# Cool-Season Turfgrass Mixtures for Minnesota Roadsides

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# Dedication

I dedicate this degree to my family and friends who have stood by me throughout my academic endeavors. I will be forever grateful for your love and support.

# ABSTRACT

Roadsides present significant challenges to maintaining functional and sustainable vegetation due to the presence of multiple stresses that can be extreme. These stresses may include drought, heat, disease, and in cold-weather climates exposure to deicing salts used during winter road maintenance practices. Mixtures of cool-season turfgrasses can be used to create high quality roadside vegetation that can withstand these stresses. The current specification for roadside turfgrass in Minnesota included outdated cultivars and was in need of reassessment. The purpose of this research was to identify the best cultivars for use on roadsides in Minnesota and to create a suitable mixture of those cultivars that would maximize establishment and survival.

In the first part of this research, cultivars of cool-season turfgrass were assessed for their ability to establish and survive on roadsides in Minnesota. This was accomplished by visually assessing fall-seeded plots on roadsides for establishment and, subsequently, survival the following spring. Successful establishment and survival were related to edaphic characteristic; specifically, soil compaction and moisture.

In cold weather climates like that in Minnesota, salt tolerance is a required trait of roadside vegetation due to the application of deicing salts in the winter. As such, the same cultivars were directly assessed for salt tolerance in nutrient solution culture amended with sodium chloride to 4, 14, and 24 dS $m^{-1}$. Assessment of salt tolerance was accomplished using digital image analysis to quantify percent green tissue remaining following the different severities of salt exposure. Following exposure to the lowest level of salt stress, no significant differences were observed between cultivars in the trial. Following moderate salt stress, significant differences were identified between cultivars of turfgrass, including between cultivars within species. Specifically, between cultivars of Kentucky bluegrass (*Poa pratensis* L.) and perennial ryegrass (*Lolium perenne* L.). However, under extreme salt stress no significant differences existed between cultivars within any given species, and trends among the mean tolerance of each species were dominant. Overall, cultivars of tall fescue (*Festuca arundinacea* Schreb.) and slender creeping red fescue (*Festuca rubra* L. ssp. *litoralis*) retained the greatest amount of green tissue.

An improved method of evaluating turfgrass seed mixtures was needed before creation of an ideal mixture for Minnesota roadsides was possible. A novel approach to design and analysis of seed mixture experiments was introduced. The method used a plant community-level approach to identify the optimal species mixture. To demonstrate the method, a simple four-species mixture experiment was established in a growth chamber using a simplex-centroid design of seed mixture proportions. Polynomial regression analysis and augmented Lagrangian numerical optimization were used to predict a mixture that would maximize total dry clippings biomass over 19 wk. From this experiment, the method was proven successful as the predicted optimal mixture of 83%

perennial ryegrass (*Lolium perenne* L.) – 17% hard fescue [*Festuca trachyphylla* (Hack.) Krajina], by seed count, was shown to produce a similar or greater amount of clippings biomass as compared to all of the design point mixtures.

That plant community-level approach to seed mixture design and analysis was applied to mixtures of nine turfgrass species on roadsides with the additional requirement that each mixture contain more than two species. A single cultivar was chosen to represent each species based on the previous evaluations of roadside establishment and survival as well as direct evaluation of salt tolerance. Mixtures were established at two roadside locations and evaluated for green canopy cover and weed encroachment over two years. Data from digital image analysis and grid-intersect counts indicated that inclusion of tall fescue in the seed mixture significantly decreased the probability of retaining at least 60% cover after two years. In contrast, inclusion of hard fescue, sheep fescue (*Festuca ovina* L.), and slender creeping red fescue each increased the probability of retaining at least 60% cover. A 2:2:1 mixture by seed count of those three species, respectively, was predicted to produce the greatest percent green cover after two years of exposure to roadside environmental stresses. That mixture was deemed best for establishment on roadsides in Minnesota.

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# Preface

This dissertation contains five chapters. The first, a review of the literature, lays a foundation upon which the following research is built. The subsequent four chapters each comprise an abstract, a brief introduction, methods, results, discussion, and conclusions for each of the four primary components undertaken as part of this research. The chapters follow, more or less, the chronological order in which the work was undertaken with each chapter building on the last. At the time of publication of this dissertation, modified versions of chapters two and three have been published in peer-reviewed journals with plans to submit chapters four and five to similar, appropriate journals. Furthermore, consideration is being given to the publication of chapter one as a review of salt tolerance of turfgrasses for roadsides, which is absent from the current formal literature.

# Chapter 1: Review of the Literature

## Salt on roads

### Reasons for application

In cold-weather regions, sodium chloride (NaCl) salt is applied to roads, sidewalks, and other impervious surfaces to aid in the removal of snow and ice during the winter. Before the 1940s, public roads in the United States were primarily maintained through plowing and the use of sand for traction control. At that time, salt was used only as an additive in sand piles to prevent them from freezing, and in 1940 the total sales of rock salt for use on roads salt was 149 million kg (Jackson and Jobbagy, 2005). Road salt was first included in routine road maintenance practices during the winter of 1941 in the state of New Hampshire; however, in the entire United States just over 4.5 million kg of NaCl rock salt were spread on all highways combined that winter (National Research Council (U.S.), 1991). By contrast, 2011 consumption of rock salt for roadway use was approximately 18 million Mg in the United States (United States Geological Survey, 2011).

The use of such extreme amounts of road salt has been supported by data suggesting that transportation safety is vastly improved by its use. Traffic accident rates during winter storms in New York, Illinois, Minnesota, and Wisconsin have been found to be 4.5 times higher prior to salt application on multi-lane divided freeways and eight times higher on two-lane highways (Kuemmel and Hanbali, 1992). Moreover, those accidents occurring after salt application have lower associated injury rates by factors of about nine and seven for multi-lane divided freeways and two lane highways, respectively. These effects are not limited to the United States alone; rather, similar results have been observed in cold-weather regions of Germany (Hanke and Levin, 1988).

## Application Methods

Application of sodium chloride road salt is typically conducted by means of mechanical spreading. In the 1950s and 1960s, application of small amounts of salt were made by shoveling it from the back of a truck onto the streets (National Research Council (U.S.), 1991). Thankfully, the state of the art in road salt application has changed drastically since then. The use of spreaders with attached spinners, also known as broadcast spreaders, has become the most common method of salt distribution from trucks (Wisconsin Transportation Information Center, 2005a). Although deicing is currently the most commonly-used method to combat winter road conditions, anti-icing is quickly gaining use in suitable situations (Wisconsin Transportation Information Center, 2005b). Anti-icing is a process in which a small amount of liquid chemical is applied to the road prior to a winter storm event to prevent the bonding of ice to the surface.

## Mode of action

The mechanism by which salt helps to melt ice can be explained by its basic chemical properties. While the NaCl molecule itself is electrically neutral, it readily dissociates in water to form $Na^+$ and $Cl^-$. Those ions act to disrupt the cohesion of water molecules, effectively producing a lower overall freezing temperature for the solution as a whole. Increasing the concentration of sodium chloride in solution has the effect of depressing the freezing temperature for concentrations up to 23.31%. At that concentration, known as the eutectic point, the freezing temperature of the solution is -21.1 °C (Salt Institute, 2011). In order for the salt to be effective, the concentration of ions in solution must remain sufficiently high so as to depress the freezing temperature of the solution below the surface temperature of the road. However, at concentrations above 23%, the addition of more NaCl has no effect due to the fact that the solubility limit of NaCl in water has been reached. As a result, the range of road surface temperatures at which the use of NaCl is practical for melting ice is limited on the lower end at approximately -9.4°C (Wisconsin Transportation Information Center, 2005a). However, alternative chemicals are available that are effective below that temperature.

## Alternative De-icing Chemicals

A variety of alternative de-icing compounds have been used previously including calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$), calcium-magnesium acetate (CMA), and a variety of alcohols, glycols, and other compounds (Blomqvist, 1998). These chemicals are often more desirable than NaCl for a variety of reasons. Both $CaCl_2$ and $MgCl_2$ both have eutectic points that occur at lower temperatures than NaCl; this property makes them suitable for application at temperatures lower than would be acceptable for NaCl. Despite their lower effective temperature the deicing capacity of these chemicals is not necessarily comparable to NaCl. For instance, in early testing by the Federal Highway Administration, it was determined that nearly two times as much CMA may be required as compared to NaCl to produce comparable rates of ice melt (National Research Council (U.S.), 1991). Each of these alternatives may be used on their own, or in conjunction with NaCl, to improve overall deicing performance (Environment Canada and Health Canada, 2001). For example, pre-wetting salt with liquid $CaCl_2$ has been suggested as a method for reducing total salt applications on roadways (Gooding and Bodnarchuk, 1994).

## Environmental Concerns

Among the primary motivations for reduction in total salt use and development of viable alternatives has been concern over the environmental impact of sodium chloride in the environment. Concentrations of sodium and chloride in surface waters in the United States have steadily increased in recent years. Kaushal et al. (2005) monitored changes in chloride concentrations in streams in the northeastern United States and showed strong increases. In New Hampshire, concentrations were observed to exceed 100 mg $L^{-1}$, on a seasonal basis, in some rural streams. In Minnesota, chloride concentrations in some lakes have reached levels as high as 386 mg $L^{-1}$ and seasonal disruptions in stratification and overturn patterns have been observed (Novotny et al., 2008). The same study showed an average increase of 1.8% in the chloride concentration of lakes in Minnesota, which was strongly correlated with the amount of road salt purchased over the same period. Similarly, Mullaney et al. (2009) measured chloride concentrations in water samples from 100 basins in the east, central and west-central areas of the glacial aquifer system of the northern United States. In 15 of the samples, chloride concentrations exceeded the United States Environmental Protection Agency chronic exposure concentration recommendation for aquatic life of 230 mg $L^{-1}$. As noted by Jackson and Jobbagy (2005) some conclusions can be drawn from the data in these studies about the path by which the saline runoff reaches the surface or ground water. Equal amounts of sodium and chloride would seem to indicate a direct path while higher ratios of chlorine to sodium may imply a dominant path involving underground flow through soils where sodium would be inhibited by a cation exchange interaction with soil particles. This interaction with soils inevitably leads to an increase in soil salinity due to high sodium concentrations wherever road salts are applied. The build up of sodium in roadside soils has been well-documented. In Britain, sodium concentrations have previously been observed to reach levels of approximately 3000 mg $kg^{-1}$. Even higher sodium concentrations of greater than 10,000 mg $kg^{-1}$ were reported in Minnesota in soils sampled to a depth of 20 cm within one meter of the roadway on interstate highways during the month of December (Biesboer and Jacobson, 1994). Based on electrical conductivities of 1:5 soil-water extracts from the top 7.5 cm of soil on roadsides in Illinois, soluble salt concentrations of as high as 50,000 ppm have been observed (Hughes et al., 1975).

## Vegetation

### Effects of salt on vegetation

It is clear that increases in soil salinity and salt spray from roads can create serious challenges for growing vegetation on roadsides. Vegetation on roadsides is required perform a number of functions including erosion control and filtration of runoff water, as well as provide habitat for wildlife and aesthetic quality. However, effects of road salt can be particularly severe due to the magnitude of exposure or a general lack of tolerance among the established species.

A formal review of the mechanisms of salinity tolerance in plants, which is not within the scope of this review, is provided by Munns and Tester (2008) in which the authors describe what is referred to as a two-phase growth response. The first, referred to as the osmotic phase, occurs as salt levels increase outside the roots of the plant causing an increase in osmotic pressure and subsequent reduction in plant growth. The second phase, referred to as the ion-specific phase, can be described as a further reduction in growth and disruption of cellular processes resulting in senescence of plant tissues. Tolerant plants may be described as either salt includers, which are able to withstand high levels of sodium ions in plant tissues, or salt excluders, which may have ion uptake selectivity in favor of potassium or uptake of sodium which is subsequently stored in vacuoles or retranslocated into the soil (Rose-Fricker and Wipff, 2001).

## Turfgrass as roadside vegetation

A vast number of species may be used in different situations on roadsides. Of particular importance is the use of turfgrasses as roadside vegetation. The use of turfgrass on roadsides first became of interest during the 1930s due to the construction of a number of large-scale highway projects including the United States Numbered Highway System and the autobahnen in Germany. Turfgrass on roadsides is required to function in the same ways as other roadside vegetation, but is typically implemented to avoid contrast with adjacent land and enhance visibility for drivers without the need for extensive mowing. Early attempts to provide a suitable grass mixture for roadsides resulted in overly complicated mixtures including between five and fifteen grasses, three to four legumes, and two herbs (Boeker, 1970). Since that time, grass mixtures for roadside have been improved and refined and some authors have noted the need for as few as four well-chosen species (Boeker, 1970). As noted by Duell and Schmidt (1974), this process was prompted by rising costs of maintenance and an increase in public awareness of environmental quality. In particular, as use of road salt increased, reports on the damage from salt spray and runoff became increasingly prevalent. Despite identifying some species as salt tolerant, Butler et al. (1974) concluded that there was great need for further research focused on salt-related plant problems.

Today, several states have specified turfgrass mixtures for roadsides specifically aimed at providing the basic services required of roadside turf as well as being salt tolerant so as to maximize persistence over time. These mixtures make use of several species, each of which has been evaluated for their respective tolerances to salt and other deicing chemicals through both field and greenhouse trials. Unfortunately, the prescribed mixtures are often not based on results of designed experiments. It is the aim of the subsequent sections of this review to provide an overview of the literature relevant to the salt tolerance of each cool-season turfgrass species species as used on roadsides in the northern United States and the relationship between the functional salt tolerance of each to its habitat of origin. A qualitative summary of the salt tolerance and roadside applicability of each of the major species discussed is provided in Table 1.

## Kentucky bluegrass

Kentucky bluegrass (*Poa pratensis* L.) is an apomictic, strongly rhizomatous, perennial grass thought to be native to areas of Europe and Asia. It was likely introduced to North America by early settlers who brought it as part of seed mixtures, hay, and bedding (Bashaw and Funk, 1987). As a species it is considered widely adaptable and tolerant of many stresses, a trait that may be attributable to its highly apomictic nature, and as a result is found to have circumpolar distribution ranging in latitudes from 30° North to above 83° North (Clausen, 1961). Once established, Kentucky bluegrass persists through extensive rhizome growth and is easily spread through seed dispersal. As a result, it is often found to occur on roadsides in the central and northeastern United States (Huff, 2003). Huff (2003) noted that this occurs despite the absence or small quantity of

Kentucky bluegrass included in roadside mixes. Duell and Schmit (1974) explained that Kentucky bluegrass is typically a minimally aggressive component of roadside mixes and may be unable to survive on roadsides due to competition with taller species. They evaluated seven cultivars of common and turf-type Kentucky bluegrass, that is those that have not been bred for improved traits and those that have, for seed stalk production and color retention during summer drought on newly-established roadsides in New Jersey. Results of the study showed that common-type Kentucky bluegrass produced fewer seed stalks, but retained greater green color during summer drought than the newer, turf-type cultivars. The authors concluded that poor soil conditions and minimal management practices create unsuitable conditions for use of fine turf-type cultivars of Kentucky bluegrass. Still, Boeker (1970) recommended using Kentucky bluegrass in roadside mixtures in Germany at rates of up to 50% by weight, in combination with just three to four other species. However, other roadside trials in Germany later determined that the importance of Kentucky bluegrass in roadside mixtures was likely overestimated (Trautmann and Lohmeyer, 1980). Similarly, Butler et al. (1974) described the succession of roadside vegetation, indicating that Kentucky bluegrass is quickly replaced by quackgrass (*Agropyron repens* (L.) Beauv.) followed by weeping alkaligrass (*Puccinellia distans* (L.) Parl.) on saline roadside soils.

Data from these trials indicate that the salt tolerance of Kentucky bluegrass may be a limiting factor in the establishment of quality turf on roadsides. In light of the low salt tolerance generally associated with Kentucky bluegrass, Ahti et al. (1980) recognized the need to identify commercially available cultivars of Kentucky bluegrass that were suitable for roadside use. Using subirrigation with NaCl solutions ranging from 0.8% to 1.25% the authors evaluated the response of several cultivars of Kentucky bluegrass to salinization of the soil. Cultivars differed significantly in their salt tolerance 97 days after initial salt treatments with ‘Nugget’ being most salt tolerant with several cultivars, including ‘Merion’ possessing equally poor salt tolerance. Hughes et al. (1975) examined the response of ‘Merion’ Kentucky bluegrass to the addition of 5,000; 10,000; and 20,000 mg $kg^{-1}$ NaCl to oven-dry soil in which plants were grown from seed. Respective reductions in biomass production of 21%, 40%, and 47%, as compared to non-salted controls, were observed. Several cultivars of Kentucky bluegrass were evaluated for salt tolerance by Greub et al. (1985) by applying 20 mL of 2.65 M NaCl solution to the soil surface of plants established in pots in a greenhouse. After three weeks, all Kentucky bluegrass cultivars had biomass productions of less than 50% the no-salt controls and, as a species, Kentucky bluegrass incurred more tissue damage than any other species in the experiment. Of the cultivars tested, ‘Merion’ and ‘Newport’ had the worst visual damage and ‘Nugget’ was the best despite showing severe chlorosis and mostly dead tissue.

Much research has been conducted in recent years to evaluate newer, fine turf varieties of Kentucky bluegrass for salt tolerance and roadside application. Rose-Fricker and Wipff (2001) evaluated entries from the 1995 National Turfgrass Evaluation Program for salt tolerance using nutrient solution culture amended with 10,000 mg $L^{-1}$ of a mixture of various salts. The observed significant differences between the entries indicated that cultivar ‘North Star’ was significantly better than all other entries in the trial with less than 35% damage after eight weeks in the salt bath. Cultivar ‘Haga’ exhibited the lowest salt tolerance with greater than 75% damage, but was not statistically different from several other entries. Koch et al. (2011) identified salinity tolerance differences between cultivars and experimental lines of Kentucky bluegrass and Kentucky bluegrass × Texas bluegrass (*Poa arachnifera* Torr.) hybrids using an overhead irrigation system. Response variables for their study included biweekly leaf clipping dry weights, weekly visual assessment of percent green canopy, as well as root and shoot dry weights at the conclusion of the study. Results indicated that breeding efforts have resulted in some improvement of salinity stress within the species but concluded that further testing of Kentucky bluegrass germplasm for salinity tolerance was necessary. Friell et al. (2013a) evaluated 13 cultivars of Kentucky bluegrass amongst 61 other entries for salt tolerance in nutrient solution culture. Salt was applied as a 5 M NaCl amendment to the nutrient solution culture and digital image analysis was used to assess tissue damage as the salinity level was increased. In that study, like the others, significant differences were observed between the entries; however, these results extended only until the salinity of the nutrient solution was increased to 14 dS $m^{-1}$. At that level, cultivars ‘Park’ and ‘Diva’ had the greatest salt tolerance with less than 50% tissue damage observed, while cultivar ‘Moonshine’ had the lowest salt tolerance.

It remains a question, however, whether or not results like those discussed above are applicable to roadside conditions. Brown and Gorres (2011) observed ‘Diva’ to persist well on roadsides in New England, but only in amended soils. Furthermore, comparison between cultivars was not possible as it was the only cultivar of Kentucky bluegrass included in that trial. No significant differences were observed by Friell et al. (2012) between cultivars established on roadsides in Minnesota, although the authors noted the possibility that those results may have been attributed to poor establishment from seed in the harsh roadside environment. Indeed, germination of Kentucky bluegrass seeds has also been shown to be inhibited by even small amounts of salt in soil solution. A greenhouse study by Liem et al. (1985) showed a significant decline in the germination rate of Kentucky bluegrass seeds in concentrations as low as 5 g $L^{-1}$. Tarasoff et al. (2007) also observed a decline in the germination rate of Kentucky bluegrass from 60% to 0% as osmotic potential of the solution surrounding the seeds was lowered from 0 to -2 MPa.

Taken together, evaluations of the applicability of Kentucky bluegrass to roadsides have produced mixed results. Generally, greenhouse studies have indicated a potential to increase salt tolerance in the species through continued breeding. However, field trials have resulted in poor establishment and persistence on roadsides. The ability for Kentucky bluegrass to succeed in low-fertility soil environments seems to be low and may limit the ability for roadside establishment (Duell and Schmit, 1974). Furthermore, previous authors have implied that the importance of Kentucky bluegrass in salt tolerant mixtures is likely restricted to its sod-forming ability (Butler et al., 1974).

## Perennial ryegrass

Perennial ryegrass (*Lolium perenne* L.) is a bunch-type grass native to south and central Europe, northern Africa, the Middle East, and southwest Asia. First cultivated in England, perennial ryegrass is described as an obligate outcrossing species that takes on a continuum of forms which can be found around the world (Terrell, 1968). It is adapted to a wide range of soil types, including a wide range of pH, but will not tolerate extreme heat, drought, or cold (Beard, 1973; Thorogood, 2003). Ryegrasses are best known for rapid seed germination and establishment, and are often used as nurse grasses for the establishment of Kentucky bluegrass (Christians, 2011). For this reason, it is often used on home lawns, parks, golf courses, general landscaping areas and roadsides (Thorogood, 2003).

Perennial ryegrass was included in early roadside grass mixtures, but was not included in the simplified mixtures recommended by Boeker (1970). He did recommend its use in areas particularly prone to erosion, but at no greater rates than 1-2 g $m^{-2}$. Although used extensively on roadsides in Sweden prior to 1963, it was later replaced by more desirable species (Langvad and Weibull, 1970). Duell and Schmit (1974) noted that some cultivars may be sufficient for roadside use but later found that, without mowing, all cultivars disappeared within 2 to 3 years of establishment. Similar decline of the species has been noted on roadsides in Germany (Trautmann and Lohmeyer, 1980). Researchers in France have also concluded that perennial ryegrass significantly interfered with the growth of minor species in mixtures and that its use should be restricted to sites requiring late seeding or possessing serious erosion potential (Henensal et al., 1980).

Nevertheless, perennial ryegrass is still found on roadsides today in many parts of the world (Humphreys, 1981; Ross, 1986; Tikka et al., 2000; Thorogood, 2003; Akbar et al., 2006). Measurements of the accumulated sodium levels in the leaf tissue of perennial ryegrass on roadsides in Britain have shown a marked increase over those collected from a non-saline pasture site nearby indicating salt tolerance in the species is necessary for successful roadside establishment (Davison, 1971). Similarly, Liem et al. (1985)

measured sodium concentrations in perennial ryegrass live plant matter and found concentrations next to the road to be 0.452 mmol kg$^{-1}$ as compared to 0.161 mmol kg$^{-1}$ in tissue collected 30 m from the road, two days after road salt was applied.

In light of observations like these, research into the salinity tolerance of perennial ryegrass has continued. On average, it is widely considered to have only medium salinity tolerance, tolerating soil solution extract electrical conductivities ($EC_e$) of 4-8 dS m$^{-1}$ (Marcum, 1999). Plants grown for 28 days in pots showed no significant change in shoot dry tissue weight, but did show significant reductions in root dry tissue weight of 30.5% and 40.3% when exposed to 4 g L$^{-1}$ or 8 g L$^{-1}$ NaCl solution, respectively (Spencer and Port, 1988). As with other species, common types have often been observed to possess greater salt tolerance than some newer turf-type cultivars. Following weekly application of 20 mL of 2.65 M NaCl solution for three weeks, common type perennial ryegrass was found to have a 25% reduction in shoot biomass production, while cultivar 'NK 200' produced a 35% reduction. These corresponded to a 34% and 59% reduction, respectively, in dry crown and root material (Greub et al., 1985). However, newer breeding lines have shown improvement over old cultivars, such as 'Linn'. Improvement has largely been attributed to an ability to accumulate up to 37% less $Na^+$ in crowns and 42% less $Na^+$ in young leaves while maintaining a $K^+/Na^+$ ratio 1300% higher (Krishnan and Brown, 2009). One year old plants of cultivars and entries from the 1999 National Turfgrass Evaluation Program perennial ryegrass trial showed significant variation when exposed for nine weeks to 17,000 mg L$^{-1}$ of a mixture of various salts in a nutrient culture system (Rose-Fricker and Wipff, 2001). Entry PST-2A6B showed the greatest salt tolerance with 84% of plants surviving, but those plants had only approximately 30% green tissue by the end of the trial. Worst among the entries was the cultivar 'Wilmington' which had just 25% of plants survive, but was not statistically different from several other cultivars in the trial based on visual ratings alone. Hughes et al. (1975) examined the response of perennial ryegrass to the addition of 5,000; 10,000; and 20,000 mg kg$^{-1}$ NaCl to oven-dry soil in which plants were grown from seed. Respective reductions in biomass production of 5%, 17%, and 44%, as compared to non-salted controls, were observed. That trial, however, did not evaluate the effects of salt on germination when sown in soil from actual roadsides. When grown in soils collected 6 m of the roadway, germination rates of perennial ryegrass increase to 87% as compared to 57% for soils taken 0.6 m from the roadway (Spencer and Port, 1988). Friell et al. (2013a) evaluated 16 cultivars of perennial ryegrass in nutrient solution culture and identified significant differences after exposure to salt concentrations as high as 14 dS $m^{-1}$ with ‘JR-522’ and ‘Accent II’ retaining more than 60% green tissue while ‘Affirmed’ retained the least green tissue with under 40%. Cultivar differences may also play a role in the ability for perennial ryegrass to germinate in saline conditions like those found on roadsides. An inverse relationship between salinity and germination percentage has been demonstrated; however, some data suggests that regardless of cultivar, germination rates above 50% may be expected for salt concentrations up to 10,000 mg $L^{-1}$ (Rose-Fricker and Wipff, 2001). Taken together, indications of this data are that significant progress has been made in terms of salt tolerance by enhancing the selectivity of ion uptake in tolerant cultivars (Krishnan and Brown, 2009).

Humphreys (1981) noted the relative importance of foliar salt spray to saline soils on roadsides and thus compared 20 cultivars in a greenhouse study using overhead irrigation techniques. Application of 0, 17, and 68 g $m^{-2}$ of salt applied with 0, 430.5, and 861 ml $m^{-2}$ water resulted in no significant differences in leaf damage, but did show differences in growth rate sensitivity with slower growth being associated with increasing salt and drought. In a controlled field study, Koch and Bonos (2011a) made use of overhead irrigation to apply 0.5 L of 10 dS $m^{-1}$ salt solution to individual plants, three times a week for a total of 70 applications over two years. Visual assessment of percent green tissue was performed for the 23 cultivars and entries in the study and it was found that entry RKS retained the greatest amount of green tissue after two years at over 60% while ‘Fiesta III’ retained the least with less than 40%. In recent years, true roadside trials including several cultivars of perennial ryegrass have been performed with each resulting in good establishment performance for the species but subsequent decline following winter months in the absence of soil amendments (Brown and Gorres, 2011; Friell et al., 2012). Of all cultivars tested, however, 'Accent II' and 'Headstart II' showed the greatest promise for successful roadside establishment.

Roadside establishment of perennial ryegrass has continued in recent years despite a plethora of recommendations to the contrary. If the species is to be implemented on roadsides, the need for significant improvement in its salt tolerance and overall ability to persist will need to be met through continued breeding. Furthermore, an understanding of its role in the succession of vegetation on roadsides will play a key role in understanding the limitations of its application, which may be restricted to early germination of a short-lived cultivar for erosion control (Boeker, 1970).

### Tall fescue

Tall fescue (*Festuca arundinacea* Schreb.) is perennial bunch-type grass that may or may not have short rhizomes, although rhizomes are normally absent with the primary exception being spaced plants grown in sandy soils (Terrell, 1979; Gibson and Newman, 2001; Meyer and Watkins, 2003). Native throughout Europe, North Africa, west and central Asia, and Siberia, it was introduced to the United States due to its importance as a pasture grass and is well-adapted to most of the eastern half of the country and parts of California and the Pacific Northwest (Gibson and Newman, 2001; Meyer and Watkins, 2003).

A number of characteristics make tall fescue well-adapted to roadside conditions including the abilities to thrive in soil pH ranging from 4.7 to 8.5, survive better in compacted soils than other cool-season grasses, and grow in alkaline and saline soils (Meyer and Watkins, 2003). Moreover, it has the best drought and heat resistance of the cool-season turfgrasses (Fry and Huang, 2004). Despite these useful adaptations, limited research has been conducted using tall fescue on roadsides. It was included in early seed mixtures described by Boeker (1970), but not recommended for use in the authors simplified species mixtures, and Duell and Schmit (1974) commented that its implementation on roadsides was largely attributable to the Soil Conservation Service.

The information that is available on the roadside establishment of tall fescue indicates that it holds great promise despite early indications that improvements in the species were necessary for successful implementation. In the 1970s, tall fescue mixed with Kentucky bluegrass (*Poa* pratensis L.) established on roadsides in Illinois was often succeeded by quackgrass (*Agropyron repens* (L.) Beauv.) which was later replaced by weeping alkaligrass (*Puccinellia distans* (L.) Parl.). In France, outstanding drought tolerance of 'Ludion' tall fescue led Henensal et al. (1980), to recommend it for use on roadsides despite observations that it maintained just average vegetative cover. This observation may be attributable to the fact that despite the more recent development of turf-type tall fescues which have superior density, the bunch-type growth habit of the species requires that the area be overseeded should significant stand losses occur (Fry and Huang, 2004).

The salt tolerance of tall fescue is well-established. It can be found in salt marshes throughout Britain (Gibson and Newman, 2001), and a number of greenhouse trials have indicated that tall fescue possesses significantly greater salt tolerance than many other turf species. When grown for ten days in one-third strength Hoagland solution amended with sodium chloride of increasing strengths up to an electrical conductivity of 17.7 dS $m^{-1}$, tall fescue was found to be the most salt tolerant of the cool-season turfgrasses (Kobayashi et al., 2004). Bañuelos and Beuselinck (2003) grew tall fescue in pots of soil with $EC_e$ of 1.8 and 7.0 dS $m^{-1}$, irrigated with water of EC 1.3, 2.7, and 6.4 dS $m^{-1}$. The authors observed no visual damage to leaf tissue despite elevated concentrations of both $Na^+$ and $Cl^-$ in the tissues, but concluded this was likely due to frequent clipping. Bowman et al. (2006a) demonstrated that differences in salinity tolerance between cultivars 'Monarch' and 'Finelawn I' were only evident at high nitrogen fertility levels when grown in a nutrient culture solution amended with 0, 40, 80, or 120 meq $L^{-1}$ of an 8:1 molar ratio mixture of NaCl and $CaCl_2$. Under a high nitrogen condition, clipping dry weight was reduced by as much as 50% while reductions of less than 20% were observed under the low nitrogen condition. Following 5 weeks of weekly application of 20 mL of 2.65 M NaCl solution, Greub et al. (1985) found that dry shoot biomass of ‘Kentucky 31’ tall fescue was reduced by just 17% as compared to a no-salt control. That same cultivar was found to be exceptionally tolerant, relative to other turfgrasses, to approximately 5 months of spray treatments using solutions of increasing salt concentration (Cordukes, 1968). Clipping yield was reduced by 48% and visual quality was assessed as a 5 out of 10, with 10 being most stressed. Friell et al. (2013a) evaluated differences between 12 cultivars of tall fescue in nutrient solution culture and found that, although the species had superior salt tolerance to all other cool-season grasses in the trial, no significant differences existed between cultivars.

Despite the abundance of greenhouse trials, few roadside trials have assessed the salt tolerance of the species. Brown and Gorres (2011) found that tall fescue was among the best species for establishment and persistence in both amended and non-amended soils in Rhode Island. After 21 months, cultivar ‘Tarheel II’ showed significantly better performance than other cultivars in the trial on soils amended with biosolids or compost; however, no significant differences were observed between cultivars on non-amended soils. The authors concluded that, as with other species, tall fescue offered no clear superiority of any cultivar in non-amended soil, indicating little or no advantage to selection of newer fine turf cultivars. Friell et al. (2012) evaluated 13 cultivars of tall fescue, among 61 other turfgrass cultivars, on roadsides in Minnesota where sodium chloride was known to cause problems with establishment. Cultivars ‘Grande II’, ‘Jaguar 4G’, ‘Wolfpack II’, and ‘SR 8650’ were among the most persistent cultivars after the winter salting season; however, few significant differences existed between cultivars.

Further research into the roadside salt tolerance of tall fescue is needed. Existing data indicates potential for faster establishment and better persistence on roadsides than many other cool-season turfgrass species. Its salt tolerance in greenhouse settings has been well established; although, few trials have evaluated differences between cultivars of tall fescue for their salt tolerance. Those which have indicate no significant differences exist between cultivars, despite overall agreement that salt tolerance exists within the species. Furthermore, additional information regarding the germination of tall fescue seeds under saline conditions could prove useful in breeding and selection of cultivars improved for salt tolerance.

### Fine fescues

The fine-leaved fescues, or fine fescues, are a taxonomically complex group of species which are typically classified into one of two complexes known as the red fescue (*Festuca rubra)* complex and the *Festuca ovina* complex. The *Festuca rubra* L. *senu lato* complex, native to Europe, has widespread distribution throughout Asia, North America, and Europe (Ruemmele et al., 2003). The species commonly used as turfgrass that belong to this complex include strong creeping red fescue (*Festua rubra* ssp. *rubra* Gaudin), slender creeping red fescue [*Festuca rubra* ssp. *litoralis* (G.F.W. Meyer) Auquier.], and Chewings fescue (*Festuca rubra* ssp. *commutata* (Thuill.) Nyman). Species in this complex, with the exception of Chewings fescue, are typically rhizomatous, although the wide variety of settings in which they are found including beaches, sand dunes, coastal rocks and cliffs, saltmarshes, gravel bars, meadows, boreal grasslands, and roadsides, has led to high variability within the species (Pavlick, 1985). These grasses are adapted to shade, drought, and sandy soils with pH from 5.5 to 6.5 (Beard, 1973). The second complex, *Festuca ovina*, includes the turfgrasses hard fescue (*Festuca trachyphylla* (Hack.) Krajina), sheep fescue (*Festuca ovina* L.), and blue fescue (*Festuca glauca* Lam.) which are widespread in Asia and in Europe, where they are native, and are also known to be highly polymorphic (Watson, 1958). The grasses in this complex are also shade and drought tolerant. Moreover, sheep fescue will tolerate a wide range of soils conditions including low fertility and tolerance of coarse soils with pH from 4.5 to 6.0 and hard fescue will tolerate a greater amount of water (Ruemmele et al., 1995).

As stated by Pavlick (1985), many of these species have seen significant use on roadsides, possibly due to their incredibly adaptive nature. In Nova Scotia, Canada, red fescues are found commonly growing on roadsides, but are also found in open pastures close to the coast, on beaches, and in the upper zone of salt marshes (Roland and Smith, 1966). Boeker (1970) recommended the use of both *Festuca rubra* and *Festuca ovina* in roadside mixtures due to their adaptation to a large range of soil and moisture conditions, widespread distribution, and the ability for *Festuca rubra* to recolonize gaps in the canopy via its rhizomatous growth habit. Duell and Schmit (1974) evaluated several species and cultivars of fine fescues for roadside performance as determined by assessing retention of green color during drought and spring green up. Of the cultivars evaluated, ‘C.P. Shade’ strong creeping red fescue and ‘Alaska Station’ sheep fescue provided the best color during drought and ‘Ruby’ strong creeping red fescue provided the best spring color. Overall, the authors noted that sheep and hard fescues were notably late in spring recovery and the creeping red fescues were slightly better than the Chewings fescues. Henensal et al. (1980) found that mixtures including slender creeping red fescue and hard fescue were best for roadsides in France, but noted that a lengthy drought adversely affected all fescue species.

