2013

Development of production practices and populations for coastal and urban use of sea oats (Uniola paniculata L.)

Kaitlin Barrios
Louisiana State University and Agricultural and Mechanical College, kbarri9@lsu.edu

DEVELOPMENT OF PRODUCTION PRACTICES AND POPULATIONS FOR COASTAL AND URBAN USE OF SEA OATS (*UNIOLA PANICULATA* L.)

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The School of Plant,
Environmental & Soil Sciences

by
Kaitlin Barrios
B.L.A., Louisiana State University, 2007
December 2013

Acknowledgements

This thesis could not have been written without the guidance and support of my major professor, Dr. Carrie Knott. Her patience and constructive comments have led me to think critically and comprehensively, and she has also allowed me the freedom to reach conclusions independently after many stumbles. I want to thank her for the lessons she taught me both in Sturgis and out; two years was not enough. Her intelligence and perseverance in a predominately male field inspires me to strive to be a better researcher and more avid science lover. And a tremendous thank you is owed to her for providing me the opportunity of an assistantship without which my graduate experience would have been greatly altered. I could not have asked for a more apt major professor.

Many thanks are owed to my additional committee members, Dr.'s Don Labonte and Raymond Schneider, for their time and input. They have provided me with fresh perspectives on my research topics thereby broadening my knowledge and scope of understanding. A particular thanks extended to Dr. Labonte for providing me with the employment opportunity and experience which in part led me to choose the plant breeding 'door' and get my proverbial foot in it.

Dr. James Geaghan played an important role in expanding my statistical education through his assistance in writing and then 'cracking the codes' of my projects. He has also been an exceptional teacher during my semesters of stats and experimental design. Additional thanks to Dr. Ron Strahan for contributing his pesticide knowledge. And a very big thanks given to those who have helped me realize my projects in the field, lab and greenhouse and turn research into reality by providing their time, brawn and expertise: Matt Vottier, Pheonah Nabukalu and Jason Stagg. I could not have completed my work without their service.

Thanks to my family and friends for their distractions and much needed support, and although they will probably never read this I greatly appreciate them just the same. I would also like to acknowledge my constant canine companion, Chilli.

Lastly, to Mother Nature who is routinely underestimated and grossly undervalued, thanks for being amazing and keeping me interested.

Table of Contents

List of Tables

List of Figures

Abstract

Sea oats (*Uniola paniculata* L.) is a perennial grass commonly used to help build and stabilize dunes in coastal restoration projects along the northern Gulf of Mexico. In Louisiana most sea oats plants transplanted to the beach as part of these projects are genetically identical. This lack of diversity can be extremely detrimental to the success of these large-scale sea oats communities because the susceptibility of one plant to a stressor is shared by all sea oats plants of the same genotype. Two main methods to reverse this lack of diversity are via plant breeding and the production of vegetative material from seed. Therefore the first two goals pertained to furthering research related to these methods. Production of sea oats from seed is encumbered by a few problems, one of which is low germination attributed to seed dormancy and/or pathogens which reside within seed, on seed surface or in immediate soil zone. The first study of this thesis assessed the impact of four commercial fungicides each at two rates on the production of sea oats seedlings from seed. The fungicide treatments azoxystrobin at 1x rate and thiophanate-methyl at 1x and 2x rates were found to increase sea oats germination and survival, as well as seedling quality. A high quality seedling was based on seedlings' shoot height, root length and fresh weight after 8 weeks of growth. The second study aimed to improve efficiency of sea oats breeding for coastal use. Due to the considerable number of years required for breeding relative to the rapid rate of coastal erosion, a reduction of time and expenses required for researchers to travel to coastal breeding and selection nurseries could increase efficiency. This reduction could be met by relocating selection nurseries to urban locations closer to researchers potentially leading to swifter releases of varieties. The second study conducted preliminary research into the possibility of relocation by assessing whether the average performances of selected populations, unselected populations and varieties of sea oats differed when produced outside of the coastal zone. Results found that unselected populations had higher means than selected populations for plant height, stem count, number of tillers, vigor and stem density. It was also found that means of varieties were lower than means of unselected populations for most variables and almost always similar to selected populations. Using previous data of height, stem count and vigor of varieties at a beach location, it was found that varieties had lower means at the urban location than

coastal. This would suggest that the performance of varieties differed at the two locations and because the performances differed of these populations and varieties; that selection studies of sea oats for coastal use should likely not occur in urban inland conditions such as those in Baton Rouge, LA, U.S. However, this study does suggest that selection for urban use of sea oats in this location could be successfully conducted. Due to the growing sector of native ornamental grasses in the southeastern U.S. in addition to previous displays and studies of ornamental traits of some sea oats lines, breeding and selection research should be conducted in order to produce exciting, original and lucrative ornamental sea oats varieties. The third and final study of this thesis assessed 312 sea oats experimental lines for ideal ornamental qualities such as reddish purple foliage, leaf width and plant form as well as strong growth performance in urban conditions. Fifty experimental lines were selected for advancement into further breeding and selection stages as part of the process of developing potential landscape varieties.

Chapter 1: Introduction

The Gulf Coast region of the United States loses more land from coastal erosion than any other area in the contiguous United States and Louisiana accounts for more than 90 percent of all wetland loss in the whole U.S. (Commission, 2011; Finkl and Khalil, 2005). In a study presented by the U.S. Geological Survey (USGS), the annual rate of land loss of coastal Louisiana from 1985 to 2006 was 39.19 ± 7.61 km^2/yr (15.13± 2.94 sq miles/yr) (Barras et al., 2008). According to the Coastal Protection and Restoration Authority (CPRA), coastal Louisiana has lost a net area greater than 1,900 square miles (or three times the size of Lake Pontchartrain) over the past 80 years (CPRA, 2012). In attempts to protect what remains and restore what has been lost, the state of Louisiana with local and federal agencies has been conducting restoration projects including: marsh creation, shoreline protection, river diversions and use of dredged material (Commission, 2011). Spending in 2010 alone on Louisiana coastal restoration was $618 million and total expenditures for 2014 have been projected at $725 million (Commission, 2011; CPRA, 2012). Projection rates differ on the amount of area that will be lost in the future but it is unanimous that without these efforts rate of land loss will increase. The largest portion of these costs is on construction (CPRA, 2012). A critical element in the construction component is vegetative material without which restoration of the natural systems would not be possible. Native coastal plants such as grasses are often used to build and stabilize sand dunes thereby creating an effective and sustainable restoration project. Plants are also cheaper to produce and install versus most manmade infrastructure (Williams, 2007). Additionally, maintenance of native vegetation is less costly as they are able to adapt to this dynamic beach environment; whereas manmade structures are static and susceptible to fracture.

The perennial grass Sea oats (*Uniola paniculata* L.) is a commonly used species for its dune building capabilities as well as its ability as a native to withstand the brutal coastal conditions of high wind velocities, evaporation rates and temperatures (Dahl and Woodard, 1977; Wagner, 1964; Woodhouse et al., 1968).

The plant material used in these projects is grown in greenhouses and nurseries from asexual propagation. When large amounts of identical, clonally propagated plants are

transplanted, genetic diversity is reduced. This lack of variability can hinder the sea oats community's ability to adapt during establishment and/or adverse conditions such as disease or environmental change leading to failure of these pricey projects (Kutner and Morse, 1996). One tactic to combat the lack of diversity in sea oats stands is through the development of improved, genetically distinct lines, and releasing them as commercially available cultivars via plant breeding (Knott et al., 2012). Although this results in higher-quality reliable material, breeding generally takes years. Production from seed is an alternative method yielding genetically diverse material which also interests growers because production costs could be reduced.

However, production of sea oats from seed is encumbered by a few challenges: limited seed production, poor seed storage, and low germination (Hester and Mendelssohn, 1987; Nabukalu, 2013). Sea oats' low germination could be attributed to seed dormancy and/or to pathogens, fungal or bacterial, which potentially reside internally, on seed surface or in the immediate soil zone (Arya and Perelló, 2010; Sharvelle, 1961). Germination reduction due to fungal infection has been reported for sea oats (Burgess et al., 2002; Hester and Mendelssohn, 1987). Fungicides are widely used in agricultural and ornamental production to protect seed which improves germination and seedling survival (Arya and Perelló, 2010). Therefore the first project in this thesis tests if commercial fungicides improve sea oats seedling production from seed for growers to provide coastal restoration projects with quality diverse seedlings.

An additional method to increase genetic diversity of sea oats incorporated into beach restoration sites is via plant breeding, therefore the LSU AgCenter initiated a sea oats breeding program in 2002. The program was successful in developing and releasing three clonal sea oats varieties, however this was accomplished after 10 years of research (Knott et al., 2012). Given the extreme rate of coastal land loss in Louisiana, sea oats varieties need to be released in a timelier manner. In order to evaluate and develop sea oats breeding populations and varieties requires lengthy travel to beach sites, which is both costly and time consuming. If selection could be performed in an environment closer to where plant breeders are stationed, efficiency of the program would greatly improve leading to swifter development of varieties and more efficient use of funds. Therefore the goal of the second study of this thesis is to begin preliminary research into determining

whether relocation of sea oats breeding nurseries and selection studies from a coastal to an urban location could be possible based on whether populations would perform similarly in both environments using populations from a previous study which were selected and unselected from a beach dune site as well as several sea oats varieties.

Sea oats are not only useful for building coastal sand dunes but are also aesthetically pleasing. Ornamental grasses are popular and important elements in landscape design. Their year-round aesthetic and ecological attributes make them unique among plant groups (Maddox and Kelly, 2009). They vary greatly in form, size, color and texture while also adding motion to the landscape (Greenlee, 1992). Grasses are also typically low maintenance, tolerant of tough growing conditions such as drought and poor soil, and resistant to many insects and diseases (Greenlee, 1992; Meyer, 1975; Ruter and Carter, 2000). Many native grasses are popular ornamentals which is exhibited by the trend of their growing use and currently the demand often exceeds supply in the southeast U.S. (Brzuszek and Harkess, 2009; Maddox and Kelly, 2009). Sea oats are adapted to coastal regions and some lines have exhibited tolerance to inland conditions as well (Knott, unpublished data). Because sea oats are native to this region while also having attractive aesthetic qualities they have the potential to be in the ornamental grasses marketplace. The last project of this thesis is an initial stage to select sea oats plants to be used in further selection studies and ultimately for ornamental use. Plants were selected in an urban environment based on exhibiting attractive characteristics or phenotypic traits of height, vigor (subjective ranking), color (particularly red/purple foliage), leaf width (subjective ranking), number of stems and tillers as well as form.

The objectives of this study were to: 1) assess the impact of commercial fungicides on sea oats germination, seedling survival, and seedling quality: 2) determine if the average performance of selected sea oats populations, unselected sea oats populations, and sea oats varieties differ when produced outside of the coastal zone and: 3) identify and select sea oats plants which exhibit ideal characteristics for ornamental use.

Chapter 2: Literature Review

Uniola paniculata

The species *Uniola paniculata* was formally identified by Carolus Linnaeus in 1753 as inhabiting coastal sand dunes, having stiff leaf blades and extensively creeping rhizomes. Historically, the species had been recognized in some form by writers in 1696, 1731 and 1739, until being identified as a member of the American genus *Uniola* in Linnaeus' *Species Plantarum* based on plant material from Virginia or the Carolinas (Wagner, 1964). The genus has since been narrowed, as interpreted by Yates (1966b) and Yates (1966c) to include only *U. paniculata* and *U. pittieri* Hack. *U. paniculata* resides generally along the eastern and southern coasts of the U.S. and has a rhizomatous growth habit and a loose panicle. The native perennial can be found almost continuously along the Atlantic and Gulf of Mexico coasts beginning in Virginia, south to Florida, south-west along the Gulf Coast, and further south to the Mexican state of Tabasco; including the Bahamas, Florida Keys and Cuba (Wagner, 1964; Yates, 1966a). In contrast, *U. pittieri* is found primarily on the Pacific Coast extending from lower California south to Mexico and Ecuador with a stoloniferous growth and contracted panicle (Gould and Shaw, 1983; Wagner, 1964; Yates, 1966b; Yates, 2003). Yates (1966b,c) also segregated some of the former Uniola taxa into the genera *Chasmanthium* and *Leptochloöpis* due to significant differences in chromosome number, leaf anatomy, embryo type, number of stamen, etc. (Estes and Tyrl, 1982; Gould and Shaw, 1983; Lonard et al., 2011; Yates, 1966b; Yates, 1966c).

Uniola paniculata is a grass commonly known as sea oats due to the resemblance of seeds to common oats (*Avena sativa*) (Williams, 2007). Sea oats is adapted to withstand brutal coastal conditions such as high wind velocities, evaporation rates and sand movement (Wagner, 1964; Woodhouse et al., 1968). Sea oats is a C4 plant, a unique photosynthetic quality which aids it to grow under high temperatures and low water or xeric situations (Brown and Smith, 1974; Dahl and Woodard, 1977; Woodhouse et al., 1968). Sea oats also tolerates drought and salt spray, a unique characteristic allowing it to dominate dunes and on which the cause has been speculated (Dahl and Woodard, 1977; Harper and Seneca, 1974). Some sources explain this tolerance to high levels of salt spray as a result of the heavily cutinized leaves that have large amounts of sclerenchyma cells which block and prevent chloride ions from negatively affecting live parenchyma tissue

(Boyce, 1954; Woodhouse et al., 1968). These cells could also help protect against desiccation and damage caused by strong winds which continually blow and beat at the leaves in coastal environments. Others point to the occurrence of the leaf ends rolling inward under dry or adverse conditions which minimizes surface area and therefore reduces contact and absorption of potentially hazardous chloride ions (Boyce, 1954; Woodhouse et al., 1968). Seneca (1972) also concluded that sea oats not only tolerates salt spray but apparently receives nutrition from it based on a study that found an increase in biomass under salt spray conditions compared to substrate salinity. However in an experiment by Valk (1977) mode of entry of salt spray was looked at by spraying just foliage with artificial sea water while covering the substrate thereby preventing entry of salt into substrate. No significant increase in biomass was observed thus suggesting that the leaves are an indirect source of significant nutrients by mechanically intercepting the salt spray (Valk, 1977). Boyce (1954) and Valk (1977) both demonstrated that salts from salt spray can wash off the leaves and into the substrate for temporary absorption. Studies have also suggested that the amount of nitrogen the plant receives affects foliar salt spray impact. Boyce (1954) found that available nitrogen in the substrate acts as a conditioning agent and dune grasses in North Carolina exhibited less resistance to salt spray injury when high levels of nitrogen were present. When lower concentrations of nitrogen are available, which is more similar to coastal dune conditions, salt spray injury is also lower (Valk, 1977; Woodhouse et al., 1968).

Sea oats dominate the front or sea-facing slope, crest of the foredune and have been observed to a lesser degree in the crest of the rear or hind dune (Wagner, 1964). Wagner (1964) compared measurements in a North Carolina park of moisture of soil and air, temperature of soil and air, pH, salt content, evaporation and salt spray in different zones within the coastal transect. The only factor he found to exhibit enough of a pattern to explain zonation of the vegetation was salt spray. However a more recent study of subtropical coastal dune complexes in southeastern Florida concluded that soil chemistry was the most influential factor in vegetation zonation (Lane et al., 2008). They found that as distance from the coast or shoreline increased, substrate pH and salt spray decreased while soil moisture, most nutrients (P, N, Ca, K, Mg, etc.), cation exchange capacity, and organic matter accumulation increased. Lane et al. (2008) also determined that salt spray

played a secondary role in determining plant species distribution and that sand movement had little effect. The reported pH's in the coastal region where sea oats is found ranges from approximately 7.6-9.6 but can vary depending on soil depth (Hester and Mendelssohn, 1990; Hester and Mendelssohn, 1991; Lane et al., 2008; Sylvia, 1989; Wagner, 1964; Will and Sylvia, 1990). Organic matter (O.M.) appears to be quite low as Wagner (1964) reported 0% O.M. for the foredune region and Lane et al. (2008) reported approximately 0.76-1.90% O.M. for soil 0-61cm (0-24in) deep and 6-19m (20-62ft) from the shoreline. The substrate in the vicinity where sea oats is typically found consists of quartz sand or quartz sand and shell fragment substrates (Judd et al., 1977; Lonard et al., 2011). Sand particle size was reported by Judd et al. (1977) as 0.18-0.25mm for about 95% of the sand on South Padre Island, Texas.

Visually sea oats has an overall hard texture with conspicuous, arching panicles. It has erect and glabrous stems that give the plant an overall upright form with a mature height reaching 1-2.5m (3.3-8.2ft) (Lonard et al., 2011; Radford, 1968; Westra and Loomis, 1966; Yates, 1966b; Yates, 2003). The stems are produced singly or in clumps with individual widths ranging 5-10mm (0.20-0.40in) thick (Lonard et al., 2011). The leaves have been described as long, thin and tapering to a point (Yates, 2003). The individual blades range from 20-80cm (7.9in to 2.6ft) in length by 4mm-1cm (0.16 – 0.39in) wide and are alternately arranged about the unbranched stem (Everitt, 2011; Lonard et al., 2011; Radford, 1968). A parallel venation pattern runs the length of the leaf with smooth and hairless margins and surfaces (Lonard et al., 2011; Radford, 1968; Yates, 1966a). The leaves are flat proximally, but distally the margins are commonly involute particularly when dry (Gould and Shaw, 1983; Lonard et al., 2011). Leaf sheaths are glabrous with small ciliate ligules measuring 2-5 mm (0.08-0.20in) long at the base (Everitt, 2011; Radford, 1968; Yates, 2003). Typically sea oats foliage is a bleached blue-green until winter, when dormant foliage fades to a straw color (Everitt, 2011; Fine, 2007; Yates, 1966a).

In natural beach environments sea oats plants are essential for building sand dunes. The extensive root and rhizome systems of sea oats respond to blowing sand to build sand dunes (Wagner, 1964; Woodhouse et al., 1968; Woodhouse and Hanes, 1967). As the sand particles are blown and deposited on the plant, growth is stimulated both from the sand

accumulation and the supply of nutrients brought by the film coated sand grains (Ehrenfeld, 1990; Wagner, 1964). Wagner (1964) noted that compared to stable sand, the influx of sand deposition increased plant vigor likely due to the steady fresh nutrient supply, regardless of leaching, that occurs through the substrate. Not only does it improve plant growth but also a cessation of sand accretion results in a deflation of substrate strata which if prolonged could actually harm plant roots and rhizomes by exposure and desiccation (Wagner, 1964). The plants' roots play an important role by spreading extensively throughout the below-ground environment to overcome low nutrient situations and gain a stronger, more stable hold for the plant in the shifting dunes (Ehrenfeld, 1990; Kearney, 1904; Wagner, 1964). Burial of the roots, rhizomes and base of the stem by windblown sand avoids desiccation and also stimulates internode elongation and bud formation resulting in an overall increase in height and density (Dahl, 1974; Ehrenfeld, 1990; Wagner, 1964). Vegetative reproduction occurs through adventitious buds that originate at the stem base below the soil surface in sequential layers (Figure 2.1) (Dahl, 1974; Wagner, 1964). The first buds are at acute angles to the stem and grow toward the surface becoming tillers (Dahl, 1974; Wagner, 1964). Subsequent buds form beneath the first and are thus forced out at more obtuse angles to eventual right angles from the stem axis developing into horizontal rhizomes about 3-15mm (0.12-0.20in) thick (Dahl, 1974; Ehrenfeld, 1990; Wagner, 1964) . From these prostrate rhizomes more adventitious buds and roots can sprout (Dahl, 1974; Wagner, 1964). The grass' shoots proliferate randomly around the stem circumference to form a mass which helps to combat the wind impact and increases sand accumulation (Dahl, 1974; Wagner, 1964; Woodhouse et al., 1968). This subsequent sand deposition also causes the shoot internode region to elongate in order to reach the surface and therefore grows upward later adding leaves from the apical meristem (Dahl, 1974; Wagner, 1964). Partial burial by shifting sand has been linked to increased aerial growth and rhizome extension (Dahl, 1974; Wagner, 1964). Both seeds and rhizome fragmentation due to natural processes, such as hurricanes, allow plants to populate new locations, typically as a pioneer species, and potentially establish distinct populations (Miller et al., 2001; Miller et al., 2003; Wagner, 1964; Williams, 2007; Woodhouse and Hanes, 1967). Research suggests that when rhizomes become fragmented

during a hurricane or other natural process they may be transported in wrack line and if kept sufficiently moist, establish new sites (Miller et al., 2001; Miller et al., 2003).

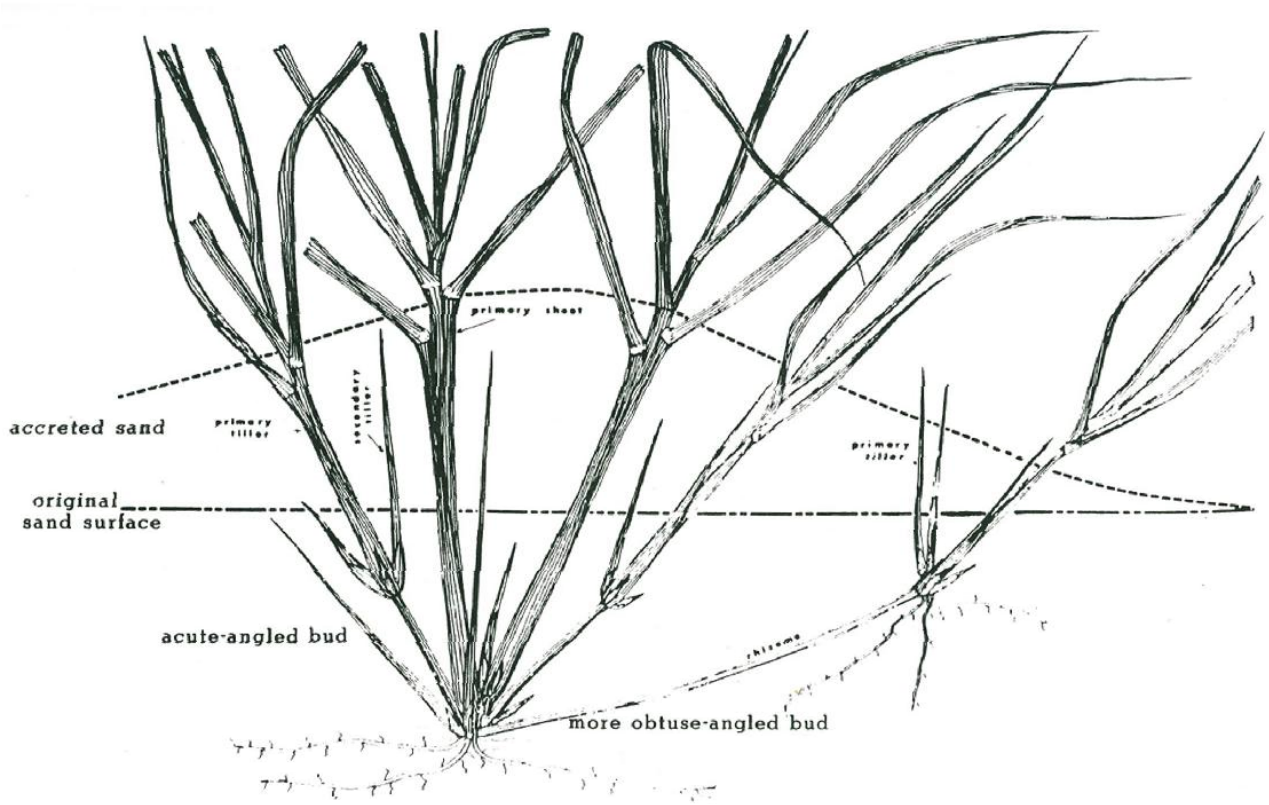


Figure 2.1 Diagram of common vegetative growth of sea oats showing stages and angles of new rhizomes as related to sand height (modified from Dahl (1974)).

