DEPARTMENT OF TRANSPORTATION

Regional Optimization of Roadside Turfgrass Seed Mixtures

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December 2018

Research Project
Final Report 2019-01

LRRB
LOCAL
ROAD RESEARCH
BOARD

Technical Report Documentation Page

1. Report No. MN/RC 2019-01	2.	3. Recipients Accession No.
4. Title and Subtitle Regional Optimization of Roadside Turfgrass Seed Mixtures		5. Report Date December 2018
		6.
7. Author(s) Eric Watkins, Florence Sessoms, Andrew Hollman, Michael Laskowski, and Kristine Moncada		8. Performing Organization Report No.
9. Performing Organization Name and Address Department of Horticultural Science University of Minnesota 1970 Folwell Ave St. Paul, MN. 55108		10. Project/Task/Work Unit No. CTS#2017016
		11. Contract (C) or Grant (G) No. (C) 99008 (WO) 232
12. Sponsoring Organization Name and Address Local Road Research Board and Minnesota Department of Transportation Research Services & Library 395 John Ireland Boulevard, MS 330 St. Paul, Minnesota 55155-1899		13. Type of Report and Period Covered Final Report
		14. Sponsoring Agency Code

15. Supplementary Notes
http://mndot.gov/research/reports/2019/201901.pdf

16. Abstract (Limit: 250 words)

Current MnDOT specifications for roadside turfgrasses suggest statewide planting of mixtures that are grouped into five broad categories such as low maintenance turf and high maintenance turf. The objective of this research was to identify turfgrasses that possess traits necessary to survive in the harsh roadside environments found throughout Minnesota. We investigated the impacts of possibly the three most limiting environmental conditions (heat, salt and ice cover) on multiple cultivars from up to fifteen individual turfgrass species. Salt stress screening revealed several species with good levels of adaptation including alkaligrass and tall fescue. In the heat stress trial, we found cultivars and selections of Canada bluegrass, tall fescue, Kentucky bluegrass, strong creeping red fescue and slender creeping red fescue were among the top performers. Finally, in our ice cover screening, tall fescue and Chewings fescue did well; however, these results did not correlate well with our typical field observations. For each of these stresses, we identified top-performing cultivars that will be evaluated in field studies with the goal of identifying optimized mixtures for stakeholders in Minnesota.

17. Document Analysis/Descriptors Turf, Grasses, Roadside flora, Vegetation, Seeds, Deicing chemicals, Salts, Thermal stresses, Icing		18. Availability Statement No restrictions. Document available from: National Technical Information Services, Alexandria, Virginia 22312	
19. Security Class (this report) Unclassified	20. Security Class (this page) Unclassified	21. No. of Pages 66	22. Price

Regional Optimization of Roadside Turfgrass Seed Mixtures

FINAL REPORT

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December 2018

Published by:

Minnesota Department of Transportation
Research Services & Library
395 John Ireland Boulevard, MS 330
St. Paul, Minnesota 55155-1899

ACKNOWLEDGMENTS

The authors would like to thank the Minnesota Department of Transportation and the Local Road Research Board for funding this research. Additionally, we thank Sam Bauer and Josh Friell for help in project design and implementation. We would also like to thank Dwayne Stenlund for his advice and input throughout the project.

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LIST OF ABBREVIATIONS

ALK – Akaligrass (*Pucinella distans*)
ALKm – Akaligrass (*Pucinella maritima*)
BLGR – Blue grama (*Bouteloua gracilis*)
BUFF – Buffalograss (*Bouteloua dactyloides*)
CAND – Canada bluegrass (*Poa compressa*)
CHF – Chewings fescue (*Festuca rubra* ssp. *fallax*)
EL – Electrolyte leakage
HDF – Hard fescue (*Festuca brevipilia*)
KBG – Kentucky bluegrass (*Poa pratensis*)
MnDOT – Minnesota Department of Transportation
NDVI – Normalized Difference Vegetation Index
PR – Perennial ryegrass (*Lolium perenne*)
PRt – Tetraploid perennial ryegrass (*Lolium perenne*)
SHF – Sheep fescue (*Festuca ovina*)
SLRF – Slender creeping red fescue (*Festuca rubra* ssp. *litoralis*)
SMBR – Smooth brome (*Bromus inermis*)
STRF – Strong creeping red fescue (*Festuca rubra* ssp. *rubra*)
TF – Tall fescue (*Schedonorus arundinaceus*)

EXECUTIVE SUMMARY

The harsh environment of roadsides can make establishing turfgrass and keeping vegetation alive difficult. There are various stresses that occur on roadsides that may result in installation failures, causing loss of time and resources. Roadside turfgrasses must cope with saline soils from salt applied to roads in the winter, heat that is intensified in urban settings, and ice sheeting that reduces oxygen availability. In this project, the University of Minnesota turfgrass research program conducted research to identify grasses that will perform best in this harsh environment.

The overall goal of this project was to quantify roadside turfgrass stress tolerances and then identify candidate turfgrasses for use throughout Minnesota. Our research strategy was to examine three specific stresses – salt, heat and ice – on turfgrass performance. We used controlled environmental conditions and testing procedures to evaluate the effect of each of these stresses separately. An important facet of this project was that we included a wide range of turfgrass species and cultivars. Species perform differently in response to roadside stresses and even cultivars within the same species do not perform the same when exposed to these stresses. Our previous work examined salt stress in a few cultivars, but we had not yet studied adaptation to stresses such as heat and ice encasement.

Salt stress. In turfgrasses, high soil concentrations of sodium chloride cause yellowing of foliage, poor cell membrane stability, and at high enough concentrations, plant death. Our findings on salt stress include the somewhat surprising result that alkaligrass, despite its reputation as salt-tolerant, did not perform better than other species, at least at moderate salt stress. However, it did maintain a significantly higher percent green cover at the highest salt concentrations than all other species except tall fescue. Newer cultivars of alkaligrass may be able to provide the adequate turf performance qualities for roadsides that older cultivars lack and so may be valuable to include in mixes in the future. Based on our salt stress experiments, smooth bromegrass, hard fescue, blue grama and prairie junegrass should not be used as roadside turfgrasses in northern climates. The two species that performed best under salt stress were tall fescue followed by perennial ryegrass; unfortunately, both of these species are susceptible to other winter-related stresses. In field trials, tall fescues have not performed well under ice cover and perennial ryegrass has poor winter hardiness.

Heat stress. Heat stress in turfgrasses is characterized by reduced vegetative growth, tissue browning and decreased membrane stability. In our experiments, we found the warm-season turfgrasses (buffalograss and blue grama) were, as expected, tolerant to heat stress. Most of the fine fescues were sensitive to heat stress, but some were able to recover once normal temperatures resumed. Chewings fescue, hard fescue and sheep fescue were affected by heat stress and the turf performance was lower than for the top-performing grasses, but most of the Chewings fescue cultivars were able to recover well. Slender creeping red fescue and strong creeping red fescue were among the top-ranked cultivars in heat tolerance and showed impressive recovery. Hard fescue and sheep fescue had difficulty recovering and would not be candidates for thriving under high-temperature conditions. Kentucky bluegrass and perennial ryegrass were both affected by heat stress; however Kentucky bluegrass cultivars displayed a higher turf performance when compared to the perennial ryegrass cultivars. The performance of ‘Tirem’ suggests it might be a good option when Kentucky bluegrass is desired in a roadside mixture.

Interestingly, the tall fescue cultivars were negatively affected by heat treatment, but all cultivars showed good recovery ability. Based on our results, our recommendations for heat-tolerant roadside turfgrasses include Canada bluegrass, tall fescue, Kentucky bluegrass, strong creeping red fescue and slender creeping red fescue cultivars and accessions.

Ice stress. Ice encasement of turfgrasses can result in low oxygen or a complete lack of oxygen, both leading to potential turfgrass death. In our ice cover experiment, tall fescue was the best-performing species. This result differs from field observations in Minnesota where it often performs very poorly. The fine fescue species also performed differently than expected. In the controlled ice trials, Chewings fescue cultivars performed better than the hard fescue and sheep fescue cultivars, which is opposite of what has been observed in field trials under severe ice cover. 'Bad River' blue grama, a warm-season grass, was completely killed in the ice cover trial. Buffalograss, the other warm-season grass in the trial, also was damaged by ice. Selection for survival to extended ice cover is important for roadside turfgrasses in conditions that lead to ice formation. Some of the results in our ice stress experiments were perplexing because they did not correspond with previous observations. It is possible that our experiment failed to replicate field conditions and we may need to refine our methods.

Conclusion. We have obtained new, unbiased data about the performance of newer cultivars and under-studied species when grown in harsh conditions similar to those found on roadsides. Our work will help select better performing species and cultivars to improve the performance of current roadside turfgrass mixes. Ultimately, our results will lead to saving public agencies significant amounts of money on re-installations, as well as reduce the environmental challenges associated with roadside vegetation failures.

CHAPTER 1: INTRODUCTION

1.1 OVERVIEW

This research project is part of a long-term effort by our team at the University of Minnesota to determine how best to ensure success of turfgrass installations under the wide-ranging conditions that occur in roadside environments. It is an unfortunate fact that many roadside turf installations fail during or just after establishment. Failed installations can happen for a number of reasons, and we believe, based on observation and previous research, that failures often are due to using the wrong species for a given site.

These failed installations most often result in needing to reseed or even resod. The most basic method for reestablishment of a failed site is to kill the existing vegetation and reseed, which has a cost of $150 to $530 per acre when using the most popular roadside seed mixtures, plus the added cost of labor and resources needed to rectify a failed installation. Replacing sod can cost nearly $20,000 per acre. The additional cost of regrading, installation and water can also be significant. Using the right turfgrass species for a specific area in the first place will lead to long-term success for roadside turfgrass installations, as well as saving limited MnDOT funds that would otherwise need to be spent to replace installation failures.

1.2 STRATEGY

The overall goal of this project was to quantify roadside turfgrass stress tolerances and to then identify candidate turfgrasses for use throughout Minnesota. In this project we examined three specific stresses – salt, heat and ice cover – that can kill turfgrasses and contribute to installation failure. We used controlled environmental conditions and testing procedures to evaluate the effect of each of these stresses separately. While our previous work examined salt stress in selected cultivars, prior to this current project we had not yet studied adaptation to stresses such as heat and ice encasement.

We wanted to include newer cultivars that had not been tested when we last screened in 2010; additionally, we wanted to test previously unstudied species. To start the project, we selected species to trial based on our previous work (Friell et al., 2012; Friell et al., 2013) and also on findings from Biesboer and Jacobson (1994) who recommended further roadside testing of blue grama and buffalograss in Minnesota given their abilities to germinate and grow in saline conditions. Based on the work in our program and other programs, we identified several other species that warranted inclusion including tall fescue, strong creeping red fescue, slender creeping red fescue, Chewings fescue, hard fescue, sheep fescue, Kentucky bluegrass, perennial ryegrass, alkaligrass, Canada bluegrass, and smooth brome.

We have obtained new, unbiased data about the performance of newer cultivars and under-studied species when grown in harsh conditions similar to those found on roadsides. Taken together, our results will help save public agencies significant amounts of money on re-installations as well as reduce the environmental challenges associated with roadside vegetation failures. We will continue to work with MnDOT to change turfgrass seed mixture recommendations based on our results.

CHAPTER 2: SALT STRESS

2.1 INTRODUCTION

Road salt performs a critical function in keeping roads clear and safe during snowy and icy conditions. In fact, 174 thousand tons of salt are applied to Minnesota's roads every winter (MnDOT, 2016). After it melts the snow and ice from roadsides and sidewalks, much of the salt runs into the storm drain. However, some of that salt will end up in the soil along the roadsides and boulevards where turfgrasses grow. The salt may even accumulate to plant-damaging levels. A study by Biesboer et al. (1994) found roadside salt concentrations up to 12,000 ppm ten feet (three meters) from the road and previous research indicates salt levels on roadsides to range from 2,500 ppm to 22,000 ppm. Friell et al. (2013) found that in a controlled environment study, salt concentrations of 14 dS m^{-1}, which is approximately in the middle of the above-mentioned range, resulted in significant damage to some cultivars.

Salt tolerance is an important measure of viability for turfgrasses on Minnesota roadsides due to the quantities of salt applied each winter. High concentrations of sodium chloride (NaCl) cause yellowing of leaf tips, poor cell membrane stability, and plant death in turfgrass. In spring, salt damage can be seen in patches of dead turfgrass along the roadsides due to too much salt in the soil. Turf that is dead cannot provide normal ecosystem functions such as protecting waterways from nutrient leaching or preventing soil erosion. To reduce turf death due to salt stress, our objective was to quantify the salt tolerance of 38 turfgrass cultivars.

2.2 MATERIALS AND METHODS

2.2.1 Species Selection

Previous research from our team found that tall fescue has the highest relative salt tolerance compared to other cool-season grasses, but may not be well suited for roadsides due to other stress factors, particularly ice tolerance (Friell, et al., 2013). Since that time, turfgrass breeders have released new cultivars of many species that show potential for use on roadsides. We chose to evaluate these newer turfgrass cultivars based on their performance under varied levels of salt stress so that we could make recommendations for turfgrass managers with saline soils.

While other studies focused on examining the salt tolerance of just a few species, we expanded beyond these few species in our current work to include 15 species. A total of 38 cultivars and accessions across these 15 species were used (Table 2.1). Species and cultivars were either common varieties available on the market or selected based on breeder input. We also chose entries that had some evidence they could perform well on roadsides.

2.2.2 Experimental Design

Plants were evaluated in a hydroponic system to eliminate variability, such as air temperature, soil composition and rainfall that are often seen in field trials. Plants were established in 4-inch (10.14 cm) pots filled with silica sand at a rate of 2 pure live seeds (PLS) per cm^2. Pots were placed in a greenhouse for 12 weeks with an average temperature of 73° F (23° C). Pots were then suspended in half strength Hoagland's solution for three weeks after establishment to adjust to the hydroponic environment. Sodium chloride salt was added every three weeks up to the specified concentration (Figure 2.1). Four concentrations of salt were used that captured the range of salt concentrations seen in Biesboer et al. (1994): 10, 14, 18 and 22 dSm^{-1}. Water was drained and refilled at the end of each exposure period.

2.2.3 Data Collection

Plant performance under salt stress was assessed by calculating percent green cover using digital images with the FieldScout TCM 500 Normalized Difference Vegetation Index (NDVI) TurfColor Meter from Spectrum Technologies (Illinois, USA). For each pot and each time point, the NVDI meter was placed on top of the pot and the NDVI measure was recorded. Plant performance under salt stress was quantified by calculating change in percent green cover to see how quickly and to what extent leaf tissue yellowed (Figure 2.2). In addition, electrolyte leakage was used to measure cell membrane stability using a modified protocol from Verslues et al. (2006).

2.2.4 Data Analysis

All data were analyzed in Program R (Version 3.5.0; R Core Team, 2018). A two-way ANOVA was used to determine differences in means. The least significant difference between cultivar means was calculated using Fisher's protected LSD. Pearson's method was used to calculate the correlation between measurements.

2.3 RESULTS

Overall, there was a high correlation between percent cover and NDVI as expected (Table 2.2). Lower correlation between electrolyte leakage and percent cover was likely due to differences between species cell membrane stability.

We found that tall fescue and perennial ryegrass maintained the highest percent green cover (Figure 2.3) and NDVI along with the lowest electrolyte leakage throughout the experiment. These data were consistent with recent work in turfgrass research that showed tall fescue maintained green color longer than other species (Friell, et al., 2013). We also found that smooth bromegrass, hard fescue, blue grama and prairie junegrass performed the worst at all salt concentrations. Significant differences between cultivars within species were observed in slender creeping red fescue (cultivars 'SeaMist' and 'Sprinkler') and prairie junegrass (cultivar 'Barkoel' and ecotype 'Minnesota').

Surprisingly, the percent green cover of alkaligrass, which is considered to be salt tolerant, was not significantly different than most of the other grasses, even under moderate salt stress. However, at the highest salt concentrations, alkaligrass maintained significantly higher percent green cover than all other species except tall fescue.

2.4 CONCLUSIONS

The design of this experiment did not allow for comparison of species or cultivars across time points. Future efforts can correct for this by using a single concentration for each tub and including a control tub. The duration of salt exposure coupled with four salt concentrations makes the results unclear whether the primary effect was from duration or concentration.

