Occurrence and Control of Large Patch (Rhizoctonia solani AG 2’2 «LP« on Seashore Paspalum (Paspalum vaginatum O. Swartz) in South Carolina

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OCCURRENCE AND CONTROL OF LARGE PATCH (RHIZOCTONIA SOLANI AG 2-2 ‘LP’ ON SEASHORE PASPALUM (PASPALUM VAGINATUM O. SWARTZ) IN SOUTH CAROLINA

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Plant and Environmental Sciences

by
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Accepted by:
Dr. S. Bruce Martin, Committee Chair
Dr. James J. Camberato
Dr. Steven N. Jeffers
ABSTRACT

Seashore paspalum is a new turfgrass being evaluated in the southeastern United States due to its tolerance to salinity and sodicity. *Rhizoctonia solani* AG 2-2 ‘LP’ was isolated from seashore paspalum exhibiting symptoms typical of Large Patch (LP). The disease was identified based on field symptoms, lesions on the plant, and the fungus identified based on cultural characteristics, multinucleate hyphal cells, and anastomosis with a known tester isolate of *R. solani* AG 2-2 ‘LP’ from zoysia grass. Two research putting greens were constructed in 2005 located in Florence, SC and near Bluffton, SC and planted to seashore paspalum cultivars SeaIsle 1, SeaIsle 2000, SeaIsle Supreme, Sea Spray, SeaDwarf, Aloha and Salam, with 3 blocks at each location. Sub-plots were treated with fungicides three times in the fall 2005 on 21 day intervals, at low label rates. Epidemics of LP occurred at both locations in fall and winter months. There were no cultivar × fungicide interactions at either location. All cultivars were affected by LP and severely damaged in non-fungicide treated plots. The most susceptible cultivar at Bluffton was SeaIsle I and at Florence SeaIsle 2000. SeaIsle Supreme, Aloha and SeaSpray were damaged less. Fungicides giving the best control were azoxystrobin and pyraclostrobin; thiophanate methyl performed well at Florence but not at Bluffton.

An irrigation quality experiment was conducted in the greenhouse to evaluate the salinity tolerance of the seven cultivars to five levels of irrigation water salinity from 0.5dS/m to 30 dS/m of electrical conductivity. Significant differences in turf quality, turf density and leaf firing were found as salinity increased. The highest levels of salinity reduced quality, density and increased leaf firing to unacceptable levels.
We conclude that LP is a major disease of seashore paspalum in transition zone climates and will require fungicide treatment for acceptable quality and that there is a limit to the irrigation water electrical conductivity that seashore paspalum can tolerate with no deterioration in turf quality.
DEDICATION

This work is dedicated to Ivana and Justa the true reason of my life. They provided me love, support, comprehension, and patience to achieve my goals. I could not make it without them.
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I am grateful with Dr. S. Bruce Martin, Chairman of my Graduate Advisory Committee, for being a phenomenal source of inspiration. His guidance, friendship, patience, and comprehension throughout Graduate School at Clemson were fundamental to my success.

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INTRODUCTION

Seashore paspalum (Paspalum vaginatum O. Swartz), described as “the environmentally friendly grass”, is among the most salt and sodium tolerant turfgrass species. This halophytic, perennial warm-season grass, which undergoes winter dormancy in colder climates, produces a beautiful turfgrass surface during the growing season. It is used mainly in mild to warm climates when the soil salinity and sodicity are high, when the drainage is a problem, and when water quality is poor. It can be used as a turfgrass for lawns, athletic fields, and golf courses but also as a ground cover to control erosion and to stabilize dunes and coasts (Duncan and Carrow 2000). Typically it is propagated vegetatively (by sod or sprigs) because this species does not produce a large amount of viable seeds and seed production is generally not reliable (Duncan and Carrow 2000); however, there is a new cultivar (‘Sea Spray’) that can be established by seed. It produces a dense and high quality turf, has excellent drought resistance and dehydration avoidance (Beard 2002), is fairly competitive against weeds, and requires less nitrogen than other warm-season grasses.

Seashore paspalum exhibits little disease incidence in its naturalized habitat, however, under high maintenance programs on modern golf courses, several diseases have been found to cause severe damage under typical management and environmental regimes. Because seashore paspalum is a relatively new grass species for golf courses in the southeastern United States, research information related to the occurrence and management of diseases on this grass as well as its shade tolerance, cold and drought tolerance is very limited.
Centers of Origin

Some authors consider seashore paspalum to be native to Asia, Africa, and Europe (Judd 1979) and introduced to the Americas. Other botanists believe that it is originated in America and naturalized into the old world (Bovo et al 1988, Echarte et al 1992, IN Duncan and Carrow 2000, Ch 4, Page 18). Though the true origin is not clear, recent studies on gene diversity and genetic distance among populations from different regions support the theory that seashore paspalum was introduced to North and South America from South Africa. Diversity among the ecotypes from South Africa is the largest while accessions from North America and South America are highly similar (Chen et al 2005).

Introduction to the United States

The wild, fine-leaf textured ecotypes of seashore paspalum found on the west coast of the Atlantic Ocean, primary coastal South Carolina and Georgia, are believed to have been introduced from Africa by the slave ships. The grass was used as a bed on the bottom of the ships that arrived in America during the 1700s and 1800s (Duncan and Carrow 2000).

During the 1950s, O. J. Noer propagated an ecotype he found from fairway 13 at the Sea Island Golf Club, Sea Island, GA and distributed it to several people interested in this grass, including those in Hawaii. The Australian cultivars Futurf and Adalyd were introduced during the 1970s into California (Duncan and Carrow 2000), and some research was conducted in California during the 1980s. The first formal breeding program for seashore paspalum was initiated at the University of Georgia by Dr. R. R. Duncan in 1993.
Taxonomy

Family Graminae

Subfamily Panicoideae

Supertribe Paniceae

Tribe Paniceae

Subtribe Setariinae

Genus Paspalum

Group Disticha

Species vaginatum

Authority Swartz

(Duncan and Carrow 2000)

The tribe Paniceae includes Axonopus (carpetgrass), Digitaria (crabgrass), Pennisetum (kikuyugrass), and Stenotaphrum (St. Augustinegrass). In the subtribe Setariinae are the genera Paspalum, Panicum, Echinochloa, Setaria, and Axonopus. Paspalum vaginatum and P. distichum are in the group Disticha which can be found naturally in saline ecosystems along seacoasts, with P. vaginatum being dispersed over a wider geographical area than P. distichum. Paspalum vaginatum is a sexually reproducing decumbent diploid species (2n=2x=20) with some self-sterility and a propensity for cross pollination between clones of diverse origin (Carpenter 1958).

Brief Botanical Description of Seashore Paspalum

Paspalum vaginatum is an aggressive warm season perennial grass with a low stoloniferous growth habit. It produces a large root volume and also a high amount of deeply occurring rhizomes and stolons. I have found rhizomes up to 60 cm deep during a
golf green renovation at Highland Park Country Club in Del Viso BA, Argentina (Canegallo unpublished 1999).

Vernation is rolled. Sheaths are large, conspicuous, and compressed. Membranous and short ligules (0.5 mm long) are present with a pubescent, broad collar. Auricles are absent (http://www.griffin.uga.edu/caes/turf/Turfgras/1113_Seashore.htm) or small (Beard 2002). Leaf blade texture is ranges widely in variation: stiff and narrow leaf-blades range from very coarse to coarse, and intermediate to fine types resemble dwarf bermudagrasses (Duble and Carrow 2000). Depending on the ecotype and fertilization, they are capable of producing a dense canopy, from light to dark green. Stems are flattened and smooth and have elongated extensively creeping rhizomes with short internodes. Stolon nodes are distinctly pubescent. Flowering culms are erect or basally decumbent, varying in height from 8 to 60 cm. that produce racemes commonly of two but sometimes three spikes (Duble and Carrow 2000). Spikelets are smooth, in two rows, ovate-lanceolate, and range in size from 3.5 to 4.0 mm long (Beard 2002).

Adaptation

*Paspalum vaginatum* occurs naturally on seacoasts of the northern and southern hemispheres in aquatic, semi-aquatic, and moist environments (Skerman and Riveros 1990, IN Duble 2000, Ch 4, Page 17). It occurs along the banks of coastal rivers where it can tolerate waterlogged conditions and periodic flooding. It has been found in the Americas - almost exclusively along the Atlantic coast - and in Australia, and South Africa where the finest leafed ecotypes presumably originated.
Cultivars

All the cultivars being used as turfgrasses are considered ecotypes, i.e. strains or selections within a given species adapted to a particular environment. Several collections of seashore paspalum have been assembled: in Argentina with 28 argentine-native ecotypes (Clausen 1989) and in the USA, first at the University of Florida Ft. Lauderdale Agricultural Center (Busey 1977) and later (between 1993 and 1999) at the University of Georgia, Griffin Experiment Station (Duncan 1999). Ecotypes from Rhodesia, Mozambique, South Africa, Argentina, Hawaii, Australia, Guam, Brazil, Thailand, Israel, Uruguay, and the states of Georgia, North Carolina, South Carolina, Florida, Texas, Arizona, California, and Louisiana in the United States are present in the University of Georgia collection. In the United States, the fine-leaf textured ecotypes were found on Sea Island, GA. Several cultivars are available in the USA market for use on golf courses, sports fields, and residential and commercial landscapes.

Stress Tolerances

Salinity and Sodicity

The increasing need to provide food to an expanding population plus the increasing demand for good quality water for the urban sector results in poorer quality water and soils being used for food production or for recreation, such as golf courses and athletic fields. Saline soils cover about 23% of the world’s cultivated land, while sodic soils cover about 10% (Szaboles 1989).

Saline soils are soils that contain excessive soluble salts that impair their productivity. They are recognized by the presence of white crusts of salts on the soil surface. Soils are considered saline when the conductivity of a saturated paste extract is more than 4 dS/m at
25 °C, the pH is less than 8.5, the sodium adsorption ratio (SAR) is less than 12, and the exchangeable sodium percentage (ESP) is less than 15. Of the total cation exchange capacity (CEC), the exchangeable Na is rarely more that 50% of the soluble cations. The relative amount of Ca, Mg, and K present in the soil solution and on the exchange complex may vary considerably. The most important anions are Cl, \( \text{SO}_4^{2-} \), and \( \text{NO}_3^- \). There are almost no \( \text{CO}_3^{2-} \) anions, but some \( \text{HCO}_3^- \) may be present. In addition, saline soils may contain salts of low solubility such as gypsum (\( \text{CaSO}_4 \cdot 2\text{H}_2\text{O} \)) and lime (\( \text{CaCO}_3 \) and \( \text{MgCO}_3 \)) (Richards 1954a).

Sodic soils are those with physical properties that are adversely affected by the presence of sodium. Sodic soils have an ESP greater than 15, conductivity less than 4 dS/m at 25 °C, SAR greater than 12, and pH readings between 8.5 and 10. As the proportion of exchangeable sodium increases, the soil tends to become more dispersed. The soil solution composition differs considerably from other soils: the majority of anions are Cl, \( \text{SO}_4^{2-} \), and \( \text{HCO}_3^- \). \( \text{CO}_3^{2-} \) may be present in small amounts; however, at higher pHs, more carbonate ions can be found, Ca and Mg are precipitated, and Na dominates the soil solution and the exchange complex (Richards 1954a).

Saline-sodic soils contain both excessive soluble salts and ESP that decrease soil productivity. The ESP is greater than 15, the conductivity of the saturation extract is greater than 4 dS/m at 25 °C, SAR is greater than 12, and pH readings are normally less than 8.5. When excess salts are leached, the properties of these soils are similar to sodic soils; when sufficient leaching occurs, pH readings will be above 8.5 and soil particles will disperse. Except when gypsum is present in the soil or the irrigation water, the drainage and leaching of salts, leads to the formation of sodic soils. (Richards 1954a).
Under humid conditions, as in coastal South Carolina, the soluble salts originally present in soil materials and those formed by the weathering of minerals generally are carried downward into the ground water and are transported by streams to the ocean. Therefore, saline soils in humid regions can be found when the soil has been subjected to seawater in river deltas and other low-lying lands near the sea. Also, soils can be made saline or sodic by frequent irrigation with water high in total salts, Na, or HCO₃⁻, particularly when soil drainage is poor (Richards 1954a).

Effects of Salinity on Physical Properties of Soils

High soluble salts or high Na concentrations in soils directly affect turfgrass roots and shoots by inhibiting germination and slowing plant growth through ion toxicity, ion imbalances, and water deficits. High soluble salts or Na indirectly affect the soil physical properties: high Na concentrations destroy soil structure and results in low water infiltration, poor drainage, and lack of oxygen (Keren 2000, Levy 2000).

Physical properties (infiltration rate, permeability, bulk density, pore size distribution, and aggregation) are affected by the ion concentration of the soil solution and the ionic composition. Sodium induces adverse soil physical properties by replacing Ca and Mg on the exchangeable complex, but the severity of adverse effects depend on the soil texture: on fine textured soils adverse effects can occur at ESP 5 to 15 while in sandy soils the values must exceed 15 (Levy 2000).

Infiltration is a process that determines how much water from rainfall or irrigation enters the soil and how much becomes runoff (Radcliffe and Rasmussen 2000). ESP and EC affect the infiltration rate of the soil. Clay swelling and deflocculation caused by dispersive cations (Na and sometimes Mg and K) alters the geometry of soil pores and for this reason
the soil permeability. Also, the energy of impacting raindrops can promote dispersion allowing a seal to form at the soil surface.

Soil permeability refers to the ability of water, oxygen, and roots to move within the micropores for good plant growth (Carrow and Duncan 1998). In sodic soils, larger pores are destroyed and the amount of pore space decreases. Soil water holding capacity is higher reducing the amount of oxygen.

Bulk density is a measure of compaction and is defined as the mass of dry soil per unit volume, including both solids and pores occupied by air and water. Due to sodic conditions, pores are fewer in number and smaller so higher bulk densities are found in sodic soils.