Sea oats panicles are branched and typically measure 20-50cm (7.9-20in) long, 5-15cm (2-6in) broad and structurally capable to weather the wind and other stresses of their environment (Harper and Seneca, 1974; Lonard et al., 2011; Radford, 1968). The tall panicles bear many spikelets, the basic unit of the inflorescence, which emerge light green around late spring to early summer and grow quickly to full size by mid-summer (Everitt, 2011; Lonard et al., 2011; Wagner, 1964). Spikelets consist of a series of florets laterally compressed on the central axis or rachis. Within each floret are an ovary and three stamens with yellow anthers 4-6mm (0.16-0.24in) long lying between the upper smaller palea and lower larger lemma (Lonard et al., 2011; Wagner, 1964; Yates, 2003). The bottommost 2-8 florets are usually sterile and the 2 or more terminal florets are incompletely developed (Gould and Shaw, 1983; Wagner, 1964). The middle florets vary in

fertility and each spikelet could have 3 to 34 florets (Lonard et al., 2011; Wagner, 1964; Yates, 2003). The florets are open briefly in early morning allowing the opportunity for dissemination of the pollen by wind and then close (Hester and Mendelssohn, 1987). Wagner (1964) observed florets in a sample of North Carolina plants had a high rate of ovules aborting of the fertile florets, resulting in an estimated ratio of 1:4 viable seed to aborted ovules. Although the spikelets have many fertile florets, observed seeds per spikelet varies but all conclude seed production is very low (Hester and Mendelssohn, 1987; Wagner, 1964; Westra and Loomis, 1966; Yates, 1966b). At maturity the abundant, characteristic stramineous colored spikelets can weigh down the panicle causing it to droop and nod. The individual spikelets measure 1.5-5cm (0.6-2in) long by 0.6-1.6cm (0.2-0.6in) and are shed whole (florets intact) from the panicle at maturity, approximately fall and/or winter (Harper and Seneca, 1974; Lonard et al., 2011; Radford, 1968; Wagner, 1964; Westra and Loomis, 1966; Yates, 2003).

Floral initiation of sea oats does not occur uniformly over regions but on a geographical gradient and has been speculated to be governed by temperature rather than photoperiod. Harper and Seneca (1974) observed floral primordial initiation in May in North Carolina and infer earlier dates at more southern latitudes. They also observed that 3 growing seasons were typically required before flowering began on new plants, whereas on Padre Island, Texas it was noted that 3-4 years of growth was required for flower initiation (Dahl, 1974; Harper and Seneca, 1974). The seed of sea oats is a caryopsis, or one-seeded dry fruit, which consists of the embryo, endosperm and fused seed coat with ovary wall (Wagner, 1964). The shape is linear and fusiform and measures 3-5mm (0.12-0.20in) long by 1-1.5mm (0.04-0.06in) wide within which the embryo measures about less than half the total length (Everitt, 2011; Gould and Shaw, 1983; Westra and Loomis, 1966; Yates, 2003). When the panicle shatters and spikelets are disarticulated whole from the panicle, the seeds remain in the florets on the rachis, become covered by sand and stay dormant until spring (Gould and Shaw, 1983; Westra and Loomis, 1966; Yates, 2003).

Wind is the main method of seed dispersal; however seasonal inundations of interdune swales can also transport spikelets and deposit them at wrack lines and depressions remote from parent stands (Ehrenfeld, 1990; Wagner, 1964). Environmental inhabitants play significant roles in seed loss. At least 18 insect species have been observed to damage

seed, such as beetles and sucking bugs, and occasionally bacteria occupy florets (especially aborted ovules) (Ehrenfeld, 1990; Wagner, 1964). These are followed by high infestations of fungi with invading hyphae and conidia masses such as *Alternaria* sp. and *Helminthosporium* sp. Additionally, seed feeders like small mammals (various mice species) and birds, such as the red-winged blackbird and song sparrow, account for around an 80% yield reduction (Wagner, 1964). The ghost crab (*Ocypode quadrata*) has also been noted to intercept spikelets and sequester them in their burrows (Wagner, 1964). In this way the seeds and plant serve as an important part of the coastal food web. Despite these many unfavorable factors, a small percentage of seeds potentially germinate the following spring. The successful seedlings sprout from a burial around 1-5cm (0.4-2in) but no deeper than 10-15cm (4-6in) below the soil surface and do so over a period of a few months (Hester and Mendelssohn, 1991; Wagner, 1964; Westra and Loomis, 1966). This coverage from sand accretion provides protection from predators, prevents desiccation and maintains an adequate temperature (Hester and Mendelssohn, 1991; Wagner, 1964). The staggered germinations are also due in part to varying degrees of sufficient endosperm to fuel emergence from burial depths (Ehrenfeld, 1990; Wagner, 1964).

The caryopsis has been reported to exhibit a degree of dormancy. Westra and Loomis (1966) found dormancy to peak about 6 months after maturation which they attributed to a diffusible inhibitor retained by the testa despite its permeability to liquid and gas. Breaking dormancy by cold stratification has shown to drastically improve germination rates in northern populations (Virginia to Carolinas), but lesser so and with varying responses in southern regions (Seneca, 1972; Woodhouse et al., 1968). Gulf Coast populations exhibited an intermediate degree of improvement in germination rates with cold stratification as compared to northern populations and those from Florida, which showed none (Seneca, 1972). The duration of germination has shown to be affected by the length of moist cold layering during winter (or artificially for 15-30 days around 4.4^0C (40^0F)) (Burgess et al., 2002; Hester and Mendelssohn, 1987; Woodhouse et al., 1968). Generally, the longer stratification, the shorter the germination period, however more time shows increases in the incidence of fungal attacks and seed decay (Burgess et al., 2002; Seneca, 1972; Wagner, 1964). The cold stratification mimics burial by sand in the dune-strand habitat (Wagner, 1964; Westra and Loomis, 1966). Studies by Hester and

Mendelssohn (1987) with Louisiana seed found that the moist and cold pre-germination treatment did not improve germination rates but did reduce duration to reach 50% germination. Westra and Loomis (1966) found moist moderate chilling temperatures to be of some effect in some seed populations from North Carolina but only if followed by warm conditions (30-40^{0}C). They indicated that chilling was not necessary if a max high temperature (around 40^{0}C) was reached, which they assumed results in the breaking of germination inhibitors within the seed (Westra and Loomis, 1966). Following the pre-germination treatment, many experiments have found consistently high rates of improved germination by alternating thermoperiod, commonly 17 hours at 18.3^{0}C (65^{0}F) and 7 hours at 35.0^{0}C (95^{0}F) (Hester and Mendelssohn, 1987; Seneca, 1972; Wagner, 1964).

Various studies have been conducted on sea oats seeds in order to improve germination success. Studies have shown that light has no effect on germination, however in laboratory experiments it commonly occurs in dark incubation (Burgess et al., 2002; Westra and Loomis, 1966). Experiments on seeds using chemical treatments such as gibberellic acid, KNO_3 and thiourea had varying results but some found to only slightly increase germination, whereas scarification by cutting into the endosperm and leaching of the caryopsis resulted in 100% germination (Westra and Loomis, 1966). Interestingly, the caryopses of sea oats, along with a few other dune-building grasses of the Atlantic and Gulf coasts, have been found to be highly sensitive to salt and do not germinate in sea water. Studies found the maximum substrate salinity tolerated during germination was between 1 and 1.5% sodium chloride and seedlings rapidly decline at NaCl concentrations between 1 and 2% (Ehrenfeld, 1990; Senaca, 1969; Seneca, 1972; Woodhouse et al., 1968). Pathogens of sea oats seeds are an additional concern since they reduce germination and could possibly be minimized with chemical treatment or plants bred with resistance. There have been only a few but significant studies on seed pathogens with the most recent observing that the highest number of incidences occurred at 21 days after germination (Nabukalu and Knott, 2013). The greatest number of pathogen incidences was also found when seed was stored at -20^{0}C at ambient moisture levels, which has been speculated to be a result of chilling injury suffered by the seed causing it to be more susceptible to attack (Nabukalu and Knott, 2013). Previous sea oats research typically has employed the seed surface sterilization step of 25% Clorox for 15 minutes and during germinations those

compromised by fungi or bacteria are removed and discarded (Hester and Mendelssohn, 1987; Woodhouse et al., 1968). Burgess et al. (2002) used a submersion in 1.3% sodium hypochlorite for 15 minutes to disinfest seed surface however seed pathogens were still observed which reduced germination percentages. They also noted a greater decay in seed caused by various fungal and bacterial pathogens which lead to a reduction in germination with longer cold stratification periods (30+ days), however there were no efforts made to identify the pathogens (Burgess et al., 2002). The authors suggested conducting studies on different treatment methods prior to stratification would likely improve germination percentages (Burgess et al., 2002).

Storage conditions for seeds have been found to affect germination percentages and depends on temperature as well as moisture (Copeland and McDonald, 2001). Recent studies investigated the effect of various storage protocols over a 12 month period to mimic commercial methods such as storage at room temperature, 4^0C, -20^0C, hermetically sealed and/or open to ambient moisture levels (Nabukalu and Knott, 2013). This objective was evaluated using germination rates and percentages. All seeds were germinated at 18.3^0C for 17 hours and then at 35^0C for 7 hours in a dark environment (Nabukalu and Knott, 2013). It was found that sea oat seeds stored at room temperature ($20\text{-}25^0$C) in hermetically sealed jars had the highest average germination (Nabukalu and Knott, 2013). The study by Nabukalu and Knott (2013) used seed harvested from Long Beach, MS to also determine average duration to reach maximum germination percentage which was 21 days. Seed moisture was tested and reported on for the first time. A range of 6-16% seed moisture content was observed and was negatively correlated with germination, a defining characteristic of orthodox type seeds (Nabukalu and Knott, 2013).

Ornamental Grasses of Louisiana/Southeast U.S.

Ornamental grasses have been growing in popularity across the southeastern United States for at least the past decade (Ruter and Carter, 2000). They hold a unique place in the landscape both aesthetically and ecologically. A few attractive qualities of ornamental grasses for a landscaper or gardener are the low maintenance which many grasses necessitate, the low water requirement and the year-round visual interest (Meyer, 1975; Ruter and Carter, 2000). A survey of Georgia landscape architects found the two most

important factors determining plant material choice were water requirement and low maintenance (Garber and Bondari, 1993). Other distinct attributes of ornamental grasses is that they vary widely in form, size, color and texture and can add motion to the landscape (Greenlee, 1992). The broad term 'grass' can include plants from many families, however botanically speaking 'true' grasses are monocots belonging to the family Poaceae (also called Gramineae) (Allen et al., 2004; Greenlee, 1992; Grounds, 2004; Loewer, 2003). Other plants commonly counted as grasses include sedges and rushes from the families Cyperaceae and Juncaceae, respectively (Allen et al., 2004; Greenlee, 1992; Grounds, 2004). Ornamental grasses are an ideal choice in the landscape for their visual interest and relief from tiresome turf which typically requires a higher input of chemicals and mowing (Greenlee, 1992). Grasses can also tolerate tough growing conditions, are very insect and disease resistant, and are prevalent over much of the global terrain (Greenlee, 1992; Meyer, 1975). Although there are no maintenance-free plants, ornamental grasses come close.

Recent trends have been towards using native plants in landscape designs and gardens for their adaptability, hardiness, low maintenance and diversity (Maddox and Kelly, 2009; Service, 2010). A recent survey study explored the use of native plants by landscape architects in the southeastern United States and found they are using a significant proportion of regional native species in their projects in efforts to increase sustainability (Brzuszek and Harkess, 2009; Maddox and Kelly, 2009). It also pointed at a potential market for expansion in the production of indigenous plant species of the southeast U.S. (Brzuszek and Harkess, 2009). Several reasons were cited for the enhanced use of natives in other regions of the country some of which were: increased awareness of environment, development laws requiring native plantings, increased availability of plant species, and the need for low water use in the landscape (Brzuszek and Harkess, 2009). Researchers advocate the use of native plants for their suitability to the regional ecosystem which are specifically intertwined structures (Maddox and Kelly, 2009; Service, 2010).

Sea oats is a native grass that has recently received interest for use as an ornamental grass in residential landscapes. Sea oats is a dune grass that prefers well drained, sandy soils (Fine, 2007; Wagner, 1964; Woodhouse et al., 1968). High or excessive soil moisture by means of a shallow water table or inundation can prove detrimental to sea oats. Hester and Mendelssohn (1989) found that growing plants as close as 30cm (12in) above the

water table negatively affected plants and more severely so than subjection to drought stress. Plants were able to recover from drought-induced stress whereas prolonged water logging resulted in plant death. This aided in explaining the observation that in Louisiana there is an absence of sea oats in depressions and low lying areas where the water table is close to the soil surface (Hester and Mendelssohn, 1989). Sea oats fair best when grown atop dune crests and therefore furthest from the water table (Oosting and Billings, 1942). Hester and Mendelssohn (1991) recommended an ideal planting height for the deep-rooted sea oats of 1.3m (4.3ft) above the water table based on established successful stands observed in Louisiana. These reports suggest that sea oats would not perform well in a residential landscape environment. However, in preliminary field trials 40 sea oats genotypes were evaluated for landscape potential at Louisiana State University's Burden Museum and Gardens in Baton Rouge, LA. Sea oats plants were transplanted in 2010 and after 3 years 28 survived and 4-5 demonstrated potential for use in residential landscape applications (Knott, unpublished data). Preliminary evaluations of sea oats genotypes also indicate that variation exists for dormant foliage color (green/red) and spikelet color (green/red) (Knott, unpublished). Using breeding techniques to maximize gene re-assortment and select for color variation, lines could be developed which display these phenotypic traits ideal for ornamental purposes.

Fungal symbiont

Most plant species worldwide have fungal associates. The affiliation between plants and fungi is one that dates back at least 400 million years and has been reported from plant fossils containing fungal endophytes (Krings et al., 2007). It is theorized that they enabled the evolutionary transition from an aquatic existence to life on land as photoautotrophs (Paracer and Ahmadjian, 2000; Selosse and Le Tacon, 1998). Additionally, benefits to host plants continue to be found such as increased growth, drought tolerance and improved resistance to various stresses, such as UV radiation, herbivores and parasitic organisms (Bacon and Hill, 1996; Cheplick and Faeth, 2009; Leuchtmann, 1993; Selosse and Le Tacon, 1998). The relationship between fungi and plants can range on a wide spectrum from extremely parasitic to mutualistic on the point of dependency. Additionally, their interaction is highly affected by the environment as well as the genetics of both the host

plant and the symbiont (Cheplick and Faeth, 2009). Without an implication as to the type of ecological relationship, a symbiosis is the close association of different species, regardless of any benefits which may or may not exist (Douglas, 1994). The term symbiosis was originally coined by plant pathologist Anton de Bary and usually applies to interactions over an extended period of time rather than those of a short, fleeting duration such as an insect pollinating a flower (Douglas, 1994). The word 'endophyte' literally translates to 'in the plant' (from Greek 'endon': within and 'phyton': plant); in this way the term potentially could be interpreted to include bacteria, fungi, algae, and insects (Schulz and Boyle, 2005; Wilson, 1995). Some debate over the semantics of the term 'endophyte' exists due to the interpretation by some of an implied mutualistic relationship even though one might not exist (Clay and Schardl, 2002; Stone et al., 2000; Wilson, 1995). However, an endophyte is generally and commonly defined as any microorganism, usually bacteria or fungus, which lives within a plant (Clay and Schardl, 2002; Stone et al., 2000; Wilson, 1995). Ecologists who study plant symbiotic interactions utilize three major classifications: parasitism, commensalism and mutualism (see table 1) (Cheplick and Faeth, 2009; Paracer and Ahmadjian, 2000). In each category the fungus benefits from the partnership, because the endophyte is an obligate symbiont and does not exist independently of its host (Bacon and Hill, 1996; Cheplick and Faeth, 2009). Whereas the effect on the host plant by the fungus can range from negative in a parasitic or pathogenic interaction, neutral in a commensalistic association, or positive as with mutualistic relationships (Cheplick and Faeth, 2009; Paracer and Ahmadjian, 2000). Because an endophyte may colonize plant tissue without causing any symptoms expressed by the host as with commensalism, the endophytic infection may go unnoticed or be asymptomatic (Petrini et al., 1993; Stone et al., 2000; Wilson, 1995). The method employed to isolate endophytes from a plant host affects the collection of taxa that is found. There is usually a numerous quantity of endophytic species found however a few dominant host-specific species or strains often prevail (Carroll, 1988; Petrini et al., 1993). Another potential complication during identification of an endophyte is of latent infections or the asymptomatic residence of a pathogenic fungus within the plant for an indefinite period of time (sometimes years) until eventually becoming evident; this has been observed with some smut diseases (Carroll, 1988; Clay and Schardl, 2002; Schulz and Boyle, 2005; Sinclair

and Cerkauskas, 1996). It is also hypothesized by some that endophytes, in contrast to known pathogens, generally have more phenotypic plasticity and therefore more options than pathogens, such as: local or extensive colonization, latency, virulence, and/or saprophytism (Belesky and Malinowski, 2000; Schulz and Boyle, 2005). Some researchers perceive the parasitism-mutualism continuum as a means to describe evolution and that this phenotypic plasticity is what propels it (Clay and Schardl, 2002; Schulz and Boyle, 2005).

Table 2.1 Range of possible fungal-plant interactions (Copied from Cheplick and Faeth (2009))

Relationship	**Symbol (Fungus, plant)**	**Defining features**	**Examples**
Parasitism	(+, -)	Beneficial to fungus, detrimental to host	Plant pathogens, *Epichloe* endophytes
Commensalism	(+, 0)	Beneficial to fungus, no effect on host	Some endophytes
Mutualism	(+, +)	Beneficial to fungus, beneficial to host	Mycorrhizae, *Neotyphodium* endophytes

Many present day fungi are found in herbaceous and woody angiosperms, grasses, trees, dicots and monocots. Endophytes may be found in any organ of the plant and have been reported worldwide in various plant parts: leaves, stems, roots, branches, bark, xylem and needles (Carroll, 1988; Schulz and Boyle, 2005; Stone et al., 2000). However, in grasses they are most often concentrated in the leaf sheath and stem (Cheplick and Faeth, 2009). The hyphae can be seen when stained under magnification and are found intercellularly or between the cells but do not penetrate them (Bacon and White, 1994; Cheplick and Faeth, 2009; Clay and Schardl, 2002). The hyphae grow in the intercellular space and likely subsist on sugars and amino acids released into the apoplast between plant cells (Clay and Schardl, 2002). Studies employing electron microscopy to observe natural associations and those induced by inoculation have found a synchronized plant and endophyte growth, where hyphae grow as the leaf does and stops when the host ceases growth (Cheplick and Faeth, 2009; Christensen et al., 2002). This pattern also partially explains why hyphae in many grasses are commonly viewed under microscope as running parallel to the leaf axis and is seldom branched (Christensen et al., 2002; Clay and Schardl, 2002). A common endophytic example is found in mycorrhizal fungi which infect the roots of many plants

(Cheplick and Faeth, 2009; Douglas, 1994). This association has been repeatedly proven to improve a plants' performance, particularly in substrate low in nutrients (Douglas, 1994). The plant's ability to uptake essential mineral nutrients, notably phosphate, from the soil is enhanced while the fungus benefits through the metabolic use of photosynthates from the plant (Cheplick and Faeth, 2009; Douglas, 1994). The symbiosis is mutualistic if the advantage of improved mineral nutrition outweighs the cost of providing photosynthetic carbon to the myccorhizal fungus (Cheplick and Faeth, 2009; Douglas, 1994). However, the myccorhizal fungi could be considered parasitic if the net cost to the plant of the symbiosis exceeds the net benefits (Cheplick and Faeth, 2009; Johnson et al., 1997). This could arise during development, by the environment, or possibly by genotypes. Predictive models which account for these complex interactions are useful in management of agriculture, forestry and restoration (Johnson et al., 1997). The species diversity of endophytes is extremely rich, possibly rivaling that of insects, as more are continued to be discovered (Arnold et al., 2000; Carroll, 1988; Clay and Schardl, 2002). Arnold et al. (2000) described fungal endophytes as ubiquitous in the plant kingdom and after examining samples from tropical plants they postulated that fungal endophytes may be hyperdiverse.

Many grasses around the world have been reported to host endophytes and more are continually being found (Carroll, 1988; Cheplick and Faeth, 2009; Paracer and Ahmadjian, 2000; Selosse and Le Tacon, 1998). Leuchtmann (1993) quantified 290 grass species as having endophytes from the family Clavicipitaceae and suggested 20-30% of grass species globally may be infected. The family of fungi that infect grasses is classified as Clavicipitaceae (phylum Ascomycota) and may be a monophyletic clade but this remains unclear (Cheplick and Faeth, 2009; Clay and Schardl, 2002). This family of fungi includes symbionts of grasses (and sometimes sedges) that can form pathogenic or mutualistic relationships with their hosts. The family consists of four tribes, three of which infect only grasses or sedges (Clay and Schardl, 2002; Kuldau et al., 1997). The most diverse tribe is the Balansieae which has traditionally been grouped into four genera: *Atkinsonella*, *Balansia*, *Epichloë* and *Myriogenospora* but contains an additional few (Clay and Schardl, 2002; Kuldau et al., 1997; White, 1997). Some of the 37 species within these genera produce toxic compounds called alkaloids just as the well-known ergot pathogens from the tribe Clavicipeae (i.e. *Claviceps*) which parasitize a wide range of grasses (Cheplick and

Faeth, 2009; Clay and Schardl, 2002). The *Claviceps* spp. which cause ergot disease are not endophytes but do produce the same toxic alkaloids and their mode of action is to replace the seed in the florets with sclerotia (Bush et al., 1997). Most of the genera in the tribe Balansieae can reproduce sexually through production of stromata and ascospores but one genus, *Neotyphodium*, consists entirely of asexual species (Cheplick and Faeth, 2009; Kuldau et al., 1997). *Neotyphodium* species have been found to have evolved from sexual *Epichloë* species (Cheplick and Faeth, 2009; Schardl and Leuchtmann, 2005; Schardl, 1996). Much research has been done on these last two genera and the grasses they infect (Bacon et al., 1997; Roberts et al., 2005; Schardl and Leuchtmann, 2005). *Neotyphodium* commonly infects globally important forage crops, cool-season grasses and turfgrasses including the plant genera *Festuca* and *Lolium*, the most common of which are tall fescue and perennial ryegrass (Bacon and Hill, 1996; Bacon et al., 1997; Roberts et al., 2005).

Transmission of endophytes can occur in either a vertical or horizontal method (Carroll, 1988; Cheplick and Faeth, 2009; Clay and Schardl, 2002; Schardl, 1996). Asexual endophytes (*Neotyphodium* spp.) are passed on vertically to other generations of plants by infecting developing seed while residing in adult host plants (see figure 2) (Cheplick and Faeth, 2009; Clay and Schardl, 2002). Horizontal transmission is a means for sexually reproducing fungi (Epichloë) to spread their spores in order to warrant successive generations and generally occurs around flower initiation by the host, which is likely due to a localized increase in sugars in the plant (Clay and Schardl, 2002; White et al., 1991). The endophyte visually emerges from the mature host to form fruiting bodies containing spores which then inhibit or abort the host's reproductive organs (which results in choke disease) (Clay and Schardl, 2002; Schardl and Leuchtmann, 2005; White et al., 1991). An additional method of horizontal transmission can be for the fungi to produce stromata which bear fruiting bodies that appear on host leaf surfaces, as observed with genera *Myriogenospora* and *Balansia* (Cheplick and Faeth, 2009). The spores disseminate from their fungal structures and are spread horizontally to infect new plants by various vectors such as wind, water, insect or other means (Bultman et al., 1995; Cheplick and Faeth, 2009; Clay, 1991).

