SCREENING AND EVALUATION OF COOL-SEASON TURFGRASSES FOR INCREASED SALINITY TOLERANCE

by

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and approved by

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ABSTRACT OF THE DISSERTATION

Screening and Evaluation of Cool-Season Turfgrasses for Increased Salinity Tolerance

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Dr. Stacy A. Bonos

The identification and development of turfgrasses with improved salinity tolerance is necessary to maintain adequate turf quality when utilizing nonpotable irrigation water. High salinity can cause salt stress injury resulting in poor turf quality. Therefore, breeders need to develop cultivars with improved salinity tolerance. However, the development of salt tolerant cultivars has been slow due in part to the fact that inheritance of salinity tolerance is complex. Previous screening techniques developed for turfgrasses have included growing plants directly in hydroponic saltwater solutions or some modification including salt solution/sand culture system however, these do not include foliar exposure to irrigation water. The goal of this thesis was to develop novel salinity screening procedures for cool-season turfgrasses to accurately mimic realistic management conditions and screen and evaluate germplasm and cultivars for salinity tolerance. The novel screening methods were compared to standard techniques to determine the feasibility of this screening method for breeding purposes. Inheritance characteristics associated with salinity tolerance will determine the effectiveness of a breeding program in developing new cultivars with increased salinity tolerance.
To achieve the objectives, a number of greenhouse and field experiments were designed between the summers of 2005 and 2010. Overhead irrigated salt spray chambers were constructed in the greenhouse and used to evaluate perennial ryegrass clones and Kentucky bluegrass cultivars for salinity tolerance at various salinity concentrations. Cultivars of three cool-season turfgrass species were established in the field and screened for salinity tolerance using overhead irrigation. Using the same field screening procedure, salinity tolerance screening was performed on a number of diverse perennial ryegrass genotypes, as well as parents and progeny from controlled crosses.

Significant differences were observed between salinity treatments in the field and greenhouse. Variation in salinity responses ranged from highly tolerant to highly susceptible. The three salinity screening techniques evaluated were highly correlated; however, methods utilizing overhead irrigation resulted in higher salinity stress compared to the hydroponic technique. Inheritance studies indicated that additive gene effects accounted for the majority of the variance associated with salinity tolerance in perennial ryegrass indicating that recurrent selection programs should be effective in developing of salt tolerant cultivars.
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LITERATURE REVIEW

Introduction

Salt-affected soils are found on every continent of the Earth. About 10% of the total land surface is affected by salinity (Carrow and Duncan, 1998; Ghassemi et al., 1995). In fact, this problem is a growing “worldwide phenomenon” and it is estimated that three hectares of arable land is lost every minute to soil salinization (Abrol et al., 1998). In addition, it has also been estimated that productivity has been reduced to zero on 10 to 20 million hectares of previously irrigated lands every year due to increased salinity levels (Choukr-Allah et al., 1996). Pessarakli and Szabolics (1999) estimate that 100 million hectares of land have become salt-affected due to the use of irrigation water with increased salts. In North America alone, about 68,500 sq mi are salt affected (equivalent to the size of Oklahoma) (Carrow and Duncan, 1998).

There are a variety of ways that salinity hazards can develop including dissolution of minerals, salts in irrigation water (wastewater) (Duncan et al., 2000), fertilizers and other soil amendments, salt intrusion in groundwater (Newport, 1997; Todd, 1974), ice melting salts applied to roadways (Marcum, 1994), flooding (Carrow and Duncan, 1998; Trenholm and Unruh, 2000), saltwater spray (Humphreys, 1982; Marcum, 1994), and even small amounts of salt in rain water. Arid and semi-arid regions are especially susceptible to accumulation of soil salts at damaging levels due to the lack of sufficient irrigation or rain to leach and remove salts that have built up over time (Carrow and Duncan, 1998).
Why is Studying Salinity Tolerance in Turfgrass Important?

Turfgrass sites are more frequently being considered as potential locations to use alternative water resources for irrigation. In fact, it has been mandated in some localities (Arizona Department of Water Resources, 1995; California State Water Resources Control Board, 1993; Carrow and Duncan, 1998; Council of Australian Governments, 2004; Duncan et al., 2009; Florida Department of Environmental Protection, 2007). Turfgrass sites are considered one of the ideal areas to use non-potable water resources for irrigation for many reasons. One reason is due to the vast amount of area that is used to grow turfgrass. According to Milesi et al. (2005), there are over 160,000 km\(^2\) of turfgrass being grown in the United States; more than three times larger than any other irrigated crop. Due to the large quantities of irrigation water necessary to maintain attractive turf with acceptable playability on golf courses and sports fields, wastewater may be an attractive alternative to irrigation with potable water (Mancino and Pepper, 1994). Potable water resources are becoming a scarce commodity due to population growth, especially in arid states in the Western United States (Dean et al., 1996; Hayes et al., 1990; Marcum, 2004; Marcum, 1994; Shannon and Grieve, 1999). An average 18-hole golf course can use between 250,000 and 1,000,000 gallons of irrigation water per day to maintain the turf (Huck et al., 2000). Multiplied across the number of golf courses nationwide, this could be a huge amount of potable water that could be reserved for human consumption (approximately 2.7 billion gallons per day) (Watson, 2006). Snow (2003) reported that as of 2003, over 1,000 golf courses across the United States use wastewater for irrigation.
Switching to wastewater irrigation provides an opportunity for turfgrass managers to decrease operating budgets (Cuthbert and Hajnosz, 1999). In an arid region, potable water is expensive due to the scarcity of rain events and available water. Irrigation water can cost an average golf course between $100,000 and $1,000,000 per year (Harivandi, 2000; Huck et al., 2000). According to Huck et al. (2000), a large savings can be achieved by irrigating turf with wastewaters that can cost 80% less than the fresh water equivalent. Transportation costs of recycled water irrigation are less in comparison to fresh water due to the fact that turfgrass sites are often present in urban areas where wastewater treatment plants are already located (Lazarova and Asano, 2005; Lazarova and Bahri, 2005).

Irrigation of turfgrass sites with wastewater sources also provides an opportunity to improve public perception of the turfgrass industry. Recently, there is a push for water conservation practices when irrigating turfgrass (Carrow, 1994; Carrow and Duncan, 1998; Gill and Rainville, 1994; Huck et al., 2000). Research into the use of wastewaters on golf courses and other turfgrass sites has a largely favorable public perception (Bruvold and Ward, 1972). Bruvold and Ward (1972) surveyed 1000 California residents to assess public opinion to wastewater use. Results indicated that public opposition to use of this water source as irrigation on golf courses was almost negligible.

Wastewater, or reclaimed water, can be defined as treated or semi-treated water from a water treatment plant that has been remediated through physical and chemical means (Lazarova, 2005) to a quality suitable for its intended use (Duncan et al., 2009). The treatment process for tertiary, or advanced, treated wastewater is summarized in Figure 1. This process usually involves the addition of salts to the water that remain after
treatment. Tertiary effluent water is likely to become the most prevalent water resource used as turfgrass irrigation due to the reasons listed above. Additionally, there are many alternative water resources that can be allocated for irrigation in order to preserve potable water resources for human consumption. A few of these sources include ponds being fed by surface runoff from surrounding terrain, graywater, and groundwater from aquifers that are deemed unsuitable for human consumption (Duncan et al. 2009).

The increased establishment of golf courses near coastlines and the impact of frequent saltwater exposure is another important reason to study salinity tolerance in turfgrasses (Carrow and Duncan, 1998; Marcum, 1994). Turfgrasses growing near saltwater can be impacted by salt spray (Humphreys, 1982; Marcum, 1994), salt intrusion in groundwater (Carrow and Duncan, 1998; Marcum, 1994; McCarty and Dudeck, 1993; Murdoch, 1987; Newport, 1997; Parker, 1975; Todd, 1974), and flooding due to tidal surges during inclement weather (Carrow and Duncan, 1998; Trenholm and Unruh, 2000). Salt spray can cause salt to accumulate on leaves and produce a foliar burn on the turfgrass verdure as well as increase the salinity of the soil (Bezona et al., 1996). Due to increased human populations in coastal regions, increased use of fresh groundwater resources in these areas has caused the saltwater to move into the groundwater aquifers. Similar to salt spray damage, flooding of turf areas with saltwater will also result in foliar burn and increased soil salinity.

In northern states, salts are used to melt ice on roadways and walkways (Greub et al., 1985; Marcum, 1994). The salts then accumulate in the turf that flank these surfaces and the soil increases salinity to levels that make growing turf extremely difficult, if not
impossible. Hughes et al. (1975) reported that growing turf on roadsides in Illinois has become challenging due to the use of sodium chloride salts on roadways.

Further motivation to study salinity tolerance in turfgrass is that turfed areas may be the perfect locations in which to use wastewaters. In addition to the fact that turf comprises such large acreage and would represent an enormous savings in potable water, turf is also not utilized for food and would lessen the potential health risks associated with irrigating edible crops with recycled water (Gill and Rainville, 1994; Lazarova and Asano, 2005). Turfgrass sites can also be looked at as a means of waste disposal since there are very few options for using reclaimed water resources (Marcum, 1994) and as a means of filtering nitrogen and other nutrients that are found in excessive quantities in recycled water when compared to freshwater (Lazarova and Asano, 2005). Anderson et al. (1981) studied the effectiveness of turf in its ability to filter out pollutants found in wastewaters. It was found that up to 64% of nitrogen contaminants were removed by the turf and soil as the irrigation water infiltrated the soil and entered the groundwater supply. Finally, soil-related issues arising from wastewater irrigation on turfed areas would represent a smaller economic and social impact when compared to food crops (Lazarova and Asano, 2005).

Consequences of Irrigating Turfgrass with Saline Water

Soil effects

One of the adverse effects of using irrigation water with increased levels of salts is decreased soil permeability. According to Carrow and Duncan (1998), soil permeability is defined as “the ability of water, oxygen, and roots to move within the soil
macropores for good turfgrass growth.” However, as Na ions, from irrigated salts, begin to dominate the CEC (Cation Exchange Capacity) sites of soil particles (through exchanges with other ions), the soil undergoes physical changes that decrease soil permeability. These changes can include the destruction of larger soil pore spaces, and reduction of pore continuity. Increased soil salinity can also drastically decrease water infiltration, percolation, and drainage. Other changes include an increase in water holding capacity of the soil, decreased soil $O_2$ due to the reduction in pore space and $O_2$ diffusion, and an increase in soil hardness (Naidu et al., 1995; Pessarakli, 1994; Rhoades and Loveday, 1990; U.S. Salinity Laboratory, 1954).

The physical changes to the soil structure in response to increased sodium on the CEC are caused by a process called “dispersion.” Due to the sodium ion’s large size and charge, clay particles begin to repel and separate from adjacent soil particles resulting in destruction of soil structure. Dispersion is the primary physical process associated with increased sodium concentrations and ultimately the main cause of soil destruction and soil issues associated with wastewater irrigation of agriculture and turfgrass sites (Ayers and Westcot, 1976; Bauder and Brock, 2001; Buckman and Brady, 1967; Chen and Banin, 1975; Frenkel et al., 1978; Hardy et al., 1983; Miller and Donahue, 1995).

Decreased infiltration rates associated with soil dispersion can be caused by the dispersion of clay particles which then clog pore spaces and decrease water flow through the soil profile (Ayers and Westcot, 1976; Miller and Donahue, 1995; Shainberg and Letey, 1984). In addition, when clay particles settle, formation of a hard structureless soil may form in pore spaces further reducing water movement (Buckman and Brady, 1967). The disruption in soil hydraulic conductivity and prevention of water infiltration may
decrease water availability to plant roots lower in the soil profile (Bauder and Brock, 2001; Buckman and Brady, 1967; Miller and Donahue, 1995) and can increase soil runoff and soil erosion (Hardy et al., 1983). The dispersive effects of sodium may also create soil crusting, where clay particles form a brick-like arrangement at the soil surface. Soil crusting further decreases soil permeability and can also prevent root penetration and emergence of new seedlings through the soil surface (Rhoades, 1977).

_Classifications of salt affected soils_

According to the U.S. Salinity Laboratory, salt affected soils can be classified into three distinct categories: saline, sodic, and saline-sodic. Saline soils, or white alkali soils, are characterized by electrical conductivity levels in excess of 4 dS m\(^{-1}\) and a sodium absorption ratio (SAR) <12 (Carrow and Duncan, 1998). Sodium absorption ratio is a measure of the amount of sodium ions in relation to calcium and magnesium ions. This measure is a way of representing the sodium status of a soil since the sodium ions are the most damaging to the soil and plants. The pH of saline soil is typically between 7 and 8.5, with sandy soils sometimes being slightly acidic.

The second type of salt affected soil is called sodic soils or black alkali soils. This condition is caused by large amounts of sodium on the CEC of the soil resulting in destruction of the soil structure. These soils are characterized by a SAR ≥ 12 and alkaline pH. Electrical conductivities of these soils are typically less than those of saline soils and are usually less than 4 dS m\(^{-1}\). Soil permeability issues due to salinity are usually associated with sodic soils because of the dispersive qualities of sodium ions in a soil’s CEC (Carrow and Duncan, 1998).
The third and final type of salt affected soil is known as saline-sodic. This soil is characterized by an electrical conductivity over 4 dS m\(^{-1}\), similar to saline soils, as well as a SAR ≥12, similar to sodic soils. These soils are typically not affected by soil destruction and instead cause damage through osmotic stress on the plants due to the osmotic potential of the soils preventing water uptake by roots (Carrow and Duncan, 1998).

**Plant effects**

Turfgrass sites irrigated with alternative water sources containing high levels of salts may have detrimental effects to the turfgrass plants causing poor turf quality and decreased playability/usability of the site. There are many symptoms of salt stress that occur on turfgrass plants after being irrigated with alternative water sources with increased salt concentrations. One of the first symptoms of salt stress on turfgrass is a reduction in overall growth. Leaves will also become a darker green, shrink in size, and stiffen. Prolonged stress will be evident as the leaves begin to wilt and burn. Finally, thinning of the turf stand will occur followed by complete death of the turfgrass stand (Horst, 1991). The injury associated with salt stress in turfgrasses is mainly due to two main causes: a rapid water (osmotic) stress and a slower ion toxicity phase (Munns and Tester, 2008). Water stress (or physiological drought) is associated with the inability of the turfgrass plants to uptake water from the soil due to the lower water potential of the salt affected soil (Marcum, 1994). Physiological drought is defined as a water stress that is imposed on plant cells due to the increased salt levels outside of the cells, the subsequent movement of water out of the cell, and a decrease in cell turgor pressure (Bernstein and Hayward, 1958; Harivandi et al., 1992). Ion toxicity can also be a
symptom of salt stress and can cause leaf burn, turf thinning, as well as other damage to the turfgrass plant (Marcum, 1994).

The stress that is evident on the turfgrasses can also be dependent on the type of salt affected soil that it is being grown in. Turfgrasses grown in saline soils are notorious for showing signs of physiological drought due to the high salt content of the soil. Ion toxicity can also be an issue in these soils with Cl⁻, B, HCO₃⁻, and SO₄²⁻ ions being the major causes of damage. Toxicity to these ions can also be an additional cause of foliar wilt due to direct damage and death of roots. Ion imbalances in saline soils are also common; resulting in plant nutrient deficiencies to Ca²⁺, K⁺, NO₃⁻, Mg²⁺, Mn, or P (Carrow and Duncan, 1998).

Symptoms of plants growing in sodic soils include poor growth and viability. Drought symptoms are also common since sodic soils cause thin black roots due to low soil oxygen levels as well as ion toxicities to elevated OH⁻ and Al ions. Foliar burn may also be a symptom of salt stress under sodic conditions due to ion toxicity to Na⁺, Cl⁻, or B. Nutrient deficiencies are also common due to high Na⁺. These deficiencies can include Ca²⁺, Mg²⁺ and K⁺ (Carrow and Duncan, 1998).

Plants growing in saline-sodic soils have damage similar to those in saline and sodic soils; however symptoms associated with saline soils are more prevalent. If sodium dominates the CEC though, plants will display damage usually related to sodic soils (Carrow and Duncan, 1998).

Another potential source of injury to turfgrasses being irrigated with saline water is the foliar burn caused by irrigating overhead. Harivandi (2004) states that in addition to salts being absorbed by plant roots, irrigation water with increased levels of sodium
salts will cause additional damage when applied overhead because salts can be directly absorbed by the leaves and cause a fertilizer-type burn. In fact, Wu et al. (1999) found that applying saltwater irrigation overhead caused significantly more damage to plants when compared with irrigation of only the roots with saltwater of the same concentration.

**Cultural Practices to Alleviate Salinity Stress**

One of the easiest ways to prevent a salt issue on a turfgrass site is to use high quality irrigation water with low concentrations of salts. Turfgrass managers can also employ cultural practices to alleviate stress on turfgrass plants associated with salt-affected turfgrass sites if high quality irrigation water is unavailable. The cultural practices chosen will depend on the classification of the salt-affected site and how the salts have impacted the soil.

Saline soils are characterized by high salt levels that prevent water uptake by the turfgrass plants. Irrigation of the site in excess of the water needs of the turfgrass plants will leach salts that have built up in the soil out of the rootzone. Ion imbalances are also common in turfgrass growing in saline soils and fertilization with the necessary nutrients can lessen associated damage (Carrow and Duncan, 1998).

Similar to saline soils, sodic soils can also benefit from leaching of the rootzone with excess irrigation. Sodic soils benefit from leaching of sodium salts since it is this salt ion that causes sodium toxicity as well as the soil permeability issues associated with this type of salt-affected site. Leaching of sodic soils, however, will not correct the soil permeability issues. In order to improve water infiltration through sodic soils, sodium must be removed from the CEC to prevent dispersion and instead promote flocculation
and improve soil structure. Use of gypsum or a sulfur based compound along with lime will replace sodium with calcium on the CEC and cause the sodium to form soluble compounds that are easily leached from the soil. Aeration of sodic soils will also facilitate leaching of sodium from the soil by improving water movement. Similar to saline soils, fertilization can help to prevent damage or stunted growth associated with nutrient imbalances (Carrow and Duncan, 1998).

Cultural practices necessary for saline-sodic soils are similar to those associated with sodic and saline soils. Again, leaching of excess salts through the soil profile by irrigating more than evapotranspiration rates is an effective approach in correcting a saline-sodic soil issue. Cultivation of the soil may improve water movement through the profile and increase the effectiveness of salt leaching if sodium ions dominate the CEC and soil dispersion has occurred. Soil structure may also be improved by amending soil with calcium sources if calcium is deficient on the CEC. Fertilization may also be beneficial in correcting nutrient deficiencies and imbalances caused by the high salinity levels (Carrow and Duncan, 1998).

In addition to all of the cultural practices listed above, another method of preventing damage due to salinity stress is to use salt tolerant turfgrass species in the place of salt sensitive species. Within each species, it is also important for the turfgrass practitioner to use salt tolerant cultivars.

**Salinity Tolerance in Plants**

According to Kramer (1984), plants have two different mechanisms of salt tolerance including: 1) highly salt resistant cytoplasm, and 2) the ability to keep salt
concentrations in the cytoplasm at low levels. Turfgrasses have evolved a number of different mechanisms in which to survive the stresses associated with salinity stress. These mechanisms include increased root growth, ion exclusion, osmotic adjustment, ion compartmentalization, formation of compatible solutes, and glandular ion secretion (Marcum, 2008a).

Root growth will typically be stimulated under moderate salinity stress in salt tolerant turfgrasses (Bernstein and Hayward, 1958; Gorham et al., 1985). Since the roots are responsible for water uptake and water is transpired through the shoot tissue, an increase in the root/shoot ratio takes place in response to the osmotic stress imposed by increased salinity levels (Donovan and Gallagher, 1985; Dudeck et al., 1983; Gorham et al., 1985). It has been shown that salt tolerant grass species have increased root growth under low to moderate salinity stress. Species such as bermudagrass (*Cynodon* spp.) (Ackerson and Youngner, 1975; Dudeck et al., 1983), seashore paspalum (*Paspalum vaginatum* Sw.) (Dudeck and Peacock, 1985a; Marcum and Murdoch, 1990a), weeping alkali grass (*Puccinellia distans* (Jacq.) Parl.) (Alshammary et al., 2004), and Manila grass (*Zoysia matrella* (L.) Merr.) (Marcum and Murdoch, 1990a) have all shown significantly higher root growth under salinity stress when compared to control plants. However, in more salt sensitive species, a reduction in root growth has been shown to occur in relation to the control plants that were not exposed to salinity stress. For example, Chewing’s fescue (*Festuca rubra* L. spp. Fallax (Thuill.) Nyman) (Khan and Marshall, 1981), Kentucky bluegrass (*Poa pratensis* L.) (Torello and Symington, 1984), and buffalograss (*Buchloe dactyloides* (Nutt.) Engelm.) (Wu and Lin, 1993) have all been studied for salinity tolerance and have shown decreases in root growth when exposed to
moderate salinity stress. In addition, seashore dropseed (Sporobolus virginicus (L.) Kunth), is a common grass that can be found growing on sand dunes next to the ocean. When the roots of this species were studied and measured after exposure to increased salinity, a linear increase in root growth was observed with increasing salinity up to concentrations of 35 dS m$^{-1}$, at which point the root growth was equal to double that of control plants (Blits and Gallagher, 1991; Marcum and Murdoch, 1992).

Excluding salt ions from shoot tissue and thereby preventing the toxic effects of these ions has been shown to be associated with the overall salinity tolerance of both C$_3$ (Qian et al., 2001; Torello and Rice, 1986) and C$_4$ (Dudeck and Peacock, 1985a; Marcum, 1999; Marcum and Murdoch, 1994) turfgrass species. The ability of turfgrasses to exclude salt ions, including Na$^+$ and Cl$^-$, from shoot tissue has been used to show cultivar differences within species with respect to salinity tolerance. For example, salt tolerant cultivars of bentgrass (Agrostis spp. L.) (Wu, 1981), bermudagrass (Ramakrishnan and Nagpal, 1973), and Chewing fescue (Hannon and Barber, 1972; Khan and Marshall, 1981) have been shown to have lower concentrations of salt ions in shoot tissue when compared to salt sensitive cultivars. It has been shown that cellular K$^+$ transporters (HKT) have a higher affinity for Na$^+$ under equal extracellular concentrations in Eucalyptus camaldulensis Dehn. (Liu et al., 2001) resulting in increased Na$^+$ concentrations in the cell. Suppression of HKT activity may significantly improve salt tolerance in plants (Chinnusamy et al., 2005) as evidenced in studies of the salinity tolerance of transgenic Arabidopsis (Rus et al., 2001) and wheat (Triticum aestivum L.) (Laurie et al., 2002).
Much of the damage associated with salinity stress is as a result of physiological drought. In order to prevent damage caused by this, turfgrasses have evolved mechanisms of osmotic adjustment, or osmoregulation, where the osmolarity of the cell cytoplasm is increased to prevent water loss (Hellebust, 1976). Increased Na$^+$ ion concentrations within a plant cell will lead to improper cellular enzyme function. One way that salt tolerant turfgrasses decrease the concentration of Na$^+$ is to maintain a high K$^+$/Na$^+$ ratio by actively pumping K$^+$ ions into the cell and Na$^+$ ions out of the cell. Marcum and Murdoch (1990a) showed this mechanism of salinity tolerance to be important in the overall salinity tolerance among C$_4$ turfgrass species. Similarly, Qian et al. (2001) illustrated osmotic adjustment as a means of differentiating two Kentucky bluegrass cultivars for salinity tolerance. The pathway for osmotic adjustment has been examined most thoroughly in Arabidopsis through the molecular genetic analysis of salt overly sensitive (sos) mutants (Zhu, 2003). The SOS signaling cascade initiates with the sensing of increased salinity concentrations by the cell membrane causing an increase in Ca$^{2+}$ signals. The Ca$^{2+}$ signals are perceived by a protein called SOS3 which in turn activates protein kinase SOS2. The antiporter protein, SOS1, is then phosphorylated by the active SOS2 resulting in pumping of Na$^+$ out of the cell cytoplasm (Chinnusamy et al., 2005). Quintero et al. (2002) found that sos2 and sos3 Arabidopsis mutants accumulated increased Na$^+$ in the cell cytosol when compared to wild type plants. Similarly, sos1 mutants also accumulated increased Na$^+$ levels. This indicates that SOS2 and SOS3 proteins are necessary in the signaling cascade to activate the Na$^+$ antiporter, SOS1.
Another method of overcoming salinity stress in turfgrasses is by accumulating damaging salt ions in vacuoles that account for 90-95% of the volume of a mature plant cell (Flowers, 1985). By compartmentalizing the salt ions, damage to essential cell function is prevented. Garbarino and Dupont (1988) found that barley (*Hordeum vulgare* L.) roots exposed to increased NaCl levels, induced K⁺/Na⁺ pumping through the membrane of the tonoplast where Na⁺ is accumulated and sequestered within the vacuole. In addition to K⁺/Na⁺ pumping, Gaxiola et al. (2001) showed that overexpression of AVP1, a vacuolar H⁺ pump, conveyed enhanced sequestration of Na⁺ in the vacuoles and maintenance of higher turgor pressure in the cells and higher relative water content in the leaves of Arabidopsis under salinity stress. It has been shown that the tonoplast Na⁺/H⁺ antiporter NHX1 gene, also responsible for sequestration of Na⁺, is stimulated by ABA which is produced in response to increased salinity levels in *Arabidopsis* (Shi and Zhu, 2002) and rice (Fukuda et al., 1999). Therefore, Shi and Zhu (2002) found that ABA deficient mutants had increased salt sensitivity due to lower NHX1 expression. Transgenic Arabidopsis (Apse et al., 1999), tomato (*Solanum lycopersicum* L.) (Zhang and Blumwald, 2001), and canola (*Brassica napus* L.) (Zhang et al., 2001) plants overexpressing the NHX1 gene have been shown to have significantly higher salt tolerance when compared to wild-type plants.