Aggregation in soils is the arrangement of primary particles into secondary structural units. Soil aggregates consist of clay particles interspersed with larger quartzitic primary particles and held together by physicochemical forces that are the product of the chemical and the microbiological environment of that particular soil. The effect of exchangeable Na in inhibiting micro-aggregation may occur by weakening the covalent association between organic materials and soil minerals and by increasing the forces that can cause particle repulsion during wetting. Slaking (the breakdown of soil aggregates when they are suddenly immersed in or placed in contact with water (Mullins 2000)) and dispersion (the further breakdown of fine soils particles and release of clay particles 2 μm or smaller) can occur as a result of Na saturation. In addition, clay dispersion is affected not only by the presence of Na but also by the type of complementary divalent cation. The presence of Mg enhances clay dispersion in soils with mixed mineralogy compared to Ca (Yousaf et al 1987). So, aggregates saturated with Na and Mg disperse at lower ESPs than those saturated with Na and Ca (Emerson and Baker 1973 from Levy 2000).
Quality of Irrigation Water

Irrigation water quality is determined by its potential to cause problems in soils and crops and is related to the management practices needed. The quality is mainly determined by the concentration and composition of the solutes present. As the use of high quality water for human consumption is being increased, several strategies must be adopted to reduce the amount of salts in water used to irrigate turfgrasses. Irrigating with a moderate to high amount of salts increases the ionic concentration around the roots of plants and Na and other interfering ions usually exceed the essential ions (Heimann 1958). The total concentration of soluble salts in irrigation waters can be expressed in terms of electrical conductivity. Almost all irrigation waters that have been used successfully have conductivity values less than 2.25 dS m⁻¹ much of the time. Using waters with higher salt concentration usually results in damaged turf, unless the species grown is highly salt tolerant. It has been found that as a result of the continual moisture extraction from the plants and evaporation, the salt concentration in the soil can be 2 to 10 times greater than the salt concentration in the irrigation water (Richards 1954c). In general, waters with conductivity values below 0.75 dS m⁻¹ are satisfactory for irrigation. Saline conditions in soils will develop when waters in the range of 0.75 dS m⁻¹ to 2.25 dS m⁻¹ are used for irrigation if management and drainage are inadequate (Richards 1954c).

The principal cations among the inorganic constituents of the irrigation waters are Ca²⁺, Mg²⁺, and Na⁺. The principal anions are CO₃²⁻, HCO₃⁻, SO₄²⁻, and Cl⁻, with NO₃⁻ occurring in low concentrations (Keren 2000). If the proportion of Na is high, the sodicity hazard will be high. Sodic soils are formed by accumulation of exchangeable Na ions and are characterized by low permeability. When the concentration of HCO₃⁻ is high, it tends react with Ca and Mg and precipitate as CaCO₃. Mg ions do not precipitate as easily as Ca ions do,
but it can accelerate the calcium precipitation by replacing it on the exchange complex. Therefore, the concentrations of Ca and Mg in the soil solution are reduced and the relative proportion of sodium is increased (Richards 1954b)

**Salinity Effects on Plants**

**Growth Responses**

Prolonged use of saline irrigation waters can lead to saline, sodic, or saline-sodic soil properties that can further challenge plant growth. According to Dudeck et al (1983), when eight cultivars of bermudagrass were evaluated, top growth was linearly reduced as salinity in the irrigation water increased. Except on common bermudagrass, root growth increased up to a point but started declining when the EC reached 10 to 20 dS m⁻¹, depending on the cultivar. On St. Augustinegrass, Meyer et al (1989) and Dudeck et al (1993) demonstrated that top growth was reduced linearly as salinity increased, except with the most salt-tolerant cultivar, which showed a quadratic response to salinity. Even though root growth declined as salinity increased, it was less affected than top growth.

Osmotic adjustments are produced in grasses subjected to increasing salinity, because solutes accumulate and induce tissue dehydration (Marcum and Murdoch 1990). Since growth depends on the maintenance of turgor, plant growth is retarded when turgor is lost unless an osmotic adjustment is produced. Leaves of plants grown with high salinity are darker green, smaller and thicker, than leaves of a non-affected plant. Plants of alkalligrass watered with different levels of sea water were darker green and stiffer and had narrower leaf blades than the control (Harivandi et al 1982). However, Mayer et al (1989) observed no chlorosis or any other change in color on two cultivars of St Augustinegrass subjected to increased salinity.
With seashore paspalum, Peacock and Dudeck (1985) and Marcum and Murdoch (1990) found that top growth decreased as salinity increased but root growth increased at intermediate salinity levels until decreasing at higher levels of EC. Also under salt stress, Na and Cl concentrations were higher in shoots than in roots and tissue water content was similar to St. Augustinegrass but higher than the other species in the study, meaning the osmotic adaptation is produced not by reducing ion concentration but by increasing the amount of water in the tissue (Marcum and Murdoch 1990) but this depended on the cultivar (Peacock and Dudeck 1985). Concerning ion concentration, salinity did not affect N content of leaf tissue but it did affect K content that decreased as salt levels increased, but the total K content was higher than in other species and Cl and Na content increased differentially, depending on the cultivar (Dudeck and Peacock 1985).

**Mechanisms of Salt Tolerance**

A simple definition of halophytes are plants that survive and complete their life cycle at high salinities where NaCl dominates among a variety of different salts like Na$_2$SO$_4$, MgSO$_4$, CaSO$_4$, MgCl$_2$, KCl, and Na$_2$CO$_3$ (Flowers et al 1977). As a true halophyte, seashore paspalum can maintain growth at high concentrations of electrolytes in the environment. Halophytes are unique in their ability to accumulate salts in concentrations equaling or exceeding that of sea water in their leaves without detriment. In the presence of low soil water potential, halophytes can maintain high cell water content (Flowers et al 1977). Peacock and Dudeck (1985) demonstrated that leaf osmotic potential decreased linearly as salt level increased in two seashore paspalum cultivars.

Glycophyte plants (sweet lover plants) react to salinity usually by ion exclusion. There is a re-absorption of Na in the roots where it accumulates, keeping it away from the leaves. High concentrations of Na in the shoots of glycophytes are found when the
mechanism fails. By contrast, there is evidence that halophytes accumulate ions in the leaves: on average, more than 90% of the sodium is in the shoots of halophytes and no less than 80% is in the leaves. Around 95% of a mature leaf cell is filled by vacuoles. The excess of ions appears to be contained within them while the ion concentration of the cytoplasm is a half or a third of the vacuolar values (Flowers et al 1977).

Traffic

Several factors affect the wear tolerance of seashore paspalum. Shoot density, leaf potassium concentration, and the total thickness of cell walls (TCW) of the leaves and the stems. (Trenholm et al 2000). The higher the shoot density, the greater is the resistance to traffic. The presence and content of potassium in leaf tissue increases wear tolerance because it increases cell turgidity and reduces tissue succulence. The cell wall appears to be an important factor in determining the wear tolerance: decreased TCW content, enhance cell wall elasticity implying a more elastic canopy.
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CHAPTER 2
RHIZOCTONIA SOLANI ON C4 GRASSES

Introduction to the Genus Rhizoctonia

First described by DeCandolle in 1815, and meaning “root rot”, the genus Rhizoctonia has been studied for years, almost since the beginning of the Science of Plant Pathology (Ogoshi 1996). A number of isolates of Rhizoctonia cause worldwide economically important diseases: pre and post emergence damping-off, root rots of seedling and adult plants, foliar blight, leaf and sheath rots, stem and leaf blights, stem rots, and crown and root rot (dry canker) on a wide range of species of plants such cereals, forage, cotton, flower bulbs, ornamental, peanut, potato, rice, sugar beet, forests, trees, vegetables, and turfgrasses (Table 2.1). In addition, there are several saprophytic species and some involved in mycorrhizal associations with orchids and other plants.

Characteristics of Rhizoctonia spp.

As there is not a strong genus concept, fungi related to R. solani have been referred as R. solani-like fungi. Moore (1987) has summarized the concept of Rhizoctonia and Rhizoctonia-like fungi, based on morphology of the septal pore apparatus and the teleomorphs: he assigned members of the genus Rhizoctonia to ascomycetes (transparent septa with central pores and two or more Woronin bodies), ustomycetes, holobasidiomycetes, and heterobasidiomycetes.
Hyphal Characteristics

*Rhizoctonia* spp. affecting turfgrasses grown in laboratory media show different color depending on the species: *R. solani* mycelium is always some shade of brown, *R. zeae* and *R. oryzae* are similarly white to salmon or pink, and *R. cerealis* is white to buff. (Sneh et al 1991, Smiley et al 2005). Primary hyphae are about 2 to 6 µm in *R. cerealis* and 5 to 11 µm in *R. solani, R. zeae, and R. oryzae*. (Smiley et al 2005). However, hyphal diameter has little value for differentiation among strains of *R. solani* (Parmeter 1967). *Rhizoctonia* species generally grow in culture with a characteristic pattern of hyphal branching. Mature hyphae commonly have side branches originating at right angles, but the branching often occurs in younger hyphae at acute angles. Side branches off of main ‘runner hyphae’ almost always occur near the distal septum on young hyphae and at any place on the cell on mature hyphae. Constriction of the hyphae at the point of origin of the branch and the formation of a septum near that point are both reliable characteristics for diagnosis.

Fungal septa, that are entire in zygomycetes and aquatic fungi, are generally perforate in the higher fungi and how these pores look is a fundamental characteristic to differentiate Ascomycetes from Basidiomycetes (Moore 1996). In the Ascomycetes, one or more Woronin bodies are present on both sides next to the pores that have rounded edges and are big enough to allow nuclei migration. Septa are homogeneous and translucent when are viewed with electron microscopy. On the other hand, Basidiomycetes have three-layered septa that can be seen with an electron microscope, which is useful to differentiate the two subkingdoms, holobasidiomycetes and heterobasidiomycetes. The dolipore/parenthesome septum is an amorphous swelling that occurs near the center of the septum that is covered by a membranous cap on each side (Sneh et al 1991) and it is a characteristic that is present on every isolate of *Rhizoctonia* spp. but it is not evident on every septum (Parmeter et al
1967). Also, and although it is a reliable characteristic for diagnosis, it must be associated with the other characteristics described above and below, because this apparatus is present in other basidiomycetes. The three elements that compose the dolipore/parenthesome septum are the dolipore, its occlusions (there are two different types) and the paraseptal parenthesome that can be imperforate, regularly perforate or vesiculate (Moore 1996). These characteristics combined, help to differentiate within all the basidiomycetes with this kind of septal-pore apparatus. The genus *Thanatephorus* (the teleomorph of *R. solani*) falls in the taxon with a regularly perforated parenthesome (P1 type) (Moore 1996).

Determination of the number of nuclei in the hyphal cells is an important characteristic for the identification of *Rhizoctonia* spp. We can divide the genus into species with multinucleate hyphal cells, including *R. solani*, *R. zeae* and immature cells of *R. oryzae* (mature cells usually have four, according to Smiley et al, 2005) and the binucleate species *R. cerealis*.

Sclerotia, or bulbils, formed among the different strains of *Rhizoctonia* vary in shape and size and color. It is a genus character by which it can be differentiated from the genus *Sclerotium*, because in *Rhizoctonia*, the difference between rind and medulla is not obvious.

**Teleomorph of *Rhizoctonia solani***

The teleomorph of *R. solani* is *Thanatephorus cucumeris* (Frank) Donk. This sexual form is rarely seen except on stems of *Solanum tuberosum* (Adams 1988) or leaves of tobacco with ‘target spot’ disease (Shew and Lucas 1991). It belongs to the subkingdom Basidiomycota, phylum Basidiomycota, class Hymenomycetes; subclass Holobasidiomycetidae; order Ceratobasidiales; family Ceratobasideaceae (Moore 1996). Hyphae of *T. cucumeris* is 12 – 17 μm in diameter, mycelium in culture is felt-like, cinnamon brown in color, sterigmata are
usually 4 in number, and about the same length as the metabasidia or shorter, with spores 7-
10 μm. (Sneh et al 1991).

Hyphal Anastomosis Groups of *Rhizoctonia solani*

More than eighty years ago, Matsumoto et al. (1921, 1932) reported the occurrence
of hyphal fusion among compatible strains of *Hypocnemus sasakii* (=R. solani). Schultz in 1936
first reported the grouping of isolates based on anastomosis reactions. Richter and Schneider
(1953) and Parameter et al. (1970) contributed with their findings by use of this method for
dividing strains of *Rhizoctonia solani* into more homogeneous groups. “The anastomosis
 group is a collection of closely related isolates grouped together based on their capability to
anastomose with one another” (Carling 1996). Isolates are assigned to anastomosis groups
by pairing with tester isolates (known AGs) and observing microscopically for hyphal fusion.
Techniques for this purpose have included use of water agar in petri dishes, cellophane
placed on agar media, agar coated slides placed on moist filter paper on Petri dishes, and
bare slides placed in a moist chamber, but the ones made in thin cultures make easier the
recognition of the hyphal fusions.

Types of hyphal fusion

Different authors have proposed different terms for describing hyphal anastomosis
reactions (Matsumoto et al, 1932; Flentje and Stretton, 1964; and Parameter et al, 1969) but a
new system of four categories was developed: ‘C3’; ‘C2’; ‘C1’; and ‘C0’ (Carling et al,1988;
McNish et al, 1993) to characterize the different degrees of compatibility between
confronted isolates.

A ‘C3’ anastomosis reaction occurs among closely related, perhaps clonal isolates or
from the same isolation, when cell walls and membranes fuse, and although anastomosing
cells and adjacent cells may die, they generally do not. Diameter of the hyphae at the
anastomosis point is equal or almost equal. A ‘C2’ anastomosis reaction occurs among related isolates within an anastomosis group but subgroups denoting different vegetative compatible populations may occur. The walls fuse but the membrane contact is uncertain. The diameter of hyphae at the anastomosis point is less than the normal hyphal diameter. Cells at the anastomosis and adjacent cells always die. A ‘C1’ anastomosis reaction occurs among very distantly related or of different anastomosis groups. There is contact between hyphae but there is no evidence of wall penetration or membrane–membrane contact. Sometimes anastomosis and adjacent cells die. A ‘C0’ category notes isolates that are not related, there is no contact interaction; in fact, they avoid each other.

Anastomosis Groups

Determination of the anastomosis group of \textit{R. solani} has become an important step to study the specific strain involved in a particular disease. In the initial work, Shultz (1936) and Ritcher and Schneider (1953) proposed to group the different strains of \textit{R. solani} based on their compatibility for hyphal fusion, using two different systems that can be compared with the present anastomosis groups (Carling 1996). Later, in 1969, Parmeter et al proposed four anastomosis groups (AG-1, AG-2, AG-3, and AG-4) and scientists started using the term “anastomosis group”.

