MORPHOLOGICAL AND PHYSIOLOGICAL LEAF ADAPTATIONS TO SEASONAL AND DIURNAL ABIOTIC STRESS FOR TWO BARRIER ISLAND SAND DUNE SPECIES

By

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A Dissertation Submitted to the Graduate Faculty of

WAKE FOREST UNIVERSITY

GRADUATE SCHOOL OF ARTS AND SCIENCES

In Partial Fulfillment of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

in the Department of Biology

August 2009

Winston-Salem, NC

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ACKNOWLEDGEMENTS

I would like to first thank my parents, Mel Pitts and Dale Joesting, for their love and support for the past decade of my college adventures as well as in all the decisions I have made for the last 31 years. I would also like to thank my wonderful supportive significant other Matt Marenberg for being my rock through the entire dissertation process and my research assistant during long hot days at the beach. I would also like to acknowledge my grandfather Elden Lee Pitts who instilled the love of science and nature in my mother and I and whose stubborn and inquisitive mind I've inherited. Without the love and support of these people, I would never have made it this far.

I would also like to thank my advisor William K. Smith for all the guidance he has given me during this dissertation research, for always being available to answer any question I had, no matter how trivial, and for giving me the opportunity to conduct research in a habitat so near and dear to my heart. I also thank my committee members, Drs. Ronald V. Dimock, Jr., Miles R. Silman, Kathy A. Kron, and Tara L. Greaver for all their assistance and advice. These people have made me a better scientist, and I have learned so much from them.

Also, thanks to the members of the Smith lab past and present, namely Z. Carter Berry for always being up for a sushi lunch and his many reviews of manuscripts and graphs, Joseph White for assistance in the field, and Cristin Walters for always being up for a "chat" break, as well as the many undergraduates who assisted in the field and the laboratory. I'd also like to thank my extended family of friends here in Winston Salem who have made the past five years some of the best in my life.

Finally, I'd like to thank my two cats, Winston and Taylor, for laying on my computer when I tried to write and laying on articles when I tried to read, and my dog Tela for being the best companion a girl could ask for on my frequent trips to the beach.

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MORPHOLOGICAL AND PHYSIOLOGICAL LEAF ADAPTATIONS TO SEASONAL AND DIURNAL ABIOTIC STRESS FOR TWO BARRIER ISLAND SAND DUNE SPECIES

Dissertation under the direction of William K. Smith, Ph.D.,
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The coastal barrier island ecosystem provides protection to the coastline from the storm and wave energy associated with extreme episodic storm events, such as hurricanes, tropical storms, and nor'easters. As well as protecting the coastline, barrier islands also produce, protect, and maintain inlets, bays, estuaries, wetlands, and salt marshes which are highly productive areas in terms of net primary production and provide important ecosystem services to the coastline. The combined effect of urban development and the predicted increased tropical cyclone activity and sea level rise associated with global climate change places barrier islands, their fauna and flora, and anthropogenic development at great risk. The sand dune ridge and swale system found on many barrier islands function as a buffer during coastal storm events and native sand dune vegetation are critical in the formation and maintenance of these systems. The creation and maintenance of artificial dune systems using native sand dune species will be an important strategy in coastal management plans, functioning to provide protection to beachfront property while maintaining natural habitat and sustaining ecosystem processes. Information regarding the leaf morphological and physiological adaptations of native dune vegetation to season and diurnal abiotic stresses will be critical in artificial dune construction to promote optimal growth conditions for native species. The aim of this dissertation research was to explore these adaptations for two common sand dune species during the growth season on a North Carolina barrier island.

The first two studies (Chapter III and IV) focus on the leaf orientation properties (i.e. leaf angle and leaf azimuth) of the clonal dune perennial *Hydrocotyle bonariensis* and its role in regulating sunlight incidence on both leaf surfaces, the influence on developing leaf anatomy, and the effect on leaf temperature and photosynthetic gas exchange. Results showed no daily variations in leaf orientation, but did reveal an increase in leaf inclination over the growth season that reduced midday sunlight incidence and an increase in leaf azimuth which tracked the progressively more southern solar azimuth, maximizing sunlight incidence in the morning and/or evening. In addition, leaf cross-sections revealed multiple adaxial palisade mesophyll layers and abaxial spongy mesophyll, corresponding to predictions based on the total amount, as well as the ratio between, sunlight incidence of both leaf surfaces. Inclined leaves had greater leaf photosynthesis, transpiration, and stomata conductance and lower leaf temperatures compared to experimental leaves held in a horizontal orientation, indicating the importance of leaf inclination in maintaining optimal leaf temperatures and facilitating leaf photosynthetic gas exchange. Furthermore, results from an experiment impeding gas exchange, thus blocking evaporative cooling, indicated an important role of transpiration in reducing leaf temperatures in *H. bonariensis*.

The last two studies (Chapter V and VI) investigate the influence of leaf structure and the abiotic environment on leaf physiology of *Hydrocotyle bonariensis*, in addition to the common shrub *Iva imbricata*, in the sand dune habitat. Leaf structure was significantly different between species and could be predicted for both species according to their position along the sand dune abiotic stress gradient. In addition, seasonal and diurnal leaf temperatures, leaf photosynthetic gas exchange, and photoinhibition risk significantly differed between species. Specifically, photoinhibition occurred during the growth season in *H. bonariensis* leaves, most pronounced at midday, while there was no photoinhibition in *I. imbricata*, and leaf photosynthesis and water use efficiency was greater while leaf temperature, transpiration, and stomata conductance were lower in *I. imbricata* compared to *H. bonariensis*. Multiple regressions revealed that leaf orientation properties, the presence of stomata on abaxial and adaxial leaf surfaces, leaf water content, vapor pressure deficit, and air temperature were important predictors of leaf physiology in *H. bonariensis*, while leaf size, presence of stomata on both leaf surfaces, leaf water content, vapor pressure deficit, and incident sunlight of the habitat were the best predictors for leaf physiology in *I. imbricata*. Furthermore, seasonal and diurnal patterns suggested that leaf orientation properties and rapid transpiration are critical in reducing photoinhibition and facilitating photosynthetic gas exchange in *H. bonariensis* while reduced leaf size, increased transpiration, and presence of water-storage cells in *I. imbricata* alleviates photoinhibition and maintains optimal conditions for leaf photosynthetic gas exchange.

In conclusion, this study is one of few that examine the relationship between leaf structure and orientation, leaf physiology, and the abiotic environment in coastal sand dune species, and provides additional evidence on the importance of leaf structure and orientation in regulating leaf-level microclimate and promoting leaf photosynthesis Results from this study will provide essential information in coastal management strategies using native vegetation to create and maintain sand dune systems.

CHAPTER I

AN INTRODUCTION TO BARRIER ISLANDS AND SAND DUNE RIDGE AND SWALE SYSTEMS

Barrier islands (BI) are dynamic, ecologically and economically significant ecotones between the continental and the oceanic landscape. These elongated bodies of sand are bordered on each end by inlets that facilitate salt- and freshwater exchange between the estuarine system behind the island and the shoreline in front of the island (Pilkey et al. 1998). BI occur along 85% of the Atlantic and Gulf coastline of the United States, comprising approximately 515 km of ocean beach in North Carolina alone (Snyder and Boss 2002; Pilkey et al. 2004). In general, Atlantic and Gulf coast BI are located 3 – 30 km from the mainland, are 2 – 5 km wide and 10 – 100 km long, and island elevation is generally 3 – 6 m above sea level, with the exception of a few areas such as Jockey Ridge, North Carolina, where dune elevations reach up to 30 m (Dolan et al. 1980). Tides along the Atlantic coast range from 1 – 3 m, average daily wave heights are 0.5 – 1 m, and storm waves generally range from 4 – 5 m with storm surges of 1 – 2 m.

BI function to provide critical physical protection to adjacent coastlines, including economically and ecologically important estuaries, bays, lagoons, and marshes, from wave and wind action associated with extreme episodic storm events, such as hurricanes and nor'easters (Snyder and Boss 2002). BI beaches are capable of radically changing shape during storm events to absorb energy and minimize damage to coastal shorelines from storm waves (Riggs 1976; Pilkey et al. 1978, 2004). During severe storms, the beach and dune ridge and swale system may be severely impacted by overwash events and flooding that can level and reconfigure sand dunes and transport sediment across the island, changing the macrotopographic characteristics, general shape, and contour of an entire island (Fahrig et al. 1993; Snyder and Boss 2002; Pilkey et al. 2004).

1. Barrier islands as a 'dynamic equilibrium'

The BI system is often described as a 'dynamic equilibrium' whose formation and maintenance is dependent upon the interaction of waves, sea level rise, beach sand erosion and accretion, and beach geometry (Pilkey et al. 1978, 1998, 2004). Due to the dynamic nature of these islands and the factors involved in their maintenance, BI are always in some continuous state of transience, when considered over a long enough time frame. BI are highly variable in their landscape morphology according to differences in geomorphic factors, climate history, and vegetation, and dynamic sand movement and the processes maintaining BI occur on several temporal scales ranging from hours to decades (Riggs 1976; Dolan et al. 1980; Ehrenfeld 1990; Pilkey et al. 1998). Although each island is unique in its morphology, BI systems are often interconnected and interdependent (Riggs 1976). For example, a BI may depend on neighboring islands for sand supply, and frequently the dynamic processes of one BI are coupled to the processes of others (Pilkey et al. 1978).

BI can be characterized into two types based on which processes, or combination of processes (i.e. waves or tides), are most influential. Wave-dominated microtidal BI, such as the Outer Banks of North Carolina, are long and narrow with widely spaced inlets (Stallins 2002). These BI are characterized by very high wave energy and low tidal ranges, and storm overwash is a frequent event, resulting in generally low-relief topography, possibly with a single line of foredunes. On the other hand, mixed-energy mesotidal BI are short and wide with closely spaced inlets, such as BI along the southeastern coast of North Carolina. These islands have low wave energies and a high tidal range, and storm overwash is relatively infrequent, commonly allowing the development of dune ridge and swale systems that function to dampen effects of coastal storm overwash.

2. Evolution and maintenance of barrier islands

Geologically speaking, BI are relatively new formations (5000 – 10000 years BP) and were a result of rapid sea level rise during the Holocene (Riggs 1976; Pilkey et al. 1998, 2004). As sea level began to rise rapidly approximately 15000 – 18000 years BP, mainland river valleys were flooded, forming estuarine systems, and shoreline retreat was initiated through erosion processes (Pilkey et al. 1978, 2004). Eroded sediment was distributed as parallel spits along the shoreline, bound to land on one end and extending into the ocean at the other end. Through the interaction of breaching and rapid sea level rise, the areas behind the spits were flooded, creating individual barrier islands. Once these islands formed, the main source of sediment shifted from the eroding shoreline to the continental shelf, and the islands began to migrate landward while the shoreline continued to retreat in response to rising sea level. The rate of sea level rise slowed approximately 4000 – 5000 years BP, resulting in a corresponding decrease in migration rates of BI and the current state of 'dynamic equilibrium' (i.e. balance among storm and wave energy, sea level, and sediment quantity) (Dolan et al. 1980; Pilkey et al. 1978, 2004).

Upon initial formation, as well as presently, BI migrate and change shape, vegetation, and landforms to maintain 'dynamic equilibrium' (Riggs 1976; Pilkey et al. 1978, 1998). To migrate, the front (ocean) side of the island must move landward through erosion processes while the back (sound) side must move landward by growth. Simultaneously, the island must maintain its elevation and volume through overwash fan deposition and dune formation (Riggs 1976; Pilkey et al. 1998).

3. Extreme episodic storm events and barrier islands

High energy coastal storms play an important role in maintaining BI systems by providing the basic energy for several environmental and geomorphic components of BI processes (Riggs 1976). The two main types of coastal storms that affect the Atlantic and Gulf Coast of the United States are tropical cyclones (e.g. tropical storms and hurricanes) and extratropical storms, often called nor'easters (Riggs 1976; Pilkey et al. 1998). Both types of storms produce high winds, and thus high storm surges and high wave heights, and heavy rains, but the most significant damage is often associated with tropical cyclones. Hurricane intensity is defined by the Saffir/Simpson scale, and there are five categories based on measurements of wind speed and central pressure, ranging from least intense to most intense. A category 1 hurricane is characterized by wind speeds of 74 – 95 mph and central pressure of 980 mmbars, and a category 5 hurricane is characterized by wind speeds greater than 155 mph and central pressures less than 919 mmbars (National Hurricane Center, http://www.nhc.noaa.gov; Pilkey et al. 1998). African waves moving westward along the tropical North Atlantic Ocean and Caribbean Sea form the majority of Atlantic tropical cyclones, and ~85% of major hurricanes (> category 3) are formed from these waves (Goldenberg et al. 2001). In addition to the storm intensity, the impact of a tropical cyclone on a BI depends on seven variables: (1) the extent of storm surge associated with the storm, (2) the direction of forward motion, (3) the tide magnitude and pattern of the BI system, (4) the size and extent of backbarrier water bodies, (5) the degree of anthropogenic development on the BI, (6) the frequency of extreme episodic storm events, and (7) the storm duration (Riggs 1976).

The greatest reshaping of the BI shoreline occurs during extreme storm events as high energy storm waves transport sand from the upper beach of the BI to the lower beach, flattening the beach and dissipating wave energy over a broader area (Pilkey et al. 1998, 2004). Additionally, storm waves may transport sand to the backside (landside) of the island (i.e. overwash events), and storm winds can blow sand from the upper beach into the dune ridge and swale system (Hayden et al. 1995; Pilkey et al. 2004). Furthermore, strong seaward-directed currents can form during storm events, temporarily rising sea level (storm surge) and increasing wave heights, thus intensifying overwash and sediment displacement (Pilkey et al. 2004).

4. Sand dune ridge and swale system

There is a very tight coupling between the geomorphology of a BI and the vegetation cover. BI vegetation communities are determined in part by island elevation, depth to freshwater, and groundwater salinity, and island topography is partly determined by the mechanism of seed dispersal and vegetative growth via rhizomes of the sand dune vegetation (Hayden et al. 1995; Pilkey et al. 2004). Over relatively short time scales, vegetation promotes sand deposition, shaping dune landforms, which in turn influence sediment mobility, pattern and frequency of overwash events, and the structure of physical gradients, all which affect vegetation diversity and cover (Stallins and Parker 2003).

The development of sand dune ridge and swale systems is associated with the abundant supply of sediment common to mixed-energy mesotidal BI (Stallins 2002). The process of dune formation depends on BI orientation with regard to prevailing winds, the rate of sea level rise, the frequency of storm events, and the vegetation community (Godfrey 1977). Embryo sand dunes form when vegetation or beach debris located on the berm slows down or blocks the wind, thus trapping blowing sand (Pilkey et al. 2004). As more sand accumulates, more dune building vegetation colonizes, trapping more sand, and the embryo dune grows. Eventually, plant species with stabilizing growth habits, such as clonal and prostrate habits, colonize the developing dune and begin to stabilize the substrate (Figure 1). The size and shape of sand dune systems are variable and depend on the amount of sand available, the size of sand grains, and the prevailing wind direction.

Sand dune vegetation has been classified into four functional groups based on their response to sand burial and their role in building and maintaining the sand dune ridge and swale system: (1) dune-building species with upright growth that traps sediment and has a positive growth response to sand accumulation (e.g. *Uniola paniculata*, *Iva imbricata*), (2) burial-tolerant species with rhizomatous growth that stabilizes the substrate and has a positive growth response to burial (e.g. *Hydrocotyle bonariensis*), (3) burial-intolerant species with a negative growth response to burial that provide additional stabilization to substrate (e.g. *Oenothera humilus*), and (4) passenger species found in sand dune communities that have no role in sediment movement (Stallins 2002, 2005).

The driving force in the sand dune ridge and swale system is the transport of sand by wind and the presence of dune-building, burial-tolerant stabilizing, and burial-tolerant stabilizing species that form and maintain dune topography (Baas 2002; Stallins 2002). The elevational contrasts of the dune system is sustained through the positive relief (i.e. dune ridges) created by dune-building and burial-tolerant stabilizing species, and the low intervening swales established by burial-intolerant stabilizing species (Stallins and Parker 2003). Thus, the vegetation of sand dune systems reinforce their presence by sustaining dune ridges that dampen overwash exposure and maintaining habitat conditions essential for their dominance (Stallins and Parker 2003).

5. The abiotic environment and sand dune vegetation ecology

The abiotic environment is an obvious selective force acting on BI sand dune vegetation both temporally and spatially. Extreme episodic storm events can immediately effect vegetation through complete destruction of the vegetation community, burial and/or exposure from sediment displacement and storm surge overwash, mechanical damage from increased wind speeds, and periodic inundation by saltwater (Figure 2). In addition, overwash events can temporarily alter salinity, water, nutrient, and edaphic conditions, negatively effecting growth and allocation in plant species (Costa et al. 1996). However, sand dune vegetation communities can recover rather quickly (1 – 3 years) following coastal storm events, due to the presence of a seed bank and/or vegetative growth from plant fragments (Snyder and Boss 2002). In addition, sand dune vegetation (especially species located in overwash zones) should possess physiological and/or morphological adaptations to survive extreme episodic storm events, although the specifics of these adaptations remain to be elucidated (Schroder et al. 1979; Fahrig et al. 1993; Snyder and Boss 2002).

The daily and seasonal abiotic stress factors of sand dune systems include salt spray and salt deposition, high incident sunlight and substrate reflectance, high air and substrate temperature, high wind resulting in dessication and sand abrasion, nutrient deficiency, sand movement (accretion and erosion), and water stress (Oosting 1954; van der Valk 1977; Barbour et al. 1985). The vegetation is subject to high incident sunlight due to lack of a plant canopy and the sparse distribution patterns, and the sandy substrate can reflect up to 34% of incident sunlight (Oosting 1954; *personal observation*). In addition, soil surface temperatures can exceed 50°C during the growing season, and air temperatures often exceed 32°C at midday (*personal observation*). The topography of the sand dune creates a mosaic pattern of soil water content, wind patterns, and exposure to salt spray deposition (Oosting 1954). High winds may cause sand abrasion and mechanical damage to plants as well as cause blowouts that expose roots and rhizomes to dessication. Soil (i.e. sand) has a low water holding capacity, thus soil nutrients are quickly leached out, and soil evaporation rates are relatively high due to high wind and temperatures. Although sand dune systems are usually not deficient in plant micronutrients (e.g. calcium, magnesium, and sodium), receiving many of these cations through salt spray, they are characteristically deficient in plant macronutrients (i.e. nitrogen, phosphorous, and potassium) essential for growth and carbon allocation.

Several of the abiotic factors of sand dune systems (i.e. sediment movement, salt spray, and storm overwash) have been shown in many studies to occur along steep gradients with increasing distance from the ocean, resulting in distinct vegetation zones (Oosting and Billings 1942; Boyce 1954; Barbour 1978; Moreno-Casasola 1986; Ehrenfeld 1990; Hesp 1991; Wilson and Sykes 1999; Stallins and Parker 2003). Based on the interaction of these factors and their influence on vegetation, the following distinct vegetation zones are characteristic of BI: (1) the berm (upper beach and embryo dunes), consisting mainly of pioneer annual plants and dune-building species, (2) the primary dunes, consisting of dune-building and burial-tolerant stabilizing grasses, herbs, and shrubs, (3) the swale, or the area between the dune crests, consisting of sparsely distributed burial-intolerant herbs and shrubs, (4) the secondary dunes, consisting of dune-stabilizing beach grasses, herbs, and perennial shrubs, (5) maritime forest behind the dunes, consisting of woody shrubs and trees, and (6) salt marsh behind the maritime forest on the landward side of the island (Ehrenfeld 1990; Pilkey et al. 2004) (Figure 3).

Sand burial has been described as a critical force driving vegetation patterns on barrier islands, filtering species along an environmental gradient of sand movement (Stallins 2002, 2005). Wind is the main agent of sand movement, picking up small loose sand particles from the beach, and carrying these particles long distances along the shore as well as perpendicular to the shore. In addition, storm surge overwash transports volumes of sand across the island, and can deposit sand in the sand dune system as storm surge recedes. Furthermore, stabilized dunes can function as a barrier for overwash, resulting in increased sediment deposition in front of the dunes. Since sand dune species vary in their response to sand burial, and sand burial occurs along an environmental gradient, a clear vegetation pattern results (Stallins and Parker 2003). Dune-building species are often found on the upper beach and the primary dune area where sediment movement and deposition from overwash is greatest, burial tolerant stabilizing species are often found in the primary dune, and burial intolerant species only occupy habitats inland where the risk of burial is reduced.

Salt spray is strongly influenced by distance from the ocean, and thus also occurs along an environmental gradient, decreasing with distance from the ocean (Oosting and Billings 1942; van der Valk 1977). As waves break along the shoreline, small droplets of seawater are caught by the wind and blown across the island and the topography of the sand dune system and the growth habit of vegetation has a significant influence on salt deposition patterns. Experiments varying the salinity of soil water and salt spray on leaves have shown that tolerance to salt spray varies among sand dune species, thus influencing BI vegetation patterns (Oosting and Billings 1942; Boyce 1954; Oosting 1954). Species with high tolerance to salt spray are commonly found in the upper beach and primary dune whereas intolerant species are limited to secondary dunes and grasslands where salt spray and salt deposition is reduced.

Many studies have attributed the characteristic sand dune vegetation patterns to the frequency of periodic saltwater inundation and sand burial due to storm overwash (Fahrig et al. 1993; Hayden et al. 1995; Costa et al. 1996; Stallins 2002, 2005, 2006; Stallins and Parker 2003). In addition to the differing tolerances to sand burial, species also vary in their tolerance to salt water inundation that often occurs as wave run-up during storm events (Hayden et al. 1995). Sand dune height may protect species behind the dunes from being inundated (if above the wave run-up height), thus creating a pattern of zonation with burial- and inundation-tolerant species occurring closer to the ocean and intolerant species occurring more inland. Moreover, plant diversity decreases as overwash probability increases, and thus, species diversity tends to be maximal at inland locations (Hayden et al. 1995, *personal observation*).

Species composition within each zone is determined by the plants' morphology, reproduction, and physiological tolerance to both abiotic stresses of the environment and extreme episodic storm events (Costa et al. 1996). Morphological leaf adaptations reported for BI vegetation include reduced leaf size, heavy cutinization, leaf pubescence, palisade mesophyll under both leaf surfaces (i.e. isobilateral symmetry), leaf gigantism, hypertrophy and succulence, amphistomatous leaves (i.e. stomata on both leaf surfaces), salt bladders, sclerophyllous leaves, prostrate growth, and leaf orientation properties (Oosting 1954; Hesp 1991; Blackman et al. 2005). Alterations in reproduction exhibited by BI species included timing of flowering, seed set, and germination, clonal and/or vegetation reproduction, large seeds, salt or burial stimulated germination and seedling growth, and saltwater-tolerant seeds (Barbour et al. 1985; Lee and Ignaciuk 1985; Fahrig et al. 1993). Physiological leaf adaptations include increased water use efficiency, adoption of the C_4 or CAM photosynthetic pathway, osmotic adaptations, ability to use mixture of salt- and freshwater and burial-stimulated growth (Barbour et al. 1985; Lee and Ignaciuk 1985; Rozema et al. 1985; Hesp 1991; Greaver and Sternberg 2006).

6. Global climate change and barrier islands

Humans have altered the composition of Earth's atmosphere and land surface through burning of fossil fuels, emission of methane and nitrous oxide, and changes in land-use due to agriculture and urbanization, resulting in an interruption of natural global energy flow (Karl and Trenberth 2003). Global temperatures are expected to warm by an interval of up to 4.9° by 2100 in the absence of climate mitigation, and the rate of change can be slowed but not stopped in the 21st century. Predictions of global climate change include altered precipitation patterns, a substantial rise in sea level (18 – 59 cm by 2100), and an increase in tropical cyclone frequency and intensity, placing low-lying coastal systems at great risk (Karl and Trenberth 2003; Titus et al. 2009).

Global climate change is expected to drastically alter global precipitation patterns. In addition to an overall ecosystem decrease in water availability, a decrease in total precipitation could result in salinization of BI freshwater sources, severely altering ecosystem services (Greaver and Sternberg 2010). The water table of most BI exists as a lens of low density freshwater floating above a layer of high density saltwater (i.e. Gyben-Hertzberg Lens), and a decrease in precipitation may cause this freshwater lens to shrink along the edges, allowing salt-water intrusion. Salinization of normally freshwater sources would result in an alteration in the vegetation community and ecosystem food webs, reducing species diversity and disrupting ecosystem processes.

Sea level has risen at a rate of 1 – 2 mm yr^{-1} in the past 100 years, and global climate change is expected to accelerate this rate through expanding ocean water and melting glaciers (Scavia et al. 2002). Global climate change has increased sea temperatures, and warmer water is more expansive than cooler water, thus increasing sea level. In addition, the melting of glaciers due to increased global temperature adds water to the ocean through runoff (Pilkey et al. 2004). A rise in global sea level will result in increased coastal flooding and inundation, as well as increased shoreline erosion (Titus et al. 2009).

The effect of global climate change on tropical cyclone frequency is difficult to identify, but elevated sea surface temperatures are predicted to result in a 5 – 10% increase in wind strength, exacerbating storm wave height and storm surge (Scavia et al. 2002; Anthes et al. 2006). There is strong support for a significant relationship between tropical cyclone intensity and changes in sea surface temperature (Emanuel 1987; Goldenberg et al. 2001; Webster et al. 2005). Warmer sea surface temperatures decrease the stability of the atmosphere, resulting in tropical cyclones that are more resistance to vertical wind shear (which modulates major hurricane frequency), and thus increasing frequency and/or intensity (Goldenberg et al. 2001; Knutson and Tuleya 2004). As stated previously, most major hurricanes are formed from African waves, making these storms strongly influenced by climate changes in the tropics (Goldenberg et al. 2001). Several studies have shown increased hurricane activity during the past 50 years (with up to 99% confidence) as well as a general increase in storm impacts along the East coast in the past 100 years (Zhang et al. 2000; Goldenberg et al. 2001; Knutson and Tuleya 2004; Emanuel 2005; Webster et al. 2005, Anthes et al. 2006).

It has been suggested that some coastal landforms, e.g. BI and wetlands, have a geomorphic threshold, or ‘tipping point’, with regard to sea level rise and increased storm intensity that, once reached, will lead to rapid and irreversible changes (Titus et al. 2009). For BI, these irreversible changes include rapid landward migration, decreased width and height, increased overwash frequency, increased barrier breaching and inlet formation, and island segmentation. In addition, coastal wetlands are predicted to lose area in response to sea level rise and changes in other climatic and environmental drivers, reducing populations of wetland-dependent bird and fish species.

7. Coastal management practices

Human development on the coast is at great risk, and management decisions will need to be made soon as to how to sustain anthropogenic development and coastal industries (e.g. fishing and seafood industry) while maintaining natural ecosystem processes. Increased anthropogenic development on BI has forced stability onto a naturally dynamic system, resulting in increased beach erosion, exploitation of freshwater resources, and addition of waste products into wetlands and thus adjusting ecological balance (Dolan et al. 1980). In addition, potential damage from a coastal storm is determined not only by its intensity but also the degree of coastal development (Zhang et al. 2000). In the face of future global climate change, developed coastal communities must either allow the shore to retreat or hold back the sea using some type of coastal engineering to stabilize the shoreline (Scavia et al. 2002). Coastal management practices aimed at protecting the shoreline generally fall into strategies designed to replace lost sand (beach replenishment), inhibit sand-carrying currents (jetties and groins), function as breakwaters (seawalls, bulkheads, sandbags, and revetments), and stabilize sediments (artificial dune construction) (Dolan 1976; Pilkey et al. 1978). The former three strategies (beach replenishment, groins and jetties, and seawalls) have been shown to have downstream negative impacts on the shoreline, whereas the latter strategy (artificial sand dunes) would promote natural geomorphological and ecosystem processes.

The three most common types of shoreline stabilization are beach replenishment, groins and jetties, and seawalls (Pilkey et al. 1978). Beach replenishment involves pumping new sand onto the beach to build dune ridges, but replenishment projects need to be repeated often, large quantities of compatible sand must be available, and often a steep beach is created, which accelerates erosion (Dolan 1976; Pilkey et al. 1978). Groins and jetties are walls built perpendicular to the shoreline to trap sand and often result in erosion of adjacent BI through sediment starvation. Seawalls are built set back and parallel from the shoreline and are designed to withstand the full impact of a tidal cycle. The harmful impacts of seawalls include increased erosion by steepening the offshore profile, increasing the intensity of longshore currents, and blocking exchange of sand between the beach and the dunes (Figure 4). In addition, studies have shown that coastal armoring, especially using seawalls, results in habitat loss and a corresponding reduction in species diversity (Dugan et al. 2008).

The coastal management strategy of creating artificial sand dunes reduces impact of storm waves, providing protection to beach-front property, while facilitating natural geological, hydraulic, and ecosystem processes (Figure 5). For example, artificial dunes constructed from geotubes (large horizontal structures made of woven biodegradable textile and filled with sediment) and planted with three native dune-building grass species on Galveston Island, Texas, prevented significant erosion during several tropical storms and minor hurricanes in 2001 – 2003 (Feagin 2005). Artificial sand dune construction often involves efforts to improve existing dunes or create new dunes through planting dune-building species and/or using sand fences that reduce wind speed and result in sediment accumulation (Pilkey et al. 2004). Along the Southeastern U.S. Atlantic and Gulf coast, *Uniola paniculata* (sea oats) and *Panicum amarum* (bitter panicum) are the dominant dune-building grasses on coastal dunes and have historically been the primary grasses used in artificial dune construction (Woodhouse and Cooper 1968; Dahl and Woodward 1977). However, an undisturbed sand dune ridge and swale system consists of additional dune-building and burial tolerant stabilizing species, and dunes constructed with natural vegetation and maintained through natural succession processes may provide greater stabilization while maintaining habitat and ecosystem processes.

Future coastal management decisions in the face of global climate change will require a basic understanding of BI biogeomorphology and the role of these processes in ecosystem health. In addition to the risk to beachfront property, poor coastal management practices place seafood and fishing industries in danger due to downstream environmental impacts on wetlands, estuaries, and other critical habitats. Advances in sedimentology (study of sand movement), morphodynamics (island evolution), paleotempestology (study of past tropical cyclones), and stratigraphy (study of rock layers) provide integral tools in understanding basic BI geomorphological processes in space and time, the stability of the shoreface, historical responses to coastal storms and sea level rise, and the impact of different coastal management practices (Liu and Fearn 2000; Scott et al. 2003; Sedgewick and Davis 2003; Schwartz and Birkemeier 2004; Stolper et al. 2005; Elko and Davis 2006; Elsner et al. 2008). Furthermore, the role native vegetation plays in morphodynamics, as well as vegetation response to coastal storm events and the abiotic environment, will be critical in modeling and predicting future impacts of sea level rise and coastal storms as well creating and maintaining artificial sand dunes using native vegetation that provides stability for anthropogenic development while sustaining ecosystem processes.

8. Study objectives

The aim of this dissertation research was to investigate the leaf morphological and physiological adaptations of two common sand dune species to the diurnal and seasonal abiotic stresses of the BI habitat. The two species chosen for this study have different growth habits and spatial distributions along the abiotic gradient of the sand dune ridge and swale system. *Hydrocotyle bonariensis* is a burial-tolerant stabilizing clonal perennial herb found in the swale, secondary dunes, and tertiary dune grasslands whereas *Iva imbricata* is a dune-building leaf-succulent shrub that occupies all habitats from the berm to the tertiary dune grassland. The results from this dissertation will be critical in coastal management strategies using native vegetation for the creation and maintenance of artificial sand dunes. Chapter II provides a detailed description of the study site, including an assessment of the vegetation community, and the two study species, including examination of leaf surface topography and internal anatomy. The first two studies (Chapter III and IV) investigate the influence of leaf orientation properties in *H. bonariensis* on sunlight incidence of both leaf surfaces, leaf temperatures, and leaf photosynthetic gas exchange. The next two studies (Chapter V and VI) determine which leaf structure and/or abiotic factors best predict leaf photosynthetic gas exchange in both species.

Literature cited

Anthes, R.A., R.W. Corell, G. Holland, J.W. Hurrell, M.C. MacCracken, and K.E. Trenberth. 2006. Hurricanes and global warming – potential linkages and consequences. American Meteorological Society 87: 623 –628.

Baas, A.C.W. 2002. Chaos, fractals and self-organization in coastal geomorphology: simulating dune landscapes in vegetated environments.

Barbour, M. 1978. Salt spray as a microenvironmental factor in the distribution of beach plants at Point Reyes, California. Oecologia 32: 213 - 224.

Barbour, M.G., T.M. DeJong, and B.M. Pavlik. 1985. Marine beach and dune plant communities. *In* Physiological Ecology of North American Plant Communities. Ed. B.F. Chabot and H.A. Mooney. Chapman and Hall, Cambridge: 296 - 322.

Blackman, C.J., G.J. Jordan, and R.J.E. Wiltshire. 2005. Leaf gigantism in coastal areas: morphological and physiological variation in four species on the Tasman Peninsula, Tasmania. Australian Journal of Botany 53: 91 - 100.

Boyce, S. 1954. The salt spray community. Ecological Monographs 24: 29 - 67.

Costa, C., C.V. Cordazzo, and U. Seeliger. 1996. Shore disturbance and dune plant distribution. Journal of Coastal Research 12: 133 - 140.

Dahl, B.E. and D.W. Woodward. 1977. Construction of Texas coastal foredunes with sea oats (*Uniola paniculata*) and bitter panicum (*Panicum amarum*). International Journal of Biometeorology 21: 267 – 275.

Dolan, R. 1976. Barrier beachfronts. *In* Barrier Islands and Beaches: Technical proceedings from the 1976 barrier island workshop. J. Clark, Ed. The Conservation Foundation: pp. 1 - 4.

Dolan, R., B. Hayden, and H. Lins. 1980. Barrier islands. American Scientist 68: 16 – 25.

Dugan, J.E., D.M. Hubbard, I.F. Rodil, D.L. Revell, and S. Schroeder. 2008. Ecological effects of coastal armoring on sandy beaches. Marine Ecology 29: 160 – 170.

Ehrenfeld, J.G. 1990. Dynamics and processes of barrier island vegetation. Aquatic Sciences 2: 437 - 480.

Elko, N.A. and R.A. Davis, Jr. 2006. Morphologic evolution of barrier islands with different coastal management. Journal of Coastal Research Special Issue 39: 127 – 131.

Elsner, J.B., T.H. Jagger, and K. Liu. 2008. Comparison of hurricane return levels using historical and geological records. Journal of Applied Meteorology and Climatology 47: 368 – 374.

Emanuel, K.A. 1987. The dependence of hurricane intensity on climate. Nature 326: 483 - 485.

Emanuel, K. 2005. Increasing destructiveness of tropical cyclones over the past 30 years. Nature 436: 686 – 688.

Fahrig, L., B. Hayden, and R. Dolan. 1993. Distribution of barrier island plants in relation to overwash disturbance: a test of life history theory. Journal of Coastal Research 9: 403 - 412.

Feagin, R.A. 2005. Artificial dunes created to protect property on Galveston Island, Texas: the lessons learned. Ecological Restoration 23: 89 – 94.

Godfrey, P.J. 1977. Climate, plant response, and development of dunes on barrier beaches along the U.S. East Coast. International Journal of Meteorology 21: 203 – 215.

Goldenberg, S.B.C., C.W. Landsea, A.M. Mestax-Nunez, and W.M. Gray. 2001. The recent increase in Atlantic hurricane activity: causes and implications. Science 293: 474 - 479.

Greaver, T.L. and L.S.L. Sternberg. 2006. Linking marine resources to ecotonal shrifts of water uptake by terrestrial dune vegetation. Ecology 87: 2389 – 2396.

Greaver, T.L. and L.S.L. Sternberg. 2010. Decreased precipitation exacerbates the effects of sea level on coastal dune ecosystems in open ocean islands. Global Change Biology 16: 1860 – 1869.

Hayden, B.P., M.C.F.V. Santos, G. Shao, and R.C. Kochel. 1995. Geomorphological controls on coastal vegetation at the Virginia Coast Reserve. Geomorphology 13: 283 - 300.

Hesp, P. 1991. Ecological processes and plant adaptations on coastal dunes. Journal of Arid Environments 32: 165 - 191.

Karl, T.R. and K.E. Trenberth. 2003. Modern global climate change. Science 302: 1719 – 1723.

Knutson, T.R. and R.E. Tuleya. 2004. Impact of CO_2-induced warming on simulated hurricane intensity and precipitation: sensitivity to the choice of climate model and convective parameterization. Journal of Climate 17: 3477 – 3495.

Lee, J.A. and R. Ignaciuk. 1985. The physiological ecology of strandline plants. Vegetatio 62: 319 - 326.

Liu, K. and M.L. Fearn. 2000. Reconstruction of prehistoric landfall frequencies of catastrophic hurricanes in Northwestern Florida from lake sediments. Quaternary Research 54: 238 – 245.

Moreno-Casasola, P. 1986. Sand movement as a factor in the distribution of plant communities in a coastal dune system. Vegetatio 65: 67 - 76.

Oosting, H.J. and W.D. Billings. 1942. Factors effecting vegetational zonation on coastal dunes. Ecology 23: 131 - 142.

Oosting, H.J. 1954. Ecological processes and vegetation of the maritime strand in the southeastern United States. Botanical Review 20: 226 – 262.

Pilkey, O.H. Jr, W.J. Neal, O.H. Pilkey Sr., and S.R. Riggs. 1978. From Currituck to Calabash: Living with North Carolina's Barrier Islands. Duke University Press, Durham. 244 pp.

Pilkey, O.H., B.A. Cowan, C.A. Webb, D.M. Bush, D.F. Pilkey, S.R. Riggs, and W.J. Neal. 1998. The North Carolina Shore and its Barrier Islands. Duke University Press, Durham. 318 pp.

Pilkey, O.H., T.M. Rice, and W.J. Neal. 2004. How to Read a North Carolina Beach: Bubble Holes, Barking Sand, and Rippled Runnels. University of North Carolina Press, Chapel Hill. 162 pp.

Riggs, S.R. 1976. Barrier islands as natural storm dependent systems. *In* Barrier Islands and Beaches: Technical proceedings from the 1976 barrier island workshop. J. Clark, Ed. The Conservation Foundation: pp. 1 - 4.

Rozema, J., P. Bijwaard, G. Prast, and R. Broekman. 1985. Ecophysiological adaptations of coastal halophytes from foredunes and salt marshes. Vegetatio 62: 499 - 521.

Scavia, D., J.C. Field, D.F. Boesch, R.W. Buddemeier, V. Burkett, D.R. Cayan, M. Fogarty, M.A. Harwell, R.W. Howarth, C. Mason, D.J. Reed, T.C. Royer, A.H. Sallenger, and J.G. Titus. 2002. Climate Change impacts on U.S. coastal and marine ecosystems. Estuaries 25: 149 - 164.

Schroeder, P.M., B. Hayden, and R. Dolan. 1979. Vegetation changes along the United States east coast following the Great Storm of 1962. Environmental Management 4: 331 - 338.

Schwartz, R.K. and W.A. Birkemeier. 2004. Sedimentology and morphodynamics of a barrier island shoreface related to engineering concerns, Outer Banks, NC, USA. Marine Geology 211: 215 – 255.

Scott, D.B., E.S. Collins, P.T. Gayes, and E. Wright. 2003. Records of prehistoric hurricanes on the South Carolina coast based on micropaleonotological and sedimentological evidence, with comparison to other Atlantic coast records. Geological Society of America Bulletin 115: 1027 – 1039.

Sedgewick, P.E. and R.A. Davis, Jr. 2003. Stratigraphy of washover deposits in Florida: implications for recognition in the stratigraphic record. Marine Geology 200: 31 – 28.

Snyder, R.A. and C.L. Boss. 2002. Recovery and stability in barrier island plant communities. Journal of Coastal Research 18: 530 - 536.

Stallins, J. A. 2002. Dune plant species diversity and function in two barrier island biogeomorphic systems. Plant Ecology 165: 183 - 196.

Stallins, J. and A.J. Parker. 2003. The influence of complex systems interactions on barrier island dune vegetation pattern and process. Annals of the Association of American Geographers 93: 13 -29.

Stallins, J. A. 2005. Stability domains in barrier island dune systems. Ecological Complexity 2: 410 - 430.

Stallins, J. A. 2006. Geomorphology and ecology: unifying themes for complex systems in biogeomorphology. Geomorphology **77**: 207 - 216.

Stolper, D., J.H. List, and E.R. Thieler. 2005. Simulating the evolution of coastal morphology and stratigraphy with a new morphological-behaviour model (GEOMBEST). Marine Geology 218: 17 – 36.

Titus, J.G., E.K. Anderson, D.R. Cahoon, S. Gill, R.E. Thieler, and J.S. Williams. 2009. Coastal sensitivity to sea-level rise: a focus on the Mid-Atlantic Region. United States Climate Change Science Program (CCSP) and the Subcommittee on Global Change Research, U.S. Environmental Protection Agency, Washington D.C., USA, 790 pp. 790.

van der Valk, A.G. 1977. The macroclimate and microclimate of coastal foredune grasslands in Cape Hatteras National Seashore. International Journal of Biometeorology 21: 227 – 237.

Webster, P.J., G.J. Holland, J.A. Curry, and H.-R. Chang. 2005. Changes in tropical cyclone number, duration, and intensity in a warming environment. Science 309: 1844 – 1846.

Wilson, J. and M.T. Sykes. 1999. Is zonation on coastal sand dunes determined primarily by sand burial or salt spray? A test in New Zealand dunes. Ecology Letters 2: 233 - 236.

Woodhouse, Jr., W.W., E.D. Seneca, and A.W. Cooper. 1968. Use of sea oats for dune stabilization in the Southeast. Shore and Beach 36: 15 – 21.

Zhang, K., B.C. Douglas, and S.P. Leatherman. 2000. Twentieth-century storm activity along the U.S. East Coast. Journal of Climate 13: 1748 – 1761.