Although alkaligrass was not significantly different in percent green cover at moderate salt stress, it did maintain significantly higher percent green cover at the highest salt concentrations than all other species except tall fescue. Alkaligrass, when planted in non-roadside conditions, does not provide adequate turf quality (Watkins et al., 2011); however, newer cultivars, such as those tested here, may be able to provide acceptable levels of turf performance to be used in roadside mixtures. Tall fescue maintained the highest percent green cover followed by perennial ryegrass. Tall fescue might be a very good option for salt-affected roadsides, however, tall fescues have not performed well under ice cover (Friell et al., 2013) so locations where ice sheeting occurs, such as poorly-drained or low-lying spots between sidewalks and curbs, may not be good locations for tall fescue. Perennial ryegrass is likely not a useful option for roadsides to do its poor winter hardiness (Hulke et al., 2007). Smooth bromegrass, hard fescue, blue grama and prairie junegrass did not maintain adequate turf cover, even at low salt concentrations and are therefore are not recommended as roadside turfgrasses.

These results show that choosing the right species is extremely important, and proper cultivar selection, while less significant, can improve the overall turf stand. Choosing species and cultivars with low salt tolerance will result in a poor turf stands on roadsides in cold climates.

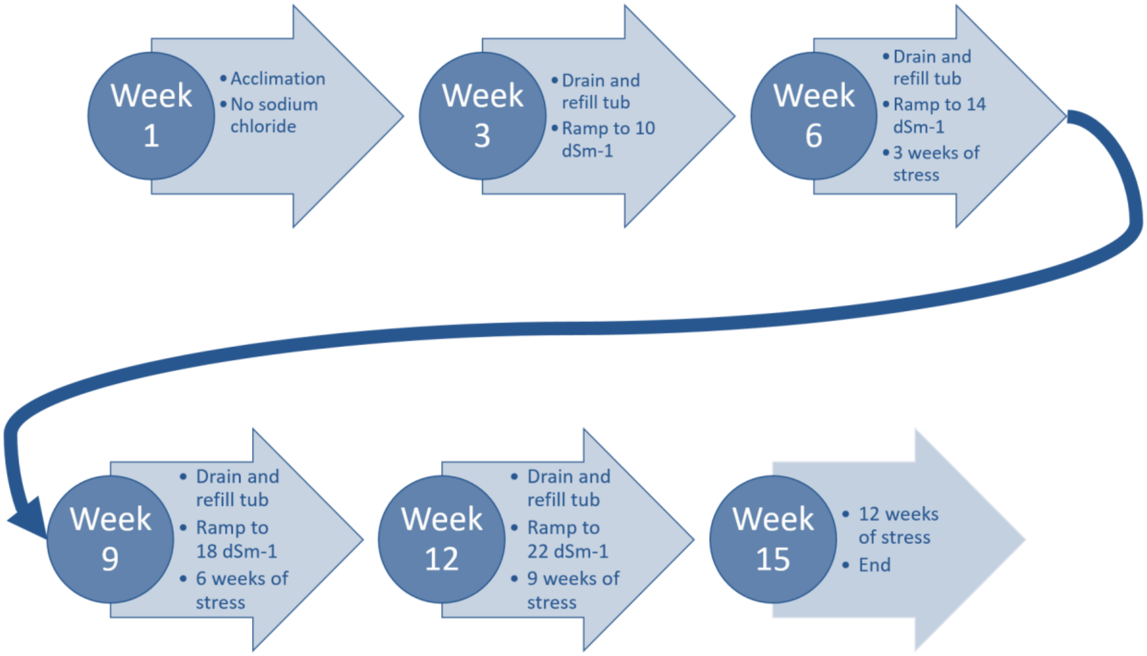

Figure 2.1: Timeline of salt concentration and stress duration.

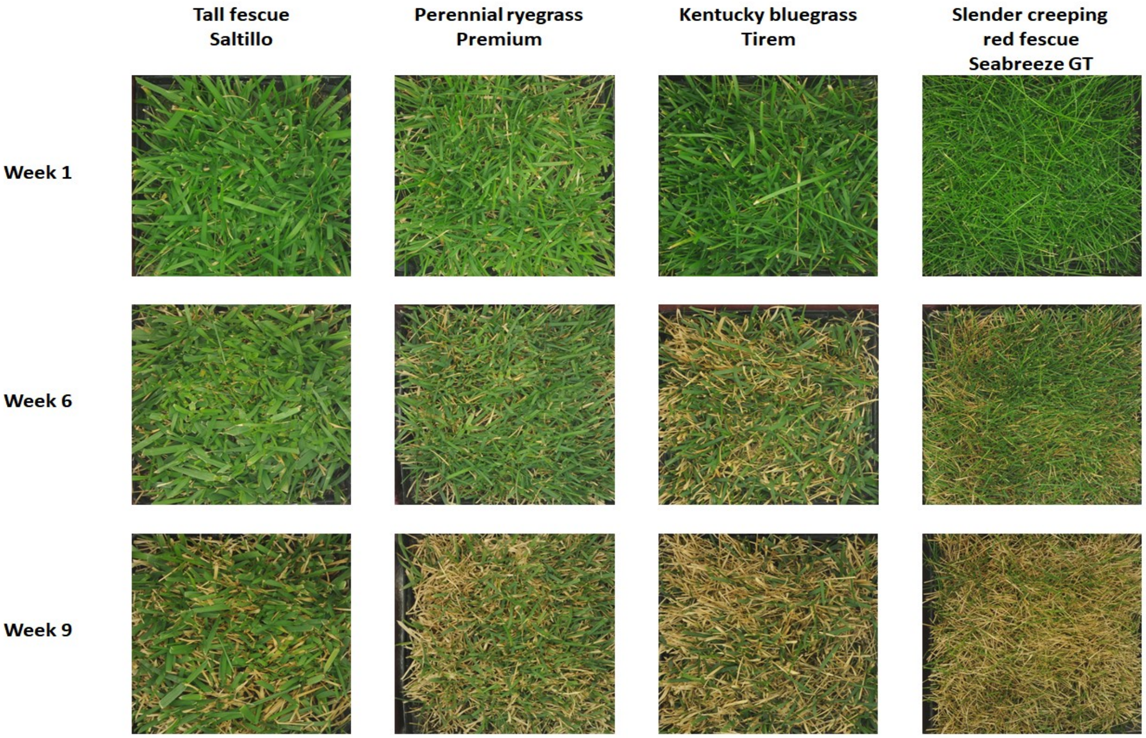

Figure 2.2: Example images of four species and how they performed over time. Week 1 was at the beginning of the experiment before any salt exposure. Week 6 was a cumulative exposure of the previous concentration of 10 dSm^{-1} and 14 dSm^{-1}. Week 9 was a cumulative exposure of previous concentrations and 18 dSm^{-1}.

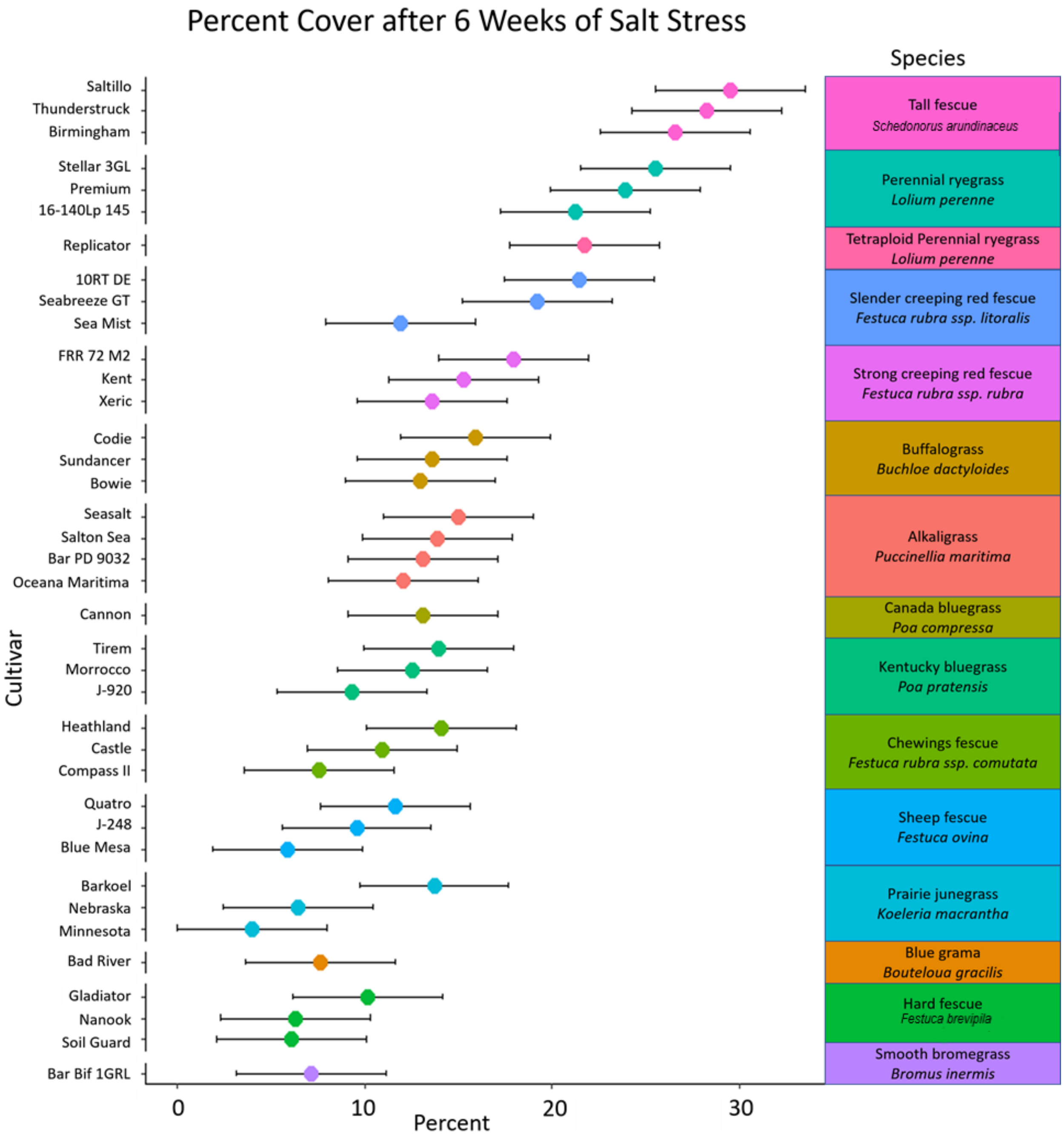

Figure 2.3: Percent cover ranked by species mean after six weeks of salt stress. This time point was chosen, because it had the greatest separation between cultivars. Bars extending left and right from the mean indicate the least significant difference (LSD) between two means, using Fisher's Least Significant Difference (LSD). Colors correspond to species.

Table 2.1: Species in this experiment; includes a total of 38 cultivars and accessions across 15 species.

Common name	Species name	Cultivar or accession names
Tall fescue	*Schedonorus arundinaceus*	Birmingham, Thunderstruck, Saltillo
Perennial ryegrass	*Lolium perenne*	16-140Lp 145, Premium, Stellar 3GL
Tetraploid Perennial ryegrass	*Lolium perenne*	Replicator
Slender creeping red fescue	*Festuca rubra* ssp. *litoralis*	SeaMist, Seabreeze GT, 10RT DE
Strong creeping red fescue	*Festuca rubra* ssp. *rubra*	Xeric, Kent, FRR 72 M2
Buffalograss	*Buchloe dactyloides*	Bowie, Sundancer, Codie
Alkaligrass	*Puccinellia maritima*	Oceana Maritima, Bar PD 9032, Salton Sea, SeaSalt
Canada bluegrass	*Poa compressa*	Cannon
Kentucky bluegrass	*Poa pratensis*	J-920, Morocco, Tirem
Chewings fescue	*Festuca rubra* ssp. *commutata*	Compass II, Castle, Heathland
Sheep fescue	*Festuca ovina*	Blue Mesa, J-248, Quatro
Prairie junegrass	*Koeleria macrantha*	Minnesota, Nebraska, Barkoel
Blue grama	*Bouteloua gracilis*	Bad River
Hard fescue	*Festuca brevipila*	Soil Guard, Nanook, Gladiator
Smooth bromegrass	*Bromus inermis*	BAR BIF 1GRL

Table 2.2: Correlation (R^2) values and significance between three plant stress measurements.

Measurement	R^2	P-value	Significance
Percent Cover – NDVI	0.79	<0.001	*
Percent Cover – Electrolyte Leakage	0.62	<0.001	*
NDVI – Electrolyte Leakage	0.64	<0.001	*

* indicates significance at $p < 0.001$

CHAPTER 3: HEAT STRESS

3.1 INTRODUCTION

Roadside turfgrasses are subjected to very harsh conditions in winter (ice and salt) and abnormally high temperatures during the summer months. Pavement and asphalt are known to produce increased air temperature that can negatively impact growth of the nearby vegetation (Mohajerani et al., 2017). Numerous researchers have studied the effect of high temperatures (heat stress) on cool-season turfgrass species (Cross et al, 2013; Li et al., 2014; Tian et al., 2015; Jespersen et al., 2016). Heat stress is characterized by reduced vegetative growth (percentage of green tissue and lower NDVI), turf quality (tissue browning) and decreased membrane stability (increased electrolyte leakage). However, most of these research studies were performed with a low number of species or cultivars (a maximum of four species) with short periods of heat stress (around 30 days), and very few of them included a recovery period under control conditions. Therefore, our objective was to identify heat stress tolerant turfgrasses that might be used on roadsides in Minnesota and similar northern climates.

3.2 MATERIALS AND METHODS

3.2.1 Plant Material and Experimental Design

Eight replications of 34 turfgrass cultivars or selections (for a total of 272 pots) were started December 8, 2016 using a total sowing density of two pure live seeds (PLS) cm^{-2} in jumbo 4-inch (10.16 cm) pots containing a mixture of sand and topsoil (1:1, v:v). Half of the pots were control plants and half were subjected to heat stress. The turfgrass cultivars and accessions consisted of three akaligrasses, one blue grama, three buffalograsses, one Canada bluegrass, three Chewings fescues, three hard fescues, three sheep fescues, three slender creeping red fescues, three strong creeping red fescues, three Kentucky bluegrasses, three perennial ryegrasses, one smooth brome, three tall fescues, and one tetraploid perennial ryegrass (Table 3.1). The greenhouse conditions consisted of 16 hours of light per day, supplemented with high-pressure sodium lighting when natural light was insufficient and kept at 70% humidity.

During establishment in the greenhouse, pots were watered daily to field capacity. Fertilization consisted of 1.7 oz (50 ml) of half strength Hoagland's solution (pH adjusted to 6.1 with a NaOH solution) containing 7.5 mM of NO_3 given weekly. Plants were manually trimmed as needed to keep a shoot height of 2 inches (5.1 cm). The four alkaligrasses had poor germination under the greenhouse conditions. Therefore, after 21 days, these cultivars were sowed again with the same amount of seeds.

After complete establishment at 12 weeks, pots were transferred into two growth chambers (control and heat treatments) set at a 14-hour photoperiod with a light intensity of 600 µmol s^{-1} m^{-2}, a day/night temperature regimen of 77°F/59°F (25°C/15°C) and a relative humidity of 40%. Each growth chamber was divided in four equal blocks each containing one replicate of each turfgrass entry. All pots were randomized within each block and were acclimated under the above conditions in the growth chamber for three weeks before the beginning of the heat stress treatment (15 weeks after seeding).

On March 28, 2017, the heat stress treatment was started in one growth chamber. The day/night temperature regimen was raised to 95°F/77°F (35°C/25°C) and the humidity was increased to 70% humidity to keep the vapor pressure deficit similar between the two growth chambers. Pots were watered as needed to field capacity to avoid the occurrence of drought stress, and fertilization continued as previously described. After 49 days of heat treatment (May 16, 2017), the chamber conditions were returned to normal for an additional recovery period of 28 days. The first heat stress experiment was ended June 12, 2017. A second experiment was started on June 29, 2017, but the 12 weeks establishment occurred in one growth chamber conditions to avoid the high temperature (>86°F [30°C]) present in the greenhouse during summer. All alkaligrasses were sowed with a double amount of seeds to match the experiment set-up of the first experiment. Plant were watered, fertilized and clipped as previously described. On August 27, 2017, plants showing heavy thrip infestation were treated with granular Marathon pesticide. Because of this pest, the turf establishment lasted longer in the second run of the experiment started November 20, 2017 (20 weeks after seeding). After 49 days of heat stress treatment (January 15, 2018), the chamber conditions returned to normal for the recovery phase (four additional weeks) of the experiment. The second run was terminated February 5, 2018.

3.2.2 Physiological Measurement

3.2.2.1 Digital Image Analysis

Digital images of all individual pots were taken weekly throughout the trial period with a customized light box designed to fit over the pots as described by Friell et al. (2013). Images were analyzed for percent green leaf coverage with ImageJ (v. 1.46r, National Institutes of Health, Bethesda, MD 20892; Schneider et al. 2012) using color threshold settings of hue = 47 to 255, saturation = 87 to 255, and brightness = 27 to 255 in a modified macro (Soldat et al., 2012).