Further research demonstrated the existence of new strains of \textit{R. solani} that did not match any of the first four AGs. Today, fourteen anastomosis groups have been described and many of these AGs have been subdivided into various ‘intra-specific’ groupings based on physical, chemical, or molecular characteristics: AG-1 with subgroups 1A, 1B and 1C; AG-2 with type 1, types -2 ‘LP’, -2 IIIB, and -2 IV; AG-3; AG-4 with subgroups HG I and HG II; AG-5; AG-6 with two subgroups HG I and GV; AG-7; AG-8; AG-9 subdivided into TX and TP subgroups; AG-10; (Sneh et al 1991); AG-11; AG-12, AG-13, and BI
(BI=bridging isolates, although Carling et al (2001) claim it should be a sub-set of AG-2). Studies of DNA base sequence homologies, indicated those homologies among AG-1, -2, -3, -4, -5, -6 and -7 ranges from 0 to 30 %, and provide evidence that the AGs are different biological species in R. solani (Adams et al 1988). Using the same technique workers have indicated that certain AG groups are more closely related than others, in other words, a more genetically homogeneous group of isolates. So we can see AG 2-1, AG 3, AG 5, AG 7, and AG-BI, are more than 90 % homologous among intraspecific isolates (Carling et al 1988, Adams et al 1988). On the other hand, AG-1, AG-2 type 2, AG-4 and AG-6 are each a heterogeneous group within the group (from 31 to 72 % homology), meaning they are genetically different.

**Rhizoctonia solani on Turfgrasses**

*Rhizoctonia solani* was first described by Kühn in 1858 and since then, it is the most studied species within the genus. In turfgrass, the disease was first observed on a bentgrass putting green in a golf course near Philadelphia, PA in 1913. But it was not until experiments were conducted in 1917 by C. V. Piper and H. S. Coe that the suspected causal agent was consistently isolated and identified *R. solani* as the cause of “Large brown patch” on a red fescue-turf garden in Philadelphia, PA. Affecting all of the major warm and cool season turf species, it is the cause of such important economic losses that are too difficult to estimate. In the absence of a host plant, *R. solani* can survive in the form of bulbils or dormant mycelium in the plant tissue or in the surface of the soil and it can survive long periods of time as a soil saprophyte.

Isolates of *R. solani* posses the following characteristics: mycelia are tan to brown color, with right angled branching, although young hyphae can produce acute angular branching,
septa near the origin of hyphal branching, constriction of hyphal branches near their origin, multinucleate hyphal cells, and formation of dolipore septa. Conidia, clamp connections, and rhizomorphs are absent, and sclerotia are not differentiated into a rind and medulla. Production of monilioid or barrel-shaped cells develop in culture as well as sclerotia (generally dark brown to black but can be white to pale buff (Couch, 1995), which can be spherical to irregular 1 to 10 mm in diameter. *Rhizoctonia solani* has a rapid growth rate and pathogenicity may or may not be present.

**Identification of *Rhizoctonia* Species on Turfgrasses**

Identification of diseases of turf induced by *Rhizoctonia* species is based on field symptoms, lesions on the plant, and microscopic examination of affected plants. After isolation of the fungus, hyphal characteristics, color of the mycelium in culture, hyphal diameter, possession of a prominent septal pore apparatus, number of nuclei in mycelia cells and placement into anastomosis groups (AG) completes the identification. *Rhizoctonia* do not produce conidia, and although a basidiomycete, clamp connections are absent.

In addition to the occurrence of different species of *Rhizoctonia* on turfgrasses, field symptoms differ depending on the turf species and the height of cut of the turf. So, certain AG’s of *R. solani* affect cool season grasses during the summer. Daily temperatures above 30 °C and night temperatures above 20 °C (Martin, *personal communication* 2006) are necessary to develop a rapid overnight outbreak of brown patch disease. Symptoms on turfgrasses mowed at very low height include patches from 5 cm to 60 cm or more in diameter (Smiley et al 2005). Under conditions favorable for disease development, large areas of the turf may become blighted. A purplish or grayish brown ring may appear at the margin of the patches (a “smoke ring” symptom). On cool season grasses that are cut at higher mowing heights,
the light brown patches are 15-30 cm in diameter, but grass in the center may be unaffected. Only occasionally will a smoke ring symptom be visible.

Disease induced by *R. solani* on warm season (C4) grasses develops during the fall or spring of the year in transition zone climates, where the grass is either approaching dormancy or emerging from it, when temperatures are 20-25 °C. Patches can be up to 8 m in diameter and smoke rings are usually absent. The disease on C4 grasses caused by *R. solani* AG 2-2 ‘LP’ is referred to as ‘large patch’. (Smiley et al 2005).

*Rhizoctonia zeae* and *R. oryzae* attack both cool and warm season grasses, during the spring, summer, and fall. Two types of symptoms have been observed: large areas with yellow turf (never turn brown), or yellow rings of all sizes, from 15 cm to 2 m in diameter. The diseases caused by *R. zeae* or *R. oryzae* have collectively been called ‘leaf and sheath spot’, mimicking the disease of rice caused by *R. oryzae* (Sneh et al. 1991).

*Rhizoctonia cerealis* affects mostly cool season grasses during the winter, causing ‘yellow patch’ (Burpee 1980) but also has been observed on warm season grasses, particularly zoysiagrass (Smiley et al. 2005). Yellow circular or irregular patches up to 1 m in diameter are the characteristic symptoms of this disease.

*R. solani* causes brown patch on cool season grasses. Symptoms include small and circular brown chocolate lesions on the leaves, with a brown band of discolored tissue delimiting the lesion borders. On warm season grasses affected with large patch disease, leaf lesions are uncommon but tip dieback is caused by the infection in the sheaths which appear water soaked with reddish brown and black lesions. Leaves can be easily pulled from their attachment to stolons, due to the girdling basal leaf lesions. Lesions produced by *R. oryzae* and *R. zeae* are similar than those caused by *R. solani*. Although often absent, leaf lesions can occur on plants infected by *R. cerealis*. 
Rhizoctonia solani on Cool Season Turfgrasses – Brown Patch

Rhizoctonia solani AG 1, 2, 2-2, 4 and 5 have been reported from isolations from cool season grasses (Martin 1984). Since then, subgroups among the various AGs have been defined further. Most of the cool season species used as turfgrass, such as Poa annua, Agrostis stolonifera, Agrostis palustris, Agrostis canina, Poa pratensis, Poa trivialis, Festuca rubra, Festuca rubra var commutata, Festuca arundinacea, and Lolium perenne have been reported in the United States to be susceptible to R. solani.

Severe epidemics of brown patch, or Rhizoctonia blight, are favored by high air temperatures (28-32 C), high relative humidity, and long periods of leaf wetness (Couch, 1995). Soil acidity favors disease development and turf receiving higher amounts of nitrogen develops more severe disease than the turf with a balanced fertilization program.

Symptoms vary depending on the turf species, mowing height, and weather conditions. Initial infections occur on the leaves, through stomata or wounds produced by the mowers. Brown patches range from 5 cm to 60 cm in diameter or more, and dark purplish smoke rings may surround them. Smoke rings are more distinctive early in the mornings (Couch 1995).

Rhizoctonia solani on Warm Season Turfgrasses – Large Patch

R. solani AG 2-2 ‘LP’, first isolated on zoysiagrass, is the strain that causes large patch on warm season grasses. It was not until 1998 when Hyakumachi et al found, based on cellular fatty acids analysis, cultural characteristics, pathogenicity and molecular biology that the strain isolated from zoysiagrass in Japan was different from the type 2-2 IIIB (reported on tall fescue, perennial ryegrass, and creeping bentgrass) and 2-2 IV (which typically causes diseases of plants in the Chenopodiaceae). Aoyagi et al (1998) confirmed the previous study,
and showed that isolates from bermudagrass, St Augustinegrass and zoysiagrass were virulent on zoysiagrass.

In the United States, several workers have identified *R. solani AG 2-2 ‘LP’* on *Cynodon* sp. (common and hybrid bermudagrass) (Burpee and Martin 1992, Martin and Lucas 1984, Li 2000), *Eremochloa ophiuroides* (centipedegrass) (Burpee and Martin 1992, Li 2000), *Stenotaphrum secundatum* (St. Augustinegrass) (Haygood and Martin 1990, Burpee and Martin 1992, Martin, and Lucas 1984, Li 2000, Li 2005), *Zoysia japonica* (zoysiagrass) (Aoyagi et al 1998, Burpee and Martin 1992, Martin, and Lucas 1984, Green et al 1993, Li 2000) and *Paspalum vaginatum* (seashore paspalum) (Canegallo and Martin *unpublished*). This disease is very important in the transition zones and it is more severe over low wet areas or on poorly drained soils. It develops when the growth of the grass is limited, either when breaking dormancy during the spring or when it is approaching dormancy during the fall. Extended periods of leaf wetness favor disease outbreaks. Cool temperatures weaken the plant and slow recovery and may promote infections to occur when plant vigor is low.

Solid large brown or yellow patches or rings of infected grasses up to several meters are visible when the disease occurs. Patches may be as small as 5 cm but they can coalesce and form large patches of up to several meters in diameter. Smoke rings are usually not observed because infections occur on or near the basal portion of leaf sheaths and induce a sheath rot. When disease is active, affected plants have a distinct yellow or bronze color, especially evident along the border of the patches. As is similar with brown patch on cool season grasses, symptoms occur on plant tissue at or above the soil surface. Leaf tip dieback can occur as a result of lesions on the sheath that makes the leaves easy to pull off the plant. Discrete lesions on leaf lamina are normally uncommon and root damage is uncertain or not confirmed (Burpee and Martin 1992).
Disease Management

Improving soil drainage and decreasing the time of leaf wetness (either mechanically or by running irrigation cycles before sunrise to remove the guttation water) will aid in reducing the severity of the disease. Irrigation cycles late in the afternoon and early in the night that increase the time of leaf wetness should be avoided (Burpee and Martin 1996). A correct and balanced fertilization program must be applied, because when conditions are favorable to the pathogen, high amounts of N available can amplify the frequency and severity of the disease (Smiley et al 2005). Thatch should be reduced if it is thicker than 1 cm. Based on my experience with seashore paspalum, increasing mowing height does not help either to prevent the disease or to heal the grass. Large patch occurs at a wide range of mowing heights.

Several fungicides among the different groups are labeled to control R. solani on warm season grasses. They include dithiocarbamates, benzimidazoles, triazoles, pyrimidine, carboximide, dicarboximide and various aromatic compounds. Azoxystrobin, pyraclostrobin, iprodione, flutalolin, chlorothalonil, thiophanate methyl are labeled but are suggested to be sprayed before disease outbreak for satisfactory control (Martin 2006).
LITERATURE CITED


Table 2.1. Diseases caused by *Rhizoctonia* species on different crops.

<table>
<thead>
<tr>
<th>Crop</th>
<th>Species of <em>Rhizoctonia</em></th>
<th>Disease</th>
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</thead>
<tbody>
<tr>
<td>Oilseed Rape and Canola</td>
<td><em>R. solani</em></td>
<td>Damping-off, Root rot, Leaf blight</td>
</tr>
<tr>
<td>Cereals</td>
<td><em>Rhizoctonia spp.</em></td>
<td>Root Rot</td>
</tr>
<tr>
<td>Cotton</td>
<td><em>R. solani AG</em> 4</td>
<td>Damping-off (pre and pot emergence), Bolls rot</td>
</tr>
<tr>
<td></td>
<td><em>R. crocorum</em></td>
<td>Copper web, Violet root rot</td>
</tr>
<tr>
<td>Flower bulbs</td>
<td><em>R. tuliparum</em></td>
<td>Gray bulb disease</td>
</tr>
<tr>
<td></td>
<td><em>R. solani AG</em> 2-2, AG 2-t, 2-1, 4 and 5</td>
<td>Rhizoctonia disease on Gladiolus, Tulip, Iris, Lily, Anemone</td>
</tr>
<tr>
<td></td>
<td><em>R. solani Kuhn</em> on Alfalfa</td>
<td>Seedling blight, Stem and Leaf Blights, Crown Bud Rot, Rot Canker</td>
</tr>
<tr>
<td>Legumes</td>
<td>*R. solani AGs 2-1 and 2-2 on Clover</td>
<td>Seedling Blight, Damping-off, Root Rot, Seedling Damping-off</td>
</tr>
<tr>
<td></td>
<td><em>R. solani AG</em> 4 and 5 on Lupin</td>
<td>Root Rot, Terminal Bud Mortality, Seedling Blight</td>
</tr>
<tr>
<td></td>
<td><em>R. solani AGs</em> 1, 2-2, 3, 4, 5</td>
<td>Aerial Blight, Root Rot, Web Blight</td>
</tr>
<tr>
<td>Ornamentals</td>
<td><em>R. solani AGs AG</em> 4, 1, <em>R. ramicola</em>, and other Binucleate</td>
<td>Pre and post-emergence damping-off, Stem rot,Foliar Blight, Web Blight</td>
</tr>
<tr>
<td></td>
<td><em>Rhizoctonia (BNR)</em></td>
<td>Root rot, Root Rot</td>
</tr>
<tr>
<td></td>
<td><em>R. solani Kuhn</em> AG 4</td>
<td>Limb rot</td>
</tr>
<tr>
<td>Peanut</td>
<td><strong>BNR AGs A, E, F, and R</strong></td>
<td></td>
</tr>
<tr>
<td>Potato</td>
<td><em>R. solani Kuhn AGs</em> 1, 2-1, 2-2, 3, 4, 5, 8, 9.</td>
<td>Rhizoctonia Canker, Black Scurf</td>
</tr>
<tr>
<td></td>
<td>*R. solani Kuhn AG 1</td>
<td>Rice Sheath Blight</td>
</tr>
<tr>
<td>Rice</td>
<td>*R. solani Kuhn AG 2-2 III B</td>
<td>Brown Sheath Blight</td>
</tr>
<tr>
<td></td>
<td><em>R. oryzae</em></td>
<td>Sheath spot</td>
</tr>
<tr>
<td></td>
<td>*R. solani Kuhn AG 2-2, 4</td>
<td>Pre and post-emergence damping-off, Web Blight</td>
</tr>
<tr>
<td>Forrest Trees</td>
<td>*R. solani Kuhn, R. endophitica, R. zeae, R. callae</td>
<td>Root rot</td>
</tr>
<tr>
<td></td>
<td>*R. solani Kuhn AG 2-2 LP</td>
<td>Large Patch of warm season grasses.</td>
</tr>
<tr>
<td>Turfgrass</td>
<td><em>R. solani Kuhn AG</em> 1, 2, 2-2, 4, and 5</td>
<td>Brown Patch of cool season grasses.</td>
</tr>
<tr>
<td></td>
<td><em>R. zeae and R. oryzae</em></td>
<td>Rhizoctonia leaf and sheath spot</td>
</tr>
<tr>
<td></td>
<td><em>R. cerealis</em></td>
<td>Yellow patch</td>
</tr>
</tbody>
</table>
CHAPTER 3
IDENTIFICATION AND MANAGEMENT OF RHIZOCTONIA SOLANI AG 2-2 ‘LP’ ON SEASHORE PASPALUM TURFGRASS IN SOUTH CAROLINA

Introduction

*Rhizoctonia solani* Kuhn (teleomorph *Thanatephorus cucumeris*) is a widely distributed soilborne pathogen causing diseases on many species of plants (Menzies 1970). On turfgrass, different anastomosis groups of *R. solani* have been isolated (AGs 1, 2, 2-2, 4 and 5) and shown to cause brown patch on cool season grasses (Martin 1984) and large patch (AG 2-2 ‘LP’) on warm season grasses (Hyakumachi et al. 1998, Aoyagi et al. 1998, Li et al. 2005).