Formation of compatible solutes within the cell cytoplasm is a way that plant cells increase the osmolarity of the cell sap and prevent water loss from the cell under saline conditions. Examples of compatible solutes in plants include glycinebetaine, proline, trigonelline, polyols, and cyclitols (Gorham, 1996). In addition, glycinebetaine has also been shown to act as an osmoprotectant by stabilizing protein structure and preventing
membrane instability (Chinnusamy et al., 2005). With the exception of weeping alkali grass, where proline has also been shown to play a minor role in salinity tolerance (Torello and Rice, 1986), glycinebetaine has been shown to be associated with salinity tolerance in C₄ turfgrass species including grama species (Bouteloua spp. Lag.), buffalograss, bermudagrass, saltgrass (Distichlis spicata (L.) Greene), centipedegrass (Eremochloa ophiuroides (Munro) Hack.), seashore paspalum, seashore dropseed, St. Augustine grass (Stenotaphrum secundatum (Walter) Kuntze), and zoysiagrass (Zoysia spp. Willd.) (Marcum, 1999; Marcum and Murdoch, 1994; Marcum and Murdoch, 1992). In these species, glycinebetaine was shown to make substantial contributions to cytoplasmic osmotic adjustment in the salt tolerant turfgrasses, however not in the salt sensitive species. For example, in the salt tolerant grass species, seashore dropseed, when grown in high saline conditions, glycinebetaine accumulated in cell cytoplasm to levels that accounted for 93% of the total cytoplasmic osmotic adjustment (Marcum and Murdoch, 1992). Similarly, when saltgrass was grown in high saline conditions, glycinebetaine accumulated to levels that accounted for 73% of the overall cytoplasmic osmotic adjustment (Marcum, 1999). It has also been shown that an Arabidopsis mutant with impaired function of a gene involved in glycinebetaine biosynthesis (S-adenosyl-L-methionine phosphoethanolamine N-methyltransferase) had hypersensitivity to salinity when compared to plants with normal gene function (Mou et al., 2002). Furthermore, creeping bentgrass (Agrostis stolonifera L.) transformed using the BADH transgene, isolated from Mountain Spinach (Atriplex hortensis L.), has shown an increase in glycinebetaine production and increased salinity tolerance. The isolated BADH gene was isolated from plants growing on the shore of a salt lake in China and confers an important
enzyme in the glycinebetaine biosynthetic pathway (Chen et al., 2004). Historically, proline had been considered to be a compatible solute that conveyed salinity tolerance. However, recent research has shown that there is in fact a negative correlation with proline concentrations in turfgrasses (Marcum, 1999; Marcum and Murdoch, 1994) and instead is associated with plant injury (Marcum, 2008a). In a study comparing two different Kentucky bluegrass cultivars, Qian et al. (2001) noted less proline in the more salt tolerant cultivar and correlated an increase proline concentrations with a significant increase in leaf firing in response to salinity stress.

Glandular ion excretion is an additional survival mechanism in some salt-adapted plant species (Flowers et al., 1977; Waisel, 1972). Turfgrasses that have salt glands include grama species, buffalograss, bermudagrass, saltgrass species, dropseed species, and zoysiagrass (Marcum, 1999; Marcum and Murdoch, 1992; Marcum and Murdoch, 1990b). Salt glands in turfgrasses consist of two cells: a basal cell that is attached to the leaf epidermis and a cap cell (Liphshchitz and Waisel, 1974). These specialized excretion glands have been found on both the abaxial and adaxial surfaces of the leaves (Liphshchitz and Waisel, 1974; Marcum, 1999; Marcum and Murdoch, 1990b) and are usually arranged in parallel rows that are flanking rows of stomata (Marcum, 2008a).

Salt glands in turfgrasses are selective in which salt ions are excreted. It has been shown that Na\(^+\) and Cl\(^-\) ions are preferentially excreted compared to other ions such as K\(^+\), Ca\(^{2+}\), and Mg\(^{2+}\) (Marcum and Murdoch, 1994; Marcum and Murdoch, 1990b). Research has been conducted that correlated overall salinity tolerance of eight turfgrass species with the rate at which salt glands are able to excrete salt ions (Marcum, 1999). In this study, highly salt tolerant seashore dropseed had five times higher Cl\(^-\) excretion rates when
compared to moderately salt tolerant bermudagrass and fifty times the excretion rate of salt sensitive buffalograss. Salt ion excretion was also highly correlated with intraspecific salinity tolerance in 57 zoysiagrass cultivars (Marcum et al., 1998) and 35 bermudagrass cultivars (Marcum and Pessarakli, 2006). Salt gland density has also been shown to be highly correlated to salinity tolerance where salt sensitive *Zosia japonica* Steud. had nearly four times fewer salt glands when compared to salt tolerant *Zoysia macrostachya* Franch & Sav (Marcum et al., 1998).

**Salinity Tolerance Screening Methods in Turfgrass**

Many techniques for screening turfgrasses for salinity tolerance have been developed to rank cultivars as well as identify germplasm that will be useful in breeding new cultivars with increased tolerance. One of the most popular methods for salinity screening is performed by growing turfgrass plants directly in a hydroponic saline solution. Hydroponic methods involve growing the plants in a nutrient solution, such as Hoagland’s solution (Hoagland and Arnon, 1950), and adding salts until the desired concentrations are achieved (Dai et al., 2009; Lee et al., 2004; Marcum, 2000; Marcum and Kopec, 1997; Marcum and Murdoch, 1994; Pessarakli and Kopec, 2008, 2009; Qian et al., 2000, 2001; Rose-Fricke and Wipff, 2001; Suplick-Ploense et al., 2002).

However, with this method, only roots are exposed to the saline solution and it does not include the foliar applications of saline water that can be expected with overhead irrigation under field conditions.

Salinity screenings have also been performed by either growing plants in soils and using flood irrigation in order to provide water treatments (Ahti et al., 1980; Poss et al.,
2010) or by irrigating by means of an ebb-and-flow bench. An ebb-and-flow bench is developed by growing plants in a container which allows for the rootzone to be flooded by the saline irrigation water and then subsequently water recedes to prevent water logging (Brown, 2010; Raymer and Braman, 2004). These methods, similar to the hydroponic technique, do not include overhead irrigation and foliar stress that can be present when irrigating turfgrass with wastewater under field conditions.

In addition to the hydroponic screening technique and flood irrigation methods, overhead irrigated methods have been developed, in the greenhouse and field, to address the apparent shortcoming of the other methods and to more accurately mimic the effects that turf managers would experience in field situations when using wastewater irrigation (Dai et al., 2008; Torello and Spokas, 1983; Walworth et al., 2009; Wu et al., 1999). These techniques include foliar stress due to the fact that irrigation is applied overhead and salts may be deposited on the verdure of the turfgrass plants. The salts deposited on the leaves could be a source of an additional stress by means of a foliar burn that can occur due to water removal from the tissue.

**Previous Research Conducted on Salinity Tolerance of Cool-Season Turfgrasses**

There is a broad range in salinity tolerance in both C$_3$ and C$_4$ turfgrasses, from very tolerant species to very sensitive species (Butler et al., 1974; Harivandi et al., 1992; Horst and Beard, 1977), however, other factors cause interactions with salinity tolerance and make comparisons among salinity tolerance research difficult. For example, tolerance to salinity has also been reported to be different due to the maturity (seedling, juvenile, or mature turf) of the turfgrass plants being exposed to the stress (Hughes et al.,
It has been shown that salinity tolerance typically increases gradually as a seedling matures (Maas and Hoffman, 1977; Maas and Nieman, 1978). Environmental factors have also been shown to interact with salinity tolerance of plants. These environmental factors can include light, temperature, and humidity levels during the experiment (Maas, 1986). For example, Hoffman and Rawlins (1971) found that high relative humidity increased the overall salinity tolerance in onions (Allium cepa L.) and radishes (Raphanus sativus L.). This may be due to the fact that the increase in humidity decreased the rate of evapotranspiration which decreased the uptake of salts into the plants. Other factors complicating the measure of salinity tolerance of plants includes the amount of time plants were exposed to the stress, the types of salts used to inflict the stress, and the type of soil used since certain salts can cause conditions in fine textured soils which can compound the effects of the salts (Harivandi, 1988; Maas, 1986; U.S. Salinity Laboratory, 1954). In order to prevent soil issues from compounding salinity effects, most salinity tolerance research has been done using hydroponics or highly porous growing media. Salinity tolerance research in turfgrass has typically been done under controlled environmental conditions, such as growth chambers or greenhouses, in order to prevent environmental issues from interacting with salinity tolerance data (Marcum, 2008b).

Salinity tolerance in turfgrasses has been measured in a number of different ways including shoot weight, relative reduction in shoot weights, root weight, root length, shoot length, visual injury, survival, and seed germination (Marcum, 2008b). Based upon research that used these criteria to quantify the overall salinity tolerance of different turfgrass species, Marcum (2008b) compiled a table summarizing the rankings of the
turfgrass species along with an estimation of the salt concentration that would result in a 50% reduction in shoot growth (Table 1).

Salinity Tolerance of Kentucky Bluegrass Cultivars

Kentucky bluegrass is a widely-used cool-season turfgrass due to its use on high maintenance golf courses and sports fields as well as home lawns and sod farms. Current cultivars of this species can exhibit high quality; however the species as a whole is considered salt-sensitive, only tolerating an electrical conductivity of < 3 or 4 dS m⁻¹ (Beard, 1973; Carrow and Duncan, 1998). Additionally, it has been shown that there is only a moderate range of salinity tolerance among Kentucky bluegrass cultivars (Marcum, 2008b). Among previous research conducted on Kentucky bluegrass cultivars, variability in the cultivar rankings can exist due to the methods of the study as well as the measurements used to quantify salinity tolerance.

Gibeault et al. (1977) performed a Kentucky bluegrass cultivar study by establishing plots on a golf course that had a preexisting soil salinity issue (EC = 11.4 dS m⁻¹). Salinity tolerance was measured with visual quality ratings and significant differences among the 11 tested cultivars were observed. Data from this study suggested that cultivars Fylking and Pennstar were among the most salt tolerant under this stress, while Merion was the most susceptible.

Ahti et al. (1980) quantified the salinity tolerance of 23 Kentucky bluegrass cultivars using visual quality ratings and flood irrigation. Plants were exposed to 14 dS m⁻¹ irrigation water for a total of 12 weeks. As mentioned above, the authors found only a moderate range in the salinity tolerance of the cultivars in this species when compared
to other species that were tested. This research resulted in findings that indicated that cultivars Nugget, KI-148, Bristol, and Parade were the most salt tolerant with Nugget having the highest salinity tolerance.

Another intensive study was carried out by Horst and Taylor (1983) that ranked 44 Kentucky bluegrass cultivars for salinity tolerance based upon germination and early growth measurements. Seeds of each cultivar were germinated on mats floating on top of tanks containing salt solutions made from equal parts of sodium chloride and calcium chloride. Measurements included total germination, days to initial germination, leaf blade length, and leaf blade fresh weights. Increasing concentrations of salts had highly significant effects on all measurements and about 70% of the tested cultivars had germination rates slowed by a factor of two compared to the controls even at the lowest salt concentration. Cultivars that exhibited the highest salinity tolerance included Arista, Nugget, Delta, Prato, Baron, Park, S-21, Pennstar, Fylking, Windsor, Victa, Brika, Banff, Cheri, and Oregon Common. Nugget, Pennstar, and Fylking were also among the most salt tolerant cultivars in previous studies (Ahti et al., 1980; Gibeault et al., 1977).

Torello and Spokas (1983) performed a field study to determine the salinity tolerance of 37 Kentucky bluegrass cultivars. A solution of sodium chloride was sprayed weekly over the plots and concentrations were increased at each irrigation. Similar to Ahti et al. (1980), only minor differences among the cultivars were observed when visual quality ratings were measured after nine weeks of treatments. Kentucky bluegrass cultivars Majestic, Princeton, and Galaxy were the top performers under the stress while Haga, Plush, and Victa were the most susceptible.
Torello and Symington (1984) studied salinity tolerance of Kentucky bluegrasses by germinating the seeds of five cultivars grown in agar under increased concentrations of sodium chloride salt. Salinity tolerance was quantified based upon the reduction in root and leaf length. In addition to the bluegrass cultivars, two cultivars of red fescue (Festuca rubra L.) and one cultivar of alkali grass (Puccinellia distans (L.) Parl.) were also tested in a similar manner and all three were determined to have higher salinity tolerance when compared to the five bluegrass cultivars. Among the bluegrass cultivars, Adelphi and Ram I consistently showed high tolerance to the salts across all measurements, while Baron was most significantly impacted by the stress. Interestingly, in an earlier study, when irrigated with water containing 15 dS m\(^{-1}\) calcium chloride, Kentucky bluegrass cultivars Ram I and Baron both exhibited less foliar damage when compared to Adelphi (Kinbacher et al., 1981).

Greub et al. (1985) studied salinity tolerance of turfgrass species and cultivars including six cultivars of Kentucky bluegrass including Nugget, Fylking, Park, Pennstar, Merion, and Newport by determining dry matter yields and using visual foliar injury ratings. This study was performed by growing plants in pots in the greenhouse which were irrigated with saltwater applied to the soil surface. When compared to the other species, Kentucky bluegrasses were among the cultivars with the greatest decrease in dry matter yields and also showed the most severe foliar damage. Within the six Kentucky bluegrass cultivars, Nugget showed significantly less foliar damage when compared to the other five cultivars indicating that, similar to Ahti et al. (1980), Nugget may have better salt tolerance than most of the tested Kentucky bluegrass cultivars.
Qian et al., (2001) measured the growth and physiological responses of two Kentucky bluegrass cultivars when exposed to increased sodium chloride and calcium chloride levels using hydroponics. Growth measurements included visual percentage leaf firing, clipping yields, root mass, and verdure mass, while physiological measurements included leaf water relations, leaf sap mineral content, and content of proline and glycinebetaine. Results of this study showed that salinity increased percent leaf firing in both cultivars and decreased all growth parameters except for verdure mass. Physiological measurements were also affected by increasing salinity. For example, leaf sap salt ion concentrations increased as salt concentration was increased. Cultivar differences also emerged with Kentucky bluegrass cultivar Limousine showing significantly higher salinity tolerance when compared to cultivar Kenblue based upon measured parameters.

Kentucky bluegrass cultivars were also studied for salinity tolerance by Rose-Fricker and Wipff (2001). Visual ratings were taken to quantify the amount of damage on 64 Kentucky bluegrass cultivars exposed to 8 weeks of salt concentrations equal to 15 dS m$^{-1}$ in hydroponic baths. Overall, this study demonstrated genetic variation in salinity tolerance in older Kentucky bluegrass cultivars. Salt tolerant cultivars from this screening included Northstar, Ascot, Moonlight, Wildwood, Sodnet, Dragon, and Blackstone and were “able to maintain green leaf tissue, active leaf growth and continued root growth.” Salt sensitive cultivars had at least 75% damage caused by the stress and were characterized by yellowing and desiccated leaves, lack of root growth, stunted shoot growth, and eventually complete plant death. Cultivars deemed salt sensitive included Kenblue, Livingston, P-105, Haga, Rita, Sidekick, Classic, Challenger, Shasta, Glade,
IBMY, H92-203, Unique, Rugby II, America, Caliber, Chicago, Arcadia, Abbey, Brilliant, Nuglade, and Rambo.

Suplick-Ploense et al. (2002) evaluated 17 bluegrass cultivars and experimental selections hydroponically for tolerance to concentrations of sodium chloride. Measurements to quantify salinity tolerance included shoot and root growth along with visual percentage leaf firing ratings. As expected, increasing concentrations of salts caused a decrease in all measurements; however data did indicate a significant interaction between salinity tolerance and environment in bluegrasses. Kentucky bluegrass selections Bensuns A-34, CS ST 1, CS ST 3, and CS ST 4 all were ranked as exhibiting the highest salinity tolerance over both years of the study after cluster analysis was performed on the relative measurements. It was also shown that aggressive and compact ecotypes exhibited higher salinity tolerance when compared to common ecotypes.

Poss et al. (2010) studied the salinity tolerance of six Kentucky bluegrass cultivars including Baron, Brilliant, Cabernet, Eagleton, Midnight, and A01-856 (a Texas x Kentucky bluegrass hybrid – [Poa arachnifera x Poa pratensis]). Plants were exposed to various levels of salinity up to a concentration of 22 dS m\(^{-1}\) using flood irrigation in tanks containing sand. Salinity tolerance was quantified by measuring cumulative growth. Based upon absolute biomass production, data indicated that Baron was among the most salt tolerant of the tested cultivars while Midnight and A01-856 were the most salt sensitive. Interestingly, Horst and Taylor (1983) also considered Baron a salt tolerant cultivar in the germination and seedling screening, however, Torello and Symington (1984) noted that Baron was one of the more sensitive cultivars in other studies.
Salinity Tolerance of Perennial Ryegrass Cultivars

Perennial ryegrasses (*Lolium perenne* L.) are used for many turf situations but probably most widely used on athletic fields and for overseeding southern golf courses. Perennial ryegrass as a species is considered to be moderately salinity tolerant, able to withstand 4-8 (Harivandi, 1988; Horst and Beard, 1977) or 6-10 dS m\(^{-1}\) (Harivandi et al., 1992). Among perennial ryegrass cultivars studied, significant differences in salinity tolerance have been found.

In addition to studying Kentucky bluegrass cultivars, Gibeault et al. (1977) also studied perennial ryegrass cultivars for salinity tolerance. The experimental procedures of this study matched those of the Kentucky bluegrass study that was presented where plots were seeded onto a salt affected golf course (EC = 11.4 dS m\(^{-1}\)). Turfgrass quality visual ratings were used to quantify the salinity tolerance of each of the seven perennial ryegrass cultivars that were studied. Data from this study indicated that cultivars Pelo, Manhattan, and NK-100 had the highest salinity tolerance while the most stressed selection was K9-124.

Rose-Fricker and Wipff (2001) also studied the impact of salinity stress on perennial ryegrass cultivars. This study ranked 45 perennial ryegrass cultivars for salinity tolerance after being exposed to salt concentrations in greenhouse water baths equal to 26 dS m\(^{-1}\) for 9 weeks. Salinity tolerance of the cultivars was determined based on a visual rating scale as well as a measurement of the percent survivors. At the end of the study, most cultivars were severely stressed and had signs of significant damage due to the salt; however, some of the cultivars maintained green leaf color as well as continued to grow. Cultivars and accessions exhibiting the highest levels of salinity
tolerance included Brightstar SLT, PST-2SLW, B-2, Manhattan 3, PST-216, Catalina, and Fiesta III. Brightstar SLT was the top performer in this study with 84.2% of the individuals tested surviving the stress. The lowest performing cultivars were Allsport, Buccaneer, MP-107, Premier, Promise, Linn, Yatsu Green, Ascend, and Wilmington with survival percentages between 40% and 25.3%.

As mentioned earlier, the way in which salinity tolerance is measured can impact the way salinity tolerance of a cultivar is perceived. For example, Marcar (1987) studied four perennial ryegrass cultivars, Vic. Cert., Tisdale, Barlata, and Linn, for germination and subsequent growth under salinity stress. Seeds were exposed to salt concentrations equal to 30 dS m\(^{-1}\) for 2 weeks and the data indicated that salinity tolerance based on germination differed from salinity tolerance based upon growth parameters after germination. Another germination study was conducted by Dudeck and Peacock (1985b) where germination of six perennial ryegrass cultivars was measured for germination under increased salinity. Seeds were treated with water of salt concentration equal to 15 dS m\(^{-1}\). Of the cultivars in this study, Pennant was the top performer with the highest amount of seeds germinating, while Fiesta had the least germination.

Horst and Dunning (1989) also conducted a laboratory experiment examining germination and seedling growth of 48 perennial ryegrass cultivars. This research was performed by germinating seeds on floating mats over hydroponic solutions containing sodium chloride and calcium chloride and measuring total germination, germination rate, leaf blade length, root length, and fresh and dry weights to quantify salinity tolerance. Data from this experiment indicated that even at the highest salt concentration, 23.4 dS m\(^{-1}\), significant differences for total germination among the cultivars were minimal with
98% of the seedlings germinating across all cultivars after 3 weeks. Other measurements were useful in separating cultivars with Premier and Citation having the highest germination rate at the most concentrated salinity level and Yorktown II having the lowest.

**Salinity Tolerance of Bentgrass Cultivars**

Although creeping bentgrass is the most popular bentgrass for golf courses in cool temperate climates, velvet bentgrass (*Agrostis canina* L.) has been shown to be useful for low cut putting greens, and colonial bentgrass (*Agrostis capilaris* L.) has utility as a fairway grass (Bonos, 2008). Creeping bentgrass is considered to be moderately salt tolerant being able to withstand salt concentrations ranging from 8 to 16 dS m\(^{-1}\) (Beard, 1973; Harivandi, 1988; Horst and Beard, 1977) or 6 to 10 dS m\(^{-1}\) (Harivandi et al., 1992). Colonial and velvet bentgrasses have significantly lower salinity tolerance and have been characterized as having poor salinity tolerance with colonial bentgrass tolerance being < 4 dS m\(^{-1}\) (Beard, 1973; Harivandi, 1988; Horst and Beard, 1977) or < 3 dS m\(^{-1}\) (Harivandi et al., 1992).

Younger et al. (1967) studied the salinity tolerance of seven creeping bentgrass cultivars under hydroponic conditions. The cultivars included in this study included Penncross, Cohanse, Seaside, Pennlu, Arlington, Old Orchard, and Congressional. Salinity treatments causing a 50% reduction in clipping weights were of concentrations from 9 to 26 dS m\(^{-1}\) with Seaside being the most salt tolerant while Penncross and Congressional were the least tolerant. Increasing salt concentrations did correlate with a decrease in relative clipping weights across all cultivars.
Marcum (2001) studied the salinity tolerance of 33 creeping bentgrass cultivars along with one colonial bentgrass and one velvet bentgrass by exposing them to salt concentrations equal to 8 dS m\(^{-1}\) for 10 weeks via hydroponics. The measurements used to quantify stress tolerance were relative dry weight of clippings, percentage of green leaf area, root dry weight, and root length. A correlation of data from each of the measurements was analyzed and all measurements were highly correlated. The cultivars with the highest salinity tolerance included Mariner, Grand Prix, Seaside, and Seaside II while the most salt sensitive creeping bentgrass cultivars were SR1119, Regent, Putter, Penncross, and Penn G-6. The most susceptible cultivars were the one colonial bentgrass (Ambrosia) and the one velvet bentgrass (Avalon [also known as SR7200]) which both survived for 5 weeks at this salinity. Interestingly, the author points out that many of the most tolerant cultivars included selections that were developed using germplasm selected from areas with salinity pressure such as Seaside, Seaside II, and Mariner, similar to Younger et al. (1967).

**Breeding for Salinity Tolerance in Turfgrasses**

The goal of a plant breeder is to develop cultivars that are resistant to abiotic and biotic stresses, however breeding for tolerance to salt stress has been slow and difficult (Rose-Fricker and Wipff, 2001). There are a number of reasons for the slow progress in breeding salt tolerant plants including: 1) an inadequate understanding of the effects of salinity on plants; 2) insufficient means of detecting and quantifying salinity; 3) unreliable selection methods for choosing breeding germplasm; 4) a poor understanding of the complex interactions of salinity and environment as it affects the plant; 5) the
vague understanding of effects of moderate salt stress on plants other than growth responses; 6) the interactions of ionic and osmotic properties of salts on plants; 7) changes in salt tolerance depending on plant development and age of plants; 8) the large number of plant physiological structures that contribute to salt tolerance; and 9) salinity tolerance is a quantitative trait controlled by many genes (Rose-Fricker and Wipff, 2001; Shannon, 1984; Winicov, 1994). Development of salinity tolerant plant cultivars is a very difficult process due to these difficulties but the challenge is being undertaken by plant breeders and geneticists.

**Heritability**

One of the first steps in developing new turfgrass cultivars with salinity tolerance is to determine the inheritance of the trait in order to optimize selection procedures. Heritability estimates are used by plant breeders as a prediction of the expected improvements after selection (Nyquist, 1991). Heritability is defined as the proportion of the variation that is observed in a progeny that is inherited (Poehlman and Sleper, 1995). Heritability can be expressed as both broad-sense and narrow-sense (Fehr, 1987). Heritability ratios are calculated based upon observations of various genotypes evaluated over multiple environments and years (Gordon et al., 1972). Traits with a high heritability value are characterized by minimal environmental influences and can be improved rapidly and with less intensive evaluation when compared with traits with lower heritability (Nyquist, 1991).

**Broad-Sense Heritability**
Broad-sense heritability is an estimate of the total genetic (additive, dominance, and epistatic) variance contributing to the observed phenotype (Dudley and Moll, 1969) which indicates to what extent the phenotype is determined by the genotype as opposed to the environment (Nyquist, 1991). Broad-sense heritability in turfgrasses was first described by Burton and Devane (1953) where tall fescue (*Festuca arundinacea* Schreb.) clones were used to estimate the broad-sense heritability of a variety of plant characteristics on a single-plant and entry mean basis. Estimates of broad-sense heritability are determined by calculating the variance components from the mean square of the analysis of variance using the following formula: $H = \frac{\sigma^2_g}{\sigma^2_g + \sigma^2_{gy} + \sigma^2_{gl} + \sigma^2_{gyl} + \sigma^2_e}$

where $\sigma^2_g = \text{the total genetic variance of clones}$, $\sigma^2_{gy} = \text{genotype x year variance}$, $\sigma^2_{gl} = \text{genotype x location variance}$, $\sigma^2_{gyl} = \text{genotype x year x location variance}$, and $\sigma^2_e = \text{variance due to experimental error}$ (Poehlman and Sleper, 1995).

