	FACW
Perennials			
Coreopsis verticillata 'Zagreb' whorled coreopsis	(Meder, 2013)	-	-
Echinacea purpurea 'Magnus Superior' purple coneflower	(Meder, 2013)	-	-
Lobelia cardinalis cardinal flower	Authors' suggestion	FACW	FACW
Grasses			
Chasmanthium latifolium river oats	Authors' suggestion	FAC	FACU
Andropogon ternarius broomsedge	(Meder, 2013)	FACU	FACU
Muhlenbergia capillaris muhly grass	(Christian et al., 2012)	FAC	FACU
Ferns			
Osmunda cinnamomea cinnamon fern	Authors' suggestion	FACW	FACW
Polystichum acrostichoides Christmas fern	Authors' suggestion	FACU	FACU
Facultative (FAC); Facultative wetland (FACW); Obligate wetland (OBL); Facultative upland (FACU) plants.usda.gov			

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