An anastomosis group (AG) is a collection of closely related isolates grouped together based on the capability of their hyphae to anastomose (fuse) with one another. Hyphal anastomosis reactions in *Rhizoctonia* spp. were first described in 1921 (Matsumoto) and 1932 (Matsumoto et al.) to differentiate among strains of *Hypochlorus sasakii* Shirai (= *R. solani*) (Parmeter and Withney 1970, Carling, 1996) and later by Schultz in 1936 (Parmeter and Withney 1970, Carling, 1996) Richter and Schneider in 1953 (Parmeter and Withney 1970, Carling, 1996) and, Parmeter in 1969 (Parmeter and Withney 1970, Carling, 1996).

Matsumoto et al. (1932), Flentje and Stretton (1964), and Parmeter et al. (1969) worked to categorize the type of reaction among strains of *R. solani*. There was not complete agreement among the three different systems developed, so Carling et al. (1988) constructed a new system of four categories (C3, C2, C1, and C0) to overcome these differences. The group C3 represents a reaction when cell wall and cytoplasmic fusion is produced with no cell death and represents closely related individuals, i.e. the same vegetatively compatible population (VCP) or possibly the same individual. The C2 category represents a reaction
among related isolates with wall fusion but without cytoplasmic fusion and death of anastomosed cells results. These isolates are the same AG but a different VCP. C1 represents a distant relationship where isolates come from heterogeneous groups or different AG groups; in these cases, there is cell wall contact but no cell wall fusion. A C0 designates no reaction between hyphae from two isolates.

Seashore paspalum (*Paspalum vaginatum* O. Swartz) is among the most salt and sodium tolerant turfgrass species (Duncan and Carrow 2000). In recent years there have been some golf courses culturing seashore paspalum in the Southeastern United States. The reasons for the interest in seashore paspalum have to do with its salinity tolerance, but also because the grass is very striking aesthetically when healthy. However, a disease similar to large patch has been observed on seashore paspalum in South Carolina and from several sites in Florida. In most cases the severity of the outbreaks warranted fungicide applications for control (Martin, unpublished). Rhizoctonia-like fungi have been observed microscopically associated with the diseased plants from these sites, and suspected to be *R. solani*. However, confirmation of the identity and further characterization of the fungi associated with the large patch symptoms, as well as proof of pathogenicity to seashore paspalum has not been conducted.

Therefore the objectives of this study were to determine if symptoms, signs, and isolates of suspected *R. solani* are consistent with a diagnosis of large patch on seashore paspalum. Further objectives were to document pathogenicity, complete Koch’s postulates and evaluate fungicides for control in field epidemics.
Materials and Methods

Large Patch has been documented on several warm-season turfgrasses in the southeastern United States (Li et al. 2005, Li 2000, Haygood and Martin 1990) Symptoms include the presence of more or less circular patches of yellow-brown turf from 50 cm up to 3 m in diameter or greater. Diseased grass shoots at the margins of patches typically are yellow, and lesions are observed near the basal portion of the leaf sheaths, near the attachment of shoots to stolons.

Samples were collected from seashore paspalum from several locations from symptomatic turf consistent with large patch disease. Solid, large brown patches up to several meters in diameter occurred on a putting green at Old Collier Golf Club, Naples, FL; on February 2005 (cultivar Sea Isle Supreme); from a practice putting green (cultivar Sea Isle 2000) at May River Golf Club, Bluffton, SC in March, 2005; and from a tee box (cultivar Sea Isle 1) at The Ocean Course, Kiawah Island, SC in May, 2005. Lesions on the basal leaf sheaths were observed under the stereo microscope at (70×) and symptomatic leaves were easily pulled off from the plant and observed under a compound microscope. The mycelium observed at the base of the leaves was identified as a *Rhizoctonia* species, based on hyphal characteristics: 90-degree hyphal branching, dolipore septae, septa occurred near the branching, and hyphal diameter approximately 10 μm.

Isolation and Identification

Leaves from the samples were surfaced sterilized for 10 s in 70% ethanol, rinsed twice in sterile deionized water, blotted dry, placed on 1.5% water agar and cultured at room temperature on a laboratory bench. After two days, colonies similar to *Rhizoctonia* spp. were observed growing from leaf pieces. Single hyphae were transferred to potato dextrose agar (PDA) amended with 200 ppm of streptomycin and 10 ppm of rifomycin, and sub-cultured
again onto PDA if necessary to rid the cultures of contaminants. After 2 weeks, the cultures
grown in the laboratory in the dark at room temperature had the typical chocolate brown
color of R. solani. Cultures also exhibited abundant aerial hyphae, brown in color, and
aggregated bulbils were few to absent in mature cultures. These characteristics in culture
were consistent with R. solani AG 2-2 ‘LP’ cultures previously collected from various warm-
season turfgrasses (Li, 2000).

Anastomosis Group Test

Three isolates of R. solani from seashore paspalum were paired with a tester isolate of
R. solani AG 2-2 ‘LP’ (isolate Rh140) recovered from Zoysia spp. This isolate was used
previously as a tester in a separate study (Li et al. 2005).

The three isolates as well as the tester isolate were grown on PDA for 4 days in the
dark at room temperature on a laboratory bench, to produce actively growing mycelia.
Microscope slides were sterilized, dipped in 95% ethanol, flamed, and covered with 1.5%
water agar solution, which results in a thin layer of water agar media on the slide. A single
plug (5 mm diameter) of the tester Rh140 was taken from the edge of the culture and was
paired with a plug taken from the edge of the culture of each unknown isolate. Plugs were
inverted on the slide 1.5 cm apart and slides were incubated on moist chambers to maintain
humidity. Petri dishes were incubated in the lab at room temperature (22- 25 C) until hyphae
from the plugs grew together and co-mingled, overlapping about 2 mm. Each pairing was
replicated three times, and the experiment was conducted three times. Slides were observed
microscopically (100× and 400×) and anastomosis was rated by counting the number of
anastomosing junctions between the tester and an unknown isolate.
Nuclear Condition in Cells of Hyphae

All isolates of \textit{R. solani} are considered to be the anamorph of \textit{Thanatephorus cucumeris} and contain multiple nuclei in the hyphal cells. The number of nuclei may vary from 3 to 28 in young cells (Sneh et al. 1991). Isolates of \textit{R. solani} collected from seashore paspalum and previously tested for anastomosis with \textit{R. solani} AG 2-2 ‘LP’ were observed under a fluorescent microscope to determine nuclear condition. (Martin 1987).

Cultures were grown on PDA for 3-4 days, and 5-mm plugs were removed from the perimeter of the colonies. Two discs from each isolate were placed on water agar covered slides as described above. Three days after transfer, colonies of \textit{R. solani} were observed on the water agar-slides. Slides were dipped for 5 min into 1 ppm of the fluorescent DNA-binding fluorochrome 4’, 6’-diamino-2-phenylindole (DAPI) in distilled water and then rinsed by dipping two times for 1 s in distilled water. Excess water was absorbed with a paper towel. A cover glass was placed on the slide and hyphae were observed under a fluorescent microscope at 400× and 1000×. Digital photographs were taken to record images and numbers of fluorescing nuclei in individual cells were counted.

Pathogenicity Test

Seashore paspalum was inoculated in a greenhouse trial to confirm Koch’s Postulates. Inoculum of \textit{Rhizoctonia solani} AG 2-2 ‘LP’ was produced using an isolate collected from May River Golf Club in September, 2006. One 100 g portion of dry oat seeds was placed in a 500 ml flask with 100 ml of deionized water and sterilized in an autoclave for 1 hr on each of two consecutive days. After sterilization, the seeds were infested with agar disks from \textit{R. solani} cultures taken from the edge of a 3-day colony grown on PDA. The flask was placed at room temperature in a lab for 14 days. Sixteen 10-cm diameter pots were planted with two seashore paspalum cultivars SeaIsle 1 and SeaIsle Supreme in the
greenhouse. In September 2006, three infested seeds were placed in each of four pots of SI 1 and four pots of SI Supreme, leaving the other 8 pots as non-inoculated controls. Pots were placed into a greenhouse, irrigated three times a day, and fertilized once a week with a fertilizer solution. Temperatures in the greenhouse ranged between 18 to 24 °C

Evaluation of Cultivars of Seashore Paspalum

An experimental putting green was built at the Pee Dee Research and Education Center (Pee Dee REC) located near Florence, South Carolina, in July 2005 for research purposes. The green was constructed following the United States Golf Association (USGA) specifications for putting greens construction. Seven cultivars of seashore paspalum (SeaIsle 1, SeaIsle 2000, SeaIsle Supreme, SeaSpray, SeaDwarf®, Aloha, and Salam) were planted in a randomized complete block design with three replications. Each plot was 3.70×6.50 m. A 60-cm buffer zone was left between plots until plots were established to prevent cross-contamination.

A natural and severe epidemic of large patch (R. solani AG 2-2 ‘LP’) occurred during late September to early October 2005 on the green, and the causal agent was isolated and identified. Every cultivar main plot was divided into seven subplots, 0.91×3.7 m and six fungicides were evaluated for curative suppression of the natural epidemic: azoxystrobin, pyraclostrobin, thiophanate methyl, chlorothalonil, flutolanil, and iprodione (Table 3.1). All of these fungicides currently are registered for control of large patch or brown patch on turfgrasses. (Martin 2006). The fungicides were sprayed three times during the epidemic 14-day intervals apart: November 2, 16, and 30. Plots were treated again on March 7, 2006 when active disease recurred. A CO2 shielded plot sprayer was used and was equipped with Teejet 8002ER flat fan nozzles; spray volume was 860 l/ha.
A similar experiment also was conducted at May River Golf Club (MRGC) in Bluffton, SC. The putting green was built in 2003 under USGA specifications. Existing seashore paspalum was killed by fumigation with methyl bromide on July 22, 2005, and was replanted on August 11, 2005 with the same seven cultivars used for the green at the Pee Dee REC in a randomized complete block design with three replications. Individual plots were 3.35×6.10 m. A 60-cm wide buffer zone was left between plots. Large patch did not occur naturally at this site, so plots were artificially inoculated.

Inoculum of *R. solani* was prepared using an isolate from seashore paspalum cultivar SeaIsle 2000 collected from MRGC in April 2005. Oat seed inoculum was prepared as described previously. Every cultivar main plot was subdivided into six 0.91×3.35 m subplots for fungicide treatments. Every subplot was inoculated in two places approximately 2 and 4 m from the end of each subplot on November 6, 2005 with four to five oat seeds of the inoculum per inoculation site. However, a natural epidemic occurred at the end of November, at about the same time symptoms in the inoculated areas were noted. Five fungicides were tested at MRGC to evaluate their curative effect: azoxystrobin, pyraclostrobin, thiophanate methyl, chlorothalonil, and flutolanil. Rates were the same as those used at the other trial (Table 3.1). The fungicides were sprayed three times during the epidemic at 14-day intervals: December 1, 15, and 29. Plots were treated again on March 9, 2006. Plots at both sites were rated for disease severity on a 1 to 9 scale, with 1 = no disease and 9 = 100% of plot area affected during and after the epidemics, from October 2005 to March 2006. Turf quality also was rated, on a 1 to 9 scale, with 1 being the worst and 9 being the best.
Results and Discussion

Field Symptoms and Pathogen Identification

A severe epidemic of a disease suspected as large patch occurred at May River Golf Club near Bluffton, SC in the fall, winter, and spring of 2005. Field symptoms on seashore paspalum were similar to large patch as it is known in other warm-season turfgrasses (Martin 2006, Couch 1995, Haygood and Martin 1990). Patches varied in size from 0.3 m up to several meters in diameter, often coalescing (Fig. 3.1, 3.2, 3.3, 3.4, and 3.5). Isolates obtained from Florida and South Carolina were all identified as R. solani AG 2-2 ‘LP’. The identification was confirmed by occurrence of lesions on the base of the leaves (Fig. 3.6), culture characteristics of colonies in vitro (Fig. 3.7), multinucleate cells in hyphae (Fig. 3.9), and positive, high frequency anastomosis between unknown isolates and a known tester isolate Rh140 (Table 3.2). Anastomosis reactions were consistent with a C2 reaction as described by Carling (1996) (Fig. 3.8).