3.2.2.2 Electrolyte Leakage (EL)

The membrane stability of each cultivar was estimated at 0 day (d), 49 d of heat stress and 28 d of recovery with the leaf cell electrolyte leakage (EL) modified protocol from Blum and Ebercon (1981). For all pots, around 0.007 oz. (0.2 g) of random leaf tissue was sampled before heat stress, at 49 days of heat treatment, and at 28 day of recovery. The samples were placed into 1.7 oz. (50 ml) autoclavable tubes filled with 0.7 oz. (20 ml) of DI water and shaken for 15 hours to 20 hours at room temperature. Initial conductivity (Ci) with a conductivity meter MC226 from Mettler Toledo was measured and the tubes were autoclaved at 250°F (121°C) for 20 minutes then shaken for an additional 15 hours to 20 hours at room temperature. The final conductivity (Cmax) was measured and the percentage of electrolyte leakage was calculated with this equation:

$$\mathrm{EL}\ (\%) = \mathrm{Ci}/\mathrm{Cmax}*100\ (1) \qquad (1)$$

3.2.2.3 Normalized Difference Vegetation Index (NVDI)

Plant performance was also evaluated weekly with the FieldScout TCM 500 Normalized Difference Vegetation Index (NDVI) TurfColor Meter from Spectrum Technologies (Illinois, USA). For each pot and each time point, the NVDI meter was placed on top of the pot and the NDVI measure was recorded.

3.2.3 Statistical Analysis

Nonlinear regression analysis was performed between the NDVI from the heat and recovery experiment run #1 and percentage of green leaf coverage using the XLSTAT (Addinsoft SARL) software. For each cultivar, the NDVI data from the 12 time points and the electrolyte leakage for the three time points were analyzed with the fit least square regression analysis and mean comparisons were obtained by Student’s t-test HSD. For the 0, 49 and 77 days after experiment starts, cultivars and accession mean separation were performed using pairwise multiple Student’s t-test. All statistical analysis were done using JMP (JMP® PRO 13. SAS Institute Inc., Cary, NC, 1989-2007).

3.3 RESULTS AND DISCUSSION

3.3.1 Correlation between the Percentage of Green and Turf Performance

A strong and significant correlation was obtained (R^2=0.68, p<0.001) between the turf performance measured by NDVI and the percentage of green (Figure 3.1) for run #1. NDVI has been extensively used in agricultural systems and forestry to evaluate vegetation density. A higher vegetation (leaves) density coupled with higher chlorophyll content will lead to a higher NDVI index (Tucker, 1979). Hence, it is not surprising to visualize this strong interaction between the NDVI measured and the percentage of green obtained by digital image analysis. Therefore, to avoid any redundancy in our data analysis, only the NDVI analysis will be presented.

3.3.2 Turfgrass Establishment

At day zero (0 day) of the first experiment (run #1), ‘10RT-DE’ (slender creeping red fescue) presented the highest NDVI data of the accessions. Twenty-four cultivars and accessions did not show any statistical difference for their NDVI. However ‘Quatro’ (sheep fescue), ‘BAR BIF 1GRL’ (smooth brome) , the three akaligrass cultivars tested (‘SeaSalt’, ‘Salton Sea’ and ‘Oceania Maritima’) and all warm-season cultivars (‘Bad River’, ‘Codie’, ‘Bowie’, ‘Bison’) presented a significant lower NDVI than ‘10RT-DE’. Despite the additional five weeks recovery due to thrip infestation and insecticide treatment, the average turf NDVI was lower in the second run (0.492) when compared to the first run (0.699). At the beginning of the second run, ‘Castle’ was the highest performing cultivar. The NDVI of all buffalograsses and ‘Bad River’ were significantly different from ‘Castle’. ‘Morocco’, ‘SeaSalt’, ‘Gladiator’, ‘16-14-Lp 145’, ‘J-920’, ‘BAR BIF 1GRL’, ‘Soil Guard’ and ‘Blue Mesa’ were also significantly different.

3.3.3 Turf Performance Response of Known Heat-Tolerant Cultivars

In our experimental set-up, four warm-season turfgrass cultivars (‘Bowie’, ‘Codie’, ‘Bison’ and ‘Bad River’) were tested. During the first run (run #1), only ‘Codie’, a buffalograss, displayed a significant higher NDVI during the heat treatment compared to the control, but this difference disappeared during the recovery period (>49 days). ‘Bowie’, ‘Bison’ and ‘Bad River’ did not show any differences between the heat stress and control treatments (Figure 3.2); ‘Bowie’ displayed a significant reduced NDVI in the recovery period of run #1. However, during the second run (run #2), ‘Bowie’, ‘Codie’, ‘Bison’ and ‘Bad River’ displayed significant higher NDVI for several time points of the heat period of the experiment; this significance disappeared during the recovery period of run #2 (Figure 3.3). Only ‘Bison’ kept a higher significant NDVI until the last time point. Altogether, these observations obtained from heat-tolerant cultivars confirmed that the heat period experimental set-up was appropriate, as only the heat-tolerant turfgrass cultivars were responding favorably to these higher temperatures.

3.3.4 Experiment 1 Turf Performance

Every week, the Normalized Difference Vegetation Index (NDVI) was estimated for each cultivar. NDVI data were compared between the first time point (before the starting date of the heat period) and at the end of the heat period (49 days). During run #1 cultivars and accessions were considered to be affected by heat if their NDVI was significantly lower 49 days from the beginning of the heat period (0 day).

3.3.4.1 Cultivars and Accessions Unaffected by Heat

During run #1 (Figure 3.2), only three cool-season turfgrass cultivars and accessions did not seem to be affected by the heat stress conditions. ‘J-248’ (sheep fescue), ‘SeaSalt’ (alkaligrass), and ‘Tirem’ (Kentucky bluegrass) did not present any differences of NDVI when compared to the first time point (0 day).

3.3.4.2 Cultivars and Accessions Affected by Heat and Unable to Recover – NDVI Results

‘Nanook’ hard fescue and ‘Quatro’ sheep fescue were extremely affected during the heat period, based on NDVI, of run #1 and were unable to recover (Figure 3.2). Similar observations were obtained for ‘Seabreeze GT’ slender creeping red fescue, ‘Morocco’ Kentucky bluegrass, and ‘Talon’ Canada bluegrass. The NDVI was also monitored in the control treatment (normal conditions for 77 days) and showed that these cultivars’ NDVI were also negatively and significantly affected. For these cultivars, their negative response to heat stress could also been amplified by the growth medium used (topsoil mixture) or light conditions (600 µmol s^{-1} m^{-2}). ‘Gladiator’ hard fescue was affected by heat and was unable to recover but the control pots did not show reduced NDVI.

3.3.4.3 Turf Performance Comparison of the Cultivars and Accessions at 49 Days

At the end of the heat stress period after 49 days (Figure 3.3), ‘Tirem’ Kentucky bluegrass displayed the highest NDVI. ‘Stellar 3GL’ and ‘Premium’ perennial ryegrasses, ‘Replicator’ tetraploid perennial ryegrass, ‘J-248’, ‘Blue Mesa’ and ‘Quatro’ sheep fescues, ‘Kent’ strong creeping red fescue, ‘SeaSalt’ and ‘Salton Sea’ akaligrasses, ‘Saltillo’ tall fescue, ‘BAR BIF 1GRL’ smooth brome, ‘Castle’ and ‘Compass II’ Chewings fescues, ‘Seabreeze GT’ slender creeping red fescue, ‘Soil Guard’, ‘Nanook’ and ‘Gladiator’ hard fescues had NDVI levels significantly different from ‘Tirem’. All entries, except ‘J-248’ and ‘SeaSalt’, also displayed a significant lower NDVI during the heat stress period (when compared to day 0). The remaining cultivars and accessions on Table 3.4, despite presenting a reduction of NDVI during the heat stress period, are not significantly different at 49 days from the highest cultivar ‘Tirem’.

3.3.5 Experiment 1 Membrane Stability

Sixteen cultivars and accessions (>50%) showed a significant increase of electrolyte leakage after 49 days of heat stress (Table 3.2). This increase in electrolyte leakage arose from a decrease permeability of the cell plasma membrane during the heat period. Most of the cultivars and accessions were able to recover except for ‘Heathland’, ‘Blue Mesa’, ‘Stellar 3GL’, ‘Birmingham’, ‘16-14-Lp 145’, ‘Nanook’ and ‘Quatro’. For these cultivars and accessions, the physiological effect of the heat stress seemed to last longer.

3.3.6 Experiment 2

3.3.6.1 Turf Performance Results

Despite the additional recovery period because of thrip infestation, the NDVI of the plants were lower at the beginning of the experiment (compare Figure 3.2 with Figure 3.3). Also, the infestation provoked a high variation of percentage of green (and NDVI) among the eight replicates for each cultivar and accession. As a result the heat stress response magnitude was smaller during the second run of the experiment and the high variability between the replicates did not allow strong significant differences. Only ‘Premium’ perennial ryegrass, ‘Nanook’ hard fescue and ‘J-248’ sheep fescue displayed a significant NDVI decrease in the second run of the experiment (Figure 3.3). Interestingly, all three cultivars were able to recover during the recovery period. ‘Nanook’ and ‘Premium’ displayed a low NDVI at the end of the experiment. During the second run, ‘J-248’ was significantly affected during the heat stress period (NVDI decreased) and was not able to recover during the recovery period. Most of the cultivars and accessions that were negatively affected (reduced NDVI) during the run #1 showed a similar, albeit not significant, tendency during run #2.

3.3.6.2 Turf Performance Comparison of the Cultivars and Accessions at 49 Days

At the end of the heat stress period after 49 days, ‘Bad River’ had the highest NDVI. ‘Tirem’ was the highest cool-season turfgrass cultivar. ‘Compass II’, ‘16-14-Lp 145’, ‘Saltillo’, ‘Heathland’, ‘Stellar 3 GL’, ‘Salton’, ‘Sea Castle’, ‘SeaSalt’, ‘Premium’, ‘Replicator’, ‘Oceania Maritima’, ‘Gladiator’, ‘Blue Mesa’, ‘Quatro’, ‘J-248’, ‘Soil Guard’ and ‘Nanook’ possessed NDVI levels significantly lower than ‘Bad River’ (Table 3.5).

3.3.6.3 Membrane Stability Results

A similar pattern can be seen of the electrolyte leakage (Table 3.3). The membrane stability of the cultivars was affected because of the infestation. All accessions and cultivars showed a higher electrolyte leakage at the beginning of run #2. Also the variability of the electrolyte leakage increased between the replicates for each cultivar and accessions. Nine turfgrass cultivars displayed a significant increase of electrolyte leakage, but most of them were able to recover except for ‘Soil Guard’ (Table 3.3).

3.4 CONCLUSION

Identifying top-performing turfgrasses for heat stress tolerance depends on a minimum of five variables: (1) turf establishment (NDVI cultivar comparison at 0 day), (2) the reduction of turf performance (NDVI) during the heat period, (3) the comparison of the turf coverage to a top ranked cultivar, (4) ability of the turfgrass to stabilize cellular membranes during heat stress, and (5) capacity of the turfgrass to recover from the heat period (NDVI and EL). Table 3.6 summarizes the observations obtained for all cultivars and accessions during for experimental heat stress and recovery period.

Our results demonstrated that several cultivars and accessions responded better to heat stress conditions than others. We also observed that a large heat stress response variability exists within species, leading to a potential species improvement by breeding strategy. The warm-season turfgrasses (buffalograss and blue grama) were tolerant during the heat stress period but had difficulties in establishing during both of the experimental set-ups. The akaligrasses also presented difficulty in germination and establishment. All the alkaligrass cultivars were affected by heat for both runs. In the second run their NDVI was statistically different from the top-ranked cultivars. However, they presented a stronger membrane stability.

The fine fescues group presented a wide-ranging response during heat stress; most of them were heat sensitive (decrease of NDVI) and showed cellular membrane instability (increased EL) after a long period of heat stress. Nevertheless, most of them were able to show recovery after 28 days of normal temperatures. Chewings fescue, hard fescue and sheep fescue were affected by heat stress and the turf performance was lower than for the highest top-ranked cultivars; however, most of the Chewings fescue cultivars recovered nicely during the recovery period. Hard fescue and sheep fescue had difficulty recovering and their membrane stability still presented some sign of stress after 28 days of control conditions. For slender creeping red fescue and strong creeping red fescue cultivars and selections, the turf coverage (NDVI) after 49 days of heat was mostly similar to top-ranked cultivars for both experiments. Additionally, these species showed impressive recovery with their NDVI and membrane stability.

Kentucky bluegrass and perennial ryegrass were both affected by heat stress (except for ‘Tirem’ in run #1). However, Kentucky bluegrass cultivars displayed a higher turf performance 49 days and stronger membrane stability when compared to the perennial ryegrass cultivars used in these experiments. The performance of ‘Tirem’ suggests it might be a good option when Kentucky bluegrass is desired in a roadside mixture.

Interestingly, in this experiment the tall fescue cultivars were affected after 49 days of heat treatment. ‘Saltillo’ was always significant lower (NDVI) when compared to the top-ranked cultivars at the end of the heat stress period. All cultivars showed a strong membrane stability during the heat stress period and a nice recovery ability.

The top species to use for long periods of heat stress condition on roadsides should be based on their establishment and recovery ability as all species were affected by heat. Based on our two experimental runs and recovery data, we propose that Canada bluegrass, tall fescue, Kentucky bluegrass, strong creeping red fescue and slender creeping red fescue cultivars and accessions be considered when heat stress is common.

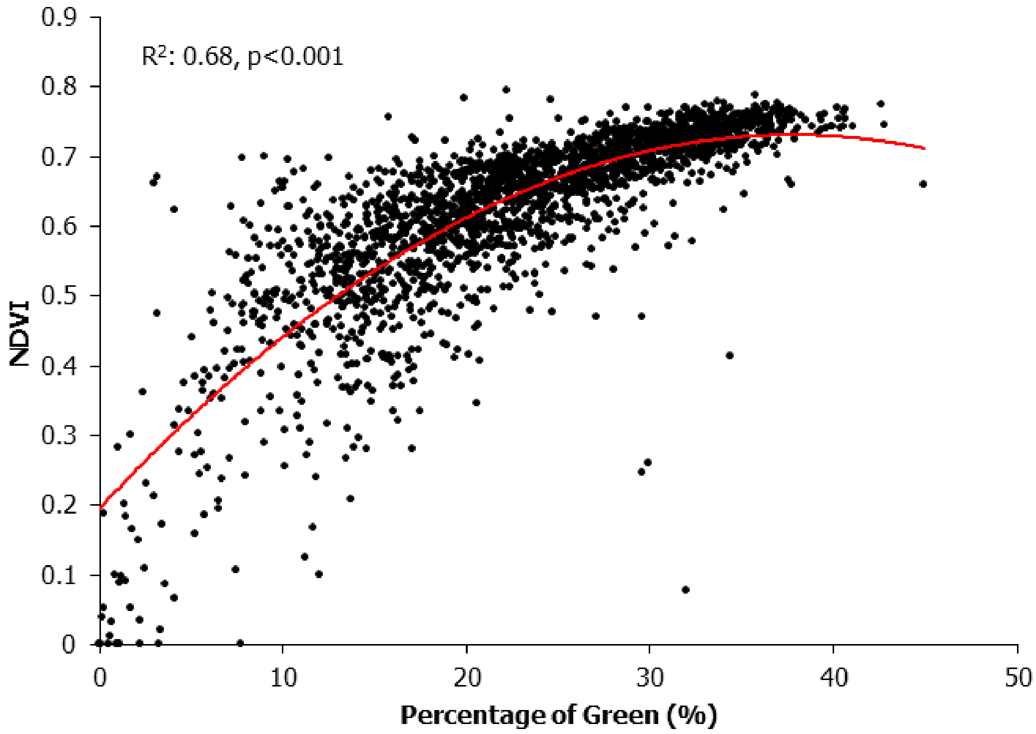

Figure 3.1: Polynomial regression model for the percentage of green and the turf performance. The model was significant at $p<0.001$.