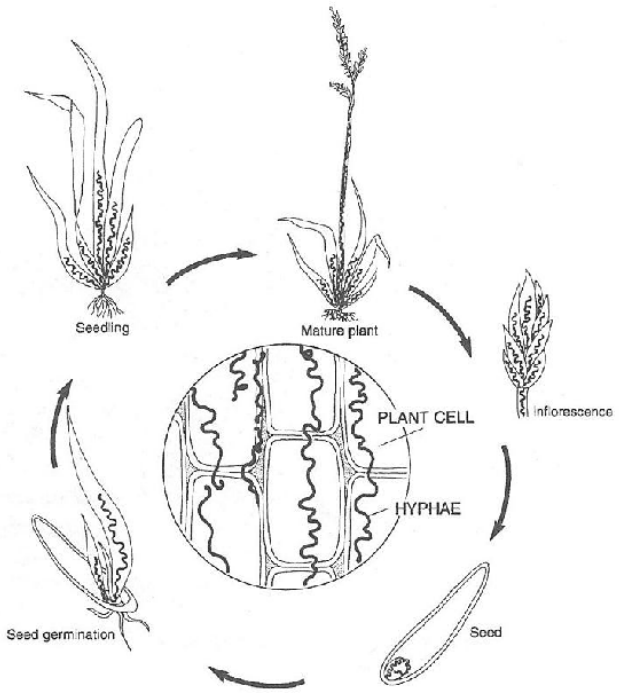


Figure 2.2 Life cycle of *Acremonium coenophialum*, vertical transmission. Copied from Paracer and Ahmadjian (2000).

Much research has been done on pastoral grasses infected with endophytes as some have a significant ecological and economic impact (Bacon et al., 1997; Cheplick and Faeth, 2009; Clay and Schardl, 2002; Roberts et al., 2005). Many of these pastoral endophytes produce alkaloids which are harmful to grazing animals, insects and nematodes (Bacon et al., 1977; Bush et al., 1997; Clay, 1991; Paracer and Ahmadjian, 2000; Schardl, 1996). In fact, the intense endophytic research focus over the past 20-30 years was mostly initiated and motivated by the realizations that a common toxic syndrome of cattle grazing on tall fescue grass (Festuca arundinaceae Schreber) and of sheep grazing on perennial ryegrass (Lolium perenne L.) was strongly correlated with endophyte infection (Bacon et al., 1977; Clay and Schardl, 2002; Leuchtmann, 1993; Schardl, 1996). The endophyte-tall fescue symbiosis has become somewhat of a model system for endophyte studies with a substantial body of data (Clay, 1991). Some of the disorders observed in cattle grazing on or fed endophyte-infected tall fescue include reduced daily weight-gain, fescue foot, fat necrosis, fescue toxicosis, reduced milk production and increased incidence of stillborn calves (Schmidt and Osborn, 1993; Thompson and Stuedemann, 1993). Beef cattle losses in

the US due to endophytes in tall fescue were estimated by Hoveland (1993) to be at least 600 million dollars. One of the first publications relating endophytes to herbivore resistance was by Bacon et al. (1977) who found a clavicipitaceous fungus at 100% frequency in tall fescue grass in pasture where cattle exhibited symptoms of fescue toxicity syndrome versus pasture with 0-50% infection frequency where cattle exhibited no symptoms. Bacon et al. (1977) identified the fungus as *Epichloë typhina* based on similarity to morphology of isolates from bent grass (*Agrostis perennans* L.). However, subsequent examinations of growth characteristics and morphology of the endophyte isolated from tall fescue reclassified the anamorphic stage as *Acremonium coenophialum* Morgan-Jones and Gams which later became *Neotyphodium coenophialum* (Bacon and Hill, 1996; Belesky and Malinowski, 2000; Glenn et al., 1996; West, 1994). The endophyte has coevolved with its host, tall fescue, within which it produces mycelia but does not sporulate and is passed on vertically via infected seeds. Tall fescue grass is also tolerant of abiotic stresses such as drought conditions, high temperatures and low soil fertility, which can be attributed to the endophyte-grass symbiosis (Bush et al., 1997; Cheplick and Faeth, 2009; West, 1994). In addition to livestock, wild herbivores such as voles, rabbits, some bird species, Canadian geese and rodent species have been found to suffer after the consumption of endophyte-infected tall fescue (Clay, 1991; Clay and Schardl, 2002; Conover and Messmer, 1996; Fortier et al., 2000; Giuliano et al., 1994). Studies have shown the animals initially do not discriminate and eat both infected and uninfected tall fescue and/or seed but following weight loss they prefer non-infected forage or seed (Clay, 1991; Clay and Schardl, 2002; Conover, 1998; Madej and Clay, 1991). It was also noted that the eating habits of wild animals is somewhat more difficult to study as they are able to move and choose other foods whereas livestock are confined to their pens with no choice but to consume endophyte-infected grass (Clay, 1991; Clay and Schardl, 2002).

Tall fescue originates from temperate and cool climatic areas with a wide native distribution throughout Europe, North Africa, and west and central Asia (Bacon and Hill, 1996; Belesky and Malinowski, 2000). However, in the United States it is a cool-season grass grown extensively in the eastern region of the country (Belesky and Malinowski, 2000). Despite growing outside of its typical adaptation zones, this perennial temperate grass is able to thrive. Considerable evidence exists that endophyte-infected tall fescue has

enhanced growth and vigor, notably improved shoot biomass, number of tillers, and nitrogen utilization, which give it a competitive advantage over non-infected plants (Bacon and Hill, 1996; Belesky and Malinowski, 2000; West, 1994). Research has shown that alkaloid production is affected by mineral nutrition in the substrate (nitrogen, phosphorous, calcium) and gaining an understanding of the mechanisms involved in the endophyte will aid management to reduce livestock toxicity (Bacon and Hill, 1996; Belesky and Malinowski, 2000; Malinowski and Belesky, 2000). One mutualism example amongst many is in the southeastern coastal plain in the state of Georgia where endophyte-infected tall fescue grew and persisted while non-infected isogenic material succumbed to stresses associated with the environment (Bouton et al., 1993; Schardl, 1996). Several studies of infected plant superiority in highly stressful environments along with livestock selectivity for non-infected plants suggest a resulting alteration of the population dynamics and biodiversity in pasture and field settings (Belesky and Malinowski, 2000; Cheplick and Faeth, 2009; Clay and Holah, 1999; Clay et al., 1993; Hill et al., 1991). These effects are still being researched however, as well as a comparison to performance of native or wild grasses infected with endophytes (Faeth et al., 2004; Faeth and Sullivan, 2003; Rudgers and Swafford, 2009). Although additional studies of native grasses and endophytes in natural ecosystems needs to be conducted in order to offer applicable generalizations, evidence suggests that infection in wild grasses does not improve competitive abilities and may decrease it (Brem and Leuchtmann, 2002; Cheplick and Faeth, 2009; Faeth et al., 2004; Faeth and Sullivan, 2003; Rudgers and Swafford, 2009).

Fungicide Research

The development of seed-treatment fungicides can be traced back to the mid-17th century when it was observed that wheat seed salvaged from salty sea water having spilled in shipwreck resulted in plants un-plagued with bunt as was other wheat seed. Farmers then began brining grain in order to control what they did not understand (Morton and Staub, 2008; Sharvelle, 1961; Torgeson, 1967). It was not until 1755 that the scientist Tillet proposed the cause of wheat bunt as a seed-borne fungal pathogen (*Tilletia tritici*, *T. laevis*) capable of being controlled with lime and/or lye (Morton and Staub, 2008; Torgeson, 1967). In the 1800's compounds such as lime sulfur, the Bourdeaux mixture,

mercury chloride, and others were developed to control plant pathogens; however their use was somewhat limited as preparation of these primitive fungicides was typically done on site with little regard for safety of the user or environment. However once patented products became available they were widely applied to crops, notably fruit and vegetable, at high rates up until the 1930's and 40's (Gianessi and Reigner, 2006). This was in part because control of the disease was unreliable leading to frequent applications and often phytotoxicity (Morton and Staub, 2008). The 1950's saw a switch to synthetic fungicides which significantly improved yields of crops such as apple and potato (Gianessi and Reigner, 2006). Since the 1940's to 2002 there has been a drastic reduction in the total amount of fungicides applied in the U.S. due to more effective and selective fungicides developed and introduced on the market over those decades (Gianessi and Reigner, 2006). In addition to the rapid evolution of chemical fungicides that the last century or so has seen, the methods of application and machinery involved has greatly advanced ranging from fumigants, seed slurries, broadcasting, incorporated, etc. (Torgeson, 1967).

Since the inception of fungicides the protection of plants has been the main goal for agricultural use, and more recently ornamental use. Yield can be significantly improved upon with the application of modern, highly efficient and selective fungicides with minimal side effects (Gianessi and Reigner, 2006; Lyr, 1995). Fungicidal application at seeding is a traditional and widely-used method to protect germinating seed from suspect fungal pathogens which can also affect seedling survival (Arya and Perelló, 2010). In particular the 'in-the-row' application method was used perhaps for the first time in 1947 to treat onion smut using a solution of formaldehyde which was dripped into the open row after seed was dropped and before row was closed (Torgeson, 1967). Pathogens targeted are potentially located on seed surface, within the seed, or the immediately surrounding soil zone, depending on the fungicide. Additionally, these pathogens could parasitize the seed prior to or during germination, or revive later and attack the seedling as a latent infection (Arya and Perelló, 2010; Sharvelle, 1961). Diseases can result from vertical or horizontal transmission of fungal spores via seed from previous years or enter seed undetected during storage (Arya and Perelló, 2010). Chemical treatments at the early stages of a plant's life when more vulnerable before they establish a well-developed root system ensures growers a greater number of surviving plants potentially leading to higher yields

(Arya and Perelló, 2010; Sharvelle, 1961). "Seed treatment is a useful and indispensable tool in our arsenal of defense against losses caused by plant diseases" (Sharvelle, 1961).

Background on Fungicides Used in Experiment

The fungicide iprodione belongs to a group of chemicals called dicarboximides and was introduced on the market in 1974 by Rhone-Poulenc (Lacroix et al., 1974; Lyr, 1995). It is used worldwide to treat fungi affecting such crops as stone fruit, grapes, vegetables, berries, ornamentals as well as turf (Lyr, 1995; Roberts and Reigart, 2013). Common diseases controlled are of the following genera: *Botrytis*, *Sclerotinia*, *Monilinia*, *Alternaria*, *Phoma*, *Rhizoctonia*, and *Fusarium*. In ornamental bulb production dicarboximides are also applied as disinfectant dips against various pathogens including *Curvularia*. Iprodione is a contact fungicide and its main mode of action is by inhibiting DNA and RNA synthesis and cell division in fungi (Lyr, 1995). Studies have reported enhanced degradation of iprodione in soils which have been previously treated with this chemical as well as developed resistance by fungi with repeat use (Walker and Welch, 1990).

The fungicide azoxystrobin belongs to the strobilurin family of fungicides which were recently developed in the 1990's (Roberts and Reigart, 2013). It originates from a natural antifungal compound, strobilurin A, emitted by mushrooms of the basidiomycete *Strobilurus tenacellus* as a defense against competitors (Anke et al., 1977; Knight et al., 1997; Lyr, 1995). The range of fungi which strobilurins are active against is broad, covering all four taxonomic groups of fungi, but selective; varying greatly in activity within genera with both preventative and curative properties (Knight et al., 1997; Lyr, 1995; Roberts and Reigart, 2013). Strobilurins are widely used in agriculture on many different types of crops, notably cereals, soybeans and corn (Morton and Staub, 2008; Roberts and Reigart, 2013). Azoxystrobin is a systemic fungicide classified by the Environmental Protection Agency (EPA) as a reduced risk pesticide and its mode of action, as a strobilurin-based fungicide, is through inhibition of mitochondrial respiration of fungi by disrupting the electron transfer at the cytochrome complex III (Becker et al., 1981; EPA, 2013; Kim et al., 2003; Lyr, 1995).

The fungicide commonly referred to as thiophanate-methyl is assimilated into the class of chemicals known as benzimidazoles (Lyr, 1995). The benzimidazoles were introduced in the 1960's and became widely used due to their unique properties including efficacy at

low doses, active against a broad spectrum of fungi and systemic action in host which provides control even after infection. This class of fungicides is also highly selective and therefore effect on non-target organisms is minimal, but they are so specific that resistance development is a major problem (Lyr, 1995; Morton and Staub, 2008). Although they are effective against a broad range of fungi including most ascomycetes and some of the basidiomycetes and deuteromycetes, activity against oomycetes has not been observed, with the exception of *Plasmodiophora brassica* (Bollen and Fuchs, 1970; Edgington et al., 1971; Lyr, 1995). Thiophanate-methyl is commonly used on turf and ornamental plants (Milne, 2004). Benzimidazoles are used as protectants since they bind tightly to plant surfaces and degrade slowly but may also move systemically within the plant after entering wounded sites lending preventive and/or curative effects to untreated parts of the plant (Lyr, 1995; Milne, 2004). Their mode of action is by inhibiting fungal mitosis at a single site during microtubule assembly (Lyr, 1995; Morton and Staub, 2008).

The chemical mefenoxam is the active isomer in metalaxyl, which has been a widely used fungicide since its introduction in 1977 (Lyr, 1995; Morton and Staub, 2008). Metalaxyl belongs to the chemical class phenylamides, one among a few classes limited to controlling plant diseases only caused by oomycetes, such as *Pythium* and *Phytophthora* spp. The fungicide mefenoxam was introduced in 1996 by Syngenta® and can be used on vegetables, ornamental crops, turf grasses, non-bearing fruit and nut trees and conifers grown in nursery or greenhouse conditions (Kegley et al., 2010; Morton and Staub, 2008; Syngenta, 2009). A notable advantage to mefenoxam over metalaxyl is its comparable effectiveness at controlling plant diseases at half the dosage rate as well as having a shorter half-life (Nuninger et al., 1996; Syngenta Crop Protection, 2005). Consequently, it poses a less estimated risk to the environment, leading to its classification as a reduced risk pesticide by the EPA. Although it has a low toxicity rating, leaching into ground water is of some concern (EPA, 2013; Syngenta Crop Protection, 2005). Mefenoxam acts systemically being absorbed and degraded rapidly within plant tissue and its mode of action is by inhibiting RNA synthesis (Lyr, 1995; Syngenta Crop Protection, 2005).

Chapter 3: Effect of Fungicides on Production of Sea oats Seedlings from Seed

Introduction

Sea oats is a perennial grass native to the Gulf of Mexico and Atlantic coastal regions of the U.S. It is adapted to withstand high wind velocities, sand movement, limited water or xeric situations, high evaporation rates and extreme temperatures (Dahl and Woodard, 1977; Wagner, 1964; Woodhouse et al., 1968; Woodhouse and Hanes, 1967). For these characteristics and its ability to build and stabilize sand dunes it is commonly used in beach restoration projects. Beach restoration is growing in size and funding. In 2010 $618 million was spent on coastal restoration in Louisiana (CPRA, 2012). The largest portion of this cost was used to construct manmade structures to protect communities and natural resources along coastal Louisiana (CPRA, 2012).

An important and sustainable element of beach restoration is the use of native coastal plants such as grasses to build and stabilize sand dunes. Installation of vegetative material is critical to restore the natural systems for an effective and long-lasting restoration project that is sustainable. Plants are cheaper to produce and install versus most manmade infrastructure (Williams, 2007). In addition, plants which are native to the coastal zone, such as sea oats, are less costly to maintain, dynamic and capable of adapting to changes in the environment; whereas manmade structures are static and susceptible to fracture.

In Louisiana most sea oats plants used in beach restoration projects are genetically identical. They are produced by vegetatively dividing and propagating asexual clonal material in greenhouses and nurseries. As large amounts of identical plants are transplanted to beaches, genetic diversity is reduced and possibly eliminated in these sea oats communities. This lack of variability is alarming. If the sea oats genotype used in large beach restoration projects is susceptible to any one stress, then the entire project is susceptible (Kutner and Morse, 1996). This could lead to widespread death of plants. To increase the genetic variation in beach restoration projects, the Louisiana State University Agricultural Center developed and released 3 genetically different sea oats varieties (Knott et al., 2012). Despite these efforts the genetic variation of large beach restoration sites will be quite low. A more desirable system would be to produce sea oats from seed (Knott et al., 2012). Production of sea oats plants from seed would not only increase genetic diversity of

sea oats plants but would also reduce production costs. Currently it takes at least one year to produce acceptable trade gallon containers of sea oats from vegetative material, typically costing $5-6. If acceptable plants could be grown from seed in one half to one quarter that time, then costs would be greatly reduced.

Large scale production of sea oats from seed has been limited in Louisiana for several reasons. First, limited seed production, possibly resulting from the majority of the florets in the spikelet being sterile or incompletely developed as well as ovules being aborted in fertile florets making acquisition of seed in sufficient quantities difficult (Gould and Shaw, 1983; Wagner, 1964; Williams, 2007). Second, is the limited knowledge of seed storage conditions which could compromise the integrity of the seed. Typically most seeds are stored at 4°C, however recent research indicates sea oats seed storage should be at room temperature in sealed jars to maximize seed longevity (Nabukalu and Knott, 2013). Finally, sea oats typically have low germination rates. These low germination rates have been attributed to seed dormancy and/or to pathogens, fungal or bacterial, that potentially reside internally, on seed surface or in the immediate soil zone which can reduce germination (Burgess et al., 2002; Hester and Mendelssohn, 1987; Nabukalu, 2013; Sharvelle, 1961). Additionally, these pathogens could parasitize the seed prior to or during germination, or revive later and attack the seedling as a latent infection (Arya and Perelló, 2010; Sharvelle, 1961).

To combat pathogens compromising germination, commercial fungicides have successfully increased production in agricultural and ornamental crop production for years. Fungicide application at seeding is a traditional and effective method of protecting seeds from fungal pathogens, thereby aiding germination and improving seedling survival in field, nursery and greenhouse conditions (Arya and Perelló, 2010).

In theory, fungicides should also be effective for reducing sea oats seed and seedling pathogens and increase efficiency of sea oats seedling production. The objectives of this study were to assess the impact of commercial fungicides on sea oats germination, seedling survival, and seedling quality, such as shoot height, root length and fresh weight.

Materials and Methods

Fungicide Applications

On May 31 and June 14, 2013 sea oats seeds from Long Beach, MS which were harvested Aug. 26, 2011 were planted under greenhouse conditions at Burden Museum and Gardens in Baton Rouge, LA, U.S. Seeds were put into standard trays (11" x 21.375") (Standard flat trays; T.O. Plastics, Inc., Seller: BWI™, Item #FG1020S7) containing 72 cell inserts (Standard Inserts 1206; T.O. Plastics, Inc., Seller: BWI™ Item#FG12067) filled with an all-purpose soilless media mix (Professional Growing Mix #8; Sun Gro®, Sunshine®, Canada). Media was moistened 3-4 days prior to seeding. Four fungicide treatments at two rates were immediately applied to seeded trays.

Ten treatments were examined. Mefenoxam (SubdueMaxx; Syngenta, Greensboro, NC) 1x rate (0.019mL/1L) and 2x rate (0.004mL/1L) and thiophanate-methyl (3336F; Cleary Chemicals LLC, Dayton, NJ) 1x rate (0.926mL/ 1L) and 2x rate (1.852mL/1L) were applied as soil drenches. Azoxystrobin (Abound; Syngenta, Greensboro, NC) 1x rate (0.785L/ha) and 2x rate (1.570L/ha) and iprodione (Rovral, Bayer Crop Science, Research Triangle Park, NC) 1x rate (1.753L/ha) and 2x rate (3.504L/ha) were applied as surface sprays. Remaining treatments were two controls with no fungicide application consisting of soaked and dry sea oats seed. The soaked seed were submerged in tap water for 15 minutes prior to seeding. The dry seed were seeded without any seed preparation treatment.

To prepare fungicide solutions, tap water was adjusted to pH of approximately 6.0 with pH Down (GH Inc., Sebastopol, CA) prior to addition of fungicides. Mefenoxam was applied at rate of 5.079L solution per m^2 (1 pint/sqft) and thiophanate-methyl at rate of 7.936L per m^2 (1.56 pints/sqft). Half of soil drench fungicides, mefenoxam and thiophanate-methyl, were applied to soilless media prior to seeding and remaining solution applied after seeding.

Surface spray fungicides, iprodione and azoxystrobin, were applied with a hand sprayer (Flo-Master, Root-Lowell Manufacturing Co., Lowell, MI; Model 4OTS, 2.5pints). To ensure even fungicide application a 100mL solution was prepared; however only approximately 50mL of fungicide solution was applied to the soil surface of each replicate prior to seeding.

Approximately 24 hours after fungicide applications, all seeded trays were watered to prevent desiccation and in accordance with label for surface spray fungicides (Rovral and

Abound). Trays were subsequently watered approximately every 3-4 days for the first 2-3 weeks followed by approximately every 7 days for remaining weeks. If media appeared dry and the tray was empty then water was applied, if water remained in the tray then watering was delayed. Irrigation was accomplished manually by pouring into the tray bottom unless media surface was noticeably dry, then water was poured over surface as well. Acephate (75%; 0.98mL per L) and spinosad (Lawn & Garden Spray Spinosad$_2$®; Green Light®, San Antonio, TX; 15.85mL per L) insecticides were sprayed to control pests on June 21 and July 17, respectively.

Data Collection

Every 7 days after planting sea oats seed, germination was measured. A seed was considered germinated if the coleoptile or radicle was visible. Germination percentage was calculated as: (observed germinations / total seed of each tray) X 100. Beginning 14 days after seeding, seedling mortality was measured every 7 days; a seedling was considered dead if it had no visible green color or became completely separated at crown. Seedling survival was calculated as: [(germinated seed – dead seedlings) / germinated seed] X 100. Fifty-six days (8 weeks) after seeding, seedling shoot height, root length, and fresh weight were measured. Shoot height was measured as the distance from media surface to the end of the longest leaf. Seedlings were then removed from trays and rinsed thoroughly in water to remove media. Root length was measured from the root tip to crown of seedling (Figure 3.1). Total fresh weight was measured for each sea oats seedling using a balance (Mettler; Delta Range®, PC 4400).

Figure 3.1 Rinsing and measuring root length of seedlings (K. Barrios 2013)

Statistical Analysis

Experiment was designed as a Randomized Complete Block Design with three replications and two blocks. Each replication was a tray that consisted of 52 sea oats seeds. Each block consisted of 30 trays and duration was 8 weeks. The two blocks were the dates the experiments were initiated: May 31 and June 14, 2013. Germination and survival data was analyzed using Analysis of variance with logistic regression (PROC GLIMMIX, SAS Institute, Cary, NC, version 9.3). Treatment and week were fixed effects; block and replicates were considered random effects. Means were adjusted with Tukey-Kramer adjustment and separated using pdmix800 at $p < 0.05$ level (Saxton, 1998). Full model analysis found week by treatment interaction was not significant, therefore a reduced model of main effects (week and treatment only) was used.

Odds ratios were used to obtain the likelihood of germination of one treatment versus another. An odds ratio is the ratio of two odds, each of a different treatment. The odds are obtained from probabilities which here are germination. The odds of an event occurring is a ratio (p / 1-p, where p is probability of success or in this case germination). If success or germination of a seed is more likely then the odds are > 1, if failure is more likely odds are < 1. By comparing odds of two treatments with a ratio the likelihood of germination success of one treatment to another can be found.

Seedling shoot height, root length and fresh weight data was analyzed with analyses of variance (ANOVA) as randomized complete block designs (PROC MIXED, SAS Institute, Cary, NC version 9.3). Treatment was specified as fixed effect; block, replicate and sea oats seedling were considered random effects. LSMEANS were separated using pdmix800 at $p < 0.05$ level (Saxton, 1998). Contrasts were also performed with SAS to compare groups of treatments.