Hurley and Funk (1985) studied variability within rough bluegrass (*Poa trivialis* L.) for traits including turf quality, leaf color, leaf texture, plant density, seed shattering, and disease susceptibility. A total of 279 clonal collections were replicated three times and studied in three locations (greenhouse, field/sun, and field/shade) and broad-sense heritability estimates were calculated on a three plant mean basis. Estimates from all measurements indicated that genetic advancements may be possible to develop improved varieties.

Estimation of broad-sense heritability has been used in turfgrasses to effectively quantify the effectiveness of breeding initiatives. Ashraf et al. (1987) studied the heritability of salinity tolerance in four grass species including red fescue, common velvetgrass (*Holcus lanatus* L.), creeping bentgrass, and orchard grass (*Dactylis*...
glomerata L.). Broad-sense heritability estimates were made using data from replicated genotypes grown at a single concentration of sodium chloride. Data from this study indicates that the broad-sense heritability estimates for all species were above 80% which means that variation in tolerance to sodium chloride stress has a large genetic component.

Broad-sense heritability estimates were also performed for salinity tolerance parameters in zoysiagrass including relative leaf firing, shoot and root growth, and sodium and potassium content (Qian et al., 2000). A total of 29 zoysiagrass cultivars and experimental lines were treated hydroponically with salt solutions with concentrations equal to 42.5 dS m\(^{-1}\). Estimates of broad-sense heritability were moderate to high (leaf firing = 0.67, shoot growth = 0.50, root growth = 0.41, sodium accumulation = 0.54, and potassium content = 0.40) and indicate that reasonable progress may be possible in a breeding program. However, due to the large amount of environmental influence, progeny testing may be important.

In addition to turfgrass, broad-sense heritability has also been estimated for the salinity tolerance in other plant species. Gregorio and Senadhira (1993) analyzed the broad-sense heritability in a rice (Oryza sativa L.) population grown under salinity stress. Seedlings were exposed to salt concentrations equal to 12 dS m\(^{-1}\) using hydroponics for 19 days. Samples were then taken from the plant shoots and analyzed for Na-K ion balance, since typical salinity tolerance in rice has been associated with the ability to exclude Na\(^+\) ions and increased absorption of K\(^+\) ions. Based on this measurement, broad-sense heritability estimates were equal to 0.37, indicating that this phenomenon is greatly affected by environmental effects.
Broad-sense heritability was also studied for various growth parameters under salinity stress in spring wheat (Ashraf, 1994). Saline water, at concentrations of 2.81 (Control), 8.0, 16.0, and 24.0 dS m\(^{-1}\), were added to pots containing six plants in the greenhouse. At maturity, plants were harvested and yield components were measured, including tiller number, number of grains per spike, and the weight of 1000 seeds. Broad-sense heritability estimates for all measurements used to quantify salinity tolerance of spring wheat were between 0.49 and 0.91 across all salinity concentrations, indicating that a large portion of the variance among these measurements under salinity stress may be attributable to genetic factors.

**Narrow-Sense Heritability**

Broad-sense heritability estimates, although useful to determine how much variation is due to genetic effects, are not as useful for breeding purposes as narrow-sense heritability estimates. The inheritance of dominance and epistatic genetic effects, which are included in the broad-sense heritability estimate, cannot be accurately predicted to be transferred to the progeny in an out-crossing species. Narrow-sense heritability estimates, however, measure the portion of additive genetic effects compared to the total observed variation (Nyquist, 1991; Poehlman and Sleper, 1995). An understanding of the ratio of the additive genetic variance to the total phenotypic variance is important in cross pollinated grasses since the most effective breeding design maximizes the use of the additive genetic variation, which is usually achieved through recurrent selection (Vogel and Pendersen, 1993).
In addition to turfgrasses, narrow-sense heritability of salinity tolerance has also been studied in other plant species. Four forage species, including forage rape (*Brassica napus* L.), berseem clover (*Trifolium alexandrinum* L.), alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pretense* L.), were studied for salinity tolerance under hydroponic conditions (Ashraf et al., 1987). Narrow-sense heritability for this trait was estimated for each of the species by measuring shoot growth in a solution of sodium chloride. Parent-progeny regression provided estimates of narrow sense heritability of 0.74 for forage rape, 0.50 for berseem clover, 0.52 for alfalfa, and 0.98 for red clover. The authors conclude that the relatively high heritability estimates suggest a recurrent selection program should be effective in a salinity tolerance breeding program due to the large proportion of additive gene effects associated with this trait.

Along with broad-sense heritability, Gregorio and Senadhira (1993) also estimated narrow-sense heritability of salinity tolerance in rice. Similar to the low broad-sense heritability estimates for Na-K ratio, narrow-sense heritability estimates were equal to 0.20. Due to these results, the authors conclude that both additive and dominant gene actions are responsible for the inheritance of salinity tolerance in rice.

Ashan et al. (1996) studied the inheritance of salinity tolerance in spring wheat (*Triticum aestivum* L.). Narrow-sense heritability estimates were calculated based on grain weight as well as ion uptake and resulted in relatively high values of 0.75 and 0.70 respectively. The results suggested to the authors that it should be possible to employ recurrent selection breeding procedures to obtain salt tolerance and high yielding recombinant wheat lines.
Combining Abilities

In addition to narrow-sense heritability estimates, the data obtained from diallel crosses can also be used to estimate general and specific combining abilities (GCA and SCA) by following the statistical methods presented by Griffing (1956). According to Sprague and Tatum (1942), combining abilities can be used to study aspects of quantitative traits. Combining ability estimates are also useful in determining the potential for specific parents to contribute resistance or susceptibility to a breeding program (Bokmeyer et al., 2009; Lonnquist, 1950; Hedge et al., 2007). General combining ability is calculated as the mean performance of progeny from a particular parent compared to the mean of all other crosses. Estimates of GCA provide researchers with an expected value for a specific cross which is equal to the sum of the GCA from the two parents involved. Specific combining ability is defined as the deviation from the expected value for a particular cross. The estimates of combining abilities can be used to define the relative impact of the additive (GCA) vs. non-additive (SCA) gene effects that are contributing to the trait as well as identify parents to include and avoid in a breeding program (Araujo and Coulman, 2002; Becelaere and Miller, 2004; Cisar et al., 1982; Falconer and Mackay, 1996).

In addition to studying heritability of the salinity tolerance trait in rice, Gregorio and Senadhira (1993) also calculated combining abilities of the parental lines used in their diallel cross. Calculations of variance components resulted in highly significant GCA and SCA values, however, the mean squares of GCA were twice as large as the mean squares of SCA. This indicates that salinity tolerance in rice, when categorized
using Na-K ion ratio, is more influenced by additive gene effects compared to non-additive gene effects.

Chen et al. (2008) studied combining ability data of barley for salinity tolerance. Diallel crosses were made between parents with contrasting salinity tolerance in the greenhouse and progeny were evaluated for $K^+$ flux under saline conditions. Data indicated that, similar to Gregorio and Senadhira (1993), both GCA and SCA had highly significant variances but GCA had a much larger mean squares value compared to SCA. The authors conclude that due to the highly additive nature of this trait in barley, a breeding program should be effective in generating novel material with increased salinity tolerance.

**Heterosis and Maternal Effects**

Heterosis is a useful measure for breeders as it gives an indication of dominance involved in the cross. A lack of heterosis in a cross can denote that gene effects are additive in nature. However, a lack of heterosis can also signify that parents involved in a specific cross can have groups of loci with opposing responses for the trait of interest, which can result in a progeny mean similar to the mid-parent mean (Falconer and Mackay, 1996). Calculating heterosis is performed by comparing the mid-parent means of a specific cross against the progeny means. Maternal effects inform breeders if the trait of interest is transferred to the progeny unequally among the egg and pollen donor plants. Maternal effects are calculated by comparing the progeny means of reciprocal crosses. Both of these measures are accomplished using a two-sample $t$ test using data obtained from a diallel cross (Kitchens, 1998).
Gregorio and Senadhira (1993) found significant maternal effects present in crosses analyzing the salinity tolerance of rice by noting a significant difference between data from reciprocal crosses. Singh and Singh (2000) studied salinity tolerance in spring wheat by measuring grain yield, 1000 grain seed weight, and biological yield under saline conditions. Similar to Gregorio and Senadhira (1993), significant differences were observed between reciprocal crosses for the grain yield and 100 grain seed weight measurements, indicating that maternal effects may be present. Significant differences were also observed between parental means and progeny means, indicating the presence of heterosis.

**Conclusion**

Salinity is a mounting issue in agriculture due to the increasing amount of salt affected land area and the decreasing availability of clean fresh water for irrigation. Turfgrass sites are attractive places to use wastewater irrigation, however this water typically has elevated levels of dissolved salts. Use of saline irrigation water can have detrimental effects to soil and plants, although turfgrasses have evolved mechanisms to alleviate this stress depending on the turfgrass species. Previous research performed on salinity tolerance in turfgrasses has included various screening techniques and has demonstrated significant differences among cool-season turfgrass cultivars, however it is necessary to screen currently available cultivars and germplasm using methods that mimic realistic field conditions in order to accurately predict responses to the salinity stress. According to previous research in turfgrasses and other plant species, salinity tolerance may be heritable as a result of additive gene effects but further research is
necessary to determine the effectiveness of a cool-season turfgrass breeding program focused on developing novel cultivars with increased salinity tolerance.
Goal of This Thesis: The goal of this thesis was to develop novel salinity screening procedures for cool-season turfgrasses to accurately mimic realistic management conditions. The novel screening methods were compared to standard techniques to determine the feasibility of this screening method for breeding purposes. The identification of cultivars and selections of cool-season turfgrass species with increased salinity tolerance using these new techniques can be used to recommend to turf managers while breeding efforts could be begin to identify tolerant parent plants for future cultivar development. Inheritance characteristics associated with salinity tolerance will determine the effectiveness of a breeding program in developing new cultivars with increased salinity tolerance.

This thesis reports the following:

1. Development and testing of a novel greenhouse screening technique for screening turfgrass plants for salinity tolerance
2. Correlation of three salinity screening techniques, including a novel greenhouse screening procedure developed at Rutgers University, a field-based screening technique, and a popular hydroponic method
3. Use of this novel greenhouse screening technique to evaluate 24 Kentucky bluegrass cultivars and selections for salinity tolerance
4. Evaluation of Kentucky bluegrass, bentgrass, and perennial ryegrass cultivars and selections using a field screening technique for salinity tolerance
5. Evaluation of inheritance characteristics of salinity tolerance in a perennial ryegrass population
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Table 1. Rankings of turfgras species based upon estimations using current literature.

Source: Marcum, 2008b.

<table>
<thead>
<tr>
<th>Relative Salinity Tolerance of Turfgrasses</th>
<th>C₄ (Warm-Season) Turfgrasses</th>
<th>Salinity Toleranceᵃ</th>
<th>C₃ (Cool-Season) Turfgrasses</th>
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<tbody>
<tr>
<td><em>Distichlis spicata</em> sp. <em>stricta</em></td>
<td></td>
<td>35 dS m⁻¹</td>
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<td><em>Sporobolus virginicus</em></td>
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<tr>
<td><em>Paspalum vaginatum</em></td>
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<td>25 dS m⁻¹</td>
<td><em>Puccinellia</em> spp.</td>
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<tr>
<td>'Sea Isle 1'</td>
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<td>'Fulits'</td>
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<td><em>Zoysia matrella</em></td>
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<td>'Salty'</td>
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<td>'Diamond'</td>
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<tr>
<td><em>Zoysia pacifica</em> (tenuifolia)</td>
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<td>20 dS m⁻¹</td>
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<td><em>Stenotaphrum secundatum</em></td>
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<td>'Seville'</td>
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<tr>
<td><em>Cynodon</em> spp.</td>
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<td>18 dS m⁻¹</td>
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<td>'FloraTex'</td>
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<td>'MS Supreme'</td>
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<tr>
<td><em>Zoysia japonica</em></td>
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<td>14 dS m⁻¹</td>
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<tr>
<td>'Meyer'</td>
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<tr>
<td><em>Pennisetum clandestinum</em></td>
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<td>12 dS m⁻¹</td>
<td><em>Agrostis stolonifera</em></td>
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<td></td>
<td></td>
<td>10 dS m⁻¹</td>
<td>'Mariner'</td>
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<td></td>
<td></td>
<td>8 dS m⁻¹</td>
<td><em>Festuca arundinaceae</em></td>
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<td>'Tar Heel II'</td>
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<td><em>Lolium perenne</em></td>
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<td></td>
<td>7 dS m⁻¹</td>
<td>'Paragon'</td>
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<td>'Fiesta III'</td>
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<td></td>
<td>6 dS m⁻¹</td>
<td><em>Agropyron cristatum</em></td>
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<td></td>
<td><em>Festuca rubra</em> spp.</td>
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<td>'Dawson'</td>
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<tr>
<td><em>Buchloë dactyloides</em></td>
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<td>5 dS m⁻¹</td>
<td><em>Bromus inermis</em></td>
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<td><em>Lolium multiflorum</em></td>
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<td><em>Lolium xhybridum</em></td>
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<td><em>Poa pratensis</em></td>
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<td>'Nugget'</td>
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<td><em>Poa arachnifera x pratensis</em></td>
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<td><em>Festuca rubra</em> spp. fallax</td>
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<td><em>Festuca brevipila</em></td>
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<td><em>Festuca elatior</em></td>
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<td><em>Festuca ovina</em></td>
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<td><em>Axonopus</em> spp.</td>
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<td>3 dS m⁻¹</td>
<td><em>Agrostis capillaris</em></td>
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<tr>
<td><em>Eremochloa atheriroides</em></td>
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<td><em>Agrostis canina</em></td>
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<td><em>Paspalum notatum</em></td>
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<td>2 dS m⁻¹</td>
<td><em>Agrostis gigantea</em></td>
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<td><em>Poa trivialis</em></td>
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<td><em>Poa annua</em></td>
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ᵃ Relative salinity tolerance is an estimate (based on literature review) of the maximum salinity (ECe in dS m⁻¹) at which the grass can acceptably grow, or the salinity at which shoot growth is reduced by approximately 50%.

ᵇ Salt-tolerant cultivar within species. Indicates published information is available for comparisons to be made among cultivars within a species.
Figure 1. Flow sheet summarizing wastewater treatment for irrigation. Source: Asano and Pettygrove, 1987.
CHAPTER 2

An Overhead Irrigation Screening Technique for Salinity Tolerance in Cool-Season Turfgrasses

Abstract. The successful use of alternative water sources on urban landscapes and golf courses will require salinity tolerant turfgrasses. The objectives of this study were to develop a greenhouse screening method using overhead irrigation, determine useful measurements for screening germplasm and to determine whether perennial ryegrass (Lolium perenne L.) genotypes with salinity tolerance can be identified. A greenhouse irrigation chamber system was developed to apply salinity treatments (1 (control), 5, 10 or 15 dS m\(^{-1}\)) for 10 weeks. Eight genotypes of each of five perennial ryegrass cultivars (Palmer III, Paragon GLR, Applaud, Brightstar SLT and Nui) were arranged in a combined split plot design with three replications in 100% sand trays. For all genotypes, relative percent green ratings, clipping yields, and root and shoot weights were significantly reduced with each level of increased salinity. Significant differences between perennial ryegrass genotypes were also observed. Relative percent green ratings were highly correlated to clipping yields (\(r^2 = 0.95\) \(p<0.001\)), shoot weights (\(r^2 = 0.89; p<0.001\)) and root weights (\(r^2=0.93; p<0.001\)) indicating percent green ratings may be used to predict more intensive measurements. This method is a novel technique utilizing overhead irrigation and should be useful in developing improved turfgrass cultivars with salinity tolerance.
INTRODUCTION

The identification and development of turfgrasses with improved salinity tolerance is necessary to maintain adequate turf quality when utilizing non-potable water for irrigation. Therefore, breeders need to develop cultivars with improved salinity tolerance. However, the development of salt tolerant cultivars has been slow (Winicov, 1998), due in part to the fact that inheritance of salinity tolerance is complex. It is assumed to be controlled by a large number of genes with quantitative inheritance that affect both morphological and physiological traits (Flowers, 2004; Neumann, 1997; Shannon, 1984). Additionally, different mechanisms of salinity tolerance have been associated with different plant growth stages (Neumann, 1997) (i.e. seedlings vs. mature plants).

Researchers and breeders have developed several procedures to select plants that can endure saline water (Bauer et al., 2009; Peabody, 2004; Rose-Fricker and Wipff, 2001). However, most of the screening techniques previously developed for turfgrasses have included growing plants directly in a hydroponic saltwater solution (Dudeck et al., 1993; Marcum, 2001; Marcum and Murdoch, 1990; Peacock et al., 1993; Qian et al., 2000; Rose-Fricker and Wipff, 2001) or some modification including a salt solution and sand culture system (Lee et al., 2002; Peel et al., 2004; Qian et al., 2000). Overhead irrigation was not used in most of these techniques so they do not include salt stress on the leaves (Qian et al., 2001; Rose-Fricker and Wipff, 2001) even though most turfgrass managers water turfgrass using overhead irrigation. Our study describes a novel, efficient salinity screening technique using overhead irrigation which exposes all plant
parts (including leaves) to high levels of salinity and may be used to screen cool-season turfgrass germplasm and identify tolerant plants for use in turfgrass breeding programs.

The objectives of this research were to 1) develop a greenhouse technique for screening turfgrass plants for salinity stress using overhead irrigation, 2) determine useful measurements for screening turfgrass germplasm and 3) determine whether the method is useful at identifying salt tolerant germplasm of perennial ryegrass (*Lolium perenne* L.).
MATERIALS AND METHODS

Spray Chamber Design

A greenhouse sand culture system was designed to overhead irrigate plants with varying concentrations of saltwater. Chambers were designed to apply a controlled rate of saltwater using an overhead sprinkler system in an effort to simulate turfgrass management under field conditions. With this design, saltwater was sprayed overhead directly onto the turfgrass plants resulting in salt exposure directly on the leaves and in the growing medium. A schematic of the design is shown in Figure 1. The saltwater solution drained into a reservoir tank that contained a circulating pump to reapply the saltwater back onto the plants. Chamber frames were constructed from pieces of wood (5.1 x 10.2 cm) (Figure 1). Clear polyethylene plastic (0.05 mm thick) (Sunbelt Plastics Centerville, GA) was used to cover the wood structure to create an entirely enclosed spray area. Plastic lattice (Brite Manufacturing Inc. Ontario, Canada) was put on top of the wood frame to allow water to drain through the system. Black plastic (0.1 mm thick) (Sunbelt Plastics Centerville, GA) was attached around the entire lower section of the chambers in order to prevent light from entering, which greatly reduced the amount of algal growth in the holding tanks. A 238 L polyethylene holding tank (Tamco Industries, Lima, OH) was placed below each platform to collect excess water and leachate. One pump (per frame) pressurized to 310 KPa, delivered saltwater at the rate of 12.11 L min\(^{-1}\) (Shurflo Cypress, CA) (Figure 1). The saltwater solution was applied to the plants using spray nozzles (3.71 L min\(^{-1}\)) (McMaster-Carr Robinsville, NJ) that were attached to the top of the frame to apply uniform salt solution onto the plants. Particle filters (5-15 micrometer) (General Electric Co. Trevose, PA) were put before the spray nozzles to
prevent sand particles and algae from clogging the overhead spray nozzles. Industrial garden hose and PVC connecters were used to connect the pumps to the overhead nozzles (Figure 1). Groco 18.93 L expansion tanks (Groco, Hanover, MD) were also used to keep the pressure of the nozzles at a constant level without fluctuating when the pumps turned on and off. A PVC “T” was used to add an additional hose to the system which was inserted back into the holding tank where it agitated the salt solutions to keep it homogenous (Figure 1). Prior to the second run of the experiment, nozzles and filters were cleaned and calibrated to maintain consistent water flow through the system. All holding tanks were initially filled with 95 L of water with half-strength Hoagland’s Solution (Hoagland and Arnon, 1950) to provide plant nutrition. A total of four chambers were built to accommodate the four salinity treatments (reported below). Plastic trays (57 x 44 x 15cm) (US Plastics, Lima, Ohio), used to hold the plants and sand medium were placed on the plastic lattice of the salt chamber for each salt treatment. Trays were removed after the irrigation water had drained through the system.

**Plant Material and Salt Treatments**

Eight genotypes from five cultivars were chosen to represent a broad range of diversity among perennial ryegrass cultivars and corresponded to several generations of breeding improvements within the species (ranked from most recent): Paragon GLR, Applaud, Brightstar SLT, Palmer III and Nui. Tillers were taken from established turf plots at the Rutgers Plant Biology Research and Extension Farm in Freehold, NJ and separated into single plants. A total of eight genotypes from each of the five cultivars of perennial ryegrass were randomly selected for screening. The individual genotypes were
grown until they were large enough to be vegetatively propagated. Once they were large enough, plants were separated into 12 individual clonal replicates of equivalent size, with approximately 3 tillers each, and established in Pro-Mix growing media (Premier Tech Ltd. Quebec, Canada) and grown for four weeks in the greenhouse.

Plants were removed from the Pro-mix and roots were washed and cut to 5.1 cm below the crown. Shoots were cut to 3.8 cm above the crown. One of the twelve replicate clones of each genotype was planted randomly into one cell of a plastic 15 cm deep tray filled with SurePlay® topdressing sand from US Silica (Mauricetown, NJ). Each tray had 40 individual cells separated by plastic dividers to prevent roots from adjacent plants growing together. At the bottom of each cell, a drainage hole was made to allow water to pass through the root zone. Each tray represented a replication. There were three replications (three trays) for each of the four salinity treatments (described below) for a total of 12 trays in the experiment. All trays were irrigated initially (using the spray chambers) with half-strength Hoagland’s solution (Hoagland and Arnon, 1950) every other day for a week to allow plants to establish in the trays before beginning salt treatments.

Salt solution treatments were made using Instant Ocean (Spectrum Brands Inc. Atlanta, GA) in order to accurately mimic the constituents of sea water and contained a mixture of the following salts: chloride, sodium, sulfate, magnesium, calcium, potassium, bromide, as well as small amounts of other salts. Instant Ocean was added at a rate of 1 dS m$^{-1}$ each irrigation day, to tap water (and Hoagland’s nutrient solution), until the final concentrations were reached. The final electrical conductivities (EC) of the four chambers were as follows: Treatment 1 (Control): 1 dS m$^{-1}$ (this treatment included
Hoagland nutrient solution so the EC did not equal 0), Treatment 2: 5 dS m\(^{-1}\), Treatment 3: 10 dS m\(^{-1}\), and Treatment 4: 15 dS m\(^{-1}\). Electrical conductivity was checked and adjusted on each irrigation day. The Hoagland’s nutrient solution was also replaced weekly to ensure the stress being observed was not due to a nutrient deficiency. Plants were irrigated every other day for 10 weeks after final EC was attained in the salt solutions. A total of 4.98 cm of water was sprayed over the plants on each irrigation day. Water pH was adjusted to 6.5 before each irrigation cycle since this is the optimum pH for perennial ryegrasses (Murphy and Mohr, 2002). The pH was adjusted by adding acid (H\(_2\)SO\(_4\)) or base (NaOH) as necessary.

The experiment took place in a greenhouse with 400 Watt HPS supplemental lighting (Kavita Canada Inc. Ontario, Canada) providing photosynthetically active radiation (PAR) in the range of 90-110 μmol m\(^{-2}\) s\(^{-1}\) as measured with an Apogee Quantum Meter (Apogee Instruments Inc. Logan, UT). Fourteen-hour day lengths were maintained for the duration of both runs of the 10 week study. Greenhouse temperatures were set to be maintained between 17°C and 24°C (average temperature = 17.5°C; Standard Deviation = 0.74) and were monitored using an Onset HOBO Pro Series data logging temperature probe (Bourne, MA). The air exchange rate in the greenhouse was 4 air exchanges per minute. Humidity was not controlled during the course of the experiment.

**Data Collection**

Percent green ratings were taken weekly on all plants to quantify the amount of leaf senescence as a result of salinity stress. Plants were cut weekly to a height of 3.8 cm.
Clippings were collected every two weeks, dried in an oven at 65°C for 48 hours, and weighed.

After 10 weeks of salt treatments, the plants were removed from the trays and the roots were washed free of sand. Plants were then split into two parts: roots (included only root tissue) and shoots (included shoots, crowns, and leaf tissue). Once separated, roots and shoots were dried at 65°C for 48 hours, and weighed. Soil samples were also taken at this point to determine the final electrical conductivity of the sand rootzone. Representative samples of sand were taken from each tray and were sent to the Rutgers Soil Testing Laboratory for soil EC analysis. Analysis was performed using the 1:2 Soil:Water Extract Method (Dellavalle, 1992).

**Statistical Analysis**

The experiment was arranged as a combined split plot with three replications, runs were the main plot, replications were nested within runs, treatments were sub plots and genotypes were considered the sub-sub plots. The 10 week experiment was repeated twice; Run 1 was initiated in March 2006 and Run 2 in October 2006.

In order to account for inherent genetic differences in growth patterns among genotypes of perennial ryegrass, all measurements were reported as a percentage of the control plants. Percentages of control values were calculated by taking the actual measured value for each salinity treatment and dividing it by the control value (Treatment 1) for the same measurement, then multiplying by 100. All data was then subjected to Analysis of Variance (ANOVA) using SAS Version 9.1 (SAS Institute, 1991). All 40 individual genotypes were used in the statistical analysis, however, due to space
restrictions, only 15 genotypes are presented (Data for all genotypes is available upon request). The 15 genotypes were chosen to represent the range of responses observed. The five most tolerant, five moderately tolerant and the five most susceptible genotypes were chosen and categorized based on their relative percent green rating at the highest salinity treatment (Trt 4) at the week 10 rating date in Run 1. The same 15 genotypes were presented for all measurements. Correlations between relative percent green ratings, clipping yields and root and shoot weights were conducted using Proc CORR in SAS.
RESULTS AND DISCUSSION

The ANOVA is summarized in Table 1. Run (P < 0.001), salinity treatment (P < 0.0001), genotype (P < 0.0001), and the interaction of genotype and salinity treatment (P < 0.001) were significant for percent green ratings, clipping yields, and root and shoot weights on most, but specifically the later, rating dates of the 10 week study. Replication was not significant for any measurement on any rating date, and therefore is not presented in the ANOVA table (Table 1). Ryegrass clones responded uniformly across replications, with little variation observed. This finding is significant for breeding programs because it indicates that the salinity screening method described here was effective at controlling environmental variation between replications. This is important because it may be possible to reduce the number of replications and increase the number of germplasm lines that could be screened for salinity tolerance using this method.