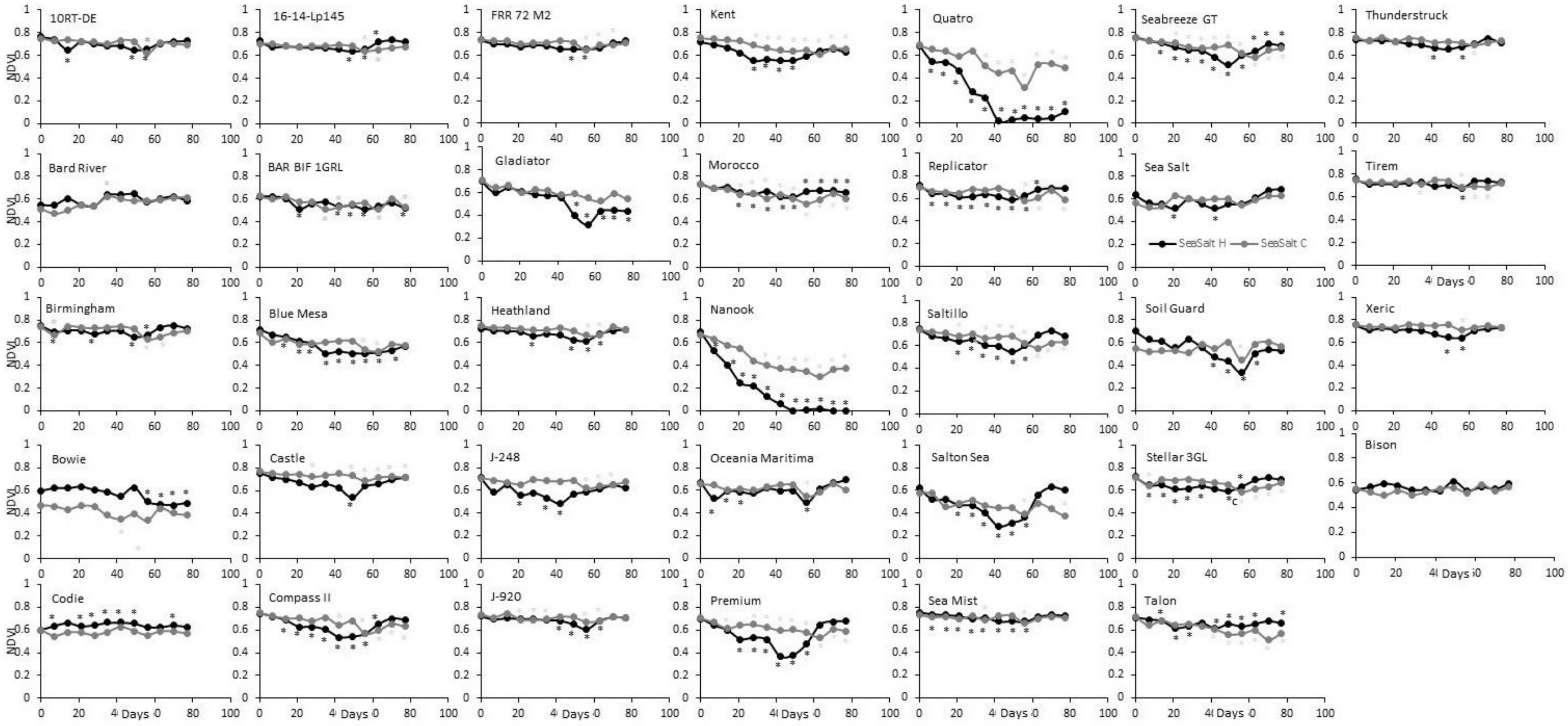

Figure 3.2: Average NDVI measured for each time point for the 34 cultivars and selections tested during the first run of the experiment. Black lines represent the NDVI measured during the heat stress experiment while gray lines measured the NDVI of the controls. A black star shows a statistical difference with the first time point of the heat stress data. A grey star shows a statistical difference with the first time point of the control data. Statistical analysis was performed using a fit least square regression analysis and mean comparisons were obtained by Tukey's honest significant difference (HSD).

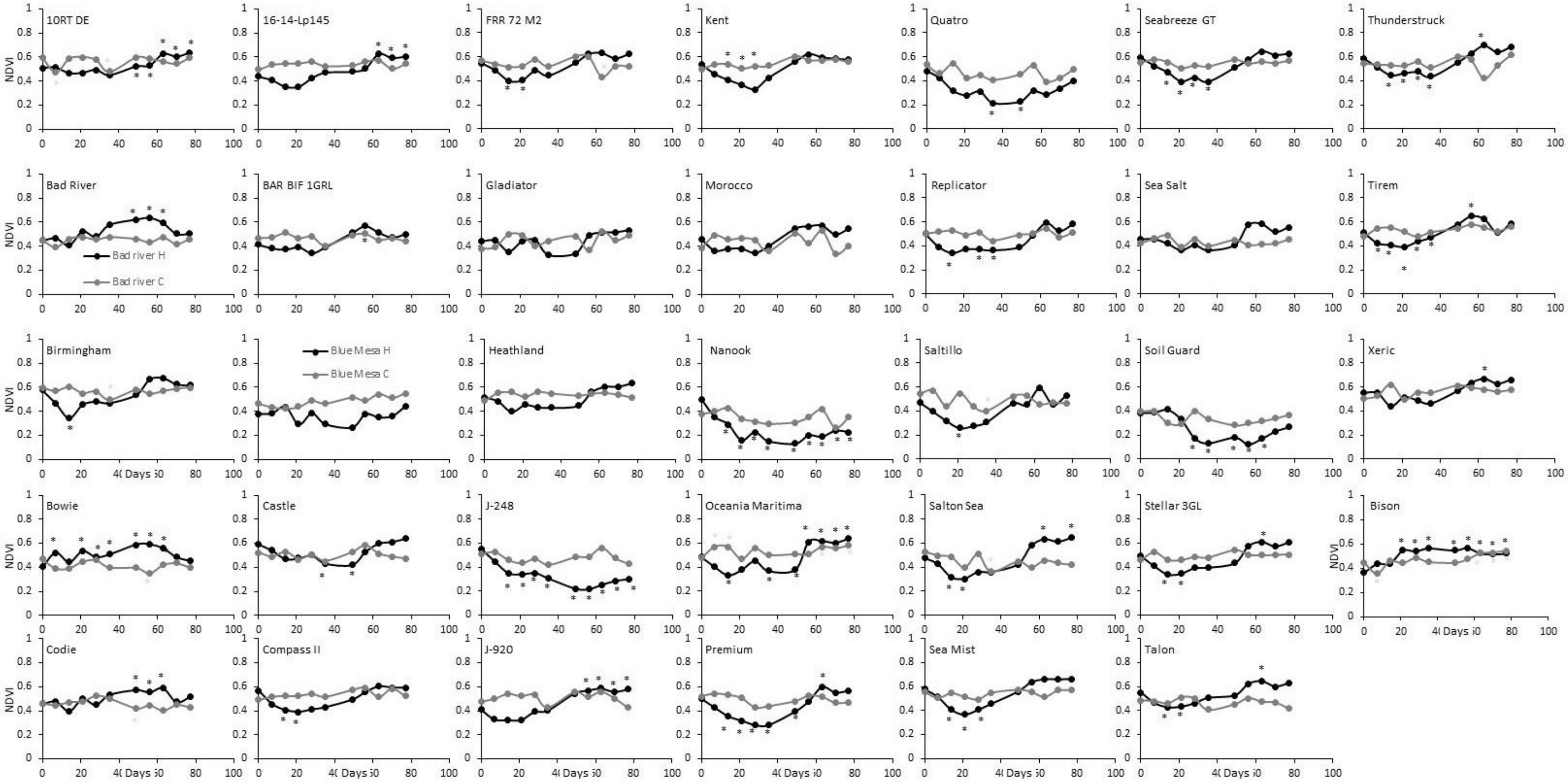

Figure 3.3: Average NDVI measured for each time point for the 34 cultivars and selections tested during the second run of the experiment. Black lines represent the NDVI measured during the heat stress experiment while the gray lines measured the NDVI of the control. A black star shows a statistical difference with the first time point of the heat stress data. A gray start shows a statistical difference with the first time point of the control data.

Table 3.1: Species in this experiment that includes a total of 38 cultivars and accessions across 15 species.

Scientific name	Common name	Cultivar
Bouteloua gracilis	Blue grama	Bad River
Buchloe dactyloides	Buffalograss	Bison
		Bowie
		Codie
Pucinella maritima	Akaligrass	Oceania Maritima
Pucinella distans	Akaligrass	Salton Sea
		SeaSalt
Poa compressa	Canada bluegrass	Talon
Festuca rubra ssp. *fallax*	Chewings fescue	Castle
		Compass II
		Heathland
Festuca brevipila	Hard fescue	Gladiator
		Nanook
		Soil Guard
Festuca ovina	Sheep fescue	Blue Mesa
		J-248
		Quatro
Festuca rubra ssp. *litoralis*	Slender creeping red fescue	10RT DE
		SeaMist
		Seabreeze GT
Festuca rubra ssp. *litoralis*	Strong creeping red fescue	FRR 72 M2
		Kent
		Xeric
Poa pratensis	Kentucky bluegrass	J-920
		Morocco
		Tirem
Lolium perenne	Perennial ryegrass	16-14-Lp 145
		Stellar 3GL
		Premium
Bromus inermis	Smooth brome	BAR BIF 1GRL
Schedonorus arundinaceus	Tall Fescue	Birmingham
		Saltillo
		Thunderstruck
Lolium perenne ssp. *perenne*	Tetraploid perennial ryegrass	Replicator

Table 3.2: Average electrolyte leakage and recovery pattern for each cultivar and accessions at 0 days, 49 days and 77 days for the heat stress pots (Exp1). Different letters indicates significant differences between the time points.

Cultivar/accession	EL (%) at 0 d	EL (%) at 49 d	EL (%) at 77 d	Recovery
Xeric	4.83	19.94	13.82	1
10RT DE	5.42 a	27.38 b	19.36 ab	4
SeaMist	5.67 a	19.66 b	10.30 a	3
Seabreeze GT	6.07 a	36.99 b	14.32 a	3
Heathland	6.21 a	22.30 b	14.05 c	2
Compass II	6.55 a	33.88 b	4.23 a	3
Kent	6.58 a	29.18 b	12.49 ab	4
FRR 72 M2	6.76 a	21.79 b	10.90 a	3
J-920	6.86 a	21.05 b	10.71 a	3
Blue Mesa	7.03 a	43.27 b	43.61 b	2
J-248	7.85 a	21.32 a	13.45a	1
Tirem	9.10	18.04	8.51	1
Saltillo	9.29	13.73	10.22	1
Stellar 3GL	9.50 a	26.57 b	21.06 b	2
Replicator	9.53 a	28.36 b	13.97 a	3
Morocco	10.12	10.45	11.78	1
Birmingham	10.20 a	23.51 b	19.95 b	2
Talon	10.24	15.15	14.28	1
Soil Guard	10.44	39.19	33.23	1
Castle	10.48	38.62	23.54	1
16-14-Lp 145	10.64 a	26.19 b	27.32 b	2
Premium	10.84 a	42.61 b	11.40 a	3
SeaSalt	10.96	18.84	13.53	1
Thunderstruck	12.23	22.19	25.06	1
BAR BIF 1GRL	12.52	16.18	15.90	1
Oceania Maritima	12.54 ab	20.05 b	7.21 a	1
Salton Sea	14.01 a	12.15 a	2.79 b	1
Nanook	14.92 a	87.83 b	-	-
Codie	17.95 a	14.43 a	36.38 b	2
Bad River	18.95 a	20.63 a	41.76 b	2
Bison	19.84 a	8.89 a	39.56 b	2
Gladiator	20.18	47.28	44.64	1
Quatro	21.31 a	74.30 b	-	-
Bowie	28.72	7.26 b	37.00 a	2

1 = indicates that the cultivar or accession EL did not seem to be affected by the heat stress treatment.

2 = indicates that the EL was affected by heat and did not recover at the end of the recovery period.

3 = indicates that EL was affected by heat and recovered at the end of the recovery period.

4 = indicates that EL was affected by heat and almost recovered (non-significantly different from 0 d and 49 d) at the end of the recovery period.

Table 3.3: Average electrolyte leakage and recovery pattern for each cultivar and accessions at 0 days, 49 days and 77 days for the heat stress pots (Exp2). Different letters indicates significant difference between the time points.

Cultivar/accession	EL at 0 d	EL at 49 d	EL at 77 d	Recovery
Talon	4.99	6.93	8.34	1
Xeric	9.27 a	18.54 b	7.25 ab	3
Saltillo	9.59	9.32	9.28	1
Morocco	9.69 a	2.43 b	8.91b	5
Tirem	10.03	6.38	19.90	1
Oceania Maritima	10.17	11.58	7.99	1
J-920	10.23	7.73	8.14	1
Heathland	10.64 a	37.80 b	10.39 a	3
Salton Sea	10.87	7.42	4.59	1
SeaSalt	12.22 a	22.86 b	10.22 a	3
Bowie	12.29 a	6.75 b	18.31a	1
10RT DE	12.39	16.16	11.19	1
Birmingham	12.51	18.00	17.11	1
Nanook	12.56 a	44.79 b	30.69 ab	4
Seabreeze GT	13.06	17.07	9.57	1
Codie	13.51	38.26	35.13	1
Kent	13.81 a	4.14 b	5.37 b	1
Blue Mesa	14.28	68.17	33.76	2
Soil Guard	14.63 a	55.52 b	57.71 b	2
Bison	15.11 a	11.56 a	35.77 b	4
Compass II	15.16	22.58	16.25	1
FRR 72 M2	15.24	16.89	11.29	1
Replicator	15.31	24.31	15.68	1
Thunderstruck	16.00	13.77	11.80	1
J-248	16.50 a	55.75 b	40.33 ab	4
Gladiator	18.45	34.45	17.54	1
Bad River	18.92 a	11.54 a	42.12 b	4
Quatro	19.60	38.81	23.64	1
Castle	20.29 ab	40.84 b	14.14 a	3
Stellar 3GL	20.96 a	31.89 b	11.19 ab	3
Premium	21.87 a	17.52 a	7.43 b	5
SeaMist	23.13	17.90	12.47	1
16-14-Lp 145	25.65	23.69	16.48	1
BAR BIF 1GRL	26.12 a	3.26 b	7.8 4 b	5

1 = indicates that the cultivar or accession EL did not seem to be affected by the heat stress treatment.
2 = indicates that the EL was affected by heat and did not recover at the end of the recovery period.
3 = indicates that EL was affected by heat and recovered at the end of the recovery period.
4 = indicates that EL was affected by heat and almost recovered (non-significantly different from 0 d and 49 d) at the end of the recovery period.
5 = indicates that the cultivar or accession recovered from heat treatment.

Table 3.4: Average NDVI for each cultivar and accessions at 0 days, 49 days and 77 days for the heat stress experiment #1. An asterisk indicates a significant difference compared to the top performing cultivars or selection. Pairwise mean separation for each time point was performed with a Student t-test using JMP software.

Cultivar/accession	0 day	49 days	77 days
Xeric	0.757	0.644	0.735
Tirem	0.758	0.707	0.728
10RT DE	0.762	0.643	0.727
FRR 72 M2	0.736	0.655	0.725
Birmingham	0.746	0.65	0.724
SeaMist	0.752	0.679	0.724
16-14-Lp 145	0.726	0.638	0.72
Castle	0.755	0.543 *	0.714
Heathland	0.726	0.626	0.714
J-920	0.721	0.564 *	0.707
Thunderstruck	0.735	0.652	0.707
Oceania Maritima	0.669 *	0.599	0.694
Stellar 3GL	0.723	0.593 *	0.694
Compass	0.747	0.540 *	0.69
Replicator	0.72	0.584 *	0.687
SeaSalt	0.635 *	0.552 *	0.687
Saltillo	0.747	0.545 *	0.685
Seabreeze GT	0.76	0.519 *	0.685
Premium	0.7	0.379 *	0.677
Morocco	0.732	0.62	0.659
Talon	0.707	0.65	0.659
Codie	0.600 *	0.66	0.628 *
Kent	0.716	0.556 *	0.624 *
J-248	0.714	0.649	0.621 *
Salton Sea	0.623 *	0.315 *	0.606 *
Bison	0.546 *	0.613	0.596 *
Bad River	0.546 *	0.646	0.586 *
Blue Mesa	0.716	0.503 *	0.569 *
Soil Guard	0.702	0.435 *	0.532 *
BAR BIF 1GRL	0.623 *	0.545 *	0.518 *
Bowie	0.597 *	0.627	0.490 *
Gladiator	0.701	0.401 *	0.437 *
Nanook	0.695	0.003 *	-
Quatro	0.686 *	0.035 *	-

Table 3.5: Average NDVI for each cultivar and accessions at 0 days, 49 days and 77 days for the heat stress experiment #2. An asterisk indicates a significant difference between the highest cultivar or accession.