Together, these results have confirmed that many species of fine fescues are particularly well suited to roadsides under a variety of conditions. One condition of particular interest, however, has been that of alkaline or saline soils. Research on the salt tolerance of fine fescues has provided variable results, possibly due to the taxonomic difficulties associated with the species. Differences between unclassified species or ecotypes within the *Festuca rubra* complex have been noted in plants collected from the lower and upper regions of salt marshes and adjacent non-saline highlands (Hannon and Bradshaw, 1968). Populations of *Festuca rubra* have been found to have widely varying salt tolerances elsewhere as well, and the genetic heritability of the trait was explored by Ashraf et al. (1986) and by Venables and Wilkins (1978) who determined that salt tolerance was a highly heritable trait in *Festuca rubra*. Observations such as this have led some authors to attribute greater or lesser degrees of salt tolerance of species within the complex to their habitat of origin (Humphreys, 1982). Typically the ranking of salt tolerance in the species is given as *Festuca rubra* ssp. *litoralis* > *Festuca rubra* ssp. *rubra* > *Festuca rubra* ssp. *commutata*. This ranking has been confirmed by a number of trials in both roadside and greenhouse environments. Following subirrigation with NaCl solution in a greenhouse setting for 71 days, 'Dawson' and 'Golfrood' slender creeping red fescues received ratings of 2.4 and 2.5, respectively, on a scale where 1 represented ideal turf and 9 represented all dead tissue (Ahti et al., 1980). That same study judged several cultivars of strong creeping red fescue to be moderately salt tolerant but deemed hard fescue and Chewings fescue to be not salt tolerant.

Physiological adaptation to saline environments in the red fescues has been demonstrated elsewhere as well. Venables and Wilkins (1978) showed high correlation between the salinity of the soil from which plant material was collected and root growth when those plants were grown in nutrient culture solution amended with sodium chloride. Furthermore, plants from all soils showed growth increases in response to the addition of up to 100 mM NaCl. Humphreys (1981) evaluated several ecotypes and cultivars of all three species of red fescue in both field and greenhouse settings. In that study, spray treatments of three different salt levels in three different amounts of water were applied to five ecotypes and three commercially available cultivars of red fescue. Applications were made three times per week for four weeks, and dry weight of green and dead tissue were recorded. Significant differences were recorded between the entries in the trial with some coastal ecotypes producing 22% green tissue at the end of treatments as compared to 'S.59', which produced the highest percent green tissue of all commercial cultivars with 8%. Field trials in the same experiment also showed that the coastal ecotypes far outperformed the commercial cultivars in terms of tissue survival under ocean salt spray stress. The level of variability observed in previous salt tolerance trials has also been observed in newer accessions from breeding programs where salt tolerant lines have accumulated 43.8% and 73.8% less $Na^+$ in crowns and new leaves, respectively, as compared to salt sensitive lines (Krishnan and Brown, 2009). The authors of that study concluded that, as with other grasses, the salt tolerance of red fescues likely lies in its ability to exclude $Na^+$ from the xylem stream. From results such as these, it is clear that there exists significant potential to improve the salt tolerance of red fescue, and possibly other fine fescue species, through incorporation of germplasm from salt tolerant ecotypes into breeding programs.

Despite the encouraging observations noted above, other authors have recommended against the use of red fescue on roadsides due to its inability to maintain green color following 5 weeks of salt treatments applied as 20 mL of 2.65 M NaCl solution, 3 times weekly (Greub et al., 1985). It should be noted, however, that the 'Ruby' strong creeping red fescue used in that study produced just a 7% decrease in dry shoot mass as compared to a no-salt control indicating that it likely remains a viable option from a functionality standpoint. That is, it may still maintain ground cover and provide functional erosion control.

It is clear that ongoing evaluation of new cultivars for salt tolerance and roadside use is imperative. Introduction of salt tolerant cultivars is one of the most efficient ways to improve turfgrass growth in salt-stressed conditions (Qian et al., 2001). Therefore, researchers have evaluated recent breeding progress for salt tolerance on roadsides and in greenhouses by comparing commercially available cultivars for salt tolerance during germination and vegetative growth. Evaluation of the germination potential of roadside grasses is important in that establishment of turfgrass sod is not always economically or practically feasible.  In those cases, establishment of seed mixtures must provide an equivalent turf quality. However, salt tolerance during germination appears to be governed by a different mechanism than during vegetative growth (Rose-Fricker and Wipff, 2001). Liem et al. (1985) compared germination percentages of seeds of several grasses including 'Moncorde' and 'Mary' Chewings fescues and 'Biljart' hard fescue. In each case, germination rates greater than 75% were obtained in solutions containing NaCl concentrations up to 10 g $L^{-1}$; however, significant reductions were observed at 20 g $L^{-1}$.

Rose-Fricker and Wipff (2001) germinated seeds of ‘Discovery’ hard fescue, and ‘Seabreeze’ and ‘Dawson’ slender creeping red fescues in solutions of increasing salinity. ‘Seabreeze’ attained 74.93% germination at salinity levels as high as 15,000 ppm while ‘Discovery’ had no germination at the same salinity, indicating differences between species in germination ability under saline conditions. Interestingly, ‘Dawson’, which is considered to be salt tolerant during vegetative growth, also had low germination percentages at 15,000 ppm salt indicating not only that significant differences can exist within fine fescue species, but that the cultivars used in seed mixes may not be equally well-suited for establishment as sod in saline soils. Brown and Gorres (2011) evaluated several cultivars of both *Festuca rubra* ssp. *rubra* and *Festuca rubra* ssp. *litoralis*, among other grasses, on roadsides in Rhode Island. The authors found that although *Festuca rubra* L. entries as a whole outperformed other species in non-amended soil, there was no clear advantage to the use of improved cultivars over common type and that low fertility was the main cause of lack of turfgrass persistence on roadsides. Friell et al. (2013a) evaluated several cultivars of strong creeping red fescue, slender creeping red fescue, sheep fescue, Chewings fescue, and hard fescue in nutrient solution culture and observed that all cultivars of slender creeping red fescues provided excellent salt tolerance. Although no significant difference between cultivars was found, ‘Sealink’ and ‘Seabreeze GT’ slender creeping red fescues had the greatest percentage of green tissue following exposure to 24 dS $m^{-1}$ salinity and all cultivars maintained greater than 50% green tissue. In that study, Chewings fescue was found to be not salt tolerant, but several cultivars of sheep fescue and blue-hard fescue as well as ‘Beacon’ hard fescue were found to be moderately salt tolerant. When the same cultivars were evaluated on roadsides, however, ‘Shoreline’ slender creeping red fescue, ‘Navigator’ strong creeping red fescue, and an advanced population of sheep fescue were among the most persistent in areas where exposure to sodium chloride was known to be a problem in the establishment of quality roadside turfgrass (Friell et al., 2012).

A significant amount of research has been performed to evaluate and improve the salt tolerance of fine fescues for roadside use. Species in the *Festuca rubra* complex, and specifically *Festuca rubra* ssp. *litoralis*, are generally considered to have superior salt tolerance to those in the *Festuca ovina* complex; however, sheep fescue, in particular, has shown promise as a suitable roadside turfgrass. The body of literature regarding the salt tolerance and roadside establishment of species in the *Festuca ovina* complex is considerably more limited than that for the *Festuca rubra* complex, indicating that further efforts to expand it may be useful. This is especially true in light of the fact that blue, sheep, and hard fescues are all known to have exceptional low maintenance characteristics and show promise for roadside establishment (Henensal et al., 1980; Ruemmele et al., 1995; Watkins et al., 2011; Friell et al., 2012).

### Bentgrasses

The genus *Agrostis* contains approximately 200 species, five of which have been adapted for turfgrass use, including creeping bentgrass (*Agrostis stolonifera* L.), velvet bentgrass (*Agrostis canina* L.), colonial bentgrass (*Agrostis capillaris* L.), dryland bentgrass (*Agrostis castellana* L.), and redtop bentgrass (*Agrostis gigantea*) (Warnke, 2003). Another minor *Agrostis* species, Idaho bentgrass (*Agrostis idahoensis* Nash) has also been developed in recent years for turf use (Brede, 1999). Of these grasses, three species are thought to have particular adaptation to roadside use and a potential for increased salt tolerance. *Agrostis stolonifera*, is highly stoloniferous, is primarily adapted to cool, humid regions, and, despite being adapted to moderately acidic soils, is generally considered to have good salinity tolerance (Warnke, 2003). Native to western Europe where it can at times be found in the upper regions of salt marshes and in adjacent non-saline fields, *Agrostis stolonifera* was first introduced to the United States as a component of a mixture known as South German Mixed Bentgrass (Duich, 1985). *Agrostis capillaris*, has a variable growth habit often exhibiting weakly stoloniferous and rhizomatous growth habits (Hitchcock and Chase, 1951; Fry and Huang, 2004; Christians, 2011). It is native to Europe and temperate Asia, and was introduced to the

United States by immigrants from England (Madison, 1971). However, taxonomic classification of this species has proven difficult in part due to differences between European and North American naming (Hitchcock and Chase, 1951). *Agrostis idahoensis*, has only recently been introduced for use as a turfgrass. Exhibiting a bunch-type growth habit, it is native to North America and is found in mountain meadows at medium to high altitudes throughout the western United States (Brede and Sellmann, 2003). The first commercially available cultivar of Idaho bentgrass, ‘GolfStar’, was introduced in 1999 and was bred from accessions collected from a heavily polluted river basin growing alongside redtop bentgrass with which it shares many characteristics (Brede, 1999). It is perhaps due to this habitat of origin that Idaho bentgrass is considered to have considerable adaptation to low maintenance, alkaline, saline, and heavy metal-impacted sites (Brede and Sellmann, 2003).

There exists a long history of using *Agrostis* species on roadsides in both Europe and North America. Early seed mixtures in Germany included both *Agrostis alba* and *Agrostis tenuis* Sibth.(Boeker, 1970). Boeker (1970) recommended the use of *Agrostis tenuis* at a minimum of 10% by mass in seed mixtures for roadsides, and noted that the species was capable of increasing its relative proportion over time during the succession process. Langvad and Weibull (1970) pointed out that until 1963, *Agrostis tenuis* was also one of the species generally used on roadsides in Sweden. The extensive use of these grasses in low-maintenance mixtures was likely due to their ability to form a short, dense canopy and in some cases an adaptation to wet, acidic soils (Duell and Schmit, 1974; Shildrick, 1980). Henensal (1980) found that *Agrostis tenuis* performed very well on roadsides in France, except under prolonged drought conditions. However, Trautman and Lohmeyer (1980) found that multiple *Agrostis* species did not fare well on roadsides in Germany, and were in significant decline within six years of establishment.

Clearly, there is disagreement in the literature as to the applicability of *Agrostis* species in creating sustainable roadside vegetation. In part, the questionable success of those species on roadsides may be attributable to variable tolerance to alkaline soils, and thus the salt tolerance of the species. *Agrostis stolonifera*, for example, may be well-adapted to salt exposure as it can be found growing in the upper levels of salt marshes and brine-flooded pastures (Hannon and Bradshaw, 1968; Venables and Wilkins, 1978). However, even in populations collected from sites such as these, significant variation in salt tolerance has been observed and may be determined, in part, by the presence or absence of suitable selective pressures (i.e. saline soils) which can lead to the evolution of a salt tolerant population (Ashraf et al., 1986). This is evident in the results of modern breeding programs where significant variability between and within species has been demonstrated. Liem et al. (1985) observed that the effect of deicing salt on seed germination was significant for both *Agrostis stolonifera* and *Agrostis capillaris*; however, the effect was seemingly much greater for *Agrostis capillaris* with germination dropping below 25% in 10 g $L^{-1}$ NaCl solution. Greub et al. (1985) also showed *Agrostis stolonifera* to be more salt tolerant than *Agrostis alba* when, after five weeks of weekly irrigation with 20 mL of 2.65 M NaCl solution, 'Seaside' creeping bentgrass was visually assessed to retain 35.6% more living green tissue as compared to *Agrostis alba*. This type of diversity was also observed by Marcum (2001) in a comparison of 35 *Agrostis* cultivars. In that study, *Agrostis stolonifera* was found to be more tolerant than *Agrostis canina* which was more tolerant than *Agrostis capillaris*; however, only a single cultivar of each of the latter two species were tested in the experiment. Nonetheless, the range of tolerances even within the *Agrostis stolonifera* species was such that, after growing for 10 weeks in a nutrient solution amended to 8 dS $m^{-1}$ with 75% NaCl : 25% $CaCl_2$ (w/w), root dry weights ranged from 59 g to 170 g. That level of diversity in the genus *Agrostis* led the author to conclude that there exists significant potential for breeding more salt tolerant cultivars of bentgrass.

Field and roadside experiments have further demonstrated the applicability of *Agrostis* species to saline environments. Foliar application of a 10 dS $m^{-1}$ saline solution over the span of two years showed significant differences in new cultivars of *Agrostis stolonifera*, *Agrostis capillaris*, and *Agrostis canina* (Koch and Bonos, 2011a). That study also showed creeping bentgrass to be more salt tolerant than either colonial or velvet bengrass. The authors of that study noted that field based methods for salinity tolerance screening correlate well with greenhouse methods, but have the additional benefit of including confounding factors such as heat and drought stress. Furthermore, this method of screening may be more applicable for roadside application as the majority of salt exposure may occur when soils are frozen or plants are dormant and foliar salt spray is the predominant form of salt exposure. Indeed, other authors have concluded that the major limiting factor for persistence of turfgrass on roadsides is fertility, not salinity, and that differences between cultivars on roadsides are irrelevant in terms of persistence (Brown and Gorres, 2011). Some evidence would suggest, however, that differences between cultivars of *Agrostis stolonifera* do exist when established in saline soils on roadsides and that the importance of proper cultivar selection can be demonstrated on saline roadsides even where no difference exists in greenhouse screenings for salt tolerance (Friell et al., 2012, 2013a). Those same studies found *Agrostis idahoensis* to be unsuitable for roadsides despite its reputation for adaptation to alkaline and low-maintenance sites.

A great amount of variation exists in *Agrostis* species for both their salt tolerance and roadside applicability. *Agrostis alba* and *Agrostis capillaris* have both been used extensively, previously, in roadside applications to produce a low-growing, dense turf capable of preventing soil erosion. However, new evidence suggests that *Agrostis stolonifera,* and specifically ‘Seaside’, ‘Seaside II’, and ‘Mariner’ cultivars, may have greater salt tolerance than the other *Agrostis* species and may be well-suited for roadside use. Due to the highly diverse salt tolerance and the compatibility of many of the *Agrostis* species, there is significant potential for improved salt tolerance within the genus in the future.

## Alkaligrass

The name alkaligrass is a generic one that has been assigned to species of the genus *Puccinellia*. In particular, the species weeping alkaligrass [*Puccinellia distans* (Jacq.) Parl.], seaside alkaligrass [*Puccinellia maritima* (Huds.) Parl.], and Lemmon alkaligrass [*Puccinellia lemmoni* (Vasey) Scribn.], and Nuttall alkaligrass [*Puccinellia airoides* (Nutt.) Wats & Coult.] have been examined for their turfgrass characteristics. Nutall, weeping, and Lemmon alkaligrasses were determined by Fults (1972) to have the greatest value for turfgrass use. Alkaligrass typically takes a cespitose form, but may have spreading stolons which root at the nodes. Due to the polymorphisms observed taxonomic classification of species within the genus can be difficult (Gray and Scott, 1977). However, the genus is often typified by the species *Puccinellia distans* (Hitchcock and Chase, 1951). Considered to be halophytes, distribution of species in the genus is typically restricted to salt marshes and saline soils in coastal regions and to altitudes lower than a few meters above sea level (Gray and Scott, 1977).

This adaptation to saline environments has generated great interest in the use of alkaligrasses for roadside vegetation. The invasion and distribution patterns of *Puccinellia distans* on roadsides due to road salt use in Britain have been previously described (Scott and Davison, 1985). Moreover, *Puccinellia distans* has been found growing along highways in Illinois where salt had destroyed other vegetation, and it has been noted that the ability of alkaligrass to withstand large amounts of soluble salts for extended periods of time may have great importance in road salt-affected areas where standing brackish water may occur in roadside basins on frozen ground (Butler et al., 1974). To that end, much research has focused on evaluating the applicability and salt tolerance of alkaligrasses for use on roadsides. Sanks (1971) noted that *Puccinellia distans* incurred minimal visual damage when treated for 8 weeks with NaCl solutions for an effective application rate of 38.2 tons lane-km$^{-1}$. Furthermore, Hughes et al. (1975) observed that after 8 weeks of NaCl solution applications at equivalent rates of up to 65,131 kg ha$^{-1}$ NaCl, forage yield of *Puccinellia distans* was reduced by just 23% as compared to a minimum of 40% for other species tested. Friell et al. (2012, 2013a) evaluated four cultivars of alkaligrass, representing both *Puccinellia distans* and *Puccinellia maritima,* on roadsides and in nutrient solution culture. The authors observed that on highway roadsides, alkaligrass incurred no visual damage following winter road salt application and, in nutrient solution culture, maintained nearly 50% green tissue after prolonged exposure to salinity levels as high as 24 dS $m^{-1}$. Ahti et al. (1980) found that 'Fults' weeping alkaligrass remained green, healty, and vigorous after 90 days exposure to subirrigation with a 0.8% NaCl solution, but did not identify the maximum salt stress that the alkaligrass could withstand. It is often the case, however, that an extended time of exposure can provide a more sensitive indicator of stress tolerance, indicating that the authors of that study likely provided a better estimate of the salt tolerance than others (Munns and Tester, 2008). Greub et al. (1985) made five weekly applications of 20 mL of 2.65 M NaCl solution which caused just a 10% and 9% decrease in shoot dry matter yield for *Puccinellia distans* and *Puccinellia airoides*, respectively, but resulted in a 21% increase for *Puccinellia lemmoni,* indicating that some species of alkaligrass may have a significant competitive advantage for growth in saline soils.

Despite the demonstrated advantage from growth in saline soils, alkaligrass is not considered to be an obligate halophyte; rather, its lack of presence in non-saline areas is likely due to lack of competitive ability over non-halophytic species in those areas (Macke and Ungar, 1971). Indeed, other studies have indicated that exposure to other stresses such as mowing, low fertility, and drought cause significant decline in stand quality for alkaligrass (Watkins et al., 2011). A number of authors have speculated that one of the main advantages of alkaligrasses is prolific seed production, which allows them to colonize disturbed soils quickly (Butler et al., 1974; Biesboer et al., 1998). Furthermore, alkaligrasses have been found to have exceptional seed germination and seedling survival rates in saline conditions (Macke and Ungar, 1971; Hughes et al., 1975; Tarasoff et al., 2007).

Alkaligrass may have great value for use as a roadside turfgrass due to its above average tolerance to soluble salts in soil. However, a lack of tolerance to other stresses as well as a relative shortage of cultivars for any given *Puccinellia* species may indicate need for further development through focused breeding programs as well as evaluation of its adaptability for roadside sod establishment.

## Other Turf Species

Two other turfgrass species, prairie junegrass [*Koeleria macrantha* (Ledeb.) Schult.] and tufted hairgrass [*Deschampsia cespitosa* (L.) P. Beauv.], have been evaluated for roadside use in recent years. Much of the increased interest in their implementation stems from evaluations of their potential as a low-maintenance turfgrass species. *Koeleria macrantha* is a bunch-type grass with widespread distribution throught Europe, North America, Asia, and parts of Africa, extending to as far north as 62° in parts of Asia (Dixon, 2000). It is described by Hitchcock and Chase (1951) to be a constituent of native pasture land throughout the western United States but can also be found in open woods and on sandy soils. Taxonomic classification of species within the genus *Koeleria* has proven difficult with some species being confused by unique naming conventions used for North American and European populations. It is generally thought that *Koeleria macrantha* is in fact the correct taxonomic classification for the species found to be widespread on both continents (Arnow, 1994). *Deschampsia cespitosa* is a dense bunch-type grass species with circumboreal distribution showing phenotypic adaptation, with differentiation often occurring along both latitude and elevation gradients (Pearcy and Ward, 1972; Davy, 1980). Often an active colonizer of disturbed sites, *Deschampsia cespitosa* is found to dominate habitats including those with moist but not flooded soils, bogs, salt marshes, poorly drained flats and basins of higher elevation forests and often on nutrient poor soils (Hitchcock and Chase, 1951; Rothera and Davy, 1986; Chambers, 1989; Barbour and Billings, 2000). The species is self-incompatible and races of several different ploidy levels have been found (Davy, 1980; Rothera and Davy, 1986). Perhaps the most notable characteristic of the species, however, is a demonstrated tolerance to multiple soil contaminants which, along with its ability to colonize disturbed sites, indicate promise for successful establishment in contaminated soils, such as those found on roadsides (Cox and Hutchinson, 1980).

Both species have been evaluated for their potential as low-maintenance turfgrasses under a number of conditions and shown promise as low-input species (Watkins et al., 2011). Still, evaluations of these species salt tolerance and adaptability for roadside use have been limited. One species of *Deschampsia*, *D. flexuosa* (L.) Trin., was in use on German roadsides as of 1968, but to what extent is not clear. Recently, Brown and Gorres (2011) evaluated *Deschampsia cespitosa* on roadsides in Rhode Island, USA and found it had poor persistence in non-amended soils. Moreover, the authors concluded that the species was not adapted to roadside use and offered no advantage over traditional roadside species. Wang et al. (2011) drenched pots of turfgrass in NaCl solutions of 0, 5, 10, 15, or 20 g $L^{-1}$ for 20 min. each week for 5 weeks and measured electrolyte leakage, tissue dry weight, and visual quality ratings to assess differences in salt tolerance between populations of *Koeleria macrantha.* Their results showed superior salt tolerance of the improved European varieties 'Barkoel' and 'Barleria' and indicated the possibility for improvement in the native North American populations. Friell et al. (2012) evaluated one cultivar and one ecotype of both *Deschampsia cespitosa* and *Koeleria macrantha* on roadsides in Minnesota, USA at locations where exposure to NaCl was a known problem for the establishment of turfgrass. The results of that study agreed with Brown and Gorres (2011) in that none of the entries were found to persist well on roadsides in non-amended soils. A greenhouse evaluation of the same cultivars and ecotypes found that after extended exposure to NaCl solutions up to 24 dS $m^{-1}$, all entries lacked sufficient salt tolerance to be suitable for roadsides; although the results of that study also found the Minnesota population of *Koeleria macrantha* to be less salt tolerant than the improved European cultivar 'Barkoel' which is in agreement with Wang et al. (2011) and indicates potential for improvement within the North American populations.

Overall, evaluation of *Deschampsia cespitosa* and *Koeleria macrantha* for salt tolerance and adaptation to roadside use is lacking. To date, however, results of all existing studies are in general agreement that neither species is well-adapted to roadside use and that both lack sufficient salt tolerance to be of use in regions where exposure to road salt is a known impediment to establishment of turfgrass as sustainable and functional roadside vegetation.

## Species Mixtures

It is clear from the results of the studies discussed above that each species has unique attributes that make it more or less well-adapted to roadside environments. However, as noted by Duell and Schmit (1974), cultivars and species that appear to be promising in the early stages of evaluation may not be the best for long-term vegetative cover, which is one of the requirements for construction of what the authors referred to as "the complete highway." This is, in part, because roadsides present significant challenges to growing turfgrass due to stressful conditions that can be unique in both form and magnitude. These stresses may include salt exposure, drought, low fertility, disease, or exposure to contaminants in runoff water. It is likely a mixture that is capable of taking advantage of the unique tolerances of each species will produce the most sustainable and functional stand of turfgrass for roadside vegetation.

It is well documented that multi-species assemblages are needed to maintain a high-functioning ecosystem (Tilman et al., 2001; Zavaleta et al., 2010; Isbell et al., 2011). Indeed, this concept extends to turfgrass communities (Watschke and Schmidt, 1992). Furthermore, it is clear that turfgrass species mixtures can provide several advantages compared to single species plantings. Mixtures of Kentucky bluegrass (*Poa pratensis* L.) and perennial ryegrass (*Lolium perenne* L.) have been shown to produce greater ground cover, higher shoot density, and faster green-up in the spring than either species planted alone (Brede and Duich, 1984a). Dunn et al. (2002) reported that mixtures including tall fescue (*Festuca arundinacea* Schreb.) often provided superior turf quality as compared to blends of only tall fescue varieties or only Kentucky bluegrass varieties, an observation which they attributed to superior disease resistance of the mixture as compared to either component species. The same study also reported superior resistance to the disease dollar spot caused by *Sclerotinia homeocarpa* in mixtures of perennial ryegrass, Kentucky bluegrass, and tall fescue. Interestingly, they noted that resistance declined over time due to the superior competitive ability of perennial ryegrass, which was the species primarily affected by the disease. As tall fescue and Kentucky bluegrass declined and were displaced by perennial ryegrass over time, disease severity increased. Such results are indicative of the fact that constituent species proportions are often among the most influential factors governing species mixture performance.

Shildrick (1980) evaluated mixtures of perennial ryegrass, timothy grass (*Phleum pretense* L.), Kentucky bluegrass, strong creeping red fescue (*Festuca rubra* ssp. *rubra*), Chewings fescue (*Festuca rubra* var. *commutata*), and colonial bentgrass (*Agrostis tenuis* L.) and observed that the proportions of each component species in the mixture largely determined its overall wear tolerance when subjected to “football-type” wear. Similarly, Shildrick (1982) also identified significant differences in sod strength and sod quality between mixtures of creeping bentgrass (*Agrostis stolonifera* L.), colonial bentgrass, Chewings fescue, slender creeping red fescue (*Festuca rubra* ssp. *litoralis*), and Kentucky bluegrass with varying proportions of each species.

Brede and Duich (1984b) observed significant reductions in visual shoot size classification when mowing began more than three weeks after seeding for mixtures of Kentucky bluegrass and perennial ryegrass that contained less than 75% Kentucky bluegrass by seed count. Furthermore, mixtures cut at 3.8 cm required at least 95% Kentucky bluegrass by seed count in the mixture in order to produce a turf stand containing equal proportions of Kentucky bluegrass and perennial ryegrass shoots at two months after seeding. Conversely, turfgrass stands mowed at 1.3 cm required just 50 to 75% Kentucky bluegrass seed in the mix to produce the same result. It is difficult to say, however, where in that range the true necessary proportions lie given that only three proportions of Kentucky bluegrass were included in the set of mixture compositions. Similarly, Stier et al. (2005) evaluated seed mixtures of Kentucky bluegrass and perennial ryegrass in varying proportions from 25% to 95% Kentucky bluegrass by weight. Mixtures containing 95% Kentucky bluegrass resulted in a greater amount of that species in the final botanical composition as compared to all other mixtures. Final compositions for the 95% Kentucky bluegrass mixtures ranged from 40% to 70% Kentucky bluegrass but mixtures containing 25% Kentucky bluegrass never produced more than 15% of that species in the sward composition. They also observed that when common types of Kentucky bluegrass were used, no more than 10% of the resulting sward was Kentucky bluegrass regardless of the initial seed proportions. Unfortunately, the study was limited in scope to athletic field applications and traffic simulation was applied to all of the plots. The authors stated that since many athletic field managers overseed, short-term competition may be more relevant than long-term competition. For roadside vegetation, however, long-term studies are of the utmost importance and although mowing is not often scheduled on an agronomic basis, it is certain that analogous relationships exist between seed count, mowing height, and timing of mowing in roadside ecosystems.

In a longer-term evaluation, Hsiang et al. (1997) analyzed the population dynamics resulting from interspecies competition in mixtures of tall fescue, Kentucky bluegrass, perennial ryegrass, and a sub-mixture of Chewings fescue and strong creeping red fescue over four years under non-wear conditions. Although not performed on roadsides, the trial was conducted in what the authors referred to as low-maintenance cultural conditions, which consisted of irrigation to prevent drought stress and nitrogen applications ranging from 50 to 100 kg ha$^{-1}$. The authors found that tall fescue generally decreased from its initial seeding ratio while perennial ryegrass generally increased from its initial seeding ratio when measured four years after establishment. The fine fescue mix and Kentucky bluegrass, however, appeared to increase or decrease to a stable equilibrium proportion of 24% for fine fescues and 42% for Kentucky bluegrass.

In a field trial of a mixture containing Kentucky bluegrass, perennial ryegrass, and colonial bentgrass in a 10:3:2 ratio, respectively, perennial ryegrass was found to decrease from 33% to less than 5% while Kentucky bluegrass increased from 22% to approximately 50% of the sward after 4 years when seeded at 48 kg ha$^{-1}$ (Engel and Trout, 1980). These results verify that even moderate competition can significantly delay the establishment of Kentucky bluegrass in a mixture. The same study showed that increased total seeding rates increased competition and further delayed the establishment of the Kentucky bluegrass.

Larsen et al. (2004) found that when Kentucky bluegrass, slender creeping red fescue, and perennial ryegrass were sown together, the per cent of total tillers recorded for each species were 31.4%, 57.7%, and 11.5% for slender creeping red fescue, perennial ryegrass, and Kentucky bluegrass, respectively. This was despite a species composition in the seed mixture of 30% slender creeping red fescue, 40% perennial ryegrass, and 30% Kentucky bluegrass, by mass.

Juska and Hanson (1959) found that during the first four years of a five year study, ‘Merion’ Kentucky bluegrass planted in monoculture provided the best turf quality as compared to other monocultures and mixtures of Kentucky bluegrass, tall fescue, red fescue, and colonial bentgrass. During the fifth year, however, quality of the Merion monostand declined due to disease. As a result, the polyculture of red fescue and Merion provided the highest quality turf over the 5-yr period. Such results are the basis of the argument for greater biodiversity in vegetative swards and the push to establish turfgrass mixtures rather than monocultures.

With this in mind it is clear that evaluation of species mixtures should play an important role in identifying vegetation suitable for roadsides. Unfortunately, most previous mixture experiments rely on selection of evenly-spaced or convenient proportions of component species and identify the best mixture entry in the trial. This provides little or no predictive ability regarding what the optimal species mixture is, should it not be one of the mixtures tested. Designs for mixture experiments have been well defined and should be used to achieve experimental results that provide for prediction and optimization of overall mixture performance as a function of species composition with respect to desirable traits (Scheffé, 1963; Bondari, 2005).

**Table 1**. Qualitative summary of salt tolerance and general roadside performance of turfgrass species discussed in the literature review, based on consensus of existing literature.

| Species | Scientific Name | Salt Tolerance[†,‡] | Roadside Performance[†,‡] |
|---|---|---|---|
| Kentucky bluegrass | *Poa pratensis* | - | - |
| perennial ryegrass | *Lolium perenne* | 0 | -- |
| tall fescue | *Festuca arundinacea* | + | + |
| slender creeping red fescue | *Festuca rubra* ssp. *litoralis* | ++ | ++ |
| strong creeping red fescue | *Festuca rubra* ssp. *rubra* | + | + |
| hard fescue | *Festuca trachyphylla* | 0 | ++ |
| sheep fescue | *Festuca ovina* | 0 | ++ |
| Chewings fescue | *Festuca rubra* ssp. *fallax* | -- | - |
| creeping bentgrass | *Agrostis stolonifera* | + | 0 |
| colonial bentgrass | *Agrostis tenuis* | 0 | + |
| redtop bentgrass | *Agrostis alba* | - | + |
| alkaligrass | *Puccinellia* spp. | ++ | - |
| prairie junegrass | *Koeleria macrantha* | - | -- |
| tufted hairgrass | *Deschampsia cespitosa* | - | -- |

[†] (--) – Very poor; (-) – Poor; (0) – Moderate; (+) – Good; (++) – Very good

[‡] Ratings based on a qualitative overview of existing literature, as described in this review. See text for citations.

# Chapter 2: Establishment and survival of 75 cool-season turfgrass cultivars on Minnesota roadsides

## Summary

Roadside vegetation is subject to several abiotic stresses including drought, heat, and compaction as well as exposure to deicing salt in cold-weather climates. Evaluation of cool-season turfgrass germplasm on roadsides is necessary to identify cultivars suitable for use as roadside vegetation. The objective of this research was to evaluate a wide variety of cultivars and selections of cool-season turfgrass for their ability to establish and persist on roadsides in Minnesota. Three replications of 75 cool-season turfgrass cultivars were established in a randomized complete block design at two locations: Roselawn Cemetery (Roseville, MN, USA) and MnROAD research facility (Albertville, MN, USA). Plots were seeded during August and September of 2010. Plots were visually assessed for establishment during the fall of 2010, and for survival in the spring of 2011. Differences between sites for establishment appeared to follow edaphic characteristics such as compaction and soil moisture. Top statistical groupings for mean establishment ratings contained numerous cultivars of perennial ryegrass (*Lolium perenne* L.) at all sites. Cultivars of alkaligrass (*Puccinellia* spp.), including ‘Fults’, ‘Salty’, ‘Oceania’, and ‘Salton Sea’, had the highest mean survival ratings at Albertville where salt application rates were highest and runoff flowed directly onto plots. Cultivars of several fine fescue species had the highest mean survival ratings at Roseville. Among them were ‘Shoreline’ slender creeping red fescue (*Festuca rubra* L. ssp. *litoralis*), ‘Beacon’ hard fescue [*Festuca trachyphylla* (Hack.) Krajina], and ‘Marco Polo’ sheep fescue (*Festuca ovina L.*), which had the highest mean survival ratings of cultivars in their respective species at Roseville. These results may be used to guide the decisions of public works employees and turfgrass breeders in the implementation and improvement of roadside turfgrasses.

## Introduction

Cool-season turfgrasses have been used in adverse environments such as roadsides with limited success. Many extreme stresses, including drought, heat, and compaction are often present and may contribute to the poor performance of turfgrasses on roadsides. In cold-weather climates, exposure to sodium chloride from road deicing practices also presents a significant challenge to the establishment and maintenance of high quality turfgrass. Turfgrass that is sown in the fall often does not survive until the following spring, and it is commonly thought that salt exposure plays a major role in the extensive turf death observed. Sodium concentrations of greater than 10,000 mg $kg^{-1}$ were reported in Minnesota in soils sampled to a depth of 20 cm within one meter of the roadway on interstate highways during the month of December (Biesboer and Jacobson, 1994). Turfgrasses planted in that type of roadside location must not only withstand the unsuitable growth conditions, but they must do so while also surviving the perennial application of deicing salt.

Although salt tolerance is a vital characteristic of roadside turfgrass in areas subject to deicing salt exposure, it has been shown to be interdependent with other growth factors. For example, Bowman et al. (2006a) demonstrated that the effect of sodium chloride (NaCl) and calcium chloride ($CaCl_2$) on tall fescue (*Festuca arundinacea* Schreb.) leaf growth is less severe with reduced nitrogen availability. Moreover, Brown and Gorres (2011) evaluated several cultivars on roadsides in Rhode Island. The authors rejected the hypothesis that exposure to deicing salt was primarily responsible for death of turfgrass on roadsides and concluded that soil fertility was the determining factor for long-term persistence. They found that although *Festuca rubra* L. entries as a whole outperformed other species in non-amended soil, there was no clear advantage to the use of improved cultivars over common type. Such results suggest that overall roadside performance of turfgrasses should not be predicted by an evaluation of salt tolerance alone, and must be evaluated on roadsides under realistic management, environmental, and edaphic conditions that more closely represent the intended application.