Figure captions

Figure I – 1: (A) Embryo dune developing on berm at Topsail Island, North Carolina. The growth habit of the dune-building shrub *Iva imbricata* blocked the wind and trapped sand, resulting in increased sand accumulation. (B) Developing sand dunes of the primary dunes. The developing dunes have been colonized by multiple dune-building species (*Iva imbricata* and *Uniola paniculata*).

Figure I – 2: Possible impacts of a coastal storm on the barrier island sand dune ridge and swale system. (A) Increased wave height combined with storm surge can result in complete destruction of the berm vegetation community. (B) Sediment displaced from storm waves and overwash can expose roots and rhizomes or (C) be deposited in the dune ridge and swale system as overwash recedes, resulting in burial. (D) Overwash may also result in temporary saltwater inundation from wave run-up.

Figure I – 3: Characteristic vegetation zones of mixed-energy mesotidal barrier islands. Species diversity increases with distance from the ocean while abiotic stress decreases.

Figure I – 4: End of seawall at Galveston Island, Texas after Hurricane Ike in 2008. The seawall resulted in sediment starvation on the beach beside the seawall, resulting in extreme erosion.

Figure I – 5: Vegetated sand dunes provide protection to property behind dunes from storm wave energy at Topsail Island while promoting ecosystem processes. (A) Dune line at Topsail Island, North Carolina before coastal storm and (B) dune line after a nor'easter caused increased storm waves in September 2008.

Figure I – 1

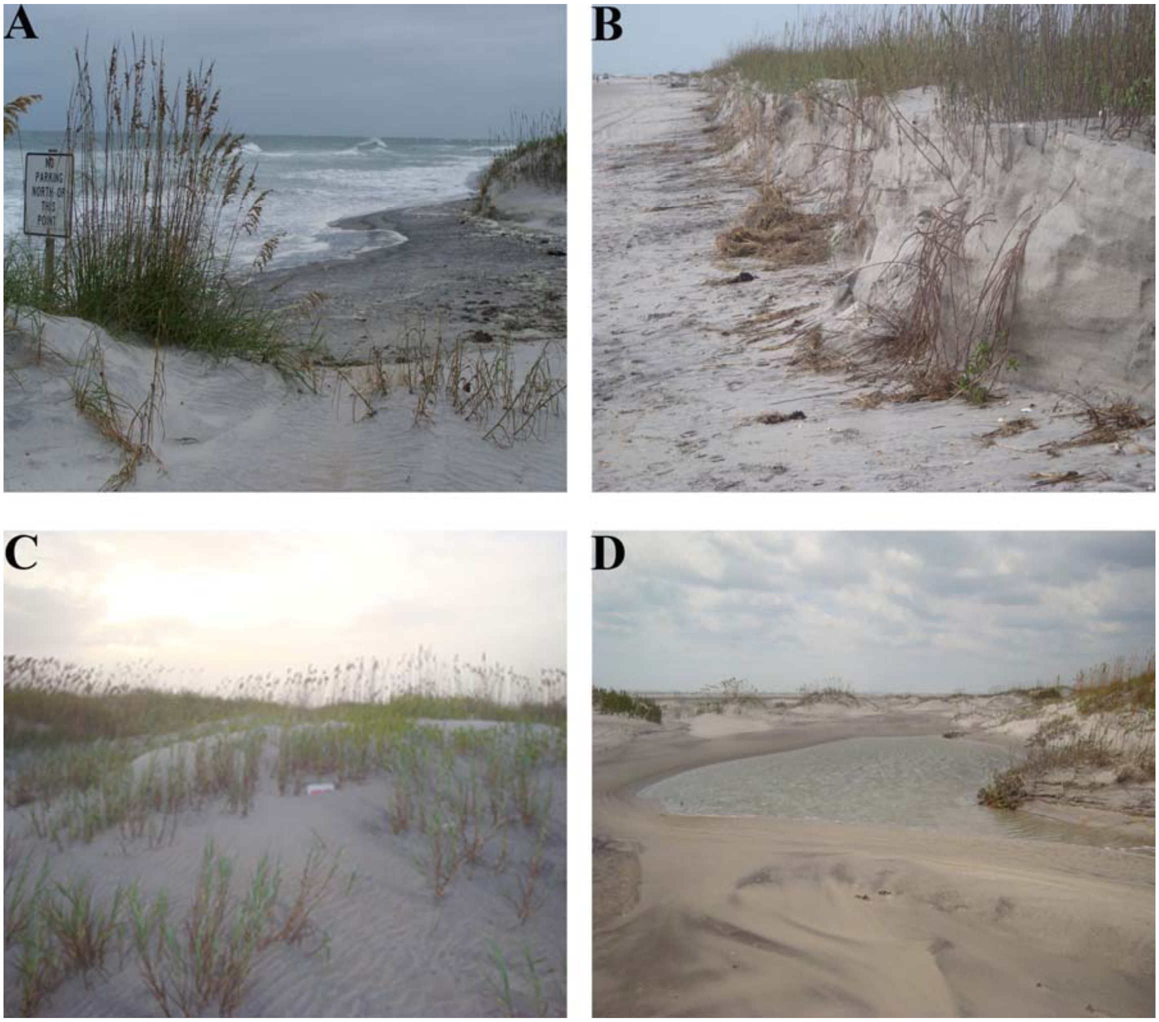


Figure I – 2

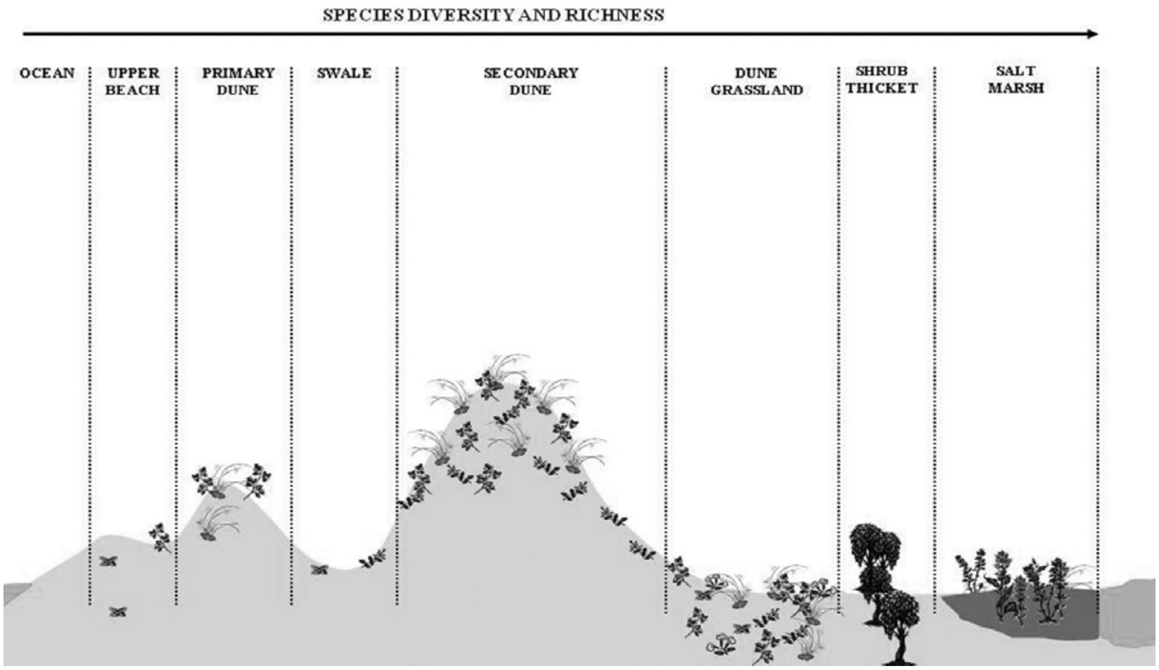

SPECIES DIVERSITY AND RICHNESS
OCEAN
UPPER BEACH
PRIMARY DUNE
SWALE
SECONDARY DUNE
DUNE GRASSLAND
SHRUB THICKET
SALT MARSH

Figure I – 3

Figure I – 4

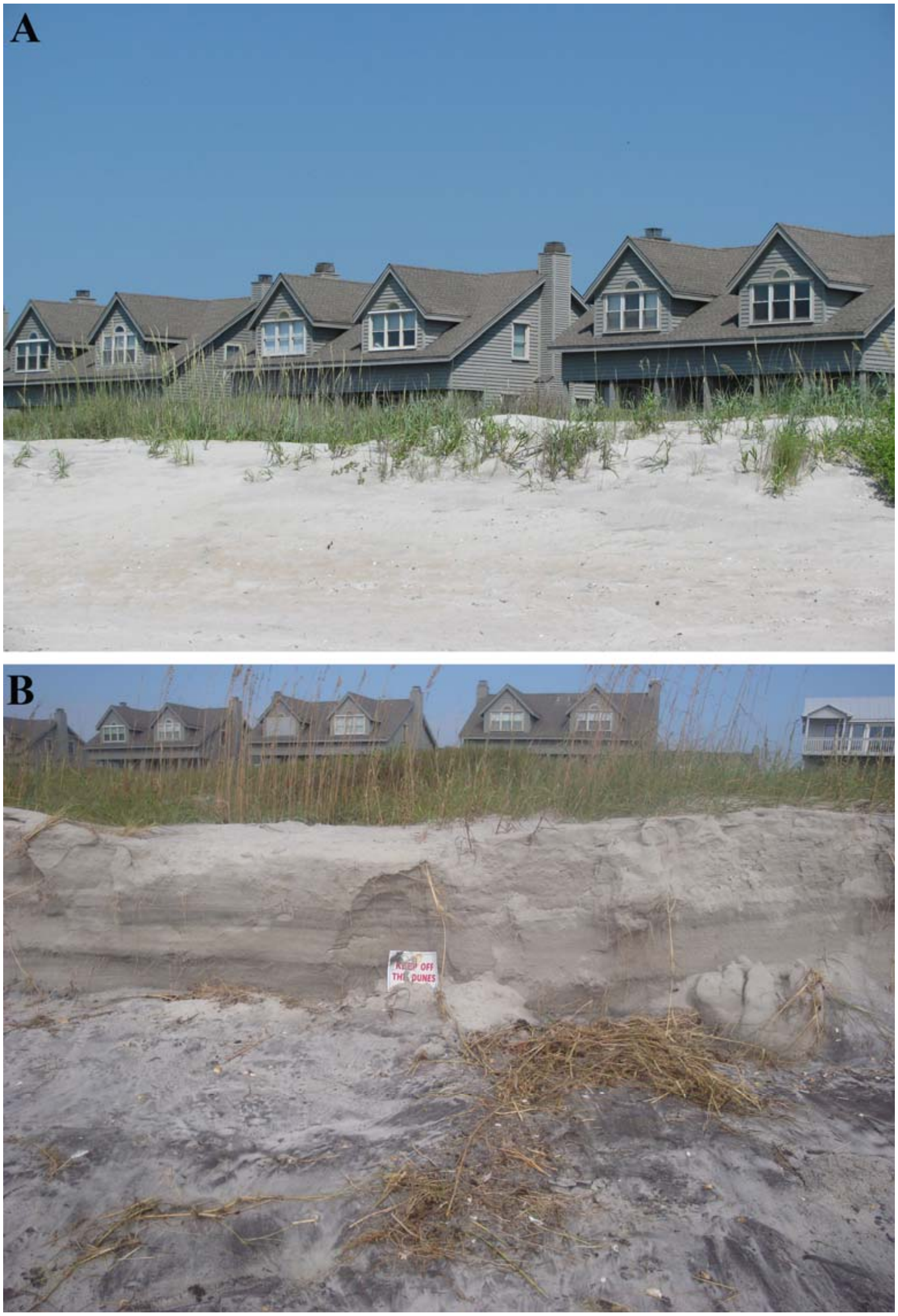


Figure I – 5

CHAPTER II

INTRODUCTION TO STUDY SITE AND STUDY SPECIES

The focus of this dissertation research was to elucidate the adaptations of two common barrier island sand dune species with different growth habits to diurnal and seasonal abiotic stresses of the coastal barrier island environment. Most research previously conducted on barrier island sand dune vegetation has concentrated on plant distribution patterns rather than the morphological and physiological adaptations of individual species or functional groups (Oosting and Billings 1942; Travis 1977; Moreno-Casasola 1986; Hayden et al. 1995; Costa et al. 1996: Wilson and Sykes 1999). Thus, the research conducted for this dissertation concentrated on the morphological and physiological leaf adaptations related to photosynthetic carbon gain and carbon allocation patterns in response to abiotic stresses such as high incident sunlight and high air and substrate temperature for *Iva imbricata* and *Hydrocotyle bonariensis* in the sand dune ridge and swale system of a North Carolina barrier island.

1. Study site

The research presented in this dissertation was conducted during the 2003 and 2007 – 2010 growth seasons on the southern end of Topsail Island, North Carolina, United States (34°20’N, 77°39’W), a barrier island off the coast of Pender County (Figure 1). Topsail Island is a low and narrow barrier island approximately 39-km long situated between New River Inlet and New Topsail Inlet. The majority of beachfront property is substantially developed with residential homes and commercial structures. However, the southern end of the island is an extended sand spit currently zoned for conservation and contains an undisturbed sand dune ridge and swale system (~ 1 – 2 km from the last home on the beachfront).

1.1. Climatology

North Carolina barrier islands are characterized by westerly winds, evenly distributed precipitation events, and seasonal evaporation cycles (Robinson 2003). The climate of Topsail Island is temperate and seasonal, and summer humidity is relatively high, with humidity between 95 and 100% recorded (North Carolina State Climate Office, http://www.nc-climate.ncsu.edu/cronos/). Mean wind gusts (non-storm related) range between 4 $m \cdot s^{-1}$ to 7 $m \cdot s^{-1}$ with maximum wind speeds of 16.5 $m \cdot s^{-1}$ recorded. At the study site, mean air temperatures ranged from 5.1°C in January to 26.9°C in July in 2003, 5.5 ºC in February to 26.1ºC in August in 2007, 6.3°C in January to 25.6°C in July in 2008, 5.8°C in January to 25.9°C in August in 2009, and 2.4°C in December to 26.8°C in June in 2010. Annual precipitation was 1616 mm, 851 mm, 1261 mm, 1925 mm, and 1281 mm in 2003, 2007, 2008, 2009, and 2010, respectively. The growth season (defined as the last spring and first fall minimum of 0°C) lasted between 212 days in 2007 and 284 days in 2003, and generally began in March and ended in November (weather data from National Climate Data Center, http://www7.ncdc.noaa.gov/IPS/cd/cd.html; station located at Wilmington International Airport (~65 km from study site) for 2003 and Surf City, NC (~11.5 km from study site) for 2007 – 2010).

The majority of the hurricane activity along the North Carolina coast occurs between August and October, and the return period for major hurricanes (category 3 or higher) for the southeastern coast of North Carolina is approximately 16 years (Blake et al. 2007). The last major hurricane to make a direct hit to the area was Hurricane Fran (category 3) in 1996. However, the North Carolina coast is generally effected by at least on tropical cyclone each year. In September 2003, tropical storm Henri impacted the North Carolina coast. In the 2007 hurricane season, the southeastern coast of North Carolina was impacted by two storms: subtropical storm Andrea in early May and tropical storm Gabrielle in early September, which made landfall in the Outer Banks. The 2008 hurricane season produced one tropical storm, Cristobal, in mid-July and one hurricane, Hannah (category 1), in early September. Hurricane Hannah had been reduced to a tropical storm by the time it was off the coast of North Carolina. The coast was only affected by tropical depression one in late May 2009, and Hurricane Earl (category 4) impacted the coast as a category 2 in early September 2010.

1.2. Vegetation community

The vegetation of Topsail Island adheres to the recognized trend found in coastal plant distribution studies, with definitive vegetation zonation and an increase in species diversity and richness with distance from the ocean. In a survey of the vegetation along two 200-m transects during the 2007 growth season (i.e. May – September), the vegetation patterns of the individual zones were found to be as follows: (1) *Iva imbricata* and *Uniola paniculata* dominating the berm, (2) beach grasses (such as *U. paniculata* and *Spartina patens*) and *I. imbricata* on the primary dune, (3) sparse vegetation in the dune swales, with species such as *I. imbricata, Erigeron canadensis,* and *Oenothera humifusa* present, (4) mixture of *I. imbricata*, *Hydrocotyle bonariensis, O. humifusa, Physalis viscosa, Strophostyles helvola* and beach grasses on the secondary dunes, and (5) beach grasses dominating the tertiary dune grassland, with representatives of *H. bonariensis* and *I. imbricata* prior to a line of *Myrica cerifera* shrubs separating the dune area from the salt marsh (Figure 2). As expected, vegetative cover increased with distance from the ocean, ranging from 5% m^{-2} vegetative cover in the upper beach to 65% m^{-2} in the tertiary dune grassland. Consistent with the other studies, species diversity (measured as the Shannon Wiener index) and species richness increased with distance from the ocean (Figure 3).

2. Study Species

The two species studied in this dissertation represent common sand dune species on southeastern U.S. barrier islands and have different growth habits and distribution patterns along the abiotic stress gradient. *Iva imbricata* (sea elder) is a dune-building perennial woody shrub with succulent leaves commonly found in the upper beach, primary dune, swale, secondary dune, and tertiary dune grassland, and is therefore susceptible to an environmental gradient of stresses such as salt spray and sand burial. *Hydrocotyle bonariensis* (pennywort) is a burial-tolerant stabilizing clonal perennial herb with rhizomatous growth and a single broad leaf ramet and is commonly only found in the dune swale, secondary dunes, and tertiary dune grassland.

For both species, leaf stomatal distribution, surface characteristics, and internal anatomy were measured on leaves collected from Topsail Island during the growth season of 2008. To measure stomatal frequency, leaf impressions of abaxial and adaxial leaf surfaces for both species were created using a thin coat of clear nail polish (NYC 271A), and cross-sections were prepared from fresh leaf samples. Images of leaf surface impressions and cross-sections were captured using an upright microscope (Zeiss Axioplan; Carl Zeiss Microimaging, Inc., Thornwood, NY) at 10x magnification with a Hamamatsu C5810 digital camera attachment (Bridgewater, NJ). Stomata frequency (# mm^{-2}) was calculated by dividing the total number of stomata in the image by the area of the field of view, and the length of individual cell layers (e.g. palisade and spongy mesophyll) were calculated to the nearest μm. In addition, the topography of leaf surfaces was examined using an Amray 1810 scanning electron microscope (SEM). Samples were dried in a Tousimis SAMDRI-PVT-3d critical point dryer (Rockville, MD) and coated with gold using a Cressington 108 sputter coater (Watford England).

2.1. Hydrocotyle bonariensis

Hydrocotyle bonariensis Comm. ex Lam. (large-leaf pennywort) is a clonal, perennial, C_3 broadleaf herb in the Eudicot clade of angiosperm plants, and can be further classified in the Asterid clade, Apiales order and the Apiaceae (carrot) family. *H. bonariensis* is indigenous to coastal sand dunes and moist, open sandy areas, from a northern limit of Virginia, U.S. to a southern limit of Chile. On southeastern North Carolina barrier island sand dune systems, this species occurs in the swale (low-lying areas behind primary dunes), secondary dunes, tertiary dune grassland, and within the shrub line. Leaves (ramets) have been shown to tolerate periodic sand burial for several days following storm events, and large clones have been reported to contain over 1500 ramets and cover over 100 m^2 (Evans 1988, 1992a).

Clones (genets) have a spreading, ground-level habit and grow and reproduce by producing alternating single, circular broad leaves (ramets) that bud from branching, almost linear rhizomes (Figure 4). Leaves are peltate (centrally attached to the petiole), 3 – 10 cm wide, and crenate (possess rounded teeth), and the petiole ranges 7 – 30 cm in length (Radford et al. 1968). Small greenish flowers with five petals, five sepals, five stamens, and an inferior ovary with two styles occur as a whorl on compound umbel, and the peduncle equals or exceeds the length of the petiole. Fruits are strongly flattened, glabrous, and measure 1 – 2 mm in length. Leaf longevity varies between 2 to 8 weeks, depending on environmental conditions (Evans 1992a). On Topsail Island, the growth season begins in March and April with seedling emergence, leaf (ramet) production, and rhizomatous growth, and ends with winter dormancy in December. Flowers are produced throughout the growing season (June to September) and fruits develop mid-August through October (Hancock 2009). Extremely low seedling survival rates (less than 1%) have been reported and thus, recruitment is primarily due to clonal growth (Evans 1992b).

Much of the research on *H. bonariensis* has focused on nitrogen translocation and resource integration along rhizomes. Nitrogen often has a patchy distribution in the sand dune habitat, and an early study indicated that nitrogen is translocated acropetally (older to younger), as well as basipetally (younger to older), along rhizomes from areas of high nitrogen availability, stimulating rhizome branching and ramet proliferation in clones located in nitrogen poor areas (Evans 1988). In addition, studies have shown that photosynthates and water can also be translocated along rhizomes (Evans 1991). The ability of *H. bonariensis* clones to translocate nitrogen, water, and photosynthates results in a net benefit in terms of fitness traits in the sand dune habitat. In addition, there is strong evidence that *H. bonariensis* clones are able to preferentially locate ramets to favorable patches of the sand dune habitat, referred to as 'clonal foraging' (Evans and Cain 1995). Furthermore, several studies have indicated that *H. bonariensis* clones are capable of adapting to their local environment, thus becoming distinct ecotypes (Overdieck and Strain 1981; Knight and Miller 2004).

Cross-sections from leaves collected at Topsail island revealed leaves with dorsiventral symmetry, i.e. multiple (three) adaxial palisade layers and abaxial spongy mesophyll (Figure 5A). Leaves were relatively thick (564 µm), and approximately 33% of the internal thickness of the leaf was composed of the three adaxial palisade layers and 51% was composed of abaxial spongy mesophyll. Stomata were relatively evenly distributed on both abaxial (0.11 ± 0.002 stomata mm^{-2}) and adaxial (0.13 ± 0.002 stomata mm^{-2}) leaf surfaces at an abaxial-to-adaxial ratio of 0.86 ± 0.012 (Figure 6A).

2.2. Iva imbricata

Iva imbricata (sea elder) is a perennial, leaf-succulent, semi-woody C_3 shrub in the Eudicot clade of angiosperms plants, and can be further classified into the Asterid clade, Asterales order, and the Asteraceae family. *I. imbricata* is commonly found on coastal sand dunes and beaches from a northern limit of Virginia to Texas and also occurs on beaches of Cuba and several islands of the Bahamas. On southeastern North Carolina beaches, *I. imbricata* is found in all vegetation zones from the upper beach, where it is an important dune-building species, to the tertiary dune grassland. Leaves have been shown to be tolerant of temporary burial by sand, temporary inundation from overwash events, and continual salt spray deposition (Oosting 1945; Colosi and McCormick 1978).

Iva imbricata shrubs are erect, reaching heights of 1 – 2 m, and are freely branched, mostly from the shrub base (Figure 7). Leaves are sessile and succulent with a thick cuticle, alternately arranged on stems, oblanecolate to elliptic-oblanceolate, 3.5 – 5.5 cm long and 0.7 – 1.2 cm wide, and serrate to dentate to entire (Radford et al. 1968). Cream-colored, imperfect tubular flowers with five stamens and two inferior ovaries are produced in racemes 5 – 30 cm long. Fruits are yellowish-brown turgid nutlets measuring 4 – 5 mm long. Recruitment is primarily by seedlings, with seedling survival rates between 70 and 100% reported along the upper beach and swale (Colosi and McCormick, 1978). However, vegetative reproduction by means of rooting, decumbent stems is prevalent in the primary dunes due to unfavorable germination conditions. On Topsail Island, *I. imbricata* seedlings emerge in early spring (March/April), flowers develop in July and August, and fruits occur from September to October. Leaves typically senesce in October and November, and winter dormancy occurs in December (Hancock 2009).

Previous research conducted on *I. imbricata* has focused on the effect of salt spray and deposition on plant growth and survival, the population structure of individuals in various dune habitats, and the role of ecological interactions in sand dune communities. Early investigations of salt spray on coastal sand dunes noted that *I. imbricata* was the only woody plant species found on sand dunes (Wells and Shunk 1938), and experiments investigating responses of dune vegetation to salt deposition determined that *I. imbricata* leaves can tolerate considerable amounts of salt deposition for several days with little to no adverse effects (Oosting 1945, Boyce 1954). A more recent study examined the population structure of *I. imbricata* in five coastal dune habitats and determined that seedling survival rates were dominated by environmental conditions in the primary dune, and thus population numbers were maintained by vegetative reproduction, while seed production limited population numbers in secondary dunes and the tertiary dune grassland (Colosi and McCormick 1978). In addition, the ecological interactions (i.e. facilitation versus competition) among species, including *I. imbricata,* has been recently investigated along the sand dune abiotic gradient (Franks 2003a, b; Franks and Peterson 2003). Results from this study indicated that facilitation between species increased with the severity of disturbance and abiotic stress and that nurse plants of *I. imbricata* maintained microsites for optimal seedling success, supporting the nucleation hypothesis.

Cross-sections of leaves collected from Topsail Island revealed thick (1300 μm) isobilateral leaves with palisade mesophyll layers present beneath adaxial and abaxial leaf surfaces (epidermis) and a central layer of water storage cells (Figure 5B). Approximately 65% of the leaf (32% adaxial and 33% abaxial) was composed of palisade mesophyll and about 31% of the leaf was composed of the central layer of water cells. Stomata were patchily distributed on both abaxial (0.12 ± 0.001 stomata mm^{-2}) and adaxial (0.14 ± 0.001 stomata mm^{-2}) leaf surfaces at an abaxial-to-adaxial stomata ratio of 0.85 ± 0.007. SEM images revealed multlicellular trichomes distributed irregularly on both leaf surfaces (Figure 8). However, the function of these trichomes has yet to be determined.

Literature cited

Blake, E.S., E.N. Rapparport, and C.W. Landsea. 2007. The deadliest, costliest, and most intense United States tropical cyclones from 1851 – 2006 (and other frequently requested hurricane facts). NOAA/NWS/NCEP/TPC/National Hurricane Center, pp. 45.

Boyce, S.G. 1954. The salt spray community. Ecological Monographs 24: 29 – 67.

Colosi, J.C. and J.F. McCormick. 1978. Population structure of *Iva imbricata* in five coastal dune habits. Bulletin of the Torrey Botanical Society 105: 175 – 186.

Costa, C., C.V. Cordazzo, and U. Seeliger. 1996. Shore disturbance and dune plant distribution. Journal of Coastal Research 12: 133 – 140.

Evans, J.P. 1988. Nitrogen translocation in a clonal dune perennial, *Hydrocotyle bonariensis*. Oecologia 77: 64 – 68.

Evans, J.P. 1991. The effect of resource integration on fitness related traits in a clonal dune perennial, *Hydrocotyle bonariensis*. Oecologia 86: 268 – 275.

Evans, J.P. 1992a. The effect of local resource availability and clonal integration on ramet functional morphology. Oecologia 89: 265 – 276.

Evans, J.P. 1992b. Seedling establishment and genet recruitment in a population of a clonal dune perennial, *Hydrocotyle bonariensis*. *In* Barrier Island Ecology of the Mid-Atlantic coast: A symposium. Ed. C.A. Cole and K. Turner. National Park Service, SE Regional Office, Atlanta Georgia: 75 – 84.

Evans, J.P. and M.L. Cain. 1995. A spatially explicit test of foraging behavior in a clonal plant. Ecology 76: 1147 – 1154.

Franks, S.J. 2003a. Competitive and facilitative interactions within and between two species of coastal dune perennials. Canadian Journal of Botany 81: 330 – 337.

Franks, S.J. 2003b. Facilitation in multiple life-history stages: evidence for nucleated succession in coastal dunes. Plant Ecology 168: 1 – 11.

Franks, S.J. and C.J. Peterson. 2003. Burial disturbance leads to facilitation among coastal dune plants. Plant Ecology 168: 13 – 21.

Hanock, T.E. 2009. Ecophysiology of Barrier Island Beach Plants: Responses in Form and Function to Daily, Seasonal and Episodic Stresses. PhD Dissertation, Department of Biology, Wake Forest University, Winston Salem, NC

Hayden, B., M.C.F.V. Santos, G. Shao, and R.C. Kochel. 1995. Geomorphological controls on coastal vegetation at the Virginia Coast Reserve. Geomorphology 13: 283 – 300.

Knight, T.M. and T.E. Miller 2004. Local adaptation within a population of *Hydrocotyle bonariensis*. Evolutionary Ecology Research 6: 103 – 114.

Moreno-Casasola, P. 1986. Sand movement as a factor in the distribution of plant communities in a coastal dune system. Vegetatio 65: 67 – 76.

Oosting, H.J. and W.D. Billings. 1942. Factors effecting vegetational zonation on coastal dunes. Ecology 23: 131 – 142.

Oosting, H.J. 1945. Tolerance to salt spray of plants of coastal dunes. Ecology 26: 85 – 89.

Overdieck, D. and B.R. Strain. 1981. Effects of atmospheric humidity on net photosynthesis, transpiration, and stomatal resistance of *Hydrocotyle umbellate* L. and *Hydrocotyle bonariensis* Lam. International Journal of Biometeorology 25: 29 – 38.

Radford, A.E., H.E. Ahles, and C.R. Bell. 1968. Manual of the Vascular Flora of the Carolinas, The University of North Carolina Press, Chapel Hill, North Carolina, pp. 1183.

Robinson, P. 2003. Atmospheric circulation and inland flooding in twentieth century North Carolina, USA: implications for climate change impacts? Natural Hazards 29: 155 – 172,

Travis, R. 1977. The effects of aspect and exposure on the growth of dune grasses in Cape Hatteras National Seashore. International Journal of Biometeorology 21: 217 – 226.

Wells, B.W. and I.V. Shunk. 1938. Salt spray: an important factor in coastal ecology. Bulletin of the Torrey Club 65: 485 – 492.

Wilson, J. and M.T. Sykes. 1999. Is zonation on coastal sand dunes determined primarily by sand burial or salt spray? A test in New Zealand dunes. Ecology Letters 2: 233 – 236.

Figure captions

Figure II – 1: (A) Location of Pender County on North Carolina coast. (B) Satellite image of Topsail Island, focusing on the southern end where study site was located. (C) Elevation, occurrence of wetlands, and main streets of Topsail Island with focus on southern end.

Figure II – 2: Mean percent cover (%) of common sand dune species and open sand in the upper beach (UB), primary dunes (PD), swale (SW), secondary dunes (SD), and tertiary dune grassland (TG) during the 2007 growth season on Topsail Island, North Carolina.

Figure II – 3: (A) Mean Shannon-Wiener (H') diversity index and (B) species richness for the upper beach (UB), primary dunes (PD), swale (SW), secondary dunes (SD), and tertiary dune grassland (TG) during the 2007 growth season on Topsail Island, North Carolina. Error bars represent standard error.

Figure II – 4: (A) *Hydrocotyle bonariensis* clones located on the leeward side of a secondary dune at Topsail Island, North Carolina. (B) Single *H. bonariensis* clone with twelve ramets (leaves). (C) Clones propagate by white rhizomes with alternating single, circular broadleaf ramets with individual root systems. (D) Small greenish flowers are produced on branching umbels.

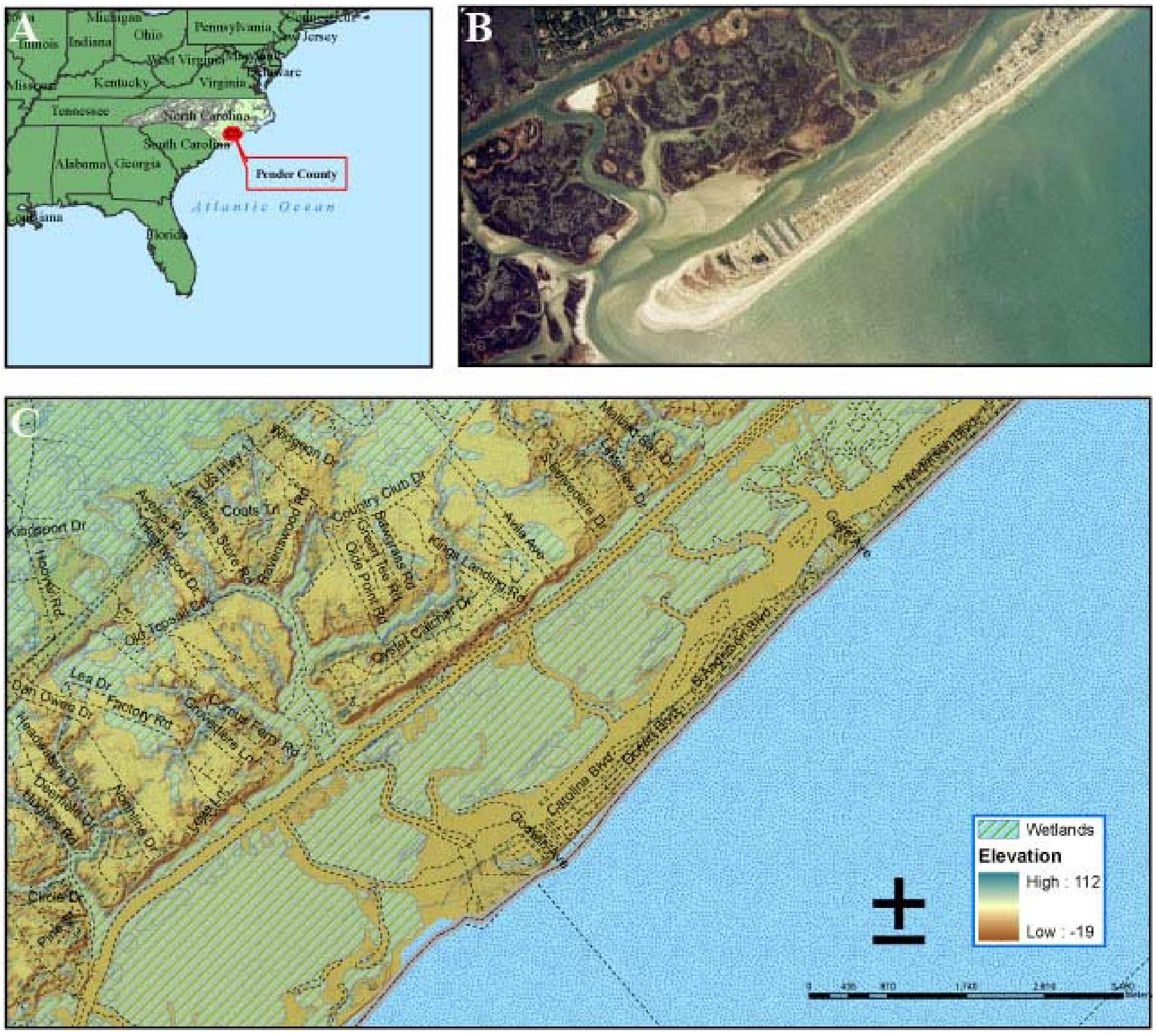
Figure II – 5: (A) Cross-section of *Hydrocotyle bonariensis*. Approximately 33.3% of the internal thickness of the leaf is composed of adaxial palisade mesophyll and 50.6% is composed of abaxial spongy mesophyll. Image by M.O. Sprague. (B) Cross-section of *Iva imbricata*. Approximately 32.2% and 33.0% of the internal thickness of the leaf is composed of adaxial and abaxial palisade mesophyll, respectively, and 30.6% is composed of a central layer of water cells.

Images are from leaf impressions and were taken at 10x on an upright light microscope and scale bars represent 5 µm.

Figure II – 6: (A) Stomata on the abaxial and adaxial leaf surface of *Hydrocotyle bonariensis*. (B) Stomata and trichomes on the abaxial and adaxial leaf surface of *Iva imbricata*. Images are from leaf impressions and were taken at 10x on an upright light microscope.

Figure II – 7: (A) *Iva imbricata* located on a primary dune at Topsail Island, North Carolina. (B) Developing cream-colored tubular flowers.