Results and Discussion

Efficient production of sea oats seedlings from seed is very important to ensuring the success of coastal restoration efforts. When sea oats germination was averaged across all 8 weeks of the experiment, seed treated with azoxystrobin 1x, thiophanate-methyl 1x and 2x, iprodione 1x, mefenoxam 1x and non-treated soaked seed had the highest germination

(Table 3.1). In contrast seed treated with mefenoxam 2x had the lowest germination of 22.4%. When comparing rates within fungicides over all weeks: azoxystrobin 1x and 2x were significantly different and germination of 1x rate was 39% more likely; mefenoxam 1x and 2x rates were significantly different and germination at 1x rate was 48% more likely (Table 3.2). However germinations were not significantly different between rates of thiophanate-methyl or iprodione, nor between untreated controls. The likelihood of germination is based on the odds of a seed germinating or not germinating which is based on probability or in this case germination percent. Using the odds of success (or germination) of each treatment in a ratio derived from statistical analyses, we can estimate the likelihood of germination with one treatment versus a different treatment. These could be useful for a grower or someone producing sea oats from seed by suggesting which rate of which fungicide would provide them with higher germination.

The higher mean germinations of treatments thiophanate-methyl 1x and 2x (even over the dry untreated control) are not surprising as it is a fungicide effective against a broad range of fungi including most ascomycetes, some basidiomycetes and deuteromycetes, but in-active against oomycetes. Previous research has observed sea oats seed to be infected by pathogens at germination with some studies reporting 13-49% pathogen incidence (Burgess et al., 2002; Hester and Mendelssohn, 1987; Nabukalu and Knott, 2013). A previous study using seed from the same location but a different year as seed in this experiment identified fungal pathogens affecting germinating seed as belonging to the fungal group (Phylum) Ascomycota (Nabukalu, 2013). This could be attributed to the success of thiophanate-methyl over untreated controls as well as other treatments. Similarly, the high mean germination of azoxystrobin 1x could be attributed to its preventative and curative properties against a broad spectrum of pathogens from all four fungal taxonomic groups. The most interesting observation is the contrast in range of activity of fungicides at high and low ends of germination rankings: thiophanate-methyl, active against the main fungal groups except for oomycetes and having higher mean germinations, versus the lowest mean germination of treatment mefenoxam 2x which is a fungicide active only against oomycetes. These findings reaffirm those of Nabukalu (2013) that the majority of fungal pathogens afflicting sea oats germination belong to the fungal group ascomycetes and not oomycetes.

Table 3.1 Average total germination percent by week for sea oats seed treated with different fungicides at 1x and 2x rates and untreated controls. Averages followed by different capitalized letter within each row are significantly different according to Tukey's test (t test, $p < 0.05$). Averages followed by different superscript letter in week means column are significantly different according to Tukey's test (t test, $p < 0.05$).

Week	Week Means		Azoxystrobin 1x		Azoxystrobin 2x		Iprodione 1x		Iprodione 2x		Mefenoxam 1x		Mefenoxam 2x		Thiophanate-methyl 1x		Thiophanate-methyl 2x		Untreated Unsoaked		Untreated Soaked	
1	12.13	b	14.5	A	10.9	D	13.2	ABC	11.8	BCD	12.8	ABCD	9.0	E	12.6	ABCD	13.6	AB	11.1	CD	12.7	ABCD
2	30.6	a	35.6	A	28.5	D	33.1	ABC	30.3	BCD	32.3	ABCD	24.4	E	32.0	ABCD	33.9	AB	28.9	CD	32.1	ABCD
3	31.6	a	35.9	A	28.8	D	33.5	ABC	30.6	BCD	32.7	ABCD	24.7	E	32.3	ABCD	34.2	AB	29.2	CD	32.4	ABCD
4	31.8	a	36.3	A	29.1	D	33.8	ABC	30.9	BCD	33.0	ABCD	24.9	E	32.7	ABCD	34.5	AB	29.5	CD	32.7	ABCD
5	32.2	a	36.6	A	29.4	D	34.1	ABC	31.2	BCD	33.3	ABCD	25.2	E	33.0	ABCD	34.9	AB	29.8	CD	33.1	ABCD
6	32.3	a	37.0	A	29.7	D	34.5	ABC	31.6	BCD	33.7	ABCD	25.5	E	33.3	ABCD	35.2	AB	30.1	CD	33.4	ABCD
7	32.5	a	37.3	A	30.0	D	34.8	ABC	31.9	BCD	34.0	ABCD	25.8	E	33.6	ABCD	35.5	AB	30.4	CD	33.7	ABCD
8	32.8	a	37.6	A	30.3	D	35.1	ABC	32.2	BCD	34.3	ABCD	26.1	E	34.0	ABCD	35.9	AB	30.8	CD	34.1	ABCD
Overall			33.2	A	26.3	D	30.8	ABC	28.0	BCD	30.0	ABCD	22.4	E	29.7	ABCD	31.5	AB	26.7	CD	29.8	ABCD

Table 3.2 Significant differences of treatment LSMeans of average germination based on pairwise comparisons adjusted using Tukey's test ($p < 0.05$). Likelihood percentages based on odds ratios.

Treatment	to Treatment	Adjusted P	Likelihood %
Azoxystrobin 1x	Azoxystrobin 2x	<.0001	39
Azoxystrobin 1x	Untreated Unsoaked	<.0001	36
Azoxystrobin 1x	Iprodione 2x	0.0046	27
Azoxystrobin 1x	Mefenoxam 2x	<.0001	71
Iprodione 1x	Azoxystrobin 2x	0.0190	24
Azoxystrobin 2x	Mefenoxam 2x	0.0483	23
Thiophanate-methyl 2x	Azoxystrobin 2x	0.0029	28
Untreated Unsoaked	Mefenoxam 2x	0.0175	26
Thiophanate-methyl 2x	Untreated Unsoaked	0.0095	26
Untreated Soaked	Mefenoxam 2x	<.0001	47
Iprodione 1x	Mefenoxam 2x	<.0001	54
Iprodione 2x	Mefenoxam 2x	0.0003	35
Mefenoxam 1x	Mefenoxam 2x	<.0001	48
Thiophanate-methyl 1x	Mefenoxam 2x	<.0001	46
Thiophanate-methyl 2x	Mefenoxam 2x	<.0001	59

Application rate was also a factor. Significant differences were observed in germination percentages of 1x rates of azoxystrobin and mefenoxam versus 2x rates (Table 3.2). Likewise, the treatment mefenoxam 2x had a mean germination significantly lower than both untreated controls. This may suggest possible phytotoxicity, which reduced germination. Another explanation could be that the fungicide destroyed a fungal symbiont conferring some advantage to the seed as with an endophyte. Many grasses worldwide have been reported to host endophytes which either parasitize their host plant or form a mutualistic relationship conferring some advantage to the host under adverse conditions. (Carroll, 1988; Paracer and Ahmadjian, 2000). This is commonly seen with tall fescue grass which is tolerant of abiotic stresses such as drought conditions, high temperatures and low soil fertility, which is attributed to the highly specific endophyte-grass symbiosis (Bush et al., 1997; Cheplick and Faeth, 2009; West, 1994). Due to the exceptional performance of sea oats in the harsh coastal environment, the possibility exists that they are host to an unknown mutualistic endophyte.

When germinations for each week were examined, it was found that all weeks were statistically similar except for week 1 (Table 3.1). Mean germination over all treatments at week one was 12% and then increased to 31% at week 2 after which weekly mean germinations increased gradually to 33% by week 8 (Table 3.1). This is interesting because previous observation evidence from six years of sea oats germination (Knott, unpublished) as well as published account by Nabukalu and Knott (2013) reported largest germination occurring three weeks after seeding. This experiment had a large increase in germination between weeks 1 and 2 followed by a slight increase. One possible explanation for this difference could be age of seed. Previous research was conducted with seed harvested approximately 6 months prior to being germinated. The present study used two year old seed because there was no seed available from the previous year due to coastal destruction from Hurricane Isaac in Aug. 2012. Younger seed has been observed to exhibit a degree of dormancy for a few months after harvest. Because seed used here seemed to be more readily germinable it could be attributed to having less degree of dormancy. Another factor could be the temperature at germination. Greenhouse temperatures for this study ranged on average from approx. 28-31^{0}C (83-88^{0}F) depending

on week of study (Table 3.3). Highest temperatures ranged from 34.7-38.6^{0}C (94.5-101.5^{0}F) and lowest were approx. 23-25^{0}C (74-77^{0}F). Nabukalu and Knott (2013) germi

nated sea oats seed in controlled conditions in an incubator where temperatures alternated from 35^{0}C (95^{0}F) for 7 hours followed by 18^{0}C (64.4^{0}F) for 17 hours. Hence ranges of temperatures differed some between this study and previous studies. In this study greenhouse conditions reached higher temperatures and did not reach the low temperatures at which incubator conditions were maintained, which could account for differences in germination rates between previous research and results from this experiment.

Table 3.3 Mean weekly temperatures in greenhouse at Burden Museum and Gardens, Baton Rouge, LA.

^{0}C (^{0}F)	May 31-June 7	June 7-14	June 14-21	June 21-28	June 28-July 5	July 5-12	July 12-19	July 19-26	July 26-Aug 2	Aug 2-9
Week (Block 1/ Block 2)	**1**	**2**	**3/1**	**4/2**	**5/3**	**6/4**	**7/5**	**8/6**	**7**	**8**
Average	29.1 (84.4)	29.5 (85.1)	29.5 (85.2)	30.2 (86.4)	28.4 (83.2)	29.1 (84.4)	28.5 (83.4)	29.4 (84.9)	29.8 (85.6)	31.1 (88.0)
High	36.5 (97.7)	38.5 (101.3)	36.9 (98.5)	37.2 (98.9)	34.7 (94.5)	36.9 (98.4)	36.1 (97.0)	36.0 (96.8)	37.3 (99.2)	38.6 (101.5)
Low	24.2 (75.6)	24.3 (75.8)	24.1 (75.4)	24.7 (76.5)	23.4 (74.1)	24.2 (75.5)	24.1 (75.4)	24.5 (76.1)	24.3 (75.7)	25.3 (77.5)

Two lab trials using no fungicide germinated seed from the same lot as the greenhouse fungicide experiment were initiated in lab incubator on April 15 and June 19, 2013 using 105 seeds each. Seeds were allowed to germinate over 4 weeks for each trial after which germination was found to be 31% for the first trial and 42% for later trial using an arithmetic mean calculation. This suggests that for the first block, germinations were similar for both greenhouse and controlled incubator conditions in laboratory. However for the second block, the lab germinations were higher than greenhouse germinations. These higher lab germinations could be attributed to surface sterilization, less stressful temperatures or less potential contact with pathogens than at greenhouse conditions. Unfortunately more replicates or trials were not performed nor were weekly germinations recorded to compare rates with the greenhouse experiment. It should be noted that seed viability was approximately 65% from an embryo viability test with 100 seed which were

soaked in a 1% solution of 2,3, 5-triphenyl tetrazolium chloride (Baskin, 1998). Further research would likely prove beneficial into determining whether the age of seed (specifically older than 6 months) or the difference in temperatures had an effect on germinating seed at lab incubator conditions and/or greenhouse conditions. Such studies could provide insight into the possibility of a difference in degree of dormancy between seed of different ages.

Seedling survival was examined because of its importance to sea oats production and growers' profitability. Sea oats survival averaged over 8 weeks ranged from 94.5-98.3%. Survival was highest for seeds treated with thiophanate-methyl 1x and 2x, azoxystrobin 1x and 2x, and mefenoxam 1x (Table 3.4). In contrast seed treated with iprodione 1x had the lowest survival at 94.5%. Azoxystrobin 1x, mefenoxam 1x, thiophanate-methyl 1x and 2x also had highest sea oats germination. This suggests that fungicides which assisted in increasing germination of sea oats seed also aided the survival of subsequent seedlings. Fungal pathogens which affect germination and seedling growth of sea oats may have been eliminated. It is interesting to note that azoxystrobin 2x had a very high survival, but a very low germination rate. This fungicide at that rate may have been phytotoxic at germination however over time the fungicide degraded whereby surviving seedlings have increased vigor. Alternatively, the fungicide could have been ineffective on a key pathogen affecting germination but possibly eliminated a different pathogen that affects seedling survival. This suggests that there are likely multiple fungal pathogens of various genera at work on sea oats seeds and seedlings.

When fungicide rates were compared for sea oats survival, rates had similar survival regardless of fungicide (data not shown). Similarly the untreated controls also had similar survival. This is interesting because it would be expected that any treatment with higher germination would correspondingly have a higher survival. However, lack of difference in survival between rates within fungicides could be attributed to fungicides degrading over weeks therefore minimizing beneficial effects between rates. Sea oats seedling survival also significantly decreased from week 1 (99.9%) to week 8 (91.4%) (Table 3.4). Although the final mean survival percentage was still higher than was expected, this could be important to growers in case they are expecting a specific or standard survival percent. One possible explanation for the significant decline could be degradation of the fungicides

and influx or growth of fungal pathogens due to higher temperatures experienced later in summer. This could be remedied by reapplication of fungicide. Further research into determining whether survival could be improved upon by reapplication of fungicides would be worthwhile.

Table 3.4 Average total percentage seedling survival by week for sea oats seed treated with different fungicides at 1x and 2x rates and untreated controls. Averages followed by different capitalized letter within each row are significantly different according to Tukey's test (t test, $p < 0.05$). Averages followed by different superscript letter in week means column are significantly different according to Tukey's test (t test, $p < 0.05$).

Week	Week Means	Azoxystrobin 1x	Azoxystrobin 2x	Iprodione 1x	Iprodione 2x	Mefenoxam 1x	Mefenoxam 2x	Thiophanate-methyl 1x	Thiophanate-methyl 2x	Untreated Unsoaked	Untreated Soaked
2	99.9 a	98.3 ABC	98.3 ABC	96.6 D	97.6 BCD	97.8 ABCD	97.4 CD	98.9 AB	98.9 A	97.0 CD	97.3 CD
3	98.0 b	97.7 ABC	97.8 ABC	95.4 D	96.8 BCD	97.1 ABCD	96.5 CD	98.6 AB	98.6 A	96.0 CD	96.3 CD
4	94.8 c	97.0 ABC	97.1 ABC	94.0 D	95.7 BCD	96.2 ABCD	95.4 CD	98.1 AB	98.1 A	94.6 CD	95.1 CD
5	93.2 cd	96.0 ABC	96.1 ABC	92.0 D	94.3 BCD	94.9 ABCD	93.9 CD	97.5 AB	97.5 A	92.9 CD	93.6 CD
6	92.0 cd	94.8 ABC	94.8 ABC	89.6 D	92.5 BCD	93.3 ABCD	91.9 CD	96.6 AB	96.6 A	90.7 CD	91.6 CD
7	91.7 cd	93.0 ABC	93.2 ABC	86.5 D	90.2 BCD	91.2 ABCD	89.4 CD	95.4 AB	95.5 A	87.9 CD	89.0 CD
8	91.4 d	90.8 ABC	91.0 ABC	82.7 D	87.2 BCD	88.5 ABCD	86.3 CD	94.0 AB	94.1 A	84.4 CD	85.8 CD
Overall		97.3 ABC	97.3 ABC	94.5 D	96.1 BCD	96.5 ABCD	95.8 CD	98.2 AB	98.3 A	95.1 CD	95.6 CD

Statistical trends observed for sea oats survival are similar to those observed for germination, specifically thiophanate-methyl 1x and 2x and azoxystrobin 1x treatments having high levels for both. Thiophanate-methyl targets mostly ascomycetes, and some basidiomycetes and deuteromycetes but not oomycetes, while azoxystrobin targets various genera from all four main fungal groups. This seems to suggest that broad-spectrum fungicides, which target mostly ascomycetes, some basidiomycetes and deuteromycetes improve both sea oats germination and survival. Iprodione 1x had the lowest survival but a relatively high germination percentage this is likely due to the anti-fungal protection which it imparted degrading over 8 weeks. Mefenoxam 2x had the lowest germination and moderate survival. This information could be useful to growers or producers in order to increase their yield of sea oats from seed. Future research should examine re-application of fungicides and their effects on seedling survival as well as efficacy of fungicides on seedlings in coastal beach restoration projects particularly in regards to their survival.

High quality seedlings are typically defined as tall, vigorous plants with healthy shoot and root systems. Therefore seedlings with high shoot height, fresh weight and root length were considered the highest quality seedlings. At the termination of this 8 week experiment, shoot height ($p < 0.0001$) and fresh weight ($p < 0.0325$) were significantly different for fungicide treatments, while significant differences for root length were not detected ($p < 0.4217$). The tallest sea oats were produced when treated with thiophanate-methyl 1x and 2x, azoxystrobin 1x and 2x, and iprodione 2x with heights ranging from 39.0-46.4cm (15.4-18.3in) (Table 3.5). These results are similar to those of germination and survival which also found highest percentages with treatments thiophanate-methyl 1x and 2x and azoxystrobin 1x. These treatments imparted benefits not only at germination but also throughout the growth and development of the sea oats seedling. In contrast, seed treated with mefenoxam 1x and 2x, iprodione 1x and both untreated controls had shortest average shoots ranging from 35.7-37.2 cm (14-14.6in). These results coincide with those of the lowest germination and the lowest survival exhibited by treatments mefenoxam 2x and iprodione 1x, respectively. This suggests that treatments mefenoxam 2x and iprodione 1x did not enhance germination nor survival and resulted in reduced seedling height.

Treatment was found to be significant for fresh weight ($p < 0.0325$), although using Tukey's adjustment and pairwise tests no significant differences of least squares means

Table 3.5 Average fresh weight, shoot height and root length of treatments after 8 weeks. LSMeans followed by different letters within each column are significantly different according to Tukey's test (t test, $p < 0.05$).

Treatment	Fresh Weight (grams)		Shoot Height (cm)		Root Length (cm)	
Azoxystrobin 1x	1.385	A	43.965	AB	40.443	A
Azoxystrobin 2x	1.259	A	40.321	ABC	40.393	A
Iprodione 1x	1.003	A	35.774	C	39.739	A
Iprodione 2x	1.181	A	39.010	ABC	40.666	A
Mefenoxam 1x	1.060	A	37.217	BC	35.449	A
Mefenoxam 2x	1.066	A	36.542	BC	36.724	A
Thiophanate-methyl 1x	1.477	A	46.385	A	41.062	A
Thiophanate-methyl 2x	1.448	A	44.710	A	37.119	A
Untreated Unsoaked	1.155	A	36.876	BC	36.718	A
Untreated Soaked	1.089	A	35.728	C	36.551	A

Table 3.6 Significant contrasts of average sea oats fresh weights based on pairwise tests ($p < 0.05$).

Treatment Groups Contrasted	F Value	Pr > F
Thiophanate-methyl 1x & 2x vs. Untreated Controls	10.46	0.0042
Thiophanate-methyl & Azoxystrobin 1x's & 2x's vs. all other treatments	19.43	0.0003
Thiophanate-methyl 1x & 2x vs. all other treatments	14.24	0.0012
Thiophanate-methyl, Azoxystrobin & Mefenoxam 1x's & 2x's & Iprodione 2x vs. all other treatments	6.49	0.0192
Thiophanate-methyl & Azoxystrobin 1x & 2x vs. Untreated Controls	8.73	0.0078

were found (Table 3.5). However, by comparing collective means of groups of treatments using contrasts some differences were observed (Table 3.6). It was found that the fresh weight overall mean of treatments thiophanate-methyl and azoxystrobin each at 1x and 2x rates was highly significantly different ($p < 0.0003$) from the mean of all other treatments (Table 3.6). Fresh weight means of treatments thiophanate-methyl and azoxystrobin 1x and 2x were also highest and ranged from 1.26-1.48 grams; whereas fresh weight means of all other treatments ranged from 1.00-1.18 grams (Table 3.5). Additionally, the fresh weight overall mean of treatments thiophanate-methyl, azoxystrobin, and mefenoxam each at 1x and 2x and iprodione 2x was significantly different ($p < 0.0192$) and higher than the mean of untreated controls and iprodione 1x. This suggests that application of fungicide at seeding (with the exception of iprodione 1x) increased seedling fresh weight after 8 weeks as compared to untreated controls. Germination, survival and seedling shoot height were also highest for treatments thiophanate-methyl 1x and 2x and azoxystrobin 1x. Treatment iprodione 1x also had lowest survival and shoot height. These similarities would suggest that treatments applied at seeding enhanced germination and benefits were sustained through seedling growth by increasing height and corresponding biomass of seedling.

The final aspect of seedling quality measured was roots because of their important effect on sea oats' dune-building capability. Unfortunately, root length means were not found to be different between treatments ($p < 0.4217$) (Table 3.5). This lack of significant differences between treatments is unexpected but could be attributed to the measuring technique. Rinsing and drying roots resulted in sample loss. Additionally stretching roots out to measure length was difficult and not uniform among samples since root anatomy varied. No additional nutrients were provided to seedlings which could have prevented significant growth and development which might have separated treatment performances. However, samples did not exhibit nutrient deficiency. Additional research into determining if fungicide treatment at seeding combined with fertilizer application to seedlings might improve sea oats seedling production.

When sea oats seed germination, survival, and seedling quality are considered together, similar trends are observed. High germination, survival, plant height and total biomass were found when sea oats seeds were treated with thiophanate-methyl 1x and 2x, and azoxystrobin 1x, as well as azoxystrobin 2x with the exception of germination (Tables 3.2,

3.4, 3.5). In contrast, seed treated with mefenoxam 2x had the lowest germination and shortest plants, and seed treated with iprodione 1x had the lowest survival, shortest plants and low biomass. It appears that seeds treated with fungicides which control most ascomycetes, and some basidiomycetes and deuteromycetes germinated higher, survived better, and resulted in more vigorous seedlings. This may indicate that the groups of pathogens which are most problematic for germinating sea oats seeds and establishing seedlings belong to the fungal group Ascomycota and possibly Basidiomycota and imperfect fungi as well. However, additional research is necessary to verify this.

Although fungicide treatments thiophanate-methyl 1x and 2x and azoxystrobin 1x seem to result in the highest yield of quality sea oats seedlings, application of these treatments must be economically feasible in order for them to be useful in seedling production systems. The increased germination over the average of untreated seed (28.25%) for seed treated with thiophanate-methyl 1x and 2x and azoxystrobin 1x were 1.45, 3.25, and 4.95%, respectively. The increased survival of quality seedlings over the average of untreated seed (95.35%) for thiophanate-methyl 1x and 2x and azoxystrobin 1x treatments were 2.85, 2.95, and 1.95%, respectively. Considering that current market value of a gallon-size sea oats seedling is $5-6, which takes approximately 1 year to produce, it can be assumed that 2 month seedlings are worth approximately $0.92. Assuming 1,000 seeds are sown for each treatment, the benefit in gross sales after 2 months of growth of using thiophanate-methyl 1x would be $21.16, of thiophanate-methyl 2x would be $37.72, and of azoxystrobin 1x would be $49.68 over untreated seed. These estimated dollar amounts were found using germination and survival percentages to calculate how many resultant seedlings would be produced from 1,000 seed multiplied by market value of 2 month-old seedling ($0.92) compared to average estimated sales of untreated seed. When the current prices of fungicides are calculated, it would cost $0.87 for thiophanate-methyl 1x, $1.74 for thiophanate-methyl 2x, and $0.01 for azoxystrobin 1x to treat 1,000 seed. It appears that sales largely outweigh the negligible costs of fungicide application at seeding therefore application of these fungicides would confer an economic advantage by increasing the amount of successful seedlings produced.