A significant run x genotype interaction was observed for all measurements (Table 1), therefore data for each run is presented separately. This interaction can be partially attributed to differential response of certain genotypes between the runs. All of the five Nui genotypes were damaged more severely by the salt treatments in the second run (fall) compared to the first run (spring), while Brightstar SLT genotypes 5 and 7 exhibited improved performance in the second run (fall) compared to the first run (spring) (Figures 2-5).

The difference in runs could be caused by the fact that the two runs were conducted at different times of the year. Although greenhouse conditions were similar during both runs of the experiment, the discrepancy between runs can be mostly attributable to the plants being at different stages of vegetative growth prior to initiation.
of the experiment because of the time of year of the experiments. The first run was performed in the spring; therefore plants were actively growing roots and shoots and were exposed to increasing day lengths in the greenhouse prior to the start of the experiment. The second run was conducted in the fall; the plants in this experiment were exposed to shorter day lengths and may have been allocating more carbohydrates to storage organs rather than shoots in preparation for winter dormancy (Turgeon, 2004) when compared to the first run. The difference in timing of the two runs may have resulted in different genotype responses. However, even though there was a significant interaction between runs, genotype responses were relatively consistent over both runs as seen in the figures described below.

Salinity irrigation treatments resulted in significant differences in electrical conductivity of the 100% sand growing medium, with higher salinity treatments exhibiting higher soil EC compared to lower salinity treatments. However, the soil ECs were relatively low [1.41 dS m$^{-1}$ (Run 1) and 1.61 dS m$^{-1}$ (Run 2) for Trt 4 (15 dS m$^{-1}$), 1.04 dS m$^{-1}$ (Run 1) and 1.12 dS m$^{-1}$ (Run 2) for Trt 3 (10 dS m$^{-1}$), 0.69 dS m$^{-1}$ (Run 1) and 0.67 dS m$^{-1}$ (Run 2) for Trt 2 (5 dS m$^{-1}$) and 0.1 dS m$^{-1}$ (Run 1) and 0.08 dS m$^{-1}$ (Run 2) for Trt 1, Control (1 dS m$^{-1}$)] since 100% sand has limited cation exchange sites. The low soil ECs observed were not considered a significant source of stress to the plant. However, all plant parts were exposed to the high EC water treatments as the water flowed through the system, so stress responses were attributed to the high EC irrigation water treatments exclusively. In all cases, increasing overhead salinity irrigation treatments resulted in decreased relative percent green ratings (Figure 2), relative clipping yields (Figure 3), relative shoot weights (Figure 4), and relative root weights (Figure 5).
These results are similar to other salinity research experiments (Dai et al., 2008; Lee et al., 2005; Marcum and Pessarakli, 2006; Qian et al., 2004), which also found similar reductions in growth parameters with increasing salinity levels.

Significant differences in relative percent green ratings (Figure 2), relative clipping yields (Figure 3), relative shoot weights (Figure 4), and relative root weights (Figure 5) were observed between genotypes (Table 1). Palmer III genotypes 8, 7, 5, 6 and Applaud 1 exhibited the highest relative percent green ratings compared to all other genotypes in all salinity treatments throughout the 10 week experiment. These genotypes represented salinity tolerant genotypes (Figure 2). Applaud genotypes 8 and 5, Paragon GLR genotype 3, and Nui genotypes 6 and 3 represented moderately tolerant genotypes. Nui 1 and 4, Brightstar SLT 5 and 7, and Paragon GLR 7 had the lowest relative percent green ratings and represented intolerant genotypes. For the most part, (excluding minor changes in ranking among a few of the genotypes either between runs or across measurements), the genotypes that exhibited the highest percent green ratings, also exhibited the highest relative clipping yields (Figure 3), and the highest relative shoot (Figure 4) and relative root weights (Figure 5). Conversely, the genotypes with lowest percent green ratings also exhibited the lowest clipping yields (Figure 3), and the lowest shoot (Figure 4) and root weights (Figure 5). There were no genotypes for any of the measurements that fell outside the range of genotypes presented.

Not surprisingly, relative percent green ratings among all 40 clones were highly correlated to relative clipping yields ($r^2 = 0.95$ $p<0.001$), relative shoot weights ($r^2 = 0.89$; $p<0.001$) and relative root weights ($r^2=0.93$; $p<0.001$). The high correlation observed between relative percent green ratings and relative clipping yield, relative shoot
weights and relative root weights suggest that plants that remain green and retain color are also maintaining growth of both roots and shoots even under relatively high salinity stress levels. Nikman and McComb (2000) suggest that measurements of the growth and survival of the plants under salinity stress is an effective measure of salinity tolerance because these are the aggregate of many physiological mechanisms within the plant. Additionally, the ability of a plant to continue growing under salinity stress is a beneficial characteristic because theoretically the plant will exhibit injury for less time and should recover from or ‘grow out’ of the stress more quickly. Qian et al. (2004) used the ability to grow under salt stress to separate Kentucky bluegrass (Poa pratensis L.) cultivars for salinity tolerance. The greenhouse salinity screening method reported here identified perennial ryegrass genotypes that were able to suffer less reduction in growth (through clipping yields, shoot weights and root weights measurements) compared to those genotypes that exhibited large reductions in growth when exposed to salinity. These results are consistent with other salinity screening methods that used clipping yields (Pessarakli et al., 2008), shoot weights (Bauer et al., 2009; Bowman et al., 2005; Marcum and Murdoch, 1990; Marcum and Murdoch, 1994; Marcum et al. 2005; Marcum and Pessarakli, 2006; Pessarakli et al., 2008; Qian et al., 2001) and/or root weights (Dai et al., 2009; Dudeck et al., 1983; Marcum et al., 2005; Qian et al., 2001; Pessarakli and Kopec, 2008) to determine salinity tolerance in turfgrasses.

The salinity screening method described here utilized overhead sprinklers to apply saline irrigation to turfgrass plants. This method was able to separate genotypes and identify salinity tolerant plants which could be used as potential parents in turfgrass breeding programs.
The maintenance of root production under salinity stress is a beneficial characteristic in turfgrasses. In many regions of the US and the world, salinity stress is found in association with drought stress. If turfgrass genotypes have the ability to maintain root growth under salinity stress it may improve water uptake and therefore could improve drought tolerance as well. Unfortunately, root biomass is also probably the most difficult characteristic/measurement to collect. In most cases, the sample must be destroyed to determine the root weight. Additionally, roots must be washed free of the soil medium and dried and weighed which increases the time and effort needed to obtain these measurements and reduces the number of samples that can be realistically processed. The high correlation observed between relative percent green ratings and relative root weights indicate that it may be possible to use relative percent green ratings to predict relative differences in root weights.

Additionally, relative percent green ratings were also strongly correlated to clipping yield and shoot weights, and could be used to deduce relative shoot responses as well. Although not as intensive as root weights, collecting data for clipping yields and shoot weights requires significantly more time and labor compared to taking percent green ratings. For breeding and selection an effective evaluation tool is needed to quickly screen germplasm because typically large numbers of plants need to be evaluated to identify a few tolerant genotypes. The time it takes to evaluate the germplasm limits the amount of germplasm that can be screened. If percent green ratings could be used effectively to infer clipping yield, and shoot and root responses to salinity it would dramatically reduce sampling time, maintain the genotype (alive), and increase the number of plants that could be evaluated. Preliminary data using this method on
Kentucky bluegrass (*Poa pratensis*) and creeping bentgrass (*Agrostis stolonifera*) indicate similar correlations of percent green ratings to clipping yields and shoot and root weights (data not shown).
CONCLUSION

The overhead irrigation spray chamber method described in this paper proved to be an effective way of applying varying levels of salinity irrigation water to turfgrass plants in a practical way that simulates typical turfgrass management systems. Relative percent green ratings were strongly correlated to relative clipping yields, and relative shoot and root weights, indicating that plants that remain green and retain color are also maintaining growth even under relatively high salinity stress levels. Some genotypes exhibited good tolerance by maintaining growth relative to their control plants while others were susceptible and exhibited a significant reduction in the growth parameters evaluated. The strong correlation between measurements also indicated that relative percent green ratings, which are easy and quick to collect, compared to clipping yields and shoot and root weights, may be used as an indicator of the more intensive measurements. The use of percent green ratings would be extremely useful for breeding programs as it would improve efficiency and allow for the screening of more germplasm. Additionally, this greenhouse salinity screening method was effective at reducing environmental variation among replicates (a task difficult to achieve under field conditions due to the influence of environmental factors). If replication could be reduced it would allow more space and time for screening additional germplasm. The utilization of this method should significantly hasten the development of cultivars with improved salinity tolerance so that alternative water sources may be used effectively to irrigate cool-season turfgrass in the future.
LITERATURE CITED


Table 1. Analysis of variance of salinity tolerance measurements (relative percent green ratings, relative clipping yields, relative shoot weights, and relative root weights) evaluated in a greenhouse using overhead irrigation at three salinity treatments (5, 10, 15 dS m$^{-1}$).

<table>
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<tr>
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<th>Relative Visual % Green Ratings</th>
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*Significant at the 0.05 probability level.  
**Significant at the 0.01 probability level.  
***Significant at the 0.001 probability level.  
†NS, not significant.
Figure 1. Schematic design of the salinity spray chamber showing water flow and materials used. A total of four identical chambers were constructed to apply salinity treatments.
Figure 2. Relative percent green ratings of perennial ryegrass genotypes taken weekly for 10 week at three salinity treatments using a greenhouse salinity screening technique. All data were represented as a percentage of the control plants that did not receive additional salt in the irrigation water. In the figure legend, Palm 5, 6, 7, and 8 represent genotypes of Palmer III. App 1, 5, and 8 represent genotypes of Applaud. Nui 1, 3, 4, and 6 represent genotypes of Nui. PGLR 3 and 7 represent genotypes of Paragon GLR. BSLT 5 and 7 represent genotypes of Brightstar SLT. Tolerant genotypes were denoted with white symbols, moderate genotypes were denoted with gray symbols, and intolerant genotypes were denoted with black symbols. Vertical bars denote LSD values at the 0.05 level and were present only when the differences between genotypes were present.
Fig 3. Relative clipping yields of perennial ryegrass genotypes taken biweekly for 10 weeks at three salinity treatments using a greenhouse salinity screening technique. All data were represented as a percentage of the control plants that did not receive additional salt in the irrigation water. In the figure legend, Palm 5, 6, 7, and 8 represent genotypes of Palmer III. App 1, 5, and 8 represent genotypes of Applaud. Nui 1, 3, 4, and 6 represent genotypes of Nui. PGLR 3 and 7 represent genotypes of Paragon GLR. BSLT 5 and 7 represent genotypes of Brightstar SLT. Tolerant genotypes were denoted with white symbols, moderate genotypes were denoted with gray symbols, and intolerant genotypes were denoted with black symbols. Vertical bars denote LSD values at the 0.05 level and were present only when the differences between genotypes were present.
Fig 4. Relative shoot weights of perennial ryegrass genotypes taken after 10 weeks at three salinity treatments using a greenhouse salinity screening technique. All data were represented as a percentage of the control plants that did not receive additional salt in the irrigation water. In the figure legend, Palm 5, 6, 7 and 8 represent genotypes of Palmer III. App 1, 5, and 8 represent genotypes of Applaud. Nui 1, 3, 4, and 6 represent genotypes of Nui. PGLR 3 and 7 represent genotypes of Paragon GLR. BSLT 5 and 7 represent genotypes of Brightstar SLT. Tolerant genotypes were denoted with white bars, moderate genotypes were denoted with gray bars, and intolerant genotypes were denoted with black bars. Vertical bars denote LSD values at the 0.05 level and were present only when the differences between genotypes were present.
Fig 5. Relative root weights of perennial ryegrass genotypes were taken after 10 weeks at three salinity treatments using a greenhouse salinity screening technique. All data were represented as a percentage of the control plants that did not receive additional salt in the irrigation water. In the figure legend, Palm 5, 6, 7, and 8 represent genotypes of Palmer III. App 1, 5, and 8 represent genotypes of Applaud. Nui 1, 3, 4, and 6 represent genotypes of Nui. PGLR 3 and 7 represent genotypes of Paragon GLR. BSLT 5 and 7 represent genotypes of Brightstar SLT. Tolerant genotypes were denoted with white bars, moderate genotypes were denoted with gray bars, and intolerant genotypes were denoted with black bars. Vertical bars denote LSD values at the 0.05 level and were present only when the differences between genotypes were present.
CHAPTER 3

Salinity Tolerance of Kentucky Bluegrass Cultivars and Selections Using an Overhead Irrigated Screening Technique

Abstract. An increasing amount of reclaimed water is being used in turfgrass management; however, reclaimed water is often high in total dissolved salts and can result in salt stress injury and poor turf quality. The objective of this study was to use an overhead irrigated greenhouse screening technique to identify Kentucky bluegrass (Poa pratensis L.) cultivars with increased salinity tolerance using growth parameters and physiological measurements. Salt treatments were applied using an overhead sprinkler system. Twenty-four Kentucky bluegrasses and Texas (Poa arachnifera Torr.) x Kentucky bluegrass hybrids were established in 100% sand tanks and evaluated at a control (1 dS m⁻¹) and three salinity concentrations (3, 6, and 9 dS m⁻¹) for a total of 10 weeks. Plants were arranged in a split plot design with 3 replications. Percent green ratings and digital images were collected weekly and clipping weights were collected biweekly. Root and shoot weights were taken at the end of the study. Significant differences were observed between salinity treatments and cultivars. Eagleton, Moonshadow, Fairfax, Cabernet, and Liberator exhibited the most salt tolerance, having the least decrease in growth parameters compared to the control plants (1dS m⁻¹) while Baron, A03-84, and A03-TB246 were the most salt sensitive when compared to control plants. Relative water content and photochemical efficiency were useful to quantify physiological differences between tolerant and susceptible cultivars.
INTRODUCTION

Water conservation is a necessary and responsible practice especially in high-water-using urban landscapes and golf courses. Decreasing potable water supplies are driving the use of reclaimed irrigation sources (Dean et al., 1996; Hayes et al., 1990; Shannon and Grieve, 1999). Turfgrass areas are the perfect environments to use reclaimed or alternative water sources (i.e. reclaimed water, seawater and/or gray water) because turf is not utilized for food. Using reclaimed water on turfgrass would reduce the demand for high quality potable water for irrigation. However, these sources are often high in total soluble salts and can result in increased soil salinity and salt stress injury leading to poor turf quality (Qian and Meccham, 2005).

The identification of turfgrasses with improved salinity tolerance is necessary to maintain adequate turf quality when utilizing non-potable water for irrigation. Therefore, breeders need to develop cultivars with improved salinity tolerance. Most of the screening techniques previously developed for turfgrasses have included growing plants directly in a hydroponic saltwater solution (Alshammary et al., 2004; Rose-Fricker and Wipff, 2001; Suplick-Ploense et al., 2002; Qian et al., 2004; Qian et al., 2001), or some modification including a salt solution/sand culture system (Poss et al., 2010; Robbins et al., 2009), however, most of these techniques do not include foliar exposure to the irrigation water even though most turfgrass managers apply irrigation water overhead. Koch and Bonos (2010) developed an overhead irrigation greenhouse salinity screening technique to screen cool-season turfgrasses and found the technique effective at differentiating perennial ryegrasses (*Lolium perenne* L.) for salinity tolerance.
Kentucky bluegrass (*Poa pratensis* L.) is a widely-used cool-season turfgrass due to its use on high maintenance golf courses and sports fields as well as home lawns and sod farms. Current cultivars of this species can exhibit high turf quality; however the species as a whole is considered salt-sensitive, only tolerating an electrical conductivity of $< 3$ to $4$ dS m$^{-1}$ (Beard, 1973; Carrow and Duncan, 1998). Additionally, it has been shown that there is only a moderate range of salinity tolerance among Kentucky bluegrass cultivars (Marcum, 2007). Rose-Fricker and Wipff (2001) performed an extensive evaluation of 64 Kentucky bluegrass cultivars for salinity tolerance, which demonstrated genetic variation in salinity tolerance in older bluegrass cultivars. However, this study was conducted ten years ago and many of the cultivars evaluated are no longer in production.

Kentucky bluegrass cultivar responses to salinity were also found to be variable between research studies (Marcum, 2007; Poss et al., 2010). For example, based on absolute biomass production, Poss et al. (2010) found that the cultivar Baron exhibited good salinity tolerance. Horst and Taylor (1983) also considered Baron a salt tolerant cultivar in a germination and seedling tolerance screening study. However, Torello and Symington (1984) found that Baron was one of the more sensitive cultivars in other studies. They identified the cultivar Adelphi as having good salt tolerance; however this cultivar was considered salt sensitive in another study (Kinbacher et al., 1981). The lack of consistency between experiments indicates that more research is needed to evaluate the salinity tolerance of Kentucky bluegrass cultivars.

In previous studies, measurements for determining the salinity tolerance of Kentucky bluegrass cultivars have included percent green ratings, clipping weights, and
shoot and root growth (Alshammary et al., 2004; Dai et al., 2009; Pessarakli et al., 2004; Qian et al., 2004; Qian et al., 2001; Suplick-Ploense et al., 2002). Percent green ratings have been analyzed both visually and digitally using digital image analysis (DIA) (Dai et al., 2008). Digital image analysis has been shown to be an effective tool in screening turfgrass for color (Karcher and Richardson, 2003; Karcher and Richardson, 2005), cover (Karcher and Richardson, 2005), drought stress (Richardson et al., 2009), as well as other turfgrass traits. An initial study conducted by Dai et al. (2008) used digital image analysis to determine salinity tolerance between experimental lines of *Poa annua*.

Although digital image analysis may take significantly longer compared to visual percent green ratings, a major benefit to using digital image analysis is that it removes “researcher bias and inaccuracies” (Karcher and Richardson, 2003). Digital image analysis has not been widely tested to evaluate salinity tolerance between Kentucky bluegrass cultivars.

The identification of turfgrass cultivars that can tolerate overhead irrigation with alternative water sources while maintaining safe, acceptable quality would result in a community and industry more accepting of voluntarily utilizing alternative water sources. The objectives of this research were to 1) evaluate the salinity tolerance of 24 Kentucky bluegrass cultivars and selections, representing different bluegrass types and breeding generations, for salinity tolerance using an overhead irrigation screening technique 2) and to compare growth parameters and physiological measurements associated with salinity responses in Kentucky bluegrass.
MATERIALS AND METHODS

Spray Chamber Design

An overhead irrigation spray chamber system was designed to apply saline water directly onto the turfgrass plants (Koch and Bonos, 2010). Briefly, the system included the use of recirculating salt spray chambers, which applied saltwater solutions to plants using spray nozzles from holding tanks placed beneath the spray chambers. Holding tanks were filled with 95 L of water with half-strength Hoagland’s Solution (Hoagland and Arnon, 1950) and salt solutions. Plastic trays (57x44x15cm) (US Plastics, Lima, OH) used to hold plants and sand growing medium (Sureplay® Topdressing Sand, US Silica, Mauricetown, NJ), were placed on a plastic lattice platform above the holding tanks.

Plant Material and Salt Treatments

A total of 24 Kentucky bluegrass cultivars, Kentucky bluegrass x Texas bluegrass hybrids, and experimental selections were chosen to represent the broad diversity among the species: Midnight, Liberator, and Rhythm represented Compact-Midnight types; Eagleton, Cabernet, Aura, and RSP, represented Mid-Atlantic types; Langara, Bedazzled, Moonshadow, Diva, A00-1400, and Argos represented Compact-America types; Lakeshore and A03-84 represented Shamrock types; Baron represented BVMG types; Bewitched and P-105 represented Compact types; Bandera, A03TB-676, and A03TB-246 represented Texas bluegrass (Poa arachnifera Torr.) hybrid selections; Julia, Jefferson, and Fairfax were also included. Tillers of these cultivars were sampled from established turf plots at the Rutgers Plant Biology Research and Extension Farm in Freehold, NJ.
Kentucky bluegrass is a highly apomictic species, so each individual plant within a cultivar is assumed to be genetically identical. Two individuals with characteristics of the repeating mother type were used to represent a cultivar. These two individual tillers from each cultivar were transplanted into flats and grown for four weeks until each contained approximately 36 tillers. When they were large enough to be vegetatively propagated, plants were divided into 12 individual replicates of equivalent size (approximately 3 tiller each), and planted into Pro-mix growing media (Premier Tech Ltd.; Quebec, Canada) and allowed to grow for an additional 4 weeks in the greenhouse.

After growing in the greenhouse, the plants were removed from the flats, washed free of Pro-mix growing medium, and roots were cut just below the crown. Shoots were cut to 3.8 cm above the crown. One of the twelve replicate plants was randomly planted into a cell of the plastic tray containing Sureplay® topdressing sand. Each tray had 48 cells made with plastic dividers to eliminate contamination of roots of adjacent plants. Holes were drilled in the bottom of each individual cell to allow for irrigation water to pass through the root zone and accumulate back into the reservoir below each spray chamber. Each tray represented a replication and included the two replicate plants of each of the 24 cultivars for a total of 48 plants per tray. There were a total of three replicate trays for each of the four salinity treatments (described below) for a total of 12 trays used in the experiment. Using the spray chambers, the trays of plants were irrigated every other day for one week prior to initiating salinity treatments using half-strength Hoagland’s solution (1950). This was done to allow the plants to begin to establish in the trays before the salt stress was induced.
Salt solution treatments were made, using an aquarium product called Instant Ocean (Spectrum Brands Inc.; Atlanta, GA), to accurately mimic the constituents of seawater. It contained a mixture of the following ions: chloride, sodium, sulfate, magnesium, calcium, potassium, and bromide as well as small quantities of other ions (Koch and Bonos, 2010). The salt was added to the nutrient solution in the holding tanks at the rate of 1 dS m\(^{-1}\) each irrigation day to tap water (and Hoagland’s nutrient solution) until the final concentrations were reached. The final electrical conductivities (EC) of the spray solution in four irrigation chambers were as follows: Treatment 1 (Control): 1 dS m\(^{-1}\) (this treatment included half-strength Hoagland nutrient solution so the EC did not equal 0), Treatment 2: 3 dS m\(^{-1}\), Treatment 3: 6 dS m\(^{-1}\), and Treatment 4: 9 dS m\(^{-1}\). Electrical conductivity of each treatment was measured and adjusted on each irrigation day. Half-strength Hoagland nutrient solution was also replaced weekly to ensure that stress symptoms due to salinity treatments were not confounded with nutrient deficiencies. Irrigation with final salt concentrations continued every other day for the duration of the 10-week study. A total of 4.98 cm of water was applied to the plants on every irrigation day. Prior to each irrigation cycle, pH was also checked and adjusted by adding acid (H\(_2\)SO\(_4\)) or base (NaOH) to maintain a pH equal to 6.5 (Murphy et al., 2004). The experiment took place in a greenhouse with the same specifications reported by Koch and Bonos (2010) with 400 Watt HPS supplemental lighting (Kavita Canada Inc., Niagara-on-the-Lake, ON) providing photosynthetically active radiation (PAR) in the range of 90 to 110 µmol m\(^{-2}\) s\(^{-1}\) as measured with an Apogee Quantum Meter (Apogee Instruments Inc., Logan, UT). Fourteen-hour daylengths were maintained for the duration of both runs of the 10 wk study. Greenhouse temperatures were set to maintain
between 17 and 24°C (Run 1: average temperature = 17.1°C; SD = 0.79, Run 2: average temperature = 17.9°C; SD = 0.71) and monitored using an Onset HOBO Pro Series data logging temperature probe (Bourne, MA). The air exchange rate in the greenhouse was 4 air exchanges per minute. Humidity was not controlled during the course of the experiment.

**Digital Image Analysis**

Digital images were taken of the turfgrass plants to quantify the percentage of each plant that remained green for the duration of the saltwater irrigation treatments. Digital images of the turf canopy were taken using an Olympus C-5500 (Olympus Optical Co.; London, UK) digital camera that was mounted at a 90° angle on a four-legged PVC stand designed specifically for this study. The camera stand was constructed of 2.54-cm-diam PVC tubes. The camera was mounted to the stand at a height of 1 m. Black plastic completely enclosed the stand on all sides to prevent light from entering. The camera’s flash was the only light used when taking the pictures. Images were taken weekly of each tray and were saved in the JPEG (joint photographic experts group, .jpg) format with a resolution of 2592 by 1944 pixels. Digital percent green values were measured for each plant using a software macro (Karcher and Richardson, 2005) written for Sigmascan Pro 5.0 (Systat Software, Inc.; Point Richmond, CA). Relative percent green values for the digital images were then calculated, similar to Koch and Bonos (2010), by comparing the percent green of treated plants against the control plants (described in more detail below). Digital images were taken of plants under the highest
salinity treatments (Treatment 4, EC = 9 dS m\(^{-1}\)) and control plants (Treatment 1, EC = 1 dS m\(^{-1}\)) and then analyzed for percent green.

**Growth Parameters Evaluated**

Visual percent green ratings (0-100%) were taken weekly on all plants to quantify the amount of leaf senescence as a result of the salinity stress. After the ratings were performed, the plants were cut to a height of 3.8 cm. Clippings were collected every 2 weeks, dried in an oven at 65°C for 48 hours, and weighed.