Koch’s Postulates (i.e. pathogenicity test) were confirmed in the greenhouse inoculation trials on two cultivars of seashore paspalum. Symptomatic leaves were detected on October 16, 2006, 17 days after inoculation, and R. solani was detected microscopically in lesions removed from inoculated plants. Lesions were dissected from symptomatic plants, and the pathogen was re-isolated on water agar. Cultures obtained were identical to the inoculum introduced and completed the requirements for proof of pathogenicity. This represents the first report of large patch caused by Rhizoctonia solani AG 2-2 ‘LP’ on seashore paspalum turfgrass. A preliminary report has been published (Canegallo et al. 2006)
Disease Management

At MRGC, field symptoms of large patch disease were detected around the end of November at the inoculation sites. Natural epidemics occurred at the same time but were not as severe as those at the Pee Dee REC. There were significant differences in the reactions of cultivars of seashore paspalum to large patch and there were significant effects of fungicides on the disease (Table 3.3). There was no interaction between fungicides and cultivars, so main effects could be evaluated across fungicides and cultivars. Surprisingly, none of the fungicides tested provided adequate control although curative control of large patch in any grass is difficult (S. B. Martin, personal communication).

At the Pee Dee REC (Figure 3.10A), the best control resulted from pyraclostrobin. There was no significant difference in control in plots treated with thiophanate methyl or azoxystrobin and they were the only treatments that were significantly different from the non-treated control. Plots treated with iprodione, flutolanil, and chlorothalonil were not significantly different in disease severity from the non-treated control. At MRGC, all fungicides provided some level of control (Figure 3.10B). Pyraclostrobin and azoxystrobin were most effective but plots treated with chlorothalonil and flutalonil were not significantly different. Plots treated with thiophanate methyl were not different from those treated with chlorothalonil or flutolanil.

A study on large patch in zoysiagrass (Ross et al. 2000) tested several fungicides and showed that azoxystrobin and flutolanil had the same lack of curative control of the disease once symptoms were evident. Tisserat (1999) conducted a study and showed excellent preventive control of large patch on zoysiagrass with azoxystrobin. In the same experiment, thiophanate methyl was tested but showed insufficient levels of disease control. On St. Augustinegrass a preventive study was done in North Carolina where two applications of
azoxystrobin and flutolanil gave the best results in disease suppression and also increased
turf quality. Treatments with pyraclostrobin showed no significant differences from the
untreated control (Tredway and Martin 2005).

Cultivars at both locations in this study showed significant differences in large patch
disease severity. At the Pee Dee Research and Education Center (Figure 3.11A), the most
susceptible cultivar was SeaIsle 2000, followed by SeaIsle 1. Salam and SeaDwarf had large
patch severity similar to SeaIsle 1. SeaIsle Supreme and SeaSpray were least susceptible and
Aloha was not significantly different from these cultivars.

At MRGC, epidemics were not as severe as at the Pee Dee REC (Figure 3.11B). SeaIsle 1 was the most susceptible cultivar, but SeaDwarf and Sea Spray were not
significantly different. The least susceptible cultivars were SeaIsle Supreme and Aloha but
SeaIsle 2000 and Salam had large patch severity ratings that were not significantly different.

All of the cultivars evaluated in this study were very susceptible to large patch
(*Rhizoctonia solani AG 2-2 LP*) under field conditions at two locations in South Carolina.
There were some significant differences among the cultivars, with SeaIsle Supreme showing
the least amount of disease at both locations, and SeaIsle 2000 the most severe disease at the
Pee Dee REC and SeaIsle 1 showing the most disease severity at MRGC. In general,
epidemics at the Pee Dee REC were more severe than at MRGC, which may be because
Florence is farther north and colder than Bluffton and large patch is more severe under
cooler conditions (Martin 2005, Smiley et al. 2005). To my knowledge this is the only trial
conducted which evaluated cultivars of seashore paspalum for large patch disease severity,
so comparison with other studies does not exist.

The fungicides sprayed after epidemics had started did not provide adequate control
of the disease. Therefore the use of preventive applications of fungicides is highly
recommended to manage large patch on seashore paspalum in South Carolina and probably elsewhere in the southeastern USA.
LITERATURE CITED


Table 3.1. Fungicide trade names, active ingredients, and rates in grams of active ingredient per 100 m² evaluated for large patch curative control on seashore paspalum putting greens at Clemson University Pee Dee Research and Education Center (Florence, SC) and May River Golf Club (Bluffton, SC)

<table>
<thead>
<tr>
<th>TRADE NAME</th>
<th>ACTIVE INGREDIENT</th>
<th>RATE (g ai/100 m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heritage TL 0.8 ME</td>
<td>Azoxyastrobin</td>
<td>2.80</td>
</tr>
<tr>
<td>Insignia 20 WG</td>
<td>Pyraclostrobin</td>
<td>7.90</td>
</tr>
<tr>
<td>Prostar 70 WP</td>
<td>Flutolanil</td>
<td>46.97</td>
</tr>
<tr>
<td>Daconil Ultrex 82.5 WG</td>
<td>Chlorothalonil</td>
<td>80.52</td>
</tr>
<tr>
<td>Chipco 26 GT 2 SC</td>
<td>Iprodione</td>
<td>22.88</td>
</tr>
<tr>
<td>Clearys 3336 50 WP</td>
<td>Thiophanate methyl</td>
<td>61.00</td>
</tr>
</tbody>
</table>
Table 3.2. Frequency of positive anastomosis reactions observed between a tester isolate, Rh140 of *R. solani* AG 2-2 ‘LP’ and three *R. solani* isolates collected from seashore paspalum at three golf courses.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Frequency of Anastomosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trial 1</td>
</tr>
<tr>
<td></td>
<td>1 2 3</td>
</tr>
<tr>
<td>The Old Collier GC</td>
<td>17 23 12</td>
</tr>
<tr>
<td>The Ocean Course</td>
<td>37 26 24</td>
</tr>
<tr>
<td>May River GC</td>
<td>21 14 18</td>
</tr>
</tbody>
</table>
Table 3.3 Analysis of variance for cultivar susceptibility and fungicide control of large patch (LP) for trials conducted at the Pee Dee Research and Education Center (Pee Dee REC), Florence, SC and at May River Golf Club, Bluffton, SC

<table>
<thead>
<tr>
<th></th>
<th>1 PEE DEE REC</th>
<th></th>
<th>2 - MAY RIVER GC</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DF</td>
<td>LP Severity&lt;sup&gt;a&lt;/sup&gt;</td>
<td>TQ&lt;sup&gt;b&lt;/sup&gt;</td>
<td>DF</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P &gt; F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blocks</td>
<td>2</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>2</td>
</tr>
<tr>
<td>Cultivar</td>
<td>6</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>6</td>
</tr>
<tr>
<td>Block * Cultivar</td>
<td>12</td>
<td>&lt; 0.01</td>
<td>0.30</td>
<td>12</td>
</tr>
<tr>
<td>Fungicide</td>
<td>6</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>5</td>
</tr>
<tr>
<td>Fungicide * Cultivar</td>
<td>36</td>
<td>0.54</td>
<td>0.27</td>
<td>30</td>
</tr>
<tr>
<td>CV</td>
<td>0.24</td>
<td>0.53</td>
<td></td>
<td>0.46</td>
</tr>
</tbody>
</table>

<sup>a</sup> Large patch severity was evaluated on a scale of 1 to 9 with 1 being least severe and 9 being most severe disease.

<sup>b</sup> Turf quality was evaluated on a scale of 1 to 9 being 1 the worst turf quality and 9 best turf quality.
Figure 3.1. Large path disease symptoms on a fairway at May River Golf Club, Bluffton, SC. December 2005.
Figure 3.2. Large path disease symptoms on a fairway at May River Golf Club, Bluffton, SC. December 2005.
Figure 3.3. Picture of a putting green at May River Golf Club, Bluffton, SC. Symptoms of large patch disease can be seen on the Seashore Paspalum approach (left) and on the bermudagrass Miniverde green (right); December 2005.
Figure 3.4. Severe symptoms of large patch where patches coalesced on a seashore paspalum fairway at May River GC, Bluffton, SC. A spot of healthy grass can be seen at the left upper corner of the picture. February 2006.
Figure 3.5. Severe symptoms of large patch where patches coalesced on a seashore paspalum fairway at May River GC, Bluffton, SC; February 2006. Patches of bermudagrass contamination can be seen in the center of the picture.
Figure 3.6. Typical large patch lesions at the bases of the leaves of a plant of seashore paspalum taken from a symptomatic area at the Pee Dee Research and Education Center, Florence, SC; October 2006
Figure 3.7. *Rhizoctonia solani* from seashore paspalum on PDA medium: typical brown culture and aerial mycelium
Figures 3.8. C2 reaction in a positive anastomosis test between an isolate of *Rhizoctonia solani* from seashore paspalum and the isolate Rh140 tester *R. solani* AG 2-2 ‘LP’ from Zoysia spp. Killing reaction of the anastomosed cell can be seen with the microscope at 400×
Figures 3.9 Mycelium of *Rhizoctonia solani* from seashore paspalum under light microscopy (left) and fluorescence microscopy after staining with DAPI, (right) where several nuclei per hyphal cell can be seen at 400×.
Figure 3.10. Summary of fungicide tests for control of large patch disease on seashore paspalum at the Pee Dee Research and Education Center, Florence, SC (3.10A) and May River Golf Club, Bluffton, SC (3.10B) between October 2005 and March 2006. Bars show means of large patch severity evaluated from 1=no disease to 9=100% of the plot affected. Means with the same letter were not significantly different.
Figure 3.11. Summary of seashore paspalum cultivar susceptibility to large patch disease from trials conducted at the Pee Dee Research and Education Center, Florence, SC (3.11A) and May River Golf Club, Bluffton, SC (3.11B) between October 2005 and March 2006. Bars represent means of large patch severity evaluated from 1=no disease to 9=100% of the plot affected. Means with the same letter were not significantly different.
CHAPTER 4
RELATIVE SENSITIVITY OF SEVEN SEASHORE PASPALUM (PAS PALUM VAGINATUM) CULTIVARS TO SALINITY IN IRRIGATION WATER

Introduction

Seashore paspalum is a warm season turfgrass with a particularly high tolerance to saline environments that can be used on golf courses, sports fields, parks, and landscapes areas (Duncan and Carrow 2000). It is considered a true halophyte, meaning that it can maintain growth at high levels of salinity in the soil. In South Carolina, it is being used at some golf courses near the coast where water quality is an issue or could be an issue in the near future. Several cultivars are being used for golf courses elsewhere in the southeastern United States, but these cultivars are natural strains or selections (ecotypes) adapted to a particular environment where they were collected and multiplied for use as turfgrasses. Cultivars of seashore paspalum have not been evaluated in transition zone climates in the southeastern United States for salinity tolerance or suitability as golf course putting greens. In this study, seven of the more popular cultivars of seashore paspalum that are being used or considered for use on golf courses in the southeastern United States were tested under greenhouse conditions to compare their tolerance to different levels of salinity in irrigation water and for performance at two field locations.
**Materials and Methods**

Seven cultivars of seashore paspalum currently being used or evaluated as golf course greens in the southeastern United States were chosen for this study.  

- **SeaIsle 1** was released in 1999 by the University of Georgia from a selection that originated in Argentina. This cultivar is recommended for use in golf course fairways and roughs, sports fields, and lawns (Duncan and Carrow 2000).

- **SeaIsle 2000** has fine leaves and a dark green color. It is marketed for golf course putting greens and tee boxes, but it can be used on fairways and roughs as well. It was selected from an old mutation of Adalyd seashore paspalum from California that was growing at a golf course near Ft. Myers, FL (Duncan and Carrow 2000).

- **SeaIsle Supreme** was collected from a single clone found on the eastern coast of the United States. This fine bladed cultivar can tolerate a wide range of mowing heights. It is recommended for use on golf courses, athletic fields, and recreational parks. It has been evaluated since 2002 at the University of Georgia – Griffin and several golf courses and athletic fields in South Carolina, Georgia, and Florida. It has been commercially available since 2005 (Dr. Paul Raymer personal communication).

- **SeaSpray** is the first seeded cultivar developed and released by Turf Seeds Inc. of Oregon and the University of Georgia. It is recommended for golf course fairways and athletic fields (Dr. Paul Raymer personal communication).

- **SeaDwarf** is a fine texture cultivar that can tolerate a wide range of mowing heights. It is recommended for use on golf courses and sports fields. It can be found at golf courses in the southern USA, Puerto Rico, and Spain (http://www.environmentalturf.com/grasses_seadwarf.html).
• Aloha was developed by the University of Florida to be used on golf courses and athletic fields (http://www.environmentalturf.com/grasses_aloha.html).

• Salam is a Hawaiian cultivar released by Southern Turf in 1998. This fine textured type is recommended for golf course fairways and sports fields (Duncan and Carrow 2000).

The seven cultivars of seashore paspalum stated above and three cultivars of hybrid bermudagrass (Cynodon dactylon × Cynodon transvaalensis) were irrigated with five different levels of salinity in artificial irrigation water under greenhouse conditions. The experiment was arranged in a blocked split-plot design with cultivars as the main plot and irrigation water salinity levels as the subplots. There were three blocks, 10 cultivars, and five salinity levels. Two trials of the experiment were conducted in a greenhouse at the Clemson University Pee Dee Research and Education Center (Pee Dee REC) in Florence, SC between January and July of 2006. Characteristics evaluated during the study were clipping yield, turf quality, turf density, and leaf firing (i.e.: damage on the leaves of the grasses produced by salts in the irrigation water, measured as a percentage of chlorotic leaf area). At the end of the study, root and shoot weights were measured and the root/shoot ratio was calculated. In addition to the seven seashore paspalum described above, three hybrid bermudagrasses cultivars (TifEagle, Tifdwarf, and MiniVerde) were used in this experiment.

Sources of the plants used in this experiment were: sprigs of SeaIsle 1, SeaIsle 2000, and SeaIsle Supreme were provided by Phillip Jennings Turf Farms, Soperton, GA; sprigs of cultivars Aloha and SeaDwarf were provided by Emerald Island Turf Farms, Avon Park, FL; sprigs of Salam from Southern Turf Nurseries, Alapaha, GA; seeds of SeaSpray were donated by Turf Seed Inc and the sprigs of the three bermudagrass cultivars were taken from research putting greens at the Pee Dee REC. The sprigs were washed free of soil and
then were surface sterilized with 5% bleach for five min and carefully rinsed for five min in deionized water two times.