Conclusion

Treatments azoxystrobin 1x and thiophanate-methyl 1x and 2x had highest germination and survival and tallest seedlings. Results strongly suggest that treating seed with thiophanate-methyl 1x and 2x and azoxystrobin 1x increases sea oats germination, survival and seedling quality. These results could translate into a monetary benefit of $21.16 for using thiophanate-methyl 1x, $37.72 for thiophanate-methyl 2x, and $49.68 for azoxystrobin 1x over untreated seed after 2 months of growth using a 1,000 seed basis and current sea oats seedling market value. These estimated increases in sales from quality 2 month old seedlings largely outweigh the costs of fungicide application at seeding of sea oats.

Chapter 4: Evaluation of Dune Populations and Commercial Varieties in an Urban Environment

Introduction

The Gulf Coast region of the United States loses more land from coastal erosion than any other area in the contiguous United States. Wetland loss in Louisiana specifically accounts for more than 90 percent of all wetland loss in the whole of the U.S. (Commission, 2011; Finkl and Khalil, 2005). Various projects in Louisiana's coastal restoration efforts include marsh creation, shoreline protection, river diversions and beneficial use of dredging materials (Commission, 2011). Employing native coastal plants such as grasses to build and stabilize sand dunes in order to reduce erosion and protect the state's shoreline is a vital component of these restoration efforts.

A native grass commonly used in beach restoration projects in the northern Gulf of Mexico is sea oats (*Uniola paniculata* L.) (Wagner, 1964; Woodhouse and Hanes, 1967). In Louisiana, sea oats installed in beach restoration are typically asexually propagated by dividing plants to produce genetically identical clones. Throughout much of the rest of the northern Gulf of Mexico, sea oats installed for beach restoration are typically produced from seed which are collected from natural beach environments near the restoration sites. The advantage of installing seedlings in restoration projects is genetic diversity, this allows for a more successful project with a stronger likelihood of survival in the event of environmental stress. In large beach restoration projects installed with clonal material if the sea oats genotype used is susceptible to any one stress, then the entire project is susceptible which could lead to widespread death of plants (Kutner and Morse, 1996). A main disadvantage of producing seedlings from seed is disruption of natural ecosystems by harvesting sea oats seed from plants in beach locations. Additionally, the unknown performance of seedlings due to sea oats being open-pollinated plants could be detrimental to restoration projects if planted directly in beach environment without screening or selection stage (Wagner, 1964).

To increase genetic diversity of sea oats incorporated into beach restoration sites the LSU AgCenter initiated a sea oats breeding program in 2002. The program was successful in developing and releasing three clonal sea oats varieties, however this was accomplished after 10 years of research (Knott et al., 2012). With extreme erosion occurring in

Louisiana, sea oats varieties desperately need to be released in a more timely manner. Evaluation and development of sea oats breeding lines and varieties requires travel to beach sites which is both costly and time consuming. If sea oats selection could be performed in an environment closer to where plant breeders are stationed, the efficiency of the sea oats breeding program would be greatly improved leading to a swifter development of varieties and more efficient use of funds.

The goal of this study was to begin preliminary research into determining whether relocation of breeding and selection studies/nurseries of sea oats from a coastal to an urban location could be possible based on whether populations would perform reliably or consistently in both environments. If this were the case, then it could be assumed that selections could be made in urban locations closer to researchers for coastal restoration use. Ideally this study could have compared performances of sea oats at the two locations (urban and coastal) simultaneously; however this was not possible due to restrictions on coastal beach area. Therefore the study was conducted at urban conditions using varietal releases for beach restoration projects as a check reference, and their performance was compared to previous performances on the coast of the same varieties. The specific objective of this study was to determine if the average performance of selected sea oats populations, unselected sea oats populations, and sea oats varieties differ when produced outside of the coastal zone.

Materials and Methods

Plant Material

Eight experimental sea oats populations, selected and unselected, were produced from seed collected in August and September of 2010 from Long Beach, Mississippi, U.S. The selected populations were evaluated in 2009 in a study at Long Beach, MS and selected based on ideal performance for coastal restoration as well as higher seed yield. Four 'selected' populations were LB10 plot 1001, LB10 plot 1038, LB10 plot 1090 and LB10 plot 1326. Four 'unselected' populations were evaluated similarly to 'selected' populations but did not perform as admirably based on selection criteria and were therefore not selected. 'Unselected' populations used were LB10 plot 1045, LB10 plot 1125, LB10 plot 1148 and LB10 plot 1220.

Germination of seeds was initiated March 7-9, 2012 on the LSU campus. Seed was surface sterilized with a solution of 25% Clorox and 0.01% tween for 15 minutes then rinsed with distilled water, placed on adequately saturated germination paper, covered with additional moistened sheets of germination paper and rolled up. Rolls were then placed in closed containers and into an incubator at alternating temperatures of 35^0C (95^0F) for 7 hours followed by 18^0C (64.4^0F) for 17 hours. Rolls were weighed periodically and necessary moisture added to prevent desiccation. Once a week rolls were checked for germinated seed. Seeds were considered germinated when either radicle or coleoptile protrusion was approximately 2 mm. Germinated seeds were removed and placed in Sun Gro® Sunshine® Professional Growing Mix #8 soilless media treated with Scotts® Banrot® fungicide at rate of 0.37 g per L water. Media was filled in tray inserts of 72 cells (Standard Inserts 1206, Manufacturer: T.O. Plastics, Inc.; Seller: BWI™ Item#FG12067). This was done for period of three weeks after which all remaining non-infected and non-germinated seeds from germination paper were placed in soilless media pretreated with Banrot® in Standard flat trays and allowed more time to germinate (11" x 21.375") (Manufacturer: T.O. Plastics, Inc.; Seller: BWI™, Item #FG1020S7). Seedlings were grown in trays under fluorescent light in the lab for approximately one week and then transferred to greenhouse conditions. Beginning May 8, 2012 seedlings which were approximately 15 cm in height were transplanted to individual 4" pots containing media amended with sand (80 pine bark: 10 peat: 10 sand). The majority of seedlings were transplanted over the next two weeks however slower growing seedlings were transplanted continuously until June 13, 2012 in order to allow them to reach adequate size. Seedlings were fertigated approximately every 8 days with Scotts® Peters Professional® 20-10-20 Peat-lite Special® fertilizer. Four inch pots containing seedlings were transported to Burden Museum and Gardens on June 23, 2012. Seedlings were placed outdoors where fertigation continued until being planted in designated plots in experimental field design on August 1, 2012.

Clonal sea oats varieties were propagated from plant beds at the Burden Museum and Gardens in Baton Rouge, LA, U.S. On Aug. 3, 2012 propagules which were approximately 2-3 stems were dug from plant beds and transplanted immediately to designated plots in experimental field design.

Plants were transplanted to field design where rows had on-center spacing of 1.2 m (4') and plants were spaced approximately 2.4 m (8') within rows. Every three rows were separated by a 2.4 m (8') flat open space to allow for mower or cart access.

Field Site

The field site for this experiment was located at Burden Museum and Gardens and was previously covered by turf grass and weeds. The site was tilled to develop a sea oats selection nursery for this study beginning July 17, 2012. Approximately 23 m^2 of sand was incorporated into approximately 2,510 m^2 area to increase soil particle size. Rows were made by disking and "hipping" soil forming row tops approximately 25-30 cm (10-12") above field level.

Soil moisture was measured at the time of each monthly assessment; two samples were collected from each of the four blocks. Within each block, one sample was taken from the middle of the block from a random row and a second sample taken from an observably wet end of the block from a random row. From each soil sample 100g was weighed in a tin as fresh weight and then placed in a constant temperature oven 132^{0}C (270^{0}F) and allowed to dry for approximately five days, then final weight was measured as dry weight. Soil moisture was calculated as:

Soil moisture % = ((fresh weight - dry weight)/dry weight) x 100.

An analysis was run on soil fertility by the LSU soil testing lab in August 2013. Levels of essential nutrients were compared to suggested levels for Dallisgrass, a perennial grass, and rice and found to be close to or within appropriate ranges. There was some variation between blocks for elements but none appeared to be problematic or cause for concern. Calcium and magnesium levels were a little high and sodium was high for all blocks. The pH of blocks ranged from 6.6-7.1 which is slightly high compared to optimal range for most plant activity (5.5-7.0). Phosphorous, potassium, sulfur and zinc levels were deemed within appropriate ranges for all blocks. See Appendix B for field site data.

Field Management

Pre-emergent herbicide Prowl® was applied at 3.504L/ha (3 pints/acre) prior to planting. Plants were watered Sept. 14, 2012 due to dry conditions and to aid acclimation. To control weeds and insects, hand weeding, herbicides, and insecticide baits were used.

Field site was sprayed Oct. 23, 2012 with a solution of herbicides Sterling Blue® 2.336L/ha (1qt/acre) and Certainty® 0.091L/ ha (1.25oz/acre). On January 28, 2013 plot was sprayed with herbicide Atrazine 4L at 4.674L/ ha (2qt/acre). On March 15, 2013 herbicide Ranger Pro® at 3.5% solution (0.039L/L or 5oz/gal) was applied with a backpack sprayer. On April 10, 2013 plot was sprayed with solution of 3 herbicides: Atrazine 4L at 4.674L/ha (2qt/acre), Sterling Blue® 2.336L/ha (1qt/acre) and Framework® at 4.674L/ha (2qt/acre). On May 17, 2013 herbicide Ranger Pro® at 3.5% solution (0.039L/L or 5oz/ gal) was applied with a backpack sprayer. On June 25, 2013 plot was sprayed with solution of 3 herbicides: Sterling Blue® 1.753L/ha (24oz/acre), Certainty® 0.091L/ha (1.25oz/ acre) and Framework® at 1.753L/ha (24oz/acre). On June 28, 2013 Hi-Yield® Improved Slug & Snail Bait (Metaldehyde 3.25%) was spread-out at 11.049kg/ha (9.901 lb/acre). On August 8, 2013 Hi-Yield® Improved Slug & Snail Bait (Metaldehyde 3.25%) was spread-out at 11.049kg/ha (9.901 lb/acre).

Data Collection

Plant height, number of stems, number of tillers, stem density, and plant vigor were measured approximately every four weeks starting at month of planting, August 2012 through December 2012 and from March 2013 through August 2013. Number of panicles was measured June 2013 through August 2013. Plant height was measured from the soil line to the tip of the longest overall leaf or leaves. Stem density was measured as the number of stems in 10.2 cm (4") quadrant beginning in May 2013. Subjective rating of vigor was on scale of 0 (dead) – 10 (most vigorous).

Statistical Analysis

Experiment was designed as Randomized Complete Block with four blocks. Each of the eight populations had 4 replicates with 10 plants per replicate totaling 40 sea oats plants per population. Each plant was considered a plot.

Plant height, stem count, tiller count, vigor and density was analyzed with analyses of variance (ANOVA) as randomized complete block designs (PROC MIXED, SAS Institute, Cary, NC version 9.3). Category (Selected, Unselected, or Check) was specified as fixed effect; block and plot were considered random effects. Repeated measures analysis using Proc Mixed was done first but interaction was found to be significant between MAT (month

after transplant) and Category for each variable. Therefore analyses were performed for each month of data collection separately, MAT: 0-4, 7-12. LSMEANS were separated using pdmix800 at $p < 0.05$ level (Saxton, 1998). Plant height, stem count and vigor were also analyzed of the three clonal check varieties at two locations: urban (data from current study) and beach (data from 2009 study) as an ANOVA (PROC MIXED, SAS Institute, Cary, NC version 9.3). Location and varieties were fixed effects; rep within location was considered a random effect. No significant interaction was found between location, variety and MAT, therefore months were not analyzed separately. LSMEANS were separated using pdmix800 at $p < 0.05$ level (Saxton, 1998).

Results and Discussion

Performances of sea oats categories (selected populations, unselected populations and varieties) were assessed on plant height, stem count, tiller count, stem density and vigor. For the variable plant height, Category was found to be significant at each month data was collected ($p < 0.0001$). The largest difference exhibited was mean heights of unselected populations which were significantly higher than means of selected populations at each month of data collection (Table 4.1). Comparatively, plant height means of varieties were also significantly higher than selected populations 6 out of the 11 months measured. At transplant mean height of varieties was significantly the tallest category, but this was due to age difference between mature clonal material and relatively younger seedling populations. One and two months following transplant as well as the last two months of the study, height means of varieties were not significantly different from either category but numerically taller than selected populations. This similarity in performance at similar months could be attributed to seasonal growth of sea oats which typically decline at the end of summer causing performances of categories to even out more. Mean heights of all categories decreased from transplanting until MAT 7 & 8 (March & April 2013). Then beginning in spring all categories generally increased in height as months progressed from April to August 2013. Unselected populations had the least decline from transplant to April 2013 (approx. 29cm) and largest increase in height (approx. 45cm) from April to August 2013. Selected populations had the least increase in height from April to August 2013 (approx. 15cm). These trends of decline during fall and winter months followed by

Table 4.1 Average height, stem count, tiller count, vigor and density by month after transplant (MAT) for sea oats lines of categories selected, unselected and checks. Averages followed by different letters within each column variable at different MAT are significantly different according to Tukey's test (t test, $p < 0.05$).

		Month after Transplant																					
		0		**1**		**2**		**3**		**4**		**7**		**8**		**9**		**10**		**11**		**12**	
		Aug. '12		Sept. '12		Oct. '12		Nov. '12		Dec. '12		Mar. '13		April '13		May '13		June '13		July '13		Aug. '13	
Height (cm)	Selected	68.16	c	49.46	b	36.00	b	30.30	b	25.43	b	21.17	b	19.45	b	20.74	b	26.29	b	31.94	b	34.63	b
	Unselected	74.21	b	65.29	a	61.76	a	58.71	a	52.32	a	45.94	a	45.43	a	50.08	a	64.62	a	78.03	a	90.08	a
	Varieties	105.92	a	47.33	ab	53.25	ab	58.17	a	58.08	a	50.08	a	50.75	a	50.33	a	59.42	a	65.08	ab	69.67	ab
Stems[1]	Selected	1.76	c	1.99	b	1.67	b	1.67	b	1.69	b	1.65	b	1.86	b	2.38	b	4.38	b	8.35	b	11.79	b
	Unselected	2.84	b	4.57	a	5.48	a	6.26	a	6.54	a	7.84	a	9.79	a	12.50	a	19.75	a	35.36	a	52.58	a
	Varieties	4.50	a	2.42	b	2.33	b	2.25	b	2.17	b	2.17	b	2.25	b	2.58	b	4.83	b	10.42	b	25.17	b
Tillers[2]	Selected	0.60	b	0.32	b	0.36	b	0.25	b	0.16	b	0.25	b	0.31	b	0.40	b	0.70	b	0.93	b	1.02	b
	Unselected	0.99	a	0.69	a	0.98	a	0.79	a	0.60	a	1.04	a	1.03	a	1.13	a	1.86	a	2.58	a	3.35	a
	Varieties	1.08	a	0.17	b	0.08	b	0.17	b	0.08	b	0.00	b	0.17	b	0.25	b	1.00	ab	1.17	b	2.25	ab
Vigor[3]	Selected	3.70	c	2.76	b	1.59	b	1.41	b	1.31	b	1.07	b	1.17	b	1.13	b	1.32	b	1.49	b	1.50	b
	Unselected	4.13	b	4.12	a	3.30	a	3.19	a	3.01	a	2.76	a	3.26	a	3.32	a	3.70	a	4.14	a	4.55	a
	Varieties	6.50	a	2.67	b	2.33	ab	2.42	ab	2.33	ab	2.00	ab	2.33	ab	2.58	a	2.75	ab	2.83	ab	3.08	ab
Density[4]	Selected															2.21	b	3.96	b	5.95	b	10.43	b
	Unselected															9.22	a	14.43	a	20.73	a	27.05	a
	Varieties															2.42	b	4.42	b	8.83	b	21.50	ab

1- Number of stems per plant
2- Number of tillers per plant
3- Subjective rating of overall vigor of plant: 0 (dead plant) to 10 (extremely full plant)
4- Number of stems in 4 in^2 area

increased growth from spring through summer are typical seasonal growth patterns for sea oats plants. However these results suggest that unselected populations tolerated cooler fall and winter months the best and were also best affected by warmer spring and summer months, notably over selected populations. Additionally, comparison of means at transplant and end of study shows unselected populations was the only category to have an increase in height (74 to 90cm). Whereas selected and variety means were shorter at end than at initiation of study. Also at end of study mean height of unselected populations was more than 2.5x the mean height of selected populations. The consistently taller means of unselected versus selected populations across months would be useful for selection studies were these populations being bred for urban conditions. The strong similarity exhibited between unselected populations and varieties is surprising because varieties were selected for coastal environments. The height means of varieties at this location were found to be similar to those of the same varieties at a beach location in Mississippi from 2009 (Table 4.2). This would suggest that heights of varieties could be used as a check (or control) of expected performance. Since the heights of selected and unselected populations were different in this location, and using the similarity of heights of varieties at urban and coastal locations, it would be expected that the heights of selected and unselected populations would be different under coastal conditions. However additional research is required to determine performances of these populations under two different environments.

Table 4.2 Average height, stem count, and vigor for three sea oats varieties at two location from different years. Averages for beach location were from data collected April-Oct. 2009 at Gulfport, MS. Averages for urban location were from this study and collected from Aug.-Dec. 2012 and March-Aug. 2013. Averages followed by different letters within rows for each variable are significantly different according to Tukey's test (t test, $p < 0.05$).

	Location			
	Beach		**Urban**	
Height (cm)	50.30	A	53.38	A
Stems[1]	3.92	A	2.27	B
Vigor[2]	5.67	A	2.35	B

1- Number of stems per plant
2- Subjective rating of overall vigor of plant: 0 (dead plant) to 10 (extremely full plant)

The next variable assessed was number of stems of each plant. Category for mean stem count was significant at each month data was collected ($p < 0.0001$). Mean stem count for unselected populations was significantly higher than selected populations each month data was collected (Table 4.1). All categories increased in stem count when comparing at transplant (MAT 0) to final month of study. At transplant each category was significantly different from one another with varieties having most stems and selected populations the fewest. But this was likely due to the size of the propagule of varietal clones versus seedlings. For all other months of data collection, unselected populations had significantly higher mean stem counts than both selected populations and varieties. At all months of data collection, besides at transplant, mean stem counts of varieties were higher than selected populations but statistically similar. At the end of the study unselected populations had approximately 52 stems which was more than 4x that of selected populations. These results are similar to trends of plant height and would suggest that unselected populations performed significantly better than both other categories and that varieties performing above selected populations. In regards to monthly changes, unselected was the only category to steadily increase in stem count with each month of assessments. Selected populations decreased slightly over 7 months from transplant to March 2013 by approximately 0.10 stems and then increased by approximately 10 stems during spring and summer until Aug. 2013. This growth trend was less dramatic than for varieties which decreased by approximately 2.3 stems from transplant to March 2013 and then increased by 23 stems until Aug. 2013. This slight drop after transplant could have been due to the expected recovery period following clonal propagation or to Hurricane Isaac which made landfall Aug. 28, 2012; however, increase of varieties was still larger than that of selected populations. The trend exhibited by selected populations was expected due to typical seasonal growth patterns of sea oats declining in winter and increasing from spring through summer. However, the steady increase without decline for the entire year in mean stem count of unselected populations was unexpected and impressive. This trait in combination with having the largest increase of mean stem count, particularly for establishment, suggests adaptability to these urban conditions and vigorous plant growth of unselected populations. The higher means of unselected populations over the other categories was also not expected. Over all months (except at transplant) there was a

statistical similarity of mean stem counts between selected populations and coastal varieties. When stem count means of varieties at this location are compared to those of the same varieties under coastal conditions, differences were observed (Table 4.2). The mean stem count estimate across varieties was significantly higher at the beach location than the urban location. This dissimilar performance of total number of stems of varieties between the locations was expected because varieties were selected for the coast and it would be expected that they would have higher means at a beach location than at urban conditions.

In addition to height and stem count, the number of tillers on each plant was counted at each assessment. Category for mean tiller count was significant at each month data was collected ($p < 0.0001$). Mean tiller count for unselected populations was significantly higher than selected populations each month data was collected (Table 4.1). Mean tiller counts of varieties were statistically similar to selected populations except at transplant when they were statistically similar to unselected populations. All categories increased in mean tiller counts when comparing assessments at transplant to the final month, but between those months all categories had some fluctuation. All categories showed a small decline from transplant until approximately Dec. 2012 and March 2013 followed by an increase from spring through summer until Aug. 2013. Unselected populations had the smallest decline in tillers (approx. 0.39) and largest increase (approx. 2.75) compared to other categories during seasonal intervals. Varieties had the largest decrease (approx. 1.08) from transplant to March and selected populations had the smallest increase (approx. 0.86) from March-Aug. 2013. The trend of decline following transplant is not surprising because tillers typically develop during times of new plant growth rather than while plant is acclimating to a new environment and establishing. This likely accounts for the increase in mean tiller count beginning in early spring until the end of summer. In general, unselected populations had a statistically higher mean number of tillers than both selected populations and varieties except at transplant and June and Aug. 2013. These results are similar to those of plant height and stem count as they suggest that varieties and selected populations performed similarly and had lower means than unselected populations.

An additional variable of interest was stem density, which is inherently related to the variable stem count. Density was measured as the number of stems in a random 10.2 cm (4”) quadrant of plant base and first taken at MAT 9 (May 2013) because plants were not

dense enough prior to that month. At each assessment starting from May to Aug. 2013 category was significant for stem density ($p < 0.0001$). Unselected populations had the highest mean densities at each month, except for Aug. when varieties was similar to both unselected and selected populations (Table 4.1). Unselected populations still had the highest mean density at final assessment which was more than double that of selected populations. All categories increased in stem density at each month. When comparing assessments at May 2013 to the final month of study, mean stem densities of selected populations had the least increase (approx. 8.22 stems) and varieties had the largest increase (approx. 19.08 stems). This outcome is unexpected because given that unselected populations had the highest mean densities it would be expected that unselected, rather than varieties, would have the largest increase in density. Stem density is related to stem count however it gives an idea of the spread or compactness of a plant. Because sea oats generally have spreading rhizome systems in coastal dune conditions, it was expected that they would grow more compactly in the heavier non-drifting inland soil. The significant difference in mean stem density and number of stems between unselected and selected populations could be attributed to the selected populations' suitability to the conditions of the coast. Results show that the relationship among categories is similar to those of plant height, stem count and number of tillers.

The final variable assessed was plant vigor which was rated subjectively on a scale from 0 to 10; 0 being a dead plant and 10 being an extremely full plant. Category was found to be significant for vigor at each month of data collection ($p < 0.0001$). Mean vigor rating for unselected populations was significantly higher than that of selected populations at each month data was collected (Table 4.1). Numerically vigor means of unselected populations were also higher than those of varieties except at transplant. Additionally, vigor means of varieties were statistically similar to both categories except for the first two months of study and one month in the spring, May 2013. Means of selected populations were lowest at each month data was collected, except at one month after transplant when varieties was slightly lower, which was likely due to adjustment following propagation. All categories declined in vigor from transplant until reaching lowest means in March 2013; then from April to Aug. 2013 vigor of all categories increased. This was somewhat expected considering plants were acclimating and establishing during the first few months after

transplant in addition to winter dormancy of perennials, and then began to 'green-out' and develop new growth in the spring season through summer. During the period of decline, unselected had the least decrease in vigor followed by selected leaving varieties with the largest decline. On the upside, unselected had the largest increase in vigor from April to Aug. 2013 and was also the only category that increased when comparing at transplant to the end of study, although slightly (+0.42). Aug. 2012 compared to Aug. 2013 showed varieties had the largest decline in vigor and both varieties and selected populations had a final vigor less than half that of their initial mean vigor. Varieties likely had the largest decline because of the inflated rating of mature clonal material at transplant relative to selected and unselected seedlings. For all categories a larger increase in vigor was expected from transplant to the end of study since individual ratings were observed to have increased by final months of study. However this is likely not reflected in mean vigor ratings because of drag by mortalities. Mortality of a plant was indicated by a vigor rating of 0 and was found using an arithmetic mean of 0 ratings divided by total number of plants (Table 4.3). Percent mortality increased over all categories with each month of data collection and total mortality was approx. 53% by the final month of study which was expected (Table 4.3). Among categories mortality differed at each MAT and selected populations had highest mortality (72%) at the final month of study followed by varieties with approx. 42% mortality and unselected populations with approx. 35% (Table 4.3). Mortality increase over months was largest for selected populations which increased from 0 to 23% after the first month of transplant. However unselected populations increased gradually and to a lesser degree from 0 to 4% after the first month. Varieties had a large increase after the first month from 0 to 25% and increased slightly throughout study, but the percentage seems higher because of a smaller sample size of 12 plants compared to approx. 160 plants each of the selected and unselected populations. While vigor is a subjective rating, it is very helpful in evaluating overall performance of a plant and hence observing trends of categories which can be compared and contrasted. Unselected was significantly higher in vigor than selected and there was less separation over months between mean vigor of varieties versus the other two categories; in other words varieties remained similar to both categories. Compared to a previous study on the coast, vigor means of varieties were higher under coastal conditions than at urban conditions (Table

4.2). This is to be expected since these varieties were selected for their admirable performance at coastal conditions. Results of vigor displayed similar relationships between categories as with plant height, stem count, tillers, and stem density.