Cultivar/accession	0 day	49 days	77 days
Thunderstruck	0.587	0.556	0.685
SeaMist	0.578	0.558	0.661
Xeric	0.552	0.567	0.661
Salton Sea	0.477	0.4235 *	0.646
10RT DE	0.510	0.521	0.637
Castle	0.591	0.422 *	0.637
Oceania Maritima	0.485	0.380 *	0.636
Heathland	0.517	0.448 *	0.632
Talon	0.551	0.527	0.626
Seabreeze GT	0.591	0.509	0.625
FRR 72 M2	0.547	0.550	0.622
Birmingham	0.576	0.537	0.616
Stellar 3GL	0.493	0.434 *	0.606
16-14-Lp 145	0.438 *	0.479 *	0.604
Compass II	0.569	0.493 *	0.592
Tirem	0.509	0.581	0.590
Replicator	0.501	0.391 *	0.586
J-920	0.414 *	0.546	0.580
Kent	0.536	0.559	0.574
Premium	0.499	0.393 *	0.565
SeaSalt	0.457 *	0.411 *	0.557 *
Morocco	0.457 *	0.544	0.545 *
Gladiator	0.441 *	0.339 *	0.534 *
Saltillo	0.474	0.465 *	0.533 *
Bison	0.361 *	0.548	0.522 *
Codie	0.458 *	0.576	0.519 *
Bad River	0.448 *	0.622	0.505 *
BAR BIF 1GRL	0.414 *	0.511	0.502 *
Bowie	0.406 *	0.588	0.457 *
Blue Mesa	0.377 *	0.268 *	0.440 *
Quatro	0.484	0.228 *	0.398 *
J-248	0.548	0.221 *	0.301 *
Soil Guard	0.386 *	0.179 *	0.266 *
Nanook	0.499	0.130 *	0.227 *

Table 3.6: Summary of all heat and recovery experiment observations. An “L” means that the cultivars were significantly lower than the highest cultivars for their NDVI. An “NA” means Non-affected by heat treatment. An “I” means a significant increase of electrolyte leakage at the end of the heat stress period. An “NR” means an absence of recovery at the end of the recovery period.

	Turf establishment		Turf response to heat 49 d (NDVI)		Turf response to heat 49 d (EL)		Turf performance 49 d (NDVI)		Turf recovery 77 d (NDVI)		Turf recovery 77 d (EL)	
Cultivar	**run #1**	**run #2**	**run #1**	**run #2**	**run #1**	**run #2**	**run #1**	**run #2**	**run #1**	**run #2**	**run #1**	**run #2**
Bad River	L	L	NA	NA				highest				
Bison	L	L	NA	NA								
Bowie	L	L	NA	NA								
Codie	L	L	NA	NA								
Oceania Maritima	L		affected	affected				L				
Salton Sea	L		affected	affected				L				
SeaSalt	L	L	NA	NA		I	L	L				
Talon			affected	affected					NR			
Castle		Highest	affected	affected		I	L	L				
Compass II			affected	affected	I		L	L				
Heathland			affected	tendency	I	I		L			NR	
Gladiator		L	affected	tendency			L	L	NR			
Nanook			affected	affected	I	I	L	L	NR	NR	NR	
Soil Guard		L	affected	affected		I	L	L				NR
Blue Mesa		L	affected	tendency	I		L	L			NR	
J-248			NA	affected		I	L	L		NR		
Quatro	L		affected	affected	I		L	L	NR		NR	
10RT DE	Highest		affected	affected	I							
SeaMist			affected	affected	I							
Seabreeze GT			affected	affected	I		L		NR			
FRR 72 M2			affected	affected	I							
Kent			affected	tendency	I		L					
Xeric			affected	NA		I						
J-920		L	affected	tendency	I							
Morocco		L	affected	tendency					NR			
Tirem			NA	affected			highest					
16-14-Lp 145		L	affected	tendency	I			L			NR	

Stellar 3GL			affected	affected	I	I	L	L	NR
Premium			affected	affected	I	I	L	L	
BAR BIF 1GRL	L	L	affected	tendency			L		NR
Birmingham			affected	affected	I				
Saltillo			affected	affected			L	L	
Thunderstruck			affected	affected					
Replicator			affected	affected	I		L	L	

CHAPTER 4: ICE COVER

4.1 INTRODUCTION

Established perennial grass systems on roadsides often experience ice cover and encasement in winter due to side-snow plowing, fluctuation of temperature, and chemical spraying resulting to snow melting and ice formation. Ice encasement of turfgrasses results in hypoxia (oxygen deficiency) and anoxia (the absence of oxygen; Andrews, 1996), both leading to potential turfgrass death. Aeration of roots in the soil can occur through pore spaces in unfrozen regions, but this is dependent on prior soil water content and root depth (Cary and Maryland, 1972). During winter, turfgrass soils surface are more saturated, and since most turfgrass roots are located close to the soil surface, there is a higher probability for root hypoxia when these wet soils freeze. Additionally, it was already observed that turfgrass shoot and crown tissues are also susceptible to hypoxia in full ice encapsulation (Andrews and Pomeroy, 1975). Moreover, turfgrasses maintained at low heights of cut may exhibit increased susceptibility due to a limited ability for leaves to protrude through the ice sheet.

It was observed that turfgrass tolerance to ice can vary based on species, cultivars and the duration of exposure (Beard, 1964, 1965; Tompkins et al., 2004; Höglind et al., 2010). Previous controlled environment trials for ice encasement have differed in the thickness of ice used from 0.5 inches (1.25 cm) (Beard,1964, 1965b; Merewitz et al., 2016) to 1.0 inch (2.5 cm) (Tompkins et al., 2004) or ice conditions being simulated with sealed containers (Aamlid et al., 2009; Tronsmo et al., 2013). To address this, we used controlled environmental conditions and testing procedures we developed to ensure complete ice cover over intact plants to evaluate the effect of ice cover on 35 turfgrasses.

4.2 MATERIALS AND METHODS

4.2.1 Plant Establishment and Growth

Four replications of 35 turfgrass cultivars or selections (Table 4.1) were seeded at 13 pure live seeds (PLS) per in^2 into SC10 Super cone-tainers (Stuewe and Sons Inc, Oregon) filled with a 2:1 (v:v) mixture of Sunshine MVP (Sun Gro Horticulture, MA, USA) to Turface MVP (Profile Products LLC, IL, USA). Cotton balls were placed in the bottom of each cone-tainer to prevent media loss. The same mix was used to cover the seeds to achieve a uniform planting depth of 0.17 in (4.4 mm). Plants were grown for 10 weeks under greenhouse conditions with a 16-hour photoperiod supplemented with high pressure sodium lamps when ambient light fell below 209 w/yd^2 (0.836 m^2). Plants were irrigated to maintain quality and fertilized weekly with a nutrient solution containing 200 ppm nitrogen, 22 ppm phosphorus, 83 ppm potassium and other micronutrients. Plants were maintained at a height of 2 in (5.1 cm) through weekly hand trimming with a scissors. The replicate of the trial was established two days after the first to allow for logistical challenges.

4.2.2 Acclimation

Cold acclimation started 10 weeks after seeding. Plants were transferred to a walk-in cooler fitted with high-output fluorescent lamps and held there for 14 days with an 8-hour photoperiod at 35.6°F (2°C). During acclimation plants were watered when necessary to maintain quality. On day 13 plants were clipped to 0.39 in (1 cm), watered to saturation and aluminum tubing (1.5 in x 2 in x 0.125 in) (3.81 cm x 5.08 cm x 3 mm) was pressed firmly to the surface of the growing media.

4.2.3 Ice Formation

After 14 days of acclimation, plants were placed in a 28.4°F (-2°C) growth chamber for 24 hours to allow for the media to freeze. Once the media was frozen, 1 in (2.5 cm) cubes of ice were added to each aluminum tube and ice water was gradually added to create a uniform 2 in (5.08 cm) depth of ice. Plants were kept at 28.4°F (-2°C) without illumination and ice water was added twice weekly to each sample to maintain a uniform ice depth. Four replications of each cultivar were removed after 4, 8, 12 and 16 weeks of ice cover and placed in a 35.6°F (2°C) growth chamber for 48 hours to thaw after which time the plants were moved back to the greenhouse for recovery. Digital images of each cone-tainer were taken at 31 days after placement in greenhouse.

4.2.4 Desiccation

Another trial and its replicate were planted following the above parameters for growth and acclimation to sample plants after being frozen but before ice encasement to verify that 28.4°F (-2°C) is not lethal to the species being tested. This second trial used the first sampling duration as the time point 24 hours after being placed in the 28.4°F (-2°C) growth chamber, referred to as time 0, and the remaining three sampling durations at 4, 8 and 12 weeks to examine the effect that being frozen without ice cover has on plant survival. Parameters after treatment durations for recovery and digital image analysis were the same as the ice cover trial.

4.2.5 Estimation of Plant-Related Pixels

Images were taken with a Nikon D300 equipped with a Nikon DX 35mm 1.8 lens. The camera was operated on manual settings (F/5.6, 1/250 sec, ISO-200, WB 5000K) in a light box equipped with four 13 watt 5000K compact fluorescent bulbs and interior dimensions of 8 in x 8 in x 12 in (20.3 cm x 20.3 cm x 30.5 cm).

Training data were developed from a subset of images that contained at least one plant from each of the species used in the study. Each image was loaded into Fiji; plant-related and non-plant-related pixels were extracted by the user, which were then exported as numeric RGB values (Schindelin et al., 2012). Training data were used to construct a random forest model using R package *randomForest* (Liaw and Wiener, 2002). The random forest model used red, green, and blue values as explanatory variables that were regressed against a binomial response of plant-related (1) and non-plant-related (0) values. The algorithm was allowed three splits at each node and grew 500 trees within the forest. The model was able to explain over 90% of the variation in the out-of-bag training data.

All images were uploaded into the R environment as high quality TIFF images. Each image was cropped in R to standardize pixel number and reduce processing time. The image arrays were then vectorized and bound to a data frame containing the x and y coordinates of each pixel from the original matrices. The random forest model was then applied to the data frame (one image). Each pixel was then vote counted (n = 500), which generated a probability of it being plant-related. A threshold of 0.8 was set to reduce the number of erroneous plant-related pixels. A for() loop was written to automate this process for all 2,240 images. The processing pipeline was written as to export the masked images that were created by reconstructing the images from the x and y coordinates into a grayscale image. These were then QCed to check that the model was functioning properly. The script also exported a data frame containing the proportion of probable plant-related pixels that was then used for data analysis.

4.2.6 Statistical Analysis

All data were analyzed in Program R (Version 3.5.0; R Core Team, 2018). To meet assumptions of analysis of covariance (ANCOVA), all data were arcsine transformed for analysis and back transformed for visualization. A mixed effects model from the *lme4* package was used to conduct the ANCOVA (Bates et al., 2015). Cultivar and trial run were treated as fixed effects, week was treated as a continuous covariate, and block was treated as a random effect. Trial and week were nested within block in the random term. Main effects and their interactions were considered significant at alpha = 0.05. Means were estimated and separated using package *emmeans* (Lenth, 2018). Means comparisons were determined using pairwise t-tests with a Bonferroni correction to improve reliability.

4.3 RESULTS

4.3.1 Ice Duration

The analysis of covariance for the ice encapsulation trials found a significant interaction between the weeks of ice cover and cultivar and a significant interaction between weeks of ice cover and the trial run (Table 4.2). Due to these interactions, weeks of ice cover were analyzed with respect to all levels of cultivar and trial runs. There was no significant interaction between cultivar and trial run so each is described individually without respect to the other. Because there was no interaction between cultivar and trial run, the analysis was done by combining all cultivars for a given trial at the designated week (Figure 4.1). The general trend was a decrease in the proportion of plant-related pixels as the duration increased for the trials. There were no significant differences between runs for weeks 4, 8, and 12, but there was a difference at week 16 (Table 4.3). Although this difference existed, whether it is biologically significant is questionable.

The effect of ice encapsulation on cultivar and duration for proportion of plant-related pixels is shown in Figure 4.2. Runs 1 and 2 were combined at each week because of the lack of significant differences for most weeks. Tables 4.5, 4.6, 4.7 and 4.8 contain the comparison of an individual treatment and the grand mean using a two-sample t-test for the 4-, 8-, 12-, and 16-week ice durations, respectively.

Several ice duration treatments performed poorly (Figure 4.2). ‘Bad River’ blue grama was killed by the ice encapsulation and had proportion of plant-related pixels of zero which was significantly below the grand mean for all ice durations. For the 4-week duration of ice, the buffalograss cultivars ‘Bowie’, ‘Codie’ and ‘Sundancer’ all had a proportion of plant-related pixels significantly lower than the grand mean with a large magnitude of difference. At the 8-week ice duration the three buffalograss cultivars are still significantly lower than the grand mean for proportion of plant-related pixels. At this time point the sheep fescue cultivars ‘Quatro’ and ‘Blue Mesa’, along with the hard fescue cultivars ‘Soil Guard’ and ‘Nanook’, show a large difference from the grand mean, which is significantly lower in the proportion of plant-related pixels. ‘J-248’ and ‘Gladiator’ – the other cultivars of sheep fescue and hard fescue, respectively – were also significantly lower than the mean, but the magnitude of the difference was not as great at 8 or 12 weeks of ice. The difference seen between cultivars within a species in their magnitude from the grand mean was also evident in the buffalograss cultivars at 12 weeks. At this time ‘Codie’ and ‘Bowie’ had a proportion of plant-related pixels approaching the grand mean, while there is still a large difference from the grand mean for ‘Sundancer’. By the 16-week ice duration, the difference in magnitude in the proportion of plant-related pixels between cultivars within a species was no longer as large.

A number of treatments performed well compared to the grand mean when examining Figure 4.2. The tall fescue cultivars ‘Birmingham’, ‘Saltillo’ and ‘Thunderstruck’ had significantly higher proportions of plant-related pixels than the grand mean with the magnitude of difference being larger than for most other treatments at 4, 8, 12 and 16 weeks of ice cover. The Chewings fescue ‘Heathland’ also had a significantly higher proportion of plant-related pixels than the grand mean for all ice cover durations and a magnitude difference that made it stand out. The two other Chewings fescue cultivars, ‘Compass’ and ‘Castle’ were only significantly greater than the grand mean at the 4-, 8- and 12-week ice durations for proportion of plant-related pixels, and the magnitude of the difference was not as great as ‘Heathland’. A difference between cultivars within a species was also seen for slender creeping red fescue: ‘SeaMist’ had a larger degree of separation from the grand mean for the proportion of plant related pixels for the 4- and 8-week ice cover treatments compared to ‘10RT DE’ and ‘Seabreeze GT’. ‘SeaMist’ was also significantly different from the grand mean for the 12-week ice cover treatment while ‘10RT DE’ and ‘Seabreeze GT’ were not. This difference in cultivar performance within a species was also seen in the strong creeping red fescues, with ‘FRR 72 M2’ having a greater difference from the grand mean for proportion of plant-related pixels at the 4-, 8- and 12-week ice cover durations than ‘Xeric’ and ‘Kent’. ‘FRR 72 M2’ was also significantly greater than the grand mean at the 16-week ice duration, while ‘Xeric’ and ‘Kent’ were not.

4.3.2 Desiccation

For the desiccation study, there were significant three-way interactions between weeks of ice cover, cultivar and trial run (Table 4.4). Because of this interaction, all combinations of weeks of ice cover by cultivar by trial run were examined. Figure 4.3 shows the effect of desiccation on cultivar and duration for the proportion of plant-related pixels. Tables 4.9, 4.10, 4.11, 4.12, 4.13, 4.14, 4.15, and 4.16 contain the comparison of an individual treatment and the grand mean using a two-sample t-test for run 1 and 2 and desiccation exposure times of 0, 4, 8 and 12 weeks.

The blue grama cultivar 'Bad River' performed below the mean performance of all cultivars at exposure times of 0, 4, 8 and 12 weeks for both run 1 and run 2. Its proportion of plant-related pixels approached 0 by the 4-week duration indicating that plants were completely killed. Alkaligrass also performed poorly in the desiccation treatment; in fact, all tested cultivars had significantly lower proportions of plant-related pixels than the grand mean for all exposure times in both runs. We also saw a poor performance for buffalograss as evidenced by reduced proportions of plant-related pixels compared to the grand mean; however, there were some differences among cultivars at certain time points.

Both 'Tirem' and 'J920' Kentucky bluegrasses performed well outperforming the grand mean in most instances. Conversely, 'Morocco' had proportion of plant-related pixels lower than the grand mean with the difference being significant for run 2 at 4 and 8 weeks. Tall fescue cultivars performed well in the desiccation treatment: the cultivars 'Birmingham', 'Saltillo' and 'Thunderstruck' had significantly higher proportions of plant-related pixels for all durations of desiccation for run 1 and for durations 4 and 8 weeks for run 2. Other species also exhibited proportion of plant-related pixels greater than the mean, but the magnitude and significance varied among the cultivars. In run 1, strong creeping red fescue 'FRR 72 M2' had a greater proportion of plant-related pixels compared to 'Xeric' and 'Kent' at 4, 8 and 12 weeks. Chewings fescue also showed differences among the cultivars: in run 1 'Heathland' had a significantly higher proportion of plant-related pixels than the grand mean for all 4 durations and in run 2 there were significant differences from the grand mean at all 4 durations for 'Compass II'.