For Trial 1, seashore paspalum cultivars were planted in 20-cm-diameter × 15-cm-deep plastic pots filled with a sterilized mix of 80% sand, 20% peat on September 1, 2005. The three bermudagrass cultivars were planted similarly on October 28, 2005. Plastic columns 10-cm-diameter x 36-cm-deep were prepared for salinity treatments and planted to each grass that was previously established grown in pots. A nylon screen was glued to the bottom of the columns to retain the soil mix. A 5-cm layer of approximately 1 to 1.5 cm diameter gravel was placed in the bottom of each column and 20 cm of the same sterilized soil mix used previously was added over the gravel layer. On January 17, 2006, 10-cm-diameter x 10-cm-deep plugs of established turfgrass were taken from the pots using a golf cup cutter and planted in the prepared columns. The turf plugs were placed on the soil mix and back-filled along the edges with soil mix to complete the planting. Fifteen columns of each of the ten cultivars were prepared (150 columns total).

Five different artificially prepared saline irrigation waters were used. Compositions were designed to mimic irrigation water typically seen in coastal South Carolina although salinity levels exceeded those usually found. Salinity levels were 0.5, 7.5, 15, 22.5, and 30 deciSiemens m⁻¹ (dS m⁻¹). A stock solution of 34.7 dS m⁻¹ was prepared (Table 4.1) and the different salinity levels were made by dilution (Table 4.2). The amount of water used to irrigate each column was calculated as follows: three columns (two with seashore paspalum and one with bermudagrass) were weighed and watered with 800 ml of water to saturation. After draining, the columns retained 400 ml water. The average amount of water lost by a column during the next 48 hours was 125 g. From this information we concluded that irrigating with 700 ml every other day would be sufficient to replace the water lost by
evapotranspiration and leach all the “old” salts out of the column and maintain a constant salinity in the soil, similar to Ashlamary et al. (2004). Irrigation was added over the top of each column using graduated bottles. This study differs from previous research that used batch solution culture systems (Dudeck et al. 1983, Dudeck and Peacock 1985, Meyer et al. 1989, Marcum and Murdoch 1990, Smith et al. 1993, Dudeck et al. 1993, Marcum and Murdoch 1994, Lee 2005). Three replicates of each salinity treatment were used for each turfgrass species.

The columns were irrigated with tap water for 16 days in trial 1 to allow the plants to root and become established before the salinization process started. The process of acclimation of the plants to the saline water was started on February 3, 2006 by irrigating all of the columns with the lowest level (0.5 dS m⁻¹) of saline solution and increasing every 4 days to the next upper level depending on treatment. The highest level (30 dS m⁻¹) was reached on February 21, 2006, when the top growth on every column was clipped and the clippings discarded. Turfgrasses in columns were clipped with scissors every week for eight weeks to a height of 1.5 cm and clippings were collected with a vacuum, dried in an oven at 70 °C, and weighed. Columns were fertilized weekly with 60 ml of a mix of 25 grams of 20-20-20 Peters fertilizer plus 3.32 grams of Epsom salt in 20 l of distilled water. The fungicide azoxystrobin (Heritage 50 WG) was sprayed at 10.53 g/100 m² every two weeks for general disease control. The study was complete after 9 weeks of salinization, on April 25, 2006. Temperatures averaged 28.4 °C during the whole trial and supplemental lights were set to provide a photoperiod of 15 h. light, 9 h. dark per day, and to increase the temperature in the greenhouse. The amount of supplemental light was reduced gradually in March and April 2006 to finish trial 1 with no supplemental light.
For Trial 2, sprigs were taken from the 20-cm-diameter plastic pots that were prepared for Trial 1 and planted in 10×10 cm cardboard pots. They were planted on January 18, 2006, and grown under greenhouse conditions until the Trial 2 was established on May 1, 2006. Grasses were planted in the columns as described for Trial 1. Columns were irrigated for 12 days with fresh tap water for establishment. Irrigation with saline solutions started on May 12, 2006. Grass was clipped, collected, dried, and weighed as in Trial 1 but every two weeks. Fertilization and fungicide applications were made as in the Trial 1. Temperatures averaged 31.2 °C and the same time of light-dark photoperiod was used as in Trial 1 but no supplemental light was used. At the end of June, 2006, the three cultivars of bermudagrass exposed to all salt levels were naturally infested by an unidentified scale insect. Even though an insecticide was sprayed repeatedly the pest could not be controlled and these cultivars were eliminated from Trial 2. There was no insect damage to any of the paspalum cultivars. The salinization treatments were complete after 9 weeks of salinization, July 16, 2006.

Evaluations based on turf quality and turf density were estimated on a scale of 1 to 9. Increasing rating numbers indicated increasing turf quality and turf density (9=green, dense and uniform grass), and increasing numbers indicated increasing severity of leaf firing with 0=no leaf firing and 9=100% of totally brown leaves (Ashlamary et al. 2005). At the end of the study and after the last trimming, the plants were taken out of the columns, washed free of soil mix, and the shoots were separated from the roots. Shoots and roots were dried at 70 °C for 72 hr and weighed. Soil samples were taken for soil analysis.
Results and Discussion

Significant differences were found in soil electrical conductivity measurement among the five different levels of the irrigation water used to irrigate the grasses under study. Saturated paste analysis from soil from each column was measured at the end of each trial and showed a significant increase in electrical conductivity as the electrical conductivity of the treatment irrigation water increased. There were no significant differences between Trial 1 and Trial 2 for this parameter. (Fig. 4.1). Analysis of variance was performed separately for each trial (tables 4.5 and 4.6) and revealed that there was no interaction between irrigation water electrical conductivity and cultivars in either Trial 1 or Trial 2, which means that cultivars were equally affected by the different concentrations of salt in the irrigation water.

Changes in irrigation water electrical conductivity applied to seashore paspalum (0.5, 7.5, 15, 22.5, and 30 dS/m) did not affect yields during both trials in this study (table 4.4). Analysis of variance performed separately for trial 1 (table 4.5) and trial 2 (table 4.6) showed that even though yield was significantly different on the different days the turf grasses were trimmed; there are no interaction between days and electrical conductivity of the irrigation water, meaning that the accumulation of salts over time did not decrease the growth of the grasses. By contrast, Marcum and Murdoch (1990 and 1994) found a significant reduction of shoot growth in seashore paspalum and other perennial warm-season turfgrasses at increasing salinities In another study with two seashore paspalum cultivars, Peacock and Dudeck (1985) showed increasing top growth at intermediate salinity levels (11.7 dS/m of the water solution) followed by decreasing growth with increasingly higher salinity. There were significant differences among the cultivars tested however. Some cultivars responded differently in Trial 1 and Trial 2 (Fig. 4.6). SI 2000, SI 1, and Aloha grew the most in the first trial with no significant differences between them. SeaDwarf and Salam had lower yields but
there was no significant differences with SeaIsle Supreme and SeaSpray (Fig. 4.6A). On the other hand, in Trial 2, no significant differences were found among SeaIsle 1, SeaIsle 2000, SeaSpray, Aloha, and Salam. SI Supreme and SeaDwarf grew the least (Fig. 4.6B).

Turf quality (Fig. 4.2) and turf density (Fig. 4.3) were affected significantly by irrigation water salinity, with both parameters decreasing as salinity increased. Similar results were obtained by Marcum and Murdoch (1990). Also, significant differences were found for turf quality among cultivars: SI Supreme had the best turf quality in Trial 1 followed by SeaDwarf, Aloha, and SI 2000 with no differences among them. SeaIsle 1, SeaSpray and Salam had the lowest quality and density, with no significant differences among them (Fig. 4.7A). SI Supreme had the best turf quality in Trial 2 and significant differences separated SI Supreme from the rest of the cultivars. SeaIsle 1, SeaDwarf, and Aloha were not different, but had better quality than Salam, SeaIsle 2000 Sea Spray in Trial 2 (Fig. 4.7B).

Turf density followed the same pattern as turf quality: the lower the electrical conductivity in the irrigation water, the better the density (Fig. 4.4). Concerning cultivars, SI Supreme and SeaDwarf had the highest density and no significant differences were noted with Aloha in Trial 1. Salam, SeaIsle 2000, and SeaIsle 1 were not different in density, but had significantly better density than SeaSpray (Fig. 4.8A). Again in Trial 2, SeaIsle Supreme and SeaDwarf had the highest turf density followed by Aloha with no significant differences among them. SeaIsle 1, Salam, and SeaIsle 2000 had no differences among them, but were significantly more dense than SeaSpray, which had the lowest density during Trial 2, similar to its performance in Trial 1 (Fig. 4.8B).

Overall and as expected, increasing salinity increased leaf firing (Figure 4.5) in contrast with results from Meyer et al. (1989), who reported no visual chlorosis in response to salt treatment. On the other hand, there was no significant differences in leaf firing among
cultivars during Trial 1, (Fig. 4.9A) but SI Supreme, Aloha and Sea Dwarf were the least affected, and SeaSpray and Salam affected the most during Trial 2 (Fig. 4.8B). No leaf firing was observed until the 5th week of Trial 1, but it was observed earlier during the Trial 2. This was possibly due to increased greenhouse temperatures in the second trial (average temperature = 31.2 C), but cooler temperatures in the first (average temperature = 28.4 C). Leaf firing would be expected to be more severe with increased transpiration rates, which would tend to accumulate salt into the shoots, killing them (Munns and Termaat 1986). As established above, in this experiment turf quality and turf density were visually reduced and leaf burning increased as irrigation water electrical conductivity increased, with visually less clipping to harvest. However yield in dry weight was not reduced by salinity. It can be explained because in halophytes, dry weight is increased by the presence of electrolytes at levels above the levels normally present in culture solutions (Flowers et al. 1977).

Trials 1 and 2 were significantly different concerning shoot weight, root weight, total plant weight, and the shoot-root ratio (Table 4.6.) Even though final shoot weight slightly decreased as salinity increased (Fig. 4.10 A and B), no significant differences were found among the five levels of the electrical conductivity in the irrigation water (Table 4.6). Dudeck et al (1983) found a reduction of bermudagrass top growth as salinity increased. Cultivars evaluated under the same parameters showed significant differences, but results were different in trial 1 and 2 (Table 4.6). In Trial 1, Seadwarf had the greatest shoot weight and it differed from Aloha, which was different from a third group that included SeaIsle 1, SeaIsle 2000, SeaIsle Supreme, and Salam. The lowest shoot weight was obtained from SeaSpray (Fig. 4.13A). In Trial 2, significant differences separated SeaIsle 2000 with the greatest shoot weight from the rest of the cultivars. SeaDwarf, SeaIsle Supreme, and SeaIsle 1 performed next, but with no differences among them. Aloha and Salam performed third and significant
differences separated them from SeaSpray, which, as in Trial 1 had the lowest shoot weight (Fig. 4.13B). Significant linear regression for Trial 2 showed a linear relationship between increasing salinity and decreasing shoot weight (Fig. 4.10A), but results in Trial 1 were not significant (Fig. 4.10A).

Concerning root weight, there were no significant differences due to salinity (Table 4.6). Although there were cultivar effects, the results differed between trials and the cultivars were affected differently by the five levels of salinity in the irrigation water (Table 4.6). Among cultivars, Seadwarf and Aloha had the highest root weight in Trial 1. There were no significant differences among Salam, SeaIsle 1, SeaIsle Supreme, and SeaIsle 2000 which weighed significantly less than SeaDwarf and Aloha. SeaSpray had the lowest root weight (Fig. 4.14A). In Trial 2, SeaIsle Supreme had the highest root weight but no significant differences were measured between SeaIsle 1 and SeaIsle 2000. Aloha, SeaDwarf and Salam were not significantly different in root weight, but as a group had greater root weight than SeaSpray, with the lowest root weight (Fig. 4.14B).

Salinity of the irrigation water did not affect shoot and root ratios and results were significantly different in Trial 1 and 2 (Table 4.6). Differences among cultivars were significant but not consistent between the two trials. In Trial 1, SeaIsle 2000 had the highest shoot/root ratio and significant differences separated it from the rest of the cultivars that did not show significant differences among them (Fig. 4.15A). In Trial 2, no significant differences were seen among the highest shoot/root ratios from SeaIsle 2000, SeaDwarf and SeaSpray, followed by SeaIsle 1, Aloha, Salam and SeaIsle Supreme (Fig. 4.15B).

In this study, we have confirmed that seashore paspalum is a salt tolerant turfgrass, because it maintains fair turf quality, density and growth under high salinity environments, as noted from the salinity levels 7.5 dS and 15 dS in the irrigation water. However, it was
demonstrated that the two highest levels of EC in the water (22.5 and 30 dS) did induce severe damage to the plants, reducing significantly the turf quality and density over all the cultivars in this study. Both are critical factors for acceptable turf (Marcum and Murdoch 1994) so this experiment showed there is a limit to the irrigation water electrical conductivity that seashore paspalum can tolerate with no deterioration in turf quality.
LITERATURE CITED


Table 4.1. Recipe of the artificial Carolina sea water stock solution prepared to irrigate the columns on both trails in the experiment of relative sensitivity of seven seashore Paspalum (*Paspalum vaginatum*) cultivars to salinity in irrigation water. The amount of salts was dissolve in 40 l of deionized water.

<table>
<thead>
<tr>
<th>Salt</th>
<th>Amount (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>605.20</td>
</tr>
<tr>
<td>MgSO4</td>
<td>80.40</td>
</tr>
<tr>
<td>MgCl2</td>
<td>119.90</td>
</tr>
<tr>
<td>CaCl2</td>
<td>32.55</td>
</tr>
<tr>
<td>NaHCO3</td>
<td>5.38</td>
</tr>
<tr>
<td>KCl</td>
<td>4.61</td>
</tr>
</tbody>
</table>

Table 4.2. Ammount of stock solution and deionized water used to obtain the five different levels of irrigation water electrical conductivity.

<table>
<thead>
<tr>
<th>Irrigation Water EC (dS m⁻¹)</th>
<th>Stock solution (l)</th>
<th>Deionized Water (l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.28</td>
<td>19.72</td>
</tr>
<tr>
<td>7.5</td>
<td>4.32</td>
<td>15.68</td>
</tr>
<tr>
<td>15</td>
<td>8.65</td>
<td>11.35</td>
</tr>
<tr>
<td>22.5</td>
<td>12.97</td>
<td>7.30</td>
</tr>
<tr>
<td>30</td>
<td>17.30</td>
<td>2.70</td>
</tr>
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</table>
Table 4.3. Analysis of variance summary for the effects of the electrical conductivity of the irrigation water (ECIW) and cultivars on seashore paspalum yield, turf quality, turf density, and leaf firing during two trials.  