Table 4.3 Percent mortality obtained from arithmetic means for total plants and by category at each month after transplant (MAT) data was collected.

	Month after Transplant										
	0 Aug. '12	**1** Sept. '12	**2** Oct. '12	**3** Nov. '12	**4** Dec. '12	**7** Mar. '13	**8** April '13	**9** May '13	**10** June '13	**11** July '13	**12** Aug. '13
Total	0.0	13.9	28.1	33.3	36.4	42.6	46.0	49.4	50.6	52.0	52.6
Selected	0.0	23.0	46.1	53.3	54.6	62.5	65.8	69.7	71.1	71.7	72.4
Unselected	0.0	4.4	11.3	15.0	20.0	24.4	28.1	31.3	32.5	34.0	34.6
Varieties	0.0	25.0	25.0	25.0	25.0	33.3	33.3	33.3	33.3	41.7	41.7

In the final months of the study (June-Aug. 2013) panicles emerged from some plants. This was an important development because panicles bear the inflorescences or reproductive structures of the plant. Emergence of panicles after one year of growth was considered a possibility at start of study but not expected. Sixteen plants from unselected populations had a total of 22 panicles at the final month of study. Whereas selected populations had just 3 plants each with one panicle and varieties had 5 plants with panicles totaling 6 panicles at the final month. These results reflect the superior performance of unselected populations exhibited with all other variables assessed. It also demonstrates the similarity in performance of selected populations and varieties. However, all categories produced some panicles which as stated was unexpected after just one year span from transplant, therefore suggesting vigorous growth.

When considering results from all variables, there were consistencies among performances and recurring relationships between categories. Means of varieties for each variable were in most cases significantly or numerically lower than means of unselected populations and almost always statistically similar to the means of selected populations. Results for unselected populations found they were significantly higher than selected populations for each variable assessed at this location. Category was significant

($p<0.0001$) for each variable assessed and therefore it could be concluded that average performances of selected and unselected sea oats populations as well as of sea oats varieties differed when produced outside of the coastal zone.

It is interesting to consider the superior performance exhibited by the unselected populations for all variables to the performances of the other two categories. This suggests that unselected populations would be better suited for urban conditions. Varieties at this urban location had lower means for stem count and vigor as compared to the same varieties under coastal conditions. As varieties were selected for their admirable performance on the coast, it would be expected for them to exhibit lower means in an environment different from their native one outside of the coastal zone. The lower performance of varieties at urban conditions compared to unselected populations and varieties at beach conditions, suggests that this selection environment may be ideal for selecting sea oats for urban conditions but not for coastal applications. However, further selection studies would be required to determine this.

Conclusion

There were recurring relationships among categories for the variables assessed which were plant height, stem count, number of tillers, stem density and plant vigor. Unselected populations were higher than selected populations for each variable assessed. Means of varieties were either significantly or numerically lower than means of unselected populations for most variables and almost always similar to the means of selected populations. Therefore it was shown that the average performances of selected and unselected sea oats populations from Long Beach, MS as well as varieties of sea oats differed from each other at this location outside of the coastal zone. These results in conjunction with previous performance data of varieties suggest that selection studies should not be conducted at urban conditions similar to those of Baton Rouge, LA for the development of sea oats populations to be used in coastal beach conditions. However, results from this study do suggest that selection in this location for an urban use of sea oats could be successfully conducted.

Chapter 5: Evaluation of Sea oats Experimental Lines for Ornamental Use

Introduction

Ornamental grasses are popular and important elements in landscape design. Their year-round aesthetic and ecological attributes make them unique among plant groups (Maddox and Kelly, 2009). They have great variety in form, size, color and texture while adding motion to the landscape (Greenlee, 1992). Grasses are also typically low maintenance, can tolerate extreme growing conditions such as drought and poor soil, and are very insect and disease resistant (Greenlee, 1992; Meyer, 1975; Ruter and Carter, 2000). Many native grasses are popular ornamental plants in the nursery industry, such as Purple muhly grass (*Muhlenbergia capillaris*) and Big bluestem (*Andropogon gerardii* Vitm.) (Maddox and Kelly, 2009). The trend towards using native plants is growing and currently the demand often exceeds supply in the southeast U.S. (Brzuszek and Harkess, 2009).

Sea oats is a grass native to the coastal regions of the east and southeast U.S. Some sea oats lines have exhibited tolerance to inland conditions in work completed at Burden Museum and Gardens in Baton Rouge, LA (Knott, unpublished data). Because sea oats are native to this region while also having attractive aesthetic qualities they have the potential to be in the ornamental grasses marketplace. However breeding plants for any trait requires multiple generations, trials and therefore time, typically years. This study is one of the first steps in developing ornamental sea oats. The objective of this study was to identify sea oats lines which exhibit ideal characteristics for ornamental use. These characteristics were based on the phenotypic traits of height, vigor (subjective ranking), color (particularly red/purple), leaf width (subjective ranking), number of stems and form.

Materials and Methods

Plant Material

Eight experimental sea oats populations were produced from seed collected in August and September of 2010 from Long Beach, Mississippi, U.S. Each plant produced for this study was considered an individual treatment or experimental breeding line and was assessed on phenotypic attributes. The 8 populations utilized for treatments originated

from: LB10 plot 1001, LB10 plot 1038, LB10 plot 1045, LB10 plot 1090, LB10 plot 1125, LB10 plot 1148 and LB10 plot 1220, and LB10 plot 1326.

Germination of seeds was initiated March 7-9, 2012 on the LSU campus. Seed was surface sterilized with a solution of 25% Clorox and 0.01% tween for 15 minutes then rinsed with distilled water, placed on adequately saturated germination paper, covered with additional moistened sheets of germination paper and rolled up. Rolls were then placed in closed containers and into an incubator at alternating temperatures of 35^0C (95^0F) for 7 hours followed by 18^0C (64.4^0F) for 17 hours. Rolls were weighed periodically and necessary moisture added to prevent desiccation. Once a week rolls were checked for germinated seed. Seeds were considered germinated when either radicle or coleoptile protrusion was approximately 2 mm. Germinated seeds were removed and placed in Sun Gro® Sunshine® Professional Growing Mix #8 soilless media treated with Scotts® Banrot® fungicide at rate of 0.37 g per L water. Media was filled in tray inserts of 72 cells (Standard Inserts 1206, Manufacturer: T.O. Plastics, Inc.; Seller: BWI™ Item#FG12067). This was done for period of three weeks after which all remaining non-infected and non-germinated seeds from germination paper were placed in soilless media pretreated with Banrot® in Standard flat trays and allowed more time to germinate (11" x 21.375") (Manufacturer: T.O. Plastics, Inc.; Seller: BWI™, Item #FG1020S7). Seedlings were grown in trays under fluorescent light in the lab for approximately one week and then transferred to greenhouse conditions. Beginning May 8, 2012 seedlings which were approximately 15 cm in height were transplanted to individual 4" pots containing media amended with sand (80 pine bark: 10 peat: 10 sand). The majority of seedlings were transplanted over the next two weeks; however, slower growing seedlings were transplanted continuously until June 13, 2012 in order to allow them to reach adequate size. Seedlings were fertigated approximately every 8 days with Scotts® Peters Professional® 20-10-20 Peat-lite Special® fertilizer. Four inch pots containing seedlings were transported to the Burden Museum and Gardens on June 23, 2012. Seedlings were placed outdoors where fertigation continued until being planted in designated plots in experimental field design on August 1, 2012.

Six clonal sea oats lines were included in this study. The 6 clonal checks were experimental sea oat breeding lines that were selected because they exhibited acceptable

landscape characteristics and performance at Burden Museum and Gardens. On Aug. 3, 2012 propagules, which were approximately 2-3 stems were dug from landscape trial beds at Burden Museum and Gardens and transplanted immediately to designated plots in experimental field design. Check lines were: Up-07-Aqop-0089-2, Up-01-LA-16J-HB-2327 (LB10 plot 1086), Up-07HBop-0007-4, Up-07-HBop-0001-7, Up-07-Aqop-0126-1, and Up-07-HBop-0009-34.

Plants were transplanted to the field where rows had on-center spacing of 1.2 m (4') and plants were spaced approximately 2.4 m (8') within rows. Every three rows were separated by a 2.4 m (8') flat open space to allow for mower or cart access.

Field Site

The field site for this experiment was located at Burden Museum and Gardens and was previously covered by turf grass and weeds. The site was tilled to develop a sea oats selection nursery for this study beginning July 17, 2012. Approximately 23 m^2 of sand was incorporated into approximately 2,510 m^2 area to increase soil particle size. Rows were made by disking and "hipping" soil forming row tops approximately 25-30 cm (10-12") above field level.

Soil moisture was measured at the time of each monthly assessment; two samples were collected from each of the four blocks. Within each block, one sample was taken from the middle of the block from a random row and a second sample taken from an observably wet end of the block from a random row. From each soil sample 100g was weighed in a tin as fresh weight and then placed in a constant temperature oven 132^0C (270^0F) and allowed to dry for approximately five days, then final weight was measured as dry weight. Soil moisture was calculated as:

Soil moisture % = ((fresh weight - dry weight)/dry weight) x 100.

An analysis was run on soil fertility by the LSU soil testing lab in August 2013. Levels of essential nutrients were compared to suggested levels for Dallisgrass, a perennial grass, and rice and found to be close to or within appropriate ranges. There was some variation between blocks for elements but none appeared to be problematic or cause for concern. Calcium and magnesium levels were a little high and sodium was high for all blocks. The pH of blocks ranged from 6.6-7.1 which is slightly high compared to optimal range for most

plant activity (5.5-7.0). Phosphorous, potassium, sulfur and zinc levels were deemed within appropriate ranges for all blocks. See Appendix B for field site data.

Field Management

Pre-emergent herbicide Prowl® was applied at 3.504L/ha (3 pints/acre) prior to planting. Plants were watered Sept. 14, 2012 due to dry conditions and to aid acclimation. To control weeds and insects, hand weeding, herbicides, and insecticide baits were used. Field site was sprayed Oct. 23, 2012 with a solution of herbicides Sterling Blue® 2.336L/ha (1qt/acre) and Certainty® 0.091L/ ha (1.25oz/acre). On January 28, 2013 plot was sprayed with herbicide Atrazine 4L at 4.674L/ ha (2qt/acre). On March 15, 2013 herbicide Ranger Pro® at 3.5% solution (0.039L/L or 5oz/gal) was applied with a backpack sprayer. On April 10, 2013 plot was sprayed with solution of 3 herbicides: Atrazine 4L at 4.674L/ha (2qt/acre), Sterling Blue® 2.336L/ha (1qt/acre) and Framework® at 4.674L/ha (2qt/acre). On May 17, 2013 herbicide Ranger Pro® at 3.5% solution (0.039L/L or 5oz/ gal) was applied with a backpack sprayer. On June 25, 2013 plot was sprayed with solution of 3 herbicides: Sterling Blue® 1.753L/ha (24oz/acre), Certainty® 0.091L/ha (1.25oz/ acre) and Framework® at 1.753L/ha (24oz/acre). On June 28, 2013 Hi-Yield® Improved Slug & Snail Bait (Metaldehyde 3.25%) was spread-out at 11.049kg/ha (9.901 lb/acre). On August 8, 2013 Hi-Yield® Improved Slug & Snail Bait (Metaldehyde 3.25%) was spread-out at 11.049kg/ha (9.901 lb/acre).

Data Collection

Plant height, number of stems, number of tillers, stem density, plant vigor, purple color and leaf width were measured approximately every four weeks starting at planting, August 2012 through December 2012 and from March 2013 through August 2013. Number of panicles was measured June 2013 through August 2013. Plant height was measured from the soil line to the tip of the longest overall leaf or leaves. Stem density was measured as number of stems in 10.2 cm (4") quadrant beginning in May 2013. Subjective rating of vigor was on scale of 0 (dead) – 10 (most vigorous). Subjective rating of purple color was a measure of intensity or overall occurrence of purple-red hue on scale of 0 (no visible purple-red, only green) – 2 (strongly visible, present on large portion of plant) beginning in October 2012. Color was also identified by matching to Munsell® Color Charts for Plant

Tissues. Subjective rating of leaf width was on scale from 1 (narrow) – 3 (wide) beginning in October 2012.

Statistical Methods

Experiment was designed as an Augmented Block with four blocks. There were 78 experimental lines evaluated per block for a total of 312 experimental lines. The 6 clonal checks had 4 replicates each with one plant per replicate totaling 24 sea oats check plants.

Plant height, stem count, tiller count, vigor, purple rating and leaf width rating was analyzed with analyses of variance (ANOVA) as an augmented block designs (PROC MIXED, SAS Institute, Cary, NC version 9.3). Checks were specified as fixed effect; block and experimental lines were considered random effects. Approximate variance estimates of experimental lines from check means for each variable were obtained using 'Solutions' option in PROC MIXED in SAS. These estimates for each experimental line were then sorted from high to low because a higher positive variance estimate would indicate a higher performance of that experimental line over checks.

Results and Discussion

Selection of ornamental plants relies on both phenotypic qualities as well as physically measureable traits. In this study the variables plant height, stem count and tiller count could be considered as empirical or objective traits assessing overall growth. Whereas the ornamental characteristics focused on in this study of reddish purple foliage, leaf width and vigor were measured using scales of subjective ratings. As a potential landscape plant, strong growth characteristics (height, stem and tiller counts, vigor) are important to the success and survival of any plant, particularly a species grown out of its native niche. Statistical analyses were run on data collected and selections were based largely but not entirely on these results. Personal observations and notes, particularly for overall appeal or attractiveness and plant form, were relied on as well.

The most unique ornamental trait observed of these experimental sea oats lines was foliage color which ranged from a typical green to pale and dark reddish purples. Color of interest was matched as close as possible to Munsell® Color Charts, however, color varied on the plant and between plants. Colors were identified as 2.5R: 4-6/2-6 and 5R: 3-5/2-6, which ranged from pale or light pink and lavender tones to very dark red purple hues.

Typically the reddish purple foliage tissue was located near the base of plant and faded up the leaves ending about halfway up the plant. In the spring there was a noticeable distinct yellow marking at the collar region of leaves on some plants. The intensity of reddish purple color exhibited by plants did fluctuate, however, throughout the year-long study (see Appendix C for exemplary photos).

The second ornamental trait of interest was leaf width which was assessed with a rating scale. This trait was not as distinct as foliage color but it was detected to differ among some plants approximately 1-2 months after initiation of the study. A subjective rating of 1-3 was used to evaluate this characteristic. A plant with a rating of 1 was considered to have narrow leaves and a 3 had the widest leaves. Wider leaves were considered more attractive because it is visually interesting and it is a trait not commonly seen in sea oats which typically have very narrow leaves. Therefore plants with higher ratings were preferred.

Performances of sea oats experimental lines were averaged collectively for each variable of plant height, stem count, tiller count, vigor, purple and leaf width. The mean of experimental lines was not found to outperform all clonal checks for any variable over all months of study (Table 5.1). Focusing on each variable, the mean of all experimental lines was second highest for purple rating when compared to means of clonal checks, ranked behind the check Up-07-HBop-0009-34 which had a mean more than double that of experimental lines. For both stem count and tiller count there were two checks (LB10-1086 and Up-07HBop-0007-4) with higher means than that of the experimental lines, and experimental lines had higher means for stem and tiller counts than four clonal checks. However for the variables vigor, plant height and leaf width the collective means of experimental lines were lower than a few checks and only outperformed checks Up-07-Aqop-0126-1 and Up-07-Aqop-0089-2. It could also be stated that the mean of experimental lines was not the lowest for any of the variables assessed. There was also no clonal check that outperformed the mean of experimental lines for all variables. These trends were also found at the final month of the study (Aug. 2013) of remaining live plants (Table 5.2). The results in tables 5.1 & 5.2 suggest that checks performed well but not always better than the mean of all the experimental lines. Over all the months the top two checks were LB10-1086 which had the highest mean for tiller count, vigor and leaf width

and check Up-07-HBop-0009-34 which had the highest mean for height and purple. The data shows no consistently highest performer across variables among clonal checks and the experimental line collective. However the number of experimental lines is large (312), especially compared to that of each clonal check (4) therefore it can be assumed that there is considerable variability among the experimental lines. The large ranges for variables from the minimum and maximum values demonstrate the magnitude of variability among the experimental lines, notably for height and number of stems, over all the months and at the final month of remaining live plants (Tables 5.1 & 5.2). These results demonstrate how the experimental lines compare collectively as a group to the clonal checks as well as providing means for each variable, but they do not directly lead to the selection of individual experimental lines for further ornamental breeding studies.

Table 5.1 Least squares means for plant height, stem count, tiller count, vigor, purple rating, and leaf width rating of checks and experimental lines over all months, $p < 0.05$.

	Line	Height (cm)	Stem Count	Tiller Count	Vigor[1]	Purple[2]	Leaf Width[3]
Checks	Up-07-Aqop-0089-2	53.14	4.04	0.70	2.34	0.31	0.86
	LB10 – 1086	74.52	12.29	1.20	3.86	0.05	1.47
	Up-07HBop-0007-4	57.50	12.50	1.14	3.07	0.00	0.89
	Up-07-HBop-0001-7	68.25	7.11	0.77	3.11	0.03	1.19
	Up-07-Aqop-0126-1	33.91	5.18	0.52	2.02	0.03	0.69
	Up-07-HBop-0009-34	80.79	5.48	0.54	3.27	0.67	1.44
	Experimental Lines						
	Mean	48.12	9.35	0.94	2.66	0.31	0.76
	Minimum	0	0	0	0	0	0
	Maximum	175	251	18	10	3	3

1- Subjective rating 0 (dead plant) – 10 (very full plant)

2- Subjective rating 0 (no purple) – 3 (intense purple color)

3 – Subjective rating 1 (very narrow leaf blades) – 3 (very wide leaf blades)

Table 5.2 Least squares means for plant height, stem count, tiller count, vigor, purple rating, and leaf width rating of checks and experimental lines at final month of study (August 2013) and only of live plants, $p < 0.05$.

	Line	Height (cm)	Stem Count	Tiller Count	Vigor[1]	Purple[2]	Leaf Width[3]
Checks	Up-07-Aqop-0089-2	124.0	32.5	5.5	5	0	1.5
	LB10 – 1086	138.3	70.0	6.0	7.7	0	2.7
	Up-07HBop-0007-4	149.0	84.5	5.0	8.5	0	1.5
	Up-07-HBop-0001-7	145.0	53.5	3.0	6.5	0	2
	Up-07-Aqop-0126-1	161.0	99.0	5.0	10	0	3
	Up-07-HBop-0009-34	165.5	34.5	3.0	6	1.5	3
Experimental Lines							
	Mean	134.1	69.5	4.7	6.5	0.3	1.7
	Minimum	6	1	0	1	0	1
	Maximum	173	251	18	10	2	3
	Mode	160	24	4	6	0	1

1- Subjective rating 0 (dead plant) – 10 (very full plant)

2- Subjective rating 0 (no purple) – 3 (intense purple color)

3 – Subjective rating 1 (very narrow leaf blades) – 3 (very wide leaf blades)

The method of selection began with output from statistical analyses followed by a merging of personal observations. The top 15% or 47 experimental lines for each variable were focused on first in the selection process (Table 5.4). For each variable, experimental lines were ranked from the highest to lowest approximated variance from the solutions table in SAS output. It was assumed that experimental lines with a high positive approximated variance would have a higher value for the variable than means of checks. For ease of selection, experimental lines were identified by entry numbers. Similar entries between lists were then identified (Table 5.3), with most frequently occurring entries over all lists to be considered the best performers meeting many of the ideal characteristics. There were three entries or experimental lines which were in the top 15% of all 6 variables measured (Tables 5.3 & 5.4). Subsequent entries selected occurred in the top 15% for 4 or 5 of the 6 variables. Additional entries that were in the top 15% of both purple and leaf width rating but not for other variables were also included because this study focuses on ornamental attributes of sea oats (Table 5.3 & 5.5). The lists of entries common across variables (Table 5.3) were then compared with observations of overall appeal and notes. Nine experimental lines which appeared in the top 15% for 2 or 3 variables measured as well as one experimental line in the top 15% for only 1 variable were added to the selection

list for a total of 50 experimental lines selected from the original 312 (Table 5.5). Hence, it can be inferred that the majority of these selections had superior performance to clonal checks and many of the other experimental lines. These selections were chosen for their ornamental appeal or aesthetic qualities in addition to exhibiting strong and vigorous growth.

Table 5.3 Lists of entry numbers or experimental lines in the top 15% for the variables plant height, stem count, tiller count, vigor, purple rating, and leaf width rating.

6 out of 6 variables	5 out of 6 variables	4 out of 6 variables	3 out of 6 variables	2 out of 6 variables	Purple and leaf width
74	94	62	11	13	11
283	98	169	64	80	13
293	218	176	70	96	80
	219	183	83	106	96
	243	194	134	116	116
	284	198	138	132	128
	295	203	140	165	139
		204	141	178	145
		207	142	210	146
		213	145	217	148
		245	155	220	152
		263	158	221	234
		285	164	228	278
		289	172	231	
		292	177	233	
		296	193	238	
		301	195	268	
			199	275	
			203	279	
			206	280	
			211	312	
			226		
			234		
			247		
			260		
			270		
			305		
			308		

Table 5.4 List of entry numbers in top 15% for each trait ranked in order from the highest to lowest approximation of variance. Entries in bold are selected sea oats experimental lines.