Most previous roadside turfgrass research has evaluated species and cultivars for roadside performance with respect to their ability to withstand many of the other abiotic stresses present on roadsides, but not necessarily in the presence of exposure to deicing salts. Boeker (1970) recommended the use of both *Festuca rubra* and *Festuca ovina* in roadside mixtures due to their adaptation to a large range of soil and moisture conditions, widespread distribution, and the ability for *Festuca rubra* to recolonize gaps in the canopy via its rhizomatous growth habit. Henensal et al. (1980) found that slender creeping red fescue, hard fescue, and colonial bentgrass (*Agrostis tenuis* Sibth.) were best for roadsides in France, but noted that a lengthy drought adversely affected all species. Duell and Schmit (1974) evaluated several species and cultivars of fine fescues for roadside performance as determined by assessing retention of green color during drought and spring green up. Of the grasses evaluated, ‘C.P. Shade’ strong creeping red fescue and ‘Alaska Station’ sheep fescue provided the best color during drought and ‘Ruby’ strong creeping red fescue provided the best spring color. Overall, the authors noted that sheep and hard fescues were notably late in spring recovery and slender creeping red fescue and strong creeping red fescue (*Festuca rubra* L. ssp. *rubra*) were slightly better than the Chewings fescues [*Festuca rubra* ssp. *fallax* (Thuill.) Nyman]. In that same study, the authors evaluated seven cultivars of common and turf-type Kentucky bluegrass for seed stalk production and color retention during summer drought on newly-established roadsides in New Jersey. They showed that common-type Kentucky bluegrass produced fewer seed stalks, but retained better green color during summer drought than the newer, turf-type cultivars. The authors concluded that poor soil conditions and minimal management practices create unsuitable conditions for use of fine turf-type cultivars of Kentucky bluegrass.

To overcome these challenges, turfgrass breeders strive to continuously improve germplasm such that plants are able to better tolerate several biotic and abiotic factors.

For example, newer breeding lines of perennial ryegrass have shown improved salt tolerance over old cultivars, such as ‘Linn’. Improvement has largely been attributed to an ability to accumulate up to 37% less $Na^+$ in crowns and 42% less $Na^+$ in young leaves while maintaining a $K^+/Na^+$ ratio 1300% higher (Krishnan and Brown, 2009). Moreover, several studies have demonstrated remarkable performance of numerous species and cultivars under a wide variety of reduced water and fertility conditions (Diesburg et al., 1997; Dernoeden et al., 1998; McKernan et al., 2001; Watkins et al., 2011).

In light of results such as these, it is important to evaluate the existing germplasm of cool-season turfgrasses for the ability to establish and persist in roadside environments where several stresses may occur concurrently or sequentially. Only once high-performing cultivars and species are identified can suitable roadside turfgrass be established and maintained. Therefore, the objective of this work was to evaluate a wide variety of cultivars and selections of cool-season turfgrass for their ability to establish and persist on roadsides in Minnesota. This was to be accomplished by visually assessing establishment following late summer sowing of 75 cultivars of cool-season turfgrass on roadsides in Minnesota, and subsequently visually assessing survival the following spring.

## Materials and Methods

### Locations

Plots were established during August and September of 2010 at two locations in the metropolitan area surrounding Minneapolis, MN, USA.  The selected research sites were: (1) Larpenteur Ave. along Roselawn Cemetery (Roseville, MN, USA) and (2) I-94 at MnROAD research facility (Albertville, MN, USA). Sites were chosen to represent distinct salt application levels, traffic volumes, runoff drainage patterns, and soil types based on information provided by the Minnesota Department of Transportation. The Roseville site was split into two sections: 1) main plot – the area opposite the sidewalk from the road measuring 1.52 meters wide by 4.57 meters deep, and 2) boulevard – the

1.52 m by 0.91 m area between the sidewalk and the road. Data for these two locations was collected separately and they were treated as distinct experimental sites due to differences resulting from the presence of a curb and storm drains on the street to which all runoff water was designed to drain, sidewalk maintenance practices, and proximity to the road. As a result, there was a total of three data sets collected and analysed in this experiment.

The portion of Larpenteur Ave. along Roselawn Cemetery is a four-lane residential street, which carries a total estimated daily traffic volume of 13,700 vehicles, and Interstate 94, which runs along the MnROAD research facility, is a four-lane divided highway that carries an estimated daily traffic volume of 63,000 vehicles (Minnesota Department of Transportation, 2010). Mean temperatures for Roseville and Albertville, MN were 17°C and 16°C, respectively, during the establishment period of August through October, 2010. Those temperatures are similar to the 1961 to 1990 regional average of 15.5°C for those three months. Precipitation during that same period totalled 34.7 cm and 33.27 cm for the Roseville and Albertville sites, respectively, as compared to the 30-year regional average of 21.7 cm. At the Albertville and Roseville sites, 21% and 23% of that precipitation fell during the first two weeks after seeding. Sunlight levels varied throughout the year and across locations, although all plots received direct sunlight for the majority of the day. The Albertville site was north-facing with an approximately 1:6 slope. Both sections of the Roseville site were primarily flat. Although salt application records were not available from the Minnesota Department of Transportation for the winter of 2010, both roads were regularly salted before, during, and after winter storm events at a minimum standard application rate ranging from 11.4 to 56.8 kg lane$^{-1}$ km$^{-1}$ with the Albertville site receiving a greater volume annually.

## Turfgrass Cultivars and Selections

Treatments in the trial were cultivars and selections. Trial entries were selected based on input from turfgrass breeders and published data from previous trials throughout the northern United States (Rose-Fricker and Wipff, 2001; Biesboer et al., 1998; Duell & Schmit, 1974; Boeker, 1970; Butler et al., 1974). In total, 75 cultivars, representing 14 species of turfgrass, were included in the trial (Table 1). Species included Kentucky bluegrass, perennial ryegrass, tall fescue, tufted hairgrass [*Deschampsia cespitosa* (L.) P. Beauv.], prairie junegrass [*Koeleria macrantha* (Ledeb.) Schult.], creeping bentgrass (*Agrostis stolonifera* L.), alkaligrass, Idaho bentgrass (*Agrostis idahoensis* Nash), hard fescue, sheep fescue, Chewings fescue, slender creeping red fescue, and strong creeping red fescue.

### Site Design and Establishment

Each site was arranged as a randomized complete block design with three replications. Plots at the Albertville site were 1.52 m by 5.48 m. Seeding rates (Table 2) were chosen for each species based on suggested rates (Christians, 2011). Because purity for the Minnesota ecotype of prairie junegrass was known to be low, the bulk seeding rate was increased to approximate the same effective seeding rate as the European cultivar ‘Barkoel’, based on breeder suggestions. This resulted in a range of seeding rates for that species only, as reflected in Table 2.

Site preparation began with a single application of glyphosate (Roundup Weather Max, Monsanto Co., St. Louis, MO, USA) on existing vegetation at a rate of 7.68 L $ha^{-1}$. Seedbed preparation began one week after glyphosate applications. Seeding dates for the trial were 18 August and 24 August 2010, for the Roseville and Albertville sites, respectively. At the Roseville site, the seedbed was prepared by roughing the surface using an Infield Rascal (ABI Inc., Osceola, IN, USA) pulled behind a utility vehicle. The device uses steel tines to drag the surface of the soil, thereby pulling out plant tissue and loosening the top 1-2 cm of soil. Loosened plant tissue was left on the surface of the soil to act as mulch. At the Albertville site, the soil was too soft to support the weight of the vehicle so seed bed preparation was accomplished by hand. Stones larger than 2.5 cm in diameter were removed by hand and the top 1-5 cm of soil was loosened by hand using a garden rake. A starter fertilizer (10-18-22) was applied to all sites at a rate of 271.1 kg $ha^{-1}$ prior to seeding.

Sowing was done by hand and plots were hand raked lightly with garden rakes to ensure good seed to soil contact. SeedAide Aero Mulch (Profile Products LLC, Buffalo Grove, IL, USA) was applied at 3360 kg $ha^{-1}$ and watered in after seeding at each site. Subsequent maintenance included occasional mowing at only the Roseville site and no regular irrigation at either location.

### Soil Characterization

Following seeding, soil samples were collected to a depth of 0.15 m. Samples were collected from every fourth plot in a zigzag pattern at 1.52 m increments from the road. Samples from each replicate were bulked together for analysis. Bulk samples were analyzed for pH, organic matter, and saturated paste extract electrical conductivity (EC) as well as extractable phosphorus, potassium, calcium, magnesium, and sodium (Table 3). Chloride was not analyzed because, as an anion, it is highly transient in soils. Soil textural analysis was also carried out for a bulk sample at all sites. Analysis was done at the University of Minnesota Soil Analytical Laboratory using standard procedures.

Twenty-four hours following a saturating rain event, soil compaction at each site was assessed using a Clegg Impact Soil Tester using a 0.5 kg missile (LaFayette Instrument Company, LaFayette, IN). The device is operated by dropping a weight with an accelerometer enclosed onto the soil surface. The peak deceleration is recorded and higher deceleration values indicate more compacted soils. Water holding capacity was assessed at the same time by measuring volumetric water content (VWC) of the soils at field capacity using a FieldScout TDR300 soils moisture sensor (Spectrum Technologies, Aurora IL). Three measurements were taken with each device within each replicate and averaged. Measurements were taken at positions where no vegetation had been established. Mean soil compaction and field capacity VWC are summarized in Table 3.

## Data Collection and Analysis

In fall 2010, establishment of turf plots was assessed visually. Each plot was assigned a rating on a 1-9 scale with 9 representing complete establishment of a dense turf canopy, and 1 representing less than 10% of the area seeded had been established. A rating of 6, or 62.5% of full scale, was considered to be acceptable establishment. Evaluation of establishment took place at the Roseville site on 6 November, 2010 and at the Albertville site on 28 October, 2010.

On 27 April, 2011 and 13 May, 2011 the Roseville and Albertville sites, respectively, were visually assessed for survival of each entry. Survival was determined by visually estimating the proportion of existing turf tissue that was established and actively growing in the fall, and which remained alive and healthy in the spring. Ratings were assigned on a 1-9 scale with 9 representing complete survival of all previously established turfgrass and 1 representing complete death of all existing turfgrass. As with establishment, a rating of 6, or 62.5% of full scale, was considered to be acceptable survival of the turfgrass plot. Evaluation of plots in this manner provided for separation of the effects of establishment and survival.

Data from all three locations were combined and analyzed for differences in establishment and survival performance as measured by the visual assessment ratings. Analysis of variance was performed using R (R Development Core Team, 2012), with subsequent means separation by Fisher's protected least significant difference (LSD) at the $\alpha=0.05$ level. Data were analyzed for significance of effects of site, replicate, and treatment. Treatment means differing by greater than the LSD value were statistically different from one another. Using this analysis, it was possible to determine if significant differences existed between cultivars and selections in their ability to establish and survive on Minnesota roadsides.

## Results

### Establishment Results

Analysis of variance of the combined data set of visual establishment ratings from all three sites (Table 4) showed a significant effect (p=0.038) of experimental site; thus, the treatment differences at the three sites were subsequently analyzed separately. Mean establishment ratings in the boulevard section at Roseville were not statistically different from those at Albertville; however, both of those sites were statistically different from the main plot section at Roseville according to means separation by Fisher's protected LSD (α=0.05). Mean visual establishment ratings for those sites were 4.7, 4.6, and 3.3 for the boulevard at Roseville, Albertville, and main plot at Roseville, respectively. Data from the boulevard at Roseville did not show a significant effect of replicate at the α=0.05 level but did show significant differences (p<0.001) between cultivars (Table 5). The main plot section at Roseville had significant effects (p<0.001) of both replicate and cultivar (Table 6). The Albertville site (Table 7) also showed significant effects of replicate (p=0.005) and cultivar (p<0.001).

Means separation (Table 8) using Fisher's protected LSD (α=0.05) showed that in the boulevard section at Roseville, perennial ryegrass cultivars 'Apple GL' and 'Caddyshack II' shared the highest mean rating of 9 for establishment, but were not statistically different from 21 other cultivars in the trial at that site. Of the 23 entries in the top statistical grouping, 14 were cultivars of perennial ryegrass. Fourteen cultivars shared the lowest mean establishment rating of 1, but were not different from 12 other cultivars in the trial at that location. Of the 26 cultivars in that lowest statistical grouping, 13 were Kentucky bluegrass cultivars while just three were cultivars of one of the fine fescue species.

Means separation by Fisher's protected LSD (α=0.05) of the cultivar treatments in the main plot section at Roseville (Table 9) showed that perennial ryegrass 'Caddyshack II' had the highest mean rating of 9 for establishment at that site. However, it was not statistically different from 10 other entries. All 11 entries in the top statistical grouping at that site were cultivars of perennial ryegrass. The lowest statistical grouping at that site contained 42 cultivars, 23 of which shared the lowest overall mean rating of 1. That list of cultivars comprised several different species, but contained all four cultivars of alkaligrass and 13 cultivars of Kentucky bluegrass. Also at that site, all three replicates were statistically different as determined by Fisher's protected LSD (α=0.05). Replicates 1, 2, and 3 had mean ratings of 3.21, 2.41, and 4.36, respectively.

The means separation results by Fisher's protected LSD (α=0.05) of the data from Albertville (Table 10) showed that perennial ryegrass cultivar 'Gray Fox' had the highest mean establishment rating of 7.7, but was not statistically different from three other cultivars, all of which were perennial ryegrasses. The second statistical grouping at that site also contained primarily perennial ryegrass cultivars, but also comprised several cultivars of tall fescue including 'Grande II', 'Coronado TDH', 'Kentucky-31 (E+)', and 'SR8650'. 'Dawson E' had the lowest mean establishment rating of 1.7 but was not statistically different from seven other cultivars, including four cultivars of Kentucky bluegrass. At the Albertville site, replicates 1 and 2 were statistically similar with means of 4.77 and 4.70, respectively. Replicate 3 was statistically different from the other two, however, with a mean establishment rating of 4.47.

## Survival Results

Analysis of variance of the combined data set of visual survival ratings from all three sites (Table 11) showed a significant effect (p=0.001) of experimental site; thus, the treatment differences for the three sites were subsequently analyzed separately. Mean survival ratings in the boulevard section at Roseville was not statistically different from the Albertville site; however, both were statistically different from the main plot section at Roseville according to means separation by Fisher's protected LSD (α=0.05). Mean visual survival ratings for those sites were 2.78, 2.77, and 1.96 for the boulevard at Roseville, Albertville, and main plot at Roseville, respectively. None of the three sites showed a significant ($p<0.05$) effect of replicate, but all did show a significant effect of cultivar (Tables 12-14).

In the boulevard section at Roseville (Table 15), ‘Shoreline’ slender creeping red fescue had the highest mean survival rating of all cultivars in the trial of 7.3. However, using means separation by Fisher’s protected LSD ($\alpha=0.05$) it was shown to be not statistically different from 14 other entries in the trial at that location. Of the 15 entries in the top statistical grouping, there were two cultivars of alkaligrass, three slender creeping red fescues, five strong creeping red fescues, two sheep fescues, one hard fescue, and one Chewings fescue. There were 15 cultivars that exhibited no survival and had mean survival ratings of 1. However, those 15 were not statistically different from 38 other entries in the trial.

Means separation by Fisher’s protected LSD ($\alpha=0.05$) of the data from the main plot section at Roseville (Table 16) showed that ‘Shoreline’ slender creeping red fescue had the highest mean survival rating of 6 but was not statistically different from 14 other entries in the trial at that location. That top statistical grouping contained at least one cultivar of each of eight different species. There were 40 cultivars that all shared the lowest mean survival rating of 1 at that site, indicating there was no survival for any of the plots of those cultivars. Moreover, those 40 were statistically different from just 10 other trial entries at that location.

Finally, means separation by Fisher’s protected LSD ($\alpha=0.05$) of the visual survival ratings at Albertville (Table 17) revealed that ‘Fults’, ‘Oceania’, ‘Salton Sea’, and ‘Salty’ alkaligrasses all shared the highest mean visual rating of 9 for survival at that site indicating complete survival of all turfgrass cover following the winter. Those four entries were all statistically different from all other entries in the trial. The second statistical grouping contained eight cultivars, of which ‘ASR 050’ slender creeping red fescue had the highest mean rating. There were 19 cultivars that shared the lowest mean rating of 1 at that site. Furthermore, those 19 cultivars were statistically different from just 25 others in the trial.

## Discussion

The objective of this work was to evaluate a wide variety of cultivars and selections of cool-season turfgrass for their ability to establish and persist on roadsides in Minnesota. This objective was accomplished by visually assessing establishment following late summer sowing of 75 cultivars of cool-season turfgrass on roadsides at three locations in Minnesota, and subsequently visually assessing survival of turfgrass the following spring. Through that process, we were able to identify significant differences between cultivars for their performance in terms of establishment and survival on Minnesota roadsides.

Significant differences of mean establishment ratings were observed between sites in this trial. The boulevard at Roseville had the highest mean establishment rating followed by Albertville, which was statistically the same. The main plot at Roseville had the lowest mean establishment across all treatments. Differences between soil physical characteristics at the site may explain some of those observed differences. Mean soil compaction, as measured using the Clegg Impact Soil Tester, was highest in the main plot at Roseville. Furthermore, soil moisture at field capacity was lowest at that site. Soil electrical conductivity and sodium concentrations, which are known to affect germination of many species (Liem et al., 1985; Tarasoff et al., 2007; Spencer and Port, 1988; Rose-Fricker and Wipff, 2001), did not display the same relationship and thus do not appear to play a role in the mean establishment of the plots at each site. This may indicate that soil structure and the ability to retain moisture in the soil are the most important factors for successful establishment of cool-season turfgrasses on roadsides in Minnesota. In the main plot section in Roseville and at Albertville, there appeared to be no relationship between mean establishment rating and compaction or soil moisture; however, differences among replicates were much smaller than those between sites. In addition, no other consistent relationships were observed between mean establishment rating and soil properties among replicates exhibiting significant differences.

In the boulevard at Roseville, main plot at Roseville, and Albertville, 61%, 100%, and 100%, respectively, of the cultivars in the top statistical groupings for mean establishment ratings were cultivars of perennial ryegrass. This result was not entirely unexpected, as perennial ryegrass is known to germinate quickly in a wide range of soil moisture conditions and establish rapidly on roadsides (Wright et al., 1978; Boeker, 1970). The results of this experiment support those of previous studies which have concluded that perennial ryegrass would be well-suited for roadsides where rapid establishment of acceptable quality turfgrass cover is required (Trautmann and Lohmeyer, 1980; Henensal et al., 1980; Boeker, 1970).

In contrast, of the entries sharing the lowest mean rating for establishment in the boulevard and main plot sections at Roseville, 50% and 61%, respectively, were cultivars of Kentucky bluegrass. The same was true of 50% of the cultivars in the lowest statistical grouping at Albertville. Kentucky bluegrass is well-adapted to moist, well-drained soils (Beard, 1973) but germination of seed is slow and decreases rapidly with increasing soil water tension (Tarasoff et al., 2007). As a result, it seems likely that establishment of those cultivars was reduced due to the lack of irrigation and the occurrence of a 27-day period of no precipitation which began approximately 4 weeks after seeding.

Alkaligrass cultivars did not exhibit acceptable establishment (mean visual rating of 6 or higher) at any of the three sites. However, they did all receive higher mean establishment ratings at Albertville. Alkaligrass is native to Europe and Asia and is often found in saltmarshes where it thrives in moist, saline soils. Previous research has shown that although alkaligrass is capable of limited germination in arid conditions, it is more likely to germinate and survive in the presence of moist conditions and is not affected during germination by increased concentrations of sodium (Tarasoff et al., 2007). Given this information, it is likely that the ability of the soil to hold more moisture increased the ability for alkaligrass to establish at Albertville, while not being affected by the increased levels of sodium at that site.

Species and cultivars that were observed to establish well did not necessarily survive the winter while those that had weak establishment sometimes had the greatest proportion of turfgrass survive. Differences among sites for mean survival rating followed in the same order as for establishment with mean survival ratings being greatest in the boulevard at Roseville followed by Albertville and the main plot at Roseville. It is possible that strength of establishment in the fall may play a role in the survival of turfgrass through the winter at the level of site. Some differences among sites may be explained by the topography and construction of the roadsides. Runoff water from the road at Albertville was allowed to run directly off the road and down the inslope of the roadside. As stated previously, salt application rates were not available from the Minnesota Department of Transportation, but were known to be higher at Albertville due to higher traffic volumes and speeds. In contrast, the curb at Roseville kept runoff water from directly running onto the plots. Instead, exposure to deicing salt at Roseville was dependent on spray from passing cars or salt bouncing off of the road during application. The cultivars and species that performed well at the Albertville site were distinct from from both of the other two sites at Roseville, as evidenced by the significant effect of site in the analysis of variance of the combined data. Specifically, alkaligrass cultivars exhibited complete survival while most other trial entries had significantly lower mean survival ratings ($p<0.05$).

All four cultivars of alkaligrass were observed to have complete survival at Albertville. That was not the case in either section at Roseville. Other authors have previously stated that autumn drought, such as that experienced in the autumn of 2010, may be critical to the survival of alkaligrass (Gray and Scott, 1977). As noted previously, the ability for the soil at Albertville to retain moisture was greater than at either of the other sites. Greater water availability during the fall drought may have increased the vigor of alkaligrass cultivars going into winter and, thus, led to greater survival. The pattern of winter death observed at Roseville is more consistent with the observations of Biesboer et al. (1998) where alkaligrass was observed to behave more like an annual grass on Minnesota roadsides and persist only by virtue of prolific seed production. It is clear, however, that in the presence of mowing, seed production is not possible. At locations like Roseville where mowing is a routine practice, it is likely that alkaligrass will quickly die if established in areas where irrigation is not present.

‘Shoreline’ slender creeping red fescue had the highest mean survival rating in both the main plot and boulevard section at Roseville. The species is capable of withstanding many of the abiotic stresses present in roadside environments, and is found in a wide range of environments (Pavlick, 1985). ‘Shoreline’ slender creeping red fescue was also in the second statistical grouping of cultivars at Albertville and had the third highest mean survival rating of all cultivars at that site, aside from the alkaligrass cultivars. This seems to be a good indication that it is capable of producing acceptable survival across a wide range of roadside environments. Interestingly, ‘Shoreline’ is the progeny of several crosses of germplasm from an English seaside ecotype with European and North American selections. It was selected for in a wide range of environments including Missouri, California, and Oregon (Seed Research of Oregon, 2013). This breeding history may give some indication of why that cultivar tolerates the variety of stresses encountered in a roadside environment including poor soil quality, drought, and exposure to deicing salt.

Two other species, hard fescue and sheep fescue, had cultivars in the top statistical grouping at both Roseville sites and the second statistical grouping at the Albertville site. Both species are well-adapted to poor soil conditions (Beard, 1973) and have been recommended for use previously on roadsides (Boeker, 1970; Duell and Schmit, 1974; Henensal, 1980). ‘Beacon’ hard fescue and ‘Marco Polo’ sheep fescue had the highest mean survival ratings for cultivars in their respective species across all study sites. Sheep fescue 67135, which is an advanced breeding population from the University of Minnesota turfgrass breeding program, had the highest mean survival rating for a non-cultivar selection. The mean rating for each of those three trial entries was highest in the boulevard at Roseville and lowest in the main plot at Roseville, with the exception of entry 67135, which was lowest at Albertville. This again follows the same general trend as establishment and survival among those sites. Yet, with the one exception of ‘Beacon’ hard fescue in the boulevard at Roseville, none of those three entries were in the top statistical grouping for establishment at any site. With such consistent survival results for those entries across all sites despite generally unacceptable establishment (mean rating less than 6) it may be expected that ‘Beacon’, ‘Marco Polo’, and 67135 may provide persistent, functional roadside turfgrass if acceptable establishment can be achieved.

## Conclusions

In this experiment, we evaluated a wide variety of cultivars and selections of cool-season turfgrass for their ability to establish and persist on roadsides in Minnesota. Seventy-five cultivars and selections of cool-season turfgrass were established at three locations and visually assessed for fall establishment and subsequently for survival the following spring. Significant differences among sites were identified for both establishment and survival, and significant differences among replicates were identified for establishment at two of the trial locations.

Mean establishment rating was related to soil compaction and field capacity VWC at each site. Mean survival rating by site followed the same trend; however, good establishment of cultivars within sites was not a guarantee of survival the following spring. Site differences appear to account for some of the variation in cultivar performance and suggest that different cultivars should be used depending on site management, environmental, and edaphic characteristics. Sites with higher anticipated salt loads may benefit from the use of cultivars of alkaligrass, given sufficient soil moisture can be maintained. All cultivars of alkaligrass had equal mean survival ratings at Albertville where salt loads were known to be highest. Sites where maintenance during establishment will not be a priority, but where survival is still desirable, will benefit from the use of one or more of several cultivars of fine fescue species. Some of these cultivars include ‘Shoreline’ slender creeping red fescue, ‘Beacon’ hard fescue, ‘Marco Polo’ sheep fescue, as well as several cultivars of strong creeping red fescue.

In this experiment we were not able to address the method or timing of salt exposure to plants in runoff water during the spring. Those factors may be critical in determining to what extent salt plays a role in observed turf death, as different species and cultivars will de-acclimate from winter at different rates. Although relative salt application rates on the road, as given by the department of transportation, did not appear to be related to mean survival rating at each site, direct evaluation of salt tolerance in these cultivars will be helpful as they will be used in areas where salt is known to be applied.

Cultivars identified as performing well in this trial may be used to guide the decisions of public works employees and turfgrass breeders in the implementation and improvement of roadside turfgrasses and may be used for the creation of roadside turfgrass species mixtures.

## Acknowledgements

We would like to thank the Minnesota Local Road Research Board and the Minnesota Department of Transportation for funding and support of this project. Also, thank you to Roselawn Cemetery and the MnROAD research facility for the use of the study area. Finally, we gratefully acknowledge the invaluable advice of our reviewers.

**Table 1**. Cultivars and selections included in the roadside salt tolerance trial.

| **Cultivar or Selection** | **Species**[†] | **Cultivar or Selection** | **Species** |
|---|---|---|---|
| Fults | AK | JR-522 | PR |
| Oceania | AK | Silver Dollar | PR |
| SaltonSea | AK | Minnesota (ecotype) | KM |
| Salty | AK | Barkoel | KM |
| Bighorn GT | BHD | 67135 | SH |
| Little Bighorn | BHD | Marco Polo | SH |
| TCP | CH | ASR 050 | SLCRF |
| Mariner | CBG | Dawson E | SLCRF |
| Providence | CBG | Seabreeze GT | SLCRF |
| Beacon | HF | Sealink | SLCRF |
| SR 5130 | CH | Shoreline | SLCRF |
| Golfstar | IBG | Cardinal | STCRF |
| Argos | KBG | Celestial | STCRF |
| Arrowhead | KBG | Epic | STCRF |
| Brooklawn | KBG | Florentine GT | STCRF |
| Diva | KBG | McAlpin | STCRF |
| Dragon | KBG | Navigator | STCRF |
| Jumpstart | KBG | OR1 | STCRF |
| Langara | KBG | PSG 5RM | STCRF |
| Midnight | KBG | PST 8000 | STCRF |
| Moonlight SLT | KBG | Shademaster III | STCRF |
| Moonshine | KBG | SR 5250 | STCRF |
| Orfeo | KBG | Corona | TF |
| Park | KBG | Coronado TDH | TF |
| Right | KBG | Dynamic II | TF |
| Accent II | PR | Endeavor II | TF |
| Apple GL | PR | Gazelle II | TF |
| Arctic Green | PR | Grande II | TF |
| Brighstar SLT | PR | Jaguar 4G | TF |
| Caddyshack II | PR | JT-158 | TF |
| Citation Fore | PR | Kentucky-31 (E+) | TF |
| Fiesta III | PR | Mustang 4 | TF |
| Grand Slam II | PR | SR 8650 | TF |
| Gray Fox | PR | Tar Heel II | TF |
| Gray Goose | PR | Wolfpack II | TF |
| Harrier | PR | Humboldt Bay (ecotype) | DC |
| Headstart II | PR | SR 6000 | DC |
| JR-521 | PR | | |

[†] AK, alkaligrass; BHD, blue hard fescue; CH, chewings fescue; CBG, creeping bentgrass; HF, hard fescue; IBG, Idaho bentgrass; KBG, Kentucky bluegrass; PR, perennial ryegrass; TF, tall fescue; KM, prairie junegrass; SH, sheep fescue; DC, tufted hairgrass; SLCRF, slender creeping red fescue; STCRF, strong creeping red fescue

**Table 2**. Seeding rates for the turfgrass species included in the salt tolerance trial.

| **Species** | **kg ha$^{-1}$** |
|---|---|
| fine fescue | 244.1 |
| tall fescue | 341.7 |
| Kentucky bluegrass | 73.2 |
| perennial ryegrass | 390.5 |
| tufted hairgrass | 97.6 |
| prairie junegrass | 146.5 – 195.3 |
| creeping bentgrass | 48.8 |
| alkaligrass | 195.3 |
| Idaho bentgrass | 146.5 |

**Table 3**. Soil chemical and physical properties for sites in the roadside turfgrass cultivar trial.

| | **Roseville[†] (Boulevard)** | | | **Roseville[‡] (Main Plot)** | | | **Albertville[§]** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Replicate** | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| **pH** | 7.7 | 7.5 | 7.4 | 7.4 | 7.2 | 7.0 | 7.8 | 8.1 | 8.0 |
| **Organic Matter, %** | 4.9 | 5.8 | 6.6 | 4.8 | 4.2 | 4.0 | 4.2 | 3.6 | 3.2 |
| **P (Bray),** mg $kg^{-1}$ | 55 | 68 | 60 | 75 | 56 | 71 | 89 | 75 | 65 |
| **P (Olsen),** mg $kg^{-1}$ | 39 | 37 | -- | -- | -- | -- | 65 | 55 | 47 |
| **K,** mg $kg^{-1}$ | 159 | 150 | 204 | 228 | 174 | 213 | 175 | 157 | 163 |
| **Saturated Paste EC,** dS $m^{-1}$ | 0.7 | 0.6 | 0.9 | 0.9 | 0.7 | 0.7 | 1.2 | 1.1 | 1.2 |
| **Ca,** mg $kg^{-1}$ | 2966 | 2547 | 3720 | 2014 | 1899 | 1798 | 2677 | 2636 | 2879 |
| **Mg,** mg $kg^{-1}$ | 185 | 168 | 192 | 243 | 249 | 226 | 280 | 256 | 300 |
| **Na,** mg $kg^{-1}$ | 106 | 15 | 38 | 187 | 94 | 37 | 302 | 299 | 385 |
| **Compaction** m $s^{-2}$ | 816.6 | 878.7 | 859.1 | 980.0 | 980.0 | 1097.6 | 715.4 | 748.1 | 630.5 |
| **Field Capacity Volumetric Water Content,** % | 29.9 | 33.1 | 31.8 | 28.7 | 25.9 | 25.6 | 35.9 | 36.2 | 40.1 |

[†] Soil texture at Roseville Boulevard was 63% sand, 17% silt, 20% clay

[‡] Soil texture at Roseville Main Plot was 51% sand, 26% silt, 23% clay

[§] Soil texture at Albertville was 40% sand, 31% silt, 29% clay

**Table 4.** Analysis of variance of combined establishment rating data from the three experimental sites of the roadside turfgrass cultivar trial.

| Source | DF | MS | Pr(>\|t\|) |
|---|---|---|---|
| Site | 2 | 146.54 | 0.0384 |
| Replicate within Site | 6 | 24.89 | < 0.001 |
| Cultivar | 74 | 40.998 | < 0.001 |
| Cultivar x Site | 148 | 3.284 | < 0.001 |
| Residuals | 444 | 1.425 | |

**Table 5.** Analysis of variance of establishment rating data for the boulevard section at the Roseville site in the roadside turfgrass cultivar trial.

| Source | DF | MS | Pr(>\|t\|) |
|---|---|---|---|
| Replicate | 2 | 0.92 | 0.605 |
| Cultivar | 74 | 23.0582 | < 0.001 |
| Residuals | 148 | 1.831 | |

**Table 6.** Analysis of variance of establishment rating data for the main plot section at the Roseville site in the roadside turfgrass cultivar trial.

| Source | DF | MS | Pr(>\|t\|) |
|---|---|---|---|
| Replicate | 2 | 71.804 | < 0.001 |
| Cultivar | 74 | 17.031 | < 0.001 |
| Residuals | 148 | 2.079 | |

**Table 7.** Analysis of variance of establishment rating data for the Albertville site in the roadside turfgrass cultivar trial.

| Source | DF | MS | Pr(>\|t\|) |
|---|---|---|---|
| Replicate | 2 | 1.95 | 0.005 |
| Cultivar | 74 | 7.47 | < 0.001 |
| Residuals | 148 | 0.37 | |

**Table 8**. Mean establishment ratings in the boulevard at Roseville for each cultivar or selection in the roadside turfgrass cultivar trial.

| Cultivar or Selection | Species[§] | Mean[†,‡] Rating | Cultivar or Selection | Species | Mean Rating |
|---|---|---|---|---|---|
| Apple GL | PR | 9 | 67135 | SH | 5.667 |
| Caddyshack II | PR | 9 | Seabreeze GT | SLCRF | 5.667 |
| Citation Fore | PR | 8.667 | Corona | TF | 5.333 |
| Grand Slam II | PR | 8.5 | Wolfpack II | TF | 5 |
| Coronado TDH | TF | 8.333 | Little Bighorn | BHD | 4.667 |
| Gray Goose | PR | 8.333 | Epic | STCRF | 4.333 |
| Silver Dollar | PR | 8.333 | PST 8000 | STCRF | 4 |
| Tar Heel II | TF | 8.333 | Florentine GT | STCRF | 3.667 |
| Accent II | PR | 8 | Humboldt Bay | KM | 3.333 |
| Arctic Green | PR | 8 | Jaguar 4G | TF | 3.333 |
| Grande II | TF | 8 | SR 5250 | STCRF | 3.333 |
| Fiesta III | PR | 7.667 | Shademaster III | STCRF | 3 |
| Headstart II | PR | 7.667 | Celestial | STCRF | 2.667 |
| JR-521 | PR | 7.667 | Fults | AK | 2.333 |
| JR-522 | PR | 7.667 | Midnight | KBG | 2.333 |
| Kentucky-31 (E+) | TF | 7.333 | Moonshine | KBG | 2.333 |
| Navigator | STCRF | 7.333 | Diva | KBG | 1.667 |
| Shoreline | SLCRF | 7.333 | Langara | KBG | 1.667 |
| Beacon | HF | 7 | Moonlight SLT | KBG | 1.667 |
| Brighstar SLT | PR | 7 | Salty | AK | 1.5 |
| Cardinal | STCRF | 7 | Brooklawn | KBG | 1.333 |
| GrayFox | PR | 7 | Park | KBG | 1.333 |
| PSG-5RM | STCRF | 7 | Providence | CBG | 1.333 |
| Dynamic II | TF | 6.667 | Argos | KBG | 1 |
| OR1 | STCRF | 6.667 | Arrowhead | KBG | 1 |
| Sealink | SLCRF | 6.667 | Dawson E | SLCRF | 1 |
| JT-158 | TF | 6.5 | Dragon | KBG | 1 |
| ASR 050 | SLCRF | 6.333 | Golfstar | IBG | 1 |
| Harrier | PR | 6.333 | Jumpstart | KBG | 1 |

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**Table 8**. Continued.

| Cultivar or Selection | Species§ | Mean†,‡ Rating | Cultivar or Selection | Species | Mean Rating |
|---|---|---|---|---|---|
| SR 5130 | CH | 6.333 | Minnesota | KM | 1 |
| SR 8650 | TF | 6.333 | Mariner | CBG | 1 |
| Bighorn GT | BHD | 6 | Oceania | AK | 1 |
| Endeavor II | TF | 6 | Orfeo | KBG | 1 |
| Gazelle II | TF | 6 | Right | KBG | 1 |
| Marco Polo | SH | 6 | Salton Sea | AK | 1 |
| McAlpin | STCRF | 6 | SR 6000 | DC | 1 |
| Mustang 4 | TF | 6 | Barkoel | KM | 1 |
| TCP | CH | 6 | | | |

† Data collected Nov 6, 2010. Three replications of plots were rated on a 1-9 scale (1 = less than 10% establishment, 6 = acceptable turf cover, 9 = complete dense turf cover) for establishment of seeded turfgrass.