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Yield</th>
<th>Turf Quality</th>
<th>Turf Density</th>
<th>Leaf Firing</th>
</tr>
</thead>
<tbody>
<tr>
<td>P &gt; F</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blocks</td>
<td>2</td>
<td>&lt; 0.01</td>
<td>0.05</td>
<td>&lt; 0.01</td>
<td>0.24</td>
</tr>
<tr>
<td>Trial</td>
<td>1</td>
<td>0.38</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>0.94</td>
</tr>
<tr>
<td>ECIW $^b$</td>
<td>4</td>
<td>0.38</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Trial$^*$ ECIW</td>
<td>4</td>
<td>0.33</td>
<td>&lt; 0.01</td>
<td>0.26</td>
<td>0.02</td>
</tr>
<tr>
<td>Error a</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cultivar</td>
<td>6</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Trial $^*$ Cultivar</td>
<td>6</td>
<td>&lt; 0.01</td>
<td>0.63</td>
<td>0.72</td>
<td>0.05</td>
</tr>
<tr>
<td>ECIW $^*$ Cultivar</td>
<td>24</td>
<td>0.80</td>
<td>0.83</td>
<td>0.41</td>
<td>0.29</td>
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<tr>
<td>Trial$^<em>$ECIW$^</em>$Cultivar</td>
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<td>0.51</td>
<td>0.16</td>
<td>0.85</td>
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<td>Error b</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CV</td>
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<td>0.20</td>
<td>0.11</td>
<td>0.12</td>
<td>0.19</td>
</tr>
</tbody>
</table>

$^a$ Analysis of variance summaries for individual trials are shown in tables 4.4 (trial 1) and 4.5 (trial 2). Yield based on g./m2/day; turf quality, turf density, and leaf firing visually evaluated on a scale 1 to 9.

$^b$ ECIW = Electrical conductivity of the irrigation water of 0.5, 7.5, 15, 22.5, and 30 dS/m.
Table 4.4. Analysis of variance summary for the effects of the electrical conductivity of the irrigation water (ECIW) and cultivars on seashore paspalum yield, turf quality, turf density, and leaf firing in the greenhouse study during trial 1.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Yield</th>
<th>Turf Quality</th>
<th>Turf Density</th>
<th>Leaf Firing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>P &gt; F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blocks</td>
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<td>&lt; 0.01</td>
<td>0.16</td>
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<td>0.08</td>
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<tr>
<td>ECIW b</td>
<td>4</td>
<td>0.11</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Error a</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cultivar</td>
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<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>0.66</td>
</tr>
<tr>
<td>ECIW * Cultivar</td>
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<td>0.68</td>
<td>0.70</td>
<td>0.10</td>
<td>0.74</td>
</tr>
<tr>
<td>Error b</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day</td>
<td>7</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>ECIW * Day</td>
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<td>0.51</td>
<td>0.52</td>
<td>0.60</td>
<td>0.03</td>
</tr>
<tr>
<td>Cultivar * Day</td>
<td>42</td>
<td>0.94</td>
<td>0.83</td>
<td>0.73</td>
<td>0.98</td>
</tr>
<tr>
<td>ECIW * Cultivar * Day</td>
<td>168</td>
<td>1.00</td>
<td>1.00</td>
<td>0.98</td>
<td>1.00</td>
</tr>
<tr>
<td>Error c</td>
<td>455</td>
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</tr>
</tbody>
</table>

CV
Table 4.5. Analysis of variance summary for the effects of the electrical conductivity of the irrigation water (ECIW) and cultivars on seashore paspalum yield, turf quality, turf density, and leaf firing in the greenhouse study during trial 2.

<table>
<thead>
<tr>
<th>Source</th>
<th>Df</th>
<th>Yield</th>
<th>Turf Quality</th>
<th>Turf Density</th>
<th>Leaf Firing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>P &gt; F</td>
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<tr>
<td>Blocks</td>
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<td>0.42</td>
<td>0.57</td>
<td>0.49</td>
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<tr>
<td>ECIW b</td>
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<td>0.62</td>
<td>0.01</td>
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<td>&lt; 0.01</td>
</tr>
<tr>
<td>Error a</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cultivar</td>
<td>6</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>ECIW * Cultivar</td>
<td>24</td>
<td>0.76</td>
<td>0.59</td>
<td>0.87</td>
<td>0.37</td>
</tr>
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<td>Error b</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day</td>
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<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>ECIW * Day</td>
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<td>0.01</td>
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<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Cultivar * Day</td>
<td>18</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>ECIW * Cultivar * Day</td>
<td>72</td>
<td>0.81</td>
<td>1.00</td>
<td>0.97</td>
<td>1.00</td>
</tr>
<tr>
<td>Error c</td>
<td>209</td>
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<tr>
<td>CV</td>
<td></td>
<td>0.27</td>
<td>0.11</td>
<td>0.12</td>
<td>0.31</td>
</tr>
</tbody>
</table>
Table 4.6. Analysis of variance summary for the effects of the electrical conductivity of the irrigation water (ECIW) and cultivars on seashore paspalum final shoot weight, root weight, total plant weight, shoot/root, and soil electrical conductivity in the greenhouse study for trials 1 and 2.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Shoot Weight</th>
<th>Root Weight</th>
<th>Shoot/Root</th>
<th>Total Weight</th>
<th>Soil EC&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blocks</td>
<td>2</td>
<td>&lt; 0.01</td>
<td>0.04</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>Trial</td>
<td>1</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>0.87</td>
</tr>
<tr>
<td>ECIW&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4</td>
<td>0.11</td>
<td>0.36</td>
<td>0.25</td>
<td>0.16</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Trial * ECIW</td>
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<td>0.42</td>
<td>0.12</td>
<td>0.05</td>
<td>0.43</td>
<td>0.02</td>
</tr>
<tr>
<td>Error a</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cultivar</td>
<td>6</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>0.28</td>
</tr>
<tr>
<td>Trial * Cultivar</td>
<td>6</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>0.45</td>
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<tr>
<td>ECIW * Cultivar</td>
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<td>0.02</td>
<td>0.46</td>
<td>0.24</td>
<td>0.32</td>
</tr>
<tr>
<td>Trial * ECIW * Cultivar</td>
<td>24</td>
<td>0.19</td>
<td>0.53</td>
<td>0.51</td>
<td>0.13</td>
<td>0.98</td>
</tr>
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<td>Error c</td>
<td>120</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CV</td>
<td></td>
<td>0.16</td>
<td>0.20</td>
<td>0.28</td>
<td>0.13</td>
<td>0.19</td>
</tr>
</tbody>
</table>

<sup>a</sup> Soil EC = Electrical conductivity of soils measured with saturated paste method.

<sup>b</sup> ECIW = Electrical conductivity of the irrigation water.
Figure 4.1. Effect of five levels of irrigation quality electrical conductivity after 9 weeks on electrical conductivity of the soil of the columns under study conducted in a greenhouse at Clemson University Pee Dee Research and Education Center, Florence, SC. Trial 1 was conducted between February and April, 2006 (4.1A) and Trail 2 between May and July 2006 (4.1B).
Figure 4.2. Effect of five levels of irrigation quality electrical conductivity after 9 weeks on the yield of seven cultivars of seashore paspalum in the greenhouse at Clemson University Pee Dee Research and Education Center, Florence, SC. Trial 1 was conducted between February and April, 2006 (4.1A) and Trail 2 between May and July 2006 (4.1B)
Figure 4.3. Effect of five levels of irrigation quality electrical conductivity after 9 weeks on the turf quality of seven cultivars of seashore paspalum in the greenhouse at Clemson University Pee Dee Research and Education Center, Florence, SC. Trial 1 was conducted between February and April, 2006 (4.2A) and Trial 2 between May and July 2006 (4.2B). Turf quality was evaluated on a scale from 1=worst to 9=best.
Figure 4.4. Effect of five levels of irrigation quality electrical conductivity after 9 weeks on the turf density of seven cultivars of seashore paspalum in the greenhouse at Clemson University Pee Dee Research and Education Center, Florence, SC. Trial 1 was conducted between February and April, 2006 (4.3A) and Trial 2 between May and July 2006 (4.3B). Turf density was evaluated on a scale from 1=worst to 9=best.
Figure 4.5. Effect of five levels of irrigation quality electrical conductivity after 9 weeks on leaf firing (i.e. chlorotic leaves due to the salt concentration in the irrigation water) on seven cultivars of seashore paspalum in the greenhouse at Clemson University Pee Dee Research and Education Center, Florence, SC. Trial 1 was conducted between February and April, 2006 (4.5A) and Trail 2 between May and July 2006 (4.5B). Leaf firing was evaluated on a scale from 1=no damage to 9=100% damage.
Figure 4.6. Evaluation of yield of seven seashore paspalum cultivars tolerance to five levels of irrigation quality electrical conductivity in a greenhouse at Clemson University Pee Dee Research and Education Center, Florence, SC. during 9 weeks. Trial 1 was conducted between February and April, 2006 and clippings were collected, dried in oven at 70 °C, and weighted every 1 week (Fig. 4.6A). Trial 2 was conducted between May and July 2006 and clippings were collected, dried in oven at 70 °C, and weighted every two weeks (Fig. 4.6B). Lines at the top of the columns represent standard deviation. Means with the same letter are no different as assessed by LSD with P<0.05.
Figure 4.7. Evaluation of turf quality of seven seashore paspalum cultivars under five levels of irrigation quality electrical conductivity in a greenhouse at Clemson University Pee Dee Research and Education Center, Florence, SC. during 9 weeks. Turf quality was evaluated on a scale from 1=worst to 9=best. Trial 1 was conducted between February and April, 2006 (4.7A), and trial 2 between May and July 2006,(4.6B). Lines at the top of the columns represent standard deviation. Means with the same letter are no different as assessed by LSD with P<0.05.
Figure 4.8. Evaluation of turf density of seven seashore paspalum cultivars tolerance to five levels of irrigation quality electrical conductivity in a greenhouse at Clemson University Pee Dee Research and Education Center, Florence, SC. during 9 weeks. Turf density was evaluated on a scale from 1=worst to 9= best. Trial 1 was conducted between February and April, 2006 (4.8A), and trial 2 between May and July, 2006 (4.8B). Lines at the top of the columns represent standard deviation. Means with the same letter are no different as assessed by LSD with P<0.05.
Figure 4.9. Effect of five levels of irrigation quality electrical conductivity after 9 weeks on leaf firing (i.e. chlorotic leaves due to the salt concentration in the irrigation water) of seven cultivars of seashore paspalum in a greenhouse at Clemson University Pee Dee Research and Education Center, Florence, SC. Trial 1 was conducted between February and April, 2006 (4.9A) and trial 2 between May and July 2006 (4.8B). Leaf firing was evaluated on a scale from 1=no damage to 9=100% damage. Lines at the top of the columns represent standard deviation. Means with the same letter are no different as assessed by LSD with P<0.05.
Figure 4.10. Effect of electrical conductivity of the irrigation water on the final shoot weight in grams of dry matter dried in oven at 70 °C during 72 h. of seven cultivars of seashore paspalum in a greenhouse at Clemson University Pee Dee Research and Education Center, Florence, SC. after 9 weeks. Trial 1 (4.10A) and trial 2 (4.10B)

\[ y = 4.75 - 0.03x \]
\[ r^2 = 0.493 \]
\[ P = 0.18 \]

\[ y = 5.63 - 0.02x \]
\[ r^2 = 0.90 \]
\[ P = 0.01 \]
Figure 4.11. Effect of electrical conductivity of the irrigation water on the final root weight in grams of dry matter dried in oven at 70 °C during 72 h. of seven cultivars of seashore paspalum in a greenhouse at Clemson University Pee Dee Research and Education Center, Florence, SC. after 9 weeks. Trial 1 (4.11A) and trial 2 (4.11B)

![Graph A](image1.png)

**Graph A**
\[ y = 1.22 - 0.004 \times \]
\[ r^2 = 0.13 \]
\[ P = 0.55 \]

![Graph B](image2.png)

**Graph B**
\[ y = 2.88 - 0.023 \times \]
\[ r^2 = 0.74 \]
\[ P = 0.06 \]
Figure 4.12. Effect of electrical conductivity of the irrigation water on the shoot and root ratio of seven seashore paspalum cultivars in a greenhouse at Clemson University Pee Dee Research and Education Center, Florence, SC. after 9 weeks. Trial 1 (4.12A) was evaluated at the end of the study on April, 2006 and trial 2 in July 2006 (4.12B).

\[ y = 4.15 - 0.039 \times \]
\[ r^2 = 0.70 \]
\[ P = 0.08 \]

\[ y = 2.00 + 0.012 \times \]
\[ r^2 = 0.5106 \]
\[ P = 0.1750 \]
Figure 4.13. Effect of five levels of irrigation quality electrical conductivity after 9 weeks of salinization on final shoot weight of seven cultivars of seashore paspalum in the greenhouse at Clemson University Pee Dee Research and Education Center, Florence, SC. Shoots were separated from the roots and dried in oven at 70 °C for 72 h. Trial 1 was evaluated in April 2006 (4.13A) and trial 2 in July 2006 (4.13B). Means with the same letter are not significantly different as assessed by LSD with P<0.05. Lines at the top of each column represent standard deviation.
Figure 4.14 Effect of five levels of irrigation quality electrical conductivity after 9 weeks of salinization on final root weight of seven cultivars of seashore paspalum in the greenhouse at Clemson University Pee Dee Research and Education Center, Florence, SC. Roots were separated from the shoots and dried in oven at 70 °C for 72 h. Trial 1 was evaluated in April, 2006 (4.14A) and trial 2 in July 2006 (4.14B). Means with the same letter are not significantly different as assessed by LSD with P,0.05. Lines at the top of each column represent standard deviation.
Figure 4.15. Effect of five levels of irrigation quality electrical conductivity after 9 weeks of salinization on shoot weight / root weight ratio after dried in oven at 70 °C for 72 h, of seven cultivars of seashore paspalum in the greenhouse at Clemson University Pee Dee Research and Education Center, Florence, SC. Trial 1 was evaluated in April, 2006 (4.15A) and trial 2 in July 2006 (4.15B). Means with the same letter are not significantly different as assessed by LSD with P,0.05. Lines at the top of each column represent standard deviation.
APPENDICES
APPENDIX A
Comparison of Seven Seashore Paspalum Cultivars under Putting Green Conditions

Materials and Methods

An experimental putting green was built at the Pee Dee REC, in July 2005. The green was constructed following the United States Golf Association (USGA) specifications for putting greens construction (Beard, 1998). Seven cultivars of seashore paspalum (‘SeaIsle 1’, ‘SeaIsle 2000’, ‘SeaIsle Supreme’, ‘SeaSpray’, ‘SeaDwarf’, ‘Aloha’, and ‘Salam’) were planted in a randomized complete block design with three replications. The plots were 3.70 x 6.50 m. Cultivars SeaIsle 1’, ‘SeaIsle 2000’, ‘SeaIsle Supreme’, ‘SeaDwarf’, ‘Aloha’, and ‘Salam’ were sprigged by hand over the surface of the green and rolled. The cultivar ‘SeaSpray’ were seeded with a Gandy vertical seeder at 680 gr/100 m2.