Figure II – 8: Distribution and morphology of trichomes on the leaf surface of *Iva imbricata*. Images were captured using an Amray 1810 scanning electron microscope at 15 kv and EDS 2006 imaging software. (A) Distribution of trichomes (examples indicated by white arrows) on the leaf surface at 49x magnification. Scale bar represents 500 µm. (B) Individual leaf trichome at 1600x magnification. Scale bar represents 20 µm.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Country Club Dr
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Figure II – 1

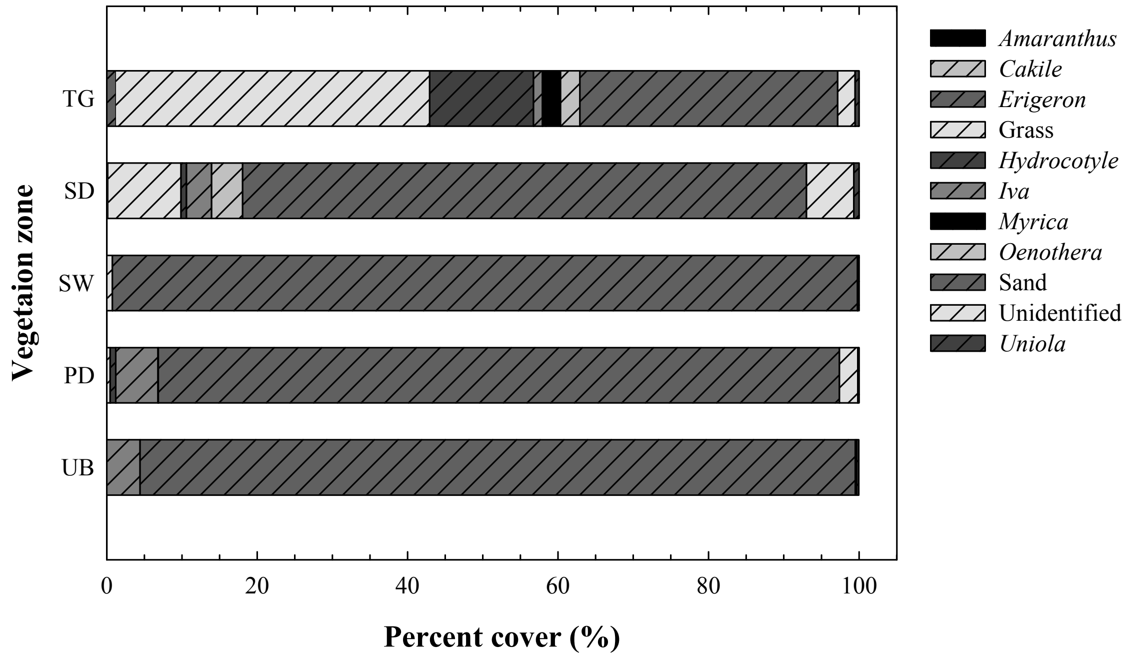
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Figure II – 2

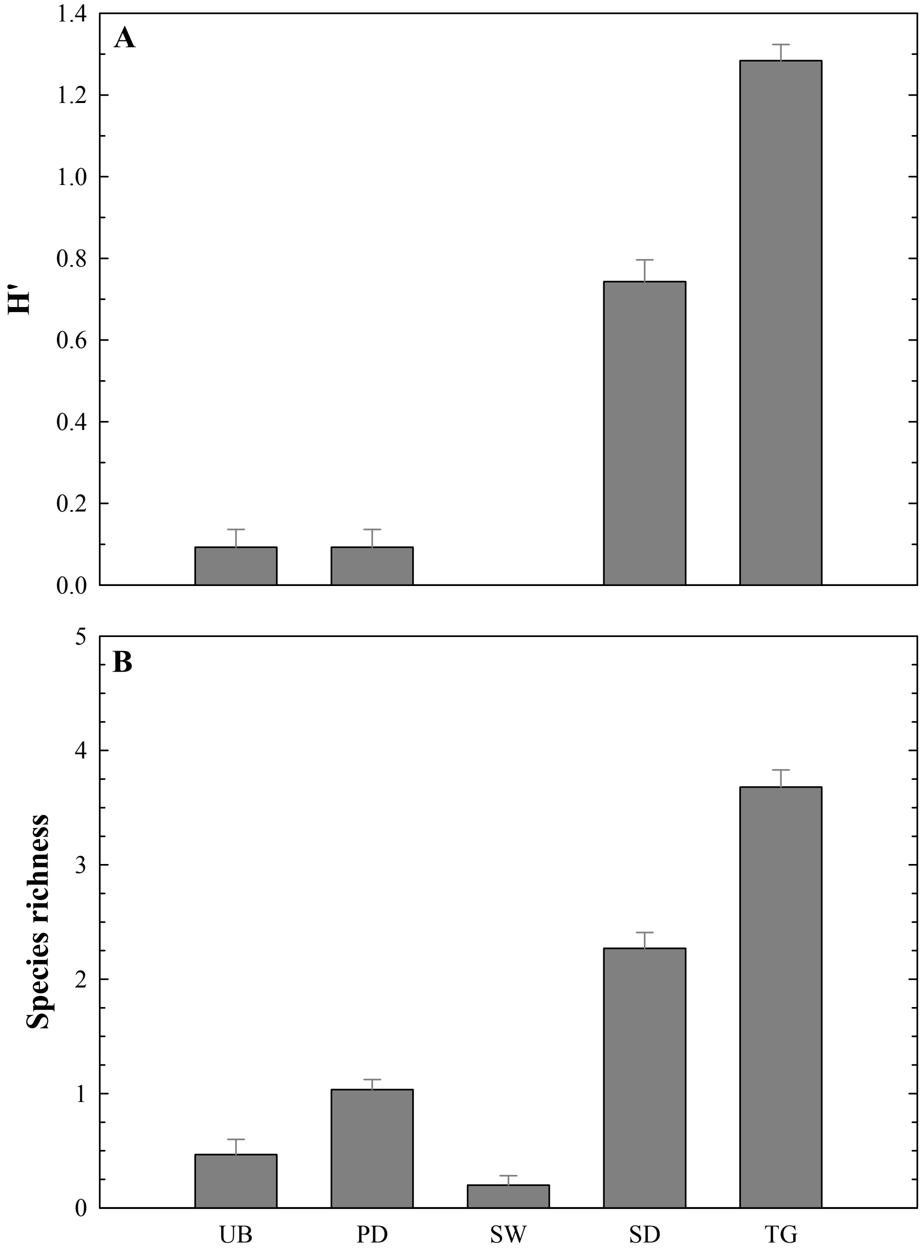
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Figure II – 3

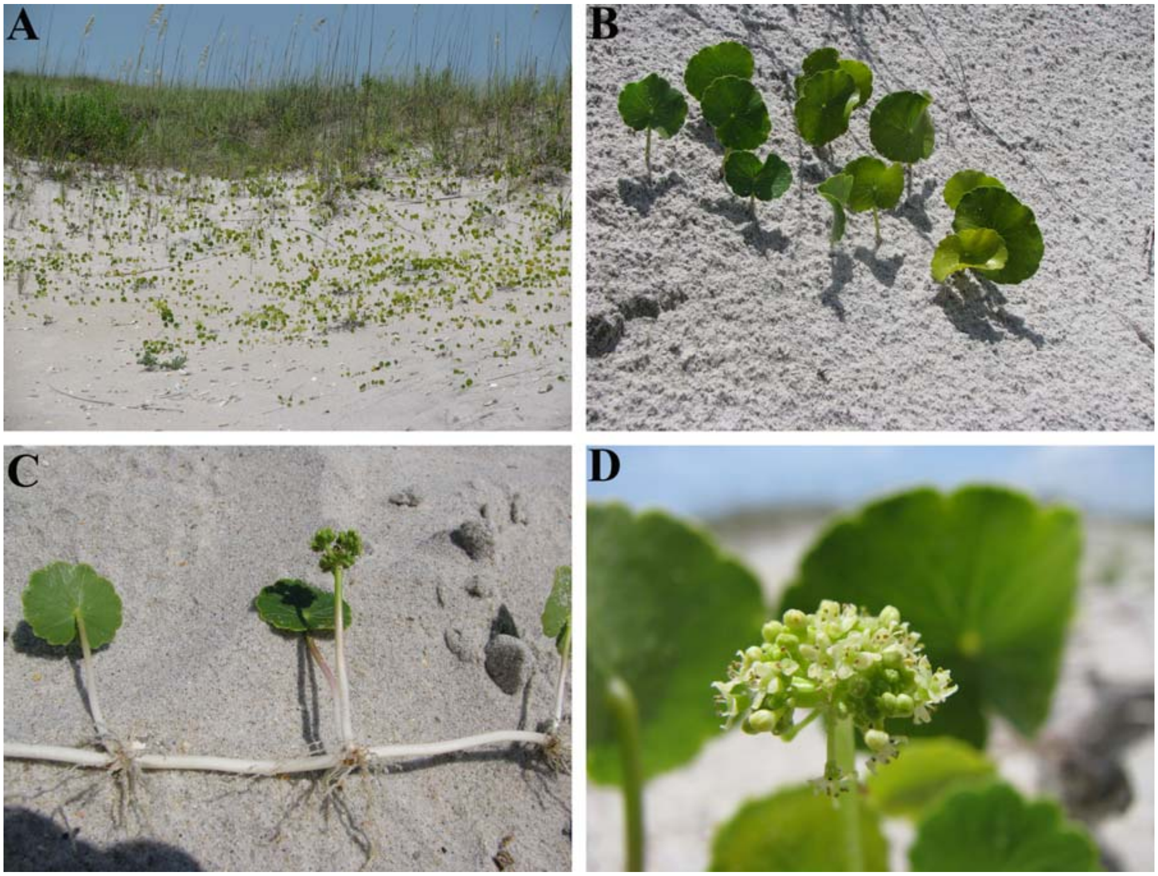

Figure II – 4

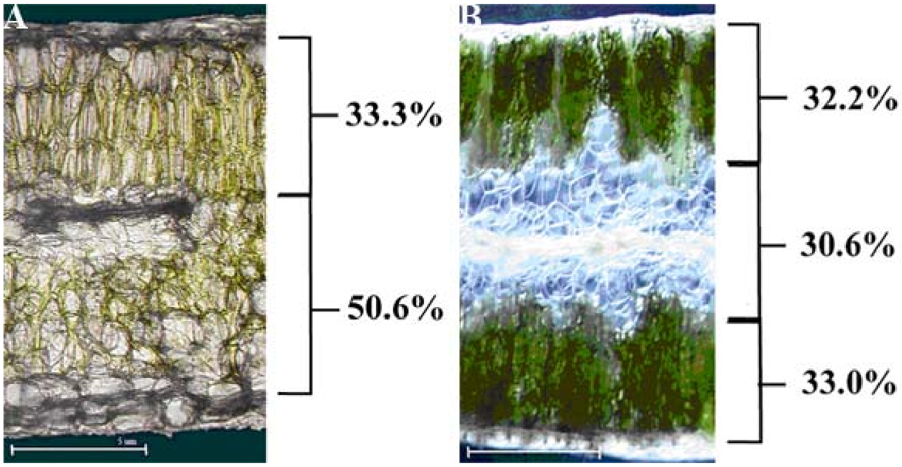

Figure II – 5

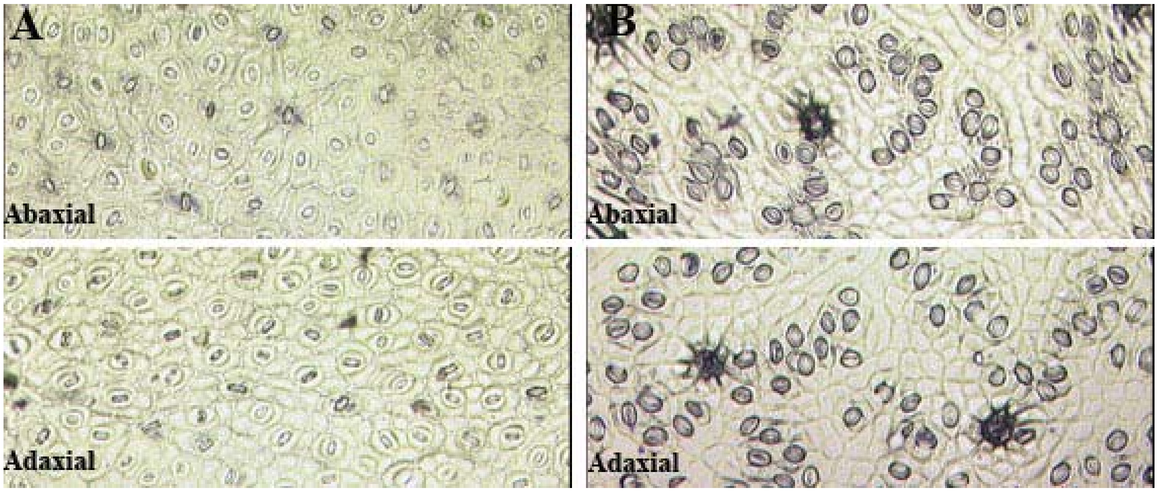

Figure II – 6

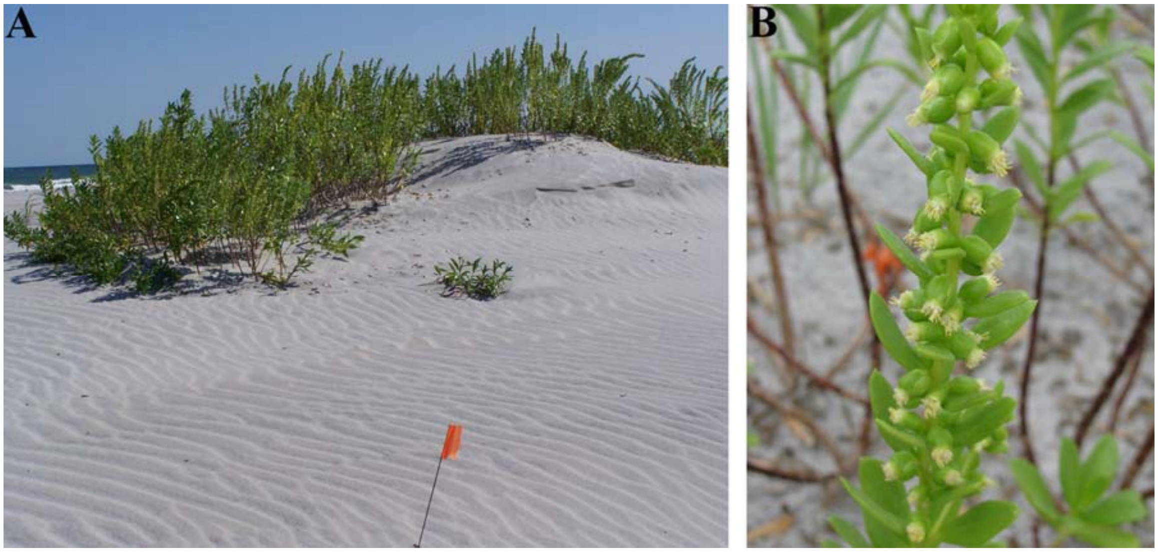

Figure II – 7

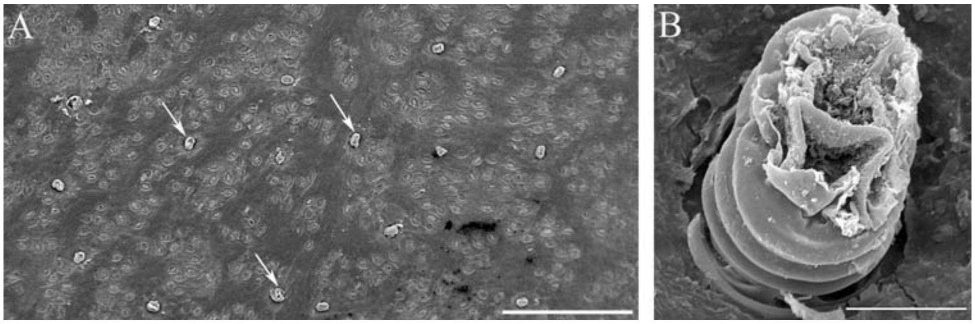

Figure II – 8

CHAPTER III

SEASONAL AND DIURNAL LEAF ORIENTATION, BIFACIAL SUNLIGHT INCIDENCE, AND LEAF STRUCTURE IN THE SAND DUNE HERB *HYDROCOTYLE BONARIENSIS*

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Abstract

A conceptual model has been proposed whereby leaf orientation and resulting sunlight exposure dictate functional leaf structure. Specifically, the model states that this relationship is driven by the absolute amount and ratio of incident sunlight on adaxial and abaxial leaf surfaces. To test this model, the relationships between corresponding values of leaf orientation and incident sunlight on both leaf surfaces were measured for the sand dune herb *Hydrocotyle bonariensis* over a growth season, along with examination of leaf structure. For mature leaves, leaf angle from horizontal and azimuth angle significantly increased over the growing season, indicating diurnal midday avoidance and seasonal maximization of incident sunlight. Consequently, seasonal changes in leaf orientation resulted in an overall decrease in midday sunlight incidence on the adaxial surface and a slight shift in the daily occurrence of peak abaxial incidence. Adaxial surfaces received three to four times more sunlight than abaxial surfaces, and leaf cross-sections revealed relatively thick (564 μm) leaves with multiple adaxial palisade layers and stomata on both leaf surfaces, as predicted by the conceptual model and measured ratio of incident sunlight on adaxial and abaxial leaf surfaces. These data provide further evidence of the relationship between leaf orientation and resulting absolute levels of sunlight incidence on both leaf surfaces, as well as their ratio, and corresponding differences in internal and external leaf structure.

Keywords: Hydrocotyle bonariensis; incident sunlight; leaf orientation; leaf structure; sand dune

1. Introduction

The quantity and quality of incident sunlight intercepted by a leaf can change dramatically over short time periods throughout a day, as well as seasonally (see Smith and Hughes 2009 for review). Too little light can result in failure of enough chlorophyll excitation to create an adequate supply of NADPH and ATP, whereas too much irradiance can result in photoinhibition (i.e. activation of the xanthophyll cycle) and damage to the photosynthetic apparatus (Zhang et al., 2002). At the whole-leaf level, sunlight exposure has been associated with major differences in leaf structure (e.g. sun/shade) and symmetry (e.g. dorsiventral and isobilateral), as well as corresponding effects on light and photosynthetic CO_2 processing (Syvertsen and Cunningham, 1979; Geller and Smith, 1982; DeLucia et al., 1991; Mott and Michaelson, 1991; Smith et al., 1997; Johnson et al., 2005; Smith and Hughes, 2009).

The interaction between leaf orientation, leaf structure, and incident sunlight has been hypothesized to promote efficient internal processing of light with CO_2 concentrations inside the leaf to maximize photosynthesis per unit leaf mass (Smith et al., 1998). Based on a survey of 209 species in Australia, 21 species from the southwestern U.S. desert, and 12 species from the central Rocky Mountains, Smith et al. (1997, 1998) proposed a conceptual model that links leaf orientation, external morphology, and internal anatomy to sunlight incidence on each leaf surface. The conceptual model predicts that native plant species in high sunlight habitats will have leaf form characteristics that reduce sunlight incidence, such as a more inclined leaf orientation, isobilateral symmetry (palisade mesophyll on both leaf sides), and amphistomy (more equal number of stomata on both leaf surfaces) (Table 1). In addition, the conceptual model predicts that the occurrence and distribution of palisade mesophyll within a leaf is strongly correlated to the ratio of incident sunlight on the adaxial and abaxial leaf surfaces (Smith et al., 1998).

Few studies have tested the supposition that the total amount of incident sunlight, plus the ratio of sunlight incidence between the two leaf sides, is correlated with leaf structural differences

(Smith et al., 1997, 1998). The present study tests the relationships between leaf orientation and incident sunlight on both leaf surfaces for *Hydrocotyle bonariensis* Comm ex. Lam, a clonal C_3 herb commonly found on barrier island sand dunes along the southeastern coast of the United States. The barrier island sand dune environment is an exposed, high sunlight habitat (no overstory shade; incident sunlight at midday greater than 1800 μmol m^{-2} s^{-1}) with a host of abiotic stress factors including sand burial and abrasion, salt spray, high wind, periodic inundation by saltwater, substrate temperatures over 50 °C, and maximum growing season air temperatures up to 40°C (Oosting and Billings, 1942; van der Valk, 1977; Ehrenfeld, 1990). Furthermore, mature *H. bonariensis* leaves have been observed in the field to possess various inclinations (leaf angle from horizontal) and azimuths (compass direction) throughout a year (Fig. 1). Based on the high sunlight exposure and abiotic stresses of the sand dune habitat and the predictions of Smith et al. (1997, 1998; Table 1), it was hypothesized that leaf orientation (leaf angle from horizontal and leaf azimuth) in *H. bonariensis* would vary both hourly and seasonally to reduce midday sun exposure when air and leaf temperatures might be excessive (similar to high light and higher stress predictions). In addition, it was expected that incident sunlight on adaxial and abaxial leaf surfaces should be significantly related to leaf orientation properties. Furthermore, because leaf structural symmetry is predicted to respond to both the total amount and ratio of incident sunlight on both leaf surfaces, the leaf structure of *H. bonariensis* should correspond to these values.

2. Materials and methods

Field studies were conducted to investigate the coupling between leaf orientation and incident sunlight, as well as their correspondence to leaf structure, by examining daily and seasonal changes in leaf orientation (leaf angle from horizontal and leaf azimuth), differences in incident sunlight levels on both leaf surfaces, and leaf structure throughout a summer growth period. *Hydrocotyle bonariensis* is a clonal sand dune species consisting of linear, shallow lateral runners with single-leaf ramets spaced approximately 10 – 15 cm apart (Fig. 1). Thus, individual leaf (ramet) measurements do not involve architectural complications on sunlight incidence such as mutual leaf shading within a crown.

2.1. Species

Hydrocotyle bonariensis (Apiaceae) Comm ex. Lam (large-leaf pennywort) is a clonal, perennial C_3 herb indigenous to the coastal sand dunes and moist, open sandy areas, from a northern limit of Virginia, USA to a southern limit of Chile. On southeastern North Carolina barrier islands, this species occurs in the swale (low-lying areas behind primary dunes), secondary dunes, dune meadows, and within the shrub line. New leaves (ramets) are produced throughout the growing season, with leaf life spans ranging from two to eight weeks depending on location (Evans, 1992*a*). Each ramet is composed of a single, circular broad leaf (~5 - 30 cm^2 area) supported by a stem with an independent, nodal root system (Fig. 1). Inflorescences develop opposite of the leaf during new ramet production, and flower and seed production occurs continuously throughout the growing season (Evans, 1992*a*, 1992*b*). Seedling survival rates less than 1% have been recorded and, thus, reproduction is primarily via vegetative clonal growth (Evans, 1992*b*). Clones (genets) grow by producing alternating single-leaf ramets on branching rhizomes (lateral runners), and individual clones have been shown to consist of over 1500 interconnected ramets and cover over 100 m^2 (Evans 1988). Clones have been shown to share water, nutrients, and photosynthates via buried rhizomes (Evans, 1988, 1992*a*, 1992*b*).

2.2. Study site

The present study was conducted during the 2003 growing season (April to mid-November, 227 growing days, as defined by last spring and first fall freeze) on the south end of Topsail

Island, North Carolina (34°20’N, 77°39’ W), a barrier island off the mainland of Pender County. The island is approximately 42 km long, and the southern end of the island is undeveloped with a mature undisturbed dune ridge and swale system. Mean daily air temperatures in the region ranged from a minimum of 5°C in January to a maximum of 27°C in July, and the area received 1616 mm of precipitation in 2003, which is a +168 mm departure from the norm (all climate data from National Oceanic and Atmospheric Association (NOAA) Climatological Data Annual Survey for 2003: North Carolina. http://www1.ncdc.noaa.gov/pub/orders/D821817A-4146-D173-22F3-FFFBB3568AA5.PDF).

2.3. Field measurements

Diurnal and seasonal changes in leaf orientation and corresponding sunlight incidence (photosynthetic photon flux density, PPFD) on both leaf surfaces were measured on single days during the early season (May 21), mid-season (June 29 and August 8), and late season (September 17) of 2003. All measurements were replicated approximately every two hours from 0800 – 1800 h (solar time). Lateral runners were randomly chosen along the ocean side of the swale and secondary dune, with approximately 4 - 5 m separating individual runners. Starting at the first mature leaf (nodes 4 - 6 from the youngest ramet at the end of the lateral runner), leaf angle and leaf azimuth were measured to the nearest degree for two to seven leaves (ramets) per runner. Leaf angle from horizontal (θ_L) was measured as the angle of the plane of the leaf blade away from horizontal (positive when more inclined upward and negative when more inclined downward) and was measured by hand to ca. ±3 degrees using a Sunnto clinometer (Langley, British Columbia, Canada) (Fig. 2a). Leaf azimuth (α_L) was measured as the difference in the compass direction of the plane of the adaxial leaf surface and North (0°) (Fig. 2b). The difference between θ_L and the angle of the sun above the horizon (θ_S, solar altitude) was calculated by taking their absolute difference (|θ_L - θ_S|,). This value represented the position of the leaf plane relative to the sun's position in the sky at a given time of the day and season. This same calculation was also used to find the absolute difference between α_L and the solar azimuth angle (α_S). Hourly angle (solar altitude) and azimuth for the sun for Topsail Island on May 21, June 29, August 8, and September 17 2003 were determined using the Astronomical Applications Department of the U.S. Naval Observatory website (http://aa.usno.navy.mil/cgi-bin/aa_altazw.pl). In the field, incident PPFD (0.4 – 0.7 µm wavelengths) was measured for both adaxial and abaxial leaf surfaces using a hand-held quantum sensor (LiCOR Inc. Model 190, Lincoln, Nebraska, USA) with the plane of the sensor positioned in the same plane and location as the leaf being measured.

2.4. Leaf anatomy and morphology

Cross-sections were made of ten mature (4 - 6 nodes below youngest ramet at end of lateral runner) *H. bonariensis* leaves collected in August and examined using an upright phase-contrast microscope (Zeiss Axioplan, Thornwood, New York, USA) with a digital camera attachment (Hamamatsu C5810, Bridgewater, New Jersey, USA). The thickness of the upper epidermis, palisade mesophyll layer, spongy mesophyll layer, lower epidermis, and entire leaf were measured to the nearest 0.1 µm. The percent of leaf thickness of each tissue type was calculated by dividing the length of the layer by the total leaf thickness. Stomata counts were also made using leaf surface impressions (with clear nail polish), as well as epidermal peels, for both adaxial and abaxial leaf surfaces (n = 250 per leaf surface). Leaf area (cm^2) and leaf thickness was also measured on leaves used for stomata counts.

2.5. Statistical analyses

There were a total of three lateral runners measured for May, eight for June, five for August, and five for September, with two to seven leaves (ramets) per runner. Individual leaves (ramets) for each runner were averaged to calculate the mean leaf inclination from horizontal (θ_L), mean leaf azimuth (α_L), mean adaxial PPFD, mean abaxial PPFD, and the ratio of adaxial-to-abaxial PPFD (*Ad/Ab*) for the runner. Each runner was then averaged to calculate mean θ_L, α_L, adaxial PPFD, abaxial PPFD, and *Ad/Ab* for each measurement period during the day, and these hourly means were used to calculated $|\theta_L - \theta_S|$ and $|\alpha_L - \alpha_S|$. Hourly means were then averaged to calculate seasonal mean θ_L, α_L, $|\theta_L - \theta_S|$, $|\alpha_L - \alpha_S|$, adaxial PPFD, abaxial PPFD, and *Ad/Ab*.

To determine if there were significant variations diurnally and/or seasonally in leaf orientation properties (θ_L and α_L) and incident sunlight on both leaf surfaces (adaxial PPFD, abaxial PPFD, and *Ad/Ab*), a repeated measures multivariate analysis of variance (MANOVA) was performed with month and time as fixed factors and a significance level of $P < 0.05$. The MANOVA was performed using SPSS version 16.0 statistical software (SPSS Inc., 2007). Additionally, the influence of leaf orientation properties (θ_L, α_L, $|\theta_L - \theta_S|$, and $|\alpha_L - \alpha_S|$) on the incident light environment of adaxial and abaxial leaf surfaces (adaxial PPFD, abaxial PPFD, and *Ad/Ab*) was examined using multiple linear regression analysis. Multiple regressions were performed using JMP version 8.0.2 statistical software (SAS Institute Inc., 2009), and the slope was determined significantly different from zero at $P < 0.05$.

3. Results

3.1. Daily changes in leaf orientation

There was no significant variation in θ_L during the day (*F*-statistic = 0.389, *P* = 0.968) (Table 2; Fig. 3A, B). Mean θ_L ranged between 47° and 56° in May, 71° and 79° in June, 80° and 84° in August, and 82° and 86° in September. For all months measured, the mean θ_S increased during the morning, reached a peak at 1200 h, and then decreased in the late afternoon (Fig. 3A, B). In general, for all months measured, $|\theta_L - \theta_S|$ was greatest in the early morning and late afternoon. The $|\theta_L - \theta_S|$ angle measured was at a minimum at 1400 h in May and at 1200 h in June, August, and September. At midday (1200 h), the $|\theta_L - \theta_S|$ angle was 23° in May, 2° in June, 9° in August, and 27° in September, suggesting that in June and August, the plane of the leaf was approximately parallel to the sun's rays at midday.

Daily α_L also showed no significant variation (*F*-statistic = 0.265, *P* = 0.812) (Table 2; Fig. 3C, D). Mean daily α_L ranged between 63° and 102° in May, 128° and 161° in June, 181° and 197° in August, and 204° and 218° in September. The α_S followed the same general daily pattern in all months measured, rising in the northeast and setting in the northwest (Fig. 3C, D), but became more southerly during mid-season, varying from 196° in May to 194° in June and September to 192° in August. The $|\alpha_L - \alpha_S|$ azimuth in May followed the same pattern as the sun azimuth, suggesting that the adaxial leaf surface faced the sun in the morning hours (0800 to 1000 h) (Fig. 3C). However, as the season progressed, the $|\alpha_L - \alpha_S|$ azimuth became more varied with little difference between the sun azimuth and the leaf azimuth between 1200 and 1400 h (Fig. 3D). Specifically, the adaxial leaf plane was approximately perpendicular to the sun's rays (as indicated by lowest $|\alpha_L - \alpha_S|$) at 1200 in June (4°) and August (22°) and at 1400 h in September (8°).

3.2. Seasonal changes in leaf orientation

There was significant increase in θ_L over the season (F-statistic = 54.195, $P < 0.001$) (Table 2; Fig. 4A). In the early season (May), leaves had a mean ± S.E. θ_L of 53 ± 3.6°. During the mid-season (June and August), θ_L became steeper with a mean inclination of 75 ± 2.3° and 82 ± 0.9°, respectively, and θ_L remained near vertical in the late season (September mean = 84 ± 1.2°). The mean zenith solar angle (maximum solar altitude at 1200 h) remained fairly constant during early- (76° in May) and mid-season (79° in June and 71° in August) and then decreased to 58° in September.

Mean α_L also varied significantly (F-statistic = 19.479, $P < 0.001$) over the growing season, increasing from almost due east (92 ± 9.1°) in May, to the southeast in June (156 ± 5.3°), to the south in August (191 ± 2.7°), and to the southwest in September (211 ± 3.1°) (Table 2; Fig. 4B). In contrast, the mean α_S varied little over the growing season, remaining to the south and ranging from 192 ± 27.9° in August to 196 ± 29.4° in May.

3.3. Abaxial and adaxial PPFD

There was significant variation in the amount of adaxial PPFD and abaxial PPFD during the day (F-statistic = 48.600 and 51.252, respectively, both $P < 0.001$; Table 2). In general, the adaxial PPFD and abaxial PPFD increased in the morning, reached a maximum between 1000 h and 1400 h, and then began to decline (Fig. 5A, B). There was a decrease in peak adaxial PPFD over the growing season. Adaxial PPFD peaked at 1200 h (solar noon) with a mean of 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in May, compared to a peak at 1000 h with a mean of 906 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in June, at 1200 h with a mean of 820 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in August, and at 1400 h with a mean of 857 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in September (Fig. 5A). For abaxial PPFD, there was a slight shift in the occurrence of peak

PPFD during the growing season from midday in the early and mid-season to early afternoon in the late season (Fig. 5B). In May, June, and August, maximum abaxial PPFD occurred at 1200 h with a mean of 332 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$, 305 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$, and 324 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$, respectively. In September, abaxial PPFD peaked at 1400 h with a mean of 335 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$.

There was significant variation in adaxial PPFD (F-statistic = 14.075, $P < 0.001$) and abaxial PPFD (F – statistic = 3.417, $P = 0.022$) over the growing season (Table 2). Adaxial PPFD was greatest in May with a mean ± S.E. of 780 ± 69.1 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$, decreased notably in June to a mean of 427 ± 32.7 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$, and then increased over the rest of the growing season, with a mean of 551 ± 27.5 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ in August and 631 ± 33.5 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ in September (Fig. 5C). Abaxial PPFD showed an opposite trend. In May, mean abaxial PPFD was 203 ± 16.6 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$, increased to 245 ± 16.1 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ in June, decreased again to 169 ± 12.2 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ in August, and then slightly increased to 195 ± 14.0 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ in September (Fig. 5C).

The ratio of sunlight incidence on both leaf surfaces (*Ad/Ab*) varied significantly (F-statistic = 3.767, $P < 0.001$) over the growth season with greatest mean *Ad/Ab* measured in May (4.1 ± 0.42), decreasing to 3.1 ± 0.23 in June, and then increasing to 3.7 ± 0.12 and 3.6 ± 0.142 in August and September, respectively (Table 2). *Ad/Ab* varied significantly during individual days (F-statistic = 8.563, $P < 0.001$) and showed similar trends during the growing season, except for May (Fig. 5D). In May, *Ad/Ab* reached a maximum value in the morning (8.2 at 1000 h) and then decreased during the remainder of the day, whereas in June, August, and September, maximum values occurred during the morning and afternoon, with slight depressions near midday indicating a reduction in incident sunlight.

There were significant relationships between leaf orientation and incident sunlight on both leaf surfaces (Table 3). Specifically, adaxial PPFD and *Ad/Ab* was significantly related to θ_L (F-statistic = 59.429 and 125.595, respectively, both $P < 0.0001$), $|\theta_L - \theta_S|$ (F-statistic = 8.677, $P = 0.0035$ and F-statistic = 33.564, $P < 0.0001$ respectively) and $|\alpha_L - \alpha_S|$ (F-statistic = 35.857 and

23.729, respectively, both $P < 0.0001$), whereas abaxial PPFD was only influenced by θ_L (F-statistic = 30.114, $P < 0.0001$) and $|\theta_L - \theta_S|$ (F-statistic = 76.816, $P < 0.0001$). Thus, the total amount and ratio of incident sunlight on adaxial and abaxial leaf surfaces appeared to be influenced by the inclination of the leaf and the position of the leaf plane according to solar angle and azimuth.

3.4. Leaf anatomy and morphology

Mean leaf thickness for *H. bonariensis* leaves collected in August was 564 µm. There were three distinct palisade layers below the adaxial epidermis which made up ca. 33% of the total leaf thickness and approximately 51% of the mesophyll thickness occurred as spongy mesophyll (Fig. 6A). Leaves were close to amphistomatous, with stomata present on both leaf surfaces (0.110 ± 0.002 stomata mm^{-2} on abaxial surfaces and 0.130 ± 0.002 stomata mm^{-2} on adaxial surfaces) at a mean abaxial-to-adaxial stomata ratio of 0.86, i.e. approximately 15% more stomata on the abaxial surface (Fig. 6B). Neither leaf size nor stomatal frequency varied over the growing season.

4. Discussion

The native habitat of *H. bonariensis* (i.e. barrier island sand dune habitat) can be characterized as high sunlight with numerous additional abiotic stress factors such as high wind, salt spray, high midday air temperatures, and sand burial and abrasion (Table 1). The results presented here showed that *H. bonariensis* leaves increased leaf angle from horizontal over the growing season to reduce midday sunlight exposure while simultaneously increasing leaf azimuth to seasonally track the sun azimuth over the growing season. Increased leaf angle, in addition to the position of the leaf plane relative to solar angle and azimuth, had significant influence on the incident sunlight on both adaxial and abaxial leaf surfaces. The adaxial leaf surface received approximately three to four times greater incident sunlight than the abaxial surface, resulting in an internal leaf structure characterized by multiple adaxial palisade layers and an abaxial spongy mesophyll layer.

4.1. Diurnal and seasonal leaf orientation

There are numerous examples of plant species in high light habitats that actively modulate θ_L and/or α_L to reduce radiation loads, especially during midday, including *Perezia nana* in the Sonoran and Chihuahuan deserts (Syvertsen and Cunningham, 1979), *Rumex densiflorus* in southeast Wyoming (Geller and Smith, 1982), *Lactuca serriola* in sun-exposed habitats in Utah (Werk and Ehleringer, 1984), *Silphium terebinthinaceum* in Illinois (Smith and Ullberg, 1989), *Silphium laciniatum* in Iowa (Zhang et al., 1991), and adult *Eucalyptus globulus* in Australia and Tasmania (King, 1997; James and Bell, 2000). The present study found that *H. bonariensis* varied θ_L and α_L seasonally, as hypothesized, but did not diurnally alter either θ_L or α_L. With the exception of a discontinuation of diurnal orientation during winter months (e.g. Nilsen, 1985), the results here may be the first example of a plant species with altered leaf orientation on a seasonal but not diurnal basis. Over the length of the growing season, *H. bonariensis* leaves increased θ_L from slightly inclined to nearly vertical and became more oriented from east to southeast as the sun position moved more southward, including the more southern position of the sun at sunrise and sunset (Fig. 7).

Changes in seasonal leaf orientation in *H. bonariensis* were suggestive of midday avoidance of direct sunlight incidence (θ_L) with alterations in α_L to maximize incident sunlight interception throughout the growth season. In May, mean θ_L was 53°, indicating that during midday leaves received relatively high irradiance loads, which was reflected in midday adaxial PPFD measured for May (1200 ± 167.2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 1200 h) (Fig. 5B, 7). Beginning in June, and continuing through August and September, the more inclined θ_L significantly reduced midday irradiance (decrease in peak adaxial PPFD), similar to the mechanisms described for solar avoiders (e.g. Geller and Smith, 1982; Werk and Ehleringer, 1984; Smith and Ullberg, 1989; Zhang et al., 1991; James and Bell, 2000). The increase in mean α_L over the growth season from east-facing (92°) in May to southwest (211°) in September tracked the sun's more southerly position over the growing season (slight shift in the occurrence of peak abaxial PPFD; Fig. 5B, C). Therefore, these results suggest that the mechanism employed by *H. bonariensis* for sun avoidance in mid- and late-season was linked primarily to an increase in θ_L that reduced irradiance incidence during midday, while simultaneously modifying α_L to track the position of the sun according to seasonal changes (Fig. 7).

4.2. Adaxial versus abaxial PPFD incidence

Studies have shown that a more inclined leaf orientation in high light habitats resulted in lower sunlight interception during midday, more symmetrical sunlight interception (morning and afternoon) on both leaf surfaces and less sunlight incidence over the entire day (Syvertsen and Cunningham, 1979; DeLucia et al,. 1991; James and Bell, 2000; Falster and Westoby, 2003). The results presented here showed that measured adaxial PPFD was greater than abaxial PPFD in all months measured during the growing season, and the adaxial surface of *H. bonariensis* leaves received approximately three to four times greater incident sunlight than the abaxial surface (Fig. 5). Furthermore, a more inclined leaf resulted in relatively constant *Ad/Ab* (3.1, 3.7, and 3.6 in June (θ_L = 75°), August (θ_L = 82°), and September (θ_L = 84°), respectively).

The current study suggests that the seasonal variation in *Ad/Ab* was due primarily to variations in adaxial PPFD (Fig. 5), with peak values decreasing as the growing season progressed (corresponding to increased θ_L). There was a significant relationship between adaxial PPFD and θ_L, $|\theta_L - \theta_S|$, and $|\alpha_L - \alpha_S|$, indicating that position of the leaf plane with regard to the sun's rays had a strong influence on incident sunlight adaxial leaf surfaces. The greatest mean adaxial PPFD occurred at 1200 h in May (1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$) when mean θ_L was 53°, while peak values in August and September were reduced (820 and 857 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively) when mean θ_L was 82° and 84°, suggesting that a more inclined leaf acted to decrease midday incident sunlight. The substantial decrease in mean adaxial PPFD in June appeared to be a result of mean θ_L. At midday (1200 h) in June, θ_L was 76° and θ_S was 78°, resulting in a $|\theta_L - \theta_S|$ of 2°, indicating that the leaf plane was nearly parallel with the sun's rays (Fig. 3B, 7). In addition, mean α_L was 156° (southeast), resulting in maximum incident sunlight exposure in the late morning on the adaxial surface, followed by decreased exposure for the remainder of the day (Fig. 7).

Furthermore, there was a slight shift in the occurrence of maximum abaxial PPFD over the growth season. Although there was no significant relationship between abaxial PPFD and α_L, the results suggested that the late-season shift in abaxial PPFD maxima could be a function of the interaction between θ_L and α_L (Fig. 7). In May, the leaf was slightly inclined (53°) and facing east (92°), resulting in increased incident sunlight during midday and the early afternoon (Fig. 7). A θ_L of 75° and α_L of 156° in June resulted in a leaf plane that was nearly parallel to the sun's rays and facing the same direction as the sun at midday, resulting in increased abaxial incident light. As the leaf turned toward the south/southwest in August and September, the leaf became more inclined (82° and 84°), and the solar azimuth became more southerly, sunlight incidence on the abaxial leaf surface was maximized during midday and the early afternoon (Fig. 7).

Daily *Ad/Ab* during the growing season offers further evidence of the influence of the leaf position according to the sun's rays on adaxial and abaxial leaf incident sunlight. In May, when α_L was east (92°) and the leaf was only slightly inclined from horizontal (θ_L = 53°), daily *Ad/Ab* was greatest in the morning (0800 – 1000 h). Since the rising sun azimuth in May was also to the east (87°), the slightly inclined leaf angle resulted in high exposure on the adaxial surface and very little exposure on the abaxial surface in the morning (Fig. 7). During the remainder of the day, the sun azimuth became more westerly and incident sunlight on the abaxial surface increased, resulting in a corresponding decrease in *Ad/Ab*. In June, August, and September, the more inclined leaf angle and more southerly leaf azimuth resulted in morning incident sunlight on the adaxial surface in June and the abaxial surface in August and September, with corresponding maximum *Ad/Ab* values and a reduction in incident light on the adaxial surface at midday, indicated by a depression in *Ad/Ab*.

4.3. Does leaf structure correspond to Ad/Ab PPFD?

Seasonal and daily changes in levels of incident PPFD due to leaf orientation have been demonstrated to generate corresponding changes in leaf structure (Smith et al., 1997, 1998; Johnson et al., 2005). More specifically, palisade mesophyll layers tend to be present under leaf surfaces exposed to high incident sunlight (James and Bell, 2000) and have been shown to transmit absorbed sunlight deeper into thicker, sun-type leaves (Vogelmann et al., 1996; Smith et al., 1997, 1998). Smith et al. (1998) proposed that the presence and distribution of palisade mesophyll is driven by the amount of incident sunlight on the adaxial leaf surface and the ratio of adaxial-to-abaxial PPFD (*Ad/Ab*). Leaves with an *Ad/Ab* between 2.7 and 3.6 were had a single abaxial palisade layer, and a more inclined leaf with an *Ad/Ab* between 3.4 and 4.5 had multiple adaxial palisade layers (Smith et al. 1998). In the present study, leaf angle ranged from 53° to 84°, resulting in an *Ad/Ab* that ranged from 3.1 in June to 4.1 in May, respectively, and a corresponding internal anatomy composed of three adaxial palisade layers, as predicted by Smith et al. (1998) (Fig. 6).

In addition, *H. bonariensis* leaves were nearly amphistomatous (abaxial-to-adaxial stomata ratio of 0.86), indicating there were approximately 15% more stomata on the abaxial surface.

However, the presence of species with completely amphistomatous leaves (e.g. abaxial-to-adaxial ratio of 1.0) is rare (Parkhurst 1994). Stomata on both leaf surfaces (instead of just abaxially) has been shown to increase supply rate of CO_2 to mesophyll cells by decreasing the diffusion distance, further enhancing photosynthesis per unit leaf surface area, especially in thick leaves (Mott and Michaelson, 1991; Parkhurst, 1994; Smith et al., 1997, 1998). The occurrence of amphistomatous leaves in high light habitats has been also proposed to provide a high internal CO_2 environment that interacts with the increased sunlight absorption by leaves, thus maximizing photosynthesis (Smith et al., 1998).

4.4. Conclusion

The results of the present study showed significant increases in leaf angle and leaf azimuth of *H. bonariensis* with negligible diurnal changes over the entire summer growth period. As a result, there were significant variations in seasonal values of adaxial and abaxial incident PPFD, as well as their ratios. Moreover, leaf structure corresponded closely to associated values of adaxial incident sunlight and in particular with the ratio of adaxial-to-abaxial PPFD as predicted in Smith et al. (1997, 1998). A more inclined leaf has been shown to effectively avoid excessive leaf temperatures and transpiration, as well as the risk of photoinhibition or photodamage in high light habitats (Geller and Smith, 1982, James and Bell, 2000). An inclined leaf has also been proposed to maximize the overlap of internal light and CO_2 inside the leaf, and thus photosynthesis per unit leaf area and leaf mass (King, 1997, Smith et al., 1997, James and Bell, 2000). Future investigation of the function of leaf orientation in *H. bonariensis* should include examination of the effect of orientation and resulting adaxial and abaxial incident sunlight on leaf physiological processes such as photosynthetic gas exchange, photoinhibition risk, and leaf temperatures.

Literature cited

DeLucia, E.H., Shenoi, H.D., Naidu, S.L., Day, T.A., 1991. Photosynthetic symmetry of sun and shade leaves of different orientations. Oecologia 87, 51 – 57.

Ehrenfeld, J.G., 1990. Dynamics and processes of barrier island vegetation. Rev. Aquatic. Sci. 2, 437 – 480.

Evans, J.P., 1988. Nitrogen translocation in a clonal dune perennial, *Hydrocotyle bonariensis*. Oecologia 77, 64 -68.

Evans, J.P., 1992*a.* The effect of local resource availability and clonal integration on ramet functional morphology in *Hydrocotyle bonariensis*. Oecologia 89, 265 – 276.

Evans, J.P., 1992*b.* Seedling establishment and genet recruitment in a population of a clonal dune perennial, *Hydrocotyle bonariensis*. In: Cole, C.A., Turner, K. (Eds.), Barrier Island Ecology of the Mid-Atlantic Coast: A Symposium, Atlanta, GA, 75 – 84.

Falster, D.S., Westoby, M., 2003. Leaf size and angle vary widely across species: what consequences for light interception? New Phytol. 158, 509 – 525.

Geller, G.N., Smith, W.K., 1982. Influence of leaf size, orientation, and arrangement on temperature and transpiration in three high-elevation, large-leafed herbs. Oecologia 53, 227 – 234.

James, S.A., Bell, D.T., 2000. Leaf orientation, light interception, and stomatal conductance of *Eucalyptus globulus* ssp. *globulus* leaves. Tree Physiol. 20, 815 – 823.

Johnson, D.M., Smith, W.K., Vogelmann, T.C., Broderson, C.R., 2005. Leaf architecture and direction of incident light influence mesophyll fluorescence properties. Am. J. Bot. 92, 1425 – 1431.

King, D.A., 1997. The functional significance of leaf angle in *Eucalyptus*. Aust. J. Bot. 45, 619 – 639.

Mott, K.A., Michaelson, O., 1991. Amphistomy as an adaptation to high light intensity in *Ambrosia cordifolia* (Compositae). Am. J. Bot. 78, 76 – 79.

Nilsen, E.T., 1985. Seasonal and diurnal leaf movements of *Rhododendron maximum* L. in contrasting irradiance environments. Oecologia 65, 296 – 302.

Oosting, H.J., Billings, W.D., 1942. Factors effecting vegetational zonation on coastal dunes. Ecology 23, 131 – 142.

Parkhurst, D.F., 1994. Diffusion of CO_2 and other gases inside leaves. New Phytol. 126, 449 – 479.