4.4 DISCUSSION

There were differences within and among species based on proportion of plant-related pixels after 31 days of recovery. In the ice cover trial, tall fescue was the best-performing species. These results differ from field observations of turf performance after extended ice cover duration in Minnesota where we often see tall fescue perform very poorly. Friell et al. (2015) found that inclusion of tall fescue in a mixture planted on a roadside in Minnesota decreased the performance of the mixture, and the authors speculated that ice cover was the primary reason for this response. Tall fescue may have done well in our trial due to its relatively fast growth rate, which may have resulted in more green tissue than slower-growing species after shoot damage occurred.

We also found contradictions from field trials for the fine fescue species that we examined. In the controlled ice trials, Chewings fescue cultivars had higher proportions of plant-related pixels than did the hard fescue and sheep fescue cultivars. This is in opposition to recent observations of field trials where ice cover damage was severe on Chewings fescue with little damage seen on other fine fescue species.

A handful of other species also had results that brought up further questions. 'Bad River' blue grama was completely killed in the ice cover trial. The follow-up desiccation trial showed that this warm-season grass was killed even when no ice was present. Buffalograss, the other warm-season grass in the trial, also was damaged by ice. Because this species is planted with burrs that can contain multiple caryopses, regrowth after damage was unable to differentiate germination of dormant caryopses from survival of the established grass. Alkaligrass also appeared to have dormant seeds germinate during the recovery period for the ice and desiccation trial.

4.5 CONCLUSION

Selection for survival to extended ice cover is important for roadsides in areas where conditions lead to ice formation. We developed a method for controlled ice cover that allowed for detection of differences within and among species of grasses used on roadsides. Unfortunately, we believe this method was not able to replicate field conditions. Refinement of our controlled ice methods is needed to better relate to and identify grasses that can tolerate ice cover that forms under field conditions and to accurately assess the potential of warm-season grasses used on roadsides.

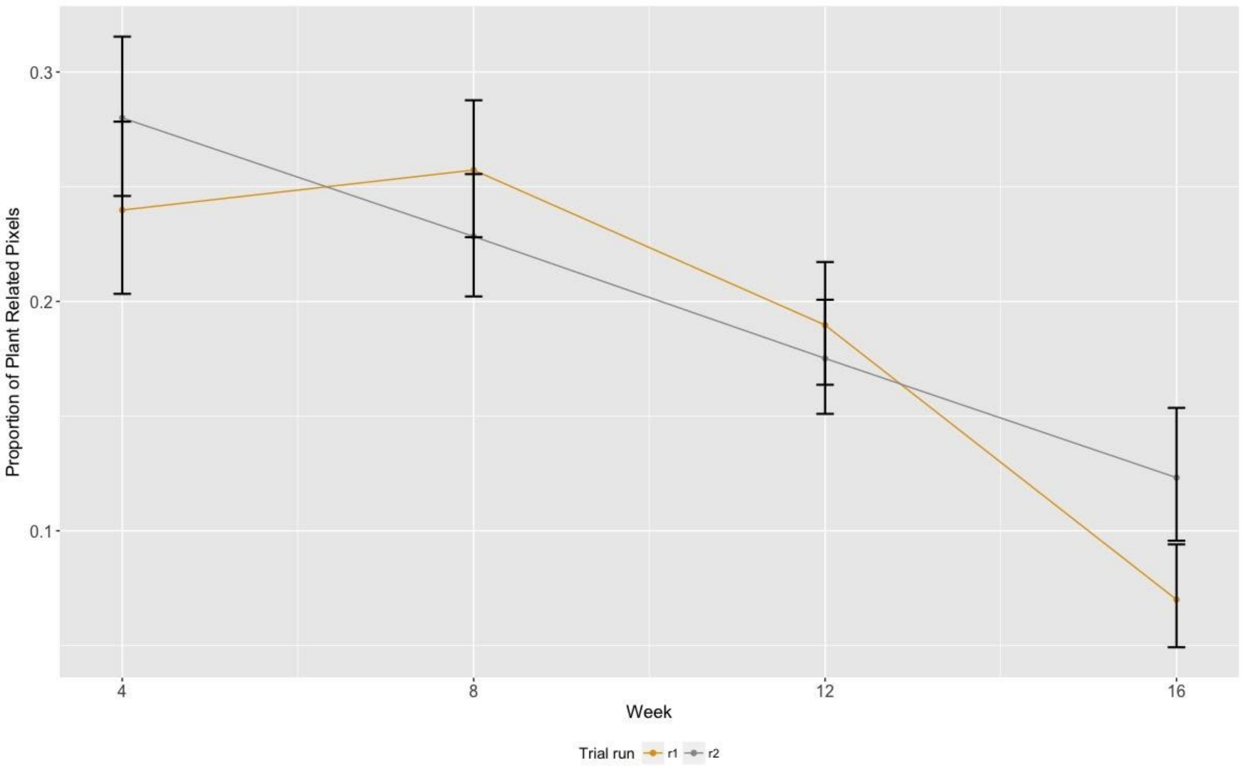

Figure 4.1: Effect of trial run and duration (week) of ice-encapsulated plants on proportion of plant-related pixels calculated from a digital image.

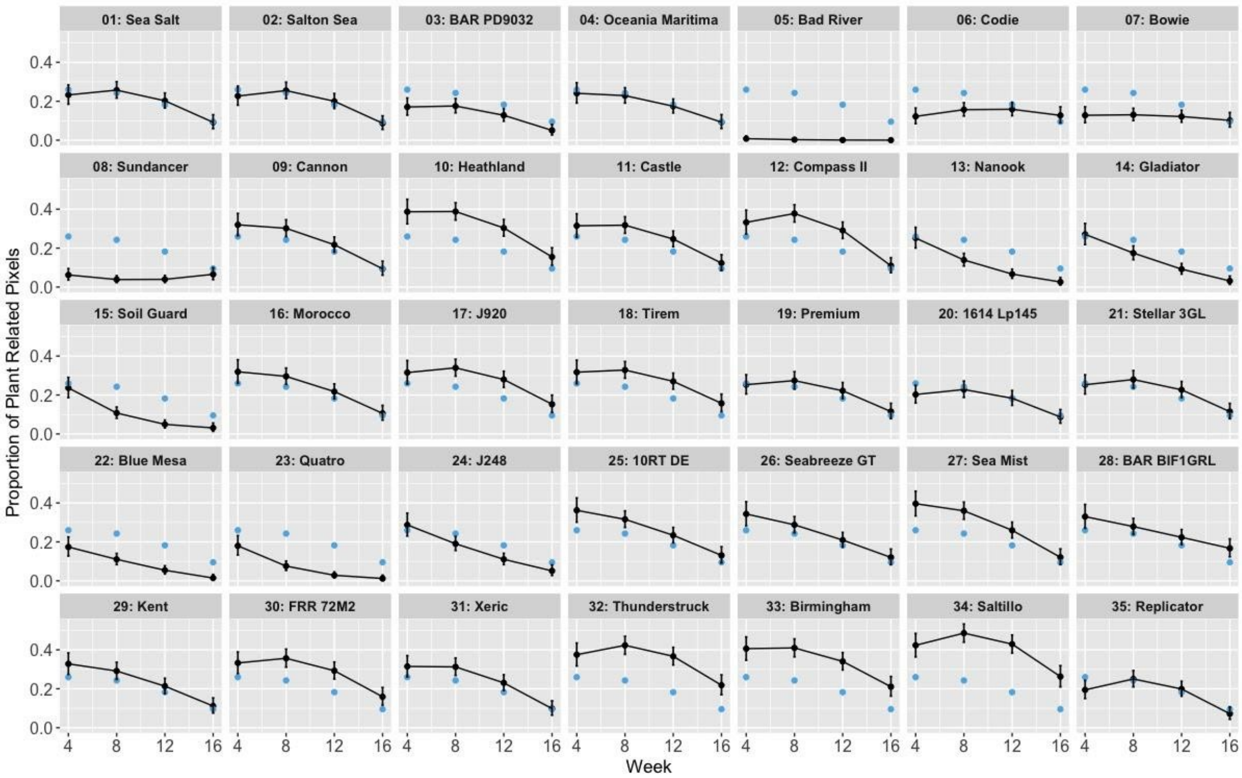

Figure 4.2: Effect of ice encapsulation on cultivar and duration (week) for proportion of plant-related pixels 31 days post treatment. Blue dots represent the 35 cultivar estimated marginal means (EMMeans) at each week. If 95% CI does not intersect the blue dot it means that the cultivar has a significantly different proportion of plant-related pixels than the mean at that time point. The 95% confidence interval gives a visual representation of the significance of its performance compared to the mean.

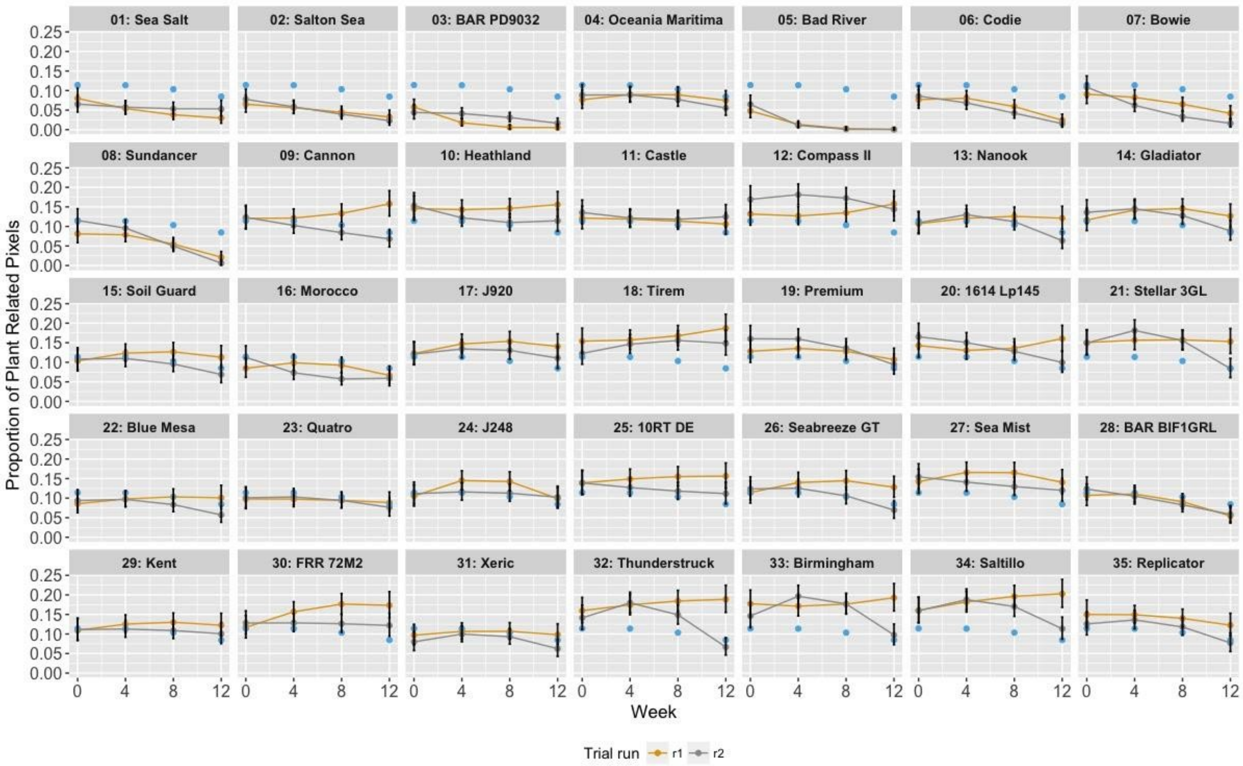

Figure 4.3: Effect of desiccated treatment on cultivar, duration (week), and trial run on the proportion of plant-related pixels 31 days post treatment. Yellow line represents trial run 1 and grey line represents trial run 2. Blue dots represent the all 35 cultivars estimated marginal means (EMMeans) at each week. If 95% CI does not intersect the blue dot it means that the cultivar has a significantly different proportion of plant-related pixels than the mean at that time point. The 95% confidence interval gives a visual representation of the significance of its performance compared to the mean.

Table 4.1: Turfgrass species and cultivars used in a controlled environment ice cover experiment.

No.	Cultivar	Common Name	Abv.	Scientific Name
	SeaSalt	Akaligrass	ALK	*Pucinella distans*
2	Salton Sea	Akaligrass	ALK	*Pucinella distans*
	BAR PD9032	Akaligrass	ALK	*Pucinella distans*
4	Oceania Maritima	Akaligrass	ALKm	*Pucinella maritima*
	Bad River	Blue grama	BLGR	*Bouteloua gracilis*
6	Codie	Buffalograss	BUFF	*Buchloe dactyloides*
	Bowie	Buffalograss	BUFF	*Buchloe dactyloides*
8	Sundancer	Buffalograss	BUFF	*Buchloe dactyloides*
	Cannon	Canada bluegrass	CAND	*Poa compressa*
10	Heathland	Chewings fescue	CHF	*Festuca rubra ssp. fallax*
	Castle	Chewings fescue	CHF	*Festuca rubra ssp. fallax*
12	Compass II	Chewings fescue	CHF	*Festuca rubra ssp. fallax*
	Nanook	Hard fescue	HDF	*Festuca brevipila*
14	Gladiator	Hard fescue	HDF	*Festuca brevipila*
	Soil Guard	Hard fescue	HDF	*Festuca brevipila*
16	Morocco	Kentucky bluegrass	KBG	*Poa pratensis*
	J-920	Kentucky bluegrass	KBG	*Poa pratensis*
18	Tirem	Kentucky bluegrass	KBG	*Poa pratensis*
	Premium	Perennial ryegrass	PR	*Lolium perenne*
20	16-14-Lp 145	Perennial ryegrass	PR	*Lolium perenne*
	Stellar 3GL	Perennial ryegrass	PR	*Lolium perenne*
22	Blue Mesa	Sheep fescue	SHF	*Festuca ovina*
	Quatro	Sheep fescue	SHF	*Festuca ovina*
24	J-248	Sheep fescue	SHF	*Festuca ovina*
	10RT DE	Slender creeping red fescue	SLRF	*Festuca rubra* ssp. *litoralis*
26	Seabreeze GT	Slender creeping red fescue	SLRF	*Festuca rubra* ssp. *litoralis*
	SeaMist	Slender creeping red fescue	SLRF	*Festuca rubra* ssp. *litoralis*
28	BAR BIF 1GRL	Smooth brome	SMBR	*Bromus inermis*
	Kent	Strong creeping red fescue	STRF	*Festuca rubra* ssp. *rubra*
30	FRR 72 M2	Strong creeping red fescue	STRF	*Festuca rubra* ssp. *rubra*
	Xeric	Strong creeping red fescue	STRF	*Festuca rubra* ssp. *rubra*
32	Thunderstruck	Tall Fescue	TF	*Schedonorus arundinaceus*
	Birmingham	Tall Fescue	TF	*Schedonorus arundinaceus*
34	Saltillo	Tall fescue	TF	*Schedonorus arundinaceus*
	Replicator	Tetraploid perennial ryegrass	PRt	*Lolium perenne* ssp. *perenne*

Table 4.2: Analysis of covariance for proportion of plant-related pixel after ice encapsulation.

Source	Sum Sq	Mean Sq	F value	Pr(>F)
Week (W)	0.02	0.02	2.63	NS
Week^2 (W^2)	0.09	0.09	12.96	***
Run (R)	0.05	0.05	7.11	**
Cultivar (Cv)	0.70	0.02	3.31	***
W:R	0.06	0.06	8.49	**
W^2:R	0.07	0.07	9.89	**
W:Cv	0.78	0.02	3.48	***
W^2:Cv	0.74	0.02	3.29	***
R:Cv	0.15	0.00	0.65	NS
W:R:Cv	0.24	0.01	1.07	NS
W^2:R:Cv	0.28	0.01	1.23	NS

*,**,*** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

† NS = Not significant at the 0.05 probability level.

Table 4.3: Pairwise comparisons for ice encapsulation trials runs within time (week) using a t-test (NOTE: differences are on an arcsine scale).

Contrast	Week	Estimate	SE	df	t ratio	p value
r1 - r2	4	-0.0458761	0.0286915	23.90185	-1.5989441	0.1229699
r1 - r2	8	0.0338173	0.022524	13.63157	1.5013856	0.1560611
r1 - r2	12	0.0188297	0.0227908	12.47942	0.8261979	0.4242065
r1 - r2	16	-0.0908388	0.0303837	20.49374	-2.9897211	0.0071106

Table 4.4: Analysis of covariance for proportion of plant-related pixels after desiccation.