After 10-weeks of salt treatments, the plants were extracted from the trays and the roots were washed completely free of all sand. Plants were then cut into two parts: roots (only root tissue) and shoots (shoots, crowns, and leaf tissue). Once separated, the roots and shoots were dried at 65°C for 48 hours and weighed. Homogenous samples of the sand growing medium were taken from each tray following the 10 week study and were analyzed by the Rutgers Soil Testing Laboratory for soil EC using the 1:2 Soil:Water Extract Method (Dellavalle, 1992).

**Statistical Analysis**

The experiment was arranged as a split plot in time (runs) with three replications; runs were the main plot, treatments were sub plots, and cultivar was considered the sub-sub plots. The 10 week experiment was repeated twice; Run 1 was initiated in February 2007 and Run 2 in December 2007. To account for inherent genetic differences in growth patterns among Kentucky bluegrass cultivars, all measurements were reported as a percentage of the control plants. Percentage of control values were calculated by taking
the actual measured value for each salinity treatment, dividing it by the control value (Treatment 1) for the same measurement, and then multiplying by 100 (Koch and Bonos, 2010). Regression data of the relative measurement (i.e. percent green, percent green as measured with DIA, etc.) was then obtained using Proc REG in SAS Version 9.1 (SAS Institute, 1991). All measurements best fit a linear regression model. Therefore, Days\textsubscript{50} values were reported based on linear regression over time. Days\textsubscript{50} represents the number of days for the relative measurement to reach 50\% of the initial relative value for that measurement (i.e. the number of days for relative percent green values to reach one half of the control value for percent green). This data was then analyzed using Proc ANOVA (Analysis of Variance) in SAS Version 9.1 and cultivar means were separated used Fishers Protected Least Significant Difference. Correlations between relative percent green ratings, clipping yields, root and shoot weights, and digital image analysis were conducted on Days\textsubscript{50} values using Proc CORR in SAS.

**Physiological Measurements**

Leaf relative water content (RWC) and photochemical efficiency were determined in order to quantify physiological responses to salinity stress for two cultivars, Diva and P-105, with contrasting salinity tolerance. The salinity tolerance of these two cultivars was determined based upon the early performance of the individual plants to the salinity treatments. For leaf RWC measurement, leaf clippings above 3.8 cm were removed and weighed (W). Clippings were then put into a de-ionized water bath in a petri dish for 24 hours at 4°C. After waiting the allotted period of time, foliar tissue was removed from the water, dried, and weighed to obtain turgid weights (TW). Samples were then oven-
dried for 72 hours at 80°C (Barrs and Weatherly, 1962) and weighed (DW). RWC is calculated by using the following equation: \[ \text{RWC(\%)} = \left( \frac{W - DW}{TW - DW} \right) \times 100. \] Treatment RWC values were then divided by the control RWC values to obtain treatment plant RWC as a percentage of the control plants. This methodology was performed for both cultivars at the end of the 10-week study for treatments 2-4. Data from two subsamples per replicate from each cultivar were collected for each treatment.

The procedures described in Merewitz et al. (2010) were used to determine leaf photochemical efficiency. Photochemical efficiency was measured as a ratio of the variable fluorescence (Fv) to the maximal fluorescence (Fm). This measurement was determined using a chlorophyll fluorescence meter (Fim 1500; Dynamax, Houston). Thirty minutes prior to taking the measurement, leaf clips were placed on fully expanded leaves of Diva and P-105 and allowed to adapt individual leaves to darkness. As with RWC, photochemical efficiency was conducted at the end of the 10 week study for treatments 2-4 and compared to control (Treatment 1) plants. Within each treatment, two subsamples from each cultivar per replication were used for this measurement.
RESULTS AND DISCUSSION

Analysis of variance indicated that run (p < 0.001), salinity treatment (p < 0.001), cultivar (p < 0.001), and the interaction of cultivar and salinity treatment (p < 0.001) were significant for Days$_{50}$ values for percent green ratings, digital image analysis, clipping weights, shoot weights, and root weights. Replication was not significant for any measurement on any rating date. There were significant differences between runs for all measurements so data is presented for each run individually. The difference in runs could be caused by the fact that the two runs were conducted at different times of the year. Although greenhouse conditions were similar during both runs of the experiment, the discrepancy between runs was mainly attributed to the plants being at different stages of vegetative growth prior to initiation of the experiment. The first run was performed in the late winter into the spring; therefore plants were beginning to actively grow roots and shoots and were exposed to increasing day lengths in the greenhouse prior to the start of the experiment. The second run began in the very early part of the winter season; the plants in this experiment were exposed to shorter day lengths and may have been allocating more carbohydrates to storage organs rather than shoots in preparation for winter dormancy (Turgeon, 2004) when compared to the first run. The difference in timing of the two runs may have resulted in different cultivar responses. However, even though there was a significant interaction between runs, cultivar responses were relatively consistent over both runs where the majority of cultivars that exhibited good salinity tolerance in the first run also performed well in the second run of the study and vice versa.
Salinity irrigation treatments resulted in significant differences in electrical conductivities of the 100% sand growing medium, with higher salinity treatments exhibiting higher soil EC compared to lower salinity treatments, similar to Koch and Bonos (2010). Analysis of the EC of the sand growing medium resulted in the following ranking:

Trt 4 – 9 dS m\(^{-1}\) (1.12 dS m\(^{-1}\) [Run 1] and 1.06 dS m\(^{-1}\) [Run 2]) > Trt 3 – 6 dS m\(^{-1}\) (0.85 dS m\(^{-1}\) [Run 1] and 0.79 dS m\(^{-1}\) [Run 2]) > Trt 2 – 3 dS m\(^{-1}\) (0.42 dS m\(^{-1}\) [Run 1] and 0.39 dS m\(^{-1}\) [Run 2]) > Control, Trt 1 – 1 dS m\(^{-1}\) (0.15 dS m\(^{-1}\) [Run 1] and 0.09 dS m\(^{-1}\) [Run 2]). These soil ECs were relatively low compared to the EC of the salt solution due to the limited cation exchange sites of the 100% sand growing medium. As observed by Koch and Bonos (2010) low soil ECs observed were not considered significant sources of stress to the plants; differences were attributable to the high EC water treatments.

**Growth Parameters**

In all cases, increasing salinity treatments resulted in decreased relative percent green ratings (Table 1), reduced clipping weights (Table 2), shoot weights (Table 3) and root weights (Table 3), and reduced percent green calculated with DIA (Table 4). These results are similar to other salinity experiments which found reductions in growth parameters with increasing salinity levels (Dai et al., 2008; Koch and Bonos, 2010; Lee et al., 2005; Marcum and Pessarakli, 2006; Qian et al., 2004).

Days\(_{50}\) values estimated from regression curves (or the number of days in which it took plants to exhibit a 50% reduction in percent green ratings [from the control], 50% reduction in clipping yield, or 50% reduction in percent green as calculated using DIA)
are reported in Tables 1, 2, and 4 respectively. The data (from 10 weeks or 70 days) best fit a linear regression model, therefore the Days$_{50}$ values were estimated based on the regression of the best fit line resulting in some Days$_{50}$ values (at least for percent green) longer than 70 days. However, we anticipate the model may change beyond 70 days as a plateau is expected when all plants senesce. Data was not collected beyond 70 days so the anticipated change in the regression model cannot be predicted. A future study may include a longer period of stress to determine the extent of individual cultivar responses to salinity stress. However, the fact that some cultivars did not reach a 50% reduction in percent green even after 70 days of salinity stress is an indication that the cultivars or selections should be able to withstand a longer period of time before showing a significant decline in growth due to salinity stress. In most instances, higher salinity treatments coincided with a more negative slope resulting in a steeper decline in Days$_{50}$ values for visual percent green ratings (Table 1), clipping yields (Table 2), and percent green data from DIA (Table 4).

There was a high correlation between all growth parameter measurements (Table 5). Generally, cultivars with high percent green Days$_{50}$ values also had higher clipping yield Days$_{50}$ values, higher shoot and root weights, and higher percent green Days$_{50}$ values from DIA. These results are similar to other studies which associated greater overall growth with improved salinity tolerance (Koch and Bonos, 2010; Nikman and McComb, 2000; Qian et al., 2004). The highest correlation was observed between visual percent green ratings and percent green calculated using DIA (Run 1: $r = 0.94$, $p < 0.001$, Run 2: $r = 0.91$, $p < 0.001$). Days$_{50}$ values for relative percent green ratings ranged from 81 days for Eagleton in Run 2 of Treatment 4 to 24 days for Baron (Table 1). The
percent green calculated from DIA ranged from 60 days for Eagleton to 11 days for Baron (Table 4). Lower percent green values for DIA compared to visual ratings may have been due to presence of the sand growing medium in the background of digital images which was included in the total pixels counted in the picture. Background color can be disregarded with visual percent green ratings where only the color of the verdure is considered in the measurement. Even though there was a slight magnitude difference, only minor differences in cultivar rankings were observed between these two measurements. These results are consistent with previous reports that DIA can be used to quantify salinity stress in turfgrass (Dai et al., 2008). It is beneficial over visual ratings as it eliminates any bias the rater might have, however, it does take significantly longer to collect and analyze the data from DIA compared to visual ratings. For breeding and screening large amounts of germplasm, a quick and efficient screening method needs to be developed. Visual ratings are quick, however, they are subjective and influenced by rater bias (Karcher and Richardson, 2003). Digital Image Analysis may be a useful tool to “screen the rater” to make sure visual ratings are collected accurately.

Days_{50} values of relative percent green ratings estimated from the regression curves are a useful indication of salinity tolerance because it is a measure of how much of the plant remains green when exposed to salt treatments. The amount of the plant that remains green with respect to salinity tolerance is one of the most important factors in overall turf quality and will be the most visible to golfers and turfgrass managers. Other research done on the salinity tolerance of turfgrass has shown that reduced percent green ratings coincided with increased salinity stress in perennial ryegrass (Koch and Bonos,
2010; Pessarakli and Kopec, 2008) and annual bluegrass (*Poa annua* L.) (Dai et al., 2008).

The lowest correlation was observed between clipping weights and percent green ratings calculated using DIA (Run 1: \( r = 0.72 \ p < 0.001 \), Run 2: \( r = 0.69 \ p < 0.001 \)). A similarly lower correlation value was also observed between clipping yields and root weights (Run 1: \( r = 0.73 \ p < 0.001 \), Run 2: \( r = 0.70 \ p < 0.001 \)). These results suggest that plants that remain green and retain color are also maintaining growth of both shoots and roots even under relatively high salinity levels (Koch and Bonos, 2010). Interestingly, clipping yield *Days*\(_{50}\) values, reflective of a reduction in growth, occurred at a faster rate than the reduction in percent green for tolerant cultivars but not susceptible cultivars. For example, tolerant cultivars had 50% reduction in clipping yields after approximately 48-55 days at the highest salinity level but retained green color for more than 60-81 days (Tables 2 and 4). Meanwhile susceptible cultivars had 50% reduction in both clipping yields and percent green in 25-40 days depending on the cultivar.

Significant differences were observed between cultivars for relative visual percent green *Days*\(_{50}\) values (Table 1), clipping yield *Days*\(_{50}\) values (Table 2), shoot and root weights (Table 3), and percent green *Days*\(_{50}\) values using DIA (Table 4). Kentucky bluegrass cultivars (Eagleton, Liberator, Fairfax, and Cabernet) were among the highest ranked cultivars for all growth parameter *Days*\(_{50}\) values and shoot and root weights across all treatments and both runs of the 10-week study (Tables 2-4). A00-1400, Bewitched, A03-84, Baron, and A03-TB246 were among the cultivars and selections with the lowest growth parameter *Days*\(_{50}\) values in all treatments and both runs.
Eagleton and Cabernet are both considered Mid-Atlantic type cultivars (Shortell et al., 2009) and exhibit good tolerance to heat and drought under field conditions. The improved salinity tolerance observed for these cultivars indicates that these cultivars may have a mechanism to tolerate a broad range of environmental (abiotic) stresses. Eagleton is also tolerant of billbug feeding (Shortell et al., 2007). Interestingly, RSP, also a Mid-Atlantic ecotype and the mother of Cabernet (Bonos et al., 2004), did not exhibit the same high salinity tolerance in this study (Tables 1-4). RSP is known to have good summer stress tolerance under field conditions (Bonos et al., 2004) yet Wang et al. (2004) found that it was most likely due to improved heat tolerance and not drought tolerance. Drought and salinity tolerance have been shown to have similar mechanisms (Sairam and Tyagi, 2004) and could be the reason for the poor salinity tolerance observed for RSP. Similarly, Aura, also a new Mid-Atlantic type cultivar with RSP as a mother did not exhibit high levels of salinity tolerance in this study.

The two Midnight-type cultivars, Midnight and Liberator exhibited differences in percent green Days$_{50}$ values in treatment 4 (9 dS m$^{-1}$) of both runs with Liberator remaining greener for 16 days longer in Run 1 and 14 days longer in Run 2. At lower salinity treatments, however, Midnight remained greener longer than Liberator. Additionally, Midnight lasted 7 and 6 days longer than Liberator in Runs 1 and 2 respectively, before a 50% reduction in clipping weights (at the highest salinity treatment) was observed. But Liberator consistently had higher shoot and root weights in all treatments across both runs. Percent green Days$_{50}$ values using DIA support the idea that Liberator has higher salinity tolerance than Midnight which lost 50% green color 9 days earlier than Liberator in Run 1 and 16 days earlier in Run 2. Rhythm (another
Midnight-type cultivar) behaved similarly to Midnight in this experiment and both exhibited above average salinity tolerance albeit lower than Liberator.

The compact-America type cultivars Moonshadow, Diva, and Langara, exhibited very good salinity tolerance. In fact, these three cultivars had the longest Days50 clipping yields (approximately 55 days) compared to all other cultivars. Argos, another compact-America type exhibited moderate salinity tolerance based on the moderate values for all growth parameter estimates (Tables 1-4). Bedazzled, another compact-America type cultivar exhibited moderate to low values for growth parameter estimates including shoot and root weights indicating below average salinity tolerance.

The Texas x Kentucky bluegrass hybrids and selections did not exhibit high salinity tolerance. Bandera and A03-TB676 exhibited moderate to low salinity tolerance, and A03TB-246 exhibited poor salinity tolerance. Poss et al. (2010) also found Texas x Kentucky bluegrass hybrids to exhibit poor salinity tolerance. Suplick-Ploense et al. (2002) found that generally Kentucky bluegrass cultivars exhibited higher salinity tolerance than hybrid (Texas x Kentucky) bluegrass. Thus, the idea of improving heat tolerance by crossing Texas x Kentucky bluegrass (Wilson et al., 2009) may not result in improved salinity tolerance.

P-105 and Julia exhibited moderate to low salinity tolerance in this study. The moderate to low salinity tolerance was substantiated by having Days50 values of 52 and 46 days respectively in Run 1 and 42 days for both cultivars in Run 2 for the visual percent green rating measurement at the highest salinity treatment (Table 1). This trend of moderate to low salinity tolerance was exhibited in the rest of the growth parameters
(Tables 2-4). Qian et al. (2004) also found P-105 to exhibit low salinity tolerance compared to other Kentucky bluegrass cultivars and selections in their study.

The poorest performing cultivars or selections in the trial were A00-1400, Bewitched, A03-84, A03TB-246 and Baron, with Baron exhibiting the least salinity tolerance among all the cultivars and selections evaluated. These cultivars and selections had the shortest Days$_{50}$ values for relative percent green ratings, relative clipping yields, and relative percent green via DIA. They also had the lowest shoot and root weights of all cultivars and selections. Baron has been evaluated for salinity tolerance in a number of studies. Several studies reported Baron as susceptible to salinity stress (Cordukes, 1981; Torello and Symington, 1984) while other studies reported Baron as having high salinity tolerance (Horst and Taylor, 1983; Kinbacher et al., 1981; Poss et al., 2010). These differences between findings may be attributable to differences in screening methods, growing conditions, and age of the germplasm being screened because the growth stage of the plant may affect the tolerance to salinity stress.

Based upon results from this study, it is clear that cultivar response to salinity stress is specific to the cultivar or selection and not the classification grouping it belongs to. Midnight and Liberator both belong to the Compact-Midnight type and also had different salinity tolerance in this study with Liberator exhibiting higher salinity tolerance than Midnight. Bedazzled and Langara belong to the Compact-America type. Langara exhibited higher salinity tolerance than Bedazzled. Within the Mid-Atlantic type, cultivars Eagleton and Cabernet ranked highest among all cultivars for all measurements with respect to the salinity tolerance while Aura and RSP were among the lowest ranked cultivars.
Physiological Measurements

Diva and P-105 were selected for physiological comparisons based on a field screening trial (data not shown) and initial differences in the first weeks of the experiment. After the results of the 10-week study were obtained, it is clear that two cultivars with a wider variation in salinity tolerance could have been chosen for this analysis. For example, Diva had significantly longer Days50 values for clipping yields and for percent green conducted visually and with DIA. But, there were no consistent statistical differences in root weights and shoot weights across treatments. However, significant differences were observed between Diva and P-105 for RWC and photochemical efficiency. Significant differences were observed for RWC between the treatments and between the two cultivars only for the final and most stressful treatment (4) (EC = 9 dS m$^{-1}$) (Figure 1). In treatment 4, Diva had significantly higher RWC compared to P-105 in both runs of the study. In run 1, Diva had significantly higher photochemical efficiency than P-105 only in treatment 4 (EC = 9 dS m$^{-1}$), while in run 2, Diva had higher photochemical efficiency in both treatments 3 (EC = 6 dS m$^{-1}$) and 4. Photochemical efficiency is a measure of the photosynthetic processes within chlorophyll; specifically the efficiency of photosystem II (Zhang et al., 2003). These results indicate that salinity tolerant cultivars, in addition to maintaining relative percent color and clipping yields longer, they are also able to maintain higher relative photochemical efficiency and higher water status within the leaves.
CONCLUSION

The spray chamber design described in this paper proved to be effective in screening Kentucky bluegrasses for salinity tolerance. Relative percent green \( Days_{50} \) values were strongly correlated to relative clipping yield \( Days_{50} \) values, relative shoot and root weights, and percent green \( Days_{50} \) values as measured with DIA indicating that plants that remain green and retain color were also maintaining growth even under relatively high salinity stress levels. Cultivars showing increased salinity tolerance, however, were able to remain green longer when compared to the other growth measurements indicating that salt tolerance may be linked to a plant's ability to continue photosynthetic processes. This observation is further supported by the physiological measurements collected in this study. Cultivars showing increased salinity tolerance by way of percent green ratings also coincided with plants that had higher photochemical efficiency and relative water content measurements. Results indicated that Kentucky bluegrass typing was not indicative of salinity tolerance with cultivars in the same types having differing salinity tolerance levels. This research indicated the need for continued evaluation of germplasm and cultivars of Kentucky bluegrass for salinity tolerance.
LITERATURE CITED


Data was analyzed as a percentage of the control plants that did not receive any additional salt in the irrigation water and regression data was determined using Proc Reg procedure in SAS. Days as (D₅₀) data was subjected to analysis of variance (ANOVA) using SAS. Cultivar means were separated using Fisher's protected least significant difference means separation test.

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| LSDadj         | 10    | 6   | 3   | 1   |

Run 2 was initiated in December 2007.
Table 2. Regression line parameters and Days$_{50}$ values for relative clipping weights of 24 Kentucky bluegrass cultivars and selections exposed to three salinity treatments for 10 weeks under greenhouse conditions.†

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<th>R²</th>
<th>D$_{35}$</th>
<th>Slope</th>
<th>SE</th>
<th>R²</th>
<th>D$_{35}$</th>
<th>Slope</th>
<th>SE</th>
<th>R²</th>
<th>D$_{35}$</th>
<th>Slope</th>
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<th>R²</th>
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†Data was analyzed as a percentage of the control plants that did not receive any additional salt in the irrigation water and regression data was determined using Proc Reg procedure in SAS. Days$_{50}$ (D$_{35}$) data was subjected to analysis of variance (ANOVA) using SAS. Cultivar means were separated using Fisher's protected least significant difference means separation test.

Run 1‡: initiated in February 2007.

Run 2§: initiated in December 2007.
Run 2 was initiated in December 2007.

Root and shoot weights were taken on the final day of the study for both runs. Data was analyzed as a percentage of the control plants that did not receive any additional salt in the irrigation water and data was subjected to analysis of variance (ANOVA) using SAS. Cultivar means were separated using Fisher's protected least significant differences means separation test.

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<th>Root Weights (% of control)</th>
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<td>6 dS m⁻¹</td>
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Table 3. Root and shoot weights of 24 Kentucky bluegrass cultivars and selections exposed to three salinity treatments for 10 weeks under greenhouse conditions.†
Table 4. Regression line parameters and $D_{50}$ values for relative digital Images of 24 Kentucky bluegrass cultivars and selections exposed to 9 dS m$^{-1}$ irrigation water for 10 weeks under greenhouse conditions.†

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†Digital images were taken weekly and analyzed for percentage green using SigmaScan Pro (v. 5.0). Data was analyzed as a percentage of the control plants that did not receive any additional salt in the irrigation water and regression data was determined using Proc Reg procedure in SAS. $D_{50}$ data was subjected to analysis of variance (ANOVA) using SAS. Cultivar means were separated using Fisher's protected least significant difference means separation test.

‡Run 1 was initiated in February 2007.

§Run 2 was initiated in December 2007.
Table 5. Pearson correlation coefficients for growth parameters of Kentucky bluegrass cultivars and selections exposed to salinity stress for 10 weeks under greenhouse conditions.†

<table>
<thead>
<tr>
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<th>Run 1‡</th>
<th>Run 2§</th>
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<td>Shoot Weights</td>
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<tr>
<td>Root Weights</td>
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*** Significant at P < 0.001
†Correlation analysis was run on relative Days₅₀ values (for visual ratings, digital images, and clipping weights) or relative percentages (for root and shoot weights) from plants receiving the highest salt concentration treatments (9 dS m⁻¹). Cultivar means were averaged over both runs of the study.
‡Run 1 was initiated in February 2007.
§Run 2 was initiated in December 2007.
Fig 1. Relative water content of Diva (salt tolerant) and P-105 (salt sensitive) Kentucky bluegrass after 10 weeks of salt treatments at 3, 6, and 9 dS m$^{-1}$. Lower-case letters, ”a” and “b”, denote Run 1 and 2, respectively. Relative water content is presented as a percentage of measured control plant values. Capital letters indicate significant differences between cultivars using Fisher’s protected least significant difference means separation test.
Fig 2. Photochemical efficiency of Diva (salt tolerant) and P-105 (salt sensitive) Kentucky bluegrass after 10 weeks of salt treatments at 3, 6, and 9 dS m\(^{-1}\). Lower-case letter, “a” and “b”, denote Run 1 and 2, respectively. Photochemical efficiency is presented as a percentage of measured control plant values. Capital letters indicate significant differences between cultivars using Fisher’s protected least significant difference means separation test.
Salinity Tolerance of Cool-Season Turfgrass Cultivars Under Field Conditions

Abstract. In order to utilize effluent or wastewater as irrigation on turfgrass sites it will require the identification of cool-season turfgrass cultivars with increased salinity tolerance. Evaluation of current cultivars and experimental selections for salinity tolerance is an important first step in making information available to turfgrass managers. An overhead irrigated field screening method was developed to closely mimic the challenges associated with irrigation of turf with saline water under summer stress conditions. A total 48 clones from each turfgrass cultivar were planted in a randomized complete block design with four replications (12 clones per replication) and were irrigated overhead with saltwater (EC = 10 dS m⁻¹). This technique effectively identified differences in salinity tolerance, of Kentucky bluegrass, bentgrass, and perennial ryegrass cultivars and selections as measured by percent green ratings. The most salt tolerant cultivars included Liberator, Eagleton, Diva and Rhythm Kentucky bluegrasses, Declaration, Kingpin, and 007 creeping bentgrasses, and RKS, Gator 3, and MSH Comp perennial ryegrasses. Cultivars and selections exhibiting the least salinity tolerance were RSP, A03-TB676, A03-84, and Julia Kentucky bluegrasses, EBM Comp and Tiger II colonial bentgrasses, SR7200 velvet bentgrass, and Fiesta III perennial ryegrass.
INTRODUCTION

In arid and semi-arid regions and in highly populated metropolitan areas, where water is a limited natural resource, irrigating with recycled water may be a viable means of coping with shortages and/or the rising cost of potable water (Harivandi, 2000). Turfgrass areas are the perfect environments to use reclaimed or alternative water sources (i.e. effluent water, seawater, and gray water) because turf is not utilized for food. However, recycled/wastewaters can have increased salt levels (Duncan et al., 2000) and their use on cool-season turfgrasses can cause salt stress injury, poor turf quality, and physiological drought. Additionally, the use of ice-melting salts on roadsides can accumulate and increase the electrical conductivity (EC) of soils (Marcum, 1994). Turfgrasses planted on the coast near saltwater can also be impacted through salt spray, salt intrusion in groundwater (Newport, 1997; Todd, 1997), and flooding of the root zones during inclement weather. The identification and subsequent use of salt tolerant turfgrass cultivars would greatly improve the turf quality of these sites and could increase the acceptance and use of effluent water on turfgrasses.

Kentucky bluegrass (*Poa pratensis* L.) and perennial ryegrass (*Lolium perenne* L.) are among the most widely used cool-season turfgrasses for home lawns and athletic fields, and bentgrasses (*Agrostis* spp. L.), the major constituent of close-cut greens, fairways, and tees on golf courses in cooler climates, are not considered salt tolerant (Harivandi et al., 1992) especially compared to many warm-season grasses, such as bermudagrass (*Cynodon dactylon* (L.) Pers.), St. Augustinegrass (*Stenotaphrum secundatum* (Walt.) Kuntze), and Seashore paspalum (*Paspalum vaginatum* Sw.) (Marcum, 2007). Kentucky bluegrass (*Poa pratensis* L.) although a popular cool-season
turfgrass for sports fields, home lawns, sod farms and some high maintenance golf courses, is considered salt-sensitive, only tolerating an electrical conductivity of $< 4$ (1) or $< 3 \text{ dS m}^{-1}$ (Carrow and Duncan, 1998). Rose-Fricker and Wipff (2001) evaluated 64 Kentucky bluegrass cultivars for salinity tolerance, however the study was conducted almost ten years ago, did not include many of the newly developed cultivars that are currently available, and did not include foliar salinity stress.