Smith, M., Ullberg, D., 1989. Effect of leaf angle and orientation on photosynthesis and water relations in *Silphium terebinthinaceum*. Am. J. Bot. 76, 1714 – 1719.

Smith, W.K., Vogelmann, T.C., DeLucia, E.H., Bell, D.T., Shepherd, K.A., 1997. Leaf form and photosynthesis: Do leaf structure and orientation interact to regulate internal light and carbon dioxide? BioScience 47, 785 – 793.

Smith, W.K., Bell, D.T., Shepherd, K.A., 1998. Associations between leaf structure, orientation, and sunlight exposure in five Western Australian communities. Am. J. Bot. 85, 56 – 63.

Smith, W.K., Hughes, N.M., 2009. Progress in coupling plant form and photosynthetic function. Castanea 74, 1 – 26.

Syvertsen, J.P., Cunningham, G.L., 1979. The effects of irradiation adaxial or abaxial leaf surface on the rate of net photosynthesis of *Perezia nana* and *Helianthus annus*. Photosynthetica 13, 287 – 293.

van derValk, A.G., 1977. The macroclimate and microclimate of coastal foredune grasslands in Cape Hatteras National Seashore. Int. J. Biometeor. 21, 227 – 237.

Vogelmann, T.C., Bornman, J.F., Yates, D.J., 1996. Focusing of light by epidermal cells. Physiol. Plantarum 98, 43 – 56.

Werk, K.S., Ehleringer, J., 1984. Non-random leaf orientation in *Lactuca serriola* L. Plant Cell Environ. 7, 81 – 87.

Zhang, H., Pleasants, J.M., Jurik, T.W., 1991. Development of leaf orientation in the prairie compass plant, *Silphium laciniatum* L. B. Torrey Bot. Club 118, 33 – 42.

Zhang, S.R., Ma, K.P., Chen, L.Z., 2002. Photosynthetic gas exchange and leaflet movement of *Robinia pseudoacacia* in relation to changing light environments. Acta. Bot. Sin. 44, 858 – 863.

Table III – 1: Predicted leaf orientation and leaf structure variables based on Smith et al. (1997, 1998) model for species occupying high incident sunlight habitats with either higher or lower additional abiotic stress (e.g. water stress, salt stress), in addition to leaf orientation and structure measured for *Hydrocotyle bonariensis*.

Leaf characteristics	**Predicted for *higher* stress**	**Predicted for *lower* stress**	**Measured**
Orientation	Vertical/cylindrical/ avoider	Horizontal/tracker	Inclined toward vertical, suggestive of avoidance
Adaxial/abaxial incident light ratio	< 2.0	> 3.5	3.1 – 4.1
Thickness (µm)	400 – 600	>600	564
Symmetry	Isobilateral	Dorsiventral	Dorsiventral
Morphology	Small and cylindrical	Large laminar broadleaf	Large laminar broadleaf
	Amphistomatous	Hypostomatous/ amphistomatous	Amphistomatous
	No bicolor	Bicolor	Weak bicolor
Anatomy	Ad/Ab palisade possible unless cylindrical	Multiple adaxial palisade possible	3 adaxial palisade layers

Table III – 2: *F*-statistics, *P*-values, and sample size for repeated measures multivariate analysis of variance (MANOVA) testing the effect of time and month (fixed factors) on leaf angle from horizontal (θ_L), leaf azimuth (α_L), adaxial photosynthetic photon flux density (PPFD), abaxial PPFD, and the ratio of adaxial-to-abaxial PPFD (*Ad/Ab*) for *Hydrocotyle bonariensis* ramets over a growth season.

Variable	**F-statistic**	***P* – value**	**Total *n***
Time (*P* < 0.001)			
θ_L	0.389	0.812	57
α_L	0.265	0.968	57
Adaxial PPFD	48.600	< 0.001	57
Abaxial PPFD	51.252	< 0.001	57
Ad/Ab	8.563	< 0.001	57
Month (*P* < 0.001)			
θ_L	54.195	< 0.001	334
α_L	19.479	< 0.001	331
Adaxial PPFD	14.075	< 0.001	321
Abaxial PPFD	3.417	0.022	261
Ad/Ab	3.767	< 0.001	254

Table III – 3: Whole model coefficient of determination (R^2), goodness of fit (r^2), *F*-statistic, and *P*-value, as well as individual effect *F*-statistics and *P*-values, from Standard Least Squares Multiple Regressions between incident sunlight [adaxial photosynthetic photon flux density (PPFD), abaxial PPFD, and the ratio of adaxial-to-abaxial PPFD (*Ad/Ab*)] and leaf orientation [leaf angle from horizontal (θ_L), leaf azimuth (α_L), absolute difference between leaf angle and sun angle ($| \theta_L - \theta_S |$), and absolute difference between leaf azimuth and sun azimuth ($| \alpha_L - \alpha_S |$)] for *Hydrocotyle bonariensis* leaves over a growth season.

	Adaxial PPFD *$R^2 = 0.35$, $r^2 = 0.995$*		**Abaxial PPFD** *$R^2 = 0.28$, $r^2 = 0.997$*		***Ad/Ab*** *$R^2 = 0.35$, $r^2 = 0.987$*	
	F-statistic	***P*-value**	**F-statistic**	***P*-value**	**F-statistic**	***P*-value**
Whole model	32.7281	< 0.0001	24.2517	< 0.0001	33.0862	< 0.0001
θ_L	59.4293	< 0.0001	30.1142	< 0.0001	125.5948	< 0.0001
α_L	0.0048	0.9447	0.5314	0.4667	0.1707	0.6798
$\| \theta_L - \theta_S \|$	8.6770	0.0035	76.8156	< 0.0001	33.5635	< 0.0001
$\| \alpha_L - \alpha_S \|$	35.8574	< 0.0001	1.2469	0.2652	23.7291	< 0.0001

Figure captions

Figure III – 1: (A) *Hydrocotyle bonariensis* clone, showing individual ramets and lateral runner growth pattern. Youngest ramets are produced at the tip (indicated by arrow). (B) Individual *H. bonariensis* ramets showing leaves of typical orientations (leaf angles and azimuths). (C) Single *H. bonariensis* runner (1) with individual ramet (2) consisting of a single broad leaf and individual root system.

Figure III – 2: (A) Graphic showing how leaf angle (θ) was measured. Leaf angle was defined as the angle of the plane of the adaxial leaf blade away from horizontal (positive when more inclined upward and negative when more inclined downward). (B) Graphic showing how leaf azimuth (α) was measured. Leaf azimuth was defined as the compass direction between an axis perpendicular to the adaxial surface of the leaf and North (0°).

Figure III – 3: The sun, mean leaf, and absolute difference between leaf and sun ($|Leaf - Sun|$) angle from horizontal in May (A) and June (B) and azimuth in May (C) and June (D) for *Hydrocotyle bonariensis* leaves measured in 2003. Diurnal trends for leaf angle and azimuth in August and September were similar to June. Larger absolute differences between mean leaf angle/azimuth and sun angle/azimuth indicate avoidance, while smaller absolute differences indicate solar tracking. Standard errors indicated by vertical bars.

Figure III – 4: Mean sun and leaf (A) angle and (B) azimuth for *Hydrocotyle bonariensis* in May (N = 54 and 54, respectively), June (N = 160 and 158), August (N = 60 and 59), and September (N = 60 and 60) 2003. Standard errors indicated by vertical bars.

Figure III – 5: (A) Daily adaxial PPFD for May (*N* = 9), June (*N* = 28), August (*N* = 10), and September (*N* = 10) 2003. (B) Daily abaxial PPFD for May (*N* = 9), June (*N* = 28), August (*N* = 10), and September (*N* = 10) 2003. (C) Mean seasonal adaxial and abaxial photosynthetic photon flux density (PPFD; $\mu mol \cdot m^{-2} \cdot s^{-1}$) for *Hydrocotyle bonariensis* leaves measured in May (*N* = 54 and 54 respectively), June (*N* = 147 and 88), August (*N* = 60 and 60), and September (*N* = 60 and 59) 2003. (D) Daily adaxial-to-abaxial PPFD ratio (*Ad/Ab*) for May (*N* = 9), June (*N* = 28), August (*N* = 10), and September (*N* = 10) 2003. Standard errors indicated by vertical bars.

Figure III – 6: (A) Cross-section of *Hydrocotyle bonariensis* leaf showing upper epidermis, multiple abaxial palisade layers, spongy mesophyll layer, and lower epidermis. Scale bar represents 5 µm. (B) Leaf peels of adaxial and abaxial surface showing stomata distribution pattern. Scale bar represents 1 µm.

Figure III – 7: Graphic showing leaf orientation (leaf angle and leaf azimuth) variations over the growing season and leaf plane relationship to the diurnal sun path. The azimuth of the rising sun, midday sun, and setting sun is estimated for each month, and the dotted line represents the incident sunlight on the leaf from the sun during morning, noon, and evening. The arrow represents the leaf azimuth (α) and the leaf angle from horizontal (θ) is represented by the attachment to the petiole.

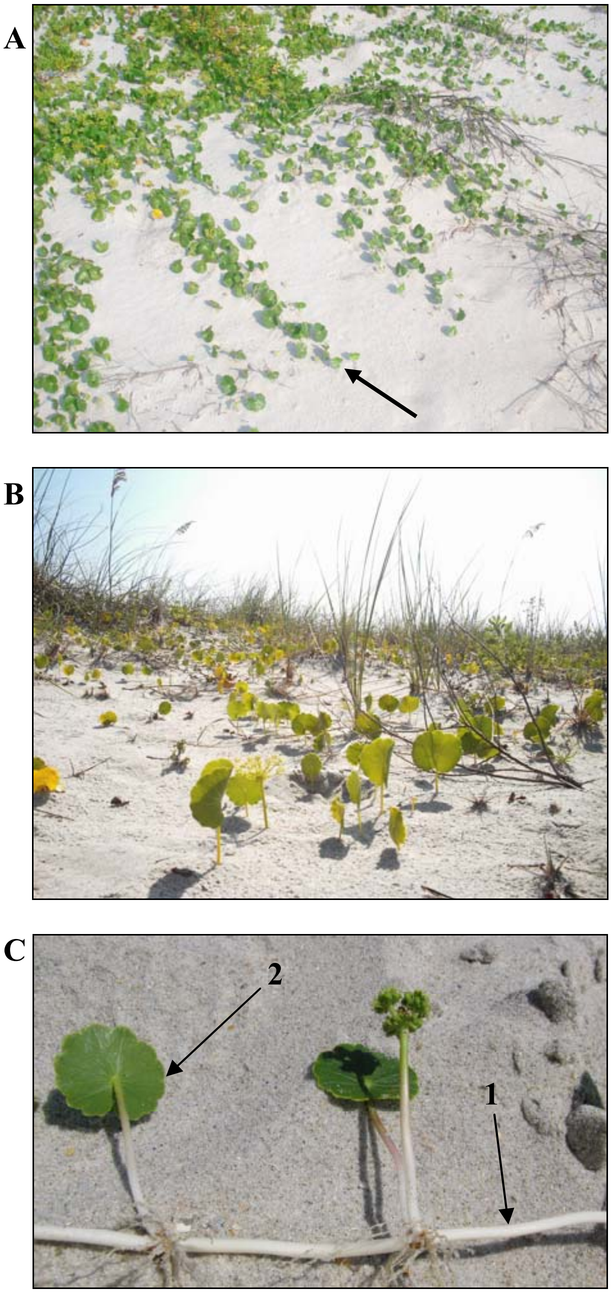

Figure III – 1

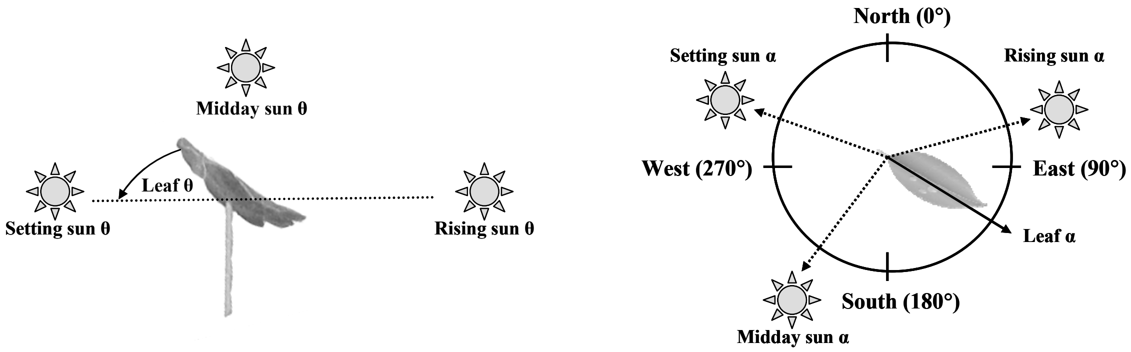
Midday sun θ
Leaf θ
Setting sun θ
Rising sun θ
North (0°)
Setting sun α
Rising sun α
West (270°)
East (90°)
Leaf α
South (180°)
Midday sun α

Figure III – 2

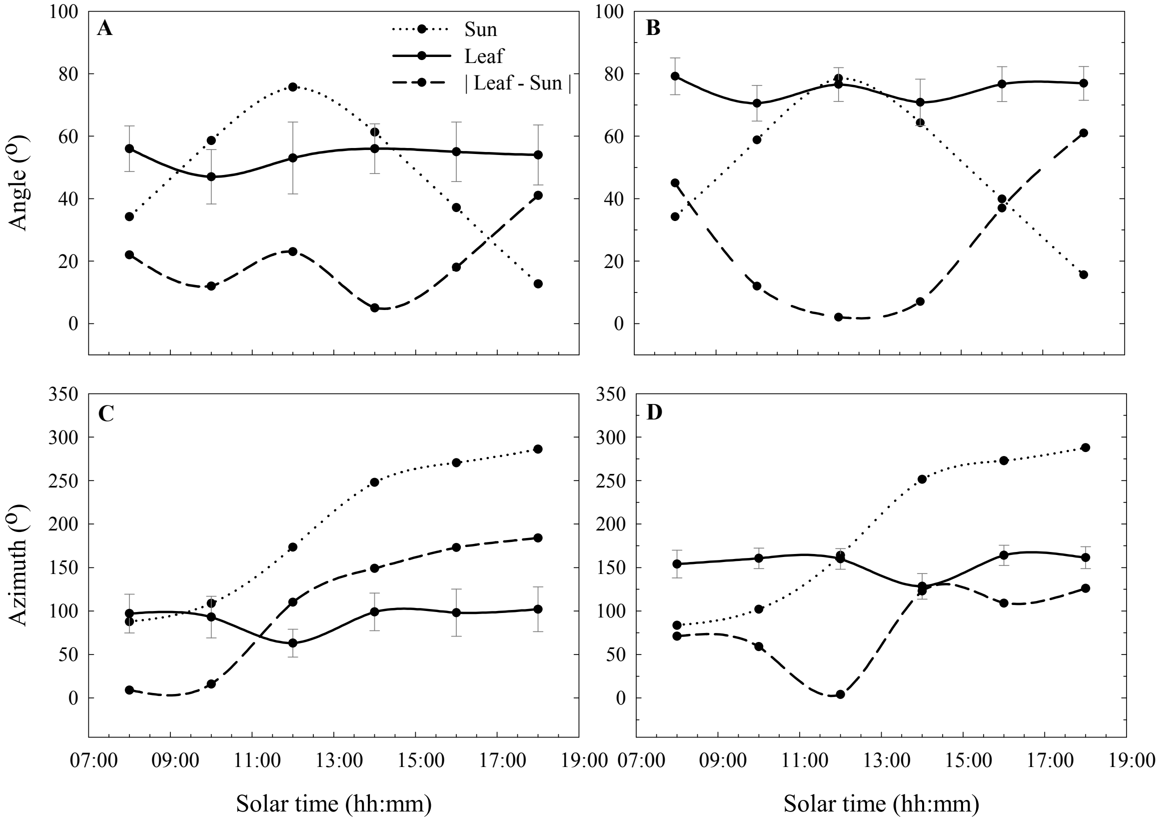
A
B
C
D
Sun
Leaf
| Leaf - Sun |
Angle (º)
Azimuth (º)
0
20
40
60
80
100
0
50
100
150
200
250
300
350
07:00
09:00
11:00
13:00
15:00
17:00
19:00
Solar time (hh:mm)
Solar time (hh:mm)

Figure III – 3

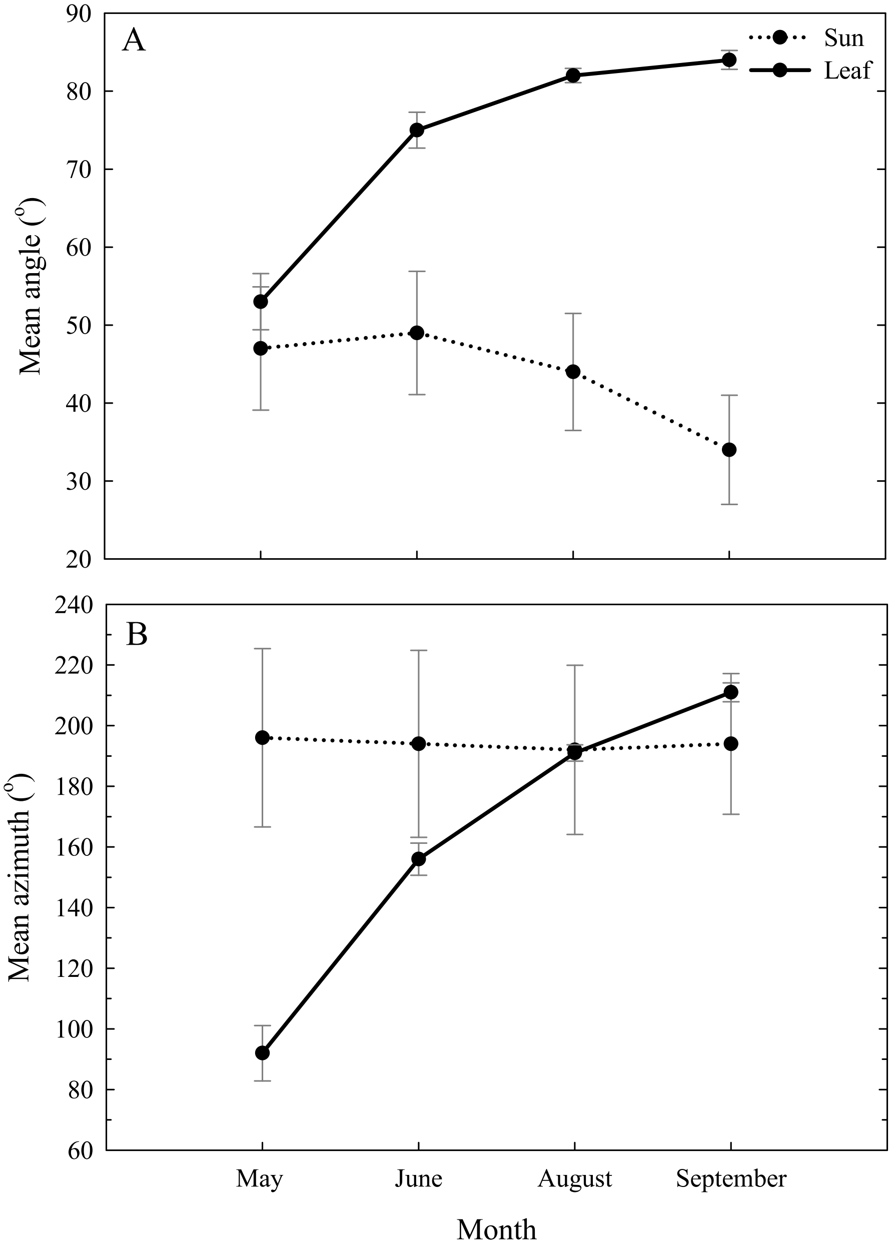
A
B
Sun
Leaf
Mean angle (°)
Mean azimuth (°)
90
80
70
60
50
40
30
20
240
220
200
180
160
140
120
100
80
60
May
June
August
September
Month

Figure III – 4

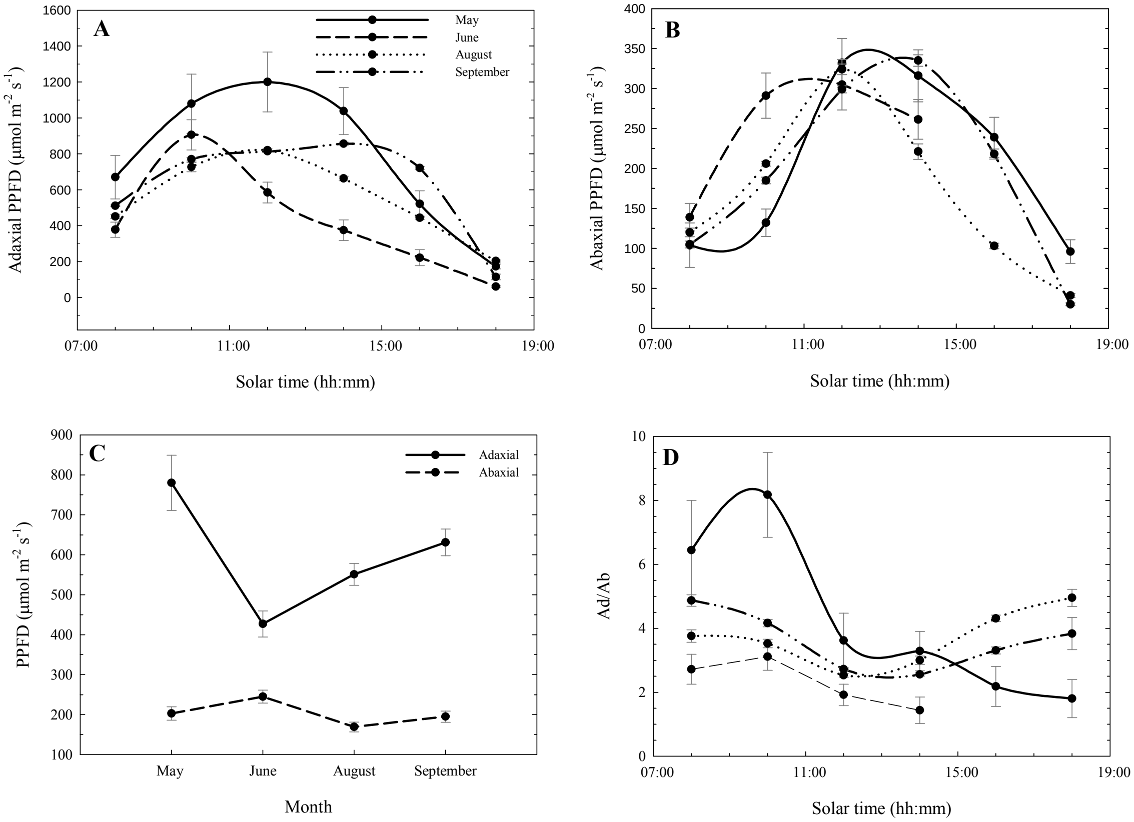
A
May
June
August
September
Adaxial PPFD (µmol m^{-2} s^{-1})
1600
1400
1200
1000
800
600
400
200
0
07:00
11:00
15:00
19:00
Solar time (hh:mm)
B
Abaxial PPFD (µmol m^{-2} s^{-1})
400
350
300
250
200
150
100
50
0
07:00
11:00
15:00
19:00
Solar time (hh:mm)
C
Adaxial
Abaxial
PPFD (µmol m^{-2} s^{-1})
900
800
700
600
500
400
300
200
100
May
June
August
September
Month
D
Ad/Ab
10
8
6
4
2
0
07:00
11:00
15:00
19:00
Solar time (hh:mm)

Figure III – 5

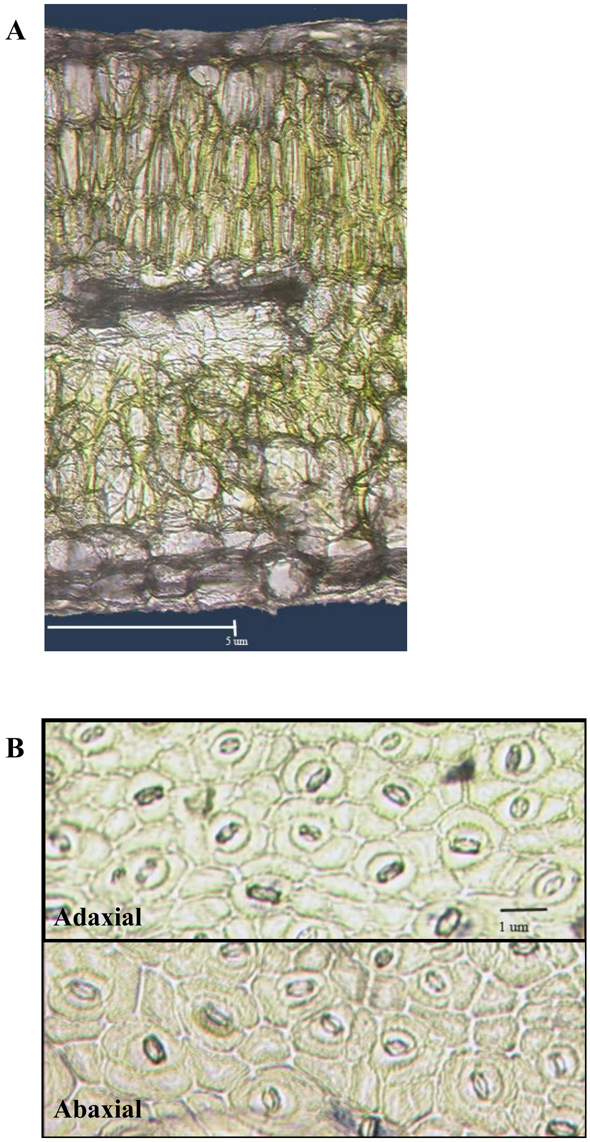

Figure III – 6

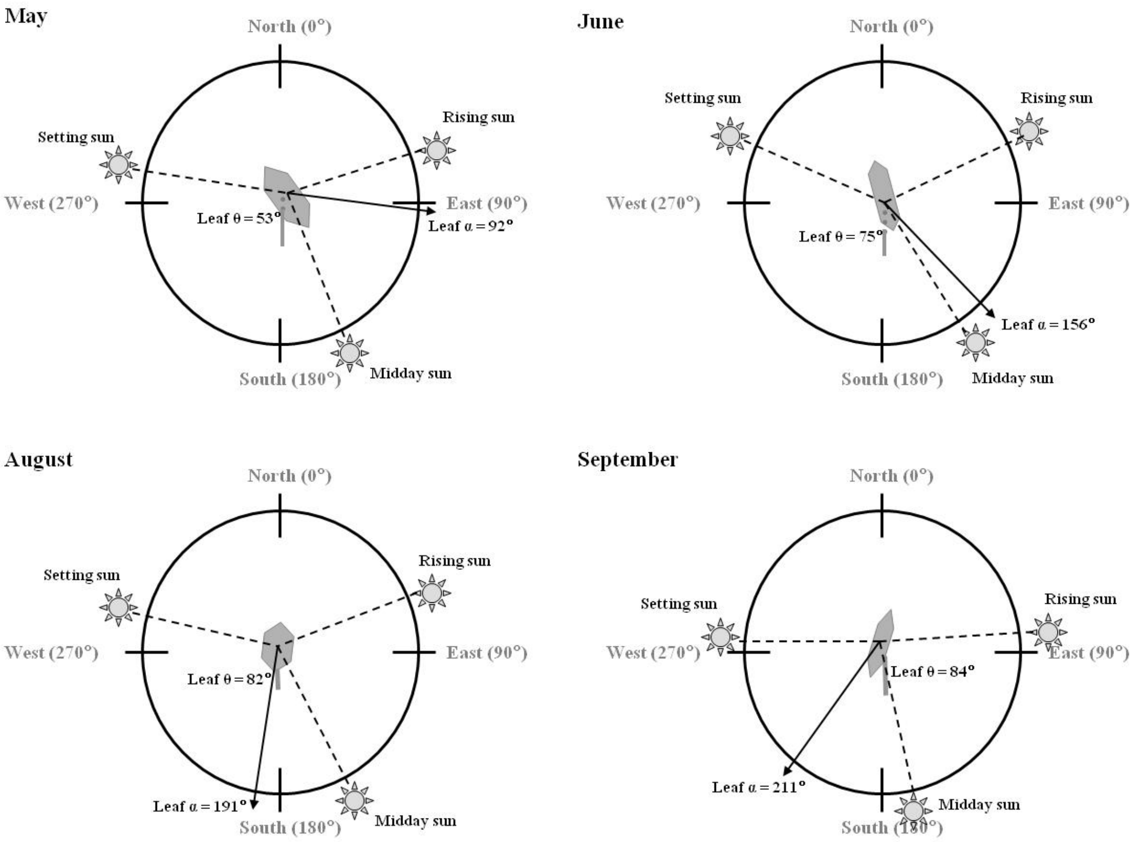
May
North (0°)
Rising sun
Setting sun
West (270°)
East (90°)
Leaf θ = 53°
Leaf α = 92°
South (180°)
Midday sun
June
North (0°)
Setting sun
Rising sun
West (270°)
East (90°)
Leaf θ = 75°
Leaf α = 156°
South (180°)
Midday sun
August
North (0°)
Rising sun
Setting sun
West (270°)
East (90°)
Leaf θ = 82°
Leaf α = 191°
South (180°)
Midday sun
September
North (0°)
Setting sun
Rising sun
West (270°)
East (90°)
Leaf θ = 84°
Leaf α = 211°
Midday sun
South (180°)

Figure III – 7

CHAPTER IV

INFLUENCE OF LEAF ORIENTATION ON LEAF PHOTOSYNTHETIC GAS EXCHANGE IN THE SAND DUNE HERB *HYDROCOTYLE BONARIENSIS*

Abstract

Leaf inclination in species of high light environments has been suggested to function to reduce risks of photoinhibition and possible damage to photosystems due to excess absorbed solar energy. Inclined leaves have been shown in the field to have reduced midday incident sunlight, lower leaf temperature and transpiration rate, and greater leaf photosynthesis compared to horizontal leaves. To test this hypothesis for the barrier island sand dune *Hydrocotyle bonariensis*, the influence of leaf inclination and transpiration on diurnal leaf temperature and photosynthetic gas exchange was investigated over a growth season. Leaf temperatures were significantly lower and leaf photosynthetic gas exchange greater in control inclined leaves compared to forced horizontal leaves in every month measured, indicating that leaf inclination functions to reduce leaf temperature and increase leaf photosynthetic gas exchange. In addition, diurnal leaf temperatures of inclined leaves more efficiently tracked ambient air temperature, especially at midday. Inclined leaves covered with petroleum jelly to impede transpiration had significantly greater leaf temperatures than uncovered control leaves, suggesting that leaf transpiration also plays an important role in maintaining optimal leaf temperatures in *H. bonariensis*. These results provide further evidence of the importance of leaf inclination and resulting incident sunlight on leaf surfaces in maximizing leaf photosynthesis, and thus daily carbon gain, in species of high light environments.

Keywords: high light; *Hydrocotyle bonariensis*; leaf orientation; leaf photosynthesis; leaf temperature

1. Introduction

Potential total daily carbon gain of a leaf is strongly influenced by the quality and quantity of incident sunlight, which can vary significantly diurnally as well as seasonally. Too little incident sunlight results in insufficient excitation energy available for chlorophyll to create the $NADPH_2$ and ATP necessary for photosynthesis, whereas too much can lead to reduction in the efficiency of energy conversion at photosystem II (i.e. photoinhibition) and irreversible photodamage to the photosynthetic apparatus (Werner et al. 1999; Zhang et al. 2002). To avoid the harmful effects of excessive absorbed irradiance, surplus solar energy can be dissipated as heat, resulting in corresponding increases in leaf temperature. However, if leaf temperatures exceed a critical upper level (42° to 45°C for most plant species), several physiological processes (e.g. enzyme activity, cytoplasmic streaming, and respiration) begin to degrade, further reducing photosynthesis and increasing risk of photoinhibition and photodamage (Gates et al. 1968). Furthermore, high leaf temperatures increase the vapor pressure difference between the leaf and the atmosphere and can result in (1) increased evaporative cooling via transpiration and decreased water use efficiency (WUE) or (2) stomatal closure and further increases in leaf temperature (van Zanten et al. 2010). Thus, plant species in high incident sunlight habitats likely possess a range of adaptations to reduce risks of photoinhibition and photodamage, such as adjustments in leaf biochemistry and photochemistry (e.g. increased xanthophyll cycle activity and presence of anthocyanins), leaf morphology and anatomy (e.g. decreased leaf size and increase in palisade mesophyll), and leaf orientation properties (e.g. inclined leaf angle) (Smith et al. 1998; Liu et al. 2003; Smith and Hughes 2009).

A survey of 209 Australian, 21 southwestern U.S. desert, and 12 Rocky Mountain native species found strong significant relationships between high sun exposure, high abiotic stress, and inclined leaf orientation (Smith et al. 1997, 1998). An inclined leaf (i.e. parallel to the sun at midday) would have decreased midday incident sunlight exposure and, thus less excess absorbed solar energy. Increased leaf angles have been shown to reduce midday incident sunlight exposure, transpiration rate, and/or leaf temperature while increasing leaf photosynthesis in *Lactuca serriola* in Utah (Werk and Ehleringer 1984), *Cistus incanus* in Mediterranean maquis (Gratani and Bombelli 1999), *Eucalyptus globulus* in Tasmania (James and Bell 2000), *Robinia pseudoacacia* in China (Zhang et al. 2002), *Acacia crassicarpa* in Australia (Liu et al. 2003), and evergreen shrubs in mexical shrubland in Mexico (Valiente-Banuet et al. 2010).

The coastal barrier island sand dune habitat is characterized by unique abiotic stress factors, e.g. blowing sand, sand erosion/accretion, periodic overwash inundation, and salt spray, in addition to high incident sunlight and growth season air and substrate temperatures. During the growth season, midday photosynthetically active radiation (PAR; 400 to 700 nm) is over 1800 μmol m^{-2} s^{-1}, daily air temperatures exceed 32°C, and sand temperatures reach over 50°C. *Hydrocotyle bonariensis* Comm ex. Lam, a broadleaf perennial herb common to the sand dune habitat, has been shown to increase leaf inclination over the growth season (from 53° in May to 84° in September) to reduce midday sunlight exposure and maximize morning and/or afternoon exposure (Joesting et al. 2011). The aim of the present study was to investigate the effect of this increase in leaf inclination on diurnal leaf temperature and leaf photosynthetic gas exchange over an entire growth season. It was hypothesized that leaf inclination in *H. bonariensis* functions to reduce midday leaf temperatures and transpiration rates while facilitating greater stomatal conductance and leaf photosynthesis.

2. Materials and methods

The objective of the present study was to compare leaf temperatures and photosynthetic gas exchange in inclined (control) and forced horizontal leaves of the common sand dune herb *Hydrocotyle bonariensis* throughout a growth season in the sand dune ridge and swale system of a North Carolina barrier island. It was hypothesized that control (inclined) leaves would have significantly lower leaf temperatures and transpiration with significantly greater stomatal conductance and leaf photosynthesis.

2.1. Study site and species

The study was conducted during the 2009 and 2010 growth season on the southern end of Topsail Island, North Carolina, United States (34°20'N, 77°39' W). Topsail Island is a barrier island approximately 42 km long and 150 to 450 m wide, and the southern end of the island is an elongated spit with an undeveloped and undisturbed sand dune ridge and swale system. The characteristic vegetation zones of barrier islands (i.e. berm or upper beach, primary dunes, swale, secondary dunes, tertiary dune grassland, shrub thicket and/or maritime forest, and salt marsh) are present (Ehrenfeld 1990). Mean air temperatures ranged from 5.8°C in January to 25.9°C in August 2009 and from 2.4°C in December to 26.8°C in June 2010, and total annual precipitation was 1925 mm and 1281 mm in 2009 and 2010, respectively (data from National Climate and Data Center, http://www.ncdc.noaa.gov/oa/ncdc.html; station at Surf City, North Carolina, 11.3 km from study site).

Hydrocotyle bonariensis (Apiaceae) is a clonal perennial herb common to the swale, secondary dunes, and tertiary dune grassland of southeastern United States coastal barrier islands. Clones spread via underground rhizomes and produce alternating single, circular broad leaves (ramets) with central petiole attachment. Leaves (ramets) have been shown to tolerate temporary sand burial following storm events and to share nutrients, water, and photosynthates via the buried rhizome (Evans 1988, 1992a). On Topsail Island, the growth season begins in April with leaf (ramet) production, rhizomatous growth, and seedling emergence. Small white flowers and nutlet-like fruits are produced in compound umbels throughout the growing season (April to September), and leaf senescence begins in December (Hancock 2009). Extremely low seedling survival rates (less than 1%) have been reported in the field; thus, recruitment is primarily due to clonal vegetative growth (i.e. ramet production) (Evans 1992b).

2.2. Leaf inclination and leaf temperature

Leaf temperature was measured diurnally on May 29, June 18, July 20, and August 16 2010 for leaves naturally inclined from horizontal (control) and experimental *H. bonariensis* leaves held in horizontal orientation. Two adjacent similar-sized, inclined leaves (ramets) were haphazardly chosen from a population of *H. bonariensis* genets located on the lee (i.e. ocean-facing) side of a single secondary dune. For each pair, one leaf was left with natural inclination (control) while the other leaf was forced into a horizontal position by tying a loop of clear fishing line around the leaf and fixing the loop to a wire buried in the sand (Figure 1A). Leaves were allowed to acclimate for approximately 12 to 24 hours prior to taking measurements.

Hourly leaf temperatures were measured for ten pairs of leaves monthly (n = 10 leaves x 2 treatments = 20 leaves x 4 months = 80) from 0900 h to 1700 h using a Raytek PM Plus handheld infrared temperature gun (Raytek Corporation, Santa Cruz, CA). All leaf temperatures were taken near the center of the leaf (but not at petiole attachment) from a distance of approximately one meter. Additional leaf temperatures were measured each month for control and horizontal leaf pairs (haphazardly selected and manipulated as described above) using leaf thermocouple wires attached to Hobo Type T thermocouple data loggers (Onset Computer Corporation, Pocasset, MA). The tip of the wire was threaded through the center of the leaf near petiole attachment (Figure 1B), and leaf temperatures were recorded at 15 min intervals from 0600 h to 2100 h (~ sunrise to sunset). There were two leaf sets for May, June, and July (n = 2 leaves x 2 treatments = 4), and four leaf sets for August (n = 4 leaves x 2 treatments = 8). Leaf temperatures from individual thermocouples were averaged to calculate control and horizontal leaf temperatures at 15 min intervals, and these 15 min leaf temperatures were averaged to determine mean hourly leaf temperature for an entire day each month for both control and horizontal leaves.

To calculate deviations of control and horizontal leaf temperatures from air temperature, air temperature was also measured monthly from May to August 2010 using a Hobo Pro Series RH/Temp data logger (Onset Computer Corporation, Pocasset, MA) attached to a 0.5 m PVC pipe covered with a Hobo rain shield. Air temperatures were measured at 15 min intervals from 0600 h to 2100 h, and these intervals were averaged to calculate hourly air temperatures, which were then compared to mean hourly leaf temperatures for control and horizontal leaves.

A repeated-measures analysis of variance (ANOVA) was performed to investigate the effect of leaf inclination on leaf temperature with treatment as the between-subjects effect and month and time as within-subject effects. The analysis was performed using JMP 8.0.2 statistical software package (SAS Institute Inc., 2009), and significance was determined at $P \leq 0.05$.

2.3. Leaf inclination and photosynthetic gas exchange

The effect of leaf inclination on midday leaf photosynthetic gas exchange was investigated for control and horizontal leaves on July 10 and August 22 2009. Six pairs of inclined leaves of similar size were haphazardly chosen along the lee side of a single secondary dune, and one leaf of each pair was forced in a horizontal orientation as previously described while the other remained inclined (control) (n = 6 leaves x 2 treatments = 12 leaves x 2 months = 24). Leaf photosynthesis (A), stomatal conductance (g), and transpiration (E) were measured at midday (1200 h – 1400 h) for each leaf set using a LICOR LI-6400 infrared gas exchange analyzer (LICOR, Lincoln, NE). Approximately half of the leaf was placed in the leaf chamber and measurements were taken with flow rate of 500 m s^{-1} and reference CO_2 of 400 ppm.

To investigate the effect of evaporative cooling via transpiration, leaf temperatures were compared in July 2010 between control leaves and leaves with both surfaces covered with petroleum jelly to block gas exchange via stomata pores, hence impeding transpiration. Four groups of six leaves of similar size and inclination were randomly chosen along the lee side of a single secondary dune, and within each group, three leaves were covered with petroleum jelly and three were left uncovered as control (n = 4 groups x 3 leaves x 2 treatments = 24). Leaf temperatures were measured near the center of the leaf as described above using a hand-held infrared temperature gun hourly from 1100 h to 1400 h.

To determine the influence of leaf inclination on photosynthetic gas exchange, a multivariate analysis of variance (MANOVA) was conducted with A, g, and E as dependent variables, treatment as a random effect, and month as a fixed effect. A repeated-measures ANOVA was performed to determine the effect of blocking transpiration (i.e. covered with petroleum jelly) on leaf temperatures with the treatment as between-subjects effect and time the within-subjects effect. Both statistical procedures were performed using the JMP 8.0.2 statistical package, and significance was considered at $P \leq 0.05$.

3. Results

3.1. Leaf inclination and leaf temperature

Mean leaf temperature (T_{leaf}) of control leaves was significantly less than mean T_{leaf} of horizontal leaves in every month measured (F = 35.5091, P < 0.0001; Table 1). More specifically, T_{leaf} of horizontal leaves were greater at midday (1100 h to 1500 h) than T_{leaf} of control leaves for each month measured (Figure 2). There was no general trend over the growth season in deviation of T_{leaf} from T_{air} for either control or horizontal leaves (Figure 3). In May, control leaves ranged from -2.0 °C at 0800 h to 1.6 °C at 1100 h above T_{air}, and remained relatively near or below T_{air} for the majority of the day. However, horizontal leaves remained between 1.3 °C (2100 h) and 3.2 °C (1600 and 1700 h) above T_{air} for the entire day in May.