Source	Sum Sq	Mean Sq	F value	Pr(>F)
Week (W)	0.00	0.00	0.59	NS
Week^2(W^2)	0.01	0.01	6.88	*
Run (R)	0.00	0.00	0.55	NS
Cultivar (Cv)	0.70	0.02	11.14	***
W:R	0.00	0.00	0.14	NS
W^2:R	0.00	0.00	0.59	NS
W:Cv	0.20	0.01	3.23	***
W^2:Cv	0.14	0.00	2.24	***
R:Cv	0.05	0.00	0.80	NS
W:R:Cv	0.09	0.00	1.49	*
W^2:R:Cv	0.11	0.00	1.68	**

*,**,*** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

† NS = Not significant at the 0.05 probability level.

Table 4.5: Means comparisons for ice-encapsulated plants between individual treatment means and the grand mean at 4 weeks. Comparisons were made using two-sample t-tests with a Bonferroni correction. (NOTE: differences are on an arcsine scale)

Contrast	Estimate	SE	df	t ratio	p value
10RT DE	0.111	0.03	882.375	3.75	0.001
16-14-Lp 145	-0.068	0.026	893.573	-2.649	0.015
Bad River	-0.447	0.028	881.996	-16.147	0
BAR BIF 1GRL	0.077	0.03	882.375	2.6	0.017
BAR PD9032	-0.109	0.026	883.942	-4.119	0
Birmingham	0.156	0.028	881.996	5.641	0
Blue Mesa	-0.105	0.03	882.375	-3.534	0.001
Bowie	-0.169	0.028	881.996	-6.105	0
Cannon	0.066	0.028	881.996	2.402	0.027
Castle	0.061	0.03	882.375	2.059	0.054
Codie	-0.178	0.028	881.996	-6.415	0
Compass II	0.08	0.03	882.375	2.693	0.014
FRR 72 M2	0.08	0.026	883.942	3.027	0.006
Gladiator	0.013	0.028	881.996	0.456	0.709
Heathland	0.137	0.03	882.375	4.61	0
J 248	0.031	0.03	882.375	1.04	0.361
J 920	0.062	0.03	882.375	2.077	0.053
Kent	0.075	0.026	883.942	2.842	0.009
Morocco	0.066	0.03	882.375	2.222	0.04
Nanook	-0.009	0.028	881.996	-0.325	0.772
Oceania Maritima	-0.021	0.028	881.996	-0.777	0.494
Premium	-0.007	0.026	893.573	-0.29	0.772
Quatro	-0.097	0.03	882.375	-3.268	0.003
Replicator	-0.078	0.026	883.942	-2.963	0.007
Saltillo	0.174	0.028	881.996	6.278	0
Salton Sea	-0.038	0.026	883.942	-1.443	0.194
SeaMist	0.146	0.03	882.375	4.93	0
Seabreeze GT	0.091	0.03	882.375	3.084	0.005
SeaSalt	-0.031	0.026	883.942	-1.187	0.294
Soil Guard	-0.027	0.028	881.996	-0.969	0.388
Stellar 3GL	-0.008	0.026	893.573	-0.302	0.772
Sundancer	-0.283	0.028	881.996	-10.212	0
Thunderstruck	0.124	0.028	881.996	4.497	0
Tirem	0.064	0.03	882.375	2.144	0.047
Xeric	0.061	0.026	883.942	2.301	0.034

Table 4.6: Means comparisons for ice-encapsulated plants between individual treatment means and the grand mean at 8 weeks. Comparisons were made using two-sample t-tests with a Bonferroni correction. (NOTE: differences are on an arcsine scale)

Contrast	Estimate	SE	df	t ratio	p value
10RT DE	0.082	0.02	882.193	3.992	0
16-14-Lp 145	-0.017	0.023	883.168	-0.74	0.487
Bad River	-0.464	0.021	881.656	-22.04	0
BAR BIF 1GRL	0.041	0.02	882.193	2.002	0.057
BAR PD9032	-0.083	0.022	882.046	-3.79	0
Birmingham	0.18	0.021	881.656	8.535	0
Blue Mesa	-0.176	0.02	882.193	-8.614	0
Bowie	-0.145	0.021	881.656	-6.905	0
Cannon	0.067	0.021	881.656	3.163	0.002
Castle	0.084	0.02	882.193	4.096	0
Codie	-0.108	0.021	881.656	-5.128	0
Compass II	0.147	0.02	882.193	7.178	0
FRR 72 M2	0.125	0.022	882.046	5.727	0
Gladiator	-0.084	0.021	881.656	-3.983	0
Heathland	0.158	0.02	882.193	7.694	0
J 248	-0.064	0.02	882.193	-3.105	0.003
J 920	0.107	0.02	882.193	5.228	0
Kent	0.055	0.022	882.046	2.522	0.016
Morocco	0.06	0.02	882.193	2.932	0.005
Nanook	-0.133	0.021	881.656	-6.311	0
Oceania Maritima	-0.016	0.021	881.656	-0.764	0.487
Premium	0.036	0.023	883.168	1.569	0.137
Quatro	-0.236	0.02	882.193	-11.54	0
Replicator	0.009	0.022	882.046	0.409	0.683
Saltillo	0.256	0.021	881.656	12.179	0
Salton Sea	0.014	0.022	882.046	0.652	0.53
SeaMist	0.128	0.02	882.193	6.261	0
Seabreeze GT	0.051	0.02	882.193	2.494	0.017
SeaSalt	0.017	0.022	882.046	0.772	0.487
Soil Guard	-0.181	0.021	881.656	-8.605	0
Stellar 3GL	0.042	0.023	883.168	1.838	0.08
Sundancer	-0.317	0.021	881.656	-15.076	0
Thunderstruck	0.193	0.021	881.656	9.18	0
Tirem	0.095	0.02	882.193	4.645	0

Table 4.7: Means comparisons for ice-encapsulated plants between individual treatment means and the grand mean at 12 weeks. Comparisons were made using two-sample t-tests with a Bonferroni correction. (NOTE: differences are on an arcsine scale)

Contrast	Estimate	SE	df	t ratio	p value
10RT DE	0.064	0.021	881.834	3.07	0.004
16-14-Lp 145	0.001	0.022	882.194	0.057	0.954
Bad River	-0.415	0.021	881.536	-19.708	0
BAR BIF 1GRL	0.051	0.021	881.834	2.475	0.02
BAR PD9032	-0.075	0.022	881.771	-3.495	0.001
Birmingham	0.182	0.021	881.536	8.652	0
Blue Mesa	-0.206	0.021	881.834	-9.939	0
Bowie	-0.085	0.021	881.536	-4.044	0
Cannon	0.044	0.021	881.536	2.084	0.049
Castle	0.079	0.021	881.834	3.792	0
Codie	-0.031	0.021	881.536	-1.496	0.158
Compass II	0.128	0.021	881.834	6.199	0
FRR 72 M2	0.13	0.022	881.771	6.048	0
Gladiator	-0.133	0.021	881.536	-6.324	0
Heathland	0.142	0.021	881.834	6.874	0
J 248	-0.102	0.021	881.834	-4.938	0
J 920	0.116	0.021	881.834	5.596	0
Kent	0.039	0.022	881.771	1.802	0.09
Morocco	0.044	0.021	881.834	2.126	0.045
Nanook	-0.18	0.021	881.536	-8.559	0
Oceania Maritima	-0.01	0.021	881.536	-0.487	0.645
Premium	0.049	0.022	882.194	2.206	0.039
Quatro	-0.272	0.021	881.834	-13.134	0
Replicator	0.021	0.022	881.771	0.973	0.351
Saltillo	0.273	0.021	881.536	12.989	0
Salton Sea	0.022	0.022	881.771	1.039	0.327
SeaMist	0.093	0.021	881.834	4.513	0
Seabreeze GT	0.034	0.021	881.834	1.65	0.12
SeaSalt	0.026	0.022	881.771	1.186	0.266
Soil Guard	-0.218	0.021	881.536	-10.341	0
Stellar 3GL	0.055	0.022	882.194	2.474	0.02
Sundancer	-0.241	0.021	881.536	-11.464	0
Thunderstruck	0.209	0.021	881.536	9.949	0
Tirem	0.105	0.021	881.834	5.092	0
Xeric	0.059	0.022	881.771	2.753	0.01

Table 4.8: Means comparisons for ice-encapsulated plants between individual treatment means and the grand mean at 16 weeks. Comparisons were made using two-sample t-tests with a Bonferroni correction. (NOTE: differences are on an arcsine scale)

Contrast	Estimate	SE	df	t ratio	p value
10RT DE	0.057	0.028	881.349	2.052	0.083
16-14-Lp 145	-0.013	0.028	881.38	-0.485	0.732
Bad River	-0.299	0.028	881.331	-10.831	0
BAR BIF 1GRL	0.108	0.028	881.349	3.908	0
BAR PD9032	-0.087	0.028	881.347	-3.143	0.004
Birmingham	0.163	0.028	881.331	5.91	0
Blue Mesa	-0.193	0.028	881.349	-6.993	0
Bowie	0.012	0.028	881.331	0.422	0.76
Cannon	-0.002	0.028	881.331	-0.059	0.953
Castle	0.045	0.028	881.349	1.63	0.181
Codie	0.052	0.028	881.331	1.874	0.113
Compass II	0.024	0.028	881.349	0.869	0.499
FRR 72 M2	0.097	0.028	881.347	3.489	0.002
Gladiator	-0.135	0.028	881.331	-4.888	0
Heathland	0.091	0.028	881.349	3.299	0.003
J 248	-0.085	0.028	881.349	-3.085	0.005
J 920	0.088	0.028	881.349	3.188	0.004
Kent	0.027	0.028	881.347	0.965	0.451
Morocco	0.018	0.028	881.349	0.644	0.649
Nanook	-0.151	0.028	881.331	-5.455	0
Oceania Maritima	-0.004	0.028	881.331	-0.144	0.912
Premium	0.033	0.028	881.38	1.177	0.349
Quatro	-0.204	0.028	881.349	-7.386	0
Replicator	-0.042	0.028	881.347	-1.522	0.207
Saltillo	0.225	0.028	881.331	8.131	0
Salton Sea	-0.014	0.028	881.347	-0.489	0.732
SeaMist	0.042	0.028	881.349	1.516	0.207
Seabreeze GT	0.041	0.028	881.349	1.475	0.214
SeaSalt	-0.005	0.028	881.347	-0.188	0.905
Soil Guard	-0.136	0.028	881.331	-4.932	0
Stellar 3GL	0.032	0.028	881.38	1.149	0.351
Sundancer	-0.054	0.028	881.331	-1.969	0.096
Thunderstruck	0.173	0.028	881.331	6.255	0
Tirem	0.095	0.028	881.349	3.425	0.002
Xeric	0.005	0.028	881.347	0.185	0.905

Table 4.9: Means comparisons for desiccated plants between individual treatment means and the grand mean at week 0 for run 1. Comparisons were made using two-sample t-tests with a Bonferroni correction. (NOTE: differences are on an arcsine scale)

Contrast	Estimate	SE	df	t ratio	p value
10RT DE	0.042	0.021	882.804	2.021	0.080
16-14-Lp 145	0.047	0.021	882.804	2.304	0.050
Bad River	-0.119	0.021	882.804	-5.783	0.000
BAR BIF 1GRL	-0.007	0.021	882.804	-0.325	0.790
BAR PD9032	-0.098	0.019	904.196	-5.143	0.000
Birmingham	0.094	0.021	882.804	4.581	0.000
Blue Mesa	-0.043	0.021	884.119	-2.085	0.073
Bowie	-0.034	0.021	882.804	-1.645	0.175
Cannon	0.015	0.021	882.804	0.716	0.615
Castle	0.016	0.021	882.804	0.768	0.596
Codie	-0.059	0.021	882.804	-2.876	0.016
Compass II	0.031	0.021	882.804	1.522	0.214
FRR 72 M2	0.009	0.021	882.804	0.440	0.770
Gladiator	0.008	0.021	882.804	0.386	0.790
Heathland	0.051	0.021	882.804	2.498	0.034
J 248	-0.010	0.021	882.804	-0.463	0.770
J 920	0.017	0.021	882.804	0.843	0.559
Kent	-0.003	0.021	882.804	-0.158	0.874
Morocco	-0.045	0.021	882.804	-2.187	0.060
Nanook	-0.007	0.021	882.804	-0.335	0.790
Oceania Maritima	-0.060	0.021	882.804	-2.914	0.016
Premium	0.026	0.021	882.804	1.246	0.339
Quatro	-0.022	0.021	882.804	-1.064	0.420
Replicator	0.057	0.024	886.378	2.438	0.037
Saltillo	0.072	0.021	882.804	3.494	0.003
Salton Sea	-0.082	0.021	882.804	-3.991	0.001
SeaMist	0.047	0.021	882.804	2.261	0.052
Seabreeze GT	0.005	0.021	882.948	0.231	0.842
SeaSalt	-0.054	0.021	882.804	-2.602	0.030
Soil Guard	-0.013	0.021	882.804	-0.622	0.667
Stellar 3GL	0.058	0.021	882.804	2.821	0.017
Sundancer	-0.051	0.021	882.804	-2.495	0.034
Thunderstruck	0.071	0.021	882.804	3.440	0.004
Tirem	0.063	0.021	882.804	3.058	0.011
Xeric	-0.023	0.021	882.804	-1.138	0.389

Table 4.10: Means comparisons for desiccated plants between individual treatment means and the grand mean at week 0 for run 2. Comparisons were made using two-sample t-tests with a Bonferroni correction. (NOTE: differences are on an arcsine scale)

Contrast	Estimate	SE	df	t ratio	p value
10RT DE	0.033	0.021	882.756	1.613	0.208
16-14-Lp 145	0.070	0.021	882.756	3.392	0.005
Bad River	-0.092	0.021	882.756	-4.465	0.000
BAR BIF 1GRL	0.010	0.021	882.756	0.475	0.754
BAR PD9032	-0.137	0.021	882.756	-6.666	0.000
Birmingham	0.043	0.021	882.756	2.107	0.083
Blue Mesa	-0.037	0.021	882.756	-1.806	0.156
Bowie	-0.013	0.021	882.756	-0.654	0.718
Cannon	0.010	0.021	882.756	0.489	0.754
Castle	0.028	0.021	882.756	1.377	0.295
Codie	-0.051	0.021	882.756	-2.499	0.037
Compass II	0.075	0.021	882.756	3.650	0.002
FRR 72 M2	0.017	0.021	882.756	0.827	0.649
Gladiator	0.029	0.021	882.756	1.414	0.291
Heathland	0.053	0.021	882.756	2.570	0.033
J 248	-0.008	0.021	882.756	-0.399	0.754
J 920	0.007	0.021	882.756	0.321	0.770
Kent	-0.008	0.021	882.756	-0.408	0.754
Morocco	-0.007	0.021	882.756	-0.321	0.770
Nanook	-0.012	0.021	882.756	-0.572	0.754
Oceania Maritima	-0.047	0.021	882.756	-2.260	0.060
Premium	0.063	0.021	882.756	3.051	0.010
Quatro	-0.026	0.021	882.756	-1.250	0.353
Replicator	0.014	0.021	882.756	0.657	0.718
Saltillo	0.062	0.021	882.756	3.032	0.010
Salton Sea	-0.066	0.021	882.756	-3.216	0.008
SeaMist	0.055	0.021	882.756	2.667	0.027
Seabreeze GT	0.011	0.021	882.756	0.511	0.754
SeaSalt	-0.090	0.021	882.756	-4.382	0.000
Soil Guard	-0.014	0.021	882.756	-0.671	0.718
Stellar 3GL	0.048	0.021	882.756	2.334	0.053
Sundancer	-0.003	0.021	882.756	-0.125	0.901
Thunderstruck	0.037	0.021	882.756	1.778	0.156
Tirem	0.009	0.021	882.756	0.446	0.754
Xeric	-0.062	0.021	882.756	-3.015	0.010

Table 4.11: Means comparisons for desiccated plants between individual treatment means and the grand mean at week 4 for run 1. Comparisons were made using two-sample t-tests with a Bonferroni correction. (NOTE: differences are on an arcsine scale)