The experiment was replicated at May River Golf Club, Bluffton, SC. The putting green was built in 2003 under USGA specifications. Prior to our experiment, it was cultured to SeaIsle 2000seashore paspalum. The green was sterilized with methyl bromide on July 22, 2005 before planting the same seven cultivars of seashore paspalum (see above, Pee Dee REC, Florence SC) on August 11, 2005, in a randomized complete block design with three replications. The plots were 3.35 x 6.10 m.

The first mowing was made with a Toro 1000 walk behind greens mower on September 7, 2005 at the Pee Dee REC and the mowing height was 12 mm. Mowing height was reduced gradually to reach 6 mm on October 3, 2005. Mowing frequency was three times a week. The last cut was made early in November, after the first frosts. There was no cut during the dormant period of November, December, January, February, and March.

At the beginning of April 2006, the green was cut at 6 mm mowing height, six times a week, and the height was reduced gradually by 0.5 mm every week to reach 3 mm on May 10, 2006. Mowing height was reduced to 2.75 mm the first week of July, when the putting
green quality and speed test started. These cutting heights were consistent with those utilized by several facilities with seashore paspalum in south Florida.

Fertilization program during the grow-in: biweekly granular fertilization was made during establishment on the six vegetative propagated cultivars. Five hundred grams of N per 100 square meters were made with a 1:1 N:K relation, using ammonium nitrate soluble fertilizer (34-0-0), potassium chloride (0-0-60), 16-4-8 slow release Country Club fertilizer and Helena 0-0-20. Phosphorus was applied at 500 grams of P₂O₅ once a week during August and September. Weekly liquid fertilizations were made on the seeded cultivar once the seeds germinated, during the first week of September 2005. The fertilization program was as follow: 114 grams of N / 100 m² once a week during the first three weeks of September. Then, the SeaSpray plots were fertilized the same as the rest of the green. No fertilization was made during the winter time.

At the beginning of the 2006 growing season, 500 g of P₂O₅/100 m² was applied twice, two weeks apart. The source was soluble 0-46-0. 500 g of N/100 m²/week was applied during April and May to complete the grow-in, using granular 16-4-8 Country Club slow release fertilizer. A N:K ratio was 1:1 using Helena 0-0-20 to complete the ratio. The fertilization program from June throughout the summer was 210 g of N/100 m²/month in two biweekly applications, using 16-4-8 slow release Country Club fertilizer.

To establish the grass during the grow-in (August, September, and October, 2005) a mechanized roller was used 3 times per week. During the putting green quality and to improve the green speed, the green was rolled once a day during the two days of the test.

Cultural practices: a triple-light verticut (1 mm deep) was made during the first two weeks of May 2006. Single, biweekly verticutting was done during the whole season until September 15, 2006. Heavy sand topdressing was made every week to level the green during
September and October 2005. At the beginning of the 2006 growing season, two heavy top
dressings were made right after the triple-verticuts, during the first two weeks of May.
During the rest of May, June, and July, weekly light top-dressings were applied. Biweekly
top-dressings were applied during August and September.

No fungicides were applied to prevent diseases during the grow-in, except on the
seeded cultivar ‘Sea Spray’, where 28 ml/100 m2 of mefenoxam 21.3 % (Subdue Maxx) was
applied. Trinexapac-ethyl (Primo Maxx) was sprayed every week from the first week of June
at a rate of 438 ml/ha. The first two applications were made at lower rates (146 ml/ha,
increasing it the following week to 292 ml/ha) to acclimate the grass to the product and
avoid turf burning. The last application was made September 18th, 2006.

Cultivars were visually rated based on turf quality, color, density (1=bad; 9=best),
dollar spot and fairy ring disease severity (1=no disease; 9=severe disease) between March
and September 2006. Greens speed were tested on the different cultivars during July,
August, and September 2006 at the Pee Dee REC, following the rules to measure ball roll
distance using the stimpmeter (Beard 1998).

Results

There were significant differences among cultivars at both locations for the
parameters evaluated. Turf quality and turf density at May River GC (Fig. A2.1.B) showed
fewer differences than at Pee Dee REC (Fig A2.1A) because at MRGC we could not reach
the putting green conditions. In addition and because of the same problem, we could not
evaluate greens speed. Sea Isle Supreme had the best turf quality at both Pee Dee REC and
May River locations followed by Salam at May River. Sea Dwarf and Sea Isle 1 ranked next
at Pee Dee REC and there were no significant differences between them. SeaSpray, Salam,
and Aloha showed acceptable turf quality at the Pee Dee REC. Sea Isle 2000 had the worst
turf quality at both locations, but it was unacceptable at Pee Dee REC. It did not recover
during the spring 2006 from the epidemics of large patch suffered during the Fall 2005.

Sea Isle Supreme had the best turf density than the rest of the cultivars either at Pee
Dee REC (Fig A2.2.A) and MRGC (Fig A2.2.B), but no significant differences were seen
respect to SeaDwarf, that showed excellent density from late spring until summer 2006.
SeaIsle 1 performed very good at the Pee Dee REC (no significant differences with the best
turf density) but the mean was significantly lower than the best one. SI 2000 showed the
worst density at Pee Dee REC, again because it was the most affected by large patch disease.
Sea Spray showed the most open turf among the cultivars evaluated.

Epidemics of dollar spot (*Sclerotinia homoeocarpa*) at the Pee Dee REC (Figure A.3.A)
were more severe than at May River (Figure A.3.B). At Pee Dee REC, SI 2000, Aloha and
Salam were the most susceptible cultivars, but there were no significant differences among
them. Aloha and Salam were damaged the most by dollar spot at May River. There were no
observed significant differences among the least affected cultivars. No dollar spot were
observed on SI 1, SI 2000, SI Supreme, Sea Spray and Sea Dwarf at May River.

All the cultivars were affected by superficial fairy rings (an unidentified
basidiomycete fungus) but significant differences among cultivars were observed in both
locations. At the Pee Dee REC, (Fig. A4A), Sea Dwarf was the most affected by this disease
but Sea Isle 2000 was most affected at May River (Fig A.4B). The infestations could be
caused by different fungi between the locations. Sea Isle Supreme was the least affected at
May River and Salam at the Pee Dee REC but with there were no significant differences
between Sea Spray, Sea Isle Supreme and Sea Isle 2000.
Green speed was only evaluated on the putting green located at Pee Dee REC.
Greens speeds were taken on July 12, July 21, Aug 16, Aug 25, Sep 8 and Sep 21, 2006, on a
2-day test. The first day double cut at 2.75 mm of mowing height early in the morning was
made and rolled after mowing. The second day, the green was mowed and rolled as during
the first day and immediately after rolling, stimp reading were taken.

There were no significant differences among cultivars on five out of the six days the
test were made. Differences among cultivars were seen only on July 21. Significant
differences between morning and afternoon were seen on July 12, July 21, and Aug 16, being
morning’s readings faster than afternoon ones; and Aug 25, when the readings during the
afternoon were faster than the ones during the morning. No significant differences were
seen on Sep 8 and Sep 21. There were no cultivars - timing interaction.
Figure A.1. Turf quality of seven cultivars of seashore paspalum under putting green conditions at Clemson University Pee Dee Research and Education Center, Florence, SC (Fig. A.1A) and May River Golf Club, Bluffton, SC (Fig. A.1B). Bi-monthly evaluations were done at the Pee Dee REC and monthly evaluations at May River GC using a scale from 1 to 9 with 9=best turf quality, from March to September, 2006. Lines at the top of the columns represent standard deviation. Means with the same letter are no different as assessed by LSD with P<0.05.
Figure A.2. Evaluation of turf density of seven seashore paspalum cultivars under putting green conditions at Clemson University Pee Dee Research and Education Center, Florence, SC (Fig. A.2A) and May River Golf Club, Bluffton, SC (Fig. A.2B). Bi-monthly evaluations were done at the Pee Dee REC and monthly evaluations at May River GC using a scale from 1 to 9 with 9=best turf density, from March to September. Lines at the top of the columns represent standard deviation. Means with the same letter are no different as assessed by LSD with P<0.05.
Figure A.3. Evaluation of dollar spot severity of seven seashore paspalum cultivars under putting green conditions at Clemson University Pee Dee Research and Education Center, Florence, SC (Fig. A.3A) and May River Golf Club, Bluffton, SC (Fig. A.3B). Bi-monthly evaluations were done at the Pee Dee REC and monthly evaluations at May River GC using a scale from 1 to 9 with 1=no disease, 9=100% disease, from March to September. Lines at the top of the columns represent standard deviation. Means with the same letter are no different as assessed by LSD with P<0.05.
Figure A.4. Evaluation of superficial fairy ring severity (unidentified basidiomycete) of seven seashore paspalum cultivars under putting green conditions at Clemson University Pee Dee Research and Education Center, Florence, SC (Fig. A.4A) and May River Golf Club, Bluffton, SC (Fig. A.4B). Bi-monthly evaluations were done at the Pee Dee REC and monthly evaluations at May River GC using a scale from 1 to 9 with 1=no disease, 9=100% disease, from March to September. Lines at the top of the columns represent standard deviation. Means with the same letter are no different as assessed by LSD with P<0.05.
APPENDIX B
Relative Sensitivity of Seven Seashore Paspalum Cultivars and Three Hybrid Bermudagrasses to Salinity in Irrigation Water During Trial 1
Table B.1. Analysis of variance summary (Trial 1) for the effects of the electrical conductivity of the irrigation water (ECIW) and cultivars on seven cultivars of seashore paspalum and three cultivars of bermudagrass yield, turf quality, turf density, and leaf firing conducted between February and April, 2006 in a greenhouse at Clemson University Pee Dee Research and Education Center, Florence, SC.

<table>
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<th>Source of Variation</th>
<th>df</th>
<th>Yield</th>
<th>Turf Quality</th>
<th>Turf Density</th>
<th>Leaf Firing</th>
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<td></td>
</tr>
<tr>
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<td>P &gt; F</td>
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<tr>
<td>Blocks</td>
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<td>&lt; 0.01</td>
<td>0.19</td>
<td>&lt; 0.01</td>
<td>0.09</td>
</tr>
<tr>
<td>ECIW</td>
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</tr>
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<tr>
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</tr>
<tr>
<td>Day</td>
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<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>0.98</td>
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<td>0.34</td>
<td>0.49</td>
</tr>
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<td>Cultivar * Day</td>
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<td>0.73</td>
<td>0.04</td>
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<td>1.00</td>
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<td>0.86</td>
</tr>
<tr>
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<td>0.84</td>
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<td>0.16</td>
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</table>
Table B.2. Analysis of variance summary (Trial 1) for the effects of the electrical conductivity of the irrigation water (ECIW) and cultivars on seven seashore paspalum and three bermudagrass cultivars final shoot weight, root weight, total plant weight, shoot/root, and soil electrical conductivity conducted between February and April 2006 in a greenhouse at Clemson University Pee Dee Research and Education Center, Florence, SC.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Shoot Weight</th>
<th>Root Weight</th>
<th>Shoot/Root</th>
<th>Total Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blocks</td>
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<td>0.04</td>
<td>0.46</td>
<td>0.07</td>
<td>0.02</td>
</tr>
<tr>
<td>ECIW b</td>
<td>4</td>
<td>0.33</td>
<td>0.35</td>
<td>0.40</td>
<td>0.06</td>
</tr>
<tr>
<td>Error a</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cultivar</td>
<td>9</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>ECIW * Cultivar</td>
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<td>0.15</td>
<td>0.30</td>
<td>0.76</td>
</tr>
<tr>
<td>Error b</td>
<td>90</td>
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<tr>
<td>CV</td>
<td></td>
<td>0.21</td>
<td>0.30</td>
<td>0.17</td>
<td>0.33</td>
</tr>
</tbody>
</table>

b ECIW = Electrical conductivity of the irrigation water.
Figure B.1. Evaluation of yield of seven seashore paspalum and three bermudagrass cultivars tolerance to five levels of irrigation quality electrical conductivity in a greenhouse at Clemson University Pee Dee Research and Education Center, Florence, SC. during 9 weeks, between February and April, 2006. Clippings were collected, dried in oven at 70 °C, and weighted every 1 week. Means with the same letter are no different as assessed by LSD with P<0.05
Figure B.2. Evaluation of turf quality of seven seashore paspalum and three bermudagrass cultivars under five levels of irrigation quality electrical conductivity in a greenhouse at Clemson University Pee Dee Research and Education Center, Florence, SC. during 9 weeks between February and April, 2006. Turf quality was evaluated on a scale from 1=worst to 9=best. Means with the same letter are no different as assessed by LSD with P<0.05.
Figure B.3. Evaluation of turf density of seven seashore paspalum and three bermudagrass cultivars under five levels of irrigation quality electrical conductivity in a greenhouse at Clemson University Pee Dee Research and Education Center, Florence, SC. during 9 weeks between February and April, 2006. Turf quality was evaluated on a scale from 1=worst to 9=best. Means with the same letter are no different as assessed by LSD with P<0.05.
Figure B.4. Evaluation of leaf firing of seven seashore paspalum and three bermudagrass cultivars under five levels of irrigation quality electrical conductivity in a greenhouse at Clemson University Pee Dee Research and Education Center, Florence, SC. during 9 weeks between February and April, 2006. Turf quality was evaluated on a scale from 1=worst to 9=best. Means with the same letter are no different as assessed by LSD with P<0.05.