‡ LSD = 2.18

§ AK, alkaligrass; BHD, blue hard fescue; CH, Chewings fescue; CBG, creeping bentgrass; HF, hard fescue; IBG, Idaho bentgrass; KBG, Kentucky bluegrass; PR, perennial ryegrass; TF, tall fescue; KM, prairie junegrass; SH, sheep fescue; DC, tufted hairgrass; SLCRF, slender creeping red fescue; STCRF, strong creeping red fescue

**Table 9**. Mean establishment ratings in the main plot at Roseville for each cultivar or selection in the roadside turfgrass cultivar trial.

| Cultivar or Selection | Species[§] | Mean[†,‡] Rating | Cultivar or Selection | Species | Mean Rating |
|---|---|---|---|---|---|
| Caddyshack II | PR | 9 | Beacon | HF | 2.667 |
| Citation Fore | PR | 8.333 | Corona | TF | 2.667 |
| Accent II | PR | 8 | Florentine GT | STCRF | 2.667 |
| Brighstar SLT | PR | 8 | PST 8000 | STCRF | 2.667 |
| Grand Slam II | PR | 8 | SR 5250 | STCRF | 2.667 |
| Gray Goose | PR | 8 | Bighorn GT | BHD | 2.333 |
| Arctic Green | PR | 7.333 | Epic | STCRF | 2 |
| Fiesta III | PR | 7.333 | Little Bighorn | BHD | 2 |
| Gray Fox | PR | 7 | Marco Polo | SH | 2 |
| JR-521 | PR | 7 | Jaguar 4G | TF | 1.667 |
| JR-522 | PR | 7 | Celestial | STCRF | 1.333 |
| Headstart II | PR | 6.667 | Fults | AK | 1.333 |
| Apple GL | PR | 6.333 | Humboldt Bay | DC | 1.333 |
| Silver Dollar | PR | 6.333 | Shademaster III | STCRF | 1.333 |
| Shoreline | SLCRF | 5.667 | Argos | KBG | 1 |
| Harrier | PR | 5.333 | Arrowhead | KBG | 1 |
| Navigator | STCRF | 5 | Brooklawn | KBG | 1 |
| ASR 050 | SLCRF | 4.667 | Dawson E | SLCRF | 1 |
| Cardinal | STCRF | 4.667 | Diva | KBG | 1 |
| 67135 | SH | 4.333 | Dragon | KBG | 1 |
| McAlpin | STCRF | 4.333 | Golfstar | IBG | 1 |
| Tar Heel II | TF | 4.333 | Jumpstart | KBG | 1 |
| TCP | CH | 4.333 | Minnesota | KM | 1 |
| Mustang 4 | TF | 4 | Langara | KBG | 1 |
| OR1 | STCRF | 4 | Mariner | CBG | 1 |
| Wolfpack II | TF | 4 | Oceania | AK | 1 |
| Grande II | TF | 3.667 | Midnight | KBG | 1 |
| SR 8650 | TF | 3.667 | Moonlight SLT | KBG | 1 |
| Coronado TDH | TF | 3.333 | Moonshine | KBG | 1 |

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**Table 9**. Continued.

| Cultivar or Selection | Species§ | Mean†,‡ Rating | Cultivar or Selection | Species | Mean Rating |
|---|---|---|---|---|---|
| Dynamic II | TF | 3.333 | Orfeo | KBG | 1 |
| JT-158 | TF | 3.333 | Park | KBG | 1 |
| Seabreeze GT | SLCRF | 3.333 | Providence | CBG | 1 |
| Sealink | SLCRF | 3.333 | Right | KBG | 1 |
| Endeavor II | TF | 3 | Salton Sea | AK | 1 |
| Gazelle II | TF | 3 | Salty | AK | 1 |
| Kentucky-31 (E+) | TF | 3 | SR 6000 | DC | 1 |
| PSG 5RM | STCRF | 3 | Barkoel | KM | 1 |
| SR 5130 | CH | 3 | | | |

† Data collected Nov 6, 2010. Three replications of plots were rated on a 1-9 scale (1 = less than 10% establishment, 6 = acceptable turf cover, 9 = complete dense turf cover) for establishment of seeded turfgrass.

‡ LSD = 2.32

§ AK, alkaligrass; BHD, blue hard fescue; CH, Chewings fescue; CBG, creeping bentgrass; HF, hard fescue; IBG, Idaho bentgrass; KBG, Kentucky bluegrass; PR, perennial ryegrass; TF, tall fescue; KM, prairie junegrass; SH, sheep fescue; DC, tufted hairgrass; SLCRF, slender creeping red fescue; STCRF, strong creeping red fescue

**Table 10**. Mean establishment ratings in the boulevard at Albertville for each cultivar or selection in the roadside turfgrass cultivar trial.

| Cultivar or Selection | Species§ | Mean†,‡ Rating | Cultivar or Selection | Species | Mean Rating |
|---|---|---|---|---|---|
| Gray Fox | PR | 7.667 | Epic | STCRF | 4.667 |
| Apple GL | PR | 7 | Mariner | CBG | 4.667 |
| Citation Fore | PR | 7 | PSG 5RM | STCRF | 4.667 |
| Harrier | PR | 7 | Florentine GT | STCRF | 4.333 |
| Caddyshack II | PR | 6.667 | Little Bighorn | BHD | 4.333 |
| Grande II | TF | 6.667 | Marco Polo | SH | 4.333 |
| Gray Goose | PR | 6.667 | McAlpin | STCRF | 4.333 |
| JR-521 | PR | 6.667 | Navigator | STCRF | 4.333 |
| JR-522 | PR | 6.667 | Shademaster III | STCRF | 4.333 |
| Arctic Green | PR | 6.333 | 67135 | SH | 4 |
| Brighstar SLT | PR | 6.333 | Golfstar | IBG | 4 |
| Coronado TDH | TF | 6.333 | Salty | AK | 4 |
| Fiesta III | PR | 6.333 | SR 5130 | CH | 4 |
| Grand Slam II | PR | 6.333 | SR 5250 | STCRF | 4 |
| Headstart II | PR | 6.333 | Celestial | STCRF | 3.667 |
| Kentucky-31 (E+) | TF | 6.333 | Fults | AK | 3.667 |
| Silver Dollar | PR | 6.333 | Humboldt Bay | DC | 3.667 |
| SR 8650 | TF | 6.333 | Oceania | AK | 3.333 |
| Corona | TF | 6 | Salton Sea | AK | 3.333 |
| Dynamic II | TF | 6 | Diva | KBG | 3 |
| Endeavor II | TF | 6 | Barkoel | KM | 3 |
| Tar Heel II | TF | 6 | Argos | KBG | 2.667 |
| Wolfpack II | TF | 6 | Brooklawn | KBG | 2.667 |
| Accent II | PR | 5.667 | Jumpstart | KBG | 2.667 |
| Gazelle II | TF | 5.667 | Midnight | KBG | 2.667 |
| JT-158 | TF | 5.667 | Moonlight SLT | KBG | 2.667 |
| Mustang 4 | TF | 5.667 | Moonshine | KBG | 2.667 |
| Sealink | SLCRF | 5.667 | Park | KBG | 2.667 |
| Jaguar 4G | TF | 5.333 | Right | KBG | 2.667 |

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**Table 10**. Continued.

| Cultivar or Selection | Species§ | Mean†,‡ Rating | Cultivar or Selection | Species | Mean Rating |
|---|---|---|---|---|---|
| Cardinal | STCRF | 5 | Arrowhead | KBG | 2.333 |
| OR1 | STCRF | 5 | Langara | KBG | 2.333 |
| PST 8000 | STCRF | 5 | Orfeo | KBG | 2.333 |
| Seabreeze GT | SLCRF | 5 | Providence | CBG | 2.333 |
| Shoreline | SLCRF | 5 | Dragon | KBG | 2 |
| TCP | CH | 5 | Minnesota | KM | 2 |
| ASR 050 | SLCRF | 4.667 | SR 6000 | DC | 2 |
| Beacon | HF | 4.667 | Dawson E | SLCRF | 1.667 |
| Bighorn GT | BHD | 4.667 | | | |

[†] Data collected Oct 28, 2010. Three replications of plots were rated on a 1-9 scale (1 = less than 10% establishment, 6 = acceptable turf cover, 9 = complete dense turf cover) for establishment of seeded turfgrass.

[‡] LSD = 0.975

[§] AK, alkaligrass; BHD, blue hard fescue; CH, Chewings fescue; CBG, creeping bentgrass; HF, hard fescue; IBG, Idaho bentgrass; KBG, Kentucky bluegrass; PR, perennial ryegrass; TF, tall fescue; KM, prairie junegrass; SH, sheep fescue; DC, tufted hairgrass; SLCRF, slender creeping red fescue; STCRF, strong creeping red fescue

**Table 11.** Analysis of variance of combined survival rating data from the three experimental sites of the roadside turfgrass cultivar trial.

| Source | DF | MS | Pr(>\|t\|) |
|---|---|---|---|
| Site | 2 | 49.76 | 0.001 |
| Replicate within Site | 6 | 1.91 | 0.618 |
| Cultivar | 74 | 18.95 | < 0.001 |
| Cultivar x Site | 148 | 4.39 | < 0.001 |
| Residuals | 444 | 2.59 | |

**Table 12.** Analysis of variance of survival rating data for the Roselawn Cemetery boulevard site in the roadside turfgrass cultivar trial.

| Source | DF | MS | Pr(>\|t\|) |
|---|---|---|---|
| Replicate | 2 | 0.56 | 0.813 |
| Cultivar | 74 | 9.85 | < 0.001 |
| Residuals | 148 | 2.71 | |

**Table 13.** Analysis of variance of survival rating data for the Roselawn Cemetery main plot site in the roadside turfgrass cultivar trial.

| Source | DF | MS | Pr(>\|t\|) |
|---|---|---|---|
| Replicate | 2 | 1.69 | 0.584 |
| Cultivar | 74 | 5.46 | 0.002 |
| Residuals | 148 | 3.14 | |

**Table 14.** Analysis of variance of survival rating data for the Albertville site in the roadside turfgrass cultivar trial.

| Source | DF | MS | Pr(>\|t\|) |
|---|---|---|---|
| Replicate | 2 | 3.48 | 0.165 |
| Cultivar | 74 | 12.41 | < 0.001 |
| Residuals | 148 | 1.91 | |

**Table 15**. Mean survival ratings in the boulevard at Roseville for each cultivar or selection in the roadside turfgrass cultivar trial.

| Cultivar or Selection | Species§ | Mean†,‡ Rating | Cultivar or Selection | Species | Mean Rating |
|---|---|---|---|---|---|
| Shoreline | SLCRF | 7.3 | Mustang 4 | TF | 2.0 |
| Navigator | STCRF | 6.7 | Shademaster III | STCRF | 2.0 |
| McAlpin | STCRF | 6.5 | Tar Heel II | TF | 2.0 |
| ASR 050 | SLCRF | 6.3 | Accent II | PR | 1.7 |
| 67135 | SH | 6.0 | Citation Fore | PR | 1.7 |
| Cardinal | STCRF | 6.0 | JR-521 | PR | 1.7 |
| Seabreeze GT | SLCRF | 6.0 | Kentucky-31 (E+) | TF | 1.7 |
| Marco Polo | SH | 5.7 | Langara | KBG | 1.7 |
| OR1 | STCRF | 5.7 | Silver Dollar | PR | 1.7 |
| SR 5130 | CH | 5.3 | Dawson E | SLCRF | 1.5 |
| Beacon | HF | 5.0 | Wolfpack II | TF | 1.5 |
| Oceania | AK | 5.0 | Apple GL | PR | 1.3 |
| PSG 5RM | STCRF | 5.0 | Arctic Green | PR | 1.3 |
| Salty | AK | 5.0 | Corona | TF | 1.3 |
| SR 8650 | TF | 4.7 | Coronado TDH | TF | 1.3 |
| Bighorn GT | BHD | 4.5 | Diva | KBG | 1.3 |
| Grande II | TF | 4.5 | Gray Fox | PR | 1.3 |
| SaltonSea | AK | 4.5 | Headstart II | PR | 1.3 |
| PST 8000 | STCRF | 4.3 | Jaguar 4G | TF | 1.3 |
| SR 5250 | STCRF | 4.3 | Jumpstart | KBG | 1.3 |
| Humboldt Bay | DC | 4.0 | Midnight | KBG | 1.3 |
| TCP | CH | 4.0 | Orfeo | KBG | 1.3 |
| Grand Slam II | PR | 3.5 | Argos | KBG | 1.0 |
| Fults | AK | 3.3 | Arrowhead | KBG | 1.0 |
| Sealink | SLCRF | 3.3 | Brighstar SLT | PR | 1.0 |
| Epic | STCRF | 3.0 | Brooklawn | KBG | 1.0 |
| Gazelle II | TF | 3.0 | Caddyshack II | PR | 1.0 |
| Little Bighorn | BHD | 3.0 | Dragon | KBG | 1.0 |
| Moonlight SLT | KBG | 3.0 | Golfstar | IBG | 1.0 |

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**Table 15**. Continued.

| Cultivar or Selection | Species§ | Mean†,‡ Rating | Cultivar or Selection | Species | Mean Rating |
|---|---|---|---|---|---|
| Dynamic II | TF | 2.7 | Harrier | PR | 1.0 |
| Gray Goose | PR | 2.7 | JT-158 | TF | 1.0 |
| Providence | CBG | 2.7 | Minnesota | KM | 1.0 |
| Celestial | STCRF | 2.3 | Mariner | CBG | 1.0 |
| Fiesta III | PR | 2.3 | Park | KBG | 1.0 |
| JR-522 | PR | 2.3 | Right | KBG | 1.0 |
| Moonshine | KBG | 2.3 | SR 6000 | DC | 1.0 |
| Endeavor II | TF | 2.0 | Barkoel | KM | 1.0 |
| Florentine GT | STCRF | 2.0 | | | |

† Data collected April 27, 2011. Three replications of plots were rated on a 1-9 scale (1 = complete death, 9 = no visible injury) for survival of existing turfgrass plant tissue.

‡ LSD = 2.66

§ AK, alkaligrass; BHD, blue hard fescue; CH, Chewings fescue; CBG, creeping bentgrass; HF, hard fescue; IBG, Idaho bentgrass; KBG, Kentucky bluegrass; PR, perennial ryegrass; TF, tall fescue; KM, prairie junegrass; SH, sheep fescue; DC, tufted hairgrass; SLCRF, slender creeping red fescue; STCRF, strong creeping red fescue

**Table 16**. Mean survival ratings in the main plot at Roseville for each cultivar or selection in the roadside turfgrass cultivar trial.

| Cultivar or Selection | Species§ | Mean†,‡ Rating | Cultivar or Selection | Species | Mean Rating |
|---|---|---|---|---|---|
| Shoreline | SLCRF | 6.0 | Brooklawn | KBG | 1.0 |
| Fults | AK | 5.7 | Caddyshack II | PR | 1.0 |
| 67135 | SH | 5.3 | Citation Fore | PR | 1.0 |
| Cardinal | STCRF | 5.3 | Corona | TF | 1.0 |
| Navigator | STCRF | 4.7 | Coronado TDH | TF | 1.0 |
| Beacon | HF | 4.0 | Dawson E | SLCRF | 1.0 |
| Bighorn GT | BHD | 4.0 | Diva | KBG | 1.0 |
| PSG 5RM | STCRF | 4.0 | Dragon | KBG | 1.0 |
| PST 8000 | STCRF | 4.0 | Endeavor II | TF | 1.0 |
| SR 5250 | STCRF | 4.0 | Gazelle II | TF | 1.0 |
| McAlpin | STCRF | 3.7 | Golfstar | IBG | 1.0 |
| OR1 | STCRF | 3.7 | Grande II | TF | 1.0 |
| SR 5130 | CH | 3.7 | Grand Slam II | PR | 1.0 |
| Celestial | STCRF | 3.3 | Humboldt Bay | DC | 1.0 |
| Headstart II | PR | 3.3 | JR-521 | PR | 1.0 |
| Accent II | PR | 2.7 | JR-522 | PR | 1.0 |
| ASR 050 | SLCRF | 2.7 | JT-158 | TF | 1.0 |
| Jaguar 4G | TF | 2.7 | Jumpstart | KBG | 1.0 |
| Seabreeze GT | SLCRF | 2.7 | Minnesota | KM | 1.0 |
| Wolfpack II | TF | 2.7 | Kentucky-31 (E+) | TF | 1.0 |
| Epic | STCRF | 2.3 | Langara | KBG | 1.0 |
| Florentine GT | STCRF | 2.3 | Mariner | CBG | 1.0 |
| Gray Fox | PR | 2.3 | Midnight | KBG | 1.0 |
| Harrier | PR | 2.3 | Moonshine | KBG | 1.0 |
| Marco Polo | SH | 2.3 | Mustang 4 | TF | 1.0 |
| Gray Goose | PR | 2.0 | Oceania | AK | 1.0 |
| Little Bighorn | BHD | 2.0 | Orfeo | KBG | 1.0 |
| Shademaster III | STCRF | 2.0 | Park | KBG | 1.0 |
| SR 8650 | TF | 2.0 | Providence | CBG | 1.0 |

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**Table 16**. Continued.

| Cultivar or Selection | Species§ | Mean†,‡ Rating | Cultivar or Selection | Species | Mean Rating |
|---|---|---|---|---|---|
| Brighstar SLT | PR | 1.7 | Right | KBG | 1.0 |
| Dynamic II | TF | 1.7 | SaltonSea | AK | 1.0 |
| Fiesta III | PR | 1.7 | Salty | AK | 1.0 |
| TCP | CH | 1.7 | Sealink | SLCRF | 1.0 |
| Arctic Green | PR | 1.3 | Silver Dollar | PR | 1.0 |
| Moonlight SLT | KBG | 1.3 | SR 6000 | DC | 1.0 |
| Apple GL | PR | 1.0 | Tar Heel II | TF | 1.0 |
| Argos | KBG | 1.0 | Barkoel | KM | 1.0 |
| Arrowhead | KBG | 1.0 | | | |

[†] Data collected April 27, 2011. Three replications of plots were rated on a 1-9 scale (1 = complete death, 9 = no visible injury) for survival of existing turfgrass plant tissue.

[‡] LSD = 2.86

[§] AK, alkaligrass; BHD, blue hard fescue; CH, Chewings fescue; CBG, creeping bentgrass; HF, hard fescue; IBG, Idaho bentgrass; KBG, Kentucky bluegrass; PR, perennial ryegrass; TF, tall fescue; KM, prairie junegrass; SH, sheep fescue; DC, tufted hairgrass; SLCRF, slender creeping red fescue; STCRF, strong creeping red fescue

**Table 17**. Mean survival ratings at Albertville for each cultivar or selection in the roadside turfgrass cultivar trial.

| Cultivar or Selection | Species§ | Mean†,‡ Rating | Cultivar or Selection | Species | Mean Rating |
|---|---|---|---|---|---|
| Fults | AK | 9.0 | Corona | TF | 2.0 |
| Oceania | AK | 9.0 | Endeavor II | TF | 2.0 |
| SaltonSea | AK | 9.0 | Florentine GT | STCRF | 2.0 |
| Salty | AK | 9.0 | Tar Heel II | TF | 2.0 |
| ASR 050 | SLCRF | 6.3 | Barkoel | KM | 2.0 |
| Mariner | CBG | 6.0 | Dragon | KBG | 1.7 |
| Marco Polo | SH | 5.3 | Gray Goose | PR | 1.7 |
| Shoreline | SLCRF | 5.3 | SR 6000 | DC | 1.7 |
| Seabreeze GT | SLCRF | 5.0 | Apple GL | PR | 1.3 |
| Sealink | SLCRF | 5.0 | Arrowhead | KBG | 1.3 |
| Beacon | HF | 4.7 | Citation Fore | PR | 1.3 |
| Grande II | TF | 4.3 | Gazelle II | TF | 1.3 |
| Cardinal | STCRF | 4.0 | Jaguar 4G | TF | 1.3 |
| Epic | STCRF | 4.0 | Jumpstart | KBG | 1.3 |
| McAlpin | STCRF | 4.0 | Midnight | KBG | 1.3 |
| TCP | CH | 4.0 | Moonlight SLT | KBG | 1.3 |
| Celestial | STCRF | 3.7 | Orfeo | KBG | 1.3 |
| Little Bighorn | BHD | 3.7 | Park | KBG | 1.3 |
| OR1 | STCRF | 3.7 | Accent II | PR | 1.0 |
| SR 5250 | STCRF | 3.7 | Arctic Green | PR | 1.0 |
| 67135 | SH | 3.3 | Argos | KBG | 1.0 |
| Bighorn GT | BHD | 3.3 | Brighstar SLT | PR | 1.0 |
| Navigator | STCRF | 3.3 | Caddyshack II | PR | 1.0 |
| Shademaster III | STCRF | 3.3 | Dawson E | SLCRF | 1.0 |
| SR 5130 | CH | 3.3 | Diva | KBG | 1.0 |
| Coronado TDH | TF | 3.0 | Dynamic II | TF | 1.0 |
| Humboldt Bay | DC | 3.0 | Fiesta III | PR | 1.0 |
| JT-158 | TF | 3.0 | Golfstar | IBG | 1.0 |
| Providence | CBG | 3.0 | Gray Fox | PR | 1.0 |

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**Table 17**. Continued.

| Cultivar or Selection | Species§ | Mean†,‡ Rating | Cultivar or Selection | Species | Mean Rating |
|---|---|---|---|---|---|
| PSG 5RM | STCRF | 3.0 | Harrier | PR | 1.0 |
| Grand Slam II | PR | 2.7 | Headstart II | PR | 1.0 |
| PST 8000 | STCRF | 2.7 | JR-521 | PR | 1.0 |
| SR 8650 | TF | 2.7 | JR-522 | PR | 1.0 |
| Wolfpack II | TF | 2.7 | Minnesota | KM | 1.0 |
| Brooklawn | KBG | 2.3 | Moonshine | KBG | 1.0 |
| Kentucky-31 (E+) | TF | 2.3 | Right | KBG | 1.0 |
| Langara | KBG | 2.3 | Silver Dollar | PR | 1.0 |
| Mustang 4 | TF | 2.3 | | | |

† Data collected May13, 2011. Three replications of plots were rated on a 1-9 scale (1 = complete death, 9 = no visible injury) for survival of existing turfgrass plant tissue.

‡ LSD = 2.23

§ AK, alkaligrass; BHD, blue hard fescue; CH, Chewings fescue; CBG, creeping bentgrass; HF, hard fescue; IBG, Idaho bentgrass; KBG, Kentucky bluegrass; PR, perennial ryegrass; TF, tall fescue; KM, prairie junegrass; SH, sheep fescue; DC, tufted hairgrass; SLCRF, slender creeping red fescue; STCRF, strong creeping red fescue

# Chapter 3: Salt Tolerance of 74 Turfgrass Cultivars in Nutrient Solution Culture

## Summary

Turfgrass may experience significant salt stress due to poor water quality, insufficient leaching, or exposure to environmental contaminants. Establishment of salt-tolerant turfgrass cultivars is one possible method of mitigating the effects of salts in irrigation water or the soil environment. The objective of this research was to evaluate the relative salt tolerance of cool-season turfgrasses in a controlled environment using digital image analysis. Six replications of 74 cool-season turfgrasses were established using recommended seeding rates in 10.16-cm by 10.16-cm pots of silica sand for 12 wk, and suspended in two 760-L tubs of half-strength Hoagland solution. Following an adaptation period, all pots were exposed to salinity levels of 4, 14, and 24 dS $m^{-1}$ successively, each for 2 wk. Digital images were collected after each exposure level using a custom light box, and analyzed for percent green tissue. Cultivars of tall fescue (Festuca *arundinacea* Schreb.) were found to be most salt tolerant, with 'Wolfpack II' and 'Jaguar 4G' performing best after the 14 and 24 dS m−1 exposure levels, respectively. Many fine fescue entries performed well, including slender creeping red fescues (Festuca *rubra* L. ssp. *litoralis)* 'Sealink', 'Seabreeze GT', and 'Shoreline'. Trial entries found to have consistent salt tolerance at all levels may be used to guide recommendations for turfgrass managers and breeders and have potential for use in salt-tolerant mixtures.

## Introduction

Turfgrass is often subject to significant salt stress as a result of poor water quality, insufficient leaching, or exposure to environmental contaminants. Establishment of salt tolerant turfgrass cultivars can help mitigate the effects of salts in irrigation water or the soil environment. Although a multitude of screening methods have been used under greenhouse conditions to evaluate turfgrass salt tolerance (Greub et al., 1985; Marcum, 2001; Rose-Fricker and Wipff, 2001; Pessarakli and Kopec, 2008; Qian et al., 2007; Koch et al., 2011; Wang et al., 2011), few have included enough entries to allow for ranking of cultivars within several different species. A variety of field screening techniques have been used to successfully identify differences between cultivars (Biesboer et al., 1998; Koch and Bonos, 2011a; Brown and Gorres, 2011; Friell et al., 2012) and are capable of capturing the wide range of simultaneous stresses experienced in the field which are not readily replicable in a greenhouse environment. However, it is often desirable to perform screenings for salt tolerance under controlled conditions in order to avoid confounding effects of weather, disease, or other environmental factors often present in a field setting. Koch and Bonos (2011b) showed that results of multiple greenhouse screening methods correlated well with those used under controlled field conditions. Moreover, Bowman et al. (2006a) demonstrated that reduced nitrogen concentrations lessened the effects of salt on leaf growth for tall fescue (*Festuca arundinacea* Schreb.) indicating that greater control over nutrient status provided by greenhouse environments can help create a more sensitive screening than some field-based methods.

Using nutrient solution culture, Rose-Fricker and Wipff (2001) observed differences in salt tolerance, as determined by a visual assessment of leaf damage, between cultivars of Kentucky bluegrass (*Poa pratensis* L.) and between cultivars of perennial ryegrass (*Lolium perenne* L.). Despite its thoroughness, this study was carried out over a decade ago, and new germplasm and cultivars have been introduced since its publication that are in need of thorough salinity tolerance screening. More recently, Koch et al. (2011) identified salinity tolerance differences between cultivars and experimental lines of Kentucky bluegrass and Kentucky bluegrass × Texas bluegrass (*Poa arachnifera* Torr.) hybrids using an overhead irrigation system. Response variables for their study included biweekly leaf clipping dry weights, weekly visual assessment of percent green canopy, as well as root and shoot dry weights at the conclusion of the study. Results indicated that breeding efforts have resulted in some improvement of salinity stress within the species but concluded that further testing of Kentucky bluegrass germplasm for salinity tolerance was necessary. Wang et al. (2011) used the unique approach of drenching grasses in saline water for 20 min. each week and measuring electrolyte leakage, tissue dry weight, and visual quality ratings to assess differences in salt tolerance between populations of prairie junegrass (*Koeleria macrantha* (Ledeb.) Schult.). Their results showed superior salt tolerance of the improved European varieties and indicated the possibility for improvement in the native North American populations.

While visual assessment methods provide for rapid data collection, they are less desirable than the quantitative data. Unfortunately, many quantitative methods such as determination of dry clipping weights, electrolyte leakage, and root mass or viability can be time consuming. In recent years, digital image analysis has proven to be a useful tool for rapid quantification of turfgrass cover and color (Richardson et al., 2001; Karcher and Richardson, 2003). Adaptation of this methodology to a greenhouse environment has been shown to provide a quantitative method of evaluation for salt tolerance trials in a controlled environment. Dai et al. (2008) identified significant differences in tolerance to sodium chloride (NaCl) among experimental lines of annual bluegrass (*Poa annua* L.) using change in percent green canopy tissue as measured by digital image analysis. Koch et al. (2011) also used digital image analysis in their assessment of Kentucky bluegrass and Kentucky bluegrass × Texas bluegrass hybrids discussed above. Both studies found digital image analysis to be a useful method for quantifying salinity stress in turfgrass.

Given the salinity tolerance differences observed between experimental lines, populations, and cultivars in many of the recent studies, it is important to directly evaluate the existing germplasm of cool-season turfgrasses for differences in salt tolerance during vegetative growth. The objective of this research was to quantitatively evaluate the relative salt tolerance of cool-season turfgrasses using nutrient solution culture in a controlled environment. This was to be accomplished using digital image analysis to quantify the amount of green turf tissue remaining when plants were subjected to salt stress.

## Methods and Materials

### Experimental Setup

Six replications of 74 cool-season turfgrasses (Table 1) representing 14 species (tall fescue, slender creeping red fescue, hard and blue hard fescues [*Festuca trachyphylla* (Hack.) Krajina], strong creeping red fescue [*Festuca rubra* L. ssp. *rubra*], alkaligrass [*Puccinellia spp.*], tufted hairgrass [*Deschampsia cespitosa* (L.) P. Beauv.], perennial ryegrass, sheep fescue [*Festuca ovina* L.] creeping bentgrass [*Agrostis stolonifera* L.], Kentucky bluegrass, prairie junegrass, Chewings fescue [*Festuca rubra* L. ssp. *fallax* (Thuill.) Nyman], and Idaho bentgrass [*Agrostis idahoensis* Nash]) were seeded in 10.16 cm x 10.16 cm square pots of silica sand. Trial entries representing cultivars, experimental lines, and ecotypes, were selected based on input from turfgrass breeders as well as published data from previous trials in the northern United States (Rose-Fricker and Wipff, 2001; Koch and Bonos, 2011a; Brown and Gorres, 2011; Friell et al., 2012). Seeding rates (Table 2) were chosen for each species based on suggested rates (Christians, 2011). Because purity of the Minnesota ecotype of prairie junegrass was known to be low, the bulk seeding rate was increased, based on breeder recommendations, to approximate the same effective seeding rate as the European cultivar 'Barkoel', resulting in the range of seeding rates for that species shown in Table 2. Plastic screen was placed in the bottom of the pots in order to allow root growth through the bottom while containing the sand within. Trial entries were established in a greenhouse for 12 wk, beginning in fall 2010, and the experiment was repeated during summer 2011 with a 14-wk establishment period. During establishment, pots were watered daily to field capacity, fertilized with a dilute fertilizer solution (Table 3) every other day, and clipped weekly to a height of 5 cm.

Following establishment, two large tubs were each filled with 760 L of half-strength Hoagland solution (Hoagland and Arnon, 1950) amended with 5.3 mg $L^{-1}$ EDTA-chelated iron. The tubs were continuously aerated, and nutrient solution within each tub was cycled from one end to the other through an external pump at a rate of 5800 L $h^{-1}$. Three replications of each cultivar were suspended in each tub in a randomized complete block design. The submerged portion of the pot was 5 cm deep with the top of the pot situated approximately 7.5 cm above the surface of the nutrient solution. During the first run of the experiment, a number of pots representing several cultivars were removed due to severe infection by *Cladosporium spp.* While in the tubs, pots were clipped every 7-10 days to a height of 5 cm and received supplemental lighting, which provided 16 hours of light exposure per day with a maximum of 1418 µmol $m^{-2}$ $s^{-1}$ photosynthetically active radiation. Plants were allowed to adapt to the nutrient culture growth conditions for four weeks before salt exposure began.

Salt exposure was initiated by supplementing the nutrient solution with 5 M NaCl solution such that the electrical conductivity (EC) of the solution reached a specified level. Solution EC was ramped at a rate of 2 dS $m^{-1}$ $d^{-1}$ to 4 dS $m^{-1}$, 14 dS $m^{-1}$, and 24 dS $m^{-1}$ successively, and held at each of the three levels for two weeks. This salinity exposure profile was chosen to approximate previous measurements of soil salinity within one meter of roadways in Minnesota during late fall and early spring (Biesboer and Jacobson, 1994). Between the specified salinity levels, the nutrient solution was drained and replaced, and the salinity level increased at a rate of 2 dS $m^{-1}$ $d^{-1}$ from the previous level to the next specified level. Pots remained in the tubs throughout the entire course of the salt exposure sequence and each data set was treated as a single observation at the end of a nested experiment. The total salt exposure for each nested experiment represented the sum of the magnitudes and exposure times prior to the data collection date, as diagrammed in Fig. 1.

## Data Collection & Analysis

Digital images were collected following the 4-wk adaptation period of each experimental run, and at the end of each subsequent 2-wk period of specified EC for a total of four data sets. Images were collected using a custom light box designed to fit over the pots. The box was designed to provide a solid color frame around the pot so as to distinguish between the background and the turf tissue. Pictures were analyzed for percent green tissue using a custom image processing script written using Image Processing Toolbox (MATLAB, 2011).

The design of the experiment allowed for comparison of cultivars within each data set collected, but not for comparison among the four data sets. Thus, the data sets collected following each of the 2-wk 4 dS $m^{-1}$, 14 dS $m^{-1}$, and 24 dS $m^{-1}$ exposure periods were treated as independent single-observation experiments, the unique salt exposure profiles for which were defined by the magnitudes and exposure times experienced prior to the data collection date. Salt exposure following collection of a data set was not relevant to the analysis of that data, and the results of each analysis played no role in the analysis of future data sets. Data collected after the 4-wk adaptation period was used to examine the effects of growth in the nutrient solution culture and to verify that no significant differences existed between the cultivars at the start of the salt exposure profile. Data from both experimental runs were analyzed together and modeled with a linear mixed effects cell means model using the *lme* function in the *nlme* package in R Project for Statistical Computing (R Development Core Team, 2012). Each model contained a single fixed effect term for cultivar. Grouping levels for the experiment were experimental run, tub within run, and replicate within tub; all of which were treated as random variables. Using linear mixed effects models, random effects are characterized as distributions and, thus, are not testable in a traditional ANOVA sense. Confidence intervals for each estimated fixed effect mean were determined at the $\alpha=0.05$ level using the *intervals* function and are calculated using the standard error of the estimates.

## Results

Analysis of the data collected following the 4-wk adaptation period (Data Set 1, Fig. 2) showed that no entries were significantly different from one another after growth in the nutrient solution culture for four weeks. This indicated that no initial differences existed between the entries' green tissue percentages, and comparison of salt tolerances could be made based using absolute values. Similarly, no entries were significantly different from one another in the data set collected following the 2-wk sustained exposure to 4 dS m$^{-1}$ (Data Set 2, Fig. 3).