Although creeping bentgrass (*Agrostis stolonifera* L.) is the most popular bentgrass for golf courses in cool temperate climates, velvet bentgrass (*Agrostis canina* L.) has been shown to be useful for low cut putting greens, and colonial bentgrass (*Agrostis capilaris* L.) has utility as a fairway grass (Bonos, 2008) in cool temperate climates. However, limited research has been performed on bentgrasses for salinity tolerance and there is some discrepancy (Marcum, 1999) as to whether creeping bentgrass is salt sensitive (Harivandi et al., 1992) or moderately tolerant (Horst and Beard, 1977). Research does agree, however, that, in general, creeping bentgrass cultivars have higher salinity tolerance than both colonial and velvet bentgrasses (Marcum, 2007; Marcum, 2000). Since 1967 only one study has been conducted on a significant number of bentgrass cultivars for salinity tolerance (Marcum, 2001). Marcum (2001) found a significant amount of diversity among creeping bentgrass cultivars for salinity tolerance and identified cultivars like Seaside II, selected from germplasm exposed to saltwater, ranked higher for salinity tolerance. However, many of the recently developed cultivars have not been evaluated for salinity tolerance.

Perennial ryegrasses are used for many turf situations but probably most widely utilized on athletic fields and for overseeding southern golf courses. Perennial ryegrass is
considered moderately salinity tolerant able to withstand 6-10 dS m$^{-1}$ (Harivandi et al., 1992). Significant variation was found between cultivars and selections for salinity tolerance (Rose-Fricker and Wipff, 2001), however, this research included a large number of experimental selections and was conducted under greenhouse conditions 10 years ago, so many of the cultivars are no longer in commercial production.

Most of the salinity screening procedures for turfgrasses were developed under greenhouse conditions (Koch and Bonos, 2010; Marcum, 2001; Pessarakli and Kopec, 2009; Pessarakli and Kopec, 2008; Poss et al., 2010; Raymer and Braman, 2004; Robins et al., 2009; Rose-Fricker and Wipff, 2001; Suplick-Ploense et al., 2002). To date, there has been no extensive research published on salinity tolerance of cool-season turfgrass cultivars under field conditions. The identification of current cultivars of perennial ryegrass, Kentucky bluegrass and bentgrasses with improved salinity tolerance in the field would provide turfgrass managers with immediate tools for dealing with salt stress injury.

The objective of this study was to determine the salinity tolerance of Kentucky bluegrass, bentgrass, and perennial ryegrass cultivars for salinity tolerance using a field screening technique.
MATERIALS AND METHODS

Individual clones of perennial ryegrass and Kentucky bluegrass were taken from mature turf plots at the Rutgers Plant Biology and Pathology Research and Extension Farm in Freehold, NJ. Bentgrass clones were taken from established plots at the Rutgers Horticultural Research Farm II in North Brunswick, NJ. A total of 22 Kentucky bluegrass cultivars and selections, 17 bentgrass cultivars and selections, and 23 perennial ryegrass cultivars and selections were evaluated (Table 1). The cultivars chosen for inclusion in this screening experiment were chosen to represent the diversity within each species. Individual clones from each cultivar from the three species were separated into one tiller each. A total of 24 individual clones from each apomictic Kentucky bluegrass cultivar and 48 individual clones from each cross-pollinated perennial ryegrass and bentgrass cultivar were planted into plastic flats filled with Pro-mix growing media (Premier Tech Ltd., Riviere-du-Loup, QC). Plants were allowed to grow for four weeks in the greenhouse and were irrigated daily and fertilized twice to grow the plants to a substantial size before planting them into the field.

Plants were then planted into a mowed spaced-plant trial at the Rutgers Plant Biology Research and Extension Farm in Freehold, NJ on a Freehold sandy loam soil. Perennial ryegrass clones were planted with 30.5 cm spacing between plants, while both Kentucky bluegrass and bentgrass clones were planted with 91.5 cm spacing between plants due to the spreading nature of these species. Six plants from each Kentucky bluegrass cultivar were planted in each replication. Twelve clones from each cultivar of the other two species were planted in each replication. A total of four replications of each cultivar were planted in a randomized complete block design. Each individual clone
of the cross-pollinated species (bentgrasses and perennial ryegrasses) represented a different genotype, so all 48 individual clones from all replicates were averaged to determine a cultivar’s response to salinity stress. Each individual clone within each apomictic Kentucky bluegrass cultivar was identical, so only 24 clones per cultivar were averaged to determine a cultivar’s salinity tolerance.

Plants were fertilized with 98 Kg N Ha$^{-1}$ at planting and fresh water irrigation was applied as necessary during establishment. The ryegrass and bluegrass trials were mowed weekly at five cm, while the bentgrass trial was mowed at 3.8 cm. After establishment, plants were irrigated with a saline solution made from equal parts of NaCl and CaCl$_2$ salts. The irrigation solution was made with an electrical conductivity (EC) of 10 dS m$^{-1}$ and was made in an 1893-liter tank with an agitator inside and a gasoline-powered pump attached. Hoses were connected to the pump and a flow meter (McMaster Carr Elmhurst, IL) was coupled to the end to regulate the flow of the irrigation water. Each plant was then overhead irrigated with 0.5 liters of the solution three times each week.

A total of 35 saltwater applications were applied to the Kentucky bluegrass and perennial ryegrass trials and 30 applications were applied to the bentgrass for each of the two years of the study (2008 and 2010). Four times per year, visual percent green ratings were taken on each individual plant beginning when salt stress was visible and variation was observed between plants. Soil samples were also taken weekly and analyzed for EC at the Rutgers Soil Testing Laboratory using the 1:2 Soil:Water Extract Method (Dellavalle, 1992). Percent green ratings were subjected to analysis of variance using Proc ANOVA in SAS Version 9.1 (SAS Institute, 1991). Each species was analyzed
separately. Replications were combined after determining the lack of a significant replication effect.
RESULTS AND DISCUSSION

Environmental Effects on Salinity Tolerance

There was a significant year effect in the analysis of variance for percent green ratings for all three species. Higher temperatures were observed between June and October in 2010 (31°C) compared to 2008 (28.5°C) and there was less rainfall in 2010 (32.5 cm) compared to 2008 (43.1 cm) (Figure 1). The higher temperatures and limited rainfall in 2010 may have also increased heat and drought stress to the turfgrass plants subsequently causing higher salinity stress reflected in the differences in percent green ratings between years (Figure 1) for all three species (Figures 2-4). When rainfall did occur, the salt leached from the soil and lowered the soil EC as can be observed in Fig. 1. A higher soil EC was maintained for a longer period of time in 2010 compared to 2008 (Figure 1). However, the soil EC in both years reached ≈3 dS m⁻¹ which is a level that would cause salt stress and damage to most cool-season turf species (Carrow and Duncan, 1998) (Figure 1). It is evident from this data that cultivars were not only exposed to salinity stress through the irrigation water but were also exposed to elevated soil ECs, and heat and drought stress conditions typical of what a turfgrass manager would face in their work environment. Interestingly, this field screening technique was found to be highly correlated to other greenhouse screening techniques for salinity tolerance that do not have the confounded heat and drought stress encountered under field conditions (Koch and Bonos, 2011) indicating that this technique should be useful in providing turfgrass managers with the useful data on the salinity tolerance of cool-season turfgrass cultivars. This is the first report of cool-season turfgrass cultivar performance under these combined conditions.
A significant year x cultivar interaction was also observed for all species, however, generally, cultivars ranked similarly between years. Significant differences between cultivars were observed for all three species (details are described below).

**Salinity Tolerance of Kentucky Bluegrass Cultivars**

Significant differences were observed between Kentucky bluegrass cultivars for salinity tolerance using this field screening technique. Liberator, Eagleton, Diva, and Rhythm were among the highest ranked cultivars for salinity tolerance based on percent green ratings in both years of the study (Figure 2). The cultivars Langara, Jefferson, Lakeshore, Bedazzled, P-105, and Bewitched exhibited moderate salinity tolerance. The cultivar Julia and experimental selections, RSP, A03-TB676 and A03-84, exhibited the least salinity tolerance in both years of the study (Figure 2). These results are similar to the results observed under greenhouse conditions (Koch et al., 2011), indicating that consistent results can be obtained by multiple screening techniques. This has also been observed in perennial ryegrass (Koch and Bonos, 2011).

All cultivars exhibited less salinity tolerance in 2010 (under more stressful conditions including heat and drought stress), except for Eagleton, Rhythm and P-105 which exhibited the same response in both years and Midnight which increased in salinity tolerance in 2010. Increased heat tolerance of these cultivars (Murphy et al., 1997; Wang and Huang, 2004) could have influenced the salinity tolerance of these cultivars under higher heat stress in 2010.

Kentucky bluegrasses have been classified into “types” (Bonos et al., 2000; Murphy et al., 2004; Shortell et al., 2009) based on growth and performance
characteristics. Interestingly, based on the results of this study, salinity tolerance of Kentucky bluegrass cultivars does not seem to be indicative of classification types. For example, Liberator, Midnight, and Rhythm are all Midnight type bluegrasses and are categorized by dark green color, good turf quality, and good heat tolerance (Murphy et al., 2004; Shortell et al., 2009). In this study, Liberator and Rhythm exhibited excellent salinity tolerance, however, Midnight exhibited only moderate salinity tolerance. Midnight was also found to have moderate tolerance in other salinity tolerance studies (Koch and Bonos, 2011; Rose-Fricker and Wipff, 2001). Additionally, Cabernet, RSP, Eagleton, and Aura are considered Mid-Atlantic type cultivars that exhibit good summer stress tolerance and deep rooting and rhizomes. Cabernet and Eagleton were among the top performing cultivars for salinity tolerance in both years, while RSP and Aura were two of the most salt sensitive cultivars. So although the classification typing can be used to identify similarities between cultivars for other defined characteristics it is not necessarily true for salinity tolerance. This supports the reason for conducting research on the salinity tolerance of Kentucky bluegrass cultivars.

**Salinity Tolerance of Bentgrass Cultivars**

Significant differences were observed between bentgrass cultivars for salinity tolerance using this field screening technique. Generally, creeping bentgrass cultivars were more tolerant of the salinity stress and higher percent green ratings than both velvet and colonial bentgrass cultivars (Figure 3). This is similar to previous research that concluded that creeping bentgrasses have increased salinity tolerance compared to colonial and velvet bentgrass cultivars (Marcum, 2007; Marcum, 2000). In this study,
velvet bentgrass cultivars exhibited better salinity tolerance than colonial bentgrass with Tiger II exhibiting the most sensitivity to salinity stress of all the bentgrass cultivars evaluated. Higher quality cultivars and selections of both species (Greenwich velvet bentgrass and EBM comp colonial bentgrass) exhibited better tolerance than SR 7200 velvet and Tiger II colonial bentgrass, respectively, indicating that turfgrass breeders may be able to improve the salinity tolerance of these species by selecting for improved overall turf quality and disease resistance. There is an additional need to improve the salinity tolerance of both velvet and colonial bentgrass if they are going to be used on golf courses in conjunction with more salt tolerant creeping bentgrass cultivars in the future.

Creeping bentgrass cultivars Declaration, Kingpin, and 007 were the most salt tolerant cultivars, while Tyee and Penneagle were among the most sensitive consistently for both years of the study. Seaside II, Alpha, Shark and L-93 exhibited moderate salinity tolerance. Most cultivars showed a decline in percent green ratings in 2010 compared to 2008 except Alpha which remained the same and Seaside II, Shark, L-93 and Tyee which had higher percent green ratings in 2010. Penncross creeping bentgrass and Greenwich velvet bentgrass showed the most declines in percent green ratings from 2008 to 2010. The top performing cultivars, Declaration, Kingpin and 007 were recently developed through an aggressive selection program for dollar spot resistance and improved turf quality (Bonos, 2005). This research indicates that the selection for improved disease resistance and turf quality may have also improved the salinity tolerance of these cultivars. Some of these cultivars, such as Declaration have been shown to also have genes associated with heat tolerance (Xu et al., 2007). Thus, the
selection for improved disease resistance may have also been a selection, in advertently, for improved overall stress tolerance. EBM comp colonial bentgrass was selected for improved brown patch resistance and this experimental selection has significantly higher percent green ratings than Tiger II colonial bentgrass which did not undergo the same selection pressure. This research indicates that there are bentgrass cultivars that exhibit improved salinity tolerance over other bentgrass cultivars suggesting that turfgrass managers have options for combating salt stress injury on golf courses.

**Salinity Tolerance of Perennial Ryegrass Cultivars**

This field screening technique was also useful for identifying salinity tolerance in perennial ryegrass cultivars (Figure 4). Similar to the other species, generally, percent green ratings were lower for perennial ryegrass cultivars in 2010 than 2008, with a few exceptions (Figure 4). Top Hat 2 had higher salinity tolerance in 2010 compared to 2008 and cultivars that were more sensitive in 2008 tended to have higher salinity tolerance in 2010 (Monterey II, Zoom, Exacta, Brightstar SLT and Amazing). This difference in salinity tolerance between years under these field conditions may be influenced by a cultivar’s ability to tolerate heat and other environmental conditions. No formal research has been conducted on the heat tolerance of these cultivars so further investigation is needed to support this hypothesis.

There was not as much genetic variation in salinity tolerance between perennial ryegrass cultivars as there was for the bluegrasses and bentgrasses. This is supported by previous research (Humphreys, 1981). Most cultivars exhibited moderate tolerance to salinity stress. But, RKS, Gator 3, and MSH Comp as well as Top Hat 2 (in 2010) all
ranked among the highest for salinity tolerance, while Fiesta III ranked the lowest in both years of the study. It may not be surprising that RKS and MSH Comp are among the top performing entries for this trait because these two experimental selections were developed for increased salinity tolerance using the field screening technique described here. This indicates that the potential exists for improving salinity tolerance in cool-season turfgrasses using this technique. This will be useful for turfgrass managers because it provides a useful method for new cultivar development.
CONCLUSION

This field screening technique proved extremely effective in determining the salinity tolerance of Kentucky bluegrass, bentgrass, and perennial ryegrass cultivars. Significant differences were observed between cultivars within all three species. In general, cultivars exhibited similar salinity tolerances in two different years. Since this was conducted under field conditions, environmental effects such as temperature and drought were present in addition to the added salinity treatments. Although these additional variables may have been responsible for slight changes in cultivar responses between years, there were no dramatic shifts in cultivar response (i.e., a cultivar going from highly tolerant to highly sensitive) indicating this was a good screening method to identify salinity tolerance in cool-season turfgrass cultivars. Additionally, these complex environmental interactions of different abiotic (and biotic) stresses are more realistic of what a turfgrass manager would face in the field. Interestingly, this field screening technique was found to be highly correlated to greenhouse screening techniques for salinity tolerance that do not have the confounded heat and drought stress encountered under field conditions indicating that this technique should be useful in providing turfgrass managers with useful data on the salinity tolerance of cool-season turfgrass cultivars. This research was conducted on individual mowed spaced-plants; however, further research should investigate the salinity tolerance of these cultivars in a mowed turf plot or solid stand on a golf course or athletic field currently in use (being trafficked) and using effluent irrigation water.
LITERATURE CITED


Supleck-Ploense, M.R., Y.L. Qian, and J.C. Read. 2002. Relative NaCl tolerance of


Wang, Z. and B. Huang. 2004. Physiological recovery of Kentucky bluegrass from

Xu, J., J. Tian, F.C. Belanger, and B. Huang. 2007. Identification and characterization of
an expansin gene AsEXP1 associated with heat tolerance in C3 Agrostis grass
Table 1. Bluegrass, bentgrass, and ryegrass cultivars and experimental selections evaluated for salinity tolerance under field conditions.

<table>
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<th>Perennial ryegrass</th>
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' TEXAS bluegrass x Kentucky bluegrass hybrid
Figure 1. Weekly soil electrical conductivity (EC) and precipitation amounts for 2008 (a) and 2010 (b) of a field salinity screening study of Kentucky bluegrass, bentgrass, and perennial ryegrass cultivars conducted in Freehold, NJ. Precipitation quantities are reported as bars with units on the left y-axis and soil EC measurements are reported as lines with units on the right y-axis. EC is measured in dS m⁻¹.
Figure 2. Percent green ratings of Kentucky bluegrass cultivars and selections evaluated for salinity tolerance in 2008 and 2010 under field conditions in Freehold, NJ. Entries with different lower-case letters and different upper-case letters represent significant differences at the 0.05 probability level for 2008 and 2010, respectively, using Fisher’s protected least significant difference means separation test.
Figure 3. Percent green ratings of bentgrass cultivars and selections evaluated for salinity tolerance in 2008 and 2010 under field conditions in Freehold, NJ. Entries with different lower-case letters and different upper-case letters represent significant differences at the 0.05 probability level for 2008 and 2010, respectively, using Fisher’s protected least significant differences means separation test.
Figure 4. Percent green ratings of perennial ryegrass cultivars and selections evaluated for salinity tolerance in 2008 and 2010 under field conditions in Freehold, NJ. Entries with different lower-case letters and different upper-case letters represent significant differences at the 0.05 probability level for 2008 and 2010, respectively, using Fisher’s protected least significant difference means separation test.
CHAPTER 5

Correlation of Three Salinity Tolerance Screening Methods for Cool-Season Turfgrasses

Abstract. The identification of turfgrasses with salinity tolerance will be important for the successful implementation of saline irrigation water use on turfgrass sites. Salinity tolerance in turfgrasses has been evaluated using different techniques including: hydroponic and overhead-irrigation methods. This study compared turfgrass response and efficiency of three different salinity screening methods: hydroponic, an overhead irrigation greenhouse method, and a field screening method. There was a significant correlation between all three methods for percent green ratings and a significant correlation between the two greenhouse techniques for dry clipping weights, dry shoot weights, and dry root weights. A difference in magnitude was observed between methods. The overhead irrigated greenhouse and field methods had lower percent green value ratings than the hydroponic method. However, similar rankings among perennial ryegrass clones were found between methods indicating that numerous methods can be used to screen turfgrass germplasm for salinity tolerance with similar results. The cost, time and available area required and reliability varied depending on the method with the field screening requiring the most area (929 cm$^2$ per plant) and cost ($23.18 per plant) and the hydroponic method requiring the most time (48.3 min per plant). However, these results indicate any of these methods should be sufficient to screen germplasm for salinity tolerance. This information will be useful to plant breeding programs choosing selection methods for germplasm screening.
INTRODUCTION

Breeding salinity tolerance into new turfgrass cultivars is of extreme importance due to increasing pressure from local governments to use wastewater as a replacement for potable irrigation water on turfgrass sites. This pressure typically occurs during times of extreme drought, when potable water needs to be conserved. Wastewater, including effluent water, may have increased salt levels (Duncan et al., 2000) which can cause salt stress injury, poor turf quality, and physiological drought on many currently available cool-season turfgrasses. Ice-melting salts (Marcum, 1994), salt spray, salt intrusion in groundwater (Newport, 1997; Todd, 1974), and flooding of the root zones on turfgrass sites planted near saltwater may also accumulate and cause increased electrical conductivity of soils. The turf quality of these areas would greatly increase from the use of salt tolerant turfgrass cultivars. Additionally, if salt tolerant turfgrass cultivars were used it may expand the use of wastewater as irrigation and reduce maintenance costs of turfgrass areas forced to use low quality irrigation water. Costs can also be further reduced because treated wastewater is typically less expensive than potable water (Cuthbert and Hajnosz, 1999).

Breeding turfgrasses for increased salinity tolerance requires an efficient screening method. First, the method must allow for the ability to quickly screen large numbers of plants. Second, cost and labor requirements must also be considered. Finally, the method must be highly reliable and repeatable. Many techniques for screening turfgrasses for salinity tolerance have been developed to identify germplasm that will be useful in breeding new cultivars with increased tolerance. One of the most popular methods for salinity screening is growing turfgrass plants in a hydroponic saline
solution (Dai et al., 2009; Lee et al., 2004; Marcum, 2000; Marcum and Kopec, 1997; Marcum and Murdoch, 1994; Pessarakli and Kopec, 2009; Pessarakli and Kopec, 2008; Qian et al., 2000; Qian et al., 2001; Rose-Fricker and Wipff, 2001; Suplick-Ploense et al., 2002). However, with this method only the roots are exposed to the saline solution and it does not include foliar applications of saline water. To address this issue, salinity screening methods using overhead-irrigation were developed, in the field and greenhouse, to more accurately mimic the effects that turf managers would experience in field situations when using wastewater irrigation (Dai et al., 2008; Koch and Bonos, 2010; Walworth et al., 2009; Wu et al., 1999). However, it is not known whether data acquired from the use of overhead screening methods is correlated to more prevalently used methods.

The objectives of this study were to 1) correlate three different turfgrass salinity screening methods to effectively screen germplasm and 2) compare the efficiency of each method for potential use in a breeding program.
MATERIALS AND METHODS

Overhead-Irrigated Greenhouse Method

The first method utilized an established overhead irrigation spray chamber system in the greenhouse (Koch and Bonos, 2010). In summary, the system included the use of recirculating salt spray chambers to apply overhead irrigation water to the plants. Eight individual clones from each of five perennial ryegrass cultivars including: Nui, Brightstar SLT, Paragon GLR, Applaud, and Palmer III were taken from mature turfgrass plots at the Rutgers Plant Biology Research and Extension Farm in Freehold, NJ for a total of 40 clones. The individual clones were separated into 12 replicates of equivalent size (approximately 3-4 tillers), planted into Pro-mix growing media (Premier Tech Ltd., Quebec, Canada) and grown for four weeks in the greenhouse. Plants were then washed free of the growing media, roots were cut to 5.1 cm, and shoots were cut to 3.8 cm. One of the twelve replicate plants from each clone was randomly planted into a cell of a divided plastic tray containing Sureplay® topdressing sand from US Silica (Mauricetown, NJ). Each tray was considered a replicate. The plants were arranged in a randomized complete block design with three replications for each salinity treatment (described below). Plants were irrigated with half-strength Hoagland’s solution (Hoagland and Arnon, 1950) for one week to allow the plants to acclimate before treatments began. Instant Ocean aquarium salt (Spectrum Brands Inc., Atlanta, GA) was then added to the solutions at the rate of 1 dS m\(^{-1}\) each day until the final concentrations were achieved. The salinity treatments were as follows: Treatment 1 (Control): 1 dS m\(^{-1}\), Treatment 2: 5 dS m\(^{-1}\), Treatment 3: 10 dS m\(^{-1}\), and Treatment 4: 15 dS m\(^{-1}\). All four solutions contained half-strength Hoagland’s Solution, so the electrical conductivity (EC)
of the control solution did not equal zero. EC and pH of each treatment was measured and adjusted on each irrigation day. Irrigation with salt solutions continued every other day for 10 weeks. The first run of the experiment was performed in the spring of 2006 and the second in the fall of 2006.

Plant measurements included visual percent green ratings taken weekly, clipping yields taken biweekly, and shoot and root weights which were taken at the conclusions of the 10-week study. Visual percent green ratings were a measure of the percentage of each plant that remained green in spite of the salinity stress. Clipping yields were measured by removing shoot growth above the 3.8 cm cutting height, drying the clippings for 48 hours at 65°C, and weighing them. Finally, plants were removed from the trays, washed free of sand, and separated at the crown. Shoot weights were calculated by drying and weighing the crowns, shoots, and remaining leaf tissue while root weights were measured by drying and weighing only the root tissue. Also at the conclusion of the study, representative soil samples were taken from each tray and soil EC was determined by the Rutgers Soil Testing Laboratory (New Brunswick, NJ) using the 1:2 Soil:Water Extract Method (Dellavalle, 1992).

The experiment was performed in a greenhouse with 400 Watt High Pressure Sodium (HPS) supplemental lighting (Kavita Canada Inc., Niagara-on-the-Lake, ON) that provided photosynthetic active radiation (PAR) in the range of 90 to 110 µmol m⁻² s⁻¹ as measured with an Apogee Quantum Meter (Apogee Instruments Inc., Logan, UT). Daylengths were maintained at 14 hours for both runs of the study. Greenhouse temperatures were set between 17 and 24°C (average temperature = 17.5°C; SD = 0.74) and were monitored with an Onset HOBO Pro Series data logging temperature probe
(Bourne, MA). Greenhouse fans provided 4 air exchanges per minute. Humidity was not controlled during the course of the experiment.

**Hydroponic Greenhouse Method**

The second salinity screening technique evaluated was a greenhouse hydroponic method. This method followed previous hydroponic studies that have been published in the literature (Dai et al., 2009; Lee et al., 2004; Marcum, 2000; Marcum and Kopec, 1997; Marcum and Murdoch, 1994; Pessarkli and Kopec, 2009; Pessarakli and Kopec, 2008; Qian et al., 2000; Qian et al., 2001; Rose-Fricker and Wipff, 2001; Suplick-Ploense et al., 2002) with some modifications. A total of four salt-tolerant perennial ryegrass clones (three from Palmer III and one from Applaud) and four salt-sensitive clones (three from Brightstar SLT and one from Paragon GLR) (for a total of eight clones), were chosen for this experiment based on their salinity tolerance in a previous study (Koch and Bonos, 2010). These eight clones were a subset of the 40 clones that were included in the first screening method. Plant preparation, prior to the start of salinity treatments, was the same as described for the previous technique. After the roots and shoots were cut to size (5.1 and 3.8 cm, respectively), plants were inserted into a sponge, which was then randomly inserted into a hole in a foam board (Dow Chemical Co., Midland, MI). Three replications of the eight individual clones were inserted into each of four foam boards; one for each salinity treatment.