Control and horizontal leaves followed the same general trend in June, July, and August, with T_{leaf} in control leaves nearer to T_{air} than forced horizontal leaves for the majority of the day in each month. Mean $T_{leaf} – T_{air}$ for control leaves was -0.1 ± 0.21 °C, 0.9 ± 0.52 °C, 1.2 ± 0.45 °C, and 2.0 ± 0.45 °C in May, June, July, and August, respectively. For horizontal leaves, mean $T_{leaf} – T_{air}$ was 2.4 ± 0.14 °C, 2.0 ± 0.51 °C, 2.3 ± 0.36 °C, and 2.2 ± 0.56 °C in May, June, July, and August, respectively.

3.2. Leaf inclination and photosynthetic gas exchange

A, g, and *E* were significantly greater in control leaves compared to horizontal leaves (F = 3.6278, P = 0.0453) but there was no significant difference between months (July and August) for either control or horizontal leaves (F = 0.7165, P = 0.5006). Mean *A, g,* and *E* in control leaves was 9.9 ± 1.28 µmol m^{-2} s^{-1}, 0.51 ± 0.123 µmol m^{-2} s^{-1}, and 8.2 ± 1.00 mmol m^{-2} s^{-1}, respectively, in July, and 9.9 ± 1.34 µmol m^{-2} s^{-1}, 0.40 ± 0.050 µmol m^{-2} s^{-1}, and 8.6 ± 0.42 mmol m^{-2} s^{-1}, respectively, in August (Figure 4). Mean *A, g,* and *E* for horizontal leaves was 7.7 ± 1.84 µmol m^{-2} s^{-1}, 0.32 ± 0.072 µmol m^{-2} s^{-1}, and 6.5 ± 0.94 mmol m^{-2} s^{-1}, respectively, in July, and 5.3 ± 0.92 µmol m^{-2} s^{-1}, 0.20 ± 0.027 µmol m^{-2} s^{-1}, and 5.9 ± 0.65 mmol m^{-2} s^{-1}, respectively, in August.

T_{leaf} of leaves covered in petroleum jelly were significantly greater than T_{leaf} of control leaves (F = 14.6687, $P < 0.0001$) but there was no significant effect of time of day for either treatment (F = 0.1346, P = 0.9388). Mean T_{leaf} of control and treatment leaves was 32.6 ± 0.55 °C and 35.9 ± 0.52 °C, respectively. Midday T_{leaf} for control leaves ranged from 31.7 ± 0.37 °C at 11:00 h to 34.1 ± 0.34 °C at 12:00 h (Figure 5). For petroleum jelly covered leaves, midday T_{leaf} ranged from 34.9 ± 0.60 °C at 13:00 h to 37.2 ± 0.35 °C at 12:00 h.

4. Discussion

The results of the present study suggest that the interaction of leaf inclination and evaporative cooling via transpiration functions to reduce leaf temperatures while facilitating photosynthetic gas exchange in *H. bonariensis* leaves in the sand dune habitat, especially during midday. Forced horizontal leaves had significantly greater leaf temperatures and lower photosynthesis, transpiration, and stomatal conductance compared to control inclined leaves. Similar results have been found in forced horizontal experiments of naturally inclined leaves of *Vitis californica* in northern California (Gamon and Pearcy 1989), *Silphium terebinthinaceum* in Illinois (Smith and Ullberg 1989), *Robinia pseudoacacia* in China (Zhang et al. 2002), and *Acacia crassicarpa* in Australia (Liu et al. 2003).

It is clear from these results that midday leaf temperatures of forced horizontal leaves were significantly greater than control leaves, supporting the hypothesis that leaf inclination in *H. bonariensis* functions to reduce leaf temperature. The primary determinant of leaf temperature is the balance between energy inputs and losses due to convective heat transport, transpiration, and thermal radiation from leaves (King 1997; Smith and Hughes 2009). A broadleaf orientated perpendicular to the sun (i.e. horizontal inclination) can absorb up to 85% of incoming PAR during midday (King 1997), suggesting that horizontal leaves may absorb more solar energy (especially during midday) than necessary for photosynthesis and thus, release more surplus energy as heat, increasing leaf temperature. However, an increase in leaf inclination to a more vertical orientation would result in low angles of illumination (i.e. leaf surface more parallel to sun) and thus, less absorbance of solar energy at midday and reduced leaf temperature.

As hypothesized, leaf photosynthesis and stomatal conductance were greater in control leaves compared to horizontal leaves, implying that inclined leaf orientation in *H. bonariensis* also functions to increase leaf photosynthetic gas exchange. Increased leaf temperatures may intensify the vapor pressure difference between the leaf and air, resulting in increased evaporative cooling via transpiration. In the present study, mean leaf temperature of forced horizontal leaves were approximately 2°C greater than air temperature whereas mean leaf temperature of control leaves were about 1°C greater than air temperature. This may have prompted partial stomatal closure during midday, especially if water supply was limited, to reduce water loss at the cost of reduced photosynthesis. Several studies have shown midday depressions in photosynthesis and stomatal conductance associated with horizontal leaf angles (Smith and Ullberg 1989; Liu et al. 2003).

However, contrary to the hypothesized reduction in evaporative cooling for inclined leaves, the results showed increased transpiration in control leaves, suggesting that transpiration also plays a significant role in maintaining optimal leaf temperatures in *H. bonariensis*. In addition, covering leaves with petroleum jelly to impede transpiration resulted in higher leaf temperatures, further indicating an important role of transpiration. Smith (1978) suggested that increased transpiration rates in large-leafed desert species separated leaf temperature from air temperature through alterations in leaf boundary layer resistance, resulting in reduced leaf temperatures. Furthermore, high transpiration rates may be adaptive in large leaved species when a stable water source is available. Water potentials rarely below -0.2 MPa have been recorded for *H. bonariensis* leaves at Topsail Island, suggesting that there is likely an adequate water supply available at Topsail Island to maintain high transpiration rates (Hancock 2009).

In conclusion, the results of the present study indicate that leaf temperature and photosynthetic gas exchange in *H. bonariensis* are maintained at optimal levels through adjustments in both leaf morphology (leaf inclination) and leaf physiology (increased transpiration rates). These results further demonstrate the importance of leaf inclination and the resulting incidence sunlight on leaf surfaces in high incident light habitats in promoting maximum leaf photosynthesis and carbon gain.

Literature cited

Ehrenfeld, J.G. 1990. Dynamics and processes of barrier island vegetation. Aquatic Sciences 2: 437 – 480.

Evans, J.P. 1988. Nitrogen translocation in a clonal dune perennial, *Hydrocotyle bonariensis*. Oecologia 77: 64 – 68.

Evans, J.P. 1992a. The effect of local resource availability and clonal integration on ramet functional morphology in *Hydrocotyle bonariensis*. Oecologia 89: 265 – 276.

Evans, J.P. 1992b. Seedling establishment and genet recruitment in a population of a clonal dune perennial, *Hydrocotyle bonariensis*. *In* C.A. Cole and K. Turner [eds.], Barrier Island Ecology of the Mid-Atlantic Coast: A Symposium, 75 – 84. U.S. Department of Interior, National Park Service, Southeast Regional Office, Atlanta, Georgia, USA.

Gamon, J.A. and R.W. Pearcy. 1989. Leaf movement, stress avoidance and photosynthesis in *Vitis californica*. Oecologia 79: 475 – 481.

Gates, D.M., R. Alderfer, and E. Taylor. 1968. Leaf temperature of desert plants. Science 159: 994 – 995.

Gratani, L. and A. Bombelli. 1999. Leaf anatomy, inclination, and gas exchange relationships in evergreen sclerophyllous and drought semideciduous shrub species. Photosynthetica 37: 573 – 585.

Hancock, T. 2009. Ecophysiology of Barrier Island Beach Plants: Responses in Form and Function to Daily, Seasonal and Episodic Stresses. PhD Dissertation, Department of Biology, Wake Forest University, Winston Salem, NC

James, S.A. and D.T. Bell. 2000. Leaf orientation, light interception and stomatal conductance of *Eucalyptus globulus* ssp. *globulus* leaves. Tree Physiology 20: 815 – 823.

Joesting, H.M., M.O. Sprague, and W.K. Smith. 2011. Seasonal and diurnal leaf orientation, bifacial sunlight incidence, and leaf form and function in the sand dune herb *Hydrocotyle bonariensis*. Environmental and Experimental Botany, *in press*.

King, D.A. 1997. The functional significance of leaf angle in *Eucalyptus*. Australian Journal of Botany 45: 619 – 639.

Liu, L-X, S-M Xu, and K.C. Woo. 2003. Influence of leaf angle on photosynthesis and the xanthophyll cycle in the tropical tree species *Acacia crassicarpa*. Tree Physiology 23: 1255 – 1261.

Smith, M. and D. Ullberg. 1989. Effect of leaf angle and orientation on photosynthesis and water relations in *Silphium terebinthinaceum*. American Journal of Botany 76: 1714 – 1719.

Smith, W.K. 1978. Temperatures of desert plants: Another perspective on the adaptability of leaf size. Science 201: 614 – 616.

Smith, W.K., T.C. Vogelmann, E.H. DeLucia, D.T. Bell, and K.A. Shepherd. 1997. Leaf form and photosynthesis: Do leaf structure and orientation interact to regulate internal light and carbon dioxide? Bioscience 47: 785 – 793.

Smith, W.K., D.T. Bell, and K.A. Shepherd. 1998. Associations between leaf structure, orientation, and sunlight exposure in five Western Australian communities. American Journal of Botany 85: 56 – 63.

Smith, W.K. and N.M. Hughes. 2009. Progress in coupling plant form and photosynthetic function. Castanea 74: 1 – 26.

Werk, K.S. and J. Ehleringer. 1984. Non-random leaf orientation in *Lactuca serriola* L. Plant, Cell and Environment 7: 81 – 87.

Werner, C., O. Correia, and W. Beyschlag. 1999. Two different strategies of Mediterranean macchia plants to avoid photoinhibitory damage by excessive radiation levels during summer drought. Acta Oecologica 20: 15 – 23.

Valiente-Banuet, A., M. Verdù, F. Valladares, and P. Garcìa-Fayos. 2010. Functional and evolutionary correlations of steep leaf angles in the mexical shrubland. Oecologia 163: 25 – 33.

van Zanten, M., T.L. Pons, J.A.M. Janssen, L.A.C.J. Voesenek, and A.J.M. Peeters. 2010. On the relevance and control of leaf angle. Critical Reviews in Plant Sciences 29: 300 – 316.

Zhang, S-R, K-P Ma, and L-Z Chen. 2002. Photosynthetic gas exchange and leaflet movement of *Robinia pseudoacacia* in relation to changing light environments. Acta Botanica Sinica 44: 858 – 863.

Table IV – 1: Leaf temperature ±SE (°C) of control (natural inclination) and forced horizontal *Hydrocotyle bonariensis* leaves in May, June, July, and August 2010.

	Control (vertical)	Forced (horizontal)
May	27.3 ± 1.00	29.7 ± 1.10
June	29.8 ± 0.93	30.9 ± 0.95
July	33.0 ± 1.05	34.3 ± 1.00
August	34.9 ± 1.27	35.1 ± 1.48

Figure captions

Figure IV – 1: (A) Control leaf left with normal inclination and experimental leaf forced horizontal using fishing line and wire. (B) Threading of leaf thermocouple wire through leaf.

Figure IV – 2: Daily leaf temperatures (T_{leaf}; °C) for control (natural inclination) and horizontal *Hydrocotyle bonariensis* leaves from 0600 h to 2100 h for a single day in May, June, July, and August 2010.

Figure IV – 3: Difference between leaf temperature and air temperature ($T_{leaf} - T_{air}$; °C) for control (natural inclination) and horizontal *Hydrocotyle bonariensis* leaves from 0600 h to 2100 h in May, June, July, and August 2010. Dashed line represents the zero value (i.e. no difference).

Figure IV – 4: Mean midday photosynthesis (A; µmol m^{-2} s^{-1}), stomata conductance (g; µmol m^{-2} s^{-1}), and transpiration (E; mmol m^{-2} s^{-1}) for control (natural inclination) and horizontal *Hydrocotyle bonariensis* leaves in July and August 2009. Error bars represent standard error.

Figure IV – 5: Mean midday leaf temperatures (T_{air}; °C) of control and treatment (covered with petroleum jelly) *Hydrocotyle bonariensis* leaves in July 2010. Error bars represent standard error.

Figure IV – 1

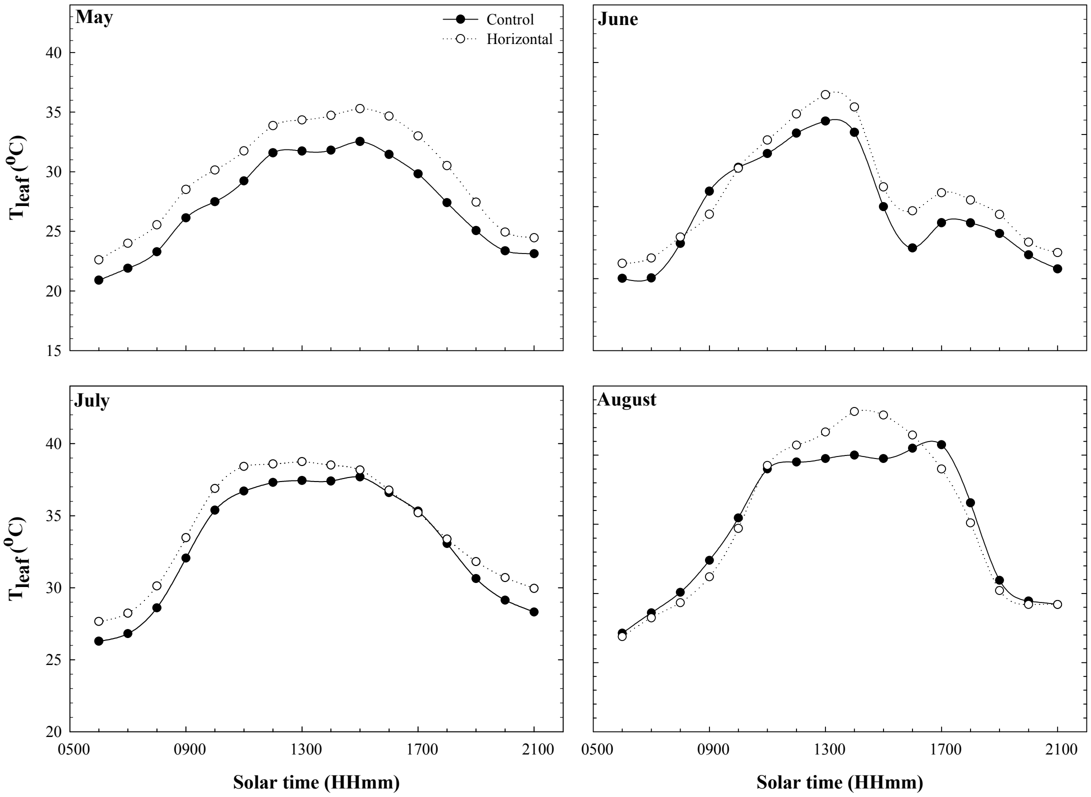
May
June
July
August
Control
Horizontal
Tleaf (0C)
15
20
25
30
35
40
0500
0900
1300
1700
2100
Solar time (HHmm)

Figure IV – 2

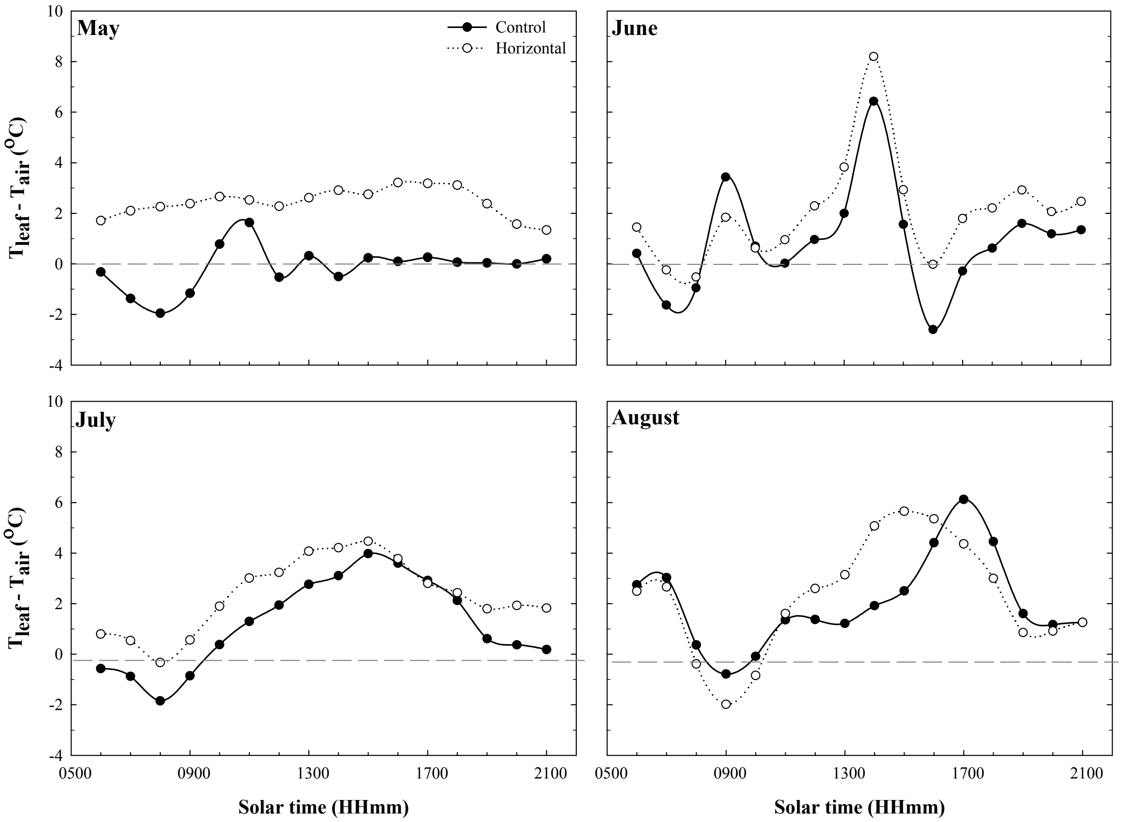
May
June
July
August
Control
Horizontal
T_{leaf} - T_{air} (^{o}C)
Solar time (HHmm)
10
8
6
4
2
0
-2
-4
0500
0900
1300
1700
2100

Figure IV – 3

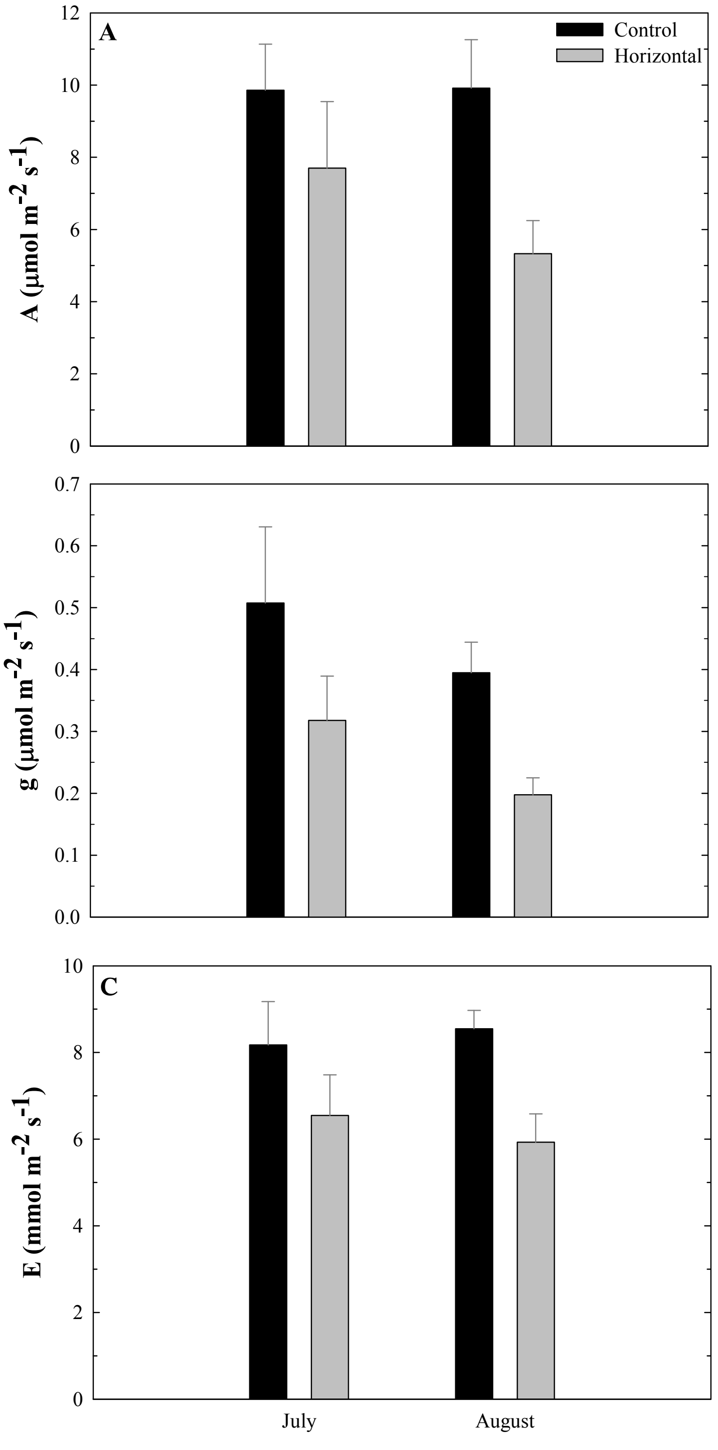
A
Control
Horizontal
A (μmol m-2 s-1)
0
2
4
6
8
10
12
g (μmol m-2 s-1)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
C
E (mmol m-2 s-1)
0
2
4
6
8
10
July
August

Figure IV – 4

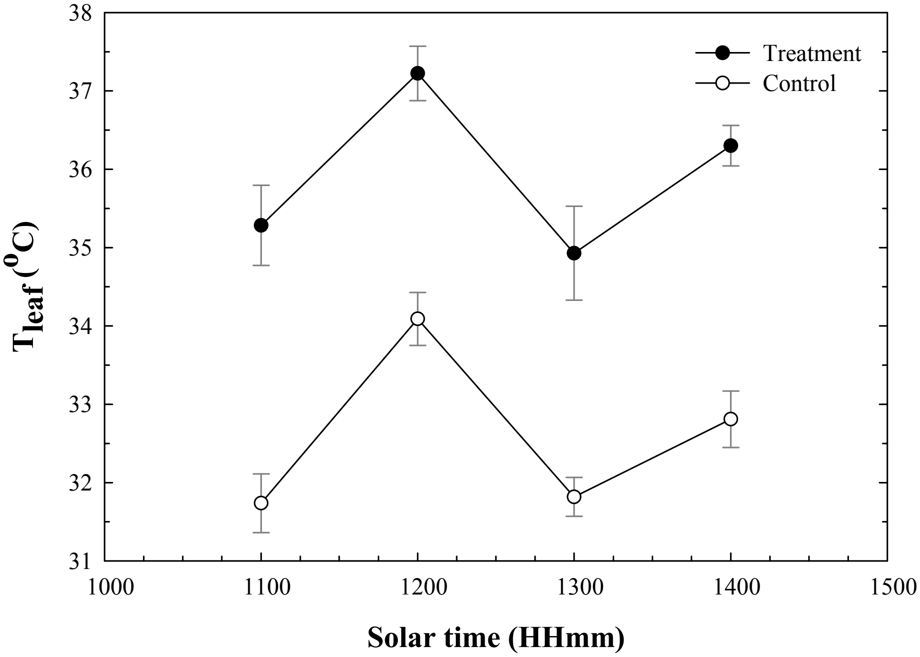
38
37
36
35
34
33
32
31
T_{leaf} (^0C)
1000
1100
1200
1300
1400
1500
Solar time (HHmm)
Treatment
Control

Figure IV – 5

CHAPTER V

DO LEAF CHARACTERISTICS PREDICT LEAF PHOTOSYNTEHTIC GAS EXCHANGE ALONG A SMALL-SCALE-STRESS GRADIENT?

Abstract

Recent research has focused on identifying easily measurable leaf traits that can be used to predict leaf-level photosynthetic gas exchange and carbon gain, but few studies have investigated these relationships for small-scale abiotic stress gradients. Measurements of leaf structure and orientation were related to photosynthetic gas exchange to determine the potential of measured leaf characteristics in predicting leaf photosynthesis for two barrier island species along a sand dune abiotic gradient. Leaf structure and orientation, leaf temperature, and photosynthetic gas exchange were measured for two native species along a 200-m transect from the tidal zone to tertiary dune grassland of a barrier island sand dune community over a growth season. Leaf structure, orientation and physiology were compared between each species, as well as to predictions from a Structure-Orientation conceptual model, and linear stepwise regressions were performed to determine which leaf characteristics were most predictive of photosynthetic gas exchange. The two species had significantly different leaf structure and physiology, and each possessed leaf characteristics predicted by the Structure-Orientation conceptual model based on their location along the sand dune abiotic stress gradient. Furthermore, leaf size, orientation, and stomatal frequency were significant predictors of leaf photosynthetic gas exchange in both species, explaining 5 – 30% of the variation. These results support the use of the Structure-Orientation conceptual model for predicting leaf structure and orientation for native species along a small-scale abiotic stress gradient. In addition, leaf characteristics in the model, e.g. leaf orientation and stomatal frequency, are important predictors of leaf photosynthetic gas exchange and should be included in model estimations of potential photosynthetic carbon gain.

Keywords: abiotic stress gradient; leaf orientation; leaf structure; leaf temperature; photosynthetic gas exchange; sand dune

1. Introduction

Recent research in plant form and function has focused on the identification of easily measurable leaf traits linked to photosynthesis and environmental responses that can be used to predict ecosystem responses to global climate change (Reich et al. 1997, 1998, 2007; Wright et al. 2005a, b). It has been recognized for some time that leaf structure and orientation can have a strong association with leaf photosynthetic carbon gain, and there has been strong support for use of a model combining leaf mass per unit area (LMA), leaf nitrogen, and leaf life-span for predicting photosynthetic potential, explaining up to up to 88% of the variation across biomes and plant functional groups (Enríquez et al. 1996; Reich et al. 1997, 1998, 2007; Wright et al. 2005a, b; Poorter et al. 2009; Smith and Hughes 2009). Although these traits are considered easily measured compared to actual measurements of photosynthesis, some of these parameters can be labor and/or time intensive (e.g. leaf life-span), expensive, and/or require specialized instrumentation and equipment (e.g. leaf nitrogen). In addition, LMA is determined by both leaf thickness and leaf density, which can vary independently according to species and/or the abiotic environment (Garnier and Laurent 1994; Niinemets 1999; Castro-Diez et al. 2000; Poorter et al. 2009; Smith and Hughes 2009) and may thus be confounded in species occurring along small-scale abiotic gradients, e.g. estuarine, riparian, or coastal sand dune systems. Furthermore, most studies have not incorporated such measurable features as leaf inclination, internal anatomy (e.g. occurrence of multiple palisade layers), the presence of water cells (i.e. succulence), or stomatal distribution on both leaf sides, all of which are common characteristics of plant species in high light and high stress habitats and have been significantly associated with photosynthetic potential (Smith et al. 1997, 1998; Smith and Hughes 2009).

The barrier island sand dune ridge and swale habitat presents a host of dynamic abiotic stress factors that can significantly vary both temporally and spatially (Stallins 2002). The combination of sand erosion and accretion, periodic seawater inundation from overwash, aerial salt exposure, high incident sunlight, reflective substrate, high air and soil temperatures, and mechanical damage from overwash, wind, and blowing sand is unique to coastal sand dunes (Ehrenfeld 1990; Snyder and Boss 2002). In addition, several abiotic factors (e.g. salt spray and sand burial) have been shown to decrease in severity with greater distance from the ocean, resulting in distinct vegetation zonation along a small-scale abiotic stress gradient (Oosting and Billings 1942; Boyce 1954; Barbour 1978; Ehrenfeld 1990; Hesp 1991). Thus, the barrier island sand dune habitat represents an ideal system for examining the use of easily measured leaf characteristics to predict leaf photosynthesis along a small-scale abiotic stress gradient.

A conceptual Structural-Orientation (SO) model has been proposed which predicts leaf structure and orientation of native species based on the incident sunlight and relative abiotic stress level of the habitat (Smith et al. 1997, 1998). The aim of the present study was to (1) verify the leaf structure and orientation predictions from the SO conceptual model, and (2) investigate the use of measured leaf characteristics to predict leaf photosynthetic gas exchange for two common barrier island sand dune species occupying different spatial positions along a small-scale abiotic stress gradient. Measurements of LMA were included in the SO model in this study based on strong support for this leaf trait in predicting leaf photosynthesis (Reich et al. 1997, 1998, 2007; Wright et al. 2005a, b). Based on the SO model, it was hypothesized that the species at the lower end of this gradient (i.e. near tidal line, relatively more abiotic stress) would have small and thick inclined leaves with top and bottom palisade layers, whereas the species at the upper end of the gradient (i.e. relatively less abiotic stress) would have large horizontal leaves with upper palisade only (Table 1; Smith et al. 1997, 1998). Furthermore, it was expected that midday values of leaf temperature and photosynthetic gas exchange would be significantly correlated to measured leaf characteristics.

2. Materials and methods

Leaf external morphology, internal anatomy, orientation, LMA, temperature, and photosynthetic CO_2 gas exchange were measured for two barrier island sand dune species to verify leaf structure and orientation predictions of a Structure-Orientation (SO) conceptual model (Table 1; Smith et al. 1997, 1998), as well as the use of measured leaf characteristics in predictions of leaf photosynthesis for two native species occupying different spatial positions along a barrier island sand dune abiotic gradient. *Iva imbricata* Walter occurs from the overwash zone (i.e. lower end of the gradient, higher abiotic stress) to the tertiary grassland whereas *Hydrocotyle bonariensis* Comm ex. Lam only occupies the upper end of this zone further away from salt spray and overwash impacts (i.e. lower abiotic stress; Figure 1).

2.1. Study site

The present study was conducted from May to September 2008 on the south end of Topsail Island, North Carolina, United States (34°20'N, 77°39' W). Topsail Island is approximately 42 km long and 150 to 450 m wide, and the southern end of the island (study site) is undeveloped with a mature, undisturbed dune ridge and swale system. The distinct vegetation zones characteristic of barrier islands (i.e. upper beach/tidal or overwash zone, primary dunes, swale, secondary dunes, tertiary dune grassland, shrub thicket, and salt marsh) are present (Ehrenfeld 1990; Figure 1). Mean monthly temperatures in 2008 ranged from a minimum of 6.3°C in January to a maximum of 25.6°C in July, and total annual precipitation was 1261 mm. The growing season (defined as total number of days between last spring minimum and first fall minimum of 0º C) totaled 217 days from March 26 to October 29 (data from National Climate and Data Center, http://www.ncdc.noaa.gov/oa/ncdc.html; station at Surf City, North Carolina, 11.3-km from study site).

2.2. Study species

Iva imbricata (Asteraceae; sea elder) is a perennial, leaf-succulent, semi-woody C_3 shrub that occurs from the upper beach to the shrub line (Figure 2A). Leaves are sessile with a thick cuticle and have been shown to be tolerant to salt spray and sand burial (Oosting 1945; Colosi and McCormick 1978). On Topsail Island, *I. imbricata* seedlings emerge in early spring (March/April), flowers develop in July and August, and fruits occur from September to October. Leaves typically senesce in October and November, and winter dormancy occurs in December (Hancock 2009). Recruitment is primarily by seedlings, with seedling survival rates between 70 and 100% reported along the upper beach and swale (Colosi and McCormick 1978). However, vegetative reproduction by means of rooting, decumbent stems is prevalent in the primary dunes due to unfavorable germination conditions (Colosi and McCormick 1978).

Hydrocotyle bonariensis (Apiaceae; large-leaf pennywort) is a clonal, perennial, C_3 broadleaf herb found in sand dune swales, secondary dunes, and tertiary dune grassland (Figure 2B). Clones (genets) have a spreading, ground-level habit and grow and reproduce by producing alternating single, circular broad leaves (ramets) that bud from branching, almost linear rhizomes. Leaf orientation in *H. bonariensis* becomes more inclined over the growing season, presumably to avoid high midday sunlight incidence, while simultaneously changing leaf azimuth to seasonally track the increasingly southern sun azimuth beyond the summer solstice (Joesting et al. *unpublished results*). Leaves (ramets) have been shown to tolerate periodic sand burial for several days following storm events and to share water, nutrients, and photosynthates via a buried rhizome (lateral runner) (Evans 1998, 1992a). On Topsail Island, the growth season begins in March and April with seedling emergence, leaf (ramet) production, and rhizomatous growth, and ends with winter dormancy in December. Flowers are produced throughout the growing season (June to September) and nutlets develop in mid-August through October (Hancock 2009). Extremely low seedling survival rates (less than 1%) have been reported and thus, recruitment is primarily due to clonal growth (Evans 1992b).

2.3. Field methods

Data were collected monthly over the 2008 growth season (May 25-26, June 13-14, July 12-13, August 14-15, and September 20-21) along a 200-m transect (i.e. abiotic stress gradient) established from the first embryo dune (defined as a small mound of sand trapped by emerging vegetation) of the upper beach to the tertiary dune grassland (Figure 1). Flags were placed at 10 m intervals beginning at 0 m, and the nearest individual of *I. imbricata* and *H. bonariensis* were selected at each 10 m interval for analysis, if an individual was located within three meters of the flag. *Iva imbricata* was present at 15 flags, spaced over the entire transect, while *H. bonariensis* occurred at five flags along the last 50 m of the transect (Figure 1B).

Instantaneous midday photosynthetic gas exchange parameters included leaf temperature (T_{leaf}), photosynthesis (A), transpiration (E), and leaf conductance (g) and were measured between 1000 h and 1500 h using an infrared gas exchange analyzer (TPS; PP Systems, Amesbury, MA) in natural sunlight with a flow rate of 300 cm^3 min^{-1}, reference CO_2 of 380 ppm, and reference H_2O vapor of 20 – 25 mbar partial pressure. For *I. imbricata*, the first mature, healthy leaf (node 3 - 5) on south-facing stems closest to the ground was randomly selected, and the entire leaf was placed in the leaf cuvette. This was replicated for five *I. imbricata* leaves on a single plant per flag, for a total of 75 leaves each month (15 flags x 5 leaves = 75 leaves x 5 months = total n = 375). A total of 10 leaves (ramets) of *H. bonariensis* were randomly chosen at each flag, for a total of 50 leaves (5 flags x 10 leaves = 50 leaves x 5 months = total n = 250). Approximately half of each *H. bonariensis* leaf (tip to mid-leaf area) was placed in the leaf chamber for measurement.

In addition to gas exchange measurements, angle of leaf attachment to the stem (leaf angle) and compass direction of the plane of the adaxial leaf surface (leaf azimuth) were quantified. Leaf angle for *I. imbricata* (*n* = 78) was measured from digital images using the Angle and Measurement tools in Image J 1.44p (National Institutes of Health, USA; http://imagej.nih.gov/ij). Leaf azimuth measurements were not made for *I. imbricata;* leaves were positioned in a whorl on the stem, i.e. facing all compass directions (random leaf azimuth), lacked a petiole attachment and consequently a pulvinus, and was therefore unable to change orientation through pulvinus twisting (Ehleringer and Forseth 1980; Koller 1990; Figure 2A). Leaf angle in *H. bonariensis* (*n* = 250) was measured in the field monthly to the nearest degree from horizontal using a Suunto Tandem clinometer (Suunto, Vantaa, Finland), and leaf azimuth was measured from North to the nearest degree using a compass. All leaves measured for gas exchange and leaf orientation properties were collected, placed in zip-lock bags, transported to the lab in an ice chest, and analyzed for leaf structure within three days of detachment.

2.4. Laboratory methods

Leaf area was measured for *I. imbricata* (*n* = 375) and *H. bonariensis* (*n* = 250) leaves using a ΔT leaf area meter (Delta-T Devices, Cambridge, UK), and leaf thickness was measured to the nearest 25 μm with electronic calipers. Leaves were dried at 65°C in a drying oven for at least 48 hours after which dry weight was measured, and LMA was computed as the ratio of dry mass to fresh unit leaf area (water saturated). To measure stomatal frequency, leaf impressions of abaxial and adaxial leaf surfaces for both species were created using a thin coat of clear nail polish (NYC 271A), and fresh leaf cross-sections were prepared to characterize internal anatomy. Images of leaf surface impressions and cross-sections were captured using an upright microscope (Zeiss

Axioplan; Carl Zeiss Microimaging, Inc., Thornwood, NY) at 10x magnification with a Hamamatsu C5810 digital camera attachment (Bridgewater, NJ). Stomata frequency (# mm^{-2}) was calculated by dividing the total number of stomata in the image by the area of the field of view. The length of palisade mesophyll and spongy mesophyll layers were measured to the nearest μm, and the percent of total leaf thickness of each layer were calculated.

2.5. Statistical analysis

To determine if there were leaf structural and/or physiological differences between *I. imbricata* and *H. bonariensis*, a multivariate analysis of variance (MANOVA) was performed with significance of $P \leq 0.05$ for leaf structure (leaf weight, leaf area, LMA, and stomatal frequency on both leaf surfaces), T_{leaf}, and photosynthetic gas exchange (*A, E*, and *g*) using PASW 17.0 statistics software (IBM Corporation, 2009). To determine which leaf variables best predicted T_{leaf}, *A*, *E*, and *g* (independently) in *H. bonariensis* and *I. imbricata*, multiple stepwise regressions were performed using JMP 8.0.2 statistics software (SAS Institute, Inc., 2008). For each analysis, the model with the lowest Akaike Information Criterion (AIC) was chosen as the best predictor for T_{leaf}, *A, E,* and *g* for each species (Fujikoshi 1985). Leaf orientation (azimuth and angle) for *I. imbricata* was not included in stepwise regressions because there was no seasonal variation in leaf angle and leaf azimuth was random (absence of petiole), and therefore would likely not respond plastically to abiotic variables.

3. Results

3.1. Leaf external morphology

Leaves of *I. imbricata* were significantly smaller, thicker, and lighter than leaves of *H. bonariensis* and also had significantly greater LMA throughout the entire growth season (Table 2). Leaves of *I. imbricata* had a mean ± SE leaf thickness of 1306 ± 14.4 µm, leaf area of 4.9 ± 0.08 cm^2, leaf weight of 0.08 ± 0.001 g, and LMA of 0.018 ± 0.0003 g cm^{-2} (Figure 3). *Hydrocotyle bonariensis* leaves had a mean leaf thickness of 583 ± 13.3 µm, leaf area of 17.5 ± 0.35 cm^2, leaf weight of 0.14 ± 0.003 g, and LMA of 0.008 ± 0.0001 g cm^{-2} (Figure 3). Both species had stomata on both abaxial and adaxial leaf surfaces. Although there were significantly more stomata per mm^2 on both abaxial and adaxial surfaces of *I. imbricata* (118 ± 1.6 mm^{-2} and 141 ± 1.6 mm^{-2}, respectively) compared to *H. bonariensis* (111 ± 1.8 mm^{-2} and 132 ± 2.1 mm^{-2}, respectively), there was no significant difference in the abaxial-to-adaxial stomata ratio (*Ab/Ad*) between species (0.85 ± 0.007 for *I. imbricata* and 0.86 ± 0.012 for *H. bonariensis*), indicating both species had approximately 15% more stomata on the adaxial leaf surface (nearly amphistomatous; Table 2).

3.2. Leaf orientation and internal anatomy

Mean ± SE seasonal leaf angle for *I. imbricata* was 40 ± 0.8°, with no variation during the growing season, and leaf azimuth did not differ from random and encompassed all compass directions from 0° to 360° (Figure 2A, 4). Cross-sections revealed isobilateral leaves with palisade mesophyll layers present beneath adaxial and abaxial leaf surfaces (epidermis) and a central layer of water storage cells (Figure 5A). Approximately 65% of the leaf (32% adaxial and

33% abaxial) was composed of palisade mesophyll and about 31% of the leaf was composed of the central layer of water cells.

Mean seasonal leaf angle for *H. bonariensis* was 70 ± 1.2°, and leaf angle increased over the growing season from a mean of 58 ± 22.2° in May to 87 ± 0.6° in September (Figure 4). Mean seasonal leaf azimuth was southeast at 161 ± 4.2°, and leaf azimuth decreased over the growth season from slightly southwest in May (200 ± 12.9°) and June (196 ± 14.2°) to southeast in July (152 ± 15.5°), August (120 ± 12.1°), and September (136 ± 13.6°). Cross-sections showed leaves with dorsiventral symmetry, i.e. multiple (three) adaxial palisade layers and abaxial spongy mesophyll (Figure 5B). Approximately 33% of the internal thickness of the leaf was composed of the three adaxial palisade layers and 51% was composed of abaxial spongy mesophyll.

3.3. Midday photosynthetic gas exchange

There was a significant difference between *I. imbricata* and *H. bonariensis* in leaf temperature (T_{leaf}), photosynthetic CO_2 exchange (*A*), transpiration (*E*), and leaf conductance *(g)* (Table 2). The mean ± SE T_{leaf} for *I. imbricata* (31 ± 0.19 °C) was significantly less than for *H. bonariensis* leaves (36± 0.19). In addition, mean ± SE *A, E,* and *g* for *I. imbricata* (12.5 ± 0.40 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 3.1 ± 0.1 $mmol \cdot m^{-2} \cdot s^{-1}$, and 175 ± 4.4 $mmol \cdot m^{-2} \cdot s^{-1}$, respectively) were significantly greater than *H. bonariensis* (3.3 ± 0.14 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 1.6 ± 0.04 $mmol \cdot m^{-2} \cdot s^{-1}$, and 71 ± 2.3 $mmol \cdot m^{-2} \cdot s^{-1}$, respectively). *Iva imbricata* maintained lower leaf temperatures relative to *H. bonariensis* over the entire growth season, as well as greater leaf photosynthetic gas exchange in all months measured except September (Figure 6).