Data collected at the point in the salt exposure profile just after the sustained 2-wk exposure to 14 dS m$^{-1}$ (Data Set 3) indicated significant differences among cultivars (Fig. 4). Results from this data set showed more significant differences among entries within the Kentucky bluegrass and perennial ryegrass species than any other data set. Perennial ryegrass 'Affirmed' retained significantly less green canopy tissue than 'Accent II' with 44.2% and 65.5%, respectively. Both 'Affirmed' and 'Citation Fore' were significantly lower than entry JR-522, which provided the greatest salt tolerance of all perennial ryegrass entries at that point. Kentucky bluegrass 'Moonshine' was observed to retain significantly less green tissue than both 'Diva' and 'Park'. Of all Kentucky bluegrass cultivars, 'Park' provided the greatest percentage of green canopy tissue with 56.4%. No other significant differences existed within species. Although the experimental design and analysis did not allow for significance tests of differences between species means, there was a clear trend of tall fescue cultivars possessing the greatest salt tolerance as is reflected in the species groupings in Fig. 4. The overall mean percent green tissue across all tall fescue entries in the data set was 80.6%. Tall fescue 'Wolfpack II' had the highest mean percent green tissue to that point in the salt exposure profile, with 82.9% green tissue followed by 'Grande II' and 'Endeavor II' with 82.6% and 82.3%, respectively. Idaho bentgrass and Chewings fescue provided the lowest mean salt tolerances by species and the Minnesota ecotype of prairie junegrass was the lowest entry overall in the data set with 27.8% green tissue.

Data collected after the 2-wk sustained exposure to 24 dS $m^{-1}$ (Data Set 4) also showed significant differences between entries (Fig. 5); however, no significant differences existed between entries within species. Overall, slender creeping red fescue entries had the highest mean percent green tissue with 52.8%. Slender creeping red fescues 'Sealink' and 'Seabreeze GT' were the best of that species with 55.1% and 55.0% green tissue, respectively. Neither of those two entries was significantly different from 'Jaguar 4G' tall fescue, which maintained the highest mean percent green tissue in the trial after the full salt exposure profile, with 56.6% green tissue. Although alkaligrass entries performed similarly to those of several other species including blue hard fescue, strong creeping red fescue, and perennial ryegrass in Data Set 3, they had a higher overall mean than each of those species with 63.5% green tissue in Data Set 4. Moreover, all cultivars of alkaligrass were statistically significantly better than both cultivars of Chewings fescue, which had an overall species mean of 3.5%. Perennial ryegrass and Kentucky bluegrass cultivars were observed to perform much worse relative to the other species after the full salt exposure profile (Data Set 4) than in the previous results (Data Set 3) with overall species means of just 15.9% and 11.3%, respectively. Of the Kentucky bluegrass entries, ‘Park’ maintained the highest percent green tissue with 15.8%, and perennial ryegrass JR-522 led all entries of that species with 23.4% green tissue remaining. ‘Radar’ Chewings fescue was the worst overall entry after the full salt exposure profile with a mean of just 3.3% green tissue.

## Discussion

This trial was conducted to quantitatively evaluate the relative salt tolerance of cool-season turfgrasses using nutrient solution culture in a controlled environment. Use of digital image analysis allowed for a more accurate measure of salinity tolerance during vegetative growth than visual rating methods used in previous studies (Marcum, 2001; Rose-Fricker and Wipff, 2001; Pessarakli and Kopec, 2008; Wang et al., 2011; Koch and Bonos, 2011b). In both the data collected following the 2-wk exposure to 14 dS $m^{-1}$ (Data Set 3) and that at the end of the 24 dS $m^{-1}$ exposure (Data Set 4), tall fescue and fine fescue entries outperformed those of other species in the trial while cultivars and ecotypes of Kentucky bluegrass and prairie junegrass were among the least salt-tolerant. These results generally agree with previous trials in both greenhouse (Alshammary et al., 2004; Wang et al., 2011) and field settings where NaCl exposure due to application of road salt is a problem (Brown and Gorres, 2011; Friell et al., 2012), although tall fescue was not observed to perform as well in roadside trials. In addition to establishment, weather, disease, and other field phenomenon not captured by greenhouse experiments, a number of other factors may explain differences between greenhouse and roadside evaluations of salt tolerance. Bowman et al. (2006a) showed that decreased nitrogen status reduced differences in salt tolerance between cultivars of tall fescue. They further demonstrated that salinity and nitrogen status in a nutrient solution culture system played a role in limiting nitrogen uptake by tall fescue plants (Bowman et al., 2006b). Their results may explain differences between observations in the field and the nitrogen-replete greenhouse environment. Also, formulation of an appropriate nutrient solution to simulate road salt exposure is complicated by the use of a variable mix of sodium, magnesium, and calcium chlorides on roads as well as the variety of anti-caking agents and corrosion inhibitors used in the treatment of the salt.

Despite tall fescue entries performing best at the two highest salt levels, cultivars of several other species were observed to be not statistically different from them. Alakaligrass cultivars 'Salton Sea', 'Salty', 'Fults', and 'Oceania' were all not statistically different from the top-performing cultivar for data taken after the 14 dS $m^{-1}$ or 24 dS $m^{-1}$ exposure periods. The same four cultivars were found to be tolerant of road salt applications when established on roadsides (Friell et al., 2012). Biesboer et al. (1998) found alkaligrass to be highly persistent on roadsides where damage due to NaCl was known to be a problem, but attributed its success to fast growth and prolific seed production. While these may be factors in its success on roadsides, results from this trial agree with others (Alshammary et al., 2004) which have shown that alkaligrass is physiologically adapted to be tolerant of exposure to NaCl.

Numerous fine fescue entries performed well following both the 14 dS m$^{-1}$ and 24 dS m$^{-1}$ exposure periods. 'Seabreeze GT', 'Sealink', 'Shoreline', and ASR050 slender creeping red fescues were the top-performing fine fescue entries following both of those salinity exposure periods, and were not significantly different from the best entry after either. 'Shoreline' slender creeping red fescue has also been observed to survive extremely well on roadsides where exposure to NaCl is a problem (Friell et al., 2012) and, as a species, slender creeping red fescue has been shown to be salt tolerant in other trials (Torello and Symington, 1984; Rose-Fricker and Wipff, 2001). While few studies have ranked cultivars within the species, Brown and Gorres (2011) concluded that in low-fertility, un-amended roadsides soils there was no clear advantage to the use of improved cultivars over common creeping red fescue (*Festuca rubra* L.) due to overall poor persistence. Results presented here indicate that under extreme, prolonged salt exposure (Data Set 4) differences between cultivars within a species are not statistically significant and that the trends among the species mean tolerances are most dominant. Taken together, results of the two studies indicate that under extreme stress, cultivar selection is of little importance relative to species selection. Results of this study confirm the ranking of red fescue species suggested by Humphreys (1981) with entries of slender creeping red fescues performing better than those of strong creeping red fescue or Chewings fescue. Interestingly, entries of Chewings fescue, which were previously found to persist well on roadsides where deicing salt is applied (Friell et al., 2012), were among the worst entries in the trial. One possible explanation for this discrepancy is the confounding effect of growth in a fully saturated media as opposed to the variable moisture levels of soils in the field. Data collected following the 4-wk adaptation period prior to salt exposure indicated no significant differences existed between entries in the trial. Although this type of culture system has been used previously to test for salt tolerance, the possibility remains that certain species or cultivars are more negatively impacted than others over time by growth under those conditions.

Despite having one of the lowest species averages in the trial, entries of Kentucky bluegrass were found to be significantly different from one another in Data Set 3, following the 14 dS $m^{-1}$ exposure period. Using an overhead spray exposure system, Koch et al. (2011) found Kentucky bluegrasses ‘Diva’ and ‘Langara’ to have very good salt tolerance. Results of this trial also show ‘Diva’ to be among the best of the Kentucky bluegrass entries with only ‘Park’ retaining a higher percent green tissue. However, ‘Langara’ retained much less green tissue despite not being significantly different from any other entry of that species. This discrepancy indicates that, as suggested by Koch et al. (2011), consideration of foliar exposure is likely an important aspect of relative salinity tolerance assessments.

The two entries of prairie junegrass performed significantly differently following the 14 dS $m^{-1}$ exposure level. Improved European cultivar ‘Barkoel’ retained a much higher percentage of green tissue than the Minnesota ecotype, although with just two entries in the trial it is not clear if the difference is an effect of breeding efforts or a difference in the habitat of origin. However, the results agree with those from Wang et al. (2011) who previously found European cultivars ‘Barkoel’ and ‘Barleria’ to possess greater salt tolerance than several native North American populations of prairie junegrass, including that from Minnesota. Together, the results indicate some potential for improvement of salt tolerance in the North American prairie junegrass populations

## Conclusions

Quantitative evaluation using digital image analysis proved to be a valuable tool for quickly assessing differences in salt tolerance between cool-season turfgrasses grown in nutrient solution culture. Using that technique, no significant differences among cultivars were observed after 4 wk growth in a nutrient solution culture system. After a minimal salt exposure sequence ending at 4 dS $m^{-1}$, no significant differences existed between any trial entries. Following a moderate salt exposure sequence ending at 14 dS $m^{-1}$, significant differences existed between trial entries as well as between some entries within species. In particular, those species that retained some of the lowest overall means for percent green tissue, such as Kentucky bluegrass and perennial ryegrass, showed the greatest number of differences between entries within the species. Following the most prolonged salt exposure, which ended at 24 dS $m^{-1}$, differences between entries within species were not significant and trends among the mean tolerances of species were most important. Based upon these results, it can be concluded that turfgrass professionals should consider the level of salt exposure expected when deciding which species to use and whether or not improved cultivars can provide improved salt tolerance. Cultivars of tall fescue performed best at the highest levels of salinity, followed closely by creeping red fescues and alkaligrass. Cultivars of Chewings fescue, Idaho bentgrass, and Kentucky bluegrass were the least salt tolerant. Results of the study generally agree with previous assessments of salt tolerance of the same species and cultivars; however, limitations of the nutrient solution culture system should be considered when conducting future assessments. Many of the cultivars that performed well in this trial had not previously been evaluated for salt tolerance and performed better than other, older cultivars. This indicates that improvements are being made in cool-season turfgrass germplasm for salt tolerance and the results of this trial may be used to guide turfgrass breeders in their future efforts. Cultivars identified as performing well in this trial may also be used by turfgrass managers for the implementation and improvement of turfgrasses for salt-affected areas and may be used for the creation of salt-tolerant turfgrass species mixtures.

## Acknowledgements

We would like to thank the Minnesota Local Road Research Board for funding this project, and gratefully acknowledge the invaluable advice of our reviewers.

**Figure 1**. Salt exposure profile stages experienced by pots for each nested experiment. Pots experienced exposure to each stage as indicated by horizontal bars before data was collected and analyzed as an individual experiment. Data set 1 was used to confirm no differences existed prior to increasing salt exposure.

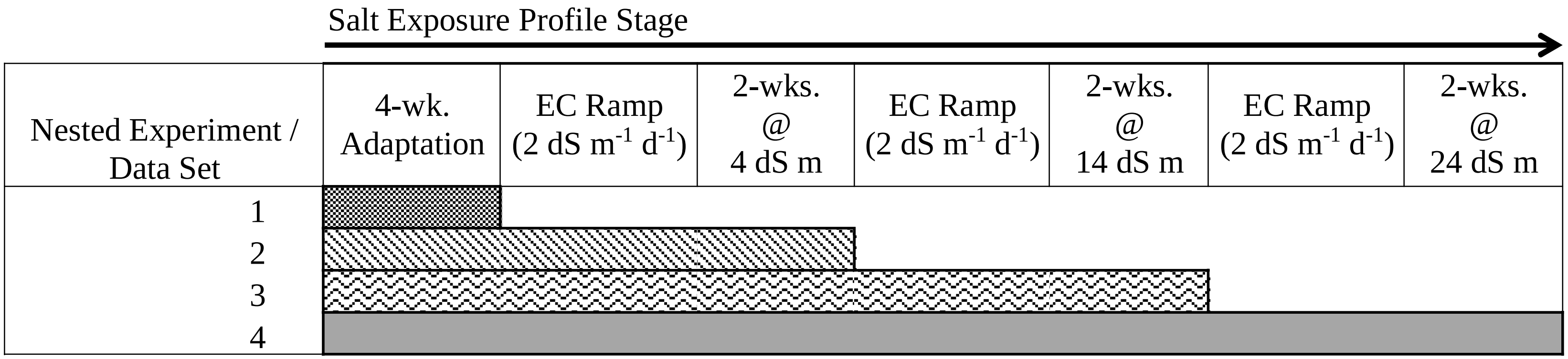

**Figure 2**. Cultivar effects and 95% confidence intervals on percent green tissue following 4-wk adaptation stage of salt exposure profile (Data Set 1). Species are separated by dotted lines. From top to bottom, species are: tall fescue, slender creeping red fescue, hard fescue, creeping bentgrass, strong creeping red fescue, blue hard fescue, alkaligrass, tufted hairgrass, perennial ryegrass, sheep fescue, Kentucky bluegrass, prairie junegrass, Chewings fescue, and Idaho bentgrass.

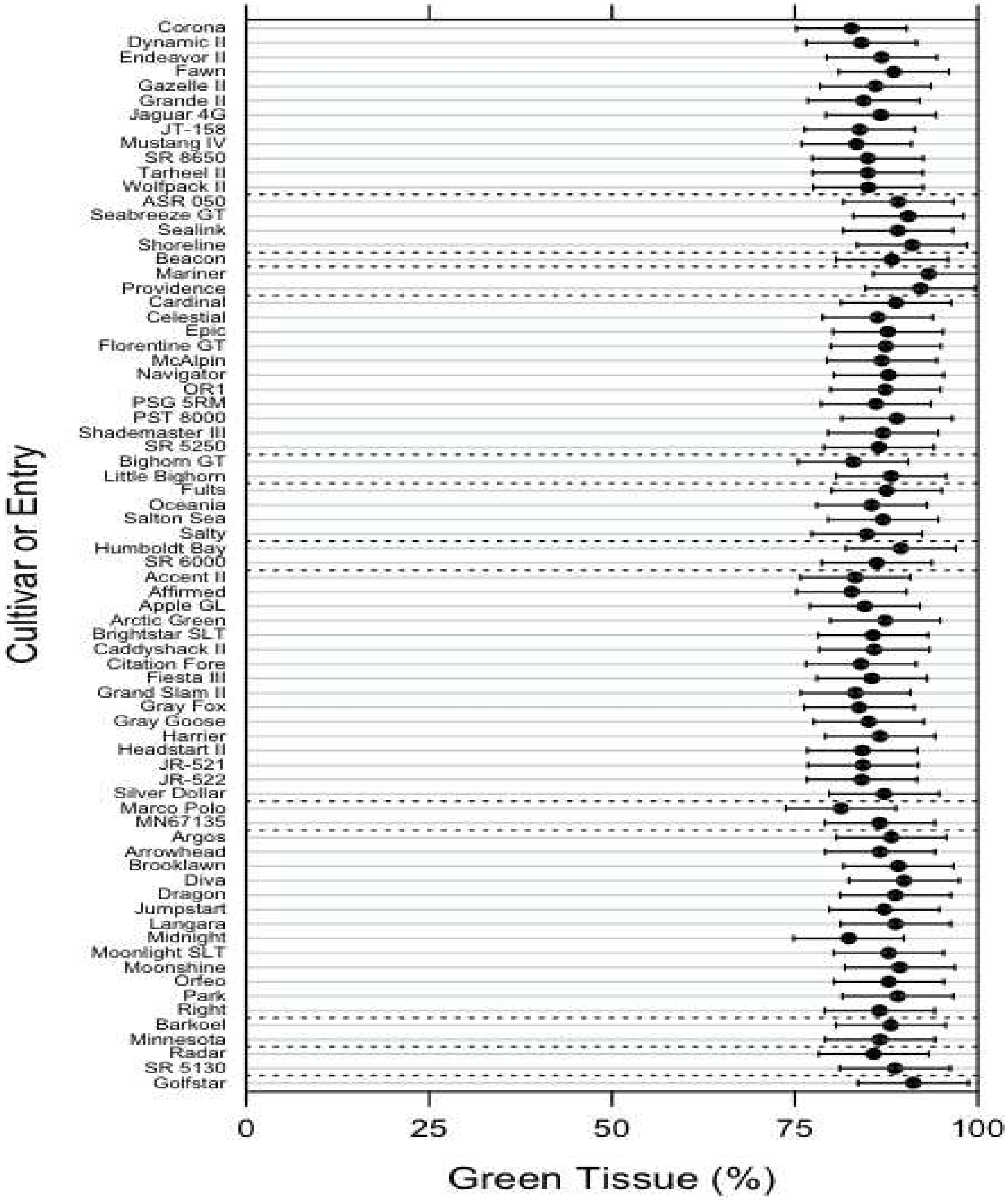

**Figure 3**. Cultivar effects and 95% confidence intervals on percent green tissue following 4 dS $m^{-1}$ stage of salt exposure profile (Data Set 2). Species are separated by dotted lines. From top to bottom, species are: tall fescue, slender creeping red fescue, hard fescue, creeping bentgrass, strong creeping red fescue, blue hard fescue, alkaligrass, tufted hairgrass, perennial ryegrass, sheep fescue, Kentucky bluegrass, prairie junegrass, Chewings fescue, and Idaho bentgrass.

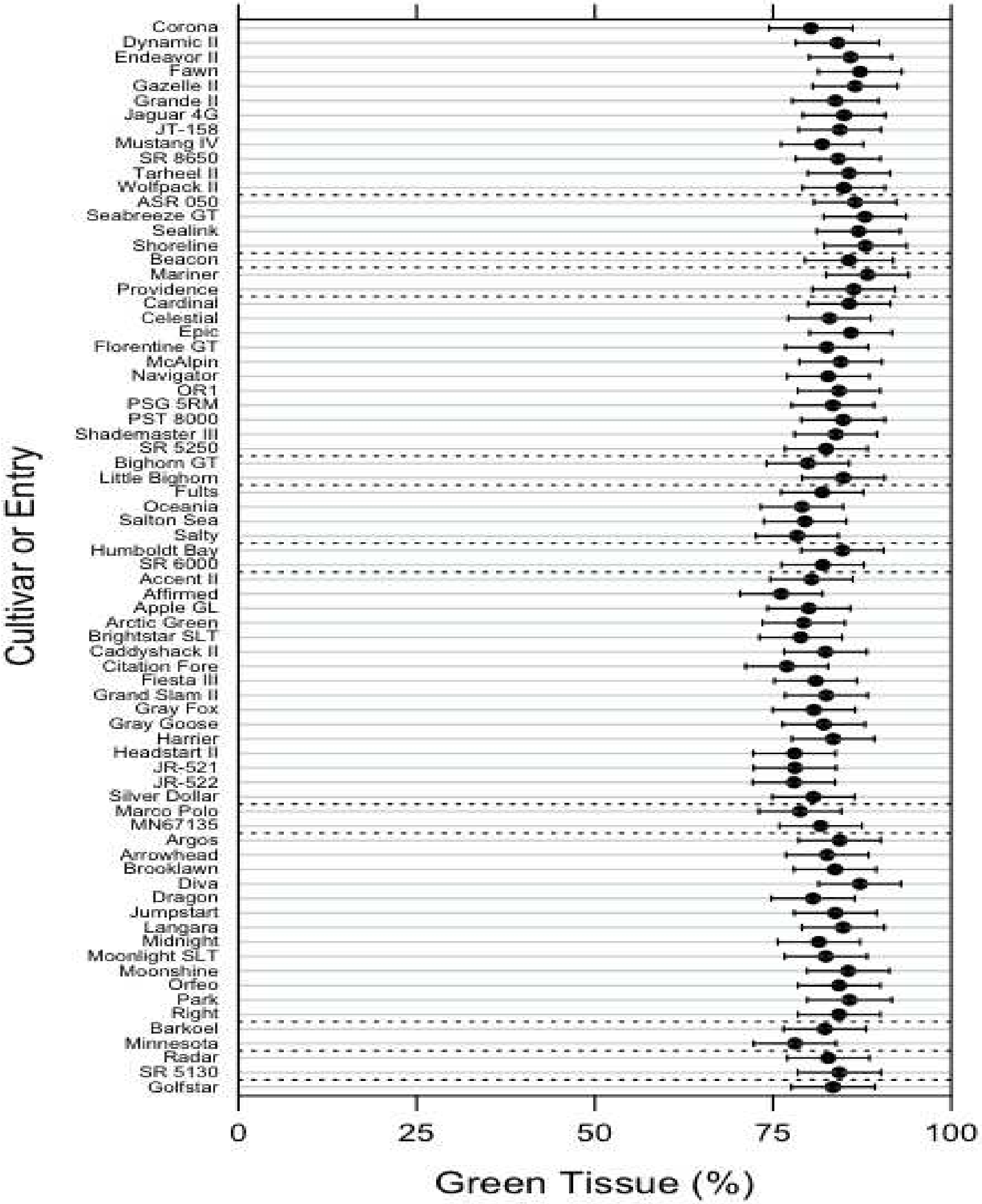

**Figure 4**. Cultivar effects and 95% confidence intervals on percent green tissue following 14 dS m$^{-1}$ stage of salt exposure profile (Data Set 3). Species are separated by dotted lines. From top to bottom, species are: tall fescue, slender creeping red fescue, hard fescue, creeping bentgrass, strong creeping red fescue, blue hard fescue, alkaligrass, tufted hairgrass, perennial ryegrass, sheep fescue, Kentucky bluegrass, prairie junegrass, Chewings fescue, and Idaho bentgrass.

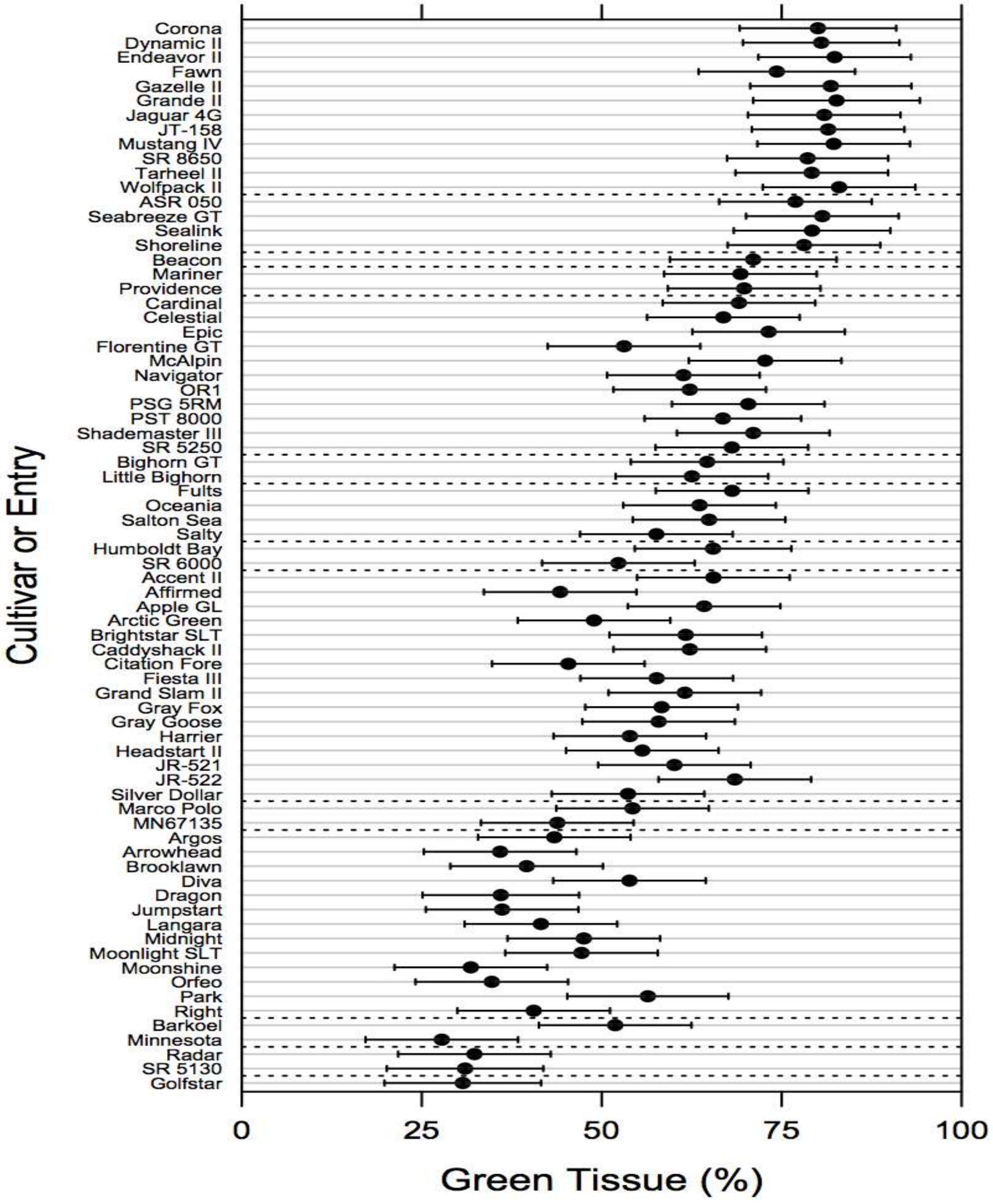

**Figure 5**. Cultivar effects and 95% confidence intervals on percent green tissue following 24 dS m$^{-1}$ stage of salt exposure profile (Data Set 4). Species are separated by dotted lines. From top to bottom, species are: tall fescue, slender creeping red fescue, hard fescue, creeping bentgrass, strong creeping red fescue, blue hard fescue, alkaligrass, tufted hairgrass, perennial ryegrass, sheep fescue, Kentucky bluegrass, prairie junegrass, Chewings fescue, and Idaho bentgrass.

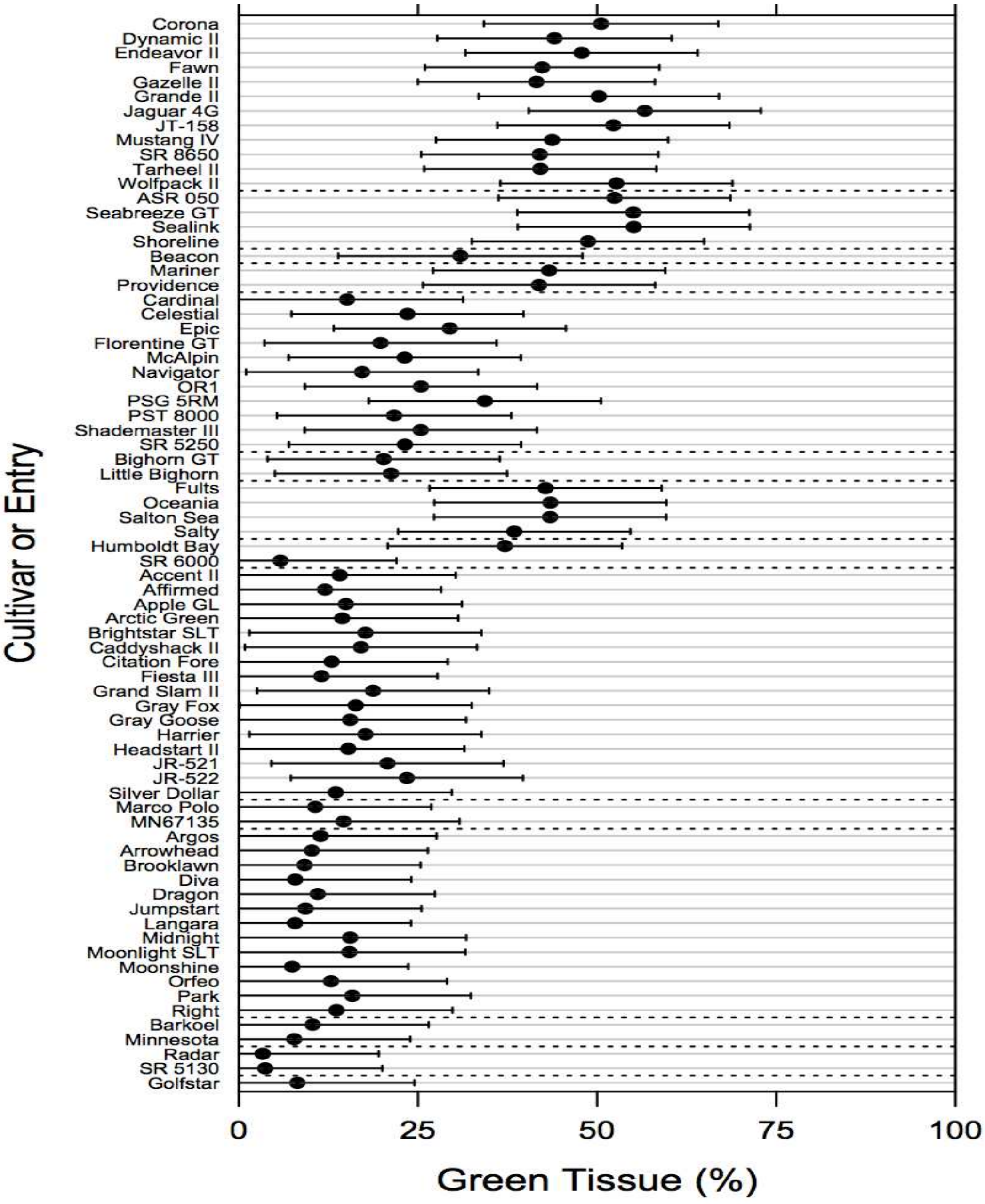

**Table 1**. Cultivars and entries included in the greenhouse salt tolerance screening.

| Cultivar or Entry | Species[†] | Cultivar or Entry | Species |
|---|---|---|---|
| Fults | AK | Grand Slam II | PR |
| Oceania | AK | Gray Fox | PR |
| Salton Sea | AK | Gray Goose | PR |
| Salty | AK | Harrier | PR |
| Bighorn GT | BHD | Headstart II | PR |
| Little Bighorn | BHD | JR-521 | PR |
| Mariner | CBG | JR-522 | PR |
| Providence | CBG | Silver Dollar | PR |
| Radar | CH | Marco Polo | SH |
| SR 5130 | CH | MN67135 | SH |
| Humboldt Bay (ecotype) | DC | ASR 050 | SLCRF |
| SR 6000 | DC | Seabreeze GT | SLCRF |
| Beacon | HF | Sealink | SLCRF |
| Golfstar | IBG | Shoreline | SLCRF |
| Argos | KBG | Cardinal | STCRF |
| Arrowhead | KBG | Celestial | STCRF |
| Brooklawn | KBG | Epic | STCRF |
| Diva | KBG | Florentine GT | STCRF |
| Dragon | KBG | McAlpin | STCRF |
| Jumpstart | KBG | Navigator | STCRF |
| Langara | KBG | OR1 | STCRF |
| Midnight | KBG | PSG 5RM | STCRF |
| Moonlight SLT | KBG | PST 8000 | STCRF |
| Moonshine | KBG | Shademaster III | STCRF |
| Orfeo | KBG | SR 5250 | STCRF |
| Park | KBG | Corona | TF |
| Right | KBG | Dynamic II | TF |
| Barkoel | KM | Endeavor II | TF |
| Minnesota (ecotype) | KM | Fawn | TF |
| Accent II | PR | Gazelle II | TF |
| Affirmed | PR | Grande II | TF |
| Apple GL | PR | Jaguar 4G | TF |
| Arctic Green | PR | JT-158 | TF |
| Brightstar SLT | PR | Mustang IV | TF |
| Caddyshack II | PR | SR 8650 | TF |
| Citation Fore | PR | Tarheel II | TF |
| Fiesta III | PR | Wolfpack II | TF |

[†] AK, alkaligrass; BHD, blue hard fescue; CBG, creeping bentgrass; CH, Chewings fescue; DC, tufted hairgrass; HF, hard fescue; IBG, Idaho bentgrass; KBG, Kentucky bluegrass; KM, prairie junegrass; PR, perennial ryegrass; SH, sheep fescue; SLCRF, slender creeping red fescue; STCRF, strong creeping red fescue; TF, tall fescue

**Table 2**. Species and seeding rates used in the salt tolerance screening.

| Species | Seeding Rate (kg ha$^{-1}$) |
|---|---|
| fine fescue | 244.1 |
| tall fescue | 341.7 |
| Kentucky bluegrass | 73.2 |
| perennial ryegrass | 390.5 |
| tufted hairgrass | 97.6 |
| prairie junegrass | 146.5 – 195.3 |
| creeping bentgrass | 48.8 |
| Idaho bentgrass | 146.5 |
| alkaligrass | 195.3 |

**Table 3.** Analysis of greenhouse fertilizer used during establishment of turfgrasses in silica sand.

| **Nutrient** | **Concentration (mg L$^{-1}$)** |
|---|---|
| nitrogen | 200.0 |
| phosphorus | 22.0 |
| potassium | 83.0 |
| iron | 2.5 |
| magnesium | 0.75 |
| boron | 0.10 |
| copper | 0.05 |
| manganese | 0.28 |
| molybdenum | 0.05 |
| sulfur | 114.0 |

# Chapter 4: Turfgrass Seed Mixture Optimization

## Summary

It is well documented that multi-species assemblages are needed to maintain a high-functioning turfgrass ecosystem. However, evaluation and analysis of turfgrass seed mixtures has historically relied upon methods that provide little or no predictive capability to identify the optimal mixture of constituent species. The objectives of this work were to: 1) introduce the use of simplex designs for mixture experiments in the design of seed mixtures for plant communities, and 2) demonstrate the identification of optimal seed mixtures through the application of basic mixture experiment methodology to a simple 4-component seed mixture analysis. Fifteen mixtures of Kentucky bluegrass (*Poa pratensis* L.), hard fescue [*Festuca trachyphylla* (Hack.) Krajina], perennial ryegrass (*Lolium perenne* L.), and tall fescue (*Festuca arundinacea* Schreb.) were chosen based on simplex designs for mixture experiments and four replications were established from seed in 20.95 cm diameter pots within a climate-controlled growth chamber. Mixtures were fertilized weekly and clipped to 5 cm every 7-19 days, with the final clipping occurring 19 wk after seeding. Total dry clipping weight was used as a response variable to which response surface and numerical optimization methods were applied to identify an optimal seed mixture. Results of the analysis showed total dry clipping weight would be maximized for a mixture consisting of 83% *Lolium perenne* and 17% *Festuca trachyphylla,* which was not part of the initial trial design. The experiment was repeated two times with the addition of this optimal mixture. Comparison of the optimized mixture to the design mixtures revealed that it produced a similar or greater amount of total clippings biomass in the two experimental repetitions. Results show that the application of simplex designs and numerical optimization to seed mixture experiments has great potential to identify seed mixtures that are optimal for specific response variables.

## Introduction

Multi-species assemblages of turfgrass provide greater resistance to change in the event of a disturbance (Danneberger, 1993). Indeed, turfgrass communities, which Beard (1973) defines as an aggregation of individual turfgrass plants that have mutual relationships with the environment as well as among the individual plants, often outperform monocultures of each of the constituent species. Mixtures of Kentucky bluegrass (*Poa pratensis* L.) and perennial ryegrass (*Lolium perenne* L.) have been shown to produce up to 11% greater ground cover, 13% higher shoot density, and faster green-up in the spring than either species planted alone (Brede and Duich, 1984a). Dunn et al. (2002) reported that mixtures including tall fescue (*Festuca arundinacea* Schreb.) often provided up to 12% better turf quality as compared to blends of only tall fescue varieties or only Kentucky bluegrass varieties, an observation which they attributed to superior disease resistance of the mixture as compared to either component species. Juska and Hanson (1959) found that during the first four years of a five year study, 'Merion' Kentucky bluegrass planted in monoculture provided the best turf quality as compared to other monocultures and mixtures of Kentucky bluegrass, tall fescue, red fescue, and colonial bentgrass. During the fifth year, however, quality of the Merion monostand declined due to disease. As a result of that disturbance, the polyculture of red fescue and Merion provided the highest quality turf over the 5-yr period. Such results are common in the turfgrass literature.