The boards containing the plants were then placed on top of 30 cm tall opaque plastic tanks (US Plastics, Lima, OH) containing half-strength Hoagland’s solution (Hoagland and Arnon, 1950) for one week. The same four salt treatments as described
for the previous method were used: Treatment 1 (Control): 1 dS m\(^{-1}\), Treatment 2: 5 dS m\(^{-1}\), Treatment 3: 10 dS m\(^{-1}\), and Treatment 4: 15 dS m\(^{-1}\). The solutions were changed weekly to prevent nutrients from becoming depleted. The salinity and pH was also checked and adjusted daily by adding or diluting salt solutions and adding acid (H\(_2\)SO\(_4\)) or base (NaOH) to maintain a pH equal to 6.5 (Murphy and Mohr, 2002). The study was conducted for 10 weeks and the entire experiment was repeated twice in the same greenhouse using the same settings as the other greenhouse method. The first run was performed in the spring of 2008 while the second was performed in the spring of 2009. The same measurements were collected as described above: percent green ratings, clipping weights, shoot weights, and root weights.

**Overhead-Irrigated Field Method**

The third screening method evaluated in this study was performed under field conditions at the Rutgers Plant Biology and Pathology Research and Extension Farm in Freehold, NJ. Similar to the first method, the same 40 perennial ryegrass clones (8 clones of each of 5 cultivars [Nui, Brightstar SLT, Paragon GLR, Applaud, Palmer III]) were used in this field experiment. The clones were separated into three replicates and established in the greenhouse for four weeks. The plants were planted into the field (Freehold sandy loam soil with 2.1% organic matter) on 30.5 cm spacing, and allowed to establish for an additional four weeks in the field before treatments began. Fertilizer was applied to the clones (98 Kg N Ha\(^{-1}\)) at planting and fresh water irrigation was applied as necessary during establishment. Perennial ryegrass clones were mowed weekly at five cm. After establishment, plants were irrigated three times each week with a saline
solution made from equal parts of NaCl and CaCl$_2$. The concentration of the solution was equal to 10 dS m$^{-1}$ and was made in an 1893 liter tank with a gasoline powered pump attached (Hights Farm Equipment Co., Monroe Twp., NJ). Using a digital flow meter (Mcmaster Carr, Elmhurst, IL) attached to a hose from the pump, 0.5 liters of the salt solution from the tank was applied over the top of each plant. A total of 35 applications were applied onto the plants for each of the two years of this study. Weekly soil samples were taken and analyzed by the Rutgers Soil Testing Laboratory (New Brunswick, NJ) using the same method as the first technique. The first run of this study was performed in the summer of 2008 and the second in the summer of 2010. Four times per year, visual percent green ratings were taken on each individual beginning when salt stress appeared and differences were observed between clones.

**Statistical Analysis**

Percent green ratings were subjected to analysis of variance (ANOVA) using PROC ANOVA (fixed effect model) in SAS version 9.1 (SAS Institute, 1991). Correlation analysis between the three methods was run on data from the highest salinity levels for each of the methods (EC = 15 dS m$^{-1}$ for overhead-irrigated greenhouse and hydroponic methods and EC = 10 dS m$^{-1}$ for the overhead-irrigated field method) using PROC CORR in SAS version 9.1 (SAS Institute, 1991). Only the final rating date was used for the correlation analysis due to the fact that the field technique did not have weekly data. The highest salinity level and final rating date were also chosen because these conditions resulted in the largest differences in salinity tolerance between the perennial ryegrass clones. Correlation analysis for percent green ratings was analyzed on
the eight common clones between all three methods and the forty common clones
between the overhead-irrigated greenhouse and field methods using PROC CORR in
SAS. A correlation analysis was also run for dried clipping weights, shoot weights, and
root weights from the first two methods. An estimation of the efficiency of the methods
was estimated on a per plant basis including all of the inputs required to conduct the
screening procedures including area, costs, and time. Area requirement estimates
included spacing between plants in the field and the greenhouse as well as the area
required for irrigation equipment (i.e. spray chambers). Monetary costs were calculated
based upon the price of all materials required to perform the studies including spray
chambers, pump tanks, hydroponic tanks, space rental fees, and salts. The price of labor,
however, was not included in this calculation, but could be determined by multiplying the
time required per plant by the hourly labor wage. Time inputs were measured to include
system construction, plant preparation, plant maintenance, irrigation, and data collection.
RESULTS AND DISCUSSION

Correlation of Three Salinity Screening Methods

Based on the ANOVA, method and clone were significant sources of variation (Table 1). These results indicated that there were significant differences between methods. The main difference was observed between the hydroponic method compared to the other two methods (Figure 1), with the hydroponic method having significantly higher percent green ratings than the overhead irrigated greenhouse and field methods. The two overhead screening methods were not significantly different from each other (Figure 1). The hydroponic method may have had higher percent green ratings (i.e. less stress) because the salt solutions were not applied to the foliage compared to the other two methods which resulted in more damage and lower percent green ratings most likely from foliar exposure to high EC irrigation water. Harivandi (2004) stated that irrigation water with increased salts may cause serious turf damage when applied overhead due to the fact that sodium can be absorbed directly through foliar tissue and cause toxicity. Other research has indicated stress associated with foliar applications of irrigation water with increased salinity in other crop species (including alfalfa and citrus) (Westcot and Ayers, 1984). Despite the magnitude differences between the methods, all techniques proved to be effective in significantly discriminating between perennial ryegrass clones (Table 1) and the eight perennial ryegrass clones exhibited similar salinity tolerance in each of the three methods as indicated by the lack of clone x method interaction (Table 1). Nelson (2008) also found that there was a significant correlation between results from greenhouse studies compared to field studies with respect to salinity tolerance of turfgrasses. These results indicate that any of these three methods can be used to screen
turfgrasses for salinity tolerance and results should be similar regardless of the method chosen. This is important because it indicates that multiple methods can be used by different breeding programs to screen turfgrasses for salinity tolerance with similar results. This could hasten cultivar development.

The highest correlation for percent green ratings was observed between the two overhead-irrigated methods (greenhouse and field) with a correlation coefficient of 0.93 \( (p = 0.0009) \) most likely because both techniques included overhead irrigation resulting in foliar stress. The overhead-irrigated field technique compared to the hydroponic method resulted in the next highest correlation coefficient of 0.82 \( (p = 0.0128) \). The overhead field method had a higher percent green value (more moderate) than the overhead irrigated greenhouse technique albeit not significant (Figure 1). This could be due to the lower concentration of the irrigation water used in the field method \( (EC = 10 \text{ dS m}^{-1}) \), which may have been less stressful to the plants than using a higher concentration. The buffering capacity of soil or other soil related effects may also have created a less stressful condition. The coefficient of 0.82 for this correlation is higher than expected since the field method included other environmental stresses such as heat which can influence salinity stress. This is remarkable considering the fact that many other traits evaluated under greenhouse conditions, including disease evaluations, have not been found to be highly correlated to field performance (Bonos et al., 2006). These results are important for breeding programs because it indicates that germplasm can be screened in the greenhouse with similar anticipated field performance which could save time, space and resources.
The least correlated methods were the overhead-irrigated greenhouse method and the hydroponic method with a correlation coefficient of 0.72 (p = 0.0436). Although still significantly correlated, these two methods had the largest difference in percent green ratings (Figure 1) which was most likely due to differences in how saline solutions were applied, as stated previously. Wu et al. (1999) also found that saltwater applications applied overhead appear to be more damaging to plants when compared to irrigation of only the roots with saltwater of the same concentration.

Only eight perennial ryegrass clones were available to correlate across all three methods, however, another correlation analysis was conducted with the forty perennial ryegrass clones in common between the overhead irrigated greenhouse method and overhead irrigated field method. The correlation was very similar ($r^2 = 0.90; p = 0.0131$ ) indicating the values obtained with the eight clones were close to what might be expected if more plants were used for the correlation analysis.

A correlation analysis was also run on dried clipping weights, shoot weights, and root weights for the overhead-irrigated greenhouse and the hydroponic methods. The two methods were significantly correlated for clipping weights ($r^2 = 0.76; p = 0.0281$), shoot weights ($r^2 = 0.70; p = 0.0469$) and root weights ($r^2 = 0.79; p = 0.0302$). The significant correlation between the overhead-irrigated greenhouse technique and the hydroponic method for clipping, shoot and root weights indicates that these methods may be used interchangeably to screen turfgrasses for salinity tolerance with consistency.

**Efficiency of the Overhead-Irrigated Greenhouse Method**
Space, time, and cost were the main factors for determining the efficiency of each salinity screening method (Table 2). The overhead-irrigated greenhouse method required 104.5 cm$^2$ per plant. Plants could be planted close together because plants were separated by plastic dividers within each of the sand-filled trays. In fact, the only real limitation for space with this method would be the size of the greenhouse. Initial costs were relatively high for this method at $18.28 per plant (Table 2). Each chamber cost approximately $500 to construct. However, after the chambers are constructed they can be used over again to screen larger numbers of plants. Therefore, the more plants you screen the lower the cost. To date, about 3,500 plants have been screened using this technique which would bring costs down to $1.06 per plant. This was the quickest method to screen germplasm requiring only 14.63 minutes per plant (Table 2). Since this screening method was performed in the greenhouse, the reliability of this method is very high because the environment is controlled (Table 2). Since the plants were planted in sand trays in the greenhouse, the plants are easily accessible for additional measurements including root measurements, which are difficult to do in field studies (Table 2).

The highly controlled environment allowed for salts to accumulate in the root zone consistently. Final ECs of 1.41 dS m$^{-1}$ in run 1 and 1.61 dS m$^{-1}$ in run 2 were obtained. These soil ECs were relatively low compared to the EC of the salt solution due to the limited cation exchange sites of the 100% sand growing medium. As observed by Koch and Bonos (2010), low soil ECs observed were not considered significant sources of stress to the plants; differences in salinity tolerance were attributable to the high EC water treatments.
Efficiency of the Hydroponic Greenhouse Method

The area requirement of the hydroponic technique was similar to the overhead-irrigated method with 104.5 cm$^2$ needed per plant with only the limitation being the size of the greenhouse (Table 2). Contrary to the other greenhouse method, the hydroponic technique does not require expensive initial construction costs which made it the least expensive (of the three methods) costing only $9.43 per plant (Table 2). However, monitoring and adjusting the pH and salinity levels required a significant time commitment. Additionally, the solutions needed to be changed weekly with this technique. Therefore, this method required the most time (of the three methods) at 48.26 minutes per plant (Table 2). This time requirement could be greatly reduced with the addition of automated nutrition and pH control; however, this is an extremely expensive addition. If this is the chosen method to screen turfgrasses for salinity tolerance, investing in automated control of these factors may be beneficial to prevent the time requirements from becoming restrictive. The reliability and precision of this method is very high, like the other greenhouse technique, because the study is performed in the greenhouse, the environment is controlled and plants are easily accessible for additional measurements (Table 2).

Efficiency of the Overhead-Irrigated Field Method

The area requirement for this technique is the largest of all the methods (929 cm$^2$ per plant) because the plants are not confined to a limited space and need to be separated by space to prevent plants from growing together (Table 2). However, field space tends to be less limiting than greenhouse space so the opportunity exists to screen the largest
number of plants compared to the other two methods. This method was the most expensive on a per plant basis because of the initial cost of purchasing the pump tank at approximately $5,000. Additionally, salt solutions are not re-circulated with this method so the salts needed to be replenished. This brought the cost to approximately $23.18 per plant. As with the overhead-irrigated greenhouse method, costs could be reduced with further screening of germplasm. To date, the authors have evaluated more than 30,000 plants with this method which reduced the cost down to $0.45 per plant. The time requirement for this method was in between the other two methods; however, if adequate weed control is not available, the time may be increased. This method is subject to changing environmental conditions, such as rain events, which could prolong the time it would take for stress to become evident. For example, large amounts of rain can leach the salts from the soil and decrease the soil EC. This occurred in the summer of 2009 when the trial received large quantities of rain with each rain event, resulting in a lack of observable salinity stress. For example, in September of 2008, soil salinity was reduced from 2.98 to 1.80 following a 4.5 cm rain event. Salt solution applications were continued after rain events to prevent plant recovery. The growing seasons of 2008 and 2010 had total rainfall of 43.1 and 32.5 cm respectively. However, despite rain events, periods without rainfall did occur and high levels of soil salinity were achieved by the end of both runs of the field study (Run 1 = 2.89dS m⁻¹; Run 2 = 2.95dS m⁻¹). This soil salinity concentration is at a level that would cause salt stress and damage to most cool-season turf species (Carrow and Duncan, 1998). Since this technique is carried out in the field, the reliability and therefore consistency is lower than the other two methods (Table 2). Although, this method most simulates the true turfgrass environment which includes
other environmental stresses (i.e. heat, drought etc.) it was still highly correlated to the greenhouse techniques which did not include these stresses indicating the greenhouse screening may be used to predict mature turfgrass field performance.
CONCLUSIONS

All three of the described techniques were significantly correlated to one another and effective in differentiating between perennial ryegrass clones for salinity tolerance. The hydroponic method did not cause as much stress as the overhead irrigated greenhouse or field methods which may be due to the lack of foliar stress with the hydroponic method. The overhead irrigated and hydroponic greenhouse methods both required similar amounts of space to conduct the experiment while the field method required a much larger area. Due to initial costs, the overhead irrigated greenhouse and field methods had higher per plant costs, but with further screening of germplasm, these costs could be reduced. The reliability of the field method was lower than the two greenhouse methods because of changing environmental conditions in the field; however the field method was designed to most accurately mimic a true turfgrass growing environment. All three of the methods described were useful for screening turfgrasses for salinity tolerance. The chosen method will depend on the time, cost, area available, and the amount or type of data collected. Due to the high correlation between techniques, it can be assumed that whichever method is chosen it should produce similar results when compared to other salinity screening methods being performed by other researchers. This knowledge could be used to help coordinate breeding efforts in different locations and may result in faster cultivar development.
LITERATURE CITED


Table 1. Analysis of variance of percent green values for three turfgrass salinity screening methods.

<table>
<thead>
<tr>
<th></th>
<th>% Green Ratings</th>
<th>(p values)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Rep</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Method</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td>Method x Year</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Clone</td>
<td>***</td>
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<tr>
<td>Clone x Year</td>
<td>NS</td>
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</tr>
<tr>
<td>Method x Clone</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Method x Clone x Year</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

NS - Not Significant
*** - Significant at the 0.001 probability level

2Salinity treatments for each method used in the correlation were: EC = 15 dS m⁻¹ for Overhead-Irrigated Greenhouse and Hydroponic methods and EC = 10 dS m⁻¹ for the Overhead-Irrigated Field method.
Table 2. Efficiency estimates of three turfgrass salinity screening methods based on area requirement, cost, time, and reliability. These estimates are calculated based upon inputs to perform this correlation study.

<table>
<thead>
<tr>
<th>Method</th>
<th>Area / Plant</th>
<th>Price / Plant</th>
<th>Time / Plant</th>
<th>Reliability and Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overhead-Irrigated Greenhouse</td>
<td>104.5 cm²</td>
<td>$18.28</td>
<td>14.63 minutes</td>
<td>High</td>
</tr>
<tr>
<td>Hydroponic Greenhouse</td>
<td>104.5 cm²</td>
<td>$9.43</td>
<td>48.26 minutes</td>
<td>High</td>
</tr>
<tr>
<td>Overhead-Irrigated Field</td>
<td>929 cm²</td>
<td>$23.18</td>
<td>20.11 minutes</td>
<td>Low</td>
</tr>
</tbody>
</table>
Figure 1. Mean percent green values of three turfgrass salinity screening methods.

Salinity treatments for each method used in the correlation were: EC = 15 dS m\(^{-1}\) for overhead-irrigated greenhouse and hydroponic methods, and EC = 10 dS m\(^{-1}\) for the overhead-irrigated field method. Letters indicate significant differences at the 0.05 level.
CHAPTER 6

Inheritance of Salinity Tolerance in Perennial Ryegrass

Abstract. An increasing amount of reclaimed water is being used in turfgrass management; however, reclaimed water is often high in total dissolved salts and can result in salt stress injury and poor turf quality. The objectives of this study were to 1) determine the broad-sense heritability estimate of salinity tolerance of 142 perennial ryegrass genotypes; 2) determine narrow-sense heritability estimates and evaluate inheritance characteristics of salinity tolerance (heterosis, maternal effects, and combining ability) of progeny from crosses between fixed resistant and susceptible clones. Six replicates of each clone were irrigated overhead with a salt solution and were rated visually for percent green. The broad-sense heritability estimate for salinity tolerance was 0.78 indicating that the majority of the phenotypic variation was due to genetic effects. A diallel cross between three salt tolerant and three salt sensitive parents was performed in 2006. Parents and progeny were established, irrigated overhead with a salt solution, and visually rated for percent green in 2007 and 2008. Narrow-sense heritability ranged from 0.72 (2007) to 0.66 (2008) indicating that the majority of the phenotypic variation was due to additive gene effects. No heterosis or maternal effects were found and General Combining Ability (GCA) accounted for more variance than Specific Combining Ability (SCA). These results support the finding that salinity tolerance in perennial ryegrass is strongly controlled by additive gene effects. These data
support a recurrent selection program concentrating additive alleles and should be an effective breeding strategy to improve salinity tolerance in perennial ryegrass.
INTRODUCTION

Water conservation is a necessary and responsible practice especially in high-water-using urban landscapes and golf courses. Decreasing potable water supplies are driving the use of reclaimed irrigation sources (Dean et al., 1996; Hayes et al., 1990; Shannon and Grieve, 1999). Turfgrass areas are the perfect environments to use reclaimed or alternative water sources (i.e. effluent water, seawater and/or gray water) because turf is not utilized for food. Using effluent water on turfgrass would reduce the demand for high quality potable water for irrigation. However, these sources are often high in total soluble salts and can result in increased soil salinity and salt stress injury leading to poor turf quality (Qian and Meccham, 2005).

Perennial ryegrass (*Lolium perenne* L.) is an important cool-season turfgrass species as it can be used in many different turfgrass situations from home lawns to highly manicured sports fields. This species is known for its fast germination and establishment which makes it exceptionally suited for the establishment and repairing of sports fields (Murphy and Park, 2004). Newer ryegrass varieties have been developed to provide a playable surface that is wear tolerant, disease resistant, and attractive. With the increasing use of saline wastewater as irrigation on turf sites and the already abundant use of perennial ryegrasses, there is an increasing necessity for new perennial ryegrass varieties with increased salinity tolerance. Perennial ryegrass is also used in conjunction with warm-season grasses as an overseeding companion grass during the winter season (Horgan and Yelverton, 2001). When warm-season grasses go dormant, perennial ryegrass is typically seeded into the dormant grass to provide a green and playable surface. Golf courses using wastewater irrigation as the sole irrigation source will need
perennial ryegrass cultivars with increased salinity tolerance to use in conjunction with warm-season grasses since many warm-season grasses are able to withstand higher salt concentrations compared to cool-season turf species (Marcum, 2008b).

One of the first steps in developing new turfgrass cultivars with salinity tolerance is to determine the inheritance of the trait in order to optimize selection procedures. Heritability estimates are used by plant breeders as a prediction of the expected improvements due to selection (Nyquist, 1991). Broad-sense heritability is an estimate of the total genetic (additive, dominance, and epistatic) variance contributing to the observed phenotype (Dudley and Moll, 1969) which indicates to what extent the phenotype is determined by the genotype as opposed to the environment (Nyquist, 1991). Broad-sense heritability in turfgrasses was first described by Burton and Devane (1953) where tall fescue (*Festuca arundinacea* Schreb.) clones were used to estimate the broad-sense heritability of a variety of plant characteristics on a single-plant and entry mean basis. Estimation of broad-sense heritability has been used in turfgrasses to effectively quantify salinity tolerance in zoysiagrass (*Zoysia* sp.) (Qian et al., 2000), red fescue (*Festuca rubra* L.), creeping bentgrass, common velvetgrass (*Holcus lanatus* L.), and orchard grass (*Dactylis glomerata* L.) (Ashraf et al., 1986). In additions to turfgrassm broad-sense heritability has also been estimated for the salinity tolerance in other plant species including rice (*Oryza sativa* L.) (Gregorio and Senadhira, 1993) and wheat (*Triticum aestivum* L.) (Ashraf, 1994).

Broad-sense heritability estimates, although useful to determine how much variation is due to genetic effects, are not as useful for breeding purposes as narrow-sense heritability estimates. The inheritance of dominance and epistatic genetic effects, which
are included in the broad-sense heritability estimate, cannot be accurately predicted to be transferred to the progeny in an out-crossing species such as perennial ryegrass. Narrow-sense heritability estimates, however, measure the portion of additive gene effects compared to the total observed variation (Nyquist, 1991). Narrow-sense heritability estimates can help to identify the selection technique that will prove to be the most efficient. Narrow-sense heritability estimates have also been used to determine genetics effects of the salinity tolerance trait in four forage species including forage rape (Brassica napus L.), berseem clover (Trifolium alexandrinum L.), alfalfa (Medicago sativa L.), and red clover (Trifolium pretense L.) (Ashraf et al., 1987). Narrow-sense heritability estimates of the salinity tolerance trait have also been studied in rice (Gregorio and Senadhira, 1993) and wheat (Ashan et al., 1996), however has not been extensively investigated in grasses.

In addition to narrow-sense heritability estimates, the data obtained from diallel crosses can also be used to estimate general and specific combining abilities (GCA and SCA) by following the statistical methods presented by Griffing (1956). The estimates of combining abilities can be used to define the relative impact of the additive vs. non-additive gene effects that are contributing to the trait. Combining ability estimates are also useful in determining the potential for specific parents to contribute resistance or susceptibility to a breeding program (Bokmeyer et al., 2009a; Lonqquist, 1950; Venkatraman et al., 2007). Gregorio and Senadhira (1993) studied the salinity tolerance trait in rice by using a diallel cross to calculate combining abilities of the parental lines by measuring the Na-K ion ratio.
The objectives of this study were to 1) determine the broad-sense heritability estimate of salinity tolerance of 142 perennial ryegrass genotypes; 2) determine narrow-sense heritability estimates and evaluate inheritance characteristics of salinity tolerance (heterosis, maternal effects, and combining ability) of progeny from crosses between fixed resistant and susceptible clones.
MATERIALS AND METHODS

Field Trials

*Field trial for broad-sense heritability.* In the late winter of 2005, one hundred and forty-two perennial ryegrass genotypes were randomly selected from experimental germplasm growing at the Rutgers Plant Biology and Pathology Research and Extension Farm in Freehold, NJ. Individual clones were planted into a single cell (5.1 x 5.1 cm) of a plastic flat containing Pro-mix growing media (Premier Tech Ltd., Riviere-du-Loup, QC). These plants were grown in a greenhouse for four weeks with daily irrigation and biweekly 10-10-10 (N-P-K) fertilization. Each individual clone was then separated into 13 vegetative replicates and allowed to grow for an additional four weeks under greenhouse conditions with the same irrigation and fertilization regiment.

The clones were planted into the field (Freehold sandy loam soil) on 30.5 cm spacing, and allowed to establish for an additional four weeks in the field before treatments began. Fertilization with 10-10-10 (N-P-K) fertilizer was applied to the clones (98 Kg N Ha⁻¹) at planting and fresh water irrigation was applied as necessary during establishment. Perennial ryegrass clones were mowed weekly at 5 cm for the duration of the study. The field was planted in a randomized complete block design with 12 blocks where each block contained the 142 individuals and represented a replication. After establishment, six blocks were irrigated with fresh water and considered the control plants, while the other six blocks were irrigated with a saline solution made from equal parts of NaCl and CaCl₂. The concentration of the solution was equal to 10 dS m⁻¹ and was made in an 1893 liter tank with a gasoline powered pump attached (Hights Farm Equipment Co., Monroe Twp., NJ). Both control and saline blocks were irrigated with
0.5 liters of the salt solution or fresh water from the tank using a digital flow meter (Mcmaster Carr, Elmhurst, IL) attached to a hose from the pump, applied over the top of each plant three times per week. A total of 35 applications were applied to the plants for each of the two years of this study. The entire study was repeated for two years with the plants being replanted the second year of the study using the same methodology described above. The last clonal replicate was used to regenerate the 12 clonal replicates for the second run of the study in 2006. Four times per year, visual percent green ratings were taken on each individual clone beginning when salt stress appeared in the saline water treatments and differences were observed between clones. Soil samples were also taken weekly and analyzed for EC at the Rutgers Soil Testing Laboratory using the 1:2 Soil:Water Extract Method (Dellavalle, 1992). The planting, irrigation, and field salinity screening procedure has been reported by Koch and Bonos (2011b).

Plant material and field trial for narrow-sense heritability and inheritance characteristics. Six perennial ryegrass clones were chosen from the 142 clones used in the broad-sense heritability field trial in the fall of 2005. Three clones were identified as salt tolerant, 9505, 9453, and 9509, and three were identified as salt susceptible, 9533, 9527, and 9530. Each of the six perennial ryegrass clones were vegetatively propagated and planted into a spaced-plant nursery in the fall of 2005 and allowed to vernalize over the winter. In the spring of 2006, plants from each of the six parental clones were crossed in a diallel mating design with reciprocals. Crosses were isolated from each other with plastic partitions to prevent crossing between other controlled crossing pairs. An additional clone of each parent was also isolated to determine if selfing would occur. No
viable seed was recovered, however, so selfing was ruled out. A total of 15 controlled crosses were made with reciprocals for a total of 30 crosses.