Contrast	Estimate	SE	df	t ratio	p value
10RT DE	0.051	0.016	882.812	3.265	0.002
16-14-Lp 145	0.024	0.016	882.812	1.528	0.164
Bad River	-0.230	0.016	882.812	-14.650	0.000
BAR BIF 1GRL	-0.006	0.016	882.812	-0.411	0.681
BAR PD9032	-0.212	0.016	884.958	-12.867	0.000
Birmingham	0.082	0.016	882.812	5.201	0.000
Blue Mesa	-0.027	0.015	898.886	-1.769	0.108
Bowie	-0.054	0.016	882.812	-3.443	0.001
Cannon	0.011	0.016	882.812	0.681	0.543
Castle	0.006	0.016	882.812	0.414	0.681
Codie	-0.058	0.016	882.812	-3.687	0.001
Compass II	0.019	0.016	882.812	1.197	0.289
FRR 72 M2	0.062	0.016	882.812	3.936	0.000
Gladiator	0.041	0.016	882.812	2.624	0.015
Heathland	0.042	0.016	882.812	2.705	0.013
J 248	0.045	0.016	882.812	2.899	0.007
J 920	0.048	0.016	882.812	3.083	0.004
Kent	0.017	0.016	882.812	1.057	0.351
Morocco	-0.025	0.016	882.812	-1.603	0.147
Nanook	0.010	0.016	882.812	0.637	0.556
Oceania Maritima	-0.041	0.016	882.812	-2.613	0.015
Premium	0.032	0.016	882.812	2.025	0.066
Quatro	-0.028	0.016	882.812	-1.770	0.108
Replicator	0.051	0.015	904.232	3.440	0.001
Saltillo	0.095	0.016	882.812	6.091	0.000
Salton Sea	-0.106	0.016	882.812	-6.791	0.000
SeaMist	0.074	0.016	882.812	4.724	0.000
Seabreeze GT	0.038	0.017	886.407	2.257	0.039
SeaSalt	-0.111	0.016	882.812	-7.070	0.000
Soil Guard	0.014	0.016	882.812	0.874	0.446
Stellar 3GL	0.061	0.016	882.812	3.917	0.000
Sundancer	-0.061	0.016	882.812	-3.908	0.000
Thunderstruck	0.086	0.016	882.812	5.466	0.000
Tirem	0.062	0.016	882.812	3.959	0.000
Xeric	-0.013	0.016	882.812	-0.814	0.469

Table 4.12: Means comparisons for desiccated plants between individual treatment means and the grand mean at week 4 for run 2. Comparisons were made using two-sample t-tests with a Bonferroni correction. (NOTE: differences are on an arcsine scale)

Contrast	Estimate	SE	df	t ratio	p value
10RT DE	0.022	0.016	882.756	1.406	0.224
16-14-Lp 145	0.056	0.016	882.756	3.574	0.001
Bad River	-0.236	0.016	882.756	-15.044	0.000
BAR BIF 1GRL	-0.012	0.016	882.756	-0.775	0.479
BAR PD9032	-0.138	0.016	882.756	-8.787	0.000
Birmingham	0.117	0.016	882.756	7.460	0.000
Blue Mesa	-0.026	0.016	882.756	-1.656	0.149
Bowie	-0.091	0.016	882.756	-5.773	0.000
Cannon	-0.017	0.016	882.756	-1.055	0.364
Castle	0.014	0.016	882.756	0.883	0.426
Codie	-0.078	0.016	882.756	-4.944	0.000
Compass II	0.097	0.016	882.756	6.210	0.000
FRR 72 M2	0.024	0.016	882.756	1.526	0.186
Gladiator	0.048	0.016	882.756	3.088	0.005
Heathland	0.015	0.016	882.756	0.931	0.411
J 248	0.005	0.016	882.756	0.323	0.785
J 920	0.032	0.016	882.756	2.071	0.068
Kent	0.000	0.016	882.756	-0.007	0.995
Morocco	-0.068	0.016	882.756	-4.364	0.000
Nanook	0.027	0.016	882.756	1.691	0.145
Oceania Maritima	-0.039	0.016	882.756	-2.516	0.023
Premium	0.069	0.016	882.756	4.379	0.000
Quatro	-0.016	0.016	882.756	-1.009	0.378
Replicator	0.035	0.016	882.756	2.250	0.045
Saltillo	0.106	0.016	882.756	6.740	0.000
Salton Sea	-0.098	0.016	882.756	-6.270	0.000
SeaMist	0.042	0.016	882.756	2.708	0.014
Seabreeze GT	0.020	0.016	882.756	1.256	0.271
SeaSalt	-0.100	0.016	882.756	-6.401	0.000
Soil Guard	-0.005	0.016	882.756	-0.303	0.785
Stellar 3GL	0.098	0.016	882.756	6.223	0.000
Sundancer	-0.028	0.016	882.756	-1.777	0.127
Thunderstruck	0.095	0.016	882.756	6.085	0.000
Tirem	0.050	0.016	882.756	3.211	0.003
Xeric	-0.021	0.016	882.756	-1.334	0.246

Table 4.13: Means comparisons for desiccated plants between individual treatment means and the grand mean at week 8 for run 1. Comparisons were made using two sample t-tests with a Bonferroni correction. (NOTE: differences are on an arcsine scale)

Contrast	Estimate	SE	df	t ratio	p value
10RT DE	0.066	0.016	882.812	4.180	0.000
16-14-Lp 145	0.038	0.016	882.812	2.440	0.024
Bad River	-0.289	0.016	882.812	-18.405	0.000
BAR BIF 1GRL	-0.033	0.016	882.812	-2.101	0.048
BAR PD9032	-0.262	0.017	886.407	-15.609	0.000
Birmingham	0.094	0.016	882.812	6.015	0.000
Blue Mesa	-0.012	0.015	904.232	-0.824	0.435
Bowie	-0.081	0.016	882.812	-5.184	0.000
Cannon	0.035	0.016	882.812	2.207	0.039
Castle	0.005	0.016	882.812	0.290	0.772
Codie	-0.093	0.016	882.812	-5.960	0.000
Compass II	0.037	0.016	882.812	2.367	0.028
FRR 72 M2	0.095	0.016	882.812	6.030	0.000
Gladiator	0.052	0.016	882.812	3.340	0.002
Heathland	0.053	0.016	882.812	3.392	0.001
J 248	0.048	0.016	882.812	3.061	0.004
J 920	0.063	0.016	882.812	4.041	0.000
Kent	0.030	0.016	882.812	1.883	0.075
Morocco	-0.030	0.016	882.812	-1.940	0.068
Nanook	0.024	0.016	882.812	1.500	0.146
Oceania Maritima	-0.036	0.016	882.812	-2.290	0.032
Premium	0.027	0.016	882.812	1.719	0.101
Quatro	-0.027	0.016	882.812	-1.714	0.101
Replicator	0.044	0.015	898.886	2.899	0.006
Saltillo	0.120	0.016	882.812	7.647	0.000
Salton Sea	-0.126	0.016	882.812	-8.035	0.000
SeaMist	0.079	0.016	882.812	5.061	0.000
Seabreeze GT	0.051	0.016	884.958	3.098	0.004
SeaSalt	-0.143	0.016	882.812	-9.090	0.000
Soil Guard	0.025	0.016	882.812	1.590	0.127
Stellar 3GL	0.069	0.016	882.812	4.383	0.000
Sundancer	-0.103	0.016	882.812	-6.553	0.000
Thunderstruck	0.105	0.016	882.812	6.670	0.000
Tirem	0.083	0.016	882.812	5.287	0.000
Xeric	-0.006	0.016	882.812	-0.384	0.722

Table 4.14: Means comparisons for desiccated plants between individual treatment means and the grand mean at week 8 for run 2. Comparisons were made using two-sample t-tests with a Bonferroni correction. (NOTE: differences are on an arcsine scale)

Contrast	Estimate	SE	df	t ratio	p value
10RT DE	0.035	0.016	882.756	2.238	0.039
16-14-Lp 145	0.050	0.016	882.756	3.208	0.003
Bad River	-0.287	0.016	882.756	-18.278	0.000
BAR BIF 1GRL	-0.023	0.016	882.756	-1.495	0.175
BAR PD9032	-0.138	0.016	882.756	-8.831	0.000
Birmingham	0.119	0.016	882.756	7.586	0.000
Blue Mesa	-0.022	0.016	882.756	-1.435	0.183
Bowie	-0.133	0.016	882.756	-8.471	0.000
Cannon	-0.021	0.016	882.756	-1.353	0.206
Castle	0.035	0.016	882.756	2.259	0.039
Codie	-0.108	0.016	882.756	-6.905	0.000
Compass II	0.113	0.016	882.756	7.192	0.000
FRR 72 M2	0.048	0.016	882.756	3.048	0.004
Gladiator	0.050	0.016	882.756	3.194	0.003
Heathland	0.023	0.016	882.756	1.441	0.183
J 248	0.027	0.016	882.756	1.717	0.121
J 920	0.054	0.016	882.756	3.445	0.001
Kent	0.021	0.016	882.756	1.332	0.207
Morocco	-0.074	0.016	882.756	-4.702	0.000
Nanook	0.026	0.016	882.756	1.667	0.129
Oceania Maritima	-0.034	0.016	882.756	-2.173	0.044
Premium	0.062	0.016	882.756	3.972	0.000
Quatro	-0.003	0.016	882.756	-0.209	0.859
Replicator	0.035	0.016	882.756	2.252	0.039
Saltillo	0.110	0.016	882.756	7.016	0.000
Salton Sea	-0.115	0.016	882.756	-7.321	0.000
SeaMist	0.053	0.016	882.756	3.362	0.002
Seabreeze GT	0.016	0.016	882.756	1.007	0.344
SeaSalt	-0.082	0.016	882.756	-5.240	0.000
Soil Guard	-0.001	0.016	882.756	-0.064	0.949
Stellar 3GL	0.090	0.016	882.756	5.742	0.000
Sundancer	-0.090	0.016	882.756	-5.746	0.000
Thunderstruck	0.081	0.016	882.756	5.152	0.000
Tirem	0.090	0.016	882.756	5.733	0.000
Xeric	-0.005	0.016	882.756	-0.341	0.777

Table 4.15: Means comparisons for desiccated plants between individual treatment means and the grand mean at week 12 for run 1. Comparisons were made using two sample t-tests with a Bonferroni correction. (NOTE: differences are on an arcsine scale)

Contrast	Estimate	SE	df	t ratio	p value
10RT DE	0.085	0.021	882.804	4.111	0.000
16-14-Lp 145	0.090	0.021	882.804	4.385	0.000
Bad River	-0.296	0.021	882.804	-14.356	0.000
BAR BIF 1GRL	-0.086	0.021	882.804	-4.183	0.000
BAR PD9032	-0.250	0.021	882.948	-12.083	0.000
Birmingham	0.133	0.021	882.804	6.440	0.000
Blue Mesa	0.001	0.024	886.378	0.050	0.960
Bowie	-0.116	0.021	882.804	-5.619	0.000
Cannon	0.087	0.021	882.804	4.201	0.000
Castle	0.010	0.021	882.804	0.485	0.687
Codie	-0.166	0.021	882.804	-8.065	0.000
Compass II	0.086	0.021	882.804	4.193	0.000
FRR 72 M2	0.108	0.021	882.804	5.220	0.000
Gladiator	0.042	0.021	882.804	2.021	0.061
Heathland	0.084	0.021	882.804	4.066	0.000
J 248	-0.002	0.021	882.804	-0.093	0.953
J 920	0.062	0.021	882.804	3.029	0.004
Kent	0.036	0.021	882.804	1.729	0.109
Morocco	-0.061	0.021	882.804	-2.955	0.005
Nanook	0.034	0.021	882.804	1.635	0.128
Oceania Maritima	-0.045	0.021	882.804	-2.176	0.043
Premium	0.011	0.021	882.804	0.548	0.659
Quatro	-0.019	0.021	882.804	-0.936	0.408
Replicator	0.036	0.021	884.119	1.735	0.109
Saltillo	0.145	0.021	882.804	7.046	0.000
Salton Sea	-0.141	0.021	882.804	-6.830	0.000
SeaMist	0.062	0.021	882.804	3.030	0.004
Seabreeze GT	0.044	0.019	904.196	2.311	0.032
SeaSalt	-0.149	0.021	882.804	-7.212	0.000
Soil Guard	0.021	0.021	882.804	1.013	0.376
Stellar 3GL	0.080	0.021	882.804	3.886	0.000
Sundancer	-0.176	0.021	882.804	-8.531	0.000
Thunderstruck	0.128	0.021	882.804	6.190	0.000
Tirem	0.125	0.021	882.804	6.089	0.000
Xeric	-0.003	0.021	882.804	-0.156	0.929

Table 4.16: Means comparisons for desiccated plants between individual treatment means and the grand mean at week 12 for run 2. Comparisons were made using two-sample t-tests with a Bonferroni correction. (NOTE: differences are on an arcsine scale)

Contrast	Estimate	SE	df	t ratio	p value
16-14-Lp 145	0.053	0.021	882.756	2.557	0.021
Bad River	-0.244	0.021	882.756	-11.847	0.000
BAR BIF 1GRL	-0.024	0.021	882.756	-1.168	0.327
BAR PD9032	-0.139	0.021	882.756	-6.767	0.000
Birmingham	0.049	0.021	882.756	2.395	0.031
Blue Mesa	-0.027	0.021	882.756	-1.300	0.283
Bowie	-0.140	0.021	882.756	-6.813	0.000
Cannon	-0.004	0.021	882.756	-0.192	0.899
Castle	0.093	0.021	882.756	4.518	0.000
Codie	-0.144	0.021	882.756	-6.974	0.000
Compass II	0.121	0.021	882.756	5.891	0.000
FRR 72 M2	0.089	0.021	882.756	4.303	0.000
Gladiator	0.034	0.021	882.756	1.656	0.156
Heathland	0.077	0.021	882.756	3.735	0.001
J 248	0.057	0.021	882.756	2.782	0.012
J 920	0.071	0.021	882.756	3.458	0.001
Kent	0.055	0.021	882.756	2.648	0.017
Morocco	-0.023	0.021	882.756	-1.094	0.356
Nanook	-0.013	0.021	882.756	-0.626	0.620
Oceania Maritima	-0.030	0.021	882.756	-1.476	0.213
Premium	0.044	0.021	882.756	2.122	0.060
Quatro	0.012	0.021	882.756	0.575	0.638
Replicator	0.014	0.021	882.756	0.661	0.614
Saltillo	0.075	0.021	882.756	3.663	0.001
Salton Sea	-0.116	0.021	882.756	-5.616	0.000
SeaMist	0.086	0.021	882.756	4.159	0.000
Seabreeze GT	-0.001	0.021	882.756	-0.059	0.953
SeaSalt	-0.036	0.021	882.756	-1.733	0.139
Soil Guard	-0.003	0.021	882.756	-0.126	0.926
Stellar 3GL	0.025	0.021	882.756	1.235	0.304
Sundancer	-0.189	0.021	882.756	-9.185	0.000
Thunderstruck	-0.007	0.021	882.756	-0.351	0.794
Tirem	0.128	0.021	882.756	6.202	0.000
Xeric	-0.015	0.021	882.756	-0.749	0.568

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

Because the unforgiving environments of roadsides make establishing and maintaining roadside turfgrass so difficult, our goal was to quantify roadside turfgrass stress tolerances. We focused on the most important stresses that included salt, heat and ice and their effects on turfgrass performance. We can use this information to identify turfgrasses we would recommend for use on roadsides in Minnesota.

We succeeded in generating new and much-needed data regarding the performance of both newer cultivars and less-studied turfgrass species in stressed conditions such as those found on roadsides in Minnesota. Switching to species and cultivars that perform better will improve the function of current roadside turfgrass mixes. In time, our results will lead to saving MnDOT significant amounts of money and time on re-installations, as well as reducing the environmental impact of roadside vegetation failures.

5.1 RECOMMENDED CHANGES TO MNDOT SPECIFICATIONS

Based on our results, we recommend MnDOT adopt the following guidelines for areas susceptible to salt stress:

1. Discontinue the use of older alkaligrass cultivars and substitute newer cultivars for better roadside turf performance.
2. Increase the use of tall fescue on roadsides, except in low-lying areas that are prone to ice sheeting.
3. Reduce the use of perennial ryegrass, smooth bromegrass, hard fescue, blue grama and prairie junegrass as turfgrasses on roadsides.

We recommend MnDOT adopt the following guidelines for areas where heat stress is a major concern:

1. Avoid alkaligrass in cases where heat stress is anticipated.
2. Choose slender creeping red fescue and strong creeping red fescue cultivars as opposed to hard or sheep fescue.
3. Select Kentucky bluegrass cultivars that have exhibited heat stress tolerance, particularly the cultivar ‘Tirem’.
4. Consider the inclusion of heat-stress tolerant cultivars of tall fescue, such as ‘Thunderstruck’ and ‘Birmingham’. Canada bluegrass could also be considered; however, the turf quality of this species is not suitable for many roadsides where aesthetics are important

The results of the project did not lead to species or cultivar recommendations for areas susceptible to ice stress. Further research is needed in this area to gain a deeper understanding of this complex abiotic stress.

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