Given the broad range of landscape segments in which turfgrass is used, there is a need to identify high-functioning species mixtures for seeding across a wide range of ecosystems, management regimes, and scales. Perhaps the most fundamental factor effecting success of vegetative communities is constituent species proportions. Shildrick (1980) evaluated mixtures of perennial ryegrass, timothy grass (*Phleum pretense* L.), Kentucky bluegrass, strong creeping red fescue (*Festuca rubra* ssp. *rubra*), Chewings fescue (*Festuca rubra* var. *commutata*), and colonial bentgrass (*Agrostis tenuis* L.) and observed that the proportions of each component species in the mixture largely determined its overall wear tolerance when subjected to “football-type” wear. Similarly, Shildrick (1982) also identified significant differences in sod strength of as much as 41% and changes in ground cover of as much as 17% between mixtures of creeping bentgrass (*Agrostis stolonifera* L.), colonial bentgrass, Chewings fescue, slender creeping red fescue (*Festuca rubra* ssp. *litoralis*), and Kentucky bluegrass with varying proportions of each species. Brede and Duich (1984a) defined regression equations to relate the proportions of Kentucky bluegrass and perennial ryegrass in a mixture to leaf area index and shoot size, mass, and number. Ebdon and Skogley (1985) used a factorial design of Kentucky bluegrass, perennial ryegrass, and Chewings fescue cultivars at different relative seeding rates to show significant differences in quality between the mixtures.

Still, it is difficult to know how environmental, management, and competitive interactions shape the botanical composition of the resulting turfgrass sward at a given point in time. As discussed by various authors (Van Dersal, 1936; Beard, 1973; Duell and Schmit, 1974; Watschke and Schmidt, 1992; Danneberger, 1993), vegetative succession, which is primarily controlled by the competitive ability of each species under conditions imposed by cultural practices, is a very real phenomenon in turfgrass polycultures. Any turfgrass professional or plant ecologist can attest that cultivars and species that appear to be dominant at one point in time may not be present at the same frequency at another time. As a result, design of a seed mixture is not necessarily informed by the plant counts in a successful, established plant community.

Examples of this concept are numerous in the turfgrass literature. In a 4-yr evaluation, Hsiang et al. (1997) analyzed the population dynamics resulting from interspecies competition between tall fescue, Kentucky bluegrass, perennial ryegrass, and a mixture of Chewings fescue and strong creeping red fescue over four years under non-wear, low-maintenance cultural conditions, which consisted of irrigation to prevent drought stress and nitrogen applications ranging from 50 to 100 kg ha$^{-1}$. The authors found that tall fescue generally decreased from its initial seeding ratio while perennial ryegrass generally increased from its initial seeding ratio when measured four years after establishment. The fine fescue mix and Kentucky bluegrass, however, appeared to increase or decrease to a stable equilibrium proportion of 24% for fine fescues and 42% for Kentucky bluegrass. In a field trial of a mixture containing Kentucky bluegrass, perennial ryegrass, and colonial bentgrass in a 10:3:2 ratio, respectively, perennial ryegrass was found to decrease from 33% to less than 5% while Kentucky bluegrass increased from 22% to approximately 50% of the sward after 4 years when seeded at 48 kg $ha^{-1}$ (Engel and Trout, 1980). Larsen et al. (2004) found that when Kentucky bluegrass, slender creeping red fescue, and perennial ryegrass were sown together, the per cent of total tillers recorded for each species were 31.4%, 57.7%, and 11.5% for slender creeping red fescue, perennial ryegrass, and Kentucky bluegrass, respectively. This was despite a species composition in the seed mixture of 30% slender creeping red fescue, 40% perennial ryegrass, and 30% Kentucky bluegrass, by mass. Stier et al. (2005) evaluated seed mixtures of Kentucky bluegrass and perennial ryegrass in varying proportions from 25% to 95% Kentucky bluegrass by weight. Mixtures containing 95% Kentucky bluegrass resulted in a greater amount of that species in the final botanical composition as compared to all other mixtures. Final compositions for the 95% Kentucky bluegrass mixtures ranged from 40% to 70% Kentucky bluegrass but mixtures containing 25% Kentucky bluegrass never produced more than 15% of that species in the sward composition. They also observed that when common types of Kentucky bluegrass were used, no more than 10% of the resulting sward was Kentucky bluegrass regardless of the initial seed proportions. However, the study was limited in scope to athletic fields under simulated traffic conditions.

The authors of that study recognized that even in a simple 2-species mixture of perennial ryegrass and Kentucky bluegrass, recommendations of constituent species proportions needed to produce acceptable turfgrass vary wildly from 40% (Minner, 1998) to as much as 85% (Brede and Duich, 1984a), by weight. Although these cannot possibly both be correct for a single set of management practices and environmental conditions, incongruous recommendations such as these are often found in the turfgrass literature involving seed mixture experimentation. Such large variability occurs, in part, because previous research has relied on selection of experimental entries containing arbitrary, convenient, or commercially available proportions of constituent species. Moreover, data analysis in mixture trials is often based on standard ANOVA methods, which identify only the best entry in the trial, thus providing little or no predictive ability regarding what the optimal species mixture is if it is not one of the mixtures tested. Conclusions from the experiment are therefore only as good as the author's choice of entries. Where regression methods have been used (Brede and Duich, 1984a; Hsiang et al., 1997), they have not been based on standardized models specifically designed for mixture experimentation.

To summarize, the typical approach to specifying seeded turfgrass species mixtures is to recommend seed mixture proportions that in some way lead to a final plant community composition that is thought to produce acceptable characteristics. Recommendations are based on analysis of the chosen entries in a trial. This approach suffers as a consequence of its dependence on entry selection, lack of predictive ability, and focus on population dynamics within the mixture rather than an objective, quantitative measure of overall mixture performance. A standardized method of seed mixture experimental design and analysis to be used under a given set of conditions seems imperative to the specification of a turfgrass community suited to a specific application and for a specific purpose. Still, focus in the literature has remained on individual species' performance rather than on developing a robust plant community-level experimental approach to seed mixture design. Ideally, such an experimental design and analysis methodology would: 1) allow for inclusion of an unlimited number of constituent species in the seed mixture, 2) provide a standardized method for identification of seed mixtures to be tested, 3) allow for expansion of the design to include other design factors, such as total seed density, and 4) be able to identify the seed mixture that optimizes performance with respect to a specific function at a specific time of interest. To date, there is a lack of such experimental methodologies in the turfgrass literature.

Such experimental designs, which are generically referred to as *simplex designs* (Scheffé, 1958, 1963), have been devised previously to deal with mixture experiments in other disciplines such as chemical engineering, mechanical testing, or industrial processes. Simplex designs allow the experimenter to consider problems that are subject to constraints $0 \le x_i \le 1$ and $\sum_{i=1}^{q} x_i = 1$ where $x_i$ is the proportion of component *i*, in a mixture of *q* components. Furthermore, they systematically identify the mixtures to be tested through consideration of the desired number of mixture components and, in some cases, the desired degree of the polynomial to be used in modeling the data during subsequent analyses.

Modifications on mixture designs have been suggested and implemented that allow for the use of alternative constraints such that $L_i \le x_i \le U_i$ where $L_i$ and $U_i$ represent the lower and upper limits, respectively, on the proportion of constituent species *i* in the mixture or for selecting which mixtures to exclude when physical considerations do not allow for testing of all points specified by the simplex design (Snee and Marquardt, 1974). Mixture experiments using simplexes have also been expanded to allow for consideration of additional variables, which Scheffé (1963) referred to as *process variables*. Several approaches to this problem have been demonstrated, and have been reviewed by Hare (1979). In the context of seed mixtures, such process variables might include soil type, fertility, moisture regime, or planting time, among others. One particularly useful modification of mixture experiment methodology is the ability to take into consideration the total amount of the mixture applied, that is, the total seeding rate (Piepel and Cornell, 1985).

That type of experimental design allows for advanced regression analysis as well. Some previous studies have used polynomial regression techniques to predict turfgrass community population dynamics (Brede, 1982; Brede and Duich, 1984a, 1984b; Hsiang et al., 1997). Typically, such analyses exhibit high levels of correlation between the predictors. To minimize this problem, Scheffé (1958, 1963) proposed a set of *canonical* *polynomials* to fit to the measured data on the simplex thus allowing the experimenter to obtain a meaningful, functional form of the relationship between the response variable and the proportions of each component in the mixture. This is especially important as it provides the aforementioned predictive capability.

A convenient extension of the functional form of a response is that of numerical optimization techniques, which may then be used to identify the exact seed mixture that is best for a given set of conditions. Numerical optimization of a function subject to constraints such as those of a mixture experiment can be accomplished using Augmented Lagrangian methods. Augmented Lagrangian algorithms are a class of algorithms designed to solve constrained optimization problems and are able to accommodate both inequality (i.e. Eq. [1]) and equality (i.e. Eq. [2]) constraints; however, a full discussion of these methods is beyond the scope of this work. The reader is directed to Powell (1969) for a rigorous explanation of such methods.

It is clear that the use of simplex designs and analysis for experiments with seed mixtures can provide all of the necessary requirements for a comprehensive analysis, as detailed above. Indeed, the use of such methodology has been suggested for agricultural experiments previously (Bondari, 2005). For a detailed discussion of mixture experiments the reader is directed to Cornell (1973, 1979, 1981) who has conducted detailed reviews of the literature on experiments with mixtures and elucidated the necessary details for implementation of simplex designs for mixture experiments, both of which are beyond the scope of this work. The objectives of the work presented here are to: 1) introduce the use of simplex designs for mixture experiments in the design of seed mixtures for plant communities, and 2) demonstrate the identification of optimal seed mixtures through the application of basic mixture experiment methodology to a simple 4-component seed mixture analysis.

## Methods & Materials

### General Approach

The methods used in this study allowed us to meet our two objectives of introducing simplex designs for seed mixture experiments and demonstration of their application to a simple mixture experiment. First, seed mixtures of four species were designed using simplex design methodology, and mixtures were grown in a climate-controlled growth chamber. Next, total dry clipping weights from the pots in the chamber were modelled and the model was used to predict a mixture that would maximize total dry clipping weight over the course of the experiment. Finally, the identified optimal mixture, as well as the original 15 design point mixtures, were then included in two more subsequent experimental runs in order to evaluate the relative performance of the identified optimal mixture. The methods and purpose of these steps are summarized in Table 1.

### Seed Mixture Design

Because a simple application of mixture design methodology was desired in order to best demonstrate its applicability, the design space was left unrestricted such that

$$0 \le x_i \le 1 \qquad [1]$$

and

$$\sum_{i=1}^{q} x_i = 1 \qquad [2]$$

where $x_i$ is the proportion of species *i* in each total mixture of *q* components. In this way, both monocultures and polycultures of the constituent species were possible. Seed mixtures to be included in the experiment were identified using a well-defined simplex design called a *simplex centroid design*. This type of design allows for straight-forward identification of test mixtures based simply on the number of constituent species. Designed mixtures are then used as treatments in the experiment. All experimental design, data preparation, and analysis was conducted using R Project for Statistical Computing (R Development Core Team, 2012) and the specified packages. The mixture design process was carried out using the *mixexp* package (Lawson, 2011), which resulted in the 15 design point seed mixtures shown in Table 2. The apparent simplicity of the design points is a result of the use of the simplex centroid design as well as the unrestricted design space characterized by Eq. [1-2]. Altering either of these design characteristics can result in a more uneven proportions or different numbers of combinations for given species. When time or space limit the total number of entries that may be included in a trial, design optimization algorithms exist which allow for selection of a subset of design points.

## Establishment and Maintenance

The mixture components selected for the trial were ‘Moonlight SLT’ Kentucky bluegrass, ‘Apple GL’ perennial ryegrass, ‘Beacon’ hard fescue, and ‘Grande II’ tall fescue. Tree pots (21.0 cm dia. X 40.6 cm tall) were filled with a 1:1 (v:v) mixture of Turface calcined, non-swelling illite and silica clay (Profile Products, Buffalo Grove, IL) to Sunshine MVP potting soil (SunGro Horticulture) and placed in a growth chamber set for a 14-hour photoperiod, 22°C temperature, and 50% humidity with a maximum 477 $\mu$mol $m^{-2}$ $s^{-1}$ photosynthetically active radiation (PAR) . Dimming of the lights over time due to aging of the growth chamber light bulbs resulted in reduced light levels during the second and third experimental runs, with a maximum of 402 and $\mu$mol $m^{-2}$ $s^{-1}$. When light levels were observed to drop to 300 $\mu$mol $m^{-2}$ $s^{-1}$ PAR during the third experimental run, the light bulbs in the chamber were replaced to restore the original light intensity.

Treatments (designed mixtures) were seeded into four replicates of pots in a randomized complete block design at a total rate of 2 pure live seeds (PLS) $cm^{-2}$. Seeding took place on 12 May 2012, 9 Mar. 2013, and 9 Nov. 2013 for the first, second and third experimental runs, respectively. Pots were topdressed with the soil media and watered to field capacity. For two weeks, the pots were watered daily to field capacity after which watering frequency was reduced to every 2 to 3 days. Liquid fertilizer was applied weekly to each pot such that each received 5.7 g N $m^{-2}$, 0.6 g P $m^{-2}$, and 2.4 g K $m^{-2}$.

## Data Collection

Beginning three weeks after establishment, pots were clipped to 5 cm every 7 to 19 d. Clippings were dried at 65°C and dry weights for the clippings from each pot were recorded. A final clipping was performed 19 wk after seeding, and dry clipping weights from all clippings were summed for each pot. The total dry clipping biomass for each pot was used as the response variable as it is a commonly used objective and quantitative indicator of overall plant community health.

## Mixture Data Modeling

The first step in data analysis was to fit the quadratic form of Scheffé's canonical polynomials to the response variable. A full regression model of the form

$$\hat{Y} = \sum_{i=1}^{q} \beta_i x_i + \sum_{i=1}^{q} \sum_{j=1}^{q} \beta_{ij} x_i x_j \quad \{i \neq j\} \qquad [3]$$

was fit to the data where $\hat{Y}$ is the total dry clipping weight response variable predicted from the regression equation, $\beta_i$ is the regression coefficient associated with the proportion of seeds of species $i$ in the seeded mixture, $\beta_{ij}$ is the regression coefficient associated with the interaction of seed proportions of species $i$ and species $j$, $x_i$ and $x_j$ are the proportions of seeds of constituent species $i$ and $j$, respectively, in the mixture, and $q$ is the total number of species in the trial. In this way, all main effects and two-way interactions of species seed proportions were included in the model. It may be noted that this is, in effect, equivalent to a standard multiple regression with no intercept term. All regressions were performed using the *lm* function in R.

Subsequently, variable selection was accomplished using backward elimination as implemented in the *step* function in R, which uses Akaike Information Criterion (AIC) for model comparisons. For this process, the largest model to be considered was defined as the full model given by Eq. [3], and the simplest model was defined to be the linear combination of all first-order terms. Homoscedasticity assumptions were checked using the *ncvTest* function in the *car* package in R (Fox and Weisberg, 2011). Having derived the final regression models for the dry clippings biomass data, lack of fit was examined using the *pureErrorAnova* function in the *alr3* package for R (Weisberg, 2010). The resulting regression models were interpreted in the typical way such that the value of the function calculated for any given combination of predictor values represented the expected function value at that point.

## Mixture Optimization

Having defined a functional form of the relationship between the constituent species proportions in the seed mixture and the total dry clipping weight, the function needed to be optimized within the constraints of the mixture problem (Eq. [1-2]). An Augmented Lagrangian Optimization Algorithm (ALOA) was chosen for this task.

The regression model derived was used in conjunction with the ALOA as implemented in the *auglag* function found in the *alabama* package for R (Varadhan, 2011). Using this type of analysis, we were able to identify the exact proportions of constituent species in the seed mixture that would maximize the value of the regression function.

## Data Display

Display of regression response data was complicated by the number of constituent species. One dimension was required for each species in the experiment, and it was not readily apparent how to display any number greater than three. For a ternary mixture, Barycentric coordinates can be used to create diagrams that may be familiar to readers due to their use in display of soil textural properties, i.e. the soil triangle. While this concept may be expanded to display higher-order mixtures in increasingly complex diagrams, it was simpler in the present context to display dry clippings biomass data as a series of ternary diagrams. In this way, the coordinates associated with each ternary diagram represented the composition of the remaining seed mixture given a fixed proportion of the fourth component, as shown in Fig. 1. A familiar analogy for this form may be to envision each ternary diagram as a slice through a pyramid. Although each diagram has the same physical size for ease of display, those associated with larger proportions of the fourth component represent smaller total proportions of the total seed mixture. In this case, the fourth component was chosen to be tall fescue thus resulting in ternary diagrams which define the relationships between Kentucky bluegrass, perennial ryegrass, and hard fescue for a fixed proportion of tall fescue. Plots were created using the *lattice* graphics package for R (Sarkar, 2008).

## Mixture Performance Comparison

At the completion of the experiment, data from the second and third experimental runs were combined and analyzed for differences in overall mixture performance as measured by total dry clippings biomass. Analysis of variance was carried out in R with means separation by Fisher’s protected least significant difference (LSD). Data from the second and third experimental runs were log-transformed. Data were analyzed for significance of effects of experimental run, replicate, and mixture. This allowed for comparison of the performance of the optimized mixture to those identified as treatments from the simplex design. By doing so it was possible to determine whether the optimization methodology had successfully optimized the mixture to provide the greatest amount of dry clippings biomass. Analysis of variance of data from the first experimental run was also carried out to assess the significance of replicate and mixture followed by means separation by Fisher’s protected LSD.

## Results

### Mixture Data Regression Equations

The final model of total dry clipping biomass following the variable selection process included all main effect terms as well as two-way interactions for *P. pratensis* × *F. tracyphylla* and *L. perenne* × *F. trachyphylla*. The regression obtained an adjusted $R^2$ value of 0.995 and lack of fit was not significant. Coefficient estimates as well as associated p-values are given in Table 3. The magnitude of each coefficient indicates the relative effect of each constituent species proportion on the overall performance of the mixture with respect to the response variable. Positive interaction terms present in the final models indicated the presence of what is often referred to as *synergism*, which is an increase in performance as a result of co-establishment between two particular species. The interaction terms are responsible for the curvilinear form of the response. Figure 2 shows the predicted response surface for dry clipping biomass as predicted from the derived regression equation. Each ternary diagram represents the relationship between *Poa pratensis*, *Lolium perenne*, and *Festuca trachyphylla* for a fixed proportion of *Festuca arundinacea* in the mixture, as indicated in each of the figures. The synergisms indicated by the regression functions are apparent in the plots and indicate that an optimal mixture of species exists at the boundaries of the design space. From these plots it is easy to see the trends associated with increasing or decreasing the proportion of each constituent species in the mixture.

### Optimization Results

Examination of the response surface figures showed that the greatest amount of dry clipping biomass was likely to be somewhere on the slice where the proportion of *F. arundinacea* was equal to zero. Furthermore, the maximum appeared to occur along the line where the proportion of *Poa pratensis* was also equal to zero. However, from the plot it was not possible to discern the exact balance of *L. perenne* and *F. trachyphylla* that would produce the predicted maximum dry clippings biomass. Application of the ALOA to the derived regression function identified the optimal mixture to be 83% *L. perenne* and 17% *F. trachyphylla*.

## Mixture Performance Comparison

Analysis of variance of data from the first experimental run showed a significant effect of mixture, but blocking was not significant (Table 4). Analysis of variance of the combined data set of the second and third experimental runs (Table 5) showed a significant effect of experimental run; thus, the two runs were subsequently analysed separately. Data from the second experimental run showed no significant blocking effect but had a significant effect of mixture (Table 6). The third experimental run (Table 7) had significant effects of both block and mixture. Means separation using Fisher’s protected LSD (Table 8) showed that, in the first experimental run, Mixture 2 produced the greatest total dry clippings biomass with 35.1 g, and was not statistically different from Mixture 8 or Mixture 11, which produced 33.3 g and 33.1 g total, respectively. Mixture 10 produced the least total dry clippings with just 25.4 g and Mixture 3 was the second worst with just 25.5 g. In the second run, Mixture 5 produced the greatest amount of dry clippings biomass with a mean of 37.4 g followed by the optimized mixture, which had a mean of 36.4 g. Four other mixtures were not statistically different from those two. Mixture 1 produced the least dry clippings with just 23.1 g. In the third run, the optimized mixture produced the greatest amount of dry clippings biomass with a mean of 14.4 g followed by Mixture 5 with 14.2 g. Those two mixtures were not statistically different from eight others in the trial. In that run, Mixture 4 produced the least total dry clippings with a mean of just 9.1 g.

When dry clippings biomass was plotted versus days after first clipping, several differences between mixtures, as well as mixture performance over time could be seen (Fig. 3-5). It is clear that relative performance of mixtures changes over time, but that in general, those that produced more clippings early on generally continued to do so throughout the trial. Clear differences in the overall trend existed between the runs. In all runs, Mixture 2 (100% perennial ryegrass) had the greatest amount of dry clippings on the first clipping date. In the first two runs, Mixture 3 (100% hard fescue) had the least dry clippings for the first two clipping dates. During the third experimental run, that same mixture had the most dry clippings during four of the last five clippings while Mixture 4 (100% tall fescue) had the least over that same time period.

## Discussion

The objectives of this study were to: 1) introduce the use of simplex designs for mixture experiments in the design of seed mixtures for plant communities, and 2) demonstrate the identification of optimal seed mixtures through the application of basic mixture experiment methodology to a simple 4-component seed mixture analysis. The first objective was accomplished through application of simplex design, polynomial regression, and numerical optimization in the study methods. The second objective was also accomplished as an optimal mixture was successfully identified and verified; however, some variability in the performance of all mixtures was evident.

During the first experimental run, Mixture 2, which comprised 100% perennial ryegrass produced the greatest amount of dry clippings biomass. This result was not entirely unexpected as perennial ryegrass germinates rapidly across a wide range of soil moisture conditions (Wright et al., 1978) and produced more clippings than any other entry in the trial on two of the first three clipping dates. The performance of Mixture 8, which comprised 50% perennial ryegrass and 50% hard fescue clearly played a large role in the optimization process, effectively weighting the composition of the optimal mixture away from the perennial ryegrass monostand and toward a larger proportion of hard fescue in the mixture.

Keeping in mind that the monoculture of hard fescue (Mixture 3) produced the second least total dry clippings in the first experimental run, it may at first seem counterintuitive that optimizing the mixture should involve substituting it for a portion of the best performing species in monoculture. Monocultures are often limited in yield, though, by what is referred to as the “rule of constant yield,” which describes the limiting relationship between plant density and biomass production due to intraspecific competition. That relationship has been shown to apply to multiple turfgrass species and can be modified by management practices and inputs such as mowing, water, shade, and nitrogen (Lush, 1990; Lush and Rogers, 1992). When intraspecies competition is much stronger than interspecies competition, and the competitive effects of the minor species on the dominant one are small, a species that fills a different realized niche in the plant community may intermix with the dominant one thereby increasing total plant density and overall yield.

Previous work has shown that intraspecies competition may play a role in limiting the density and yield of perennial ryegrass monocultures. Brede and Duich (1984a) showed, using polynomial regression, that percent ground cover for a turfgrass sward consisting purely of perennial ryegrass would have a negative regression coefficient on the term for the square of the total seeding rate indicating the existence of a small amount of intraspecies competition. Culleton et al. (1986) showed no increase in perennial ryegrass yield between sowing rates of 11.3, 22.6, and 33.9 kg ha$^{-1}$ when grown as a forage crop. Furthermore, the capacity for coexistence of perennial ryegrass in binary mixtures with strong creeping red fescue (*Festuca rubra* L. ssp. *rubra*), annual bluegrass (*Poa annua* L.), and rough bluegrass (*Poa trivialis* L.) seeds has been previously demonstrated (Snaydon and Howe, 1986). Taken together, these studies outline one possible mechanism by which the interaction of two species outperformed the best monoculture.

The reason that hard fescue, and neither Kentucky bluegrass nor tall fescue, was the species to complement perennial ryegrass in the final optimal mixture is not immediately obvious. However, it is useful to consider the various resources for which plants are competing including water, nutrients, and light, among others. It is possible that competition for light was a factor in weighting the species proportions toward the inclusion of hard fescue with the perennial ryegrass. Germinating perennial ryegrass plants would have created a partially closed canopy and shaded the soil surface to some extent thereby increasing competition for light. Fine fescue species, including hard fescue, have been shown to exhibit greater germination and ground cover during establishment under shade than Kentucky bluegrass and tall fescue (Gardner and Taylor, 2002). Competition for nitrogen is also one possible reason that hard fescue persisted better with perennial ryegrass than the other two species did. Snaydon and Howe (1986) showed that *Festuca* species were able to persist better than *Poa* species when seeded into an existing perennial ryegrass stand. The authors concluded that the primary mode of competition with perennial ryegrass was belowground competition for nutrients, with the *Fescue* species best able to withstand competition from the perennial ryegrass and the *Poa* species least able. Tall fescue has been shown to perform better under low-nitrogen conditions than Kentucky bluegrass (Walker et al., 2007), and hard fescue has been shown to outperform both species under generally low-input conditions.

Interestingly, Mixture 11, which comprised 1/3 perennial ryegrass, 1/3 Kentucky bluegrass, and 1/3 hard fescue, was the third best mixture in the first experimental run. This was perhaps foretelling of the variability of results seen in the second and third experimental runs where Mixture 5 and the optimal mixture traded places for the top two spots and were followed by the perennial ryegrass monoculture in both cases. This, along with the performance of Mixture 11 in the first experimental run, seems to indicate that there is a delicate balance in the relationship between the three species in those mixtures. It may be advisable to consider using data from two experimental runs for the optimization analysis, if possible, in order to take the observed run-to-run variability into account in the regression model.

The strength of the optimization technique lies in its ability to use species interactions to predict an optimal species assemblage, or mixture. The result of the optimization process highlights the shortcomings of using only traditional ANOVA techniques to identify the “best” mixture. Had the optimization techniques not been used, the conclusion of the first experimental run would have been that planting a monoculture of perennial ryegrass produces the greatest possible dry clippings biomass. Instead, the optimized mixture performed as well or better than any of the mixtures in the original trial during both of the subsequent experimental runs. It is important to keep in mind that the response variable used may be any quantitative measure of performance, but should be chosen carefully and specifically for the intended application.

The applications of this type of mixture experimental design and analysis go beyond just the identification of a best mixture to use under given conditions. It may also have applications in breeding species mixtures for optimal function as opposed to breeding cultivars of individual species to be later mixed together. Plant breeding approaches focused on selection of plant groups as opposed to individuals were first suggested by Harper (1977), and discussed by others (Denison et al., 2003; Weiner et al., 2010). The approach is based on the idea that there are inherent tradeoffs between the competitive ability of a cultivar against other genotypes in the plant community, and its overall potential when grown in monoculture (Donald, 1968). That type of tradeoff emphasizes the importance of methods such as the optimization approach used here in assessing mixtures of plants where a mixture will be used in the final application. That is, simply mixing together cultivars shown to perform well in monoculture is not necessarily sufficient for the creation of a high-performing mixture.

## Conclusions

In this study, we introduced the use of simplex design and analysis techniques for seed mixture experimentation. In addition, we proposed that numerical optimization could identify a mixture that performed as well or better than any mixture in the original trial. Those methods were applied to a simple 4-species experiment in a climate controlled growth chamber and showed that, of the species used, a mixture of 83% perennial ryegrass – 17% hard fescue was predicted to produce more dry clippings biomass than any of the mixture treatments in the original trial design. That mixture produced a similar or greater amount of clippings than all other mixtures in two subsequent experimental runs indicating that the method is a viable approach to seed mixture design experimentation. Use of data from several experimental runs for the modelling and optimization process may help remove some of the variability in the relative performance of the optimal mixture with respect to other top-performing mixtures in the trial. The methods used have potential for application in multiple areas including seed mixture design and plant breeding.

Future research in this area should include the expansion to a greater number of species, restricted ranges for constituent species proportions, and experimentation in varied environments. Moreover, consideration of the overall seeding rate will likely be of importance in future studies. Each of those investigations may address the generalizability of the results obtained from the optimization method.

## Acknowledgements

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**Table 1.** Summary of general experimentation and analysis approach for the growth chamber 4-species mixture experiment.

| Experimental Run | Mixtures Included | Analysis Methods | Analysis Purpose |
|---|---|---|---|
| 1 | 1-15 | Polynomial regression | Define functional form of the response |
| | | Numerical optimization | Predict optimal seed mixture from response function |
| 2 | 1-15, Optimal | ANOVA | Verify significant effect of mixture treatments |
| | | Means Separation | Compare performance of optimal mixture to design point mixtures |
| 3 | 1-15, Optimal | ANOVA | Verify significant effect of mixture treatments |
| | | Means Separation | Compare performance of optimal mixture to design point mixtures |

**Table 2.** Proportions of constituent species in each mixture identified as a design point in the simplex centroid design for the growth chamber experiment for seed mixture optimization.

| | Constituent Species Proportion | | | |
|---|---|---|---|---|
| Mixture ID | *Poa pratensis* | *Lolium perenne* | *Festuca trachyphylla* | *Festuca arundinacea* |
| 1 | 1 | 0 | 0 | 0 |
| 2 | 0 | 1 | 0 | 0 |
| 3 | 0 | 0 | 1 | 0 |
| 4 | 0 | 0 | 0 | 1 |
| 5 | ½ | ½ | 0 | 0 |
| 6 | ½ | 0 | ½ | 0 |
| 7 | ½ | 0 | 0 | ½ |
| 8 | 0 | ½ | ½ | 0 |
| 9 | 0 | ½ | 0 | ½ |
| 10 | 0 | 0 | ½ | ½ |
| 11 | ⅓ | ⅓ | ⅓ | 0 |
| 12 | ⅓ | ⅓ | 0 | ⅓ |
| 13 | ⅓ | 0 | ⅓ | ⅓ |
| 14 | 0 | ⅓ | ⅓ | ⅓ |
| 15 | ¼ | ¼ | ¼ | ¼ |

**Table 3.** Marginal effects summary for mixture experiment regression of total dry clipping biomass in the 4-species mixture experiment.

| Constituent species | $\beta_i$ | Pr(>\|t\|) |
|---|---|---|
| *P. pratensis* | 27.56 | < 2e-16 |
| *L. perenne* | 34.96 | < 2e-16 |
| *F. trachyphylla* | 24.88 | < 2e-16 |
| *F. arundinacea* | 27.37 | < 2e-16 |
| *P. pratensis* × *F. trachyphylla* | 10.35 | 0.025 |
| *L. perenne* × *F. trachyphylla* | 15.40 | 0.002 |

**Table 4.** Analysis of variance of dry clippings biomass for the first experimental run of the growth chamber 4-species mixture experiment.

| Source | DF | MS | Pr(>\|t\|) |
|---|---|---|---|
| Rep | 3 | 8.762 | 0.128 |
| Mix | 14 | 33.770 | < 0.001 |
| Residuals | 42 | 4.372 | |

**Table 5.** Analysis of variance of combined, log-transformed dry clippings biomass data from the second and third experimental runs of the growth chamber 4-species mixture experiment.

| Source | DF | MS | Pr(>\|t\|) |
|---|---|---|---|
| Run | 1 | 53.288 | < 0.001 |
| Block within Run | 6 | 0.331 | < 0.001 |
| Mix | 15 | 0.095 | < 0.001 |
| Mix x Run | 15 | 0.060 | < 0.001 |
| Residuals | 90 | 0.021 | |

**Table 6.** Analysis of variance of log-transformed dry clippings biomass for the second experimental run of the growth chamber 4-species mixture experiment.

| Source | DF | MS | Pr(>\|t\|) |
|---|---|---|---|
| Rep | 3 | 0.037 | 0.085 |
| Mix | 15 | 0.196 | < 0.001 |
| Residuals | 45 | 0.016 | |

**Table 7.** Analysis of variance of log-transformed dry clippings biomass for the third experimental run of the growth chamber 4-species mixture experiment.

| Source | DF | MS | Pr(>\|t\|) |
|---|---|---|---|
| Rep | 3 | 0.153 | 0.002 |
| Mix | 15 | 0.194 | < 0.001 |
| Residuals | 45 | 0.025 | |

**Table 8.** Mean total dry clipping biomass produced by each mixture during the each experimental run of the growth chamber 4-species mixture experiment.

| Run 1 | | Run 2 | | Run 3 | |
|---|---|---|---|---|---|
| Mixture (Treatment) | Mean†,‡ (g) | Mixture (Treatment) | Mean§,‡ (g) | Mixture (Treatment) | Mean¶,‡ (g) |
| 2 | 35.1 a | 5 | 37.4 a | Optimized | 14.4 a |
| 8 | 33.3 ab | Optimized | 36.4 ab | 5 | 14.2 a |
| 11 | 33.1 ab | 2 | 35.5 abc | 2 | 14.1 a |
| 9 | 31.3 bc | 8 | 34.1 abc | 11 | 14.1 a |
| 12 | 30.7 bcd | 12 | 33.9 abc | 15 | 14.0 a |
| 15 | 30.5 bcde | 14 | 33.2 abc | 3 | 13.8 a |
| 5 | 30.4 bcde | 9 | 33.0 bc | 9 | 13.6 a |
| 14 | 30.0 cde | 15 | 31.9 c | 8 | 13.4 ab |
| 6 | 28.4 cdef | 11 | 31.9 c | 14 | 13.0 ab |
| 4 | 28.1 def | 10 | 27.3 d | 12 | 12.9 ab |
| 1 | 28.0 def | 13 | 27.2 d | 1 | 11.7 bc |
| 13 | 27.5 ef | 4 | 27.0 d | 10 | 11.5 bc |
| 7 | 26.2 f | 6 | 25.7 de | 7 | 10.4 cd |
| 3 | 25.5 f | 7 | 25.5 de | 13 | 10.2 cd |
| 10 | 25.4 f | 3 | 24.8 de | 6 | 9.7 d |
| | | 1 | 23.1 e | 4 | 9.1 d |

† Mean total dry clipping weight from four replications of mixture treatments seeded on 5 Dec. 2012 and collected every 7-19 d for 16 wk.

‡ Means in each column followed by the same letter are not statistically different using Fisher’s Protected LSD (α=0.05).

§ Mean total dry clipping weight from four replications of mixture treatments seeded on 9 Mar. 2013 and collected every 7-19 d for 16 wk.

¶ Mean total dry clipping weight from four replications of mixture treatments seeded on 9 Nov. 2013 and collected every 7-19 d for 16 wk.

**Figure 1.** Generic ternary diagram showing relationships between *P. pratensis*, *L. perenne*, and *F. trachyphylla* with a fixed proportion, *Fa*, of *F. arundinacea* in a 4-species seed mixture. Coordinates represent species proportions corresponding to (*P. pratensis*, *F. trachyphylla*, *L. perenne*, *F. arundinacea*).