Ripened seed was harvested from each of the thirty individual parents, dried and threshed. Seedlings were germinated in the greenhouse and 96 seedlings from each parent were randomly selected and transplanted into plastic flats containing Pro-mix growing media (Premier Tech Ltd., Riviere-du-Loup, QC). They were grown for four weeks while receiving fertilization biweekly and daily irrigation. The seedlings were then planted into a mowed spaced-plant field trial at the Rutgers Plant Biology and Pathology Research and Extension Farm (Freehold sandy loam) with 30.5 cm spacing between each perennial ryegrass plant. A total of four replications were planted in a randomized complete block design. Each replication consisted of 24 genotypes from each cross and 2 clonal replicates of each of the six parents. Fertilization with 10-10-10 (N-P-K) fertilizer was applied to the clones (98 Kg N Ha\(^{-1}\)) at planting and fresh water irrigation was applied as necessary during establishment. Plants were mowed weekly at 5 cm for the duration of the study. Diseases were prevented as needed throughout the study with fungicide applications to avoid interaction of damage associated with diseases and damage from salinity stress.

Saltwater irrigation was initiated four weeks after planting. The irrigation solution was prepared and applied as described above. The whole trial was repeated in 2007 and 2008. A total of 35 salt water applications were applied in each year of the study. Four times per year, visual percent green ratings were taken on each individual beginning when salt stress appeared and differences were observed between plants. Weekly soil
samples were collected from the field and analyzed for EC at the Rutgers Soil Testing Laboratory using the 1:2 Soil:Water Extract Method (Dellavalle, 1992).

**Statistical Analysis**

*Broad-Sense Heritability.* All data was first transformed by calculating the percent green rating values of plants receiving saltwater applications as a percentage of the percent green rating values of plants receiving fresh water irrigation. This was done to account for inherent genetic differences between perennial ryegrass clones as well as eliminating the effects of other stresses confounding the effects of the saltwater irrigation applications. This data was then averaged over the rating dates for the two runs of the study. Broad-sense heritability estimates were determined from restricted maximum likelihood variance and covariance components using the random model PROC MIXED in SAS (SAS Institute, Cary, NC) (Bokmeyer et al., 2009b; Bonos, 2006; Bonos et al., 2005; Bonos et al., 2004). All of the effects were considered random due to the fact that the salinity tolerance of each perennial ryegrass clone was unknown. Heritability estimates were calculated on a clonal mean (Hc) and on a single-plant basis (Hsp) according to the following formulas: 

\[
Hc = \frac{\sigma_c^2}{(\sigma_c^2 + \sigma_{cy}^2/y + \sigma_e^2/ry)}; Hsp = \frac{\sigma_c^2}{(\sigma_c^2 + \sigma_{cy}^2 + \sigma_e^2)}
\]

where \(\sigma_c^2\) = the total genetic variance of clones, \(\sigma_{cy}^2\) = clone x year variance, and \(\sigma_e^2\) = experimental error (clone x replication x year) (Poehlman and Sleper, 1995).

*Narrow-Sense Heritability.* Narrow-sense heritability estimates were calculated based on data obtained from the diallel crosses using mid-parent progeny regression analysis (Bokmeyer et al., 2009a; Bonos, 2006; Han et al., 2006; Poehlman and Sleper, 1995).
The visual percent green rating means of the F\textsubscript{1} progeny from each cross were regressed against the average visual percent green ratings of the two parents. The slope of the resulting regression line was equal to the narrow-sense heritability (Falconer and MacKay, 1996). The data from the two runs of this study were analyzed separately.

**Heterosis and Maternal Effects.** Heterosis, the comparison between progeny and midparent means, and maternal effects, the comparison of progeny of reciprocal crosses, were evaluated for significance with a two-sample *t* test using data obtained from the diallel crosses (Kitchens, 1998).

**Combining Abilities.** Responses of diallel crosses were analyzed for general and specific combining abilities using Griffing’s (1956) method 1 (includes parents, F\textsubscript{1} progeny, and reciprocals) model 1 (fixed effects). The combining ability analysis model was: 

\[
X_{ijk} = u + g_i + g_j + s_{ij} + r_{ij} + b_k + e_{ijk},
\]

where \(X_{ijk}\) = observed salinity tolerance of the \(ij^{th}\) cross in the \(k^{th}\) block, \(u\) = population mean, \(g_i\) = GCA effect of the \(i^{th}\) parent, \(g_j\) = GCA effect of the \(j^{th}\) parent, \(s_{ij}\) = SCA effect for the \(ij^{th}\) cross, \(r_{ij}\) = reciprocal effect for the \(ij^{th}\) cross, \(b_k\) = effect of the \(k^{th}\) block, \(e_{ijk}\) = residual effect. Analysis was accomplished using DIALLEL-SAS05 (Zhang et al., 2005). Diallel analysis assumes that inbred parents were used; however, this assumption is violated due to the heterozygous nature of cross-pollinated perennial ryegrass and may lead to an inaccurate estimation of combining ability.
RESULTS AND DISCUSSION

Effects of Salt Water Applications. Saline water applications reduced visual percent green of perennial ryegrass clones. Some clones exhibited only a minor reduction in percent green values, some exhibited near complete loss of green verdure, and the majority of the clones exhibited salinity stress between the two extremes (Figure 1). The population distribution approached a bell shaped curve. The damage to plants may have been due to a combination of effects caused by the irrigation applications. Firstly, by applying the salt water overhead, foliar injury can occur. Harivandi (2004) stated that irrigation water with increased salts may cause serious turf damage when applied overhead due to the fact that sodium can be absorbed directly through foliar tissue and cause toxicity. Other research has indicated stress associated with foliar applications of irrigation water with increased salinity in perennial ryegrass (Koch and Bonos, 2011a) and other crop species (including alfalfa (*Medicago sativa* L.) including citrus (*Citrus sp.* L.)) (Westcot and Ayers, 1984). Secondly, perennial ryegrass clones were exposed to increased soil salinity levels in each of the 4 years (2005 = 2.72, 2006 = 3.07, 2007 = 3.19, 2008 = 2.98 dS m$^{-1}$). These levels have been shown to be high enough to cause salt stress and damage to most cool-season turf species (Carrow and Duncan, 1998).

Broad-sense Heritability. Clone accounted for the largest variability in the analysis of variance (Table 1). The large variance associated with the clone variable indicates that salinity tolerance in perennial ryegrass is largely under genetic control. Environmental variation was accounted for by evaluating six replications over a period of two years (Burton and Devane, 1953). The broad-sense heritability for the salinity tolerance among
perennial ryegrass clones was 0.78, however, on a single plant basis, the broad-sense heritability estimate is 0.49 (Table 1). This indicates that 78% of the variation observed in salinity tolerance was attributable to genetic differences between perennial ryegrass clones however, when only one replicate is used it reduces that to only 48% suggesting that the environment does play a role in salinity tolerance and that replication will dramatically increase the likelihood of finding salinity tolerant plants. Since the estimated heritability calculated on a single plant is moderate, selection of individuals without evaluation over multiple replications and over multiple years may prove to be inefficient in improving salinity tolerance in perennial ryegrass.

Broad-sense heritability estimates of overall salinity tolerance in this study were higher than the heritability estimates of percentage leaf firing of zoysiagrass under salt stress ($H^2 = 0.67$) (Qian et al., 2000). The lower heritability estimate in zoysiagrass may be attributable to the fact that salinity was increased to 42.5 dS m$^{-1}$, which, in comparison, was well above the salinity levels used in the study. The discrepancy in heritability between the two turf species may also be attributable to the differences between the mechanisms of salt tolerance between the two species, where it has been thought that perennial ryegrass may rely mainly on the exclusion of salt ions from the plant cells as well as osmotic adjustment (Marcum, 2008a). However, zoysiagrass has an additional mechanism in which specialized salt glands remove excess salts from the plant (Marcum and Murdoch, 1990), which may lead to more complex inheritance.

Gregorio and Senadhira (1993) analyzed the broad-sense heritability in a rice population grown under salinity stress using hydroponics with concentrations equal to 12 dS m$^{-1}$. Heritability estimates based upon measurements on Na-K ion balance were equal
to 0.37, indicating that this phenomenon is greatly affected by environmental effects. However, broad-sense heritability estimates of wheat growth parameters under salinity stress were between 0.49 and 0.91 for all measurements (Ashraf, 1994) indicating that similar to this perennial ryegrass population, a large portion of the variance may be attributable to genetic factors. Heritability of the salinity tolerance trait may be greatly affected by the measurements used to quantify resistance to the trait. It is important to note that the heritability estimates reported here are only relevant for the population of perennial ryegrasses used in this study and the particular environments studied.

*Narrow-sense Heritability.* Narrow-sense heritability estimates were calculated using mid-parent progeny regression analysis where the slope of the regression line was equal to the heritability estimate (Falconer and Mackay, 1996). The two years of the study were analyzed independently to evaluate the repeatability of the heritability estimate and were equal to 0.72 for 2007 and 0.66 for 2008 (Fig. 2). Narrow-sense heritability estimates are lower than the broad-sense heritability estimates due to the fact that narrow-sense heritability excludes epistatic and dominant gene effects and only calculates the contribution of additive gene effects. These additive effects are more important to plant breeders because additive effects can be selected for with reasonable certainty and that an improvement should be made in the next generation (Henderson, 1963). The moderately high narrow-sense heritability estimates reported in this study indicate that additive gene effects account for the majority of the genetic factors that contribute to overall salinity tolerance and may suggest that a breeding program using recurrent selection methods should be effective in concentrating additive genes resulting in progeny with increased
salinity tolerance. These results are similar to narrow-sense heritability estimates of salinity tolerance based on grain weight and ion uptake in wheat (Ashan et al., 1996). Narrow-sense heritability estimates of salinity tolerance based on Na-K ratios in rice shoots (Gregorio and Senadhira, 1993) were significantly lower than estimates obtained in this study, which may indicate more environmental interaction or increased contributions of non-additive genetic effects. This discrepancy may also be due to differences in how salinity tolerance was measured.

**Heterosis and Maternal Effects.** Progeny means were not significantly different from the mid-parent means for any of the controlled crosses based on the two-sample \( t \) test statistic (data not shown) (Kitchens, 1998). This indicated that heterosis was not present in any of these crosses and supports the narrow-sense heritability estimate that additive gene effects may be the major gene effect conveying salinity tolerance in perennial ryegrass. Another possible explanation for the lack of heterosis could be the fact that salinity tolerant and susceptible parents can have groups of loci conveying opposing responses, thereby negating the effects of the parents and resulting in progeny with salinity tolerance between the two parents (Bonos et al. 2003; Falconer and MacKay, 1996). Maternal effects, calculated by comparing the progeny means from reciprocal crosses, were not significant for any of the diallel crosses (data not shown). These data indicate that inheritance of salinity tolerance was not related to maternal factors in this perennial ryegrass population. Sing and Sing (2000) studied salinity tolerance in wheat by measuring grain yield, 1000 grain seed weight, and biological yield under saline conditions and analyzed data for the presence of heterosis and maternal effects.
Significant heterosis and maternal effects were present in all measurements indicating a significant contribution of non-additive genetic effects. The difference between perennial ryegrass and wheat, with respect to the presence of heterosis and maternal effects, is not surprising because the selfing nature of wheat is preferential towards accumulation of non-additive genetic effects while the outcrossing nature of perennial ryegrass is preferential towards accumulation of additive genetic effects.

*Combining Ability.* Diallel cross data was combined over both runs (2007 and 2008) of the study and subjected to analysis of variance using Diallel-SAS. The mean squares of the ANOVA are presented in Table 2. The main effect of cross was significant indicating that the genetic factors that are transmitted from parents to the offspring do influence the level of salinity tolerance. General Combining Ability (GCA) and Specific Combining Abilitiy (SCA) effects were both significant (Table 2) which indicates that additive and non-additive gene effects were involved in the phenotypic expression of salinity tolerance. Although both GCA and SCA were statistically significant, the mean squares for GCA were much larger than the mean squares for SCA which indicates that GCA is more important in predicting the performance of the progeny than SCA (Becelaere and Miller, 2004; Cisar et al., 1982). General combining ability is an indirect measure of additive gene effects, so the statistically significant GCA in this study further indicates that additive gene effects are important for salinity tolerance. All six of the parents had very significant GCA values (Table 3). The three tolerant parents, 9505, 9453, and 9509, all have significantly positive GCA values, meaning that each contributed towards tolerance in the progeny. Parent 9505, had the highest GCA value which indicates that
this parent contributed the most towards salinity tolerance. Similarly, each of the susceptible parents, 9533, 9527, and 9530, each had significantly negative GCA values, meaning that each contributed towards susceptibility to salinity in the progeny. The parent with the most negative GCA, 9530, contributed the most towards susceptibility. These data are supported by the narrow-sense heritability estimates and lack of heterosis suggesting that additive gene effects play an important role in salinity tolerance in perennial ryegrass.

Although GCA was more significant, SCA was also statistically significant (Table 2). Specific combining ability estimates the gene effects that are attributable to non-additive gene effects, including dominance and epistatic effects (Sprague and Tatum, 1942). A significant SCA designates a discrepancy in predicted progeny values (higher or lower) on the basis of the two parents involved in the specific cross based on the GCA values. A significant negative SCA effect of a specific cross indicates a lower tolerance to salinity than what can be predicted from the GCA effects of the parents, while a significant positive SCA indicates a higher tolerance to salinity compared to the prediction based on the GCA effects. According to Table 4, many of the crosses included in the diallel have significant SCA effects. These data indicate that more complex types of inheritance or different types of gene action may be involved in salinity tolerance inheritance contingent on the individual contributions of each parent. Although GCA accounts for a higher proportion of the mean squares, the significant SCA values suggest that progeny testing may be required before choosing parents to include in a breeding program focused on developing novel salt tolerant perennial ryegrass cultivars. In addition, parents identified as having significantly negative SCA values should be
omitted from a breeding program since these individuals contain genes for susceptibility and will contribute negatively to salinity tolerance.
CONCLUSION

This study illustrated that salinity tolerance in perennial ryegrass is controlled largely by genetic factors. The moderate to high broad-sense heritability estimates, calculated on a clonal basis, indicate that the majority of the phenotypic variation seen in the perennial ryegrass population with respect to the salinity stress was due to genetic factors. However, the moderate heritability calculated on a single plant basis identifies that a significant amount of the variation was due to environmental interaction. It is because of this interaction that replication and evaluation over multiple environments may be useful for identifying genotypes with superior performance (Dudley and Moll, 1969). Narrow-sense heritability estimates and the fact that GCA accounted for a larger portion of the sum of squares when compared to SCA indicated that the majority of the genetic effects are additive gene effects. However, because of the significant SCA, it may be necessary to perform progeny testing to prevent crosses that will have a negative impact on the progeny’s salinity tolerance. The lack of maternal effects and heterosis also further the notion that additive genes account for the majority of the genotypic effects involved in salinity tolerance in perennial ryegrass. The data from this study suggest that salinity tolerance in perennial ryegrass is predominantly controlled by additive genetic effects and an effective breeding program would select individuals after careful progeny testing and evaluation over multiple replications and environments.
LITERATURE CITED


Table 1. Analysis of variance of salinity tolerance of 142 perennial ryegrass clones averaged over two years (2007 and 2008).

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Mean square</th>
<th>F value</th>
<th>P &gt; F</th>
<th>Variance Component†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>1</td>
<td>1514.66</td>
<td>23.02</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Rep (year)</td>
<td>10</td>
<td>1548.03</td>
<td>23.15</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Clone</td>
<td>141</td>
<td>1565.96</td>
<td>23.80</td>
<td>&lt;0.0001</td>
<td>102.0100</td>
</tr>
<tr>
<td>Clone x year</td>
<td>141</td>
<td>341.89</td>
<td>5.20</td>
<td>&lt;0.0001</td>
<td>46.0039</td>
</tr>
<tr>
<td>Error = clone x year x rep</td>
<td>1410</td>
<td>65.78</td>
<td>5.20</td>
<td>&lt;0.0001</td>
<td>65.7834</td>
</tr>
</tbody>
</table>

Hc‡ = 0.78
Hsp = 0.49

† Variance components determined using restricted maximum likelihood (REML) of Proc Mixed in SAS (SAS Institute, Cary, NC)
‡ 95% confidence interval for 6-plant mean heritability = 0.86-0.62; calculated according to Knapp and Bridges (1987).
Table 2. Analysis of variance for salinity tolerance of a diallel cross of six perennial ryegrass parents evaluated in a field trial in 2007 and 2008.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Mean square†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>1</td>
<td>846.6 *</td>
</tr>
<tr>
<td>Rep(year)</td>
<td>6</td>
<td>250.8</td>
</tr>
<tr>
<td>Cross</td>
<td>35</td>
<td>11900.9 ***</td>
</tr>
<tr>
<td>GCA§</td>
<td>5</td>
<td>61956.4 ***</td>
</tr>
<tr>
<td>SCA§</td>
<td>15</td>
<td>1457.6 ***</td>
</tr>
<tr>
<td>Reciprocal</td>
<td>15</td>
<td>135.5</td>
</tr>
<tr>
<td>Maternal</td>
<td>5</td>
<td>143.2</td>
</tr>
<tr>
<td>Non-maternal</td>
<td>10</td>
<td>131.7</td>
</tr>
<tr>
<td>Cross x year</td>
<td>35</td>
<td>199.8</td>
</tr>
<tr>
<td>GCA x year</td>
<td>5</td>
<td>274.7</td>
</tr>
<tr>
<td>SCA x year</td>
<td>15</td>
<td>294.8 **</td>
</tr>
<tr>
<td>Reciprocal x year</td>
<td>15</td>
<td>103.6</td>
</tr>
<tr>
<td>Maternal x year</td>
<td>5</td>
<td>117.6</td>
</tr>
<tr>
<td>Non-maternal x year</td>
<td>10</td>
<td>96.7</td>
</tr>
<tr>
<td>Error</td>
<td>210</td>
<td>140.8</td>
</tr>
</tbody>
</table>

† *, **, and *** indicate significant F tests at P < 0.05, 0.01, and 0.001, respectively.
§ GCA, general combining ability
§§ SCA, specific combining ability
Table 3. Characterization and general combining ability (GCA) of salinity tolerance of six perennial ryegrass parents crossed in a diallel design and evaluated in 2007 and 2008.

<table>
<thead>
<tr>
<th>Parent ID</th>
<th>Tolerance designation</th>
<th>% green rating‡</th>
<th>GCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>9505</td>
<td>T</td>
<td>58.4</td>
<td>6.1</td>
</tr>
<tr>
<td>9453</td>
<td>T</td>
<td>61.2</td>
<td>9.7</td>
</tr>
<tr>
<td>9509</td>
<td>T</td>
<td>62.9</td>
<td>12.0</td>
</tr>
<tr>
<td>9533</td>
<td>S</td>
<td>47.0</td>
<td>-8.9</td>
</tr>
<tr>
<td>9527</td>
<td>S</td>
<td>48.0</td>
<td>-7.7</td>
</tr>
<tr>
<td>9530</td>
<td>S</td>
<td>45.2</td>
<td>-11.2</td>
</tr>
<tr>
<td>LSD (P ≤ 0.05)</td>
<td></td>
<td>6.1</td>
<td>3.9</td>
</tr>
</tbody>
</table>

*** indicates significant F tests at P ≤ 0.001 level.
† T, tolerant; S, susceptible. Resistance and susceptibility to salinity stress were determined from a previous field trial.
‡ Average % green rating combined from 2007 and 2008.
Table 4. Estimation of specific combining ability (SCA) effects in a diallel cross of six perennial ryegrass parents using combined data from 2007 and 2008.

<table>
<thead>
<tr>
<th>Parents</th>
<th>9505 (T$^\dagger$)</th>
<th>9453 (T)</th>
<th>9509 (T)</th>
<th>9533 (S)</th>
<th>9527 (S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9453 (T)</td>
<td>0.55$^\dagger$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9509 (T)</td>
<td>-2.41**</td>
<td>-3.42***</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9533 (S)</td>
<td>-3.42***</td>
<td>3.02***</td>
<td>-1.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9527 (S)</td>
<td>-0.26</td>
<td>-4.81***</td>
<td>2.96***</td>
<td>3.31***</td>
<td></td>
</tr>
<tr>
<td>9530 (S)</td>
<td>-2.14*</td>
<td>1.10</td>
<td>-2.17*</td>
<td>3.85***</td>
<td>2.14*</td>
</tr>
</tbody>
</table>

*, **, and *** indicate significant F tests at P < 0.05, 0.01, and 0.001, respectively
$^\dagger$ SCA effects
$^\ddagger$ T, tolerant; S, susceptible. Resistance and susceptibility to salinity stress were determined from a previous field trial.
Figure 1. Distribution of perennial ryegrass clone responses to salinity stress taken as a percentage of control plants receiving fresh water evaluated in a field trial and averaged over 2 years.

Mean = 71.9%, s.d. = 11.5
Figure 2. Mid-parent-progeny regression of six perennial ryegrass parents crossed in a diallel mating design evaluated for salinity tolerance observed in a field trial in 2007 (a) and 2008 (b).
CHAPTER 7

Thesis Conclusion

The necessity to use low quality irrigation water will become more important as fresh water resources are depleted and become increasingly scarce, so development of cultivars that surpass current tolerance levels is necessary. This fact also elucidates the necessity to continue to perform screenings at increasing salinity concentrations. In fact, it may be beneficial to perform screenings at salinity concentrations far above levels found currently in wastewaters in order to always exceed the stress and prevent scenarios where salinity concentrations of irrigation water increase at a faster rate compared to cultivar development.

This research effectively ranked many currently available cool-season turfgrass cultivars for levels of salinity tolerance using novel salinity screening procedures that accurately mimicked field conditions. This effectively served as a baseline indication of the status of salinity tolerance of the turfgrass species studied as well as the range of tolerance levels within each species. It also identified current turfgrass cultivars that field managers could plant in situations where low quality irrigation water is to be used.

All of the measurements that were used to quantify the salinity tolerance of the turfgrasses in these studies proved to be highly correlated, so it may be possible to limit measurements when screening germplasm in the future. In fact, since visual percent green ratings were the least intensive and nondestructive measurement, it is recommended that this measurement be used as the primary approach in quantifying
salinity tolerance. The fact that visual ratings are nondestructive is especially useful for a breeding program since the plants deemed tolerant to the stress can then be used for selection and subsequent cultivar development. Visual percent green ratings were also highly correlated to an analysis of percent green performed using digital images. Digital image analysis can also be used in future germplasm screenings as a method of determining the accuracy of the rater by using an objective measure to test the subjective visual percent green ratings. The procedure of testing raters may also be useful in standardizing ratings scales when using this system on a much larger scale or other situations where multiple researchers will be involved with collecting data from the germplasm. The novel overhead irrigated techniques that were developed proved to be effective in determining the salinity tolerance of turfgrass germplasm. In addition to these methods more accurately mimicking realistic conditions, these novel techniques proved to be more useful for use in a breeding program when compared to the more popular hydroponic techniques because of the ease of use and the large quantities of germplasm that can be evaluated for this trait at minimal costs.

Based on the inheritance characteristics calculated in the perennial ryegrass population studied in this research, salinity tolerance seems to be controlled by mainly additive genes. This indicates that a recurrent selection breeding program should be effective at concentrating additive alleles and creating novel cultivars with higher salinity tolerance compared to the previous generation. However, due to the fact that these inheritance estimates are only representative on the perennial ryegrass population being studied, it may be necessary to perform similar genetic studies on other perennial ryegrass populations as well as other turfgrass species in order to understand the
heritability of the trait outside of the studied population. Although these findings indicated the majority of the phenotypic variance was due to genetic effects, environmental interactions were still significantly associated with this trait, illustrated by the fact that runs were significantly different from one another in both greenhouse and field studies and that the heritability was low on a single plant basis. This interaction indicated the necessity to repeat these screenings in multiple environmental conditions such as varying temperatures and humidity.

Recurrent selection has been employed and has proven effective in efforts to improve the salinity tolerance of new cool-season turfgrass cultivars using the novel screening techniques described through this research. Through a single generation of selection and breeding of perennial ryegrass germplasm, using the overhead irrigation procedures described through this research, germplasm was successfully selected and experimental cultivars have been developed that surpass the salinity tolerance levels of currently available cultivars. Screening of improved germplasm exhibiting exceptional turf quality, disease resistance, and abiotic stress resistance, for increased salinity tolerance will allow for a faster method of developing elite cultivars with improved salinity tolerance. Future research to further improve the salinity tolerance of cultivars as well as the overall quality of the tolerance associated with new cultivars, should include the selection of germplasm that have shown tolerance at all growth stages including germination, establishment, and mature plants. Tolerance to salinity stress has been shown to be unrelated among different growth stages in other plant species so future screening of germplasm could benefit from germinating seedlings under saline conditions and continuing the stress throughout the life cycle of the turfgrasses to ensure that
germplasm selected for inclusion in a breeding program will be tolerant to the stress at all growth stages. Collection of germplasm for use in a breeding program should be focused on salt affected sites including land areas adjacent to salt water sources, arid lands with perpetual salinity issues, as well as old turf areas that have a long history of irrigation with low quality water with increased salinity. Germplasm collected from these sites will have an increased likelihood of having superior tolerance to the salinity stress and may expedite the development of elite cultivars with increased salinity tolerance.

Future research may include further cultivar evaluations among the species to determine the most tolerant cultivars within each species and also determine maximum and minimum current tolerance levels for each species. It will also be important to continue tolerance screenings as novel cultivars are developed in order to maintain accurate cultivar recommendations to turfgrass practitioners for use on salt affected sites. Further investigation of salinity tolerance of turfgrasses in soils with different soil amendments may also clarify how different soils affect the accumulation of salts and interact with plants under salinity stress.

Additional research is necessary to determine which tolerance mechanisms are responsible for the largest gains associated with overall salinity tolerance in turfgrasses. This would provide breeders with a specific trait that would achieve the largest gain in salinity tolerance with each generation of screening and breeding and result in cultivar development with larger increases in salinity tolerance when compared to the previous generation.
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