3.4. Predictions based on leaf characteristics

For *I. imbricata*, LMA and stomatal frequency of both leaf surfaces were the best predictors of T_{leaf} and photosynthetic gas exchange, based on AIC values from multiple linear stepwise regressions (Table 3). Thirty percent of the variation in leaf temperature was explained by a combination of leaf area, leaf weight, LMA, adaxial stomatal frequency, and *Ab/Ad*. The best fit model for *g* was the combination of leaf area, leaf weight, LMA, and stomatal frequency of both leaf surfaces, explaining 19% of the variation. Both *E* and *A* were best predicted by a model including leaf area, leaf weight, LMA, and *Ab/Ad* and explained 26% and 20% of the variation, respectively.

For *H. bonariensis*, T_{leaf} was best predicted by leaf weight, leaf angle, and adaxial stomatal frequency, explaining 14% of the variation throughout the growth season (Table 3). Leaf orientation properties (leaf angle and leaf azimuth), leaf area, and abaxial stomatal frequency were the best predictors of photosynthetic gas exchange in *H. bonariensis*. Specifically, *g* was best predicted by a combination of LMA, leaf orientation, and abaxial stomatal frequency, *E* was best predicted by combination of leaf area, leaf orientation, and abaxial stomatal frequency, and *A* was best predicted by leaf area and leaf orientation. The models explained 5, 11, and 13% of the variation in *A, E,* and *g*, respectively.

4. Discussion

To verify leaf structure and orientation predictions of a conceptual Structure-Orientation (SO) model according to position along a small-scale stress gradient (Table 1; Smith et al. 1997, 1998), as well as the use of measured leaf characteristics in predicting leaf photosynthesis, the leaf structure, orientation, and photosynthetic gas exchange of two barrier island sand dune species occupying different spatial positions along a 200-m transect were measured. It was hypothesized that the species located at the lower end of the gradient near the tidal line (i.e. *I. imbricata*) would have leaf structure and orientation similar to predictions from the SO model for high incident light and higher stress habitats while the species at the upper end of the gradient (i.e. *H. bonariensis*) would have characteristics predicted for lower stress species. In addition, it was expected that these leaf characteristics would be related to and predictive of leaf-level temperatures, stomatal conductance, transpiration, and photosynthesis.

4.1. Verification of the Structure-Orientation conceptual model

The results of the present study appeared to support the use of the SO model for predicting leaf structure and leaf orientation along a small-scale stress gradient (Table 1). The species at the lower end of the gradient (i.e. relatively higher stress), *I. imbricata*, had small leaves with upper and lower palisade mesophyll layers (isobilateral), as predicted for leaves of high sun exposure and higher stress habitats. In contrast, leaves of the species at the upper end of the gradient (i.e. relatively lower stress), *H. bonariensis*, were large (17.5 ± 0.35 cm^2 vs. 4.9 ± 0.08 cm^2 in *I. imbricata*) and laminar with three layers of adaxial palisade mesophyll and an adaxial spongy mesophyll layer (dorsiventral symmetry), corresponding to predictions for leaves adapted to high sunlight exposure, but a lower stress habitat (Smith et al. 1997, 1998). In addition, *I. imbricata* had relatively greater midday photosynthesis (12.5 ± 0.40 $\mu mol \cdot m^{-2} \cdot s^{-1}$) compared to *H. bonariensis* (3.3 ± 0.14 $\mu mol \cdot m^{-2} \cdot s^{-1}$), as predicted by the SO model.

Both species had inclined leaf orientation, as predicted for leaves in high incident sunlight and high stress habitats. Mean leaf angle in *I. imbricata* remained at 40 ± 0.8° whereas *H. bonariensis* leaf angle became more inclined over the growing season, ranging from 58 ± 22.2° in May to 87 ± 0.6° in September. The inclined leaf angle found in both species is likely an adaptation to the high incident sunlight of the sand dune habitat, which varies little along such a small-scale abiotic stress gradient. In addition, there is no overstory in most barrier island vegetation zones (with exception of shrub line and maritime forest), and the sandy substrate of the study site reflects up to 34% of full sunlight. Therefore, species are likely exposed to the risks associated with absorbing excess incident sunlight, such as increased leaf temperatures, photoinhibition, and oxidative photodamage. A more inclined leaf orientation has been found to reduce incident sunlight at midday while increasing morning and evening exposure, thus reducing the risks of high incident sunlight, in *Rumex densiflorus* in southeast Wyoming (Geller and Smith 1982), the prairie forb *Silphium terebinthinaceum* in Illinois (Smith and Ullberg 1989), and *Eucalyptus globulus* in Australia (James and Bell 2000).

Both species also had stomata present on both abaxial and adaxial leaf surfaces, with approximately 15% more stomata on the adaxial surface compared to the abaxial leaf surface (nearly amphistomatous), as predicted by the SO model for species in high incident sunlight habitats. The presence of a significant number of stomata on adaxial and abaxial leaf surfaces increases the CO_2 supply rate by decreasing diffusion distance**,** especially in thick leave**s** (Mott and Michaelson 1991; Parkhurst 1994; Smith et al. 1997, 1998). Furthermore, the occurrence of amphistomatous leaves in high light habitats has been proposed to provide a high internal CO_2 environment that interacts with the increased sunlight absorption by leaves in these habitats, thus maximizing leaf photosynthesis (Smith et al. 1997, 1998; Smith and Hughes 2009).

4.2. Predicting photosynthetic gas exchange from leaf traits

The results of the present study suggest that leaf size (i.e. leaf area, leaf weight, and LMA) and stomatal frequency of both leaf surfaces were the best predictors of leaf temperature and photosynthetic gas exchange in *I. imbricata*, explaining between 19 to 30% of the variation. The reduced leaf size likely functions to maintain optimum leaf temperatures and high photosynthetic gas exchange throughout most of the growth season (the decrease in photosynthetic gas exchange in September was likely due to preparation for October leaf senescence). A smaller broadleaf dimension has been shown to increase convective heat exchange and thus the vapor pressure difference between the leaf and the atmosphere, resulting in a thinner leaf boundary layer, lower leaf temperatures, and reduced transpiration rates in high incident sunlight and relatively high abiotic stress habitats, such as in perennial desert plants of the Sonoran Desert (Smith 1978) and desert sand dune species in Egypt (Hegazy and El Amry 1998).

However, *I. imbricata* leaves had relatively high midday transpiration rates, suggesting that evaporative cooling via transpiration also plays a critical role in maintaining leaf temperatures and photosynthetic gas exchange in the sand dune habitat. The presence of stomata on both leaf surfaces would increase transpiration (through same manner as increased CO_2 supply rate), but to maintain high transpiration rates as well as high photosynthetic rates, there must be a relatively steady source of freshwater available to *I. imbricata* on barrier island sand dune habitats. Hancock (2009) measured xylem water potentials for several sand dune species during the growth season and found that *I. imbricata* xylem water potentials remained above -0.6 mPa during the growth season, further suggesting that there is a relatively constant water source available for rapid transpiration. In addition, the central layer of water storage cells in the leaves may function as an additional water supply for photosynthetic reactions and evaporative cooling via transpiration when freshwater sources become scarce (von Willert et al. 1990; Egbert et al. 2008).

Leaf area, leaf orientation properties (leaf angle and azimuth), and stomatal frequency of both leaf surfaces were the best predictors of T_{leaf} and photosynthetic gas exchange in *H. bonariensis*, explaining 5 – 14% of the variation. A more inclined leaf has been shown to effectively avoid excessive leaf temperatures and transpiration, as well as the risk of photoinhibition and photodamage, and to maximize photosynthesis per unit leaf area and leaf mass (Geller and Smith 1982, King 1997, Smith et al. 1997, James and Bell 2000). Also, a previous study showed that the leaf orientation properties in *H. bonariensis* maximize light capture in the morning and evening while avoiding high sunlight exposure during midday (Joesting et al. 2011). Therefore, it would be expected that leaf photosynthetic gas exchange would be relatively high during morning and evening hours and lower during midday when incident sunlight is minimized by the inclined leaf orientation. The present study found that midday leaf photosynthesis in *H. bonariensis* was relatively low compared to *I. imbricata* (seasonal mean of 3.3 ± 0.14 μmol m^{-2} s^{-1}), supporting this hypothesis. However, it should be noted that gas exchange was measured once a day during midday (1000 h to 1600 h) and likely do not reflect maximum rates that may be achieved by *H. bonariensis* during morning and/or evening hours.

4.4. Conclusion

The results of the present study provide support for the use of the SO model to predict leaf structure and orientation for species occurring along a small-scale stress gradient (Smith et al. 1997, 1998). Specifically, the present study showed that leaf characteristics measured for *I. imbricata* better fit the higher-stress microhabitat as predicted by the SO model whereas *H. bonariensis* leaf characteristics fit the lower-stress situation. Furthermore, the leaf characteristics measured were significant predictors of leaf photosynthetic gas exchange, explaining 5 – 30% of the variation in photosynthetic gas exchange over the growing season. Thus, these results support the inclusion of measurements stomatal frequency, internal anatomy, and leaf orientation in leaf form and function analyses to provide more accurate predictions of leaf photosynthesis, especially for species occurring along small-scale abiotic gradients.

Literature cited

Barbour, M. 1978. Salt spray as a microenvironmental factor in the distribution of beach plants at Point Reyes, California. Oecologia 32: 213 - 224.

Boyce, S. 1954. The salt spray community. Ecological Monographs 24: 29 - 67.

Castro-Diez, P., J.P. Puyravaud, and J.H.C. Cornelissen. 2000. Leaf structure and anatomy as related to leaf mass per area variation in seedlings of a wide range of woody plant species and types. Oecologia 124: 476 – 486.

Colosi, J., and J.F. McCormick. 1978. Population structure of *Iva imbricata* in five coastal dune habitats. Bulletin of the Torrey Botanical Club 105: 175 – 186.

Egbert, K. J., C.E. Martin, and T.C. Vogelmann. 2008. The influence of epidermal windows on the light environment within the leaves of six succulents. Journal of Experimental Botany 59: 1863 - 1873.

Ehleringer, J. and I. Forseth. 1980. Solar tracking by plants. Science 210: 1094 – 1098.

Ehrenfeld, J.G. 1990. Dynamics and processes of barrier island vegetation. Aquatic Sciences 2: 437 – 480.

Enríquez, S., C.M. Duarte, K. Sand-Jensen, and S.L. Nielsen. 1996. Broad-scale comparison of photosynthetic rates across phototropic organisms. Oecologia 108: 197 – 206.

Evans, J.P. 1988. Nitrogen translocation in a clonal dune perennial, *Hydrocotyle bonariensis*. Oecologia 77: 64 – 68.

Evans, J.P. 1992a. The effect of local resource availability and clonal integration on ramet functional morphology in *Hydrocotyle bonariensis*. Oecologia 89: 265 – 276.

Evans, J.P. 1992b. Seedling establishment and genet recruitment in a population of a clonal dune perennial, *Hydrocotyle bonariensis*. *In* C.A. Cole and K. Turner [eds.], Barrier Island Ecology of the Mid-Atlantic Coast: A Symposium, 75 – 84. U.S. Department of Interior, National Park Service, Southeast Regional Office, Atlanta, Georgia, USA.

Fujikoshi, Y. 1985. Selection of variables in 2-group discriminant-analysis by error rate and Akaike Information. Journal of Multivariate Analysis 17: 27 – 37.

Garnier, E. and G. Laurent. 1994. Leaf anatomy, specific mass and water content in congeneric annual and perennial grass species. New Phytologist 128: 725 – 736.

Gates, D.M., R. Alderfer, and E. Taylor. 1968. Leaf temperatures of desert plants. Science 159: 994 – 995.

Geller, G.N. and W.K. Smith. 1982. Influence of leaf size, orientation, and arrangement on temperature and transpiration in three high-elevation, large-leafed herbs. Oecologia 53: 227 – 234.

Hancock, T. 2009. Ecophysiology of barrier island beach plants: responses in form and function to daily, seasonal and abiotic stresses. Ph.D. dissertation, Wake Forest University, Winston Salem, North Carolina, USA.

Hegazy, A.K. and M.I. El Amry. 1998. Leaf temperature of desert sand dune plants: perspectives on the adaptability of leaf morphology. African Journal of Ecology 38: 34 – 43.

Hesp, P.A. 1991. Ecological processes and plant adaptation on coastal dunes. Journal of Arid Environments 21: 165 – 191.

James, S.A. and D.T. Bell. 2000. Leaf orientation, light interception and stomatal conductance of *Eucalyptus globulus* ssp. *globulus* leaves. Tree Physiology 20: 815 – 823.

Joesting, H.M., M.O. Sprague, and W.K. Smith. 2011. Seasonal and diurnal leaf orientation, bifacial sunlight incidence, and leaf form and function in the sand dune herb *Hydrocotyle bonariensis*. Environmental and Experimental Botany, *in press*.

King, D.A. 1997. The functional significance of leaf angle in *Eucalyptus*. Australian Journal of Botany 45: 619 – 639.

Koller, D. 1990. Light-driven leaf movements. Plant, Cell, and Environment 13: 615 – 632.

Mott, K.A. and O. Michaelson. 1991. Amphistomy as an adaptation to high light intensity in *Ambrosia cordifolia* (Compositae). American Journal of Botany 78: 76 – 79.

Niinemets, Ü. 1999. Research review: Components of leaf dry mass per area – thickness and density – alter leaf photosynthetic capacity in reverse directions in woody plants. New Phytologist 144: 35 – 47.

Oosting, J. and W.D. Billings. 1942. Factors effecting vegetational zonation on coastal dunes. Ecology 23: 131 - 142.

Oosting, J. 1945. Tolerance to salt spray of plants of coastal dunes. Ecology 26: 85 – 89.

Parkhurst, D. 1994. Tansley Review No. 65: Diffusion of CO_2 and other gases inside leaves. New Phytologist 126: 449 – 479.

Poorter, H., Ü. Niinemets, L. Poorter, I.J. Wright, and R. Villar. 2009. Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. New Phytologist 182: 565 – 588.

Reich, P.B., M.B. Walters, and D.S. Ellsworth. 1997. From tropics to tundra: Global convergence in plant functioning. Proceedings of the National Academy of Science USA 94: 13730 – 13734.

Reich, P.B., D.S. Ellsworth, and M.B. Walters. 1998. Leaf structure (specific leaf area) modulates photosynthesis-nitrogen relations: evidence from within and across species and functional groups. Functional Ecology 12: 948 – 958.

Reich, P.B., I.J. Wright, and C.H. Lusk. 2007. Predicting leaf physiology from simple plant and climate attributes: a global GLOPNET analysis. Ecological Applications 17: 1982 – 1988.

Smith, M. and D. Ullberg. 1989. Effect of leaf angle and orientation on photosynthesis and water relations in *Silphium terebinthinaceum*. American Journal of Botany 76: 1714 – 1719.

Smith, W.K. 1978. Temperature of desert plants: another perspective on the adaptability of leaf size. Science 201: 614 – 616.

Smith, W.K., T.C. Vogelmann, E.H. DeLucia, D.T. Bell, and K.A. Shepherd. 1997. Leaf form and photosynthesis: Do leaf structure and orientation interact to regulate internal light and carbon dioxide? Bioscience 47: 785 – 793.

Smith, W.K., D.T. Bell, and K.A. Shepherd. 1998. Associations between leaf structure, orientation, and sunlight exposure in five Western Australian communities. American Journal of Botany 85: 56 – 63.

Smith, W.K., and Hughes, N.M. 2009. Progress in coupling plant form and photosynthetic function. Castanea 74: 1 – 26.

Snyder, R.A. and C.L. Boss. 2002. Recovery and stability in barrier island plant communities. Journal of Coastal Research 18: 530 - 536.

Stallins, J.A. 2002. Dune plant species diversity and function in two barrier island biogeomorphic systems. Plant Ecology 168: 183 – 196.

Witkowski, E.T.F. and B.B. Lamont. 1991. Leaf specific mass confounds leaf density and thickness. Oecologia 88: 486 – 493.

Wright, I.J., P.B. Reich, J.H.C. Cornelissen, D.S. Falster, E. Garnier, K. Hikosaka, B.B. Lamont, W. Lee, J. Oleksyn, N. Osada, H. Poorter, R. Villar, D.I. Warton, and M. Westoby. 2005a. Assessing the generality of global leaf trait relationships. New Phytologist 166: 485 – 496.

Wright, I.J., P.B. Reich, J.H.C. Cornelissen, D.S. Falster, P.K. Groom, K. Hikosaka, W. Lee, C.H. Lusk, Ü. Niinemets, J. Oleksyn, N. Osada, H. Poorter, D.I. Warton, and M. Westoby. 2005b. Modulation of leaf economic traits and trait relationships by climate. Global Ecology and Biogeography 14: 411 – 421.

von Willert, D.J., B.M. Eller, M.J.A. Werger, and E. Brinckmann. 1990. Desert succulents and their life strategies. Vegetatio 90: 133 – 143

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Table V – 1: Predicted leaf orientation, leaf external morphology, leaf internal anatomy, leaf mass per area (LMA), and leaf photosynthetic potential for species occupying high incident light level habitats of either lower or higher abiotic stress, and results from the present study indicating which species best fits each characteristic stress level. Based on Smith et al. (1998) Structure-Orientation model and Poorter et al. (2009) review of variation in leaf mass per area (LMA).

	High incident sunlight			
	Lower stress		**Higher stress**	
	Predicted	**Species**	**Predicted**	**Species**
Orientation (Leaf angle)	Horizontal / tracker		Inclined / avoider	*H. bonariensis* (58° to 87°) *I. imbricata* (40°)
Thickness (µm)	> 600	*I. imbricata* (1300)	400 – 600	*H. bonariensis* (564)
Symmetry	Dorsiventral	*H. bonariensis*	Isobilateral	*I. imbricata*
Morphology	Large laminar broadleaf	*H. bonariensis* (17.5 cm^2)	Small and cylindrical	*I. imbricata* (4.9 cm^2)
Stomata distribution	Hypo- or amphistomatous	*I. imbricata* and *H. bonariensis*	Amphistomatous	*I. imbricata* and *H. bonariensis*
Anatomy	Multiple adaxial palisade possible	*H. bonariensis* (three layers)	Adaxial and abaxial palisade	*I. imbricata* (upper and lower)
LMA*	Lower	*H. bonariensis* (0.008 g·cm^{-2})	Higher	*I. imbricata* (0.018 g·cm^{-2})
Photosynthetic potential*	Lower	*H. bonariensis* (3.3 µmol·m^{-2}·s^{-1})	Higher	*I. imbricata* (12.5 µmol·m^{-2}·s^{-1})

*Two species relative to each other

Table V – 2: Mean square, *F*-ratio, and *P*-value from multivariate analysis of variance (MANOVA) comparing leaf structure, leaf temperature (T_{leaf}), and leaf photosynthetic gas exchange [stomatal conductance (*g*), transpiration (*E*), and photosynthesis (*A*)] between *Iva imbricata* and *Hydrocotyle bonariensis* (all *df* = 1).

Variable	**Mean Square**	***F*-ratio**	***P*-value**
Leaf weight (g)	0.391	303.056	< 0.001
Leaf area (cm^2)	22973.572	1615.191	< 0.001
Leaf mass per area (g cm^{-2})	0.016	1092.344	< 0.001
Abaxial stomatal frequency (mm^{-2})	0.007	8.914	0.003
Adaxial stomatal frequency (mm^{-2})	0.013	12.053	< 0.001
Abaxial-to-adaxial stomatal ratio	0.031	1.174	0.279
T_{leaf} (°C)	3746.114	297.628	< 0.001
g (mmol m^{-2} s^{-1})	259.071	204.722	< 0.001
E (mmol m^{-2} s^{-1})	1392802.496	314.047	< 0.001
A (µmol m^{-2} s^{-1})	10162.214	309.305	< 0.001

Table V – 3: Best fit model for prediction of leaf-level photosynthetic gas exchange [photosynthesis (*A*; µmol·m^{-2}·s^{-1}), transpiration (*E*; mmol·m^{-2}·s^{-1}), and stomata conductance (*g*; mmol·m^{-2}·s^{-1})] from leaf morphological characteristics [leaf weight (g), leaf area (cm^{2}), leaf mass per area (*LMA*; g·cm^{-2}), adaxial and abaxial stomatal frequency (mm^{-2}), and ratio of abaxial-to-adaxial stomatal frequency (*Ab/Ad*)], and leaf orientation (leaf angle and leaf azimuth; *H. bonariensis* only) for *Iva imbricata* and *Hydrocotyle bonariensis* based Akaike Information Criterion (AIC) from Linear Stepwise Regressions.

Response	**Best Fit Model**	**AIC**	**r^2**
Iva imbricata			
T_{leaf}	Leaf area x leaf weight x LMA x adaxial stomatal frequency x *Ab/Ad*	1727.86	0.30
g	Leaf area x leaf weight x LMA x abaxial stomatal frequency x adaxial stomatal frequency	3829.86	0.19
E	Leaf area x leaf weight x LMA x *Ab/Ad*	1095.94	0.26
A	Leaf area x leaf weight x LMA x *Ab/Ad*	2211.18	0.20
Hydrocotyle bonariensis			
T_{leaf}	Leaf weight x angle x adaxial stomatal frequency	1231.91	0.14
g	LMA x angle x azimuth x abaxial stomatal frequency	2400.43	0.13
E	Leaf area x angle x azimuth x abaxial stomatal frequency	372.377	0.11
A	Leaf area x angle x azimuth	1045.75	0.05

Figure captions

Figure V – 1: (A) Schematic illustrating characteristic vegetation zones present at study site. (B) Diagram showing location of *Iva imbricata* and *Hydrocotyle bonariensis* individuals used for present study. *Iva imbricata* was present from 20 – 90-m, 110 – 140-m, 160 – 170-m, and 190-m (15 flags), and *H. bonariensis* was present from 160 – 200-m (5 flags).

Figure V – 2: (A) *Iva imbricata* shoot showing typical whorled leaf arrangement, random leaf azimuths, and leaf angles. (B) Individual *Hydrocotyle bonariensis* ramet (leaf) with near vertical leaf inclination.

Figure V – 3: Mean (A) leaf dry weight (g), (B) leaf area (cm^2), and (C) leaf mass per area (*LMA*; $g{\cdot}cm^{-2}$) for *Iva imbricata* (black bars) and *Hydrocotyle bonariensis* (grey bars) during May, June, July, August, September, and the 2008 growth season. Error bars represent Standard Error.

Figure V – 4: Mean leaf angle (°) for *Iva imbricata* (black bars) and *Hydrocotyle bonariensis* (gray bars) for the 2008 growth season. Mean leaf angle for *H. bonariensis* also shown for May, June, July, August, and September 2008 to illustrate seasonal variation in leaf angle. Error bars represent Standard Error.

Figure V – 5: (A) Cross-section of *I. imbricata* leaf, showing adaxial and abaxial palisade layers and central layer of water cells. (B) Cross-section of *H. bonariensis* leaf, showing multiple (three) adaxial palisade layers and spongy mesophyll layer (photograph by M.O. Sprague). Scale bar represents 5 µm.

Figure V – 6: Mean (A) leaf temperature (T_{leaf}; °C), (B) leaf conductance (*g*; mmol·m^{-2}·s^{-1}), (C) transpiration (*E*; mmol·m^{-2}·s^{-1}), and (D) photosynthesis (*A*; µmol·m^{-2}·s^{-1}) for *Iva imbricata* (solid bar) and *Hydrocotyle bonariensis* (gray bar) during May, June, July, August, September, and the 2008 growth season. Error bars represent Standard Error.

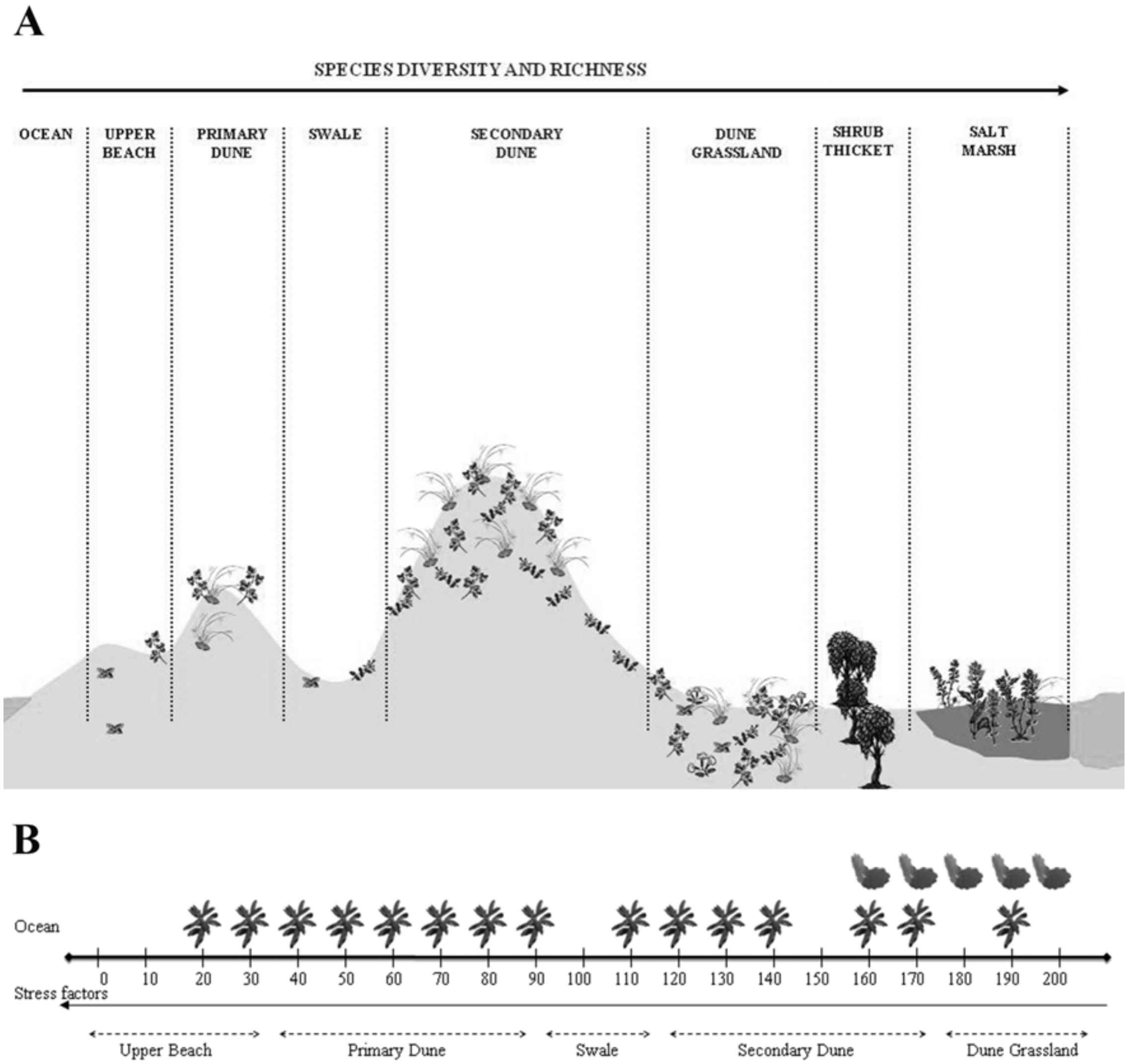

A
SPECIES DIVERSITY AND RICHNESS
OCEAN
UPPER BEACH
PRIMARY DUNE
SWALE
SECONDARY DUNE
DUNE GRASSLAND
SHRUB THICKET
SALT MARSH
B
Ocean
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
Stress factors
Upper Beach
Primary Dune
Swale
Secondary Dune
Dune Grassland

Figure V – 1

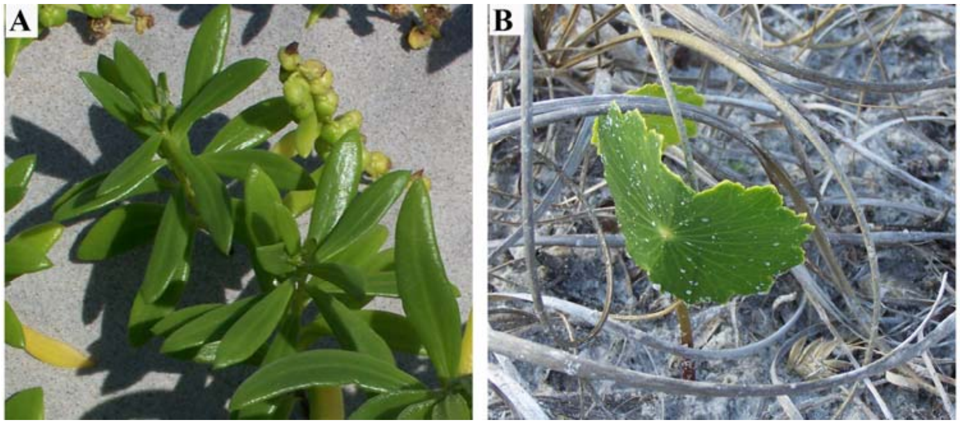


Figure V – 2

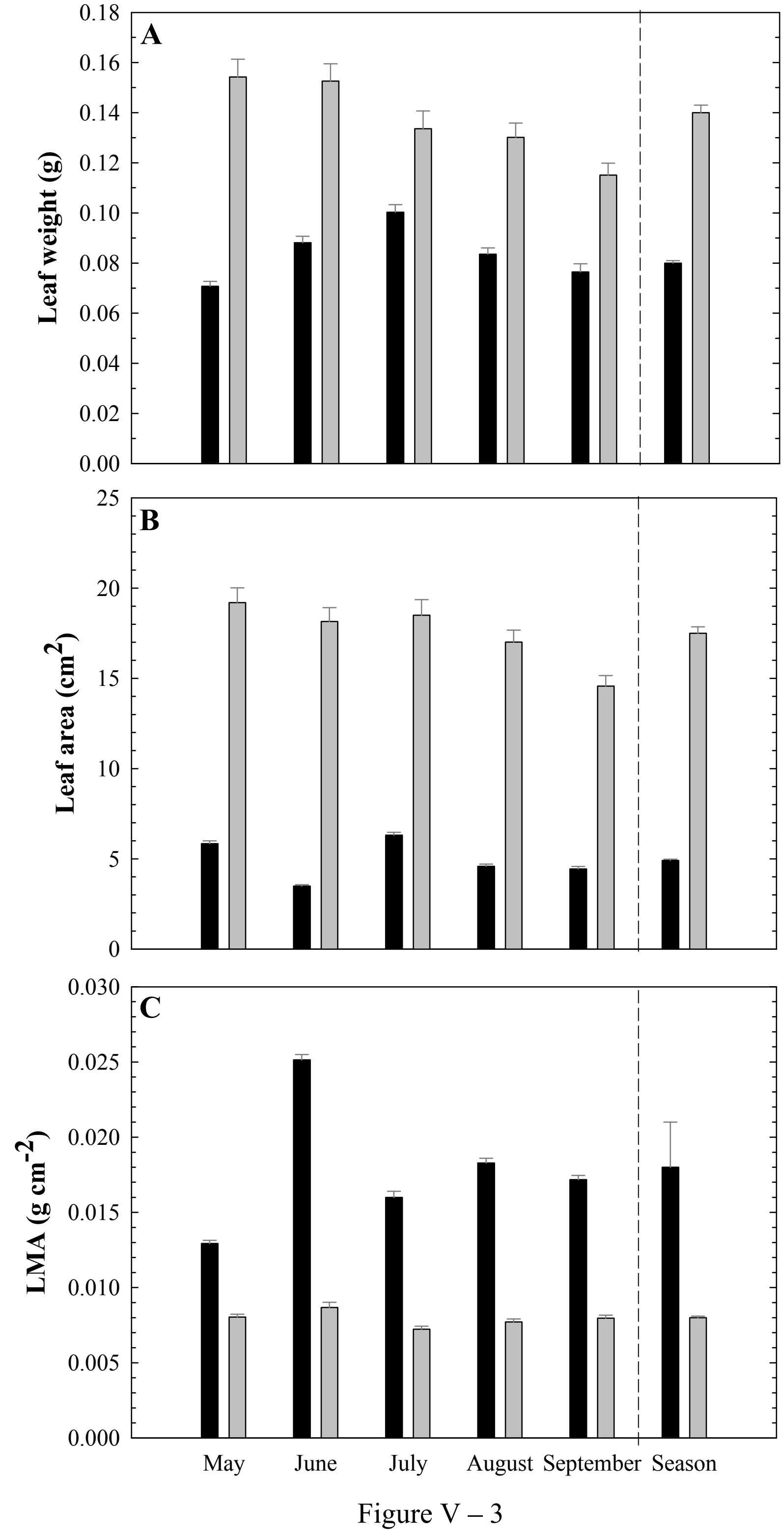
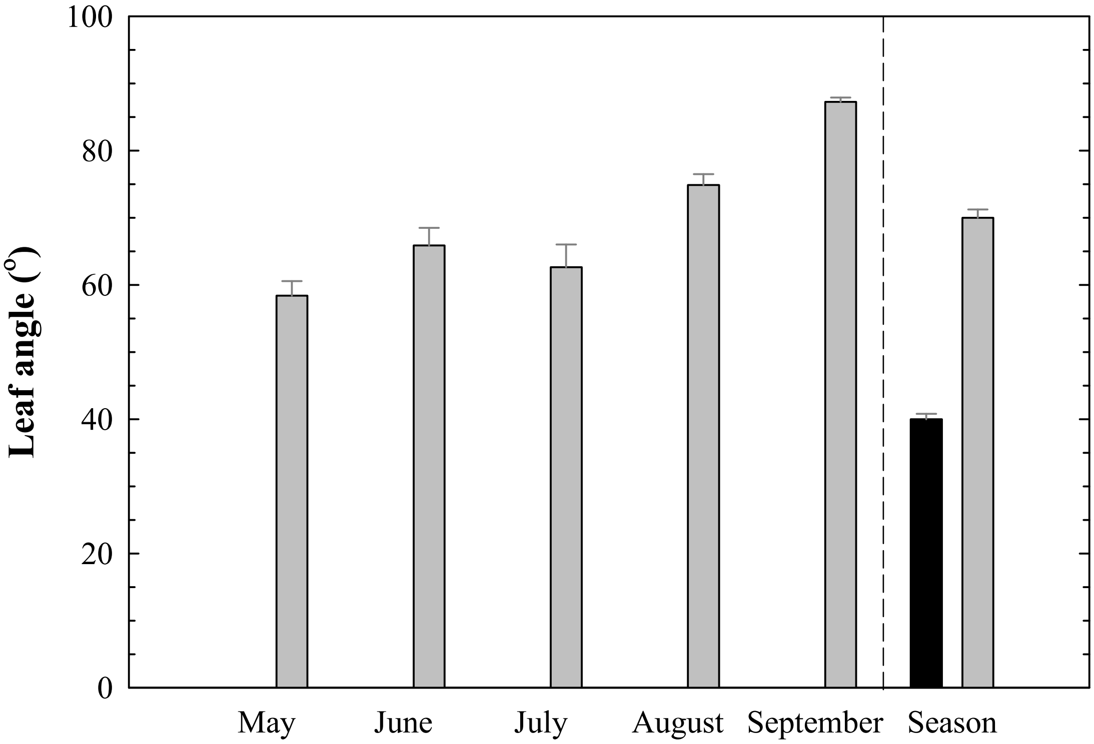

A
Leaf weight (g)
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0.16
0.18
B
Leaf area (cm^2)
0
5
10
15
20
25
C
LMA ($g\ cm^{-2}$)
0.000
0.005
0.010
0.015
0.020
0.025
0.030
May
June
July
August
September
Season

Figure V – 3



Leaf angle (°)
100
80
60
40
20
0
May
June
July
August
September
Season

Figure V – 4

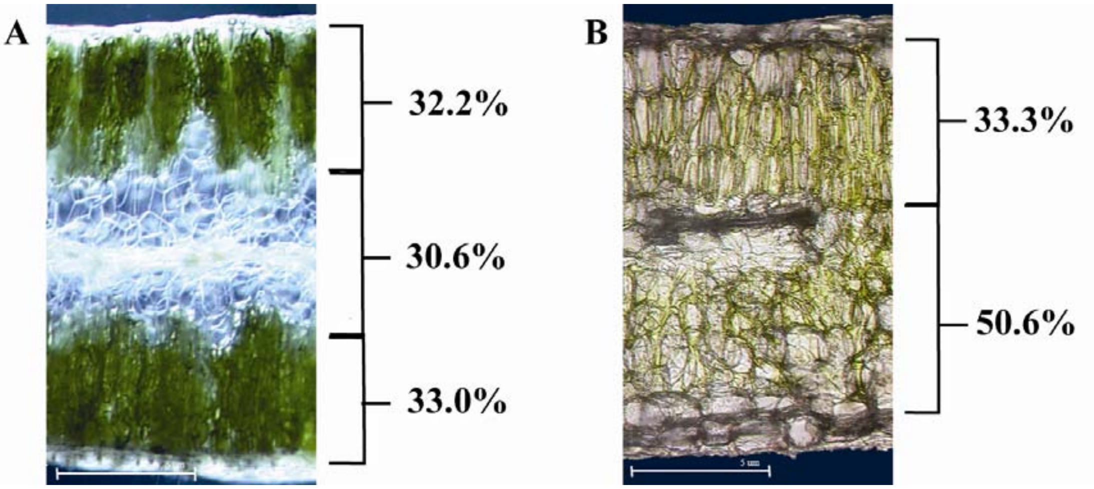


Figure V – 5

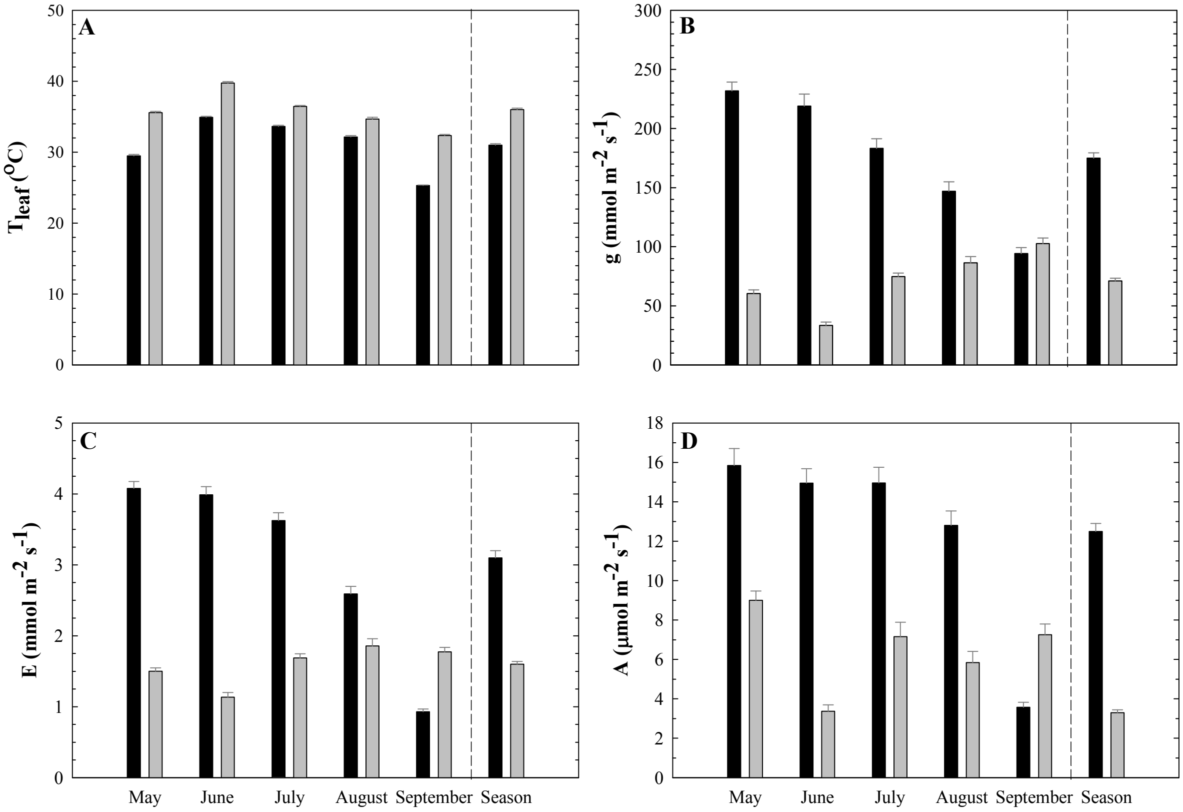

A
B
C
D
Tleaf (°C)
g (mmol m-2 s-1)
E (mmol m-2 s-1)
A (µmol m-2 s-1)
0
10
20
30
40
50
0
50
100
150
200
250
300
0
1
2
3
4
5
0
2
4
6
8
10
12
14
16
18
May
June
July
August
September
Season

Figure V – 6

CHAPTER VI

INFLUENCE OF LEAF STRUCTURE, LEAF ORIENTATION, AND ABIOTIC ENVIRONMENT ON LEAF PHYSIOLOGY IN TWO BARRIER ISLAND SAND DUNE SPECIES WITH DIFFERENT GROWTH HABITS

Abstract

It has been long recognized that leaf structure and orientation have a significant influence on diurnal photosynthetic gas exchange through manipulations in the leaf-level microclimate. The barrier island sand dune system can be characterized as a high incident sunlight habitat with a host of additional abiotic stress, suggesting that native species possess leaf adaptations to enhance leaf photosynthetic gas exchange and diurnal carbon gain. This hypothesis was tested by comparing leaf temperatures, leaf photosynthetic gas exchange, and photosystem II efficiency (proxy for photoinhibition) between two common sand dune species with significantly different leaf structure and orientation for single days in June, July, and August 2009 on a North Carolina barrier island. Leaf physiology differed significantly between large inclined leaves of the clonal herb *Hydrocotyle bonariensis* and small succulent leaves of the shrub *Iva imbricata*. Multiple step-wise regressions revealed significant influence of diurnal air temperature, vapor pressure deficit, and incident sunlight on leaf physiology, and there were significant variations diurnally and between months in incident sunlight on adaxial and abaxial leaf surfaces, leaf temperature, photosynthetic gas exchange, and occurrence of photoinhibition for both species. Seasonal and diurnal patterns suggested that leaf orientation properties and rapid transpiration are critical in reducing photoinhibition in *H. bonariensis* while reduced leaf size and presence of water-storage cells in *I. imbricata* alleviates photoinhibition and facilitates leaf photosynthesis. These results provide further evidence of the importance of leaf structure and orientation in regulating leaf-level microclimate and enhancing leaf photosynthesis for species in high light environments.