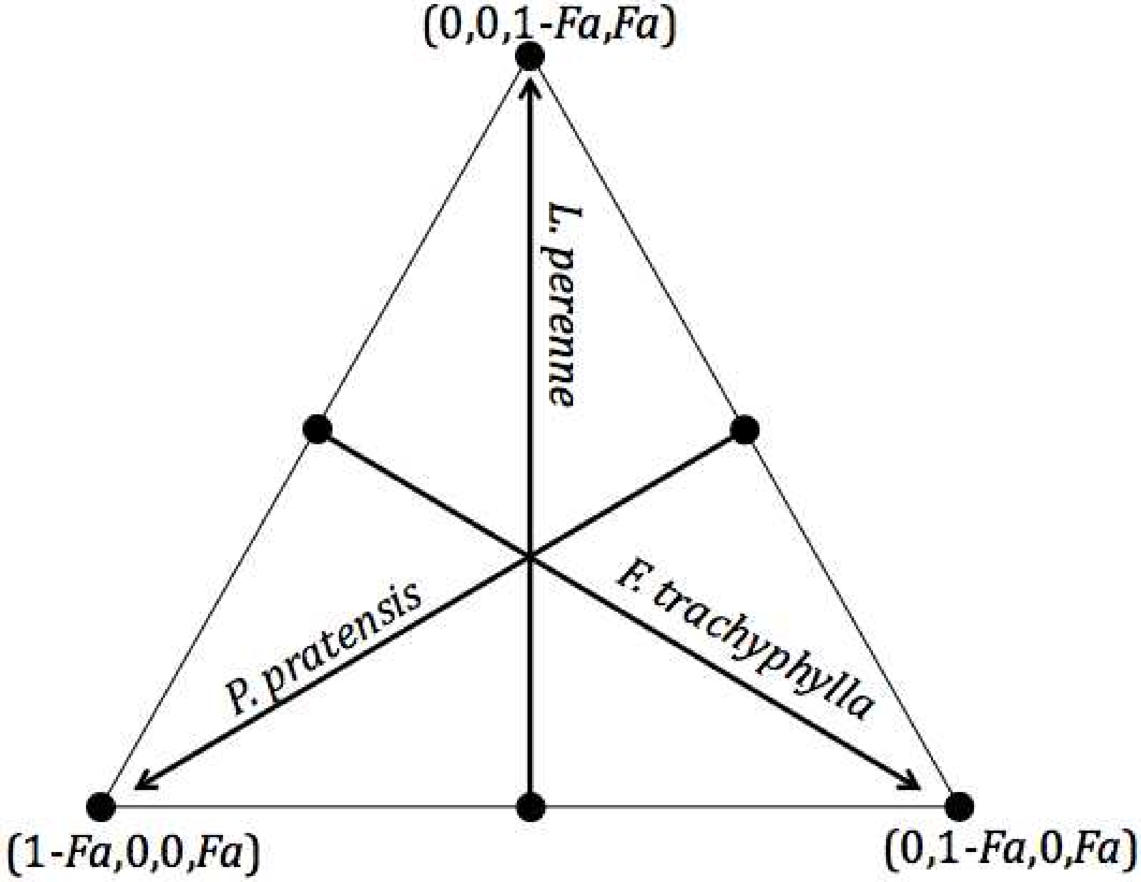


**Figure 2.** Predicted response surface for total dry clipping biomass as a function of constituent species in a seed mixture. Proportions of *F. arundinacea* are fixed as shown with all other species varying as shown in Fig. 1.

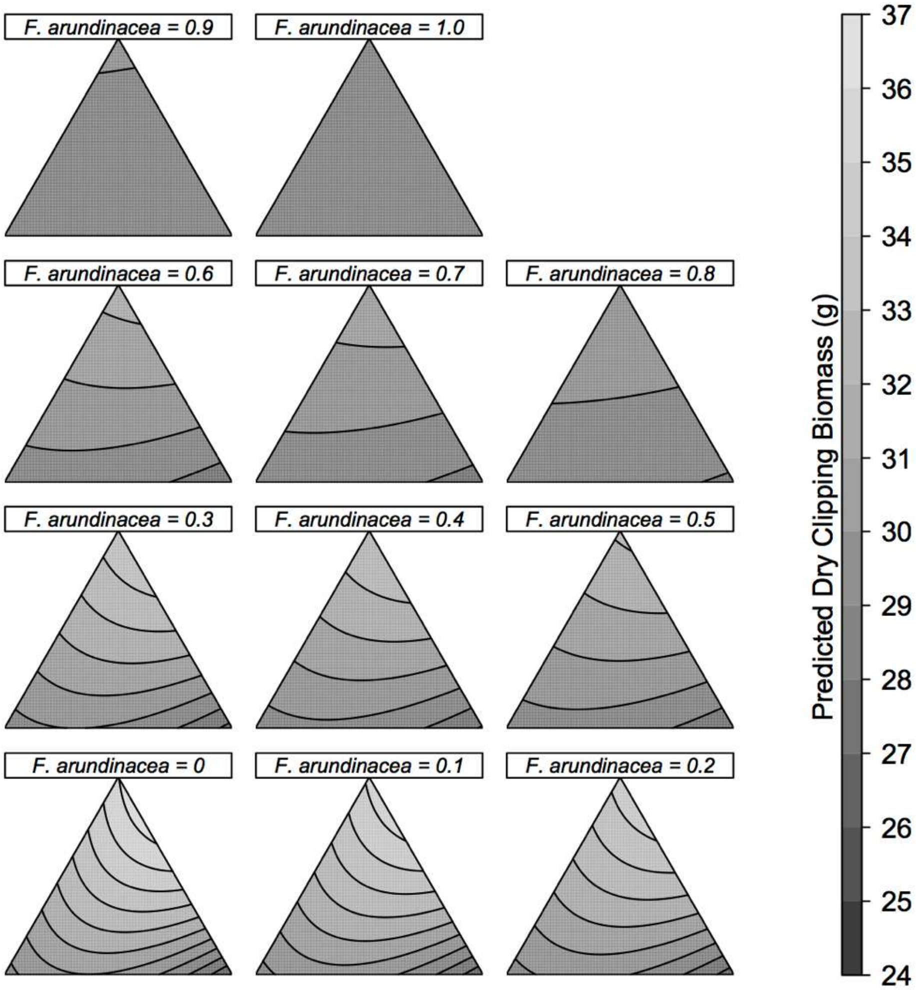


**Figure 3.** Dry clippings biomass versus days after first clipping for the first experimental run of the growth chamber 4-species mixture experiment.

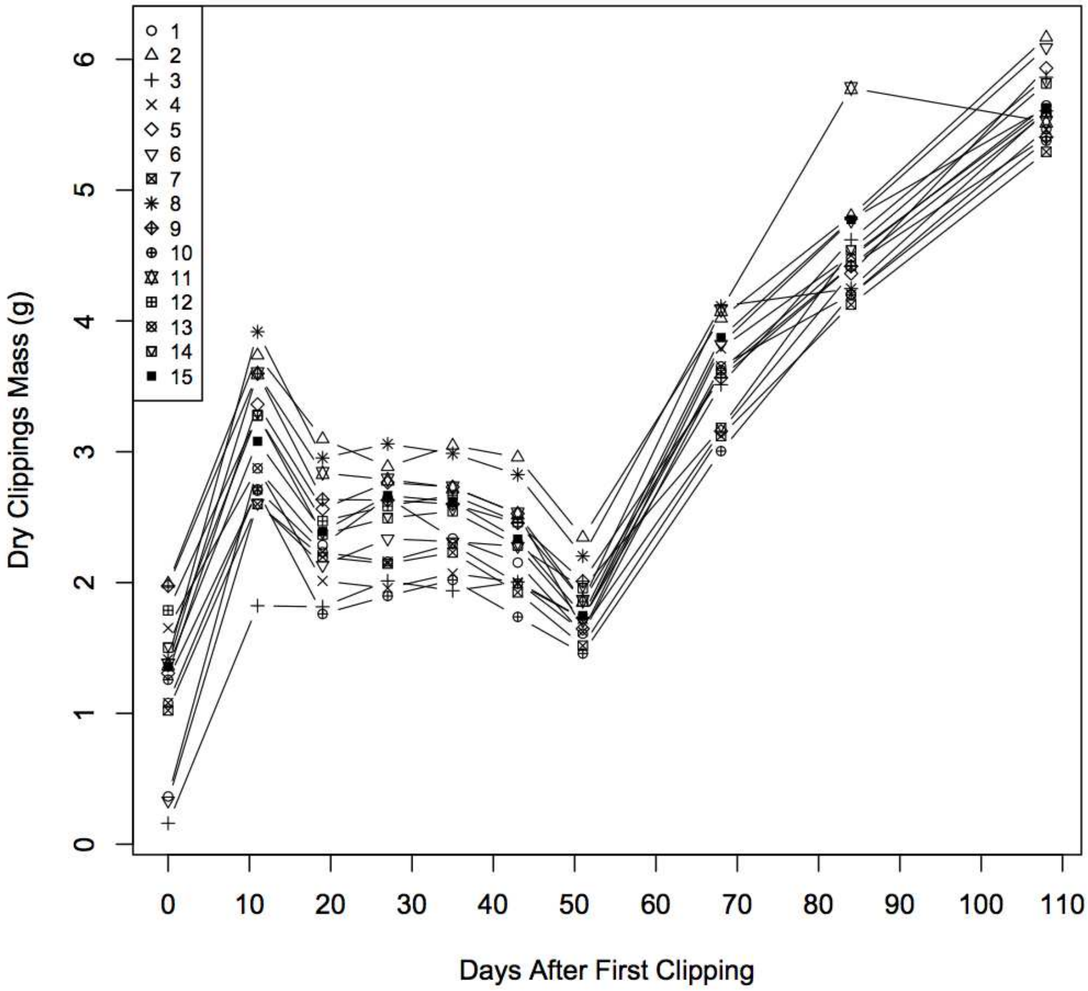


**Figure 4.** Dry clippings biomass versus days after first clipping for the second experimental run of the growth chamber 4-species mixture experiment.

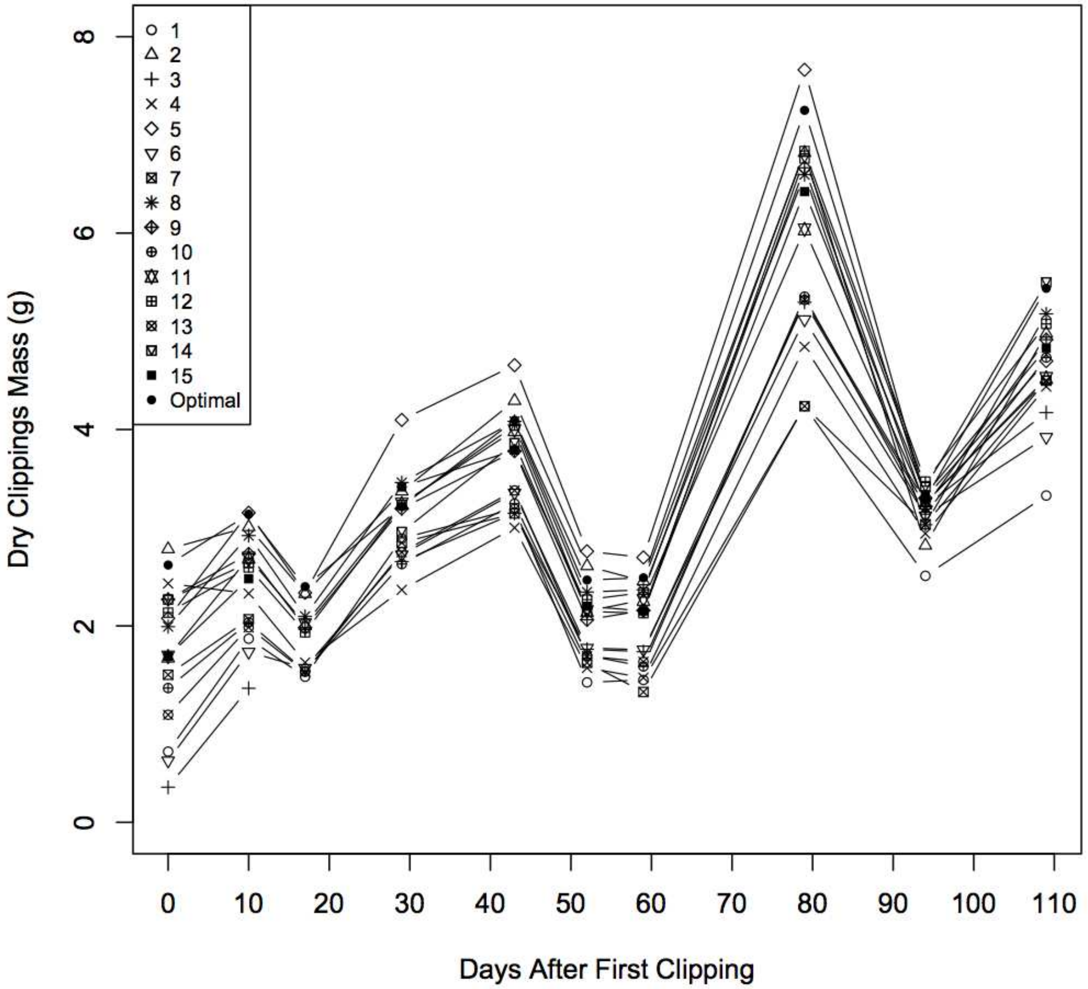
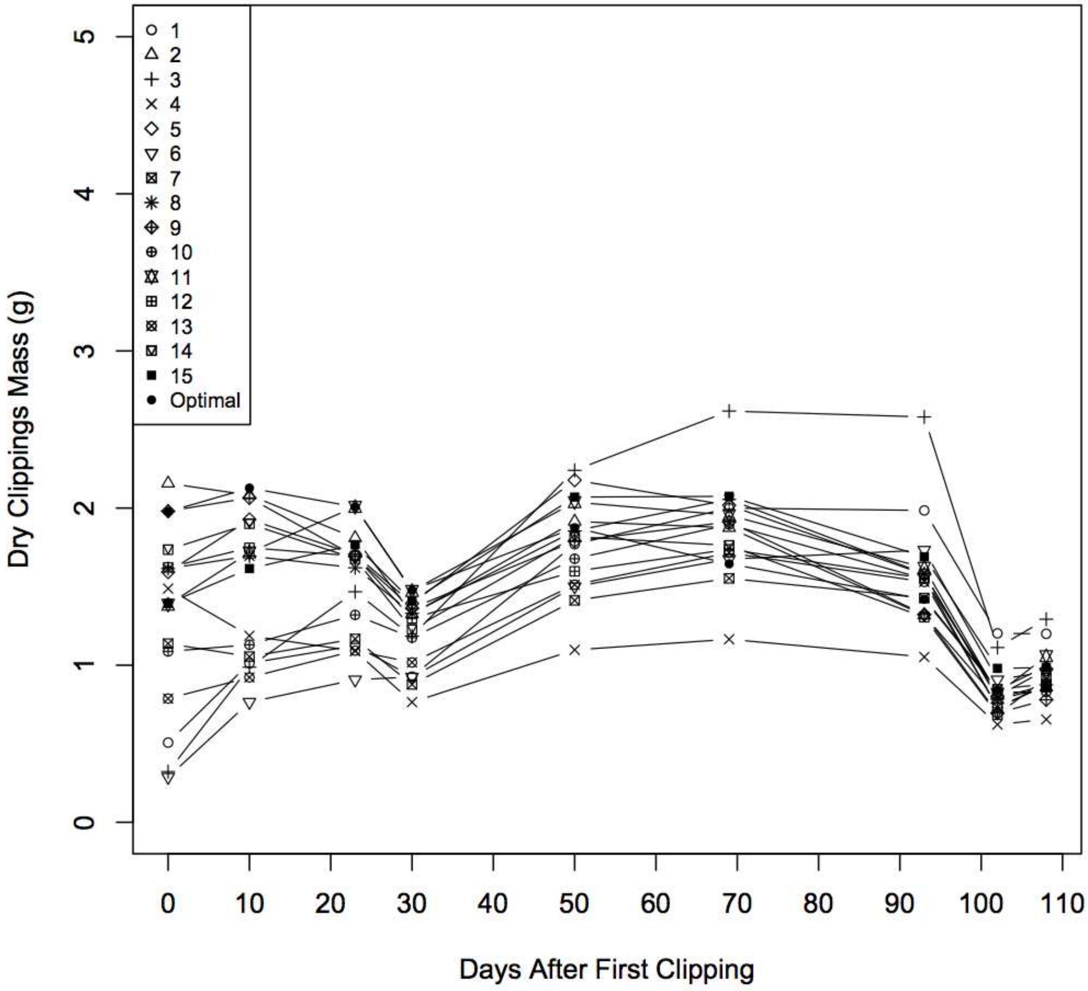


**Figure 5.** Dry clippings biomass versus days after first clipping for the third experimental run of the growth chamber 4-species mixture experiment.



# Chapter 5: Cool-season Turfgrass Species Mixtures for Roadsides in Minnesota

## Summary

Roadside turfgrass mixtures are subject to a wide variety of extreme stresses including heat, drought, low fertility, ice cover, and road salt exposure. Historically, most roadside turfgrass experiments have focused on single species performance for their response to this multitude of stresses. Furthermore, the currently specified turfgrass seed mixtures for roadsides in Minnesota have not been based on the results of recent, designed experiments. A new approach to evaluating turfgrass species mixtures for roadsides in Minnesota is needed in order to improve the current mixture recommendation. The objectives of this experiment were to 1) assess the performance of several mixtures of cool-season turfgrass for survival on roadsides in Minnesota, 2) quantitatively evaluate the influence of individual species on the survival of turfgrass mixtures on roadsides, and 3) identify a suitable mixture of cool-season turfgrass species for Minnesota roadsides. In fall 2011, three replications of 51 cool-season turfgrass mixtures, comprising nine species of cool-season turfgrass, were established at two roadside locations in the metropolitan area surrounding St. Paul, MN, USA. Survival of the established mixtures was assessed using digital image analysis to determine percent living ground cover during both spring and summer 2012, and again using a grid-intersect method during spring 2013. The log-odds of a plot retaining at least 60% living turfgrass cover in spring 2013 decreased by 1.78 for mixtures including tall fescue (*Festuca arundinacea* L.). Strong creeping red fescue (*Festuca rubra* L. ssp. *rubra*) also produced a decrease in the log-odds of success but was not significant. Slender creeping red fescue (*Festuca rubra* L. ssp. *litoralis*) increased the log-odds of success by 1.049, but was not significant while hard fescue [*Festuca trachyphylla* (Hack.) Krajina] and sheep fescue (*Festuca ovina* L.) increased the log-odds of success by 0.95 and 0.96, respectively, and both estimates were significant at the 90% confidence level. Quantitative analysis of survival percentage in spring 2013 indicated that the best mixture for roadsides in Minnesota comprises 20% slender creeping red fescue, 40% hard fescue, and 40% sheep fescue. These results can be used by public works officials to implement and improve roadside turfgrass mixtures in Minnesota, and may be helpful to turfgrass breeders interested in breeding mixtures of turfgrass.

## Introduction

Roadsides present significant and unpredictable challenges to maintaining vegetative cover due to stressful conditions that can be extreme. Drought, low fertility, disease, ice cover, and exposure to road salt are just a few examples of the many stresses placed on roadside vegetation. Moreover, the mid-continental position of locations like Minnesota results in a highly variable environment including a wide range of temperature, humidity, and soil moisture conditions. These conditions necessitate vegetation with a distinct set of plant community characteristics that must be considered when identifying proper vegetative cover.

Turfgrasses are typically implemented on roadsides to avoid contrast with adjacent land use, prevent erosion, and enhance visibility for drivers without the need for extensive mowing. Their use as roadside vegetation first became of interest during the 1930s due to the construction of a number of large-scale highway projects including the United States Numbered Highway System and the Autobahnen in Germany (Hottenstein, 1969; Weingroff, 2013). However, no single species of turfgrass possesses a superlative tolerance to all of the concurrent stresses experienced by roadside vegetation. It is well documented that multi-species assemblages are needed to maintain a high-functioning ecosystem (Tilman et al., 2001; Zavaleta et al., 2010; Isbell et al., 2011). Watschke and Schmidt (1992) reviewed the literature showing that, indeed, this concept extends to turfgrass communities. It is therefore likely that a mixture capable of taking advantage of the unique tolerances of several species will produce the most sustainable and functional roadside turfgrass.

Selection of the proper species proportions for use on roadsides, however, has been troublesome. This is, in part, because, most previous mixture experiments have not been conducted in a roadside environment and have not used cultivars chosen based on their ability to establish and survive on roadsides. Most trials of cool-season turfgrass species mixtures in the current literature have evaluated the effects on sward composition of treatments such as golf cart or foot traffic, mowing frequency and height, and fertility regime. These factors, while important in other contexts, are not entirely relevant in roadside ecosystems. Moreover, such practices are neither easily specified nor often followed in roadside vegetation maintenance practices. Those mixture trials have largely shown that significant changes in performance can be generated by altering mixture species proportions (Engel and Trout, 1980; Shildrick, 1980, 1982; Brede and Duich, 1984a, 1984b; Hsiang et al., 1997; Dunn et al., 2002; Larsen et al., 2004); although, most entries in those trials were selected based on mixtures that were convenient or commercially available.

Fortunately, the roadside turfgrass mixture work that has been done has provided strong indications as to which species may be useful in that environment. Many early attempts to provide a suitable grass mixture for roadsides resulted in overly complicated mixtures including between five and fifteen grasses, three to four legumes, and two herbs (Boeker, 1970). Since that time, grass mixtures for roadsides have been improved and refined to use as few as four well-chosen species (Boeker, 1970). Some basic principles for creating cool-season turfgrass mixtures for roadsides were laid out by Blaser (1963) who concluded that seed mixtures should not contain any species with aggressive seedlings such as ryegrasses or cereals, and found that all mixtures containing tall fescue and Kentucky bluegrass performed well in roadside trials. Henensal et al. (1980) conducted a roadside study of polystands in a clay soil outside of Paris, France in what was described as a marine climate, altered to some extent. The authors reported that including more than 10% perennial ryegrass (*Lolium perenne* L.) in the mixture impeded growth of all species in the mixture and that performance over time was generally poor. Moreover, they noted that polystands consisting of ‘Dawson’ slender creeping red fescue, ‘Biljart’ hard fescue [*Festuca trachyphylla* (Hack.) Krajina], and ‘Tracenta’ colonial bentgrass (*Agrostis capillaris* L.) performed best and concluded that, in that environment, roadside mixtures should be based on those three species. Butler et al. (1974) identified individual species as being salt tolerant, but did not evaluate mixtures and concluded that there was great need for further research focused on salt-related plant problems. Brown and Gorres (2011) evaluated the effect of soil amendments on several monocultures and one experimental species mixture from the Rhode Island Department of Transportation on roadsides in Rhode Island. The mixture performed similarly to monostands of strong creeping red fescue (*Festuca rubra* L. ssp. *rubra*) for turf cover throughout the entire study and across several soil amendments.

It is clear that there is further need for studies aimed at identifying the best cool-season turfgrass mixture for roadsides. Nevertheless, focus has largely remained on single-species performance under the multitude of stresses present on roadsides. Today, several states have specified turfgrass mixtures for roadsides with the goal of providing basic ecosystem services required of roadside turfgrass such as providing safe driving conditions, preventing soil erosion, and filtering runoff water. In addition, it is ideal that the mixtures should maximize persistence over time. Those mixtures make use of several species, but given the demonstrated difficulty in the design and analysis of roadside mixture trials, they are seldom based on results of recent, designed experiments. The need exists for more quantitative, *in situ* evaluations of mixture performance. That includes the need to specify a method for identifying superior turfgrass species mixtures. Such a method should: 1) systematically define mixtures to be included in the trial, 2) quantify the effect of each species on the success of a mixture including any synergisms or interferences with other species that may be present, and 3) identify the best possible mixture composition based on the quantitative effects of each species.

In the present study we have taken a system-level statistical approach, similar to that suggested by Friell et al. (2013b), which allowed us to identify a best mixture of cool-season turfgrass species for roadside establishment in Minnesota, USA based on carefully designed entries and quantitative measures of survival. We selected cultivars based on their ability to establish and survive on roadsides in Minnesota (Friell et al., 2012) and direct assessments of their salt tolerance during vegetative growth (Friell et al., 2013a). While our results are applicable under the environmental conditions of the study, our approach is generalizable such that the wide range of stressful conditions and maintenance practices, which cannot be consistently well defined on roadsides, may be accounted for at any given location. Ultimately, implementation of this novel approach to cool-season turfgrass seed mixture experimentation provided a sound methodology by which to accomplish the objectives of our study: 1) assess the performance of several mixtures of cool-season turfgrass for survival on roadsides in Minnesota, 2) quantitatively evaluate the influence of individual species on the survival of turfgrass mixtures on Minnesota roadsides, and 3) identify a suitable mixture of cool-season turfgrass species for Minnesota roadsides.

## Methods and Materials

### General Approach

First we identified mixtures of cool-season turfgrass that performed well on roadsides in Minnesota. We then quantified the extent to which individual species were responsible for the superior performance. Finally we used that information to define the best possible turfgrass seed mixture for roadside environments like those in the study.

The mixtures included in the trial were designed using an extreme vertices simplex design (Snee and Marquardt, 1974). Extreme vertices designs systematically identify mixtures to be design points in a trial based on the total number of species in the trial and the constraints placed on proportion of each species. Each of those mixtures, in effect, becomes a treatment for the experiment and is seeded into a plot. Given a large number of species included in the trial, the set of identified treatments is likely to exceed the physical space available for the trial. In such a case, a design optimization algorithm is used to select a subset of the design points that allow for the greatest amount of information to be gained from the experiment. In this type of experiment, the overall seeding rate is held constant so as to avoid confounding the effects of total seeding rate with effects of individual species seeding rate.

The response data were examined in three ways. First, the effects of mixture, time, and weed cover on percent living ground cover were assessed using linear mixed effects modelling. The purpose of this was simply to identify any obvious trends in mixture performance and provide a check for the results of the second and third analysis steps. Second, logistic regression was used to quantify the extent to which the presence or absence of a species in an applied seed mixture increased or decreased the long-term success of the plot. This allowed for the identification of species that may be considered important to the success of a plot. Finally, a polynomial function specifically designed for simplex experiments (Scheffé, 1963) was fit to survival response data. The fitting was done in a least squares sense, using the species proportions as the independent variables. Fitting the polynomials was equivalent to carrying out multiple linear regression with the intercept term forced to be equal to zero. The resulting regression equation defined a response surface for the survival of all possible mixtures in the design space, from which, the best possible mixture was predicted.

### Study Sites

The experiment was conducted at two sites in the metropolitan area surrounding St. Paul, MN, USA. The selected research sites were: (1) Larpenteur Ave. along the University of Minnesota campus (St. Paul, MN, USA) and (2) County Highway 14 (Centerville, MN, USA). The experiment was conducted at two separate sites, which were chosen to represent two of the many different types of roadsides where mowed turfgrass would typically be used in Minnesota. The sites were both established in fall

2011, and maintained through spring 2013. The Larpenteur Ave. site was located in the area between the curb and the sidewalk adjacent to the road on the south side of a four-lane divided urban street. The Highway 14 site was located in the area between the curb and a bicycle trail adjacent to the road on the west side of a two-lane suburban road. Both sites received full sun during the day and were not shaded. Temperatures in the top two inches of soil at those sites may regularly exceed 35° C during sunny summer days.

From late fall to early spring, sodium chloride road salt is applied to the roads during snow and ice storms as needed. Salt is typically scattered from the back of a snow plow truck through an auger mechanism. Minimum recommended salt application rates for sections of road such as these range from 11.4 to 45.4 kg lane$^{-1}$ km$^{-1}$, but may be significantly higher depending on local road and weather conditions (Minnesota Local Road Research Board, 2012). Vegetation is exposed to the salt either because it may scatter onto the plot area during application, or be dissolved in runoff water during spring thaws. However, at both locations a curb prevents much of the road runoff water from reaching the plots.

### Mixture Design

Nine species of cool-season turfgrass were identified as constituent species for the roadside turfgrass mixture experiment including creeping bentgrass (*Agrostis stolonifera* L.), Kentucky bluegrass, alkaligrass, strong creeping red fescue, slender creeping red fescue, hard fescue, sheep fescue (*Festuca ovina* L.), tall fescue, and Chewings fescue. A single, commercially-available cultivar was chosen to represent each species in the trial. Cultivars were selected based on previous assessments of their ability to establish and survive on Minnesota roadsides and direct assessment of their salt tolerance during vegetative growth in a controlled environment (Friell et al., 2012, 2013a).

Seed mixtures of those species were systematically designed as an extreme vertices simplex using the *Xvert* algorithm of Snee and Marquardt (1974) as implemented in the SAS software function *adxxvert*. Constituent species seed proportions were limited to 40% of the total mixture, by seed count, so as to force the inclusion of more than two species but not require so many species so as to make the mixtures overly complicated, as described by Boeker (1970). In addition, a minimum of 5% was required for inclusion as a constituent species in the mixture. Species proportions were specified as percent of total pure live seed (PLS) in the mixture and the overall total seeding rate was fixed at 2 PLS cm$^{-2}$, which is a generally accepted total density for seeding a closed-canopy turfgrass sward. Average 1000-seed weight and germination percentage were determined for each species, and viability-adjusted seed weight was used as a proxy measurement to provide the correct PLS count for each mixture.

Available space at the roadside sites allowed for the inclusion of 51 mixtures in the trial. Of the mixtures defined by the algorithm, 50 were selected (Table 1) and used as treatments in the experiment and the rest were discarded. Those 50 mixtures were chosen using a D-optimality criterion with *proc optex* in SAS software, and each was assigned a treatment number for identification. A mixture representing the currently specified salt-tolerant mixture for the Minnesota Department of Transportation (MnDOT) was also included in the trial. For that mixture, species proportions from MnDOT road construction standards were used with each species represented by its respective cultivar used in this trial. That mixture was not assigned an identification number; rather was identified by the name “MNDOT” in the trial.

## Experiment Design and Establishment

Plots were laid out in a randomized complete block design with three replications at each site. The replications ran parallel to the road, and each plot was defined to be 1.52 m parallel to the road by 0.91 m perpendicular to the road. Plots at both sites were sprayed with a broadleaf herbicide [a.i. Triisopropanalamine Salt of 2,4-Dichlorophenoxyacetic Acid; 1-methylheptyl (4-amino-3,5-dichloro-6-fluoro-2-pyridyloxyl) acetate; Triethylamine Salt of 3,5,6-Trichloro-2-Pyridinyloxyacetic Acid] at a rate of 4.77 L ha$^{-1}$ and with glyphosate at a rate of 7.72 L ha$^{-1}$ to eliminate existing vegetation. Soil was tilled to a depth of 15 cm and smoothed using hand rakes. Dead plant material was left on the surface of the plots. Starter fertilizer was applied to provide 26.8 kg N $ha^{-1}$, 21.3 kg P $ha^{-1}$, and 49.0 kg K $ha^{-1}$ to help ensure good establishment. Soil samples were collected to a depth of 10.16 cm at points 45.7 cm away from the curb and every 19.6 m along the length of the experiment. Soil samples were bulked together by replication, and submitted to the University of Minnesota Research Analytical Lab for analysis of pH, organic matter, and saturated paste extract electrical conductivity (EC) as well as extractable phosphorus and potassium. Soil texture was also analysed for a bulk sample from each experimental site. In April 2012, soil samples were collected again in the identical way, bulked together by replicate, and analysed for extractable calcium, magnesium, and sodium, using standard procedures (Table 3).

Each of the 51 seed mixtures was randomly assigned as a treatment to a single plot in each replication. Seed was applied to plots by shaking mixtures in jars and hand seeding followed by light raking. Both sites were covered using Futerra erosion control blankets (Profile Products, LLC., Buffalo Grove, IL, USA) and water was applied by a water truck as needed during establishment. Following establishment, no additional fertilizer or water was applied at either site.

### Data Collection

Percent green ground cover was used as a measure of mixture performance. To assess the percent green ground cover, digital images were collected of each plot at both roadside test sites using a 0.91 m x 0.91 m x 0.91 m custom-built light box containing 16 fluorescent lights during the weeks of April 22, June 17, July 15, August 19, and October 28 in 2012. Pictures were taken from 0.91 m above the ground and stored for later analysis. In addition, weed cover was visually assessed on a 1-9 scale with 1 representing complete weed coverage, 9 representing no weeds present in the plot, and 6 representing approximately 33% weed cover.

In June 2013, a final data collection was made using a grid-intersect method wherein a 9.14 cm x 15.24 cm grid of 100 points was overlain on each plot and grid intersections located on living turfgrass plant tissue were counted. The total proportion of points counted for each plot was used as a measurement of mixture performance. The grid intersect method was chosen for the final data collection because of extensive death of turfgrass in a majority of the plots which resulted in a sparse pattern of surviving plants and extensive weed encroachment. Due to difficulty in distinguishing among the five different fine leaf fescues, species identification was not recorded for the plants counted in each grid.

## Data Analysis

Digital images were analyzed for percent green ground cover using a custom script written in Image Processing Toolbox (MATLAB, 2011). By quantifying the percent of the ground area covered in green plant tissue, we were able to assess the relative performance of the mixtures in the presence of several roadside stresses. The resulting data set was analyzed using R Environment for Statistical Computing (R Development Core Team, 2012). Percent cover data from both locations were combined and arcsin transformed. Data from the first two dates (hereafter, 2012 spring data) were analyzed together using a linear mixed-effects model for repeated measures in R, and data from the final three dates (hereafter, 2012 summer data) were analyzed together in the same way. Pooling of data into two seasonal groups was done in order to isolate data collection dates representing spring survival from those that occurred during extreme heat and drought.

For each of the spring and summer data sets, a full model was created including main effects for weed cover, time, and mixture, as well as all two-way interactions. Site and replication were used as random effects, which are characterized as distributions in a linear mixed effects model, and are therefore not testable in a traditional ANOVA sense. Variable selection for the fixed effects was carried out using backward elimination with a model containing all three main effects as the smallest acceptable model. The term for weed cover was included as a covariate in the full models to account for the fact that the method of digital image analysis used, which is based exclusively on image hue information, is not capable of distinguishing between green weeds and green turfgrass. Thus, it was desirable to control for weed cover statistically. Where a better fit was achieved, a weighted variance was used and was created as a function of the fitted values using the *varPower* function in R. Comparisons of sequential models were made using Akaike's Information Criterion (AIC). In the resulting regression equation, positive regression coefficients indicate an increase of percent green turfgrass cover as a result of the mixture while negative values indicate a decrease in percent green turfgrass cover. Using this method of analysis, the relative magnitudes of the regression coefficients were used to rank each mixture in the trial as to its ability to successfully maintain green vegetative cover. Confidence intervals at the 95% level were calculated using the standard error for the estimates for each mixture coefficient. Mixtures that performed well were compared against the results of later quantitative analyses.

For the spring 2013 grid counts, data from both locations were combined and the proportion of intersects with surviving turfgrass plants in each plot was used to threshold the data. Plots that retained 60% or greater survival were designated as successful and assigned a 1, while those with less than 60% survival rate were designated as failures and assigned a 0. The threshold level of 60% was chosen both because that level of cover is likely to retain a high level of functionality and because it is consistent with the often-used 1-9 scale where 6, or 62.5% of full scale, is considered acceptable in turfgrass research (Skogley and Sawyer, 1992).