Rank	Height	# of Stems	#of Tillers	Vigor	Purple	Leaf Width
1	**283**	**284**	**285**	**218**	**146**	**11**
2	**204**	**285**	**284**	**203**	**11**	134
3	**289**	**218**	**218**	**284**	134	**141**
4	**243**	**193**	**213**	**285**	**152**	**70**
5	**164**	**194**	**243**	**283**	132	**176**
6	**218**	**203**	**194**	**289**	**140**	**140**
7	**11**	**263**	226	**204**	142	**293**
8	**292**	308	275	**292**	**148**	**295**
9	**141**	**292**	**198**	**219**	114	**128**
10	260	**198**	**301**	**301**	138	**62**
11	**70**	206	195	**263**	**139**	**233**
12	**295**	195	308	**295**	**145**	**94**
13	262	226	**292**	**243**	**128**	**245**
14	**62**	**289**	**263**	**293**	**198**	**284**
15	**293**	275	206	**213**	**141**	290
16	**203**	231	158	**193**	**296**	83
17	**219**	**283**	280	**183**	37	**234**
18	**94**	**301**	**293**	206	112	**145**
19	**284**	**243**	270	**62**	**116**	**148**
20	**245**	312	247	260	123	**152**
21	221	**295**	**193**	**176**	78	184
22	**301**	**183**	64	**207**	**234**	138
23	**98**	280	**268**	308	**238**	142
24	**140**	199	155	**70**	**13**	**219**
25	**285**	**219**	276	**74**	64	221
26	134	155	**295**	172	**74**	**296**
27	166	228	**98**	**94**	211	**164**
28	**176**	165	**245**	247	**293**	**243**
29	**213**	**62**	**289**	195	158	**283**
30	57	270	217	**198**	242	**116**
31	178	305	231	199	**283**	**74**
32	**234**	**74**	312	**98**	294	**183**
33	**145**	279	165	178	**106**	**96**
34	**296**	**207**	**203**	**164**	185	177
35	**263**	158	83	305	**278**	**218**
36	138	**268**	**176**	220	**169**	**13**
37	**207**	**213**	**74**	210	287	**204**
38	**74**	**204**	**183**	270	**94**	**207**
39	286	247	**169**	165	**96**	211
40	205	**293**	**283**	**245**	**98**	**80**
41	132	217	172	**194**	188	**278**
42	210	237	**106**	228	305	69
43	211	260	52	279	309	**146**
44	**233**	83	**94**	226	**80**	64
45	142	172	102	177	155	**98**
46	220	**169**	177	**296**	**194**	**139**
47	302	**238**	**219**	**169**	199	181

Table 5.5 List of the 50 selected sea oats experimental lines; 'x' indicates in top 15% for specific variable.

Population of Origin	Plot #	Entry #	Height	Stems	Tillers	Vigor	Purple	Leaf Width
LB10 - 1038	4079	**74**	x	x	x	x	x	x
LB10 - 1220	2003	**283**	x	x	x	x	x	x
LB10 - 1220	3002	**293**	x	x	x	x	x	x
LB10 - 1090	3008	**94**	x		x	x	x	x
LB10 - 1090	3038	**98**	x		x	x	x	x
LB10 - 1148	3046	**218**	x	x	x	x		x
LB10 - 1148	3062	**219**	x	x	x	x		x
LB10 - 1125	2002	**243**	x	x	x	x		x
LB10 - 1220	2016	**284**	x	x	x	x		x
LB10 - 1220	3019	**295**	x	x	x	x		x
LB10 - 1038	3050	**62**	x	x		x		x
LB10 - 1045	2058	**169**		x	x	x	x	
LB10 - 1045	3032	**176**	x		x	x		x
LB10 - 1045	4002	**183**		x	x	x		x
LB10 - 1148	1017	**194**		x	x	x	x	
LB10 - 1148	1046	**198**		x	x	x	x	
LB10 - 1148	2001	**203**	x	x	x	x		
LB10 - 1148	2014	**204**	x	x		x		x
LB10 - 1148	2040	**207**	x	x		x		x
LB10 - 1148	3003	**213**	x	x	x	x		
LB10 - 1125	2022	**245**	x		x	x		x
LB10 - 1125	4007	**263**	x	x	x	x		
LB10 - 1220	2025	**285**	x	x	x	x		
LB10 - 1220	2063	**289**	x	x	x	x		
LB10 - 1220	2090	**292**	x	x	x	x		
LB10 - 1220	3035	**296**	x			x	x	x
LB10 - 1220	3077	**301**	x	x	x	x		
LB10 - 1001	2009	**11**	x				x	x
LB10 - 1001	2024	**13**					x	x
LB10 - 1038	4031	**70**	x			x		x
LB10 - 1090	1050	**80**					x	x
LB10 - 1090	3023	**96**					x	x
LB10 - 1090	4024	**106**			x		x	
LB10 - 1090	4090	**112**					x	
LB10 - 1326	1034	**116**					x	x
LB10 - 1326	2047	**128**					x	x
LB10 - 1326	3055	**139**					x	x
LB10 - 1326	3070	**140**	x				x	x
LB10 - 1326	3076	**141**	x				x	x
LB10 - 1326	4022	**145**	x				x	x
LB10 - 1326	4032	**146**					x	x
LB10 - 1326	4054	**148**					x	x
LB10 - 1326	4083	**152**					x	x
LB10 - 1045	2012	**164**	x			x		x

(Table 5.5 continued)

Population of Origin	Plot #	Entry #	Height	Stems	Tillers	Vigor	Purple	Leaf Width
LB10 - 1048	1008	**193**		x	x	x		
LB10 - 1125	1005	**233**	x					x
LB10 - 1125	1014	**234**	x				x	x
LB10 - 1125	1053	**238**		x			x	
LB10 - 1125	4053	**268**		x	x			
LB10 - 1220	1052	**278**					x	x

Additional observations were noted during the study such as fluctuation in foliage color throughout the year. Some plants showed more intense purple foliage when temperatures became cooler and in the spring this foliage tissue became a dark purple and died. This could indicate color is affected by some abiotic factor such as temperature or even possibly nutrients since there were significant rainfalls in Dec. 2012. However plants which exhibited purple foliage tended to originate from a few populations, notably 'LB10 1220' and 'LB10 1326,' which would suggest a genotypic effect as well. It was also noted during the study that many plants which exhibited strong reddish purple leaves had fewer stems and leaves and were not as vigorous in growth which is also reflected some in Table 5.4.

It was also observed that plants with noticeably wider leaves generally had a different form. They appeared to have a fountain or weeping shape with the ends of their leaves touching down to the ground more than plants with thin, narrow leaves. Another similarity in phenotypic traits was the observance that many of the plants with strong purple appearance were often very straight, rigid, and upright in form. This suggests that these traits could be correlated, however further studies are needed to determine whether it is exhibited by subsequent generations implying a genotypic control.

In addition to ornamental traits, this study evaluated experimental lines on their overall performance in an urban environment outside of their native coastal zone. Variables which assessed overall plant growth were vigor, plant height, number of stems, and tiller count. Thirty out of the 50 selected experimental lines were in the top 15% for height and vigor. Twenty six of the 50 selected lines were in the top 15% for number of tillers and half of selected lines were in the top 15% for number of stems. This suggests that selected experimental lines not only survived this very different environment from their native

coastal conditions for one year, but also quickly adapted and displayed a successful growth performance. Sea oats are useful in an urban environment as landscape plants.

It is interesting to take a broader perspective and compare population origins of these selected entries. Two of the top 3 selections were from the population 'LB10 1220' which was an unselected population from a sea oats study at Long Beach, MS. This population was also strongly represented in this study by having the most experimental lines selected versus other populations with 10 out of the total 50 selected. Other populations highly represented in selections were 'LB10 1148' and 'LB10 1326' each with 9 experimental lines. This information could be useful for future breeding work when including parental contributions of ornamental characteristics. Of the total selected experimental lines in this study, 30 originated from unselected populations versus 20 experimental lines originating from populations which were selected in a previous beach study at Long Beach, MS. Additionally it appears experimental lines that were selected were distributed fairly randomly and evenly over field design with blocks 1, 2, 3, and 4 having 9, 15, 15, and 11 plants, respectively, indicating uniformity across the study.

Although selected experimental lines were based on top performers across variables assessed, there is variability among experimental lines with strong growth versus lines with strong phenotypic ornamental characteristics. However, high variability and diversity is favorable for early stages of breeding and selection. Out of the fifty selected experimental lines, the top five selections would be plots 4079, 2003, 3002, 3008, and 3038 based on ranking in the top 15% for most variables including the ornamental traits of purple foliage and leaf width. Further breeding would ideally develop sea oats varieties that are both visually attractive and impressive as urban landscape plants but also full and vigorous in growth.

Conclusion

Selection of sea oats experimental lines for potential use as landscape plants was conducted following evaluations of traits assessing growth and ornamental characteristics for one year. Basis of selection of experimental lines was on statistical analyses which used performance of clonal check material as comparison, as well as personal observations. Fifty from the original 312 experimental lines were selected for exhibiting ideal ornamental

traits for use in further breeding research and selection stages to enhance sea oats potential as becoming an attractive landscape plant in the native grasses marketplace.

Chapter 6: Summary and Overall Conclusions

Sea oats (*Uniola paniculata* L.) is a perennial grass commonly used in coastal restoration projects along the northern Gulf of Mexico for its dune building capabilities. Use of vegetative material is cheaper than manmade material and necessary to restore the natural systems for an effective and sustainable restoration project, however these restoration projects have one sizeable flaw. Most of the sea oats transplanted to beaches in Louisiana are genetically identical. This drastically reduces genetic diversity in these large-scale sea oats communities, thereby putting the fate of the entire project at risk. Two prominent methods to increase genetic diversity of sea oats along the coast are via plant breeding and production of vegetative material from seed. Two goals of this thesis pertained to furthering research related to these methods.

Production of sea oats plants from seed for restoration projects would increase genetic diversity and reduce production costs however its faces a few problems, one of which is low germination rate. This has been attributed to seed dormancy and/or to pathogens, fungal or bacterial, that potentially reside internally, on seed surface or in the immediate soil zone. Fungicide application at seeding is a traditional and effective method of protecting seeds from fungal pathogens, thereby aiding germination and improving seedling survival. Therefore the first study of this thesis assessed the impact of commercial fungicides on production of sea oats seedlings when applied at seeding on germination, seedling survival, and seedling quality. It was found that the fungicides azoxystrobin at 1x rate and thiophanate-methyl at 1x and 2x rates increased sea oats germination, survival, and seedling quality. A monetary benefit of applying these fungicides was found via increased sales which also largely outweighed estimated costs of applying these beneficial fungicides. This information provides growers protocols for increasing amount of high quality sea oats seedlings from seed thereby increasing genetic diversity in coastal restoration projects. Further research is needed to assess survival and performance of seedlings once installed in the dune environment.

Plant breeding produces high-quality genetically diverse sea oats varieties for restoration projects but typically requires several years, possibly 10 or more, of research. Because current breeding and selection studies occur in coastal beach environments far

from where researchers are stationed, efficiency of the program is reduced due to costly and time-consuming travel by locating studies inland. The second study of this thesis conducted preliminary research into that possible solution through the assessment of performances of selected populations, unselected populations and varieties of sea oats when produced outside of the coastal zone. It was shown that average performances of selected and unselected sea oats populations as well as commercial varieties of sea oats differed from each other when produced outside of the coastal zone. It was interesting to note, however, that for all the 5 variables measured to assess performance, unselected populations performed significantly higher than selected populations. It was strongly suggested that selection studies should not be conducted in urban conditions similar to those of Baton Rouge, LA for the development of sea oats populations for coastal use. However, this study does suggest that selection in this location for urban use of sea oats could be successfully conducted.

Previous studies have found sea oats lines display ornamental qualities, such as reddish purple foliage and a different plant form or overall shape, and a tolerance to urban conditions out of their native coastal zone. Ornamental and native grasses are popular and important elements in landscape design, and in the southeast U.S. there is a growing demand for native grasses. Sea oats are low maintenance, hardy grasses while also having aesthetically pleasing qualities, therefore breeding and selection work could produce exciting ornamental landscape varieties. Therefore the third study of this thesis was the first step in the process of developing potential ornamental varieties. A total of 312 experimental lines were assessed for one year for phenotypic traits and growth performance. Clonal check material was used for comparison and 50 experimental lines were selected for exhibiting ideal ornamental traits for use in further breeding and selection stages.

The studies presented in this thesis have shown that production methods of sea oats plants from seed for coastal use can be improved by use of certain fungicides, that selection studies of sea oats for coastal use should not be conducted out of the coastal zone, and that there are promising sea oats lines to be used in further ornamental breeding and selection stages to develop potential varieties for urban landscapes.

Literature Cited

Allen, C.M., D.A. Newman, and H.H. Winters, 2004. Grasses of Louisiana. Allen's Native Ventures, LLC, Pitkin, La.

Anke, T., F. Oberwinkler, W. Steglich, and G. Schramm, 1977. Strobilurins: new antifungal antibiotics from basidiomycete Strobilurus tenacellus (pers ex fr) sing. J. Antibiotics 30:806-810.

Arnold, A.E., Z. Maynard, G.S. Gilbert, P.D. Coley, and T.A. Kursar, 2000. Are tropical fungal endophytes hyperdiverse? Ecol. Lett. 3:267-274.

Arya, A. and A.E. Perelló, 2010. Management of fungal plant pathogens. CABI, Wallingford.

Bacon, C.W. and N. Hill, 1996. Symptomless grass endophytes: products of coevolutionary symbioses and their role in the ecological adaptations of grasses, p. 155-178. In: Redlin, S. C. and Carris, L. M. (eds.), Endophytic fungi in grasses and woody plants : systematics, ecology, and evolution. APS Press, St. Paul, Minn.

Bacon, C.W., N.S. Hill, and I. International Symposium on Acremonium/Grass, 1997. Neotyphodium/grass interactions. Plenum Press, New York.

Bacon, C.W., J.K. Porter, J.D. Robbins, and E.S. Luttrell, 1977. Epichloe-Typhina from toxic tall fescue grasses. Appl. Environ. Microbiol. 34:576-581.

Bacon, C.W. and J.F. White, 1994. Stains, media and procedures for analyzing endophytes, p. 47-56. In: Bacon, C. W. and White, J. F. (eds.), Biotechnology of endophytic fungi of grasses. CRC Press, Boca Raton.

Barras, J.A., J.C. Bernier, and R.A. Morton, 2008. Land area change in coastal Louisiana: A multidecadal perspective (from 1956 to 2006): U.S. Geological Survey Scientific Investigations Map 3019, p. 14. In: Interior, U. S. D. o. t. and Survey, U. S. G. (eds.).

Baskin, C.C.B.J.M., 1998. Seeds : ecology, biogeography, and evolution of dormancy and germination. Academic Press, San Diego, Calif.

Becker, W.F., G. Vonjagow, T. Anke, and W. Steglich, 1981. Oudemansin, strobilurin-A, strobilurin-B and myxothiazol: new inhibitors of the bc1 segment of the respiratory chain with an e-beta-methoxyacrylate system as common structural element. Febs Letters 132:329-333.

Belesky, D. and D. Malinowski, 2000. Abiotic stresses and morphological plasticity and chemical adaptations of Neotyphodium-infected tall fescue plants, p. 455-484. In: Bacon, C. W. and White, J. F. (eds.), Microbial Endophytes. M. Dekker, New York.

Bollen, G.J. and A. Fuchs, 1970. On the specificity of the in vitro and in vivo antifungal activity of benomyl. Neth. J. Plant Pathol. 76:299-312.

Bouton, J.H., R.N. Gates, D.P. Belesky, and M. Owsley, 1993. Yield and persistence of tall fescue in the Southeastern Coastal-Plain after removal of its endophyte. Agron. J. 85:52-55.

Boyce, S.G., 1954. The Salt Spray Community. Ecol. Monogr. 24:29-67.

Brem, D. and A. Leuchtmann, 2002. Intraspecific competition of endophyte infected vs uninfected plants of two woodland grass species. Oikos 96:281-290.

Brown, W.V. and B.N. Smith, 1974. The Kranz Syndrome in Uniola (Gramineae). Bul. Torrey Botanical Club 101:117-120.

Brzuszek, R.F. and R.L. Harkess, 2009. Green industry survey of native plant marketing in the southeastern United States. HortTechnology 19:168-172.

Bultman, T.L., J.F. White, T.I. Bowdish, A.M. Welch, and J. Johnston, 1995. Mutualistic transfer of Epichloe spermatia by Phorbia flies. Mycologia 87:182-189.

Burgess, T., F. Blazich, and D. Nash, 2002. Seed Germination of Southern Seaoats (*Uniola paniculata*) as Influenced by Stratification, Temperature, and Light. J. Environ. Hort. 20:180-183.

Bush, L.P., H.H. Wilkinson, and C.L. Schardl, 1997. Bioprotective alkaloids of grass-fungal endophyte symbioses. Plant Physiol. 114:1-7.

Carroll, G., 1988. Fungal endophytes in stems and leaves, from latent pathogen to mutualistic symbiont. Ecol. 69:2-9.

Cheplick, G.P. and S.H. Faeth, 2009. Ecology and evolution of the grass-endophyte symbiosis. Oxford University Press, Oxford, New York.

Christensen, M.J., R.J. Bennett, and J. Schmid, 2002. Growth of Epichloe/Neotyphodium and p-endophytes in leaves of Lolium and Festuca grasses. Mycol. Res. 106:93-106.

Clay, K., 1991. Endophytes as antagonists of plant pests, p. 331-357. In: Andrews, J. H., Hirano, S. S., and International Symposium on the Microbiology of the, P. (eds.), Microbial ecology of leaves. Springer-Verlag, New York.

Clay, K. and J. Holah, 1999. Fungal endophyte symbiosis and plant diversity in successional fields. Science 285:1742-1744.

Clay, K., S. Marks, and G.P. Cheplick, 1993. Effects of insect herbivory and fungal endophyte infection on competitive interactions among grasses. Ecol. 74:1767-1777.

Clay, K. and C. Schardl, 2002. Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. Am. Nat. 160:S99-S127.

Commission, L.W. 2011. Coastal Restoration Spending in Louisiana: Economic Impact Analysis.

Conover, M.R., 1998. Impact of consuming tall fescue leaves with the endophytic fungus, Acremonium coenophialum, on meadow voles. J. Mammal. 79:457-463.

Conover, M.R. and T.A. Messmer, 1996. Feeding preferences and changes in mass of Canada geese grazing endophyte-infected tall fescue. Condor 98:859-862.

Copeland, L.O. and M.B. McDonald, 2001. Principles of seed science and technology. Kluwer Academic Publishers, Boston, Mass.

CPRA. 2012. Fiscal Year 2013 Annual Plan: Integrated Ecosystem Restoration and Hurricane Protection in Coastal Louisiana. Coastal Protection and Restoration Authority of Louisiana. 28 May 2013. <http://www.lacpra.org/assets/docs/2012%20Plans/Final_FY_2013_Annual_Plan_3-19-2012_Web_Format.pdf>.

Dahl, B.E., 1974. Stabilization and reconstruction of Texas coastal foredunes with vegetation. Final report to U.S. Army Corps of Engineers, Coastal Engineering Research Center, Fort Belvoir, Virginia, 22060. Gulf Universities Research Consortium, Galveston.

Dahl, B.E. and D.W. Woodard, 1977. Construction of Texas coastal foredunes with sea oats (*Uniola paniculata*) and bitter panicum (*Panicum amarum*). Intl. J. Biometeorol. 21:267-275.

Douglas, A.E., 1994. Symbiotic interactions. Oxford University Press, Oxford ; New York.

Edgington, L.V., K.L. Khew, and G.L. Barron, 1971. Fungitoxic Spectrum of Benzimidazole Compounds. Phytopathol. 61:42-44.

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

EPA, 2013. Reduced Risk/Organophosphate Alternative Decisions for Conventional Pesticides, p. 12. In: Agency, E. P. (ed.).

Estes, J.R. and R.J. Tyrl, 1982. The generic concept and generic circumpscription in the Triticeae: an end paper., p. 145-164. In: Brunken, J. N., American Society of Plant Taxonomists., Ecological Society of America., and Botanical Society of America. (eds.), Grasses and grasslands : systematics and ecology. University of Oklahoma Press, Norman.

Everitt, J.H., 2011. Grasses of South Texas : a guide to their identification and value. Texas Tech University Press, Lubbock, Tex.

Faeth, S.H., M.L. Helander, and K.T. Saikkonen, 2004. Asexual Neotyphodium endophytes in a native grass reduce competitive abilities. Ecol. Lett. 7:304-313.

Faeth, S.H. and T.J. Sullivan, 2003. Mutualistic Asexual Endophytes in a Native Grass Are Usually Parasitic. The Am. Naturalist 161:310-325.

Fine, G. 2007. Plant Guide- sea oats. USDA NRCS Golden Meadow Plant Materials Center. <http://www.plant-materials.nrcs.usda.gov/pubs/lapmcpg7412.pdf>.

Finkl, C.W. and S.M. Khalil, 2005. Saving America's wetland: Strategies for restoration of Louisiana's coastal wetlands and barrier islands. J. Coast. Res.:2-6.

Fortier, G.M., N. Bard, M. Jansen, and K. Clay, 2000. Effects of tall fescue endophyte infection and population density on growth and reproduction in prairie voles. J. Wildl. Mgt. 64:122-128.

Garber, M.P. and K. Bondari, 1993. Trends in Plant Material Requirements of Landscape Architects. J. Environ. Hort. 11:110-115.

Gianessi, L. and N. Reigner, 2006. The importance of fungicides in U.S. crop production. Outlooks on Pest Management 17:209-213.

Giuliano, W.M., C.L. Elliott, and J.D. Sole, 1994. Significance of Tall Fescue in the diet of the Eastern Cottontail. The Prairie naturalist. 26:53-60.

Glenn, A.E., C.W. Bacon, R. Price, and R.T. Hanlin, 1996. Molecular phylogeny of Acremonium and its taxonomic implications. Mycologia 88:369-383.

Gould, F.W. and R.B. Shaw, 1983. Grass systematics. 2nd. Texas A & M University Press, College Station.

Greenlee, J., 1992. The encyclopedia of ornamental grasses : how to grow and use over 250 beautiful and versatile plants. Rodale Press ; Distributed in the book trade by St. Martin's Press, Emmaus, Pa.

Grounds, R., 2004. Gardening with ornamental grasses. Horticulture Publications, Boston, Mass.

Harper, J.R. and E.D. Seneca, 1974. A Preliminary Study of Flowering in Uniola paniculata Along the North Carolina Coast. Bul. Torrey Botanical Club 101:7-13.

Hester, M.W. and I.A. Mendelssohn, 1987. Seed Production and Germination Response of Four Louisiana Populations of Uniola paniculata (Gramineae). Amer. J. Bot. 74:1093-1101.

Hester, M.W. and I.A. Mendelssohn, 1989. Water Relations and Growth Responses of Uniola paniculata (Sea Oats) to Soil Moisture and Water-Table Depth. Oecologia 78:289-296.

Hester, M.W. and I.A. Mendelssohn, 1990. Effects of Macronutrient and Micronutrient Additions on Photosynthesis, Growth Parameters, and Leaf Nutrient Concentrations of Uniola paniculata and Panicum amarum. Botanical Gaz. 151:21-29.

Hester, M.W. and I.A. Mendelssohn, 1991. Expansion Patterns and Soil Physicochemical Characterization of Three Louisiana Populations of Uniola paniculata (Sea Oats). J. Coast. Res. 7:387-401.

Hill, N.S., D.P. Belesky, and W.C. Stringer, 1991. Competitiveness of Tall Fescue as influenced by *Acremonium coenophialum*. Crop Sci. 31:185-190.

Hoveland, C.S., 1993. Importance and economic significance of the Acremonium endophytes to performance of animals and grass plant. Agr., Ecosystems & Environ. 44:3-12.

Johnson, N.C., J.H. Graham, and F.A. Smith, 1997. Functioning of mycorrhizal associations along the mutualism-parasitism continuum. New Phytol. 135:575-586.

Judd, F.W., R.I. Lonard, and S.L. Sides, 1977. The Vegetation of South Padre Island, Texas in Relation to Topography. The Southwest. Naturalist 22:31-48.

Kearney, T.H., 1904. Are Plants of Sea Beaches and Dunes True Halophytes? Botanical Gaz. 37:424-436.

Kegley, S.E., B.R. Hill, O. S., and C. A.H., 2010. PAN Pesticide Database-Chemicals: Mefenoxam. Pesticide Action Network, North America, San Francisco, CA.

Kim, Y.S., E.W. Dixon, P. Vincelli, and M.L. Farman, 2003. Field resistance to strobilurin (Q(o)I) fungicides in Pyricularia grisea caused by mutations in the mitochondrial cytochrome b gene. Phytopathol. 93:891-900.

Knight, S.C., V.M. Anthony, A.M. Brady, A.J. Greenland, S.P. Heaney, D.C. Murray, K.A. Powell, M.A. Schulz, C.A. Spinks, P.A. Worthington, and D. Youle, 1997. Rationale and perspectives on the development of fungicides. Annu. Rev. Phytopathol. 35:349-372.