Keywords: abiotic environment; *Hydrocotyle bonariensis*; incident sunlight; *Iva imbricata*; leaf structure; photoinhibition; photosynthetic gas exchange; sand dune

1. Introduction

Plant species are often exposed to more solar energy then can be used in photochemistry, and this surplus of energy can result in a reversible reduction in daily photosynthesis (i.e. photoinhibition) and/or irreversible damage to photosynthetic machinery. In many habitats (e.g. deserts, alpine tree-line, and coastal sand dunes), high incident sunlight is combined with additional abiotic stress factors (e.g. high air temperature and water stress), enhancing risks of photoinhibition and photodamage (Powles 1984; Araus and Hogan 1994; Xu et al. 2007). Thus, native plant species of high light and high abiotic stress habitats possess a variety of leaf adaptations to reduce the occurrence of photoinhibition and photodamage and maximize diurnal leaf photosynthesis, including adjustments in biochemistry and photochemistry (e.g. increased xanthophyll cycle activity and presence of anthocyanins), morphology and anatomy (e.g. decreased leaf size and pubescence), and orientation properties (e.g. inclined leaf angle) (Powles 1984; Smith et al. 1997, 1998; Liu et al. 2003; Xu et al. 2007; Smith and Hughes 2009).

It has been accepted for some time that leaf structure and orientation can have a strong association with daily leaf photosynthesis. Strong significant relationships have been described between the abiotic environment, leaf structure and orientation, and leaf photosynthesis for 242 species from Australia, the US southwest desert, and the Rocky Mountains (Smith et al. 1997, 1998). These relationships suggest that there is a corresponding increase in the presence of native species with small, thick, inclined leaves with increased incident sunlight and abiotic stress of the habitat. Inclined leaf orientation has been shown to effectively reduce leaf temperatures, transpiration, and photoinhibition while maximizing photosynthesis per unit leaf area and leaf mass in high light environments (Geller and Smith 1982; Werk and Ehleringer 1984; James and Bell 2000; Liu et al. 2003). Similarly, reduced leaf size has been shown to lower leaf temperatures and reduced transpiration rates, thus facilitating leaf photosynthesis (Smith 1978; Hegazy and El Amry 1998).

The barrier island sand dune system is characterized by high incident sunlight, growth season air temperatures, and vapor pressure deficit, in addition to unique abiotic stress factors such as salt spray, reflective substrate, periodic salt water inundation, sand accretion/erosion, and blowing sand (Ehrenfeld 1990; Hesp 1991). Thus, native sand dune species likely possess leaf adaptations that maximize leaf photosynthesis and reduce photoinhibition. The aim of the present study was to investigate the influence of leaf structure, leaf orientation, and the abiotic environment on leaf physiology for two common sand dune species with contrasting growth habits. *Hydrocotyle bonariensis* is a single large-leafed clonal perennial shown to vary leaf orientation during the growth season while *Iva imbricata* is perennial semi-woody shrub with small succulent leaves. Leaf temperatures, maximum quantum yield of photosystem II (proxy for photoinhibition), and leaf photosynthetic gas exchange were compared between species and related to measurements of the abiotic environment and leaf structure. Specifically, it was hypothesized that (1) leaf temperature, photosystem II efficiency, and leaf photosynthetic gas exchange would significantly differ between species based on differences in leaf structure and orientation, (2) there would be significant seasonal and diurnal variation in leaf temperature, photosystem II efficiency, and leaf photosynthetic gas exchange for both species, and (3) diurnal leaf physiology would be significantly related to measurements of the abiotic environment and leaf structure in both species.

2. Methods and materials

2.1. Study species

Hydrocotyle bonariensis Comm ex. Lam (Apiaceae) is a clonal, perennial C_3 herb common to the swale, secondary dunes, and tertiary dune grassland of southeastern United States barrier islands. Clones spread via underground rhizomes and produce alternating single, circular broadleaf ramets with central petiole attachment, reaching up to 0.5-m in height. Leaf orientation in *H. bonariensis* has been shown to become more inclined over the growing season to avoid midday sunlight incidence, while simultaneously changing leaf azimuth to seasonally track the increasingly southern sun azimuth (Joesting et al. 2011). On Topsail Island, the growth season begins in March with leaf (ramet) production, rhizomatous growth, and seedling emergence. Small white flowers and nutlet-like fruits are produced in compound umbels throughout the growing season, and leaf senescence begins in December (Hancock 2009). Extremely low seedling survival rates (less than 1%) have been reported in the field; thus, recruitment is primarily due to clonal vegetative growth (Evans 1992).

Iva imbricata Walter (Asteraceae) is a perennial, semi-woody C_3 shrub common in barrier island sand dune habitats from the upper beach zone to the shrub line. Sessile leaves with a thick cuticle and central layer of water-storage cells (i.e. succulent) occur alternately on stems and individual shrubs with multiple branches can reach up to 1-m in height. On Topsail Island, seedlings and vegetative sprouts emerge in early spring (March/April), flowers develop in July and August, and berries occur from late September through November. Leaf senescence initiates in October, and winter dormancy begins in December (Hancock 2009). Recruitment is primarily by seedlings, with seedling survival rates between 70 and 100% reported along the upper beach and the swale. However, vegetative reproduction by means of rooting, decumbent stems is prevalent in primary dunes due to unfavorable germination conditions (Colosi and McCormick 1978).

2.2. Study site

The present study was conducted during the 2009 growth season on the southern end of Topsail Island, North Carolina, United States (34°20'N, 77°39'W). The southern end of the island is an elongated spit with an undeveloped and undisturbed dune ridge and swale system. The characteristic vegetation zones of barrier islands (i.e. berm or upper beach, primary dunes, swale, secondary dunes, tertiary dune grassland, shrub thicket or maritime forest, and salt marsh) are present (Ehrenfeld 1990). Mean air temperatures ranged from 5.8°C in January to 25.9°C in August, and total annual precipitation was 1925 mm for 2009 (data from National Climate and Data Center, http://www.ncdc.noaa.gov/oa/ncdc.html; station at Surf City, North Carolina, 11-km from study site).

A 200-m transect was established from the first embryo dune on the upper beach inland through the primary dunes, swale, secondary dunes, and tertiary dune grassland. Six ramet groups (clones) of *H. bonariensis* and five individual shrubs of *I. imbricata* were randomly chosen along the transect to represent the extent of each species distribution. Clones of *H. bonariensis* were located in the last 60-m of the transect (i.e. swale, secondary dune, and tertiary dune grassland) and *I. imbricata* shrubs were distributed along the entire transect. All leaf-level measurements (i.e. structure and orientation, sunlight incidence, photosynthetic gas exchange, and photosystem II efficiency) were conducted on the same *H. bonariensis* clones and *I. imbricata* individual shrubs.

2.3. Characterization of abiotic environment

Air temperature (T_{air}), relative humidity (RH), and photosynthetically active radiation (PAR; 400 – 700 nm) were measured on single days in June, July, and August 2009 in the primary dune, swale, secondary dune, and tertiary dune grassland. Measurements were taken monthly at 15-min intervals from 0700 h to 2100 h (ca. sunrise to sunset). T_{air} and RH were measured 0.5-m from the ground with Hobo Pro Series RH/Temp data loggers (Onset Computer Corporation, Pocasset, MA) attached to Hobo rain shields. Vapor pressure deficit (VPD) is a measure of how close air is to saturation; zero indicates completely saturated air and increases from zero progressively drier air. VPD was calculated as the difference between saturation vapor pressure (VP_{sat}) and actual vapor pressure of the air (VP_{air}), with VP_{sat} calculated as:

$$VP_{sat} = \exp\left[\frac{16.78T_{air} - 116.9}{T_{air} + 273.3}\right]$$

and VP_{air} as:

$$VP_{air} = \frac{VP_{sat} \times RH}{100}$$

Diurnal PAR was measured approximately 0.5-m from the ground with LI-190 PAR sensors (LI-COR Inc., Lincoln, NE) attached to Hobo 4-Channel External data loggers via UTA interface for voltage amplification (Onset Computer Corporation, Pocasset, MA). Measurements from each sensor (4 PAR and 4 Temp/RH) were averaged to find the mean for each 15-min interval for the entire transect, and these 15-min means were averaged to find mean hourly T_{air}, VPD, and PAR. In addition, sand temperature (T_{sand}) was measured every two hours from 0900 h to 1700 h in June, July, and August 2009 at various locations along the 200-m transect using a handheld infrared temperature gun (Raytek Corporation, Santa Cruz, CA).

2.4. Leaf structure and orientation

Leaf area was measured within 48 hours for *H. bonariensis* (*n* = 204) and *I. imbricata* (*n* = 185) leaves collected from the field using a ΔT leaf area meter (Delta-T Devices, Cambridge, UK). Leaf thickness was measured to the nearest 25 μm with electronic calipers, and fresh mass was measured to the nearest gram. Leaves were dried at 65°C in a drying oven for 48 to 72 hours after which dry mass was measured to the nearest gram. Leaf mass per area (LMA) was computed as the ratio of dry mass to leaf area (water saturated). The succulence index for each species was calculated as the ratio of leaf water content (fresh mass – dry mass) to leaf area. The angle of leaf attachment to the stem (leaf angle) was measured for *H. bonariensis* in the field to the nearest degree from horizontal using a Suunto Tandem clinometer (Suunto, Vantaa, Finland), and leaf angle for *I. imbricata* was measured from digital images using the Angle and

Measurement tools in ImageJ 1.44p (National Institute of Health, http://imagej.nih.gov.ij). In addition, the compass direction of the adaxial leaf plane from North (leaf azimuth) was measured for *H. bonariensis* to the nearest degree using a compass. Leaf azimuth was not measured for *I. imbricata*; leaves are sessile and distributed alternately as a whorl on the stem, thus leaf azimuth was random.

2.5. Leaf-level incident light environment

Incident sunlight incidence (PAR) on adaxial and abaxial leaf surfaces of *H. bonariensis* and *I. imbricata* was measured monthly in June, July, and August 2009. Incident sunlight exposure was measured on each leaf surface every two hours from 0900 h to 1700 h using a hand-held LI-250 photometer (LICOR, Lincoln, NE). The sensor was placed in the same plane as the leaf, and incident light was averaged over 30-sec.

2.6. Leaf temperature and photosynthetic gas exchange

Leaf temperature (T_{leaf}), photosynthesis (A), transpiration (E), and stomatal conductance (g) were measured in June, July, and August 2009 on randomly selected single leaves for each species every two hours from 0900 to 1700 h using a LICOR LI-6400 portable gas exchange system (LICOR, Lincoln, NE) with a flow rate of 500 m s^{-1} and reference CO_2 of 380 ppm. For both species, a new leaf was selected for each measurement period, thus T_{leaf}, A, E, and g are considered clone and individual shrub level for *H. bonariensis* (n = 6 leaves (ramets)/clone x 5 times/day = 30 leaves x 4 months = 120) and *I. imbricata* (n = 5 leaves/shrub x 5 times/day = 25 leaves x 4 months = 100), respectively. Water use efficiency (*WUE*) was calculated as the ratio of photosynthesis (*A*) to transpiration (*E*). All leaves were collected and immediately placed on ice for leaf structure analysis.

2.7. Photosystem II efficiency

Photosystem II efficiency (F_v/F_m) was measured as a proxy for photoinhibition using chlorophyll fluorescence techniques. Measurements of excess absorbed solar energy remitted as light, or chlorophyll fluorescence, can provide meaningful information about the efficiency of photochemistry and heat dissipation (Maxwell and Johnson 2000). When photosystem II absorbs incident sunlight and all electrons have been accepted, the reaction center is said to be closed, and the proportion of closed reaction centers leads to reduction in photochemistry and an increase in fluorescence yield. Thus, the efficiency of photosystem II can be estimated by comparing fluorescence yield in the absence of light (i.e. all reaction centers open) and in high intensity light (i.e. all reaction centers closed). Healthy non-stressed leaves have F_v/F_m values between 0.75 and 0.83, and a reduction in F_v/F_m indicates increased photoinhibition, i.e. reduced leaf photosynthesis (Björkman and Demmig 1987; Araus and Hogan 1994).

Measurements of F_v/F_m were repeated on the same leaf each month (i.e. leaf-level for both species) from June to August 2009 at two hour intervals from 0900 h to 1700 h for *H. bonariensis* (n = 6 leaves x 4 months = 24 leaves) and *I. imbricata* (n = 5 leaves x 4 months = 20 leaves) using a FMS-2 fluorescence analyzer (Hansatech Institute, Cambridge, UK). Prior to measurement, each leaf was dark-adapted in the field for at least 30 min with a FMS-2 leaf clip. After dark-adaptation, a two second, 3 mmol·m^{-2}·s^{-1} saturating pulse of light was administered to the leaf through the fiber-optic cable of a Hansatech FMS2 fluorometer. All leaves were collected and immediately placed on ice for leaf structure analyses.

2.8. Statistics

The effect of time and month on abiotic variables (T_{air}, VPD, PAR, and T_{sand}) was analyzed using repeated-measures Analysis of Variance (ANOVA) performed in JMP 8.0.2 (SAS Institute Inc., 2009). A separate ANOVA was performed for sand temperature due to the use of different measurement periods and techniques for this variable. Species differences in leaf structure (leaf area, leaf fresh and dry mass, LMA, leaf thickness, and succulence index), leaf-level light environment (adaxial PAR, abaxial PAR, ratio of adaxial-to-abaxial PAR, or *Ad/Ab*), T_{leaf}, photosynthetic gas exchange (*A, g, E, and WUE*), and F_v/F_m were investigated using Wilcoxon Rank-Sum tests performed in JMP 8.0.2. The effect of time and month on *Fv/Fm* and leaf-level light environment for each species individually was explored using the Friedman test in PASW 17.0.2 (IBM, SPSS Statistics, 2009). In addition, Kruskal-Wallis analysis was used to test the effect of time and month on the photosynthetic gas exchange for each species individually and was performed in JMP 8.0.2. Non-parametric tests were selected because data did not meet normality assumptions and could not be transformed into a normal distribution. For all analyses, significance was determined at $P \leq 0.05$.

A Stepwise Multiple Regression analysis was performed to determine the influence of abiotic factors (T_{air}, VPD, PAR, and T_{sand}) and leaf structure and orientation (Table 2) on photosynthetic gas exchange (*g, A*, *E*, and *WUE*) and F_v/F_m for *H. bonariensis* and *I. imbricata* separately in JMP 8.0.2. The combination of variables (up to 8) with the lowest corrected Akaike Information Criteria was selected as the best fit model for the response variable. Leaf orientation measurements were not included in analysis for *I. imbricata*; leaves were sessile, and thus incapable of alterations in leaf angle in response to abiotic factors.

3. Results

3.1. Abiotic environment

There was a significant effect of month on T_{air}, VPD, and PAR ($F = 16.2105$, $P < 0.0001$), as well as T_{sand} ($F = 31.5709$, $P < 0.0001$). Mean daily T_{air} was greatest in August, followed by June and July (Table 1). Mean VPD and PAR were greatest in July, followed by June and August, and mean daily T_{sand} was greatest in June, followed by August and July. There was also a significant effect of time on T_{air}, VPD, and PAR ($F = 19.7019$, $P < 0.0001$) and T_{sand} ($F = 12.3566$, $P < 0.0001$). Maximum daily T_{air} and VPD occurred during midday (1100 h – 1400 h) in June (1200 h), July (1400 h), and August (1300 h) (Figure 1). Maximum daily PAR occurred at 1300 h each month measured, with the greatest peak in August, followed by July and June. Maximum T_{sand} also occurred at 1300 h in each month measured. VPD, PAR, and T_{sand} were reduced in August by cloud cover in late morning (1000 h – 1200 h) and a rain event in the afternoon (1400 h – 1700 h).

3.2. Leaf structure and orientation

H. bonariensis leaves were significantly larger and heavier than *I. imbricata* leaves (Table 2). However, *I. imbricata* leaves were significantly thicker than *H. bonariensis* leaves and had significantly greater LMA. In addition, *I. imbricata* leaves were significantly more succulent (greater succulence index) than *H. bonariensis* leaves. Leaves of *H. bonariensis* were inclined with a mean leaf angle of 77 ± 0.8° and faced towards the South with a leaf azimuth of 181 ± 7.1°, while *I. imbricata* had slightly inclined leaves with mean leaf angle of 40 ± 0.8° and random leaf azimuth.

3.3. Leaf level-light environment

There was no significant difference in mean adaxial PAR between *H. bonariensis* and *I. imbricata* (Table 3). For *H. bonariensis,* there was no significant variation in adaxial PAR between months, but there was significant variation in adaxial PAR with time of day (Table 4). Maximum daily incident light on the adaxial leaf surface of *H. bonariensis* shifted from late morning in June and July (1100 h) to midday in August (1300 h) (Figure 2). There was significant variation in adaxial PAR for *I. imbricata* leaves with time of day and between months (Table 4). Mean adaxial PAR in June, July, and August was 1319 ± 102.1 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, 1262 ± 86.1 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and 894 ± 116.3 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively, and maximum incident sunlight on adaxial leaf surfaces occurred at midday in June (1300 h) and in the afternoon in July and August (1500 h) (Figure 2).

Mean abaxial PAR was significantly greater in *H. bonariensis* than *I. imbricata* (Table 3). There was significant variation in abaxial PAR during the day as well as between months for both *H. bonariensis* and *I. imbricata* (Table 4). Mean incident sunlight exposure on the abaxial leaf surface of *H. bonariensis* was greatest in June (418 ± 29.3 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), followed by July (360 ± 27.1 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and August (229 ± 40.9 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), and maximum incident light exposure on abaxial leaf surfaces occurred in the afternoon in June and August (1500 h) and at midday in July (1300 h) (Figure 2). For abaxial *I. imbricata* leaf surfaces, mean daily PAR was 149 ± 10.1 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in June, 171 ± 9.2 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in July, and 132 ± 16.2 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in August, and maximum sunlight exposure occurred in the afternoon (1500 h) in June, July, and August.

Mean *Ad/Ab* was significantly less in *H. bonariensis* compared to *I. imbricata* (Table 3). There was significant variation in *Ad/Ab* with time but no significant difference between months for *H. bonariensis* leaves (Table 4). Mean *Ad/Ab* was 4.2 ± 0.40 and was greatest in the morning and decreased throughout the day in each month measured (Figure 2). Mean *Ad/Ab* for *I. imbricata* leaves was 8.1 ± 0.40 and did not vary significantly with time or between months.

3.4. Photosynthetic gas exchange

Leaf temperature (T_{leaf}) was significantly greater and leaf photosynthesis (A) significantly less in *H. bonariensis* leaves relative to *I. imbricata* leaves (Table 3). For both species, there was significant variation in T_{leaf} during the day but no variation between months (Table 5). For *H. bonariensis* leaves, maximum T_{leaf} occurred in late morning (1100 h) in June, at midday (1300 h) in July, and in the afternoon (1500 h) in August (Figure 3). Maximum diurnal T_{leaf} in *I. imbricata* leaves occurred during late morning (1100 h) in June and July and in the afternoon (1500 h) in August. There was significant variation in A both during the growing season and during the day for *H. bonariensis* leaves (Table 5). Mean A was 14.8 ± 1.35 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in June, 8.8 ± 0.77 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in July, and 9.3 ± 0.82 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in August, and maximum diurnal A occurred at late morning (1100 h) in June and July and in the afternoon (1300 h) in August (Figure 3). There was no diurnal variation in A for *I. imbricata* leaves, but there was variation between months (Table 5). There was a general decrease in A over the growth season, from 16.9 ± 1.23 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in June to 12.2 ± 0.92 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in July to 9.8 ± 0.87 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in August.

Stomatal conductance (g) and transpiration (E) was significantly greater in *H. bonariensis* leaves, and water use efficiency (WUE) was significantly less, compared to *I. imbricata* leaves (Table 3). For both species, mean g did not significantly vary between months or during the day (Table 5; Figure 4). There was significant variation in E diurnally and between months for both species. Mean E increased over the growth season in *H. bonariensis* leaves, from 5.5 ± 0.31 $mmol \cdot m^{-2} \cdot s^{-1}$ in June to 6.4 ± 0.5 $mmol \cdot m^{-2} \cdot s^{-1}$ in July to 10.7 ± 0.87 $mmol \cdot m^{-2} \cdot s^{-1}$ in August. Diurnal trends for E differed over the growth season in *H. bonariensis*, staying relatively consistent during June, increasing to a maximum at midday (1300 h) followed by a decrease in July, and decreasing during the day in August (Figure 4). For *I. imbricata* leaves, E increased over the growth season from 3.9 ± 0.36 $mmol \cdot m^{-2} \cdot s^{-1}$ in June to 4.5 ± 0.24 $mmol \cdot m^{-2} \cdot s^{-1}$ in July to 9.0 ± 0.73 $mmol \cdot m^{-2} \cdot s^{-1}$ in August. Maximum diurnal E occurred in the late morning (1100 h) in

June, in the afternoon (1500 h) in July, and in the morning (0900 h) in August (Figure 4). *WUE* showed no variation during the day but did significantly differ between months for both species (Table 5; Figure 4). In general, *WUE* decreased in *H. bonariensis* leaves during the growth season from 2.7 ± 0.19 in June, 1.4 ± 0.10 in July, and 1.0 ± 0.08 in August. Similarly in *I. imbricata* leaves, *WUE* decreased during the growth season from 4.6 ± 0.34 in June to 2.7 ± 0.13 in July to 1.2 ± 0.16 in August.

3.5. Photosystem II efficiency

Mean photosystem II efficiency (F_v/F_m) was significantly greater in *I. imbricata* compared to *H. bonariensis* (Table 3). There was significant variation in F_v/F_m diurnally and seasonally for both species (Table 4). For *H. bonariensis* leaves, mean F_v/F_m was 0.74 ± 0.001 in June and decreased in July and August to 0.67 ± 0.015 and 0.68 ± 0.019, respectively, whereas mean F_v/F_m was similar in June (0.82 ± 0.007) and July (0.82 ± 0.006) and then decreased in August (0.80 ± 0.004) for *I. imbricata* leaves. During the day, F_v/F_m of *H. bonariensis* leaves reached minimum values at midday (1300 h) in June and August and in late morning (1100 h) in July (Figure 5). Minimum F_v/F_m for *I. imbricata* occurred in the late morning (1100h) in June and in the afternoon (1500 h) in July and August.

3.6. Primary predictors of leaf physiology

Stepwise multiple regressions revealed that abiotic factors and leaf structure had significant influence on seasonal and diurnal leaf temperature, leaf photosynthetic gas exchange, and photosystem II efficiency, explaining 8 – 92% of the variation (Table 6). VPD was the best predictor of T_{leaf} in both species. For *H. bonariensis*, a combination of T_{air}, VPD, and the succulence index best predicted *A*, whereas leaf dry weight best predicted *A* for *I. imbricata*.

VPD had an important influence on *g* for both species, and was combined with leaf area in *H. bonariensis* and leaf thickness in *I. imbricata*. A combination of T_{air} and leaf area was the best fit model for *E* in *H. bonariensis*, and PAR, VPD, and leaf fresh weight was the best fit model for *E* in *I. imbricata*. T_{air}, VPD, and leaf thickness best predicted *WUE* in *H. bonariensis* leaves while PAR and VPD best predicted *WUE* in *I. imbricata*. F_v/F_m was best predicted by a combination of PAR, leaf water weight, and leaf azimuth *H. bonariensis* and LMA in *I. imbricata*.

4. Discussion

The present study investigated differences in seasonal and diurnal patterns of leaf photosynthetic gas exchange and photoinhibition for two common barrier island sand dune species with significantly different leaf structure and leaf orientation properties. Multiple step-wise regressions revealed that diurnal and seasonal variation in leaf temperature, photosynthetic gas exchange, and photosystem II efficiency in both species were strongly influenced by corresponding changes in the abiotic environment. Diurnal measurements suggested that *H. bonariensis* and *I. imbricata* were most susceptible to reduced photosynthesis and photoinhibition each month during midday (1100 – 1400 h) when daily PAR, air temperature, VPD, and sand temperatures were greatest, corresponding to midday reduction in photosynthesis and increased photoinhibition at midday in *H. bonariensis*. July was the driest month (i.e. greatest VPD) and also had the greatest mean incident sunlight, corresponding to reduced photosynthetic gas exchange in both species and increased photoinhibition in *H. bonariensis* in July, and air temperature and midday incident sunlight was greatest in August, corresponding to increased transpiration rates and reduced photosystem II efficiency in both species in August.

4.1. Influence of leaf orientation on regulating leaf-level light environment

The two species differed significantly in diurnal sunlight incidence on both leaf surfaces in every month measured. While mean incident sunlight on the adaxial leaf surfaces did not differ between species, the maximum adaxial incidence occurred at different times of the day and diurnal abaxial incidence was significantly greater in *H. bonariensis*, resulting in a significantly lower *Ad/Ab.* In addition, *Ad/Ab* significantly decreased during the day for *H. bonariensis* while there was no significant variation in diurnal *Ad/Ab* for *I. imbricata*. Significant relationships have been described between leaf orientation properties and sunlight incidence on, as well as the ratio between, both leaf surfaces for species in high light environments (Smith et al. 1997, 1998; Joesting et al. 2011), suggesting that the differences between species leaf-level light environment found in this study are a result of different leaf orientation properties. Mean leaf angle in *H. bonariensis* was more inclined from horizontal compared to *I. imbricata* (77° vs. 40°, respectively), and the adaxial leaf plane of *H. bonariensis* generally faced South (181°) whereas leaf azimuth in *I. imbricata* was random.

Numerous studies have shown that adjustments in leaf angle and/or leaf azimuth functions to reduce midday sunlight incidence while maximizing incidence in the morning and evening (Syvertsen and Cunningham 1979; Geller and Smith 1982; Werk and Ehleringer 1984; Smith and Ullberg 1989; Zhang et al. 1991; King 1997; James and Bell 2000). Specifically, increased leaf angle over the growth season for *H. bonariensis* in the sand dune system has been shown to regulate diurnal and seasonal sunlight incidence on both leaf surfaces (Joesting et al. 2011). The results of the present study showed maximized sunlight incidence in late morning in June and July on adaxial leaf surfaces, and an increase in incidence on abaxial leaf surfaces corresponding to a decrease in *Ad/Ab* during the day (~4 to 1.5 times greater) for inclined *H. bonariensis* leaves. However, results suggested that the slightly inclined leaf of *I. imbricata* exposed the adaxial leaf surface to full incident sunlight, especially during midday, with little to no exposure on the abaxial surface. Maximum incident sunlight on adaxial leaf surfaces occurred at midday during maximum diurnal PAR, abaxial sunlight incidence as well as *Ad/Ab* did not vary diurnally, and adaxial leaf surface received approximately eight times the amount of diurnal incidence compared to abaxial leaf surfaces.

4.2. Influence of leaf structure on leaf-level microclimate and physiology

The diurnal and seasonal patterns in leaf physiology found in *H. bonariensis* and *I. imbricata* in this study suggests that leaf structure and orientation play an important role in modifying leaf-level microclimate, thus enhancing leaf photosynthesis, for species in barrier island sand dune communities. As predicted, the different leaf structure and orientation properties of the two species resulted in significantly different patterns in leaf physiology. Leaf temperature, stomata conductance, and transpiration were significantly greater in large inclined *H. bonariensis* leaves relative to small succulent *I. imbricata* leaves, while leaf photosynthesis and water use efficiency were significantly less. In addition, there were significant seasonal and diurnal variations in leaf physiology for both species, as hypothesized, and the abiotic environment and leaf structure had a significant influence on leaf temperatures and photosynthetic gas exchange in both species.

Air temperature and VPD had significant influence on diurnal patterns of leaf photosynthetic gas exchange in *H. bonariensis*. Thus, it would be expected for this species to possess leaf adaptations that manipulate leaf boundary layer resistance and reduce the VPD gradient between the leaf and the atmosphere. The results suggested that leaf inclination, leaf azimuth, and rapid transpiration rates interact to optimize leaf photosynthesis in *H. bonariensis*. As previously discussed, a more inclined leaf reduces midday sunlight incidence when diurnal PAR, air temperature, and VPD are greatest, thus effectively avoiding excessive leaf temperatures while maximizing photosynthesis per unit leaf area and leaf mass (Geller and Smith 1982; King 1997; Smith et al. 1997, 1998; James and Bell 2000). An inclined leaf would increase convective heat exchange and decrease the proportion of leaf area exposed to incident sunlight, thus decreasing surface temperatures and boundary layer resistance and reducing the VPD gradient between the leaf and the atmosphere (Schuepp 1993).

In addition, diurnal transpiration rates were relatively high during the growth season, ranging from 4 to 13 mmol m^{-2} s^{-1}, and step-wise regressions indicated the important influence of leaf water content on leaf photosynthesis (i.e. succulence index), suggesting that increased transpiration rates in *H. bonariensis* also play an important role in maintaining leaf photosynthesis. Rapid transpiration has been suggested to be adaptive in large leaves if there is a relatively stable water source available (Smith 1978), and water potentials rarely below -0.2 MPa have been recorded for *H. bonariensis* leaves at Topsail Island, suggesting that there may be an adequate water supply available at Topsail Island to maintain high transpiration rates (Hancock 2009).

Diurnal PAR and VPD were significantly related to leaf temperature and photosynthetic leaf gas exchange for *I. imbricata* leaves, although leaf structure also had a significant influence on leaf physiology. Specifically, leaf size and leaf water content were significantly related to leaf gas exchange, suggesting that the small leaf size and presence of a central layer of water cells of *I. imbricata* (succulence) functions to maximize photosynthesis per unit leaf area. A reduced leaf size in high sunlight habitats increases convective heat exchange and is more coupled to air temperatures, resulting in lower leaf temperatures (Gates 1967, Smith 1978). Thus, the VPD gradient between the leaf and the atmosphere is decreased, reducing the force driving transpiration (Smith 1978). During the growth season, *I. imbricata* had relatively reduced diurnal transpiration (2 – 6 mmol m^{-2} s^{-1}) and high WUE (2 – 6), with the exception of August when seasonal midday (1100 – 1400 h) air temperatures and PAR were greatest and VPD was high. The presence of leaf water-storage cells likely function as an additional water supply for photosynthetic reaction and evaporative cooling via transpiration (von Willert et al. 1990; Egbert et al. 2008), which is supported by significant influence of leaf fresh weight on transpiration, relatively high WUE, and relatively stable diurnal transpiration and photosynthesis found for *I. imbricata* in the present study.

4.3. Influence of leaf structure and orientation on photoinhibition risk

Although *I. imbricata* leaves were exposed to greater diurnal incidence sunlight on adaxial leaf surfaces, photosystem II efficiency (F_v/F_m) was significantly greater during the day in every month measured relative to *H. bonariensis* leaves, and *H. bonariensis* leaves showed significant midday depressions in diurnal F_v/F_m. In addition, F_v/F_m was significantly influenced by a combination of PAR, leaf water weight, and leaf azimuth for *H. bonariensis*, explaining 67% of the variation, while LMA was the only measured variable related to F_v/F_m in *I. imbricata* and only explained 8% of the variation. Mean monthly values of *I. imbricata* leaves remained between 0.80 and 0.82, well within the range considered optimal for non-stressed plants (Björkman and Demmig 1987; Araus and Hogan 1994), whereas monthly values of *H. bonariensis* ranged from 0.67 to 0.74, indicating that photoinhibition occurred in *H. bonariensis* leaves, but not in *I. imbricata* leaves, during the measurement period.

Leaf azimuth of *H. bonariensis* and daily PAR were important predictors of photosystem II efficiency. Leaf azimuth has been shown to play an important role in regulating daily sunlight incidence in *H. bonariensis* (Joesting et al. 2011), and it is likely the interaction of leaf azimuth (i.e. maximization of morning/evening incidence) with leaf inclination (i.e. reduction of midday incidence) that influences photoinhibition. Leaf orientation has been shown to decrease risk of photoinhibition and photodamage in large leafed alpine herbs of Wyoming (Geller and Smith 1982) and adult *Eucalyptus globulus* in Australia (James and Bell 2000). In addition, leaf water weight was significantly related to F_v/F_m in *H. bonariensis*, supporting the role of transpiration in lowering leaf temperatures and thus, reducing photoinhibition.

In addition to adjustments in leaf orientation and transpiration, there is likely an important role of photoprotection in reducing photoinhibition, especially in *H. bonariensis*. Decreases in F_v/F_m can be a result of increases in fluorescence emissions in the absence of light (F_0; all reaction centers open), indicating photodamage, or decreases in the fluorescence emission in the absence of photochemical quenching (F_m; all reaction centers closed), suggesting photoprotection (Araus and Hogan 1994; Maxwell and Johnson 2000). During the day, F_0 remained fairly consistent in *H. bonariensis* leaves while there was midday depression of F_m (*data not shown*), suggesting that the decrease in F_v/F_m found in the present study is related to increased photoprotection (Araus and Hogan 1994; Larcher 2000; Xu et al. 2007).

Leaves of *I. imbricata* showed no evidence of photoinhibition in the current study, supported by the relatively enhanced daily leaf photosynthesis measured (8 – 21 $\mu mol \cdot m^{-2} \cdot s^{-1}$). As discussed previously, reduced leaf size in high light environments has been related to decreased leaf temperatures through closely coupling leaf and air temperatures (Gates 1967; Smith 1978). However, none of the abiotic or leaf structure variables measured was strongly related to F_v/F_m, and the best fit model of LMA only explained 8% of the variation. Thus, it remains unresolved what contributes to the increased photosystem II efficiency found for *I. imbricata* in coastal sand dune habitats. Cross-sections of *I. imbricata* leaves reveal adaxial and abaxial palisade layers (isobilateral symmetry) with a central layer of water-storage cells (*data not shown*). Palisade mesophyll layers have been shown to transmit absorbed sunlight deeper into thicker, sun-type leaves and be densely packed with chloroplasts, and central water storage cells may function as reflective surfaces within the leaf (Vogelmann et al. 1996, Smith et al., 1997, 1998). Therefore, the reduction in photoinhibition found in the present study could be related to increased sunlight absorbance by palisade layers in *I. imbricata* leaves.

4.4. Conclusion

The results of the present study provide further evidence for the significant contribution of leaf structure and orientation in regulating leaf-level microclimate and enhancing daily leaf photosynthetic gas exchange in high light and high abiotic stress environments. Furthermore, these results indicate that adjustments in leaf orientation, reductions in leaf size, and increased transpiration are important leaf adaptations in native barrier island sand dune species. Leaf orientation in *H. bonariensis* regulated diurnal sunlight incidence, and the combination of leaf inclination and rapid transpiration maintained optimal leaf temperature, therefore reducing photoinhibition and facilitating photosynthesis. The reduced leaf size and presence of water-storage cells in *I. imbricata* contributed to reducing the vapor pressure gradient between the leaf and the atmosphere, enhancing leaf photosynthesis and alleviating photoinhibition.

The variables measured in the present study explained most of the variation in leaf temperature and leaf photosynthetic gas exchange. However, the variation of several physiological variables, such as leaf photosynthesis and photosystem II efficiency in *I. imbricata*, were poorly explained in the present study, suggesting that there are additional influential variables that were not measured. Thus, the role of other abiotic stresses (e.g. leaf salt deposition, blowing sand), adjustments in photochemistry and biochemistry, and internal leaf structure needs to be further explored to provide a better understanding of how these two species adapt to the unique combination of abiotic stresses of barrier island sand dune habitats.

Literature cited

Araus, J.L. and K.P. Hogan. 1994. Leaf structure and patterns of photoinhibition in two neotropical palms in clearings and forest understory during the dry season. American Journal of Botany 81: 726 – 738.

Björkman, O. and B. Demmig. 1987. Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77-K among vascular plants of diverse origins. Planta 170: 489 – 504.

Colosi, J. and J.F. McCormick. 1978. Population structure of *Iva imbricata* in five coastal dune habitats. Bulletin of Torrey Botanical Club 105: 175 – 186.

Egbert, K.J., C.E. Martin, and T.C. Vogelmann. 2008. The influence of epidermal windows on the light environment within the leaves of six succulents. Journal of Experimental Botany 59: 1863 – 1873.

Ehrenfeld, J. 1990. Dynamics and processes of barrier island vegetation. Aquatic Sciences 2: 437 – 480.

Evans, J.P. 1992. Seedling establishment and genet recruitment in a population of a clonal dune perennial, *Hydrocotyle bonariensis*. In Barrier Island Ecology of the Mid-Atlantic Coast, editors C.A. Cole and K. Turner, pp.. 75 – 84.

Gates, D.M., R. Alderfer, and E. Taylor. 1967. Leaf temperatures of desert plants. Science 159: 994 – 95.

Geller, G.N. and W.K. Smith. 1982. Influence of leaf size, orientation, and arrangement on temperature and transpiration of three high-elevation, large leafed herbs. Oecologia 53: 227 – 234.

Hancock, T. 2009. Ecophysiology of barrier island beach plants: responses in form and function to daily, seasonal and abiotic stresses. Ph.D. dissertation, Wake Forest University, Winston Salem, North Carolina, USA.

Hegazy, A.K. and I. El Amry. 1998. Leaf temperature of sand dune plants: perspectives on the adaptability of leaf morphology. African Journal of Ecology 36: 34 – 43.

Hesp, P. 1991. Ecological processes and plant adaptations on coastal dunes. Journal of Arid Environments 21: 165 – 191.

James, S.A. and D.T. Bell. 2000. Leaf orientation, light interception, and stomatal conductance of *Eucalyptus globulus* ssp. *globulus* leaves. Tree Physiology 20: 815 – 823.

Joesting, H.M., M.O. Sprague, and W.K. Smith. 2011. Seasonal and diurnal leaf orientation, bifacial sunlight incidence, and leaf structure in the sand dune herb *Hydrocotyle bonariensis*. Environmental and Experimental Botany, *in press*.

King, D.A. 1997. The functional significance of leaf angle in *Eucalyptus*. Australian Journal of Botany 45: 619 – 639.

Larcher, W. 2000. Temperature stress and survival ability of Mediterranean sclerophyllous plants. Plant Biosystems 134: 279 – 295.

Liu, L., S. Xu, and K.C. Woo. 2003. Influence of leaf angle on photosynthesis and the xanthophyll cycle of the tropical tree species *Acacia crassicarpa*. Tree Physiology 23: 1255 – 1261.

Maxwell, K. and G.N. Johnson. 2000. Chlorophyll fluorescence – a practical guide. Journal of Experimental Botany 51: 659 – 669.

Powles, S.B. 1984. Photoinhibition of photosynthesis induced by visible light. Annual Review of Plant Physiology 35: 15 – 44.

Schuepp, P.H. 1993. Tansley Review No. 59: Leaf boundary-layers. New Phytologist 125: 477 – 507.

Smith, W.K. 1978. Temperatures of desert plants: another perspective on the adaptability of leaf size. Science 201: 614 – 616.

Smith, W.K., T.C. Vogelmann, E.H. DeLucia, D.T. Bell, and K.A. Shepherd. 1997. Leaf form and photosynthesis. Bioscience 47: 785 – 793.

Smith, W.K., D.T. Bell, and K.A. Shepherd. 1998. Associations between leaf structure, orientation, and sunlight exposure in five Western Australian communities. Australian Journal of Botany 85: 56 – 63.

Smith, W.K. and N.M. Hughes. 2009. Progress in coupling plant form and photosynthetic function. Castanea 74: 1 – 25.

Smith, M. and D. Ullberg. 1989. Effect of leaf angle and orientation on photosynthesis and water relations in *Silphium terebinthinaceum*. American Journal of Botany 76: 1714 – 1719.

Syvertsen, J.P. and G.L. Cunningham. 1979. The effects of irradiating adaxial or abaxial leaf surface on the rate of net photosynthesis of *Perezia nana* and *Helianthus annus*. Photosynthetica 13: 287 – 293.

Vogelmann, T.C., J.N. Nishio, and W.K. Smith. 1996. Leaves and light capture: light propagation and gradients of carbon fixation within leaves. Trends in Plant Sciences 1: 65 – 70.

von Willert, D.J., B.M. Eller, M.J.A. Werger, and E. Brinckmann. 1990. Desert succulents and their life strategies. Vegetatio 90: 133 – 143.

Werk, K.S. and J. Ehleringer. 1984. Non-random leaf orientation in *Lactuca serriola* L. Plant Cell and Environment 7: 81 – 87.

Xu, S.-M, L.-X. Liu, K.C. Woo, and D.-L. Wang. 2007. Changes in photosynthesis, xanthophyll cycle, and sugar accumulation in two North Australia tropical species differing in leaf angles. Photosynthetica 45: 348 – 354.

Zhang, H.Z., J.M. Pleasants, and T.W. Jurik. 1991. Development of leaf orientation in the prairie compass plant, *Silphium laciniatum*. Bulletin of the Torrey Botanical Society 118: 33 – 42.

Table VI – 1: Mean ±SE diurnal photosynthetically active radiation (PAR; µmol m^{-2} s^{-1}), air temperature (T_{air}; °C), vapor pressure deficit (VPD; kPa), and sand temperature (T_{sand}; °C) for study site in June, July, and August 2009.