A generalized linear model was fit to the thresholded binomial response data including terms for site, replication, and the inclusion or exclusion of each species. Species inclusion was a categorical variable with the value of 1 if the species was in the seed mixture applied to that plot and 0 if it was not. The model was fit using the *glm* function in R. The thresholded grid count data analysis allowed us to quantify the effect of including individual species in the mixtures on the log-odds of retaining 60% or greater living turfgrass cover. Log-odds is defined as $\log[p/(1-p)]$ where $p$ is the probability of 60% or more of the intersects in a plot having a living turfgrass plant beneath it. Positive and negative coefficients for each term reflect an increase or decrease, respectively, in the probability of a plot meeting those criteria due to the inclusion of each species in the planted mixture.

The third analysis allowed us to quantify the effect of the proportion of each species in the mixture on the survival rates of plants in the roadside turfgrass mixtures. For the analysis, the proportion of grid intersects with surviving turfgrass plants from the combined 2013 grid count data were arcsin transformed and modeled using zero-intercept multiple linear regression. A full model of all main effects and two-way interactions of species proportions in the seed mixture was created and backward elimination was carried out to determine the best model using AIC as a model comparison metric. The data for the current MNDOT standard mix was removed from the data set prior to this analysis since its species proportions were outside of the allowed range for all other mixtures in the trial. The resulting regression equation defined a response surface for the percent survival of all possible mixtures in the design space. From it, the best possible mixture was predicted by identifying the combination of species proportions that maximized the value of the function.

## Results

The final model for the 2012 spring image data contained fixed effect terms for all three main effects as well as a *weed* × *week* interaction term. Mixture 5, which had a regression coefficient of 0.176, was found to have the largest effect on increasing the arcsine transformed percent green ground cover in the spring of 2012 (Fig. 1); however, it was statistically different from just 10 other mixtures in the trial based on the 95% confidence intervals. It, along with the next two best performing mixtures, mixture 8 and mixture 24, which had regression coefficients of 0.167 and 0.158, respectively, contained the maximum 40% of slender creeping red fescue. Mixture numbers 29, 30, 31, 32, and the MNDOT standard mixture had the lowest regression coefficients in the trial during that period. Each of those entries were found to decrease the arcsine transformed percent green ground cover with slopes of -0.053, -0.063, -0.003, and -0.014, respectively.

The final model for the 2012 summer data contained all three main effect terms, and was modeled using a weighted variance as a function of the fitted values. Separation of the regression coefficients for each mixture term using their respective 95% confidence intervals (Fig. 2) showed mixtures 17, 10, and 5 to have the three largest regression coefficients of 0.123, 0.112, and 0.093 in summer 2012. The standard MNDOT mixture had the third lowest regression coefficient of all trial entries during that time of -0.067.

Logistic regression of the thresholded grid count data revealed significant effects of including some species, but not others (Fig. 3). Of particular note was the coefficient on the term for inclusion of tall fescue, which decreased the overall log-odds by 1.78 and was significant at the 99% confidence level. Strong creeping red fescue also produced a decrease in the log-odds of success of -0.238, but was not significant (p=0.73) at the 95% confidence level. All other species produced increases in the log-odds of success, the largest of which were slender creeping red fescue, hard fescue, and sheep fescue. Slender creeping red fescue increased the log-odds of success by 1.049, but was not significant at the 95% confidence level (p=0.1). Log-odds of success were increased by inclusion of hard fescue and sheep fescue by 0.95 and 0.96, respectively, and both estimates were significant at the 90% confidence level (p=0.09 and p=0.07).

Through multiple linear regression of the arcsin transformed grid count survival proportions onto the seed mixture species proportions, we quantified the effect of each species proportion on overall mixture performance. The final model (Table 4) contained a single interaction term for slender creeping red fescue × alkaligrass interference. The model had a residual standard error of 0.2461 on 290 degrees of freedom and adjusted $R^2$ of 0.806. The analysis revealed that the strongest effects on survival percentage were seen as a result of including hard fescue, sheep fescue, and slender creeping red fescue for which the regression coefficients were 0.85, 0.67, and 0.65, respectively. Each was significant above the 99% confidence level. Conversely, tall fescue had no significant effect on increasing the proportion of surviving turfgrass plants, and Chewings fescue had a small effect with a coefficient of 0.247, which was significant above the 99% confidence level. Based on these results, the best possible mixture for the roadside sites, within the maximum 40% PLS constraint would comprise 40% hard fescue, 40% sheep fescue, and 20% slender creeping red fescue.

## Discussion

Throughout the data, it is possible to find evidence of each of the mixtures in the trial doing well at one point, as evidenced by the lack of many significant differences in the mixture comparisons. This is in large part due to the previous work that has been done which allowed us to design mixtures that all had promise for success on roadsides. There were some, however, that consistently out-performed others. Through our analysis we were able to determine which mixtures those were and the extent to which each species was responsible for the superior performance. This represents a significant step forward in the design and analysis of roadside and other turfgrass mixture experiments.

Each of the top three performing mixtures in spring 2012 (5, 8, and 24) contained the maximum allowed 40% slender creeping red fescue. The species has long been suggested for use in roadside mixtures (Boeker, 1970). It has been reported to have superior salt tolerance to other fescue species, including other *Festuca rubra* L. subspecies, which is attributable to its evolutionary history and habitat of origin (Hannon and Bradshaw, 1968; Humphreys, 1982) and has been demonstrated previously in salt spray and roadside trials (Humphreys, 1981). More importantly, Brown and Gorres (2011) and Friell et al. (2012) showed several cultivars of slender creeping red fescue to have higher mean survival rates on roadsides than most other species, including in Minnesota. With this in mind, it is not surprising that mixtures with the highest mean green ground cover contained a considerable percentage of the species. However, it is important to note that other mixtures contained 40% slender creeping red fescue as well and did not perform as well. This fact indicated that either the remaining species in the successful mixtures performed well relative to those in the less successful ones, or interactions among the included species played a significant role in their success or failure.

Interestingly, the worst three mixtures in the spring of 2012 (29, 30, and 31) each contained 40% alkaligrass, which has previously been suggested to be naturalized on some highways, and persistent on some roadsides (Butler et al., 1974; Friell et al., 2012). Although our mixture experiment results do not seem to agree with those observations, they are in agreement with previous work in which alkaligrass has not performed well under other low-input management similar to roadsides (Watkins et al., 2011). In fact, alkaligrass has been suggested for use only as a cover crop on some roadsides due to its lack of competitiveness in mixtures (Biesboer et al., 1998). The fact that alkaligrass may do well as a roadside cover crop, and at times as a monoculture, but not as a significant component of a mixture highlights the importance of evaluating turfgrass species mixtures, rather than individual cultivars, where mixtures are to be used in the final application.

Results of the summer 2012 analysis showed a different ranking of the mixtures, than the spring months. Many of those changes may reflect the intense heat and drought experienced during the summer of 2012. During the three-month period of August-October 2012 precipitation totals were 8.15 cm. The average high temperature for the month of July was 32.2 °C and an overall average temperature of 26.8 °C, which is 3.5°C above average, making it the second hottest month on record in the Minneapolis-St. Paul metropolitan area for the period 1873-2012 (Minnesota Climatology Working Group, 2012). Moreover, mowing did not cease during that time as the plots were under the control of the University of Minnesota grounds crew and it was not possible to request specific maintenance practices. Mowing during times of heat and drought stress is known to cause undue damage to the turfgrass plant (Parr et al., 1984; Liu and Huang, 2002) and may be responsible for a large proportion of the failed plots observed, particularly at the Larpenteur Ave. location where mowing occurred more frequently. Those conditions are representative of the intense stresses experienced by roadside vegetation.

Each of the top three mixtures from the summer 2012 period (17, 10, and 5) included 40% of one of hard fescue, sheep fescue, or slender creeping red fescue, or 40% of more than one of these species. Sheep fescue has been recommended for use on roadsides due to its drought tolerance (Duell and Schmit, 1974), and all three species have been found to have overall good adaptation to low-input and roadside conditions (Henensal et al., 1980; Watkins et al., 2011; Friell et al., 2012). Sheep and hard fescues are known to be especially drought tolerant (Ruemmele et al., 1995) which explains their ability to persist despite just 18.8 cm of precipitation between 18 June and 28 Oct. 2012.

Three of the five mixtures with the highest mean green cover percentages during that period contained 20% or more of tall fescue. Tall fescue is known to possess rooting characteristics that make it well-adapted to avoid or tolerate drought stress (Huang and Fry, 1998), both alone and in combination with heat stress (Jiang and Huang, 2001) or mowing (Richie et al., 2002). Although these traits may have contributed to the superior performance of those mixtures, not all mixtures containing large proportions of tall fescue seed performed well. One possible cause of this may be a decrease in the proportion of tall fescue in the some mixtures, relative to the seed mixture, during germination and establishment as a result of intraspecies and interspecies competition or environmental stresses. Hsiang et al. (1997) observed such decreases when tall fescue was seeded above 25% by weight in mixtures under athletic field conditions; although, species dynamics are likely to be somewhat different under roadside conditions.

The results of the logistic regression analysis demonstrate the importance of selecting species for use on roadsides that can withstand several different stresses. The winter of 2012-2013 resulted in significant turfgrass death, which is likely attributable to ice and snow cover. Tall fescue, in particular, is known to be susceptible to ice damage.

Laboratory tests have shown 33 d to be the average period of ice encasement which results in 50% death of forage-type tall fescue plants ($LD_{50}$) (Gudleifsson, 2010). The greater than 90 d of ice cover experienced during the winter of 2012-2013 was well above the 33 d $LD_{50}$ and disproportionately harmed tall fescue, which likely resulted in the negative regression coefficient for that species.

The change in the apparent importance of tall fescue in the mixtures from summer 2012 to spring 2013 was a result of long-term fluctuation in environmental conditions. This demonstrates the necessity for including multiple species in a mixture with each species possessing a unique stress tolerance. Furthermore, it indicated that species used in the ideal seed mixture should be based on the final grid count data because those data were collected following exposure to several different stresses including salt, ice, drought, heat, and mowing.

The identified best possible mixture contained 40% hard fescue, 40% sheep fescue, and 20% slender creeping red fescue, which was in agreement with the species compositions of many of the mixtures that had performed consistently well during the earlier analyses. It is interesting to note that the only interaction term in the model was for slender creeping red fescue × alkaligrass. Those two species are found to coexist at equilibrium in nature except under drought stress when slender creeping red fescue typically outcompetes alkaligrass (Gray and Scott, 1977). The lack of precipitation during summer 2012 likely led to this interference term and a decline in alkaligrass when planted with slender creeping red fescue. From the present data, it is not possible to say whether a better mix exists with proportions outside of the 5-40% restricted range defined during the design of the experiment. However, it may be possible to further simplify the identified optimal mixture. Sheep fescue and hard fescue are very closely related species (Ruemmele et al., 2003) and breeding history of the sheep fescue cultivar ‘Marco Polo’ used in this experiment suggests that it may be the progeny of a hard fescue × sheep fescue cross (National Grass Review Board, 2010). With this in mind, it may be possible to replace one species with the other to further simplify the mixture.

## Conclusions

Mixtures of fine fescues performed well in the roadside turfgrass species mixture trial. Those mixtures that contained Kentucky bluegrass or creeping bentgrass in combination with large proportions of alkaligrass, did not perform well. However, in some cases the inclusion of these species in a mixture dominated by fine fescues produced a quality turfgrass sward.

Inclusion of tall fescue in mixtures was found to significantly decrease the probability of maintaining 60% survival after two years. Conversely, inclusion of hard fescue and sheep fescue increased the probability significantly. A response surface method incorporating multiple linear regression was used to successfully identify a theoretical best possible mixture, within the constraints of the experimental design. The results indicate that seed mixtures containing 40% hard fescue, 40% sheep fescue, and 20% slender creeping red fescue have the best chance for survival on roadsides. These results can be used by public works officials to implement and improve roadside turfgrass mixtures, and may be helpful to turfgrass breeders interested in breeding mixtures of turfgrass.

Future work on turfgrass mixture evaluations should include similar quantitative assessments of individual species contributions to overall mixture performance. Based on the results of this work, such experiments may include fewer species, but should be broader in the ranges of those species' possible mixture proportions. Further work towards describing the competitive interactions of fine fescue species can be useful in understanding the results achieved here. Although the cultivars used in this experiment have been previously evaluated for establishment and survival on Minnesota roadsides, comparison of our findings with results using other cultivars and in other locations will be useful in determining the extent to which our results may be generalized.

## Acknowledgements

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**Figure 1.** Regression coefficients and 95% confidence intervals for the effect of each mixture term in the model of arcsin-transformed ground cover for spring 2012 observations in the roadside turfgrass mixture experiment.

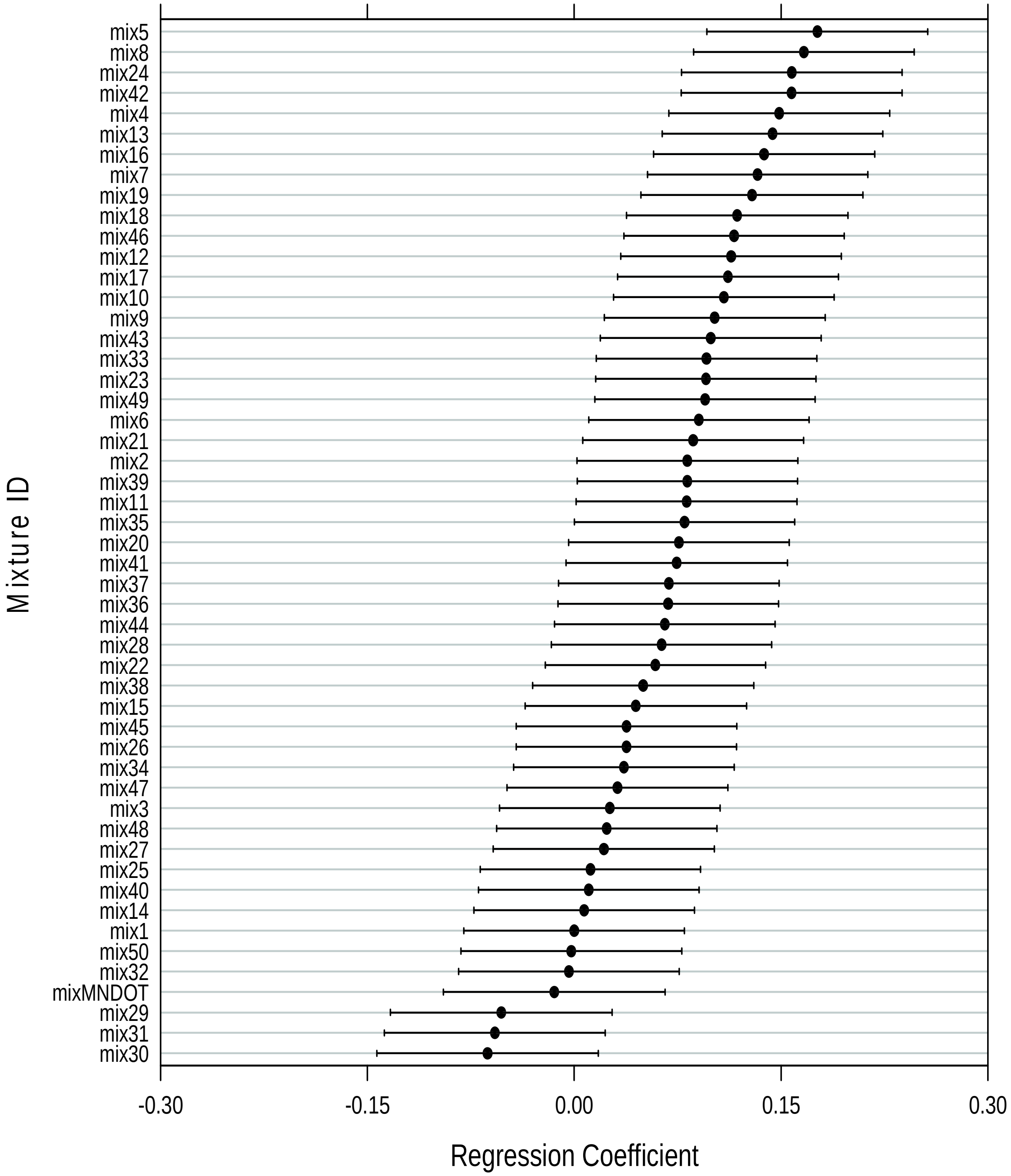

**Figure 2.** Regression coefficients and 95% confidence intervals for the effect of each mixture term in the model of arcsin-transformed ground cover for summer 2012 observations in the roadside turfgrass mixture experiment.

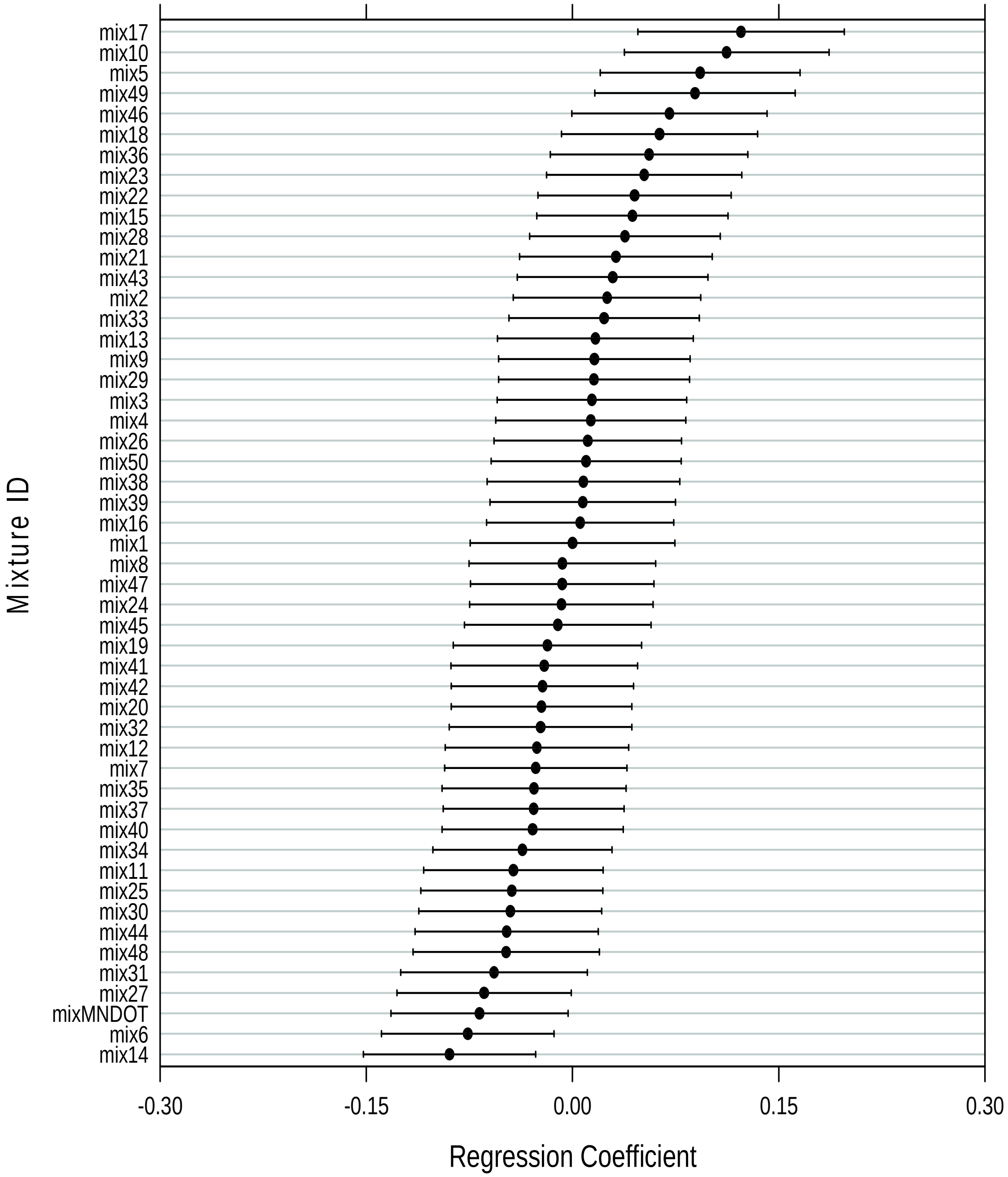

**Figure 3.** Regression coefficients and 95% confidence intervals from the model of grid count data showing the effect on log-odds of retaining greater than 60% survival based on inclusion of individual species in the roadside mixture experiment including alkaligrass (ALK), creeping bentgrass (CBG), Chewings fescue (CHF), hard fescue (HDF), Kentucky bluegrass (KBG), sheep fescue (SHF), slender creeping red fescue (SLCRF), strong creeping red fescue (STCRF), and tall fescue (TF).

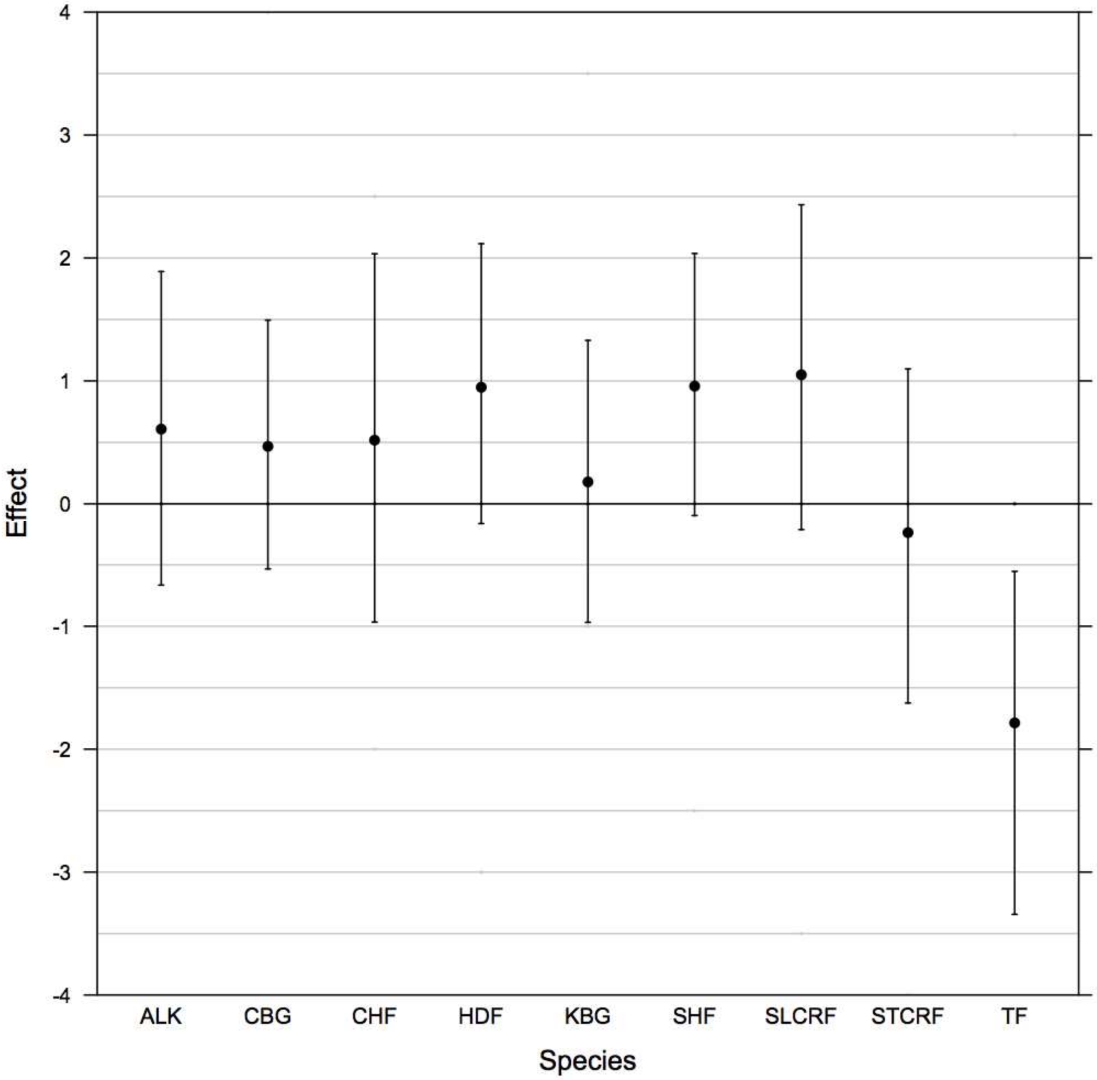

**Table 1.** Species and cultivars included in the roadside turfgrass mixture experiment.

| Species | Cultivar |
|---|---|
| *A. stolonifera* | ‘Mariner’ |
| *P. pratensis* | ‘Moonlight SLT’ |
| *P. distans* | ‘Salty’ |
| *F. rubra* ssp. *rubra* | ‘Navigator’ |
| *F. rubra* ssp. *litoralis* | ‘Shoreline’ |
| *F. trachyphylla* | ‘Beacon’ |
| *F. ovina* | ‘Marco Polo’ |
| *F. arundinacea* | ‘Grande II’ |
| *F. rubra* ssp. *fallax* | ‘Radar’ |

**Table 2.** Constituent species proportions, based on pure live seed count, that were included in the roadside turfgrass mixture experiment.

| | **Species†** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Mixture ID** | **STCRF** | **ALK** | **KBG** | **CBG** | **SHF** | **HDF** | **SLCRF** | **TF** | **CHF** |
| 1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.20 | 0.40 | 0.40 |
| 2 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.40 | 0.00 | 0.40 | 0.20 |
| 3 | 0.00 | 0.00 | 0.00 | 0.00 | 0.20 | 0.00 | 0.00 | 0.40 | 0.40 |
| 4 | 0.00 | 0.00 | 0.00 | 0.00 | 0.20 | 0.40 | 0.20 | 0.00 | 0.20 |
| 5 | 0.00 | 0.00 | 0.00 | 0.00 | 0.40 | 0.00 | 0.40 | 0.20 | 0.00 |
| 6 | 0.00 | 0.00 | 0.00 | 0.20 | 0.00 | 0.40 | 0.00 | 0.00 | 0.40 |
| 7 | 0.00 | 0.00 | 0.00 | 0.20 | 0.00 | 0.40 | 0.40 | 0.00 | 0.00 |
| 8 | 0.00 | 0.00 | 0.00 | 0.40 | 0.00 | 0.00 | 0.40 | 0.00 | 0.20 |
| 9 | 0.00 | 0.00 | 0.00 | 0.40 | 0.40 | 0.00 | 0.00 | 0.00 | 0.20 |
| 10 | 0.00 | 0.00 | 0.00 | 0.40 | 0.40 | 0.00 | 0.00 | 0.20 | 0.00 |
| 11 | 0.00 | 0.00 | 0.07 | 0.07 | 0.40 | 0.40 | 0.07 | 0.00 | 0.00 |
| 12 | 0.00 | 0.00 | 0.20 | 0.00 | 0.00 | 0.00 | 0.40 | 0.00 | 0.40 |
| 13 | 0.00 | 0.00 | 0.20 | 0.00 | 0.00 | 0.40 | 0.00 | 0.00 | 0.40 |
| 14 | 0.00 | 0.00 | 0.20 | 0.40 | 0.00 | 0.20 | 0.20 | 0.00 | 0.00 |
| 15 | 0.00 | 0.00 | 0.40 | 0.00 | 0.00 | 0.20 | 0.00 | 0.40 | 0.00 |
| 16 | 0.00 | 0.00 | 0.40 | 0.00 | 0.00 | 0.20 | 0.40 | 0.00 | 0.00 |
| 17 | 0.00 | 0.00 | 0.40 | 0.07 | 0.00 | 0.40 | 0.07 | 0.07 | 0.00 |
| 18 | 0.00 | 0.00 | 0.40 | 0.07 | 0.40 | 0.00 | 0.07 | 0.07 | 0.00 |
| 19 | 0.00 | 0.00 | 0.40 | 0.20 | 0.00 | 0.00 | 0.00 | 0.00 | 0.40 |
| 20 | 0.00 | 0.00 | 0.40 | 0.20 | 0.00 | 0.00 | 0.40 | 0.00 | 0.00 |
| 21 | 0.00 | 0.07 | 0.00 | 0.07 | 0.40 | 0.00 | 0.00 | 0.07 | 0.40 |
| 22 | 0.00 | 0.07 | 0.07 | 0.40 | 0.00 | 0.00 | 0.00 | 0.40 | 0.07 |
| 23 | 0.00 | 0.20 | 0.00 | 0.00 | 0.40 | 0.00 | 0.00 | 0.40 | 0.00 |
| 24 | 0.00 | 0.20 | 0.00 | 0.00 | 0.40 | 0.00 | 0.40 | 0.00 | 0.00 |
| 25 | 0.00 | 0.40 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.20 | 0.40 |
| 26 | 0.00 | 0.40 | 0.00 | 0.00 | 0.00 | 0.00 | 0.40 | 0.20 | 0.00 |
| 27 | 0.00 | 0.40 | 0.00 | 0.00 | 0.00 | 0.40 | 0.00 | 0.00 | 0.20 |
| 28 | 0.00 | 0.40 | 0.00 | 0.00 | 0.00 | 0.40 | 0.00 | 0.20 | 0.00 |
| 29 | 0.00 | 0.40 | 0.00 | 0.40 | 0.00 | 0.10 | 0.10 | 0.00 | 0.00 |

(continued on next page)

**Table 2.** Continued.

| | **Species†** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Mixture ID** | **STCRF** | **ALK** | **KBG** | **CBG** | **SHF** | **HDF** | **SLCRF** | **TF** | **CHF** |
| 30 | 0.00 | 0.40 | 0.20 | 0.00 | 0.40 | 0.00 | 0.00 | 0.00 | 0.00 |
| 31 | 0.00 | 0.40 | 0.40 | 0.07 | 0.07 | 0.00 | 0.07 | 0.00 | 0.00 |
| 32 | 0.10 | 0.00 | 0.00 | 0.40 | 0.00 | 0.00 | 0.00 | 0.10 | 0.40 |
| 33 | 0.12 | 0.00 | 0.00 | 0.12 | 0.12 | 0.12 | 0.12 | 0.40 | 0.00 |
| 34 | 0.20 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.40 | 0.40 | 0.00 |
| 35 | 0.20 | 0.00 | 0.00 | 0.40 | 0.00 | 0.40 | 0.00 | 0.00 | 0.00 |
| 36 | 0.20 | 0.00 | 0.40 | 0.00 | 0.00 | 0.00 | 0.00 | 0.20 | 0.20 |
| 37 | 0.20 | 0.00 | 0.40 | 0.40 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 38 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.00 | 0.00 | 0.00 | 0.00 |
| 39 | 0.20 | 0.40 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.40 | 0.00 |
| 40 | 0.33 | 0.33 | 0.00 | 0.00 | 0.00 | 0.00 | 0.33 | 0.00 | 0.00 |
| 41 | 0.40 | 0.00 | 0.00 | 0.00 | 0.00 | 0.40 | 0.20 | 0.00 | 0.00 |
| 42 | 0.40 | 0.00 | 0.00 | 0.00 | 0.20 | 0.00 | 0.40 | 0.00 | 0.00 |
| 43 | 0.40 | 0.00 | 0.00 | 0.20 | 0.00 | 0.20 | 0.00 | 0.00 | 0.20 |
| 44 | 0.40 | 0.00 | 0.00 | 0.40 | 0.00 | 0.00 | 0.20 | 0.00 | 0.00 |
| 45 | 0.40 | 0.00 | 0.07 | 0.00 | 0.40 | 0.00 | 0.07 | 0.07 | 0.00 |
| 46 | 0.40 | 0.00 | 0.20 | 0.00 | 0.00 | 0.00 | 0.00 | 0.40 | 0.00 |
| 47 | 0.40 | 0.00 | 0.40 | 0.00 | 0.00 | 0.20 | 0.00 | 0.00 | 0.00 |
| 48 | 0.40 | 0.20 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.40 |
| 49 | 0.40 | 0.20 | 0.00 | 0.00 | 0.00 | 0.20 | 0.00 | 0.20 | 0.00 |
| 50 | 0.40 | 0.40 | 0.00 | 0.00 | 0.20 | 0.00 | 0.00 | 0.00 | 0.00 |
| MNDOT | 0.00 | 0.20 | 0.60 | 0.00 | 0.00 | 0.00 | 0.20 | 0.00 | 0.00 |

†STCRF, strong creeping red fescue; ALK, alkaligrass; KBG, Kentucky bluegrass; CBG, creeping bentgrass; SHF, sheep fescue; HDF, hard fescue; SLCRF, slender creeping red fescue; TF, tall fescue; CHF, Chewings fescue

**Table 3.** Soil physical and chemical properties for sites in the roadside turfgrass mixture experiment.

| Soil Property | Site |  |  |  |  |  |
|---|---|---|---|---|---|---|
|  | Centerville[†] |  |  | Larpenteur[‡] |  |  |
|  | Rep 1 | Rep 2 | Rep 3 | Rep 1 | Rep 2 | Rep 3 |
| P (Bray), mg $kg^{-1}$ | 59 | 64 | 78 | 53 | 52 | 50 |
| P (Olsen), mg $kg^{-1}$ | 35 | 36 | 49 |  |  |  |
| K, mg $kg^{-1}$ | 100 | 91 | 93 | 172 | 148 | 137 |
| Organic Matter, % | 1.1 | 1.4 | 2.1 | 4.7 | 4.5 | 5.5 |
| pH | 8.2 | 7.9 | 7.8 | 6.8 | 6.6 | 6.5 |
| Saturated Paste EC (Establishment), dS $m^{-1}$ | 2.4 | 2.3 | 2.6 | 2.6 | 2.4 | 2.5 |
| Ca, mg $kg^{-1}$ | 3244 | 3055 | 2836 | 2921 | 2790 | 2512 |
| Mg, mg $kg^{-1}$ | 114 | 126 | 467 | 174 | 164 | 180 |
| Na, mg $kg^{-1}$ | 450 | 468 | 514 | 292 | 292 | 291 |

† Soil texture at Centerville was 55% sand, 18% silt 27% clay

‡ Soil texture at Larpenteur was 45% sand, 28% silt, 27% clay

**Table 4.** Marginal effects summary of multiple regression of final grid count data onto seed mixture species proportions.

| Species † | Coefficient | Std Error | Pr(>\|t\|) |
|---|---|---|---|
| STCRF | 0.426 | 0.080 | 2.00e-07 |
| SLCRF | 0.648 | 0.092 | 1.82e-11 |
| HDF | 0.853 | 0.080 | < 2e-16 |
| SHF | 0.667 | 0.082 | 2.11e-14 |
| CHF | 0.247 | 0.082 | 0.00298 |
| TF | 0.018 | 0.082 | 0.81967 |
| KBG | 0.570 | 0.081 | 2.32e-11 |
| CBG | 0.466 | 0.083 | 4.60e-08 |
| ALK | 0.600 | 0.098 | 3.61e-09 |
| SLCRF × ALK | -1.004 | 0.626 | 0.11016 |

† STCRF, strong creeping red fescue; ALK, alkaligrass; KBG, Kentucky bluegrass; CBG, creeping bentgrass; SHF, sheep fescue; HDF, hard fescue; SLCRF, slender creeping red fescue; TF, tall fescue; CHF, Chewings fescue

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