Knott, C.A., M.D. Materne, P.K. Subudhi, N. Baisakh, H. Utomo, and S.A. Harrison, 2012. Registration of Sea Oats Cultivars 'LA12-201', 'LA12-202', and 'LA12-203'. J. of Plant Registrations 6:289-293.

Krings, M., T.N. Taylor, H. Hass, H. Kerp, N. Dotzler, and E.J. Hermsen, 2007. Fungal endophytes in a 400-million-yr-old land plant: infection pathways, spatial distribution, and host responses. New Phytol. 174:648-657.

Kuldau, G.A., J.S. Liu, J.F. White, M.R. Siegel, and C.L. Schardl, 1997. Molecular systematics of Clavicipitaceae supporting monophyly of genus Epichloe and form genus Ephelis. Mycologia 89:431-441.

Kutner, L.S. and L.E. Morse, 1996. Reintroduction in a changing climate, p. 23-48. In: Falk, D. A., Millar, C. I., and Olwell, M. (eds.), Restoring diversity : strategies for reintroduction of endangered plants. Island Press, Washington, D.C.

Lacroix, L., G. Bic, L. Burgaud, M. Guillot, R. Leblanc, R. Riottot, and M. Sauli, 1974. Study on the antifungal properties of a new group of derivatives of hydantoin, particularly 26019 R.P. Phytiatrie-Phytopharmacie 23:165-174.

Lane, C., S.J. Wright, J. Roncal, and J. Maschinski, 2008. Characterizing environmental gradients and their influence on vegetation zonation in a subtropical coastal sand dune system. J. Coast. Res. 24:213-224.

Leuchtmann, A., 1993. Systematics, distribution, and host specificity of grass endophytes. Natural Toxins 1:150-162.

Loewer, P., 2003. Ornamental Grasses for the Southeast. Cool Springs Press, Nashville, Tennessee.

Lonard, R.I., F.W. Judd, and R. Stalter, 2011. Biological Flora of Coastal Dunes and Wetlands: Uniola paniculata L. J. Coast. Res. 27:984-993.

Lyr, H.B.P., 1995. Modern selective fungicides : properties, applications, mechanisms of action. G. Fischer, Jena; Stuttgart; New York.

Maddox, V. and L. Kelly, 2009. Ornamental Grasses for the Midsouth Landscape. In: Service, M. S. U. E. (ed.).

Madej, C.W. and K. Clay, 1991. Avian seed preference and weight-loss experiments: the effect of fungal endophyte-infected tall fescue seeds. Oecologia 88:296-302.

Malinowski, D.P. and D.P. Belesky, 2000. Adaptations of endophyte-infected cool-season grasses to environmental stresses: Mechanisms of drought and mineral stress tolerance. Crop Science 40:923-940.

Meyer, M.H., 1975. Ornamental Grasses, decorative plants for home and garden. Charles Scribner's Sons, New York.

Miller, D.L., M. Thetford, and L. Yager, 2001. Evaluation of sand fence and vegetation for dune building following overwash by hurricane Opal on Santa Rosa Island, Florida. J. Coast. Res. 17:936-948.

Miller, D.L., L. Yager, M. Thetford, and M. Schneider, 2003. Potential use of Uniola paniculata rhizome fragments for dune restoration. Restor. Ecol. 11:359-369.

Milne, G.W.A., 2004. Pesticides : an international guide to 1800 pest control chemicals. Ashgate, Aldershot, Hampshire, England; Burlington, VT.

Morton, V. and T. Staub. 2008. A Short History of Fungicides. APSnet Features. <http://www.apsnet.org/publications/apsnetfeatures/Pages/Fungicides.aspx>.

Nabukalu, P. 2013. Development of an efficient sea oats breeding program for coastal restoration. Louisiana State University, Baton Rouge, PhD Diss.

Nabukalu, P. and C. Knott, 2013. Effect of seed storage environment on sea oats (*Uniola paniculata*) germination. Ecol. Restor. 31:16-19.

Nuninger, C., G. Watson, N. Leadbitter, and H. Ellgehausen. 1996. CGA329351: introduction of the enantiomeric form of the fungicide metalaxyl. Proc. Brighton Crop Protection Conf. 1:41-46.

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

Paracer, S. and V. Ahmadjian, 2000. Symbiosis : an introduction to biological associations. Oxford University Press, New York; Oxford.

Petrini, O., T.N. Sieber, L. Toti, and O. Viret, 1993. Ecology, metabolite production, and substrate utilization in endophytic fungi. Natural Toxins 1:185-196.

Radford, A., 1968. Uniola L., p. 65-67. In: Ahles, H. B., CR (ed.), Manual of Vascular Flora of the Carolinas. Univ. of NC Press, Chapel Hill, NC.

Roberts, C.A., C.P. West, and D.E. Spiers, 2005. Neotyphodium in cool-season grasses. 1st. Blackwell Pub., Ames, Iowa.

Roberts, J.R. and J.R. Reigart, 2013. Recognition and Management of Pesticide Poisonings. 6th ed. U.S. Environmental Protection Agency.

Rudgers, J.A. and A.L. Swafford, 2009. Benefits of a fungal endophyte in Elymus virginicus decline under drought stress. Basic and Appl. Ecol. 10:43-51.

Ruter, J.M. and A.B. Carter. 2000. Ornamental grass evaluations at NESPAL. Proc. Southern Nursery Assn. Res. Conf. 45:398-400.

SAS Institute. 2002-2011. SAS for Windows. Version 9.3. Inst., S., Cary, NC.

Saxton, A.M., 1998. A macro for converting mean separation output to letter groupings in Proc Mixed, p. 1243-1246, Proc. 23rd SAS Users Group Intl. SAS Institute.

Schardl, C. and A. Leuchtmann, 2005. The Epichloë endophytes of grasses and the symbiotic continuum, p. 475-503. In: Dighton, J. W., James F.; Oudemans, Peter (ed.), The Fungal community : its organization and role in the ecosystem. Taylor & Francis, Boca Raton, FL.

Schardl, C.L., 1996. Epichloe species: Fungal symbionts of grasses. Annu. Rev. Phytopathol. 34:109-130.

Schmidt, S.P. and T.G. Osborn, 1993. Effects of endophyte-infected tall fescue on animal performance. Agr., Ecosystems & Environ. 44:233-262.

Schulz, B. and C. Boyle, 2005. The endophytic continuum. Mycol. Res. 109:661-686.

Selosse, M.A. and F. Le Tacon, 1998. The land flora: a phototroph-fungus partnership? Trends Ecol. Evol. 13:15-20.

Senaca, E.D., 1969. Germination Response to Temperature and Salinity of Four Dune Grasses from the Outer Bank of North Carolina. Ecol. 50:45-53.

Seneca, E.D., 1972. Germination and Seedling Response of Atlantic and Gulf Coasts Populations of Uniola paniculata. Amer. J. Bot. 59:290-296.

Service, N.R.C., 2010. Why Natives? Plant materials technical note. In: Agriculture, U. S. D. o. (ed.).

Sharvelle, E.G., 1961. The nature and uses of modern fungicides. Burgess Pub. Co., Minneapolis.

Sinclair, J.B. and R.F. Cerkauskas, 1996. Latent infection vs. endophytic colonization by fungi, p. 3-29. In: Redlin, S. C. and Carris, L. M. (eds.), Endophytic fungi in grasses and woody plants : systematics, ecology, and evolution. APS Press, St. Paul, Minn.

Stone, J., C. Bacon, and J. White, 2000. An overview of endophytic microbes: endophytism defined, p. 3-29. In: Bacon, C. W. and White, J. F. (eds.), Microbial Endophytes. M. Dekker, New York.

Sylvia, D.M., 1989. Nursery Inoculation of Sea Oats with Vesicular-Arbuscular Mycorrhizal Fungi and Outplanting Performance on Florida Beaches. J. Coast. Res. 5:747-754.

Syngenta, 2009. Fungicide label: Subdue Maxx. Greensboro, NC.

Syngenta Crop Protection, I. 2005. Mefenoxam: The active ingredient in Ridomil Gold®. Syngenta Group. <http://www.syngentacropprotection.com/Env_Stewardship/futuretopics/Mefenoxam_12_16_05.pdf>.

Thompson, F.N. and J.A. Stuedemann, 1993. Pathophysiology of fescue toxicosis. Agr., Ecosystems & Environ. 44:263-281.

Torgeson, D.C., 1967. Fungicides; an advanced treatise. Academic Press, New York.

Valk, A.G.v.d., 1977. The Role of Leaves in the Uptake of Nutrients by Uniola paniculata and Ammophila breviligulata. Chesapeake Science 18:77-79.

Wagner, R.H., 1964. The Ecology of Uniola paniculata L. in the Dune-Strand Habitat of North Carolina. Ecol. Monogr. 34:79-96.

Walker, A. and S.J. Welch, 1990. Enhanced biodegradation of dicarboximide fungicides in soil. Acs Symposium Series 426:53-67.

West, C.P., 1994. Physiology and drought tolerance of endophyte-infected grasses, p. 87-99. In: Bacon, C. W. and White, J. F. (eds.), Biotechnology of endophytic fungi of grasses. CRC Press, Boca Raton.

Westra, R.N. and W.E. Loomis, 1966. Seed Dormancy in Uniola paniculata. Amer. J. Bot. 53:407-411.

White, J.F., 1997. Systematics of the graminicolous Clavicipitaceae: applications of morphological and molecular approaches. In: Bacon, C. W. and Hill, N. S. (eds.), *Neotyphodium*/ grass interactions.

White, J.F., A.C. Morrow, G. Morganjones, and D.A. Chambless, 1991. Endophyte-host associations in forage grasses. 14. Primary stromata formation and seed transmission in Epichloe-Typhina developmental and regulatory aspects. Mycologia 83:72-81.

Will, M.E. and D.M. Sylvia, 1990. Interaction of rhizosphere bacteria, fertilizer, and vesicular-arbuscular mycorrhizal fungi with sea oats. Appl. Environ. Microbiol. 56:2073-2079.

Williams, M.J. 2007. Native Plants for Coastal Restoration: What, When, and How for Florida. USDA, NRCS. <http://www.fl.nrcs.usda.gov/programs/pmc/flplantmaterials.html>.

Wilson, D., 1995. Endophyte- the evolution of a term, and clarification of its use and defintion. Oikos 73:274-276.

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

Woodhouse, W.W. and R.E. Hanes, 1967. Dune stabilization with vegetation on the outer banks of North Carolina. U.S. Army, Coastal Engineering Research Center, Washington.

Yates, H.O., 1966a. Morphology and Cytology of Uniola (Gramineae). The Southwest. Naturalist 11:145-189.

Yates, H.O., 1966b. Revision of Grasses Traditionally Referred to Uniola, I. Uniola and Leptochloöpsis. The Southwest. Naturalist 11:372-394.

Yates, H.O., 1966c. Revision of Grasses Traditionally Referred to Uniola, II. Chasmanthium. The Southwest. Naturalist 11:415-455.

Yates, H.O., 2003. *Uniola* L., p. 22-24. In: Barkworth, M. E. and Flora of North America Editorial, C. (eds.), Flora of North America. north of Mexico, Magnoliophyta : Commelinidae (in part): Poaceae, part 2. Oxford University Press, New York; Oxford.

Appendix A: Fungicide tables of likelihood, contrasts, and means

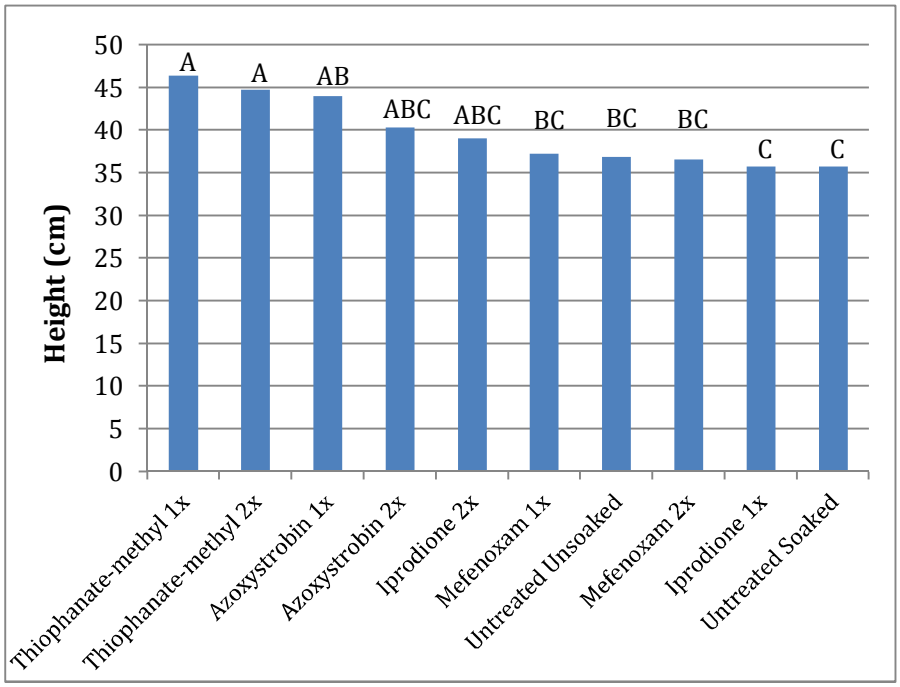
Table A.1 Significant differences of treatment LSMeans for survival percentage based on pairwise comparisons adjusted using Tukey's test ($p < 0.05$).

Treatment	to Treatment	Adjusted P	Likelihood %
Azoxystrobin 1x	Iprodione 1x	0.0192	108
Azoxystrobin 2x	Iprodione 1x	0.0280	113
Thiophanate-methyl 1x	Untreated Unsoaked	0.0019	187
Thiophanate-methyl 2x	Untreated Unsoaked	0.0010	194
Thiophanate-methyl 1x	Untreated Soaked	0.0086	159
Thiophanate-methyl 2x	Untreated Soaked	0.0051	165
Thiophanate-methyl 1x	Iprodione 1x	<.0001	229
Thiophanate-methyl 2x	Iprodione 1x	<.0001	235
Thiophanate-methyl 2x	Iprodione 2x	0.0381	134
Thiophanate-methyl 1x	Mefenoxam 2x	0.0321	146
Thiophanate-methyl 2x	Mefenoxam 2x	0.0213	152

Table A.2 Significant contrasts of average sea oats shoot heights based on pairwise tests ($p < 0.05$).

Treatment Groups Contrasted	F Value	Pr > F
All fungicide treatments vs. Untreated Controls	12.06	0.0023
Thiophanate-methyl 1x & 2x vs. Untreated Controls	37.72	<.0001
Thiophanate-methyl & Azoxystrobin 1x's & 2x's vs. all other treatments	51.76	<.0001
Thiophanate-methyl 1x & 2x vs. all other treatments	39.16	<.0001
Azoxystrobin 1x & 2x vs. Untreated Controls	14.92	0.0010
Thiophanate-methyl, Azoxystrobin & Mefenoxam 1x's & 2x's & Iprodione 2x vs. all other treatments	23.15	0.0001
Thiophanate-methyl, Azoxystrobin & Iprodione 1x's & 2x's vs. all other treatments	26.98	<.0001

Figure A.1 Average shoot heights by treatment. Treatments with different letters are significantly different according to Tukey's test (t test, $p < 0.05$).



Appendix B: Climate and field data

Table B.1 Climate data from the Burden Museum and Gardens, Baton Rouge, LA. Data was collected hourly.

	Precipitation (mm)		Precipitation (in)		°C Temperature			°F Temperature		
Month	**Total**	**Average**	**Total**	**Average**	**Average**	**High**	**Low**	**Average**	**High**	**Low**
Oct-12	1.4	0.0	0.05	0.00	17.8	29.5	1.8	64.0	85.2	35.3
Nov-12	48.8	0.1	1.92	0.00	14.3	29.1	0.9	57.8	84.3	33.7
Dec-12	73.2	0.1	2.88	0.00	13.9	26.4	-1.5	57.0	79.5	29.3
Jan-13	26.4	0.0	1.04	0.00	12.5	26.1	1.1	54.5	79.0	34.0
Feb-13	3.2	0.0	0.12	0.00	13.4	24.4	1.7	56.1	75.9	35.0
Mar-13	67.2	0.1	2.64	0.00	14.3	28.3	-3.3	57.7	82.9	26.0
Apr-13	220.0	0.3	8.66	0.01	19.3	29.5	3.9	66.7	85.1	38.9
May-13	222.2	0.3	8.75	0.01	22.7	33.1	3.9	72.9	91.6	39.0
Jun-13	85.8	0.1	3.38	0.00	28.2	36.7	20.6	82.8	98.0	69.1
Jul-13	115.8	0.2	4.56	0.01	27.3	35.3	19.6	81.2	95.6	67.2
Aug-13	114.0	0.2	4.49	0.01	27.5	36.6	18.7	81.5	97.9	65.7

Table B.2 Soil moisture percentage of each block measured each month data collected.

	MAT	2	3	4	7	8	9	10	11	12
Block	**Sample**[1]	**Oct. '12**	**Nov.**	**Dec.**	**March '13**	**April**	**May**	**June**	**July**	**Aug. '13**
1	1	17.58	9.73	19.17	17.10	17.02	14.87	14.72	7.69	9.27
	2	25.84	10.76	30.36	14.44	15.01	14.39	12.21	4.28	5.62
2	1	15.83	9.84	25.61	15.82	20.35	18.35	19.83	5.08	7.33
	2	27.42	6.26	33.08	10.80	23.20	21.62	22.03	4.21	5.09
3	1	20.94	14.39	27.88	26.91	19.71	28.78	19.74	8.65	10.30
	2	34.62	16.36	36.56	28.89	36.64	28.51	27.05	9.55	21.40
4	1	22.89	10.39	27.99	17.91	21.94	19.48	21.34	6.37	10.72
	2	27.22	16.45	30.00	24.00	28.38	28.78	25.80	9.48	9.69
Overall MAT mean		24.04	11.77	28.83	19.48	22.78	21.85	20.34	6.91	9.93

1- Samples taken from middle (1) and end (2) of blocks from a random row within the block.

Table B.3 Soil fertility analysis of random samples from each experimental block performed by Louisiana State University Soil Testing Lab August 2013.

Field Block	**Calcium, ppm**	**Copper, ppm**	**Magnesium, ppm**	**pH (1:1 Water)**	**Phosphorus, ppm**	**Potassium, ppm**	**Sodium, ppm**	**Sulfur, ppm**	**Zinc, ppm**
B-1	901.318	0.945	196.822	6.880	9.429	40.549	20.610	14.275	1.372
B-2	855.337	1.010	169.223	7.070	23.700	54.240	6.418	6.205	1.961
B-3	999.000	1.573	220.602	6.800	23.394	96.813	55.601	15.708	2.954
B-4	720.669	0.907	123.186	6.600	18.405	55.379	4.866	7.283	2.091

Note: ppm is equivalent to mg/Kg for soil and plant samples and is equivalent to mg/L for water samples. For a description of methods used, visit web site at: http://www.stpal.lsu.edu

Appendix C: Ornamental selections

Figure C.1

Figure C.2

Figure C.3

Figure C.4

Clonal check

Figure C.5

Figure C.6

Figure C.7

Figures C.1-C.7 Examples of selected experimental lines for potential use as urban ornamental sea oats at 9 months after transplant (May 2013) (all photos by author).

Figure C.8

Figure C.9

Figure C.10

Figure C.11

Figures C.8-C.14 Examples of selected experimental lines for potential use as urban ornamental sea oats at final month of study/ 12 months after transplant (August 2013) (all photos by author).

Figure C.12

Figure C.13

Figure C.14

Essen Lane

4076	4061	4046		4031	4016	4001		3076	3061	3046		3031	3016	3001		2076	2061	2046		2031	2016	2001		1076	1061	1046		1031	1016	1001
4077	4062	4047		4032	4017	4002		3077	3062	3047		3032	3017	3002		2077	2062	2047		2032	2017	2002		1077	1062	1047		1032	1017	1002
4078	4063	4048		4033	4018	4003		3078	3063	3048		3033	3018	3003		2078	2063	2048		2033	2018	2003		1078	1063	1048		1033	1018	1003
4079	4064	4049		4034	4019	4004		3079	3064	3049		3034	3019	3004		2079	2064	2049		2034	2019	2004		1079	1064	1049		1034	1019	1004
4080	4065	4050		4035	4020	4005		3080	3065	3050		3035	3020	3005		2080	2065	2050		2035	2020	2005		1080	1065	1050		1035	1020	1005
4081	4066	4051		4036	4021	4006		3081	3066	3051		3036	3021	3006		2081	2066	2051		2036	2021	2006		1081	1066	1051		1036	1021	1006
4082	4067	4052		4037	4022	4007		3082	3067	3052		3037	3022	3007		2082	2067	2052		2037	2022	2007		1082	1067	1052		1037	1022	1007
4083	4068	4053		4038	4023	4008		3083	3068	3053		3038	3023	3008		2083	2068	2053		2038	2023	2008		1083	1068	1053		1038	1023	1008
4084	4069	4054		4039	4024	4009		3084	3069	3054		3039	3024	3009		2084	2069	2054		2039	2024	2009		1084	1069	1054		1039	1024	1009
4085	4070	4055		4040	4025	4010		3085	3070	3055		3040	3025	3010		2085	2070	2055		2040	2025	2010		1085	1070	1055		1040	1025	1010
4086	4071	4056		4041	4026	4011		3086	3071	3056		3041	3026	3011		2086	2071	2056		2041	2026	2011		1086	1071	1056		1041	1026	1011
4087	4072	4057		4042	4027	4012		3087	3072	3057		3042	3027	3012		2087	2072	2057		2042	2027	2012		1087	1072	1057		1042	1027	1012
4088	4073	4058		4043	4028	4013		3088	3073	3058		3043	3028	3013		2088	2073	2058		2043	2028	2013		1088	1073	1058		1043	1028	1013
4089	4074	4059		4044	4029	4014		3089	3074	3059		3044	3029	3014		2089	2074	2059		2044	2029	2014		1089	1074	1059		1044	1029	1014
4090	4075	4060		4045	4030	4015		3090	3075	3060		3045	3030	3015		2090	2075	2060		2045	2030	2015		1090	1075	1060		1045	1030	1015

Block 4 **Block 3** **Block 2** **Block 1**

Figure C.15 Field plot layout at Burden Museum and Gardens, Baton Rouge, LA. Highlighted numbers are ornamental selections. Plot numbers represent individual plants and hatched areas represent open grass space for cart access.

Vita

Kaitlin Barrios hails from Baton Rouge, LA, U.S. She completed her Bachelor of Landscape Architecture degree from Louisiana State University in spring of 2007. While pursuing her degree she received the LA TOPS and Dean's List Scholarships. Following graduation she gained an internship at a landscape architectural firm in Costa Rica after which she moved abroad for one academic year to work as an English-teaching assistant in Spain. Upon returning to the U.S., she worked at plant nurseries and garden retail centers in Austin, TX and Baton Rouge, LA. Seeking to further her plant and ecological knowledge in an academic setting she returned to LSU and enrolled in 2010 for two semesters of undergraduate courses. This transition allowed her to enroll in and become a candidate for a Master of Science degree in the School of Plant, Environmental and Soil Sciences (SPESS). Her master's studies have allowed her the pursuit of a horticultural and agronomic education while gaining scientific research skills and experience. She received a graduate research assistantship at the start of her master's degree in spring 2011 under Dr. Carrie Knott working in the Coastal Plant Breeding Program. Additionally she has received several scholarships from the Louisiana Garden Club and SPESS. She plans to graduate in December of 2013 and seek employment locally, out-of-state, or internationally in order to continue her studies of plant breeding, ideally in ornamentals.