	June	**July**	**August**
PAR	972 ± 47.4	1082 ± 46.9	809 ± 48.4
T_{air}	29.7 ± 0.28	29.6 ± 0.32	30.5 ± 0.25
VPD	1.7 ± 0.07	2.4 ± 0.08	1.3 ± 0.08
T_{sand}	38.1 ± 0.80	35.4 ± 0.81	35.8 ± 0.87

Table VI – 2: Mean ± SE leaf structure [leaf area (cm^2), leaf dry weight (g), leaf mass per area (LMA; g cm^{-2}), leaf thickness (mm), and succulence index] and orientation [leaf angle from horizontal (°) and leaf azimuth (°)] and chi-square (χ^2) value and *P*-value from Wilcoxon Rank Sum analyses for differences in leaf structure between the two study species, *Hydrocotyle bonariensis* and *Iva imbricata* (all *df* = 1).

	H. bonariensis	***I. imbricata***	χ^2	***P*-value**
Leaf area	12.2 ± 0.26	4.4 ± 0.09	282.980	< 0.0001
Leaf dry weight	0.10 ± 0.003	0.07 ± 0.001	55.543	< 0.0001
LMA	0.008 ± 0.0002	0.016 ± 0.0002	284.610	< 0.0001
Leaf thickness	0.53 ± 0.007	1.28 ± 0.018	287107	< 0.0001
Succulence index	0.032 ± 0.0005	0.088 ± 0.0022	154.608	< 0.0001
Leaf angle	77 ± 0.8	40 ± 0.8	---	---
Leaf azimuth	181 ± 7.1	random	---	---

Table VI – 3: Mean ±SE, degrees of freedom (*df*), Chi-square (χ^2), and *P*-value from Wilcoxon Rank-Sum analysis comparing leaf-level light environment [adaxial photosynthetically active radiation (PAR; µmol m^{-2} s^{-1}), abaxial PAR (µmol m^{-2} s^{-1})], and ratio of adaxial-to-abaxial PAR (*Ad/Ab*)], Photosystem II efficiency (F_v/F_m), leaf temperature (T_{leaf}; °C), and leaf photosynthetic gas exchange [photosynthesis (*A*; µmol m^{-2} s^{-1}), leaf conductance (*g*; µmol m^{-2} s^{-1}), transpiration (*E*; mmol m^{-2} s^{-1}), and water use efficiency (*WUE*)] between *Hydrocotyle bonariensis* and *Iva imbricata* (all *df* = 1).

Variable	***H. bonariensis***	***I. imbricata***	χ^2	***P*-value**
Adaxial PAR (µmol m^{-2} s^{-1})	1105 ± 62.0	1162 ± 61.2	0.506	0.4769
Abaxial PAR (µmol m^{-2} s^{-1})	342 ± 20.1	151 ± 7.1	57.318	< 0.0001
Ad/Ab	4.2 ± 0.40	8.2 ± 3.91	48.173	< 0.0001
F_v/F_m	0.70 ± 0.009	0.82 ± 0.003	91.964	< 0.0001
T_{leaf} (°C)	34.2 ± 0.28	33.1 ± 0.33	8.216	0.0042
A (µmol m^{-2} s^{-1})	10.9 ± 0.66	12.9 ± 0.68	4.873	0.0273
g (µmol m^{-2} s^{-1})	0.51 ± 0.045	0.36 ± 0.027	9.424	0.0021
E (mmol m^{-2} s^{-1})	7.2 ± 0.37	5.7 ± 0.38	11.528	0.0007
WUE	1.7 ± 0.12	2.9 ± 0.25	17.456	< 0.0001

Table VI – 4: Degrees of freedom (*df*), chi-square (χ^2) value, and *P*-value from Friedman's test for the effect of month and time on adaxial photosynthetically active radiation (PAR; µmol m^{-2} s^{-1}), abaxial PAR (µmol m^{-2} s^{-1}), ratio of adaxial-to-abaxial PAR (*Ad/Ab*), and Photosystem II efficiency (F_v/F_m) of *Hydrocotyle bonariensis* and *Iva imbricata* leaves.

Variable	**Effect**	***df***	χ^2	***P*-value**
Hydrocotyle bonariensis				
Adaxial PAR	Month	2	7.280	0.260
	Time	4	29.506	< 0.0001
Abaxial PAR	Month	2	18.320	< 0.0001
	Time	4	19.529	0.001
Ad/Ab	Month	2	3.120	0.210
	Time	4	21.365	< 0.0001
F_v/F_m	Month	2	9.083	0.011
	Time	4	25.400	< 0.0001
Iva imbricata				
Adaxial PAR	Month	2	13.083	0.001
	Time	4	24.171	< 0.0001
Abaxial PAR	Month	2	6.505	0.039
	Time	4	36.743	< 0.0001
Ad/Ab	Month	2	1.083	0.582
	Time	4	0.229	0.994
F_v/F_m	Month	2	11.083	0.004
	Time	4	22.368	< 0.0001

Table VI – 5: Degrees of freedom (*df*), chi-square (χ^2) value, and *P*-value from Kruskal-Wallis analyses for the effect of month and time on leaf temperature (T_{leaf}; °C), leaf conductance (*g*; μmol m^{-2} s^{-1}), photosynthesis (*A*; μmol m^{-2} s^{-1}), transpiration (*E*; mmol m^{-2} s^{-1}), and water use efficiency (*WUE*) for *Hydrocotyle bonariensis* and *Iva imbricata* leaves.

Variable	**Effect**	***df***	**χ^2**	***P*-value**
Hydrocotyle bonariensis				
T_{leaf}	Month	2	3.094	0.2129
	Time	4	38.091	< 0.0001
A	Month	2	12.611	0.0018
	Time	4	11.317	0.0232
g	Month	2	2.471	0.2907
	Time	4	3.763	0.4390
E	Month	2	23.496	< 0.0001
	Time	4	10.294	0.0358
WUE	Month	2	32.133	< 0.0001
	Time	4	4.353	0.3603
Iva imbricata				
T_{leaf}	Month	2	1.906	0.3855
	Time	4	30.549	< 0.0001
A	Month	2	17.303	0.0002
	Time	4	6.020	0.1977
g	Month	2	2.852	0.2403
	Time	4	9.035	0.0602
E	Month	2	42.650	< 0.0001
	Time	4	15.456	0.0038
WUE	Month	2	42.650	< 0.0001
	Time	4	2.280	0.6844

Table VI – 6: Best fit model, corrected Akaike Information Criteria score (AIC_c), and goodness of fit (r^2) from Stepwise Multiple Regression analyses for predicting leaf temperature (T_{leaf}) and leaf photosynthetic gas exchange [stomatal conductance (*g*), photosynthesis (*A*), transpiration (*E*), water use efficiency (*WUE*), and photosystem II efficiency (F_v/F_m)] from leaf structure and abiotic environment variables. Best fit model with up to eight variables was selected based on minimum AIC_c value.

Variable	**Best fit model**	**AICc**	r^2
Hydrocotyle bonariensis			
T_{leaf}	Vapor pressure deficit	61.75	0.92
A	Air temperature x vapor pressure deficit x succulence index	106.55	0.47
g	Vapor pressure deficit x leaf area	103.79	0.48
E	Air temperature x leaf area	15.87	0.39
WUE	Air temperature x vapor pressure deficit x leaf thickness	21.29	0.77
F_v/F_m	PAR x leaf water weight x azimuth	-61.97	0.67
Iva imbricata			
T_{leaf}	Vapor pressure deficit	74.88	0.86
A	Leaf dry weight	129.05	0.19
g	Vapor pressure deficit x leaf thickness	15.91	0.47
E	PAR x vapor pressure deficit x leaf fresh weight	123.40	0.59
WUE	PAR x vapor pressure deficit	42.64	0.34
F_v/F_m	Leaf mass per area	-114.69	0.08

Figure captions

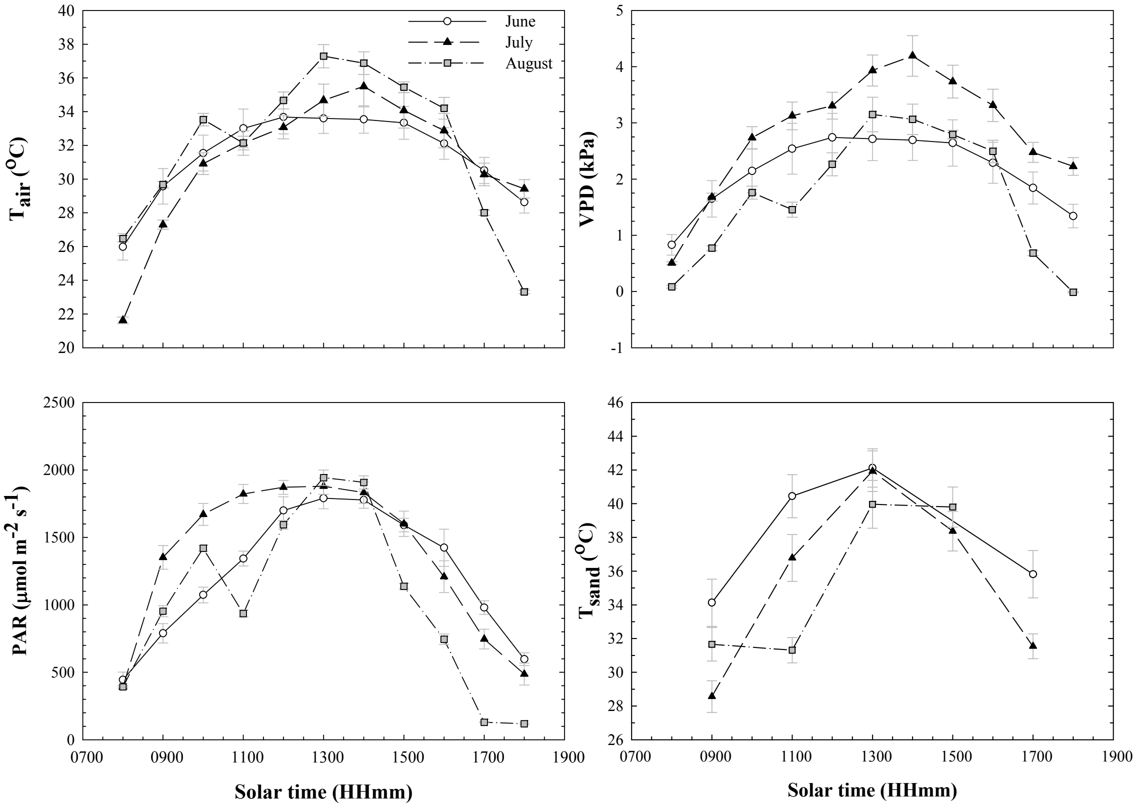
Figure VI – 1: Diurnal (0800 h to 1800 h) air temperature (T_{air}), vapor pressure deficit (VPD), photosynthetically active radiation (PAR), and sand temperature (T_{sand}) for a barrier island sand dune community in June, July, and August 2009. Bars represent standard error.

Figure VI – 2: Diurnal (0900 h to 1700 h) adaxial photosynthetically active radiation (PAR), abaxial PAR, and the ratio of adaxial-to-abaxial PAR for *Hydrocotyle bonariensis* and *Iva imbricata* leaves in June, July, and August 2009. Bars represent standard error.

Figure VI – 3: Diurnal (0900 h to 1700 h) photosystem II efficiency (F_v/F_m) for *Hydrocotyle bonariensis* and *Iva imbricata* leaves in June, July, and August 2009. Bars represent standard error.

Figure VI – 4: Diurnal (0900 h to 1700 h) leaf temperature (T_{leaf}) and leaf photosynthesis (A) for *Hydrocotyle bonariensis* and *Iva imbricata* leaves in June, July, and August 2009. Bars represent standard error.

Figure VI – 5: Diurnal (0900 h to 1700 h) stomata conductance (g), transpiration (E), and water use efficiency (WUE) for *Hydrocotyle bonariensis* and *Iva imbricata* leaves in June, July, and August 2009. Bars represent standard error.



June
July
August
T_{air} (°C)
40
38
36
34
32
30
28
26
24
22
20
VPD (kPa)
5
4
3
2
1
0
-1
PAR (μmol m^{-2} s^{-1})
2500
2000
1500
1000
500
0
T_{sand} (°C)
46
44
42
40
38
36
34
32
30
28
26
0700
0900
1100
1300
1500
1700
1900
Solar time (HHmm)
Solar time (HHmm)

Figure VI – 1

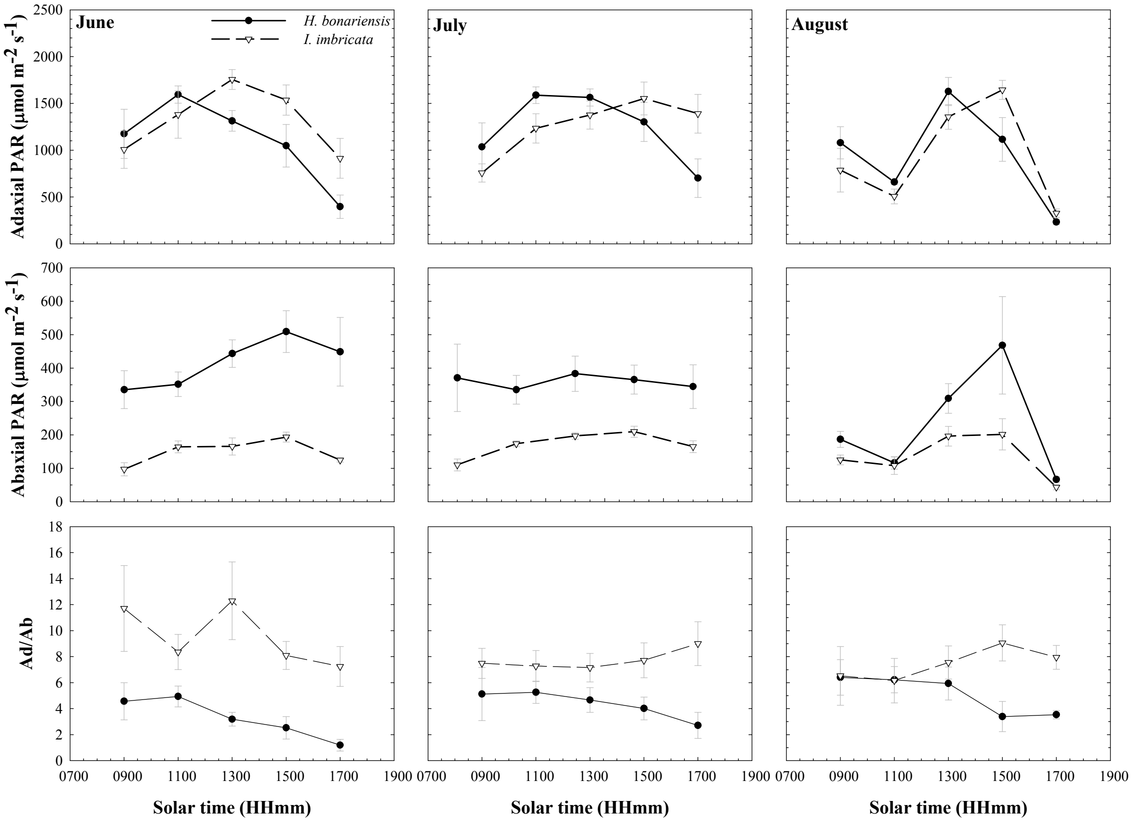

June
July
August
H. bonariensis
I. imbricata
Adaxial PAR (μmol m-2 s-1)
Abaxial PAR (μmol m-2 s-1)
Ad/Ab
0
500
1000
1500
2000
2500
0
100
200
300
400
500
600
700
0
2
4
6
8
10
12
14
16
18
0700
0900
1100
1300
1500
1700
1900
Solar time (HHmm)

Figure VI – 2

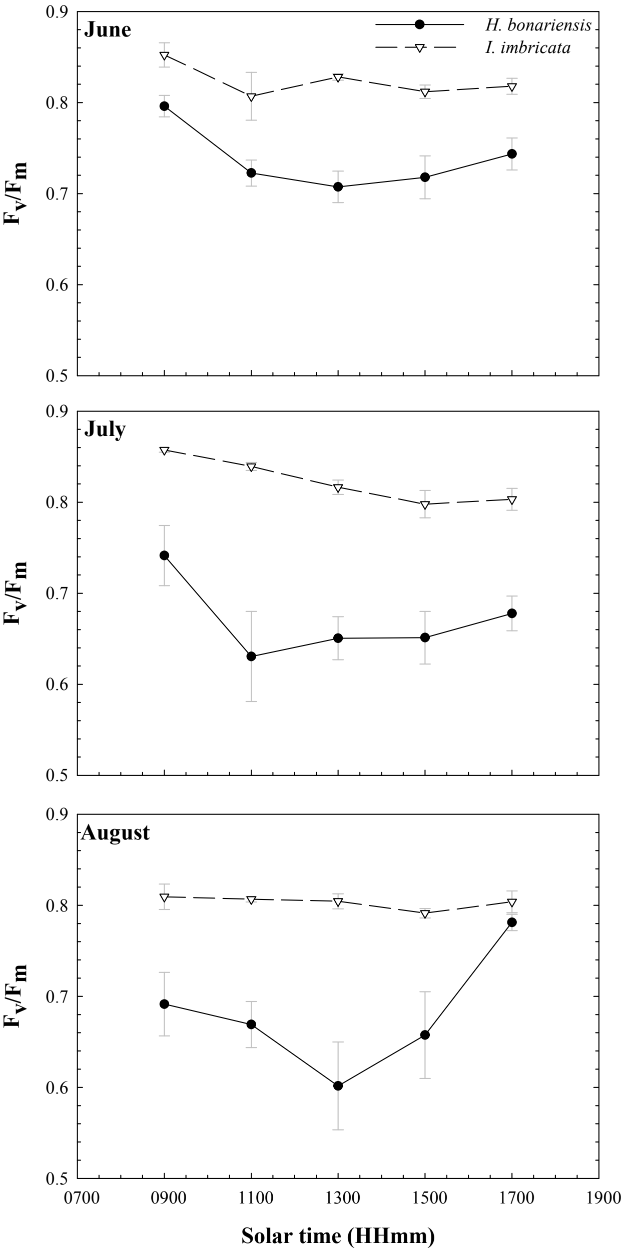

June
July
August
H. bonariensis
I. imbricata
F_v/F_m
0.9
0.8
0.7
0.6
0.5
0700
0900
1100
1300
1500
1700
1900
Solar time (HHmm)

Figure VI – 3

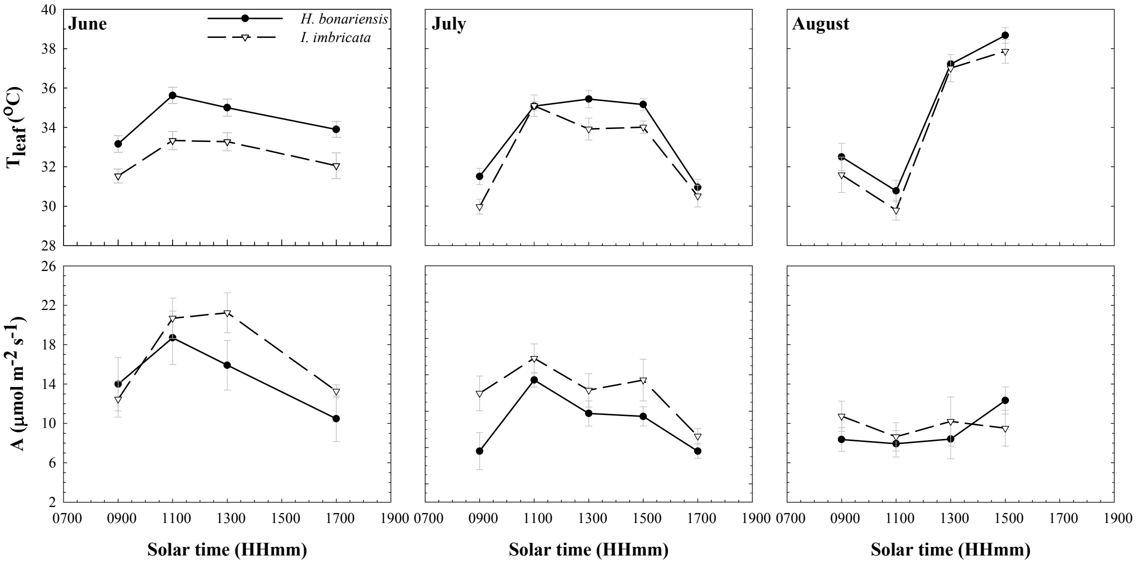

June
July
August
H. bonariensis
I. imbricata
T_{leaf} (°C)
40
38
36
34
32
30
28
A (µmol m^{-2} s^{-1})
26
22
18
14
10
6
2
0700
0900
1100
1300
1500
1700
1900
Solar time (HHmm)

Figure VI – 4

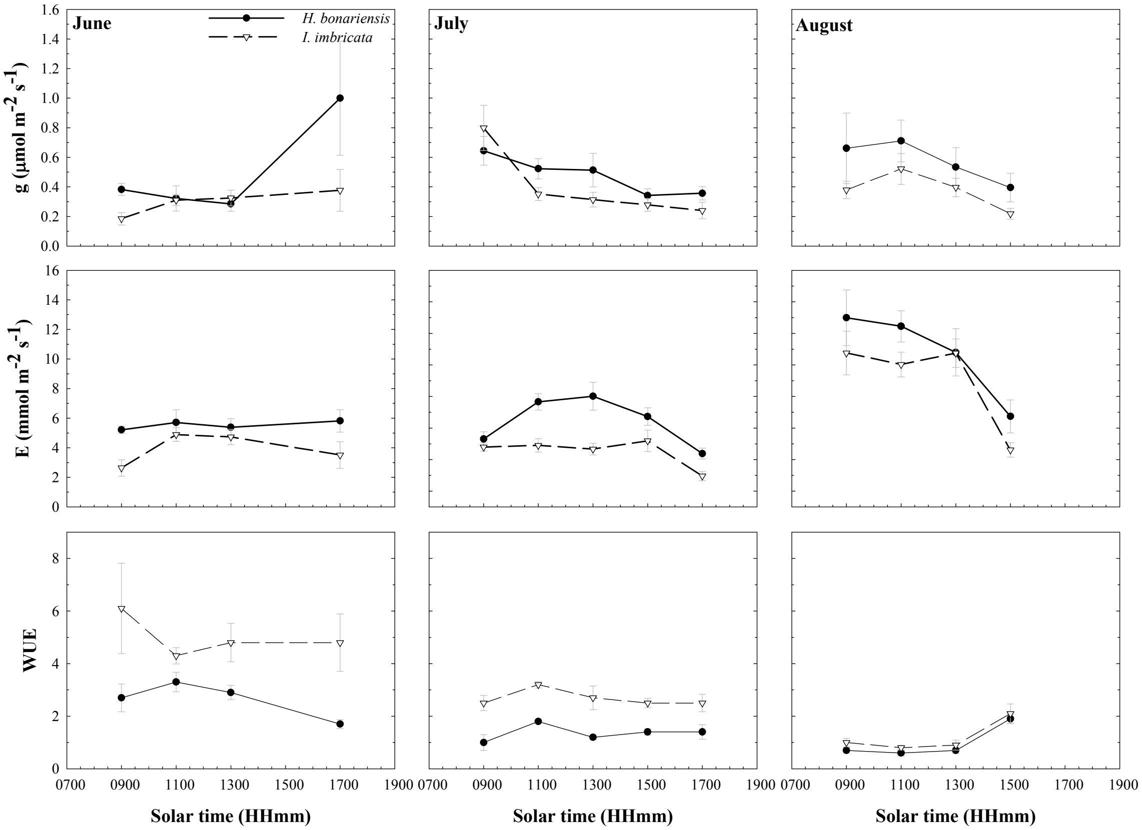

June
July
August
H. bonariensis
I. imbricata
g (µmol m^{-2} s^{-1})
E (mmol m^{-2} s^{-1})
WUE
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
0
2
4
6
8
10
12
14
16
0700
0900
1100
1300
1500
1700
1900
Solar time (HHmm)

Figure VI – 5

CHAPTER VII

CONCLUSIONS AND FUTURE RESEARCH

The aim of the research presented in this dissertation was to elucidate the leaf morphological and physiological adaptations of two common species of the sand dune habitat to the seasonal and diurnal abiotic stresses of the barrier island. Previous studies have provided evidence of the strong influence of abiotic factors, e.g. wind-mediated sand movement, salt spray and deposition, and overwash events, on vegetation patterns in the sand dune system (Oosting and Billings 1942; Boyce 1954; Barbour 1978; Moreno-Casasola 1986; Ehrenfeld 1990; Wilson and Sykes 1999; Stallins and Parker 2003), in addition to studies documenting common morphological, physiological, and reproductive adaptations of native sand dune species (Barbour et al. 1985; Lee and Ignaciuk 1985; Hesp 1991; Fahrig et al. 1993; Blackman et al. 2005; Greaver and Sternberg 2006). However, few studies have specifically addressed the relationship between leaf morphology, leaf physiology, and the abiotic environment for coastal sand dune species. It was hypothesized that the leaf physiology of the two species studied (the clonal herb *Hydrocotyle bonariensis* and semi-woody shrub *Iva imbricata*) would be significantly associated with measurements of leaf structure, leaf orientation, and the abiotic environment, and the results of the present study provide support for this hypothesis.

The overall hypothesis of this dissertation was based on the strong evidence suggesting significant relationships between leaf structure, leaf orientation, incident sunlight, and the abiotic stress level of the habitat. A conceptual framework predicting leaf structure and orientation, as well as potential leaf photosynthesis, was proposed based on the strong associations found for 242 native Australian, Rocky Mountain, and southwestern U.S. desert species, and it is this conceptual framework that provided the basis of the hypotheses in the dissertation (Smith et al. 1997, 1998; Table 5.1). The two species differed significantly in leaf structure and orientation and occupied different spatial positions along the abiotic stress gradient, suggesting that they were subject to different degrees of abiotic stress. *Hydrocotyle bonariensis* was only found at the upper end (i.e. lower stress) of the gradient while *Iva imbricata* was found in all vegetation zones along the abiotic gradient (i.e. higher stress). Therefore it was expected that *H. bonariensis* would have characteristics similar to lower stress species in high incident sunlight habitats whereas *I. imbricata* would be similar to higher stress species, and this hypothesis was supported by the dissertation results. In addition, the leaf physiology of both species was significantly associated with measurements of the abiotic environment and leaf structure (Chapter V and VI).

1. Morphological and physiological leaf adaptations in *Hydrocotyle bonariensis*

Hydrocotyle bonariensis is found in the swale, secondary dunes, and tertiary dune grasslands of the sand dune habitat, placing its habitat at the less stressful end of the abiotic gradient. Thus, it was predicted that *H. bonariensis* would have leaf characteristics similar to species in high incident light and relatively lower stress predicted by the conceptual framework proposed by Smith et al. (1997, 1998), and measured leaf characteristics did reveal a broad leaf with dorsiventral leaf symmetry and stomata on both leaf surfaces. It was also expected that leaf orientation of *H. bonariensis* would significantly vary diurnally and seasonally, thus significantly influencing diurnal patterns of sunlight incidence on both adaxial and abaxial leaf surfaces, and that leaf structure would correspond to predictions based on the total amount, as well as the ratio between, incidence of both leaf surfaces (Chapter III). In addition, leaf orientation was expected to function to reduce midday leaf temperatures and transpiration while facilitating leaf photosynthesis and stomata conductance (Chapter IV). It was also hypothesized that leaf temperatures and photosynthetic gas exchange would be significantly associated with measurements of leaf structure and the abiotic environment (Chapter V and VI).

Results indicated that leaf orientation did vary significantly during the season, but there was no significant variation during the day. This finding was relatively novel and may be the first example of a plant species which alters leaf orientation seasonally but not diurnally. As hypothesized, leaf orientation in *H. bonariensis* significantly regulated the sunlight incidence of both leaf surfaces, suggesting that the inclined leaf angle from horizontal reduces midday incidence while the increased leaf azimuth over the growth season tracks the increasingly southern solar azimuth over the growth season to maximize morning and/or evening incidence. Additionally, results suggested that the total amount of incidence, as well as the ratio, on adaxial and abaxial leaf surfaces had influence on the development leaf internal structure. Experimental manipulations of leaf inclination in *H. bonariensis* indicated that leaf orientation functions to reduce leaf temperatures and facilitate photosynthesis and stomata conductance, as hypothesized, and the results also suggested that rapid transpiration in *H. bonariensis* also plays an important role in maintaining leaf temperature and photosynthetic gas exchange.

The abiotic environment (i.e. air temperature and vapor pressure deficit) exerted a strong influence on leaf temperature, leaf photosynthetic gas exchange, and photosystem II efficiency in *H. bonariensis* leaves. Leaf structure was also associated with leaf physiology, but leaf structure (i.e. leaf weight, leaf area, leaf orientation, and stomata frequency on both leaf surfaces) alone only explained 5 – 14% of the variation in leaf temperature and photosynthetic gas exchange. When the abiotic environment was combined with leaf structure, 39 – 92% of the variation was explained. Specifically, the vapor pressure deficit, air temperature, leaf orientation, leaf size, and leaf water content were most strongly associated with leaf physiology.

The results from this dissertation suggest that the variations in leaf orientation in response to incident sunlight and the ability to maintain relatively rapid transpiration rates are important adaptations in *H. bonariensis* in the sand dune ridge and swale system. The inclined leaf functions to reduce midday sunlight incidence and avoid excessive leaf temperatures and rapid transpiration helps maintain optimal leaf temperatures. In addition, although *H. bonariensis* leaves were shown to be subjected to photoinhibition, especially during midday, results suggested that leaf inclination and transpiration, as well as a possible role of photoprotective processes, were important in reducing the occurrence and extent of photoinhibition.

2. Morphological and physiological leaf adaptations in *Iva imbricata*

Iva imbricata was found in all habitats along the sand dune abiotic stress gradient, thus it was predicted that leaf characteristics would be similar to those predicted for high light and relatively higher abiotic stress species of the conceptual framework proposed by Smith et al. (1997, 1998). The results of this dissertation provide evidence to support this hypothesis; leaves were small and thick with isobilateral symmetry, a central layer of water storage cells, and stomata on both leaf surfaces. In addition, both leaf structure and the abiotic environment exerted significant influence on leaf physiology in *Iva imbricata*. Leaf structure alone explained 19 – 30% of the variation in leaf temperature and photosynthetic gas exchange, and the addition of abiotic variables significantly explained 19 – 86% of the variation. Specifically, compared to *H. bonariensis*, leaf structural variables (i.e. leaf size, leaf water content, and stomata on both leaf surfaces) were more strongly associated with leaf physiology, and the two abiotic variables that had a significant effect on physiology were vapor pressure deficit and the incident sunlight of the habitat (Chapter V and VI).

These results suggest that the reduced leaf size, presence of water storage cells, and rapid transpiration rates are important adaptations of *I. imbricata* in the sand dune ridge and swale system. The reduced leaf size likely functions to couple leaf temperatures to air temperatures and decrease the water vapor concentration gradient between the leaf and the atmosphere. In addition, the rapid transpiration measured during the growth season would correspond to an increase in evaporative cooling, reducing leaf temperatures. Furthermore, the presence of leaf water-storage cells could provide additional water supply for photosynthetic reaction and evaporative cooling via transpiration. Photoinhibition in *I. imbricata* leaves did not occur in the current study, suggesting that the leaf adaptations described above do enhance leaf photosynthesis and carbon gain in this species. However, none of the measured leaf structure variables or abiotic factors was significantly related to photosystem II efficiency, and there was no suggested role of photoprotective processes in alleviating photoinhibition in *I. imbricata* leaves. Thus, the mechanism of reduced photoinhibition in *I. imbricata* in the sand dune habitat remains to be elucidated.

3. Conclusions

The results presented in this dissertation documented several important leaf morphological and physiological adaptations to seasonal and diurnal abiotic factors (i.e. high incident sunlight, air temperature, substrate temperatures, and vapor pressure deficit) common to the barrier island sand dune habitat for *H. bonariensis* and *I. imbricata.* In addition, the results provide further evidence of the tight coupling between leaf structure and orientation, leaf physiology, and the abiotic environment. These results provide essential information to coastal management plans for the utilization of native species in the creation and maintenance of artificial sand dunes. Specifically, these results will be critical in maintaining these species through the growth season and promoting optimal conditions for carbon gain, and thus growth and reproduction.

Several unresolved questions remain for both species, including the role of photoprotection in reducing photoinhibition risk in *H. bonariensis* and the function of trichomes in *I. imbricata*. However, in terms of using these two species in artificial dune creation and maintenance, the principal unresolved issues are the effect of spatially variable abiotic factors on plant carbon gain and allocation and the recovery and resilience of both species to extreme episodic storm events. Understanding the influence of spatial abiotic factors (e.g. salt spray and sand movement) and extreme storm events will be critical in the coastal management plans using these species in the creation and maintenance of artificial sand dunes.

Literature cited

Barbour, M. 1978. Salt spray as a microenvironmental factor in the distribution of beach plants at Point Reyes, California. Oecologia 32: 213 - 224.

Barbour, M.G., T.M. DeJong, and B.M. Pavlik. 1985. Marine beach and dune plant communities. *In* Physiological Ecology of North American Plant Communities. Ed. B.F. Chabot and H.A. Mooney. Chapman and Hall, Cambridge: 296 - 322.

Blackman, C.J., G.J. Jordan, and R.J.E. Wiltshire. 2005. Leaf gigantism in coastal areas: morphological and physiological variation in four species on the Tasman Peninsula, Tasmania. Australian Journal of Botany 53: 91 - 100.

Boyce, S. 1954. The salt spray community. Ecological Monographs 24: 29 - 67.

Ehrenfeld, J.G. 1990. Dynamics and processes of barrier island vegetation. Aquatic Sciences 2: 437 – 480.

Fahrig, L., B. Hayden, and R. Dolan. 1993. Distribution of barrier island plants in relation to overwash disturbance: a test of life history theory. Journal of Coastal Research 9: 403 - 412.

Greaver, T.L. and L.S.L. Sternberg. 2006. Linking marine resources to ecotonal shrifts of water uptake by terrestrial dune vegetation. Ecology 87: 2389 – 2396.

Hesp, P.A. 1991. Ecological processes and plant adaptation on coastal dunes. Journal of Arid Environments 21: 165 – 191.

Lee, J.A. and R. Ignaciuk. 1985. The physiological ecology of strandline plants. Vegetatio 62: 319 - 326.

Moreno-Casasola, P. 1986. Sand movement as a factor in the distribution of plant communities in a coastal dune system. Vegetatio 65: 67 – 76.

Oosting, J. and W.D. Billings. 1942. Factors effecting vegetational zonation on coastal dunes. Ecology 23: 131 - 142.

Smith, W.K., T.C. Vogelmann, E.H. DeLucia, D.T. Bell, and K.A. Shepherd. 1997. Leaf form and photosynthesis: Do leaf structure and orientation interact to regulate internal light and carbon dioxide? Bioscience 47: 785 – 793.

Smith, W.K., D.T. Bell, and K.A. Shepherd. 1998. Associations between leaf structure, orientation, and sunlight exposure in five Western Australian communities. American Journal of Botany 85: 56 – 63.

Stallins, J. and A.J. Parker. 2003. The influence of complex systems interactions on barrier island dune vegetation pattern and process. Annals of the Association of American Geographers 93: 13 -29.

Wilson, J. and M.T. Sykes. 1999. Is zonation on coastal sand dunes determined primarily by sand burial or salt spray? A test in New Zealand dunes. Ecology Letters 2: 233 – 236.

Curriculum Vitae

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September 2005 – August 2006	Research Assistant Arabidopsis Biological Resource Center Dr. Randy Scholl, Director Department of Plant Biology

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June 2005 – August 2005	Science Exploration Instructor Upward Bound Program Ayanna M. Jordan, Director College of Education Ohio University Athens, Ohio
April 2003 – June 2005	Teaching and Research Assistant / M.S. Student Forest Ecophysiology Lab Dr. Kim J. Brown, Advisor Department of Environmental and Plant Biology Ohio University Athens, Ohio

Publications

Joesting, H.M., M.O. Sprague, and W.K. Smith (2011). Seasonal and diurnal leaf orientation, bifacial sunlight incidence, and leaf form and function in the sand dune herb *Hydrocotyle bonariensis*. Experimental and Environmental Botany: *in press.*

Joesting, H.M., B.C. McCarthy, and K.J. Brown (2008). Determining the shade tolerance of American chestnut using morphological and physiological leaf parameters. Forest Ecology and Management 257: 280 – 286.

Joesting, H.M., B.C. McCarthy, and K.J. Brown (2007). The photosynthetic response of American chestnut seedlings to differing light conditions. Canadian Journal of Forest Research 37: 1714 - 1722.

Presentations/Abstracts

"Testing two leaf form and function models along a small-scale abiotic gradient on a barrier island", 95th Annual Ecological Society of America (ESA) National Meeting, Pittsburgh, Pennsylvania, August 1 – 6, 2010 (talk)

"Coastal Barrier Island Network (CBIN): Management strategies for the future", The Coastal Society's 22nd International Conference, Wilmington, North Carolina, June 13 – 16, 2010 (talk)

"Testing a conceptual leaf form and function model using two sand dune species", 25th Annual Perspectives in Biology Symposium, Wake Forest University, Winston Salem, North Carolina, November 3 – 4, 2009 (poster)

"Functional significance of leaf orientation in the sand dune herb *Hydrocotyle bonariensis* Comm ex. Lam", 94th Annual Ecological Society of America (ESA) National Meeting, Albuquerque, New Mexico, August 2 – 7, 2009 (talk)

Brown, K.J., **H.M Joesting,** and B.C. McCarthy (2005). Within-canopy variance in leaf photosynthesis and leaf density within a mature stand of American Chestnut. (National Ecological Society of America, Montreal, Canada)

"Physiology and leaf characteristics of American chestnut seedlings, saplings, and mature trees", Ohio University Creativity and Research Fair, May 12, 2005 (poster)

"Thinning promotes the performance of American chestnut seedlings in the understory of southeastern Ohio forests", 89th Annual Ecological Society of America (ESA) National Meeting, Portland, Oregon, August 1 – 6, 2004 (poster)

"Photosynthetic response of American chestnut seedlings to varying light", Midwest American Society of Plant Biologists (ASPB) Sectional Meeting, Columbus, Ohio, March 19 – 20, 2004 (talk)

Techniques and skills

Ecophysiology Gear and Maintenance (LI-6400 carbon dioxide gas exchange analyzer, C:N combustion analyzer, 8000M Ball Grinder, Decagon par sensors, LAI-2000, Hemispherical photography, Porometer, TPS photosynthesis system, Hansatech FMS Fluorometer, Pressure bomb, Hobo data loggers, Temperature and humidity sensors)

Microscopy Techniques (Widefield transmitted light, laser scanning confocal, epifluorecence, scanning electron microscopy)

Software (Microsoft Excel, Microsoft Word, Microsoft PowerPoint, Microsoft Publisher, Photosynthesis Assistant, GLA Software, Sigma Plot, Scion Image, Image J, Adobe Photoshop, Adobe PageMill, Dreamweaver, FV2000, ArcGIS)

Statistical Software (SPSS, NCSS, SAS, Model II for R)

Invited Talks and Seminars

"Structure vs. Physiology: Leaf adaptation to abiotic stress in coastal sand dune species", Department of Biology Seminar, Wake Forest University, April 20, 2011

Guest Lecture, "Geographical Information System (GIS): Using GIS in Biogeography", BIO 348 (Biogeography), Department of Biology, Wake Forest University, December 2008

"Physiology and leaf characteristics of American chestnut seedlings, saplings, and mature trees," Department of Environmental and Plant Biology Colloquium, Ohio University, May 19, 2005

Guest Lecture, "American Chestnut and the Chestnut Blight: An Introduction to Fungal Pathogens", PBIO 103 (Plants and People), Department of Environmental and Plant Biology, Ohio University, May 2004

Awards and Honors

Graduate Fellowship, Department of Biology, Wake Forest University, August 2006 - Present

Outstanding Teaching Assistant Award, Department of Environmental and Plant Biology, Ohio University, June 3, 2005
Ohio University Creativity and Research Fair, 1st Place for Poster Presentation, May 12, 2005

Summer Scholar Associate (SSA) Fellowship, Summer 2004

Graduate Fellowship, Department of Environmental and Plant Biology, Ohio University, March 2003 – June 2005

Professional Affiliations

The Coastal Barrier Island Network (CBIN), 2008 – 2011
The Ecological Society of America (ESA), 2003 – 2006, 2009 – 2010
The Botanical Society of America (BSA), 2009 – 2010

Grants and Contracts

Alumni Student Travel Fund, Graduate School, Reynolda Campus, Wake Forest University, Summer 2010, Awarded: $300.00

Elton C. Cocke Travel Fund, Department of Biology, Wake Forest University, Summer 2010, Awarded: $500.00

Vecellio Grant, Department of Biology, Wake Forest University, Spring 2010, Awarded: $934.00.

Alumni Student Travel Fund, Graduate School, Reynolda Campus, Wake Forest University, Summer 2009, Awarded: $300.00

Elton C. Cocke Travel Fund, Department of Biology, Wake Forest University, Summer 2009, Awarded: $500.00

Vecellio Grant, Department of Biology, Wake Forest University, Spring 2009, Awarded: $1500.00

Vecellio Grant, Department of Biology, Wake Forest University, Spring 2008, Awarded: $1500.00.

Vecellio Grant, Department of Biology, Wake Forest University, Spring 2007, Awarded: $1321.81.

Graduate Student Senate (GSS) Travel Grant, Ohio University, Spring 2004, Awarded: $300.

Committee and Community Service

Graduate Committee, Graduate Student Representative, Department of Biology, Wake Forest University, August 2008 – July 2010

Forsyth County Science Fair Judge, February 26, 2009 and February 19, 2010

Women in Science and Engineering (WISE) Planning Committee, Environmental and Plant Biology, Ohio University, April 2005

Ohio Southeastern District Science Fair Judge (Grades 5-12), March 5, 2005

Program/Education/Outreach Committee, The American Chestnut Foundation, Ohio State Chapter, October 2004 – June 2005

Women in Science and Engineering (WISE) Planning Committee, Environmental and Plant Biology, Ohio University, April 2004

Recruitment Committee, Graduate Student Representative, Department of Environmental and Plant Biology, Ohio University, 2003 – 2005