Screening and Selection for Salt Tolerance in Native Warm Season Grasses
This preliminary study focused on a) surveying the concentrations of sodium and other metals along the rights-of-way of several of the most heavily traveled and salted roadways, b) development of a selection and screening method for salt tolerance in 6 species of native warm season grasses, and c) establishment of outdoor garden plots and field sites to further test the ability of native warm season grasses to grow under highly saline conditions. Salt levels along roadways were found to vary from very low to very high concentrations during the winter months but were found to decrease to levels that probably will allow germination and growth of tolerant grasses. Two species of grasses, blue grama and buffalo grass, were found to be tolerant of saline environments. Two roadside prairie restoration sites were established to further test the ability of mixtures of grasses to tolerate highly saline soils.
SCREENING AND SELECTION FOR SALT TOLERANCE IN
NATIVE WARM SEASON GRASSES

Final Report

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The contents of this report reflect the views of the authors who are responsible for the facts and accuracy of the data presented herein. The contents do not necessarily reflect the views or policies of the Minnesota Department of Transportation. This report does not contain a standard or specified technique.
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Summary

Introduced cool season grasses of European and Asian origin have traditionally been planted adjacent to roadways in Minnesota. Native species are now under consideration by the Minnesota Department of Transportation for this application because these species have desirable characteristics that make them suitable for roadside use. These characteristics include: 1) germination of seedlings or an initial flush of growth from overwintering plants that typically occurs in late May-June, after roadside salt accumulations and debris have been flushed from soils by spring rains; 2) deep root systems enabling them to reduce soil erosion and possibly draw water away from the road-bed; and 3) a generally short stature that may reduce or eliminate the need for mowing.

This study surveyed roadside salt concentrations and other metals for a period of one year, examined salt tolerance in 6 species of native grasses, and established two field sites for future studies of salt tolerance in native grasses. Salt concentrations ranged from ca. 1000 ppm to very high levels of $2.2 \times 10^4$ ppm along roadsides in the Minneapolis-St. Paul metro area and were highest during the winter months with decreasing concentrations occurring during the spring and summer months. Spring and summer concentrations were found to be low enough to allow for germination and establishment of some species. Blue grama and buffalograss, of the six native species tested, were found to be able to germinate and grow under highly saline conditions. Both may be excellent candidates for future roadside testing. Two roadside plots, at T.H. 62 and T.H. 55, and at I-394 and T.H. 100, have been planted to a mixture of several native warm-season grasses for further salt testing in the field.
Further research should combine testing of suspected salt tolerant native species in the laboratory and begin field testing of various mixtures of short stature native grass mixtures adjacent to heavily traveled highways in the state.
Introduction

Vegetation, especially turf grasses, must be established adjacent to highway rights-of-way to control soil erosion, improve highway appearance, reduce hazards, and improve water flow to ditches or drainages. An inspection of vegetation within 1 to 2 meters of rights-of-way of high volume roadways (>50,000 vehicles per day) in the cities of Minneapolis/St. Paul in Minnesota indicates that areas are either largely devoid of vegetation or that grasses planted along roadways have been replaced by undesirable, weedy non-grass species.

Introduced\(^1\) cool-season grasses, such as *Bromus* or *Poa* spp., have traditionally been planted adjacent to improved roadways in Minnesota. However, native\(^2\) warm-season grasses are currently under consideration by the Minnesota Department of Transportation (Mn/DOT) for this application because these species possibly have characteristics that would make them suitable for roadside use. These characteristics include: 1) germination of seedlings or an initial flush of growth from overwintering plants that typically occurs in late May-June, after roadside salt accumulations and debris have been flushed from soils by spring rains; 2) deep root systems enabling them to reduce soil erosion and possibly draw water away from the road-bed; and 3) a generally short stature that may reduce or eliminate the need for mowing.

This project was designed to gather preliminary data on the usefulness of short stature native grasses in areas that are exposed to relatively high levels of salt during winter months in Minnesota. The tasks of this research were to: 1) to survey the concentrations of sodium and other metals on the rights of way of several heavily traveled and salted highways in the metropolitan area of Minneapolis/St. Paul;

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\(^1\)Introduced refers to species of European or Asian origin.

\(^2\)Native refers to species indigenous to the midwestern USA circa 1850.
2) to develop a selection scheme for increased salt tolerance in two short native grass species; 3) establish an outdoor garden plot to measure the ability of six native species of warm season grasses to tolerate elevated levels of soil salinity over one winter, and; 4) to establish several field sites where short grass mixtures can be evaluated in the future.

Methods and Materials

Task 1. A survey of the concentrations of sodium and other metals along the rights-of-way of several of the most heavily traveled and salted highways in the metropolitan area of Minneapolis/St. Paul. Soils were sampled for one year beginning in February of 1992 through January of 1993. Sample sites chosen as representative of heavily traveled highways were: a) Site 1: the intersection between highways I-35W and I-694; b) Site 2: the intersection between T.H. 7 and highway I-494; c) Site 3: the intersection between highways I-494 and I-35W; d) Site 4: I-35W right-of-way between T.H. 62 and I-94; and e) Site 5: I-94 right-of-way from its intersection with I-35E to Lexington Parkway. This sites are indicated by site number on a map of the metropolitan area in Appendix A.

Samples were collected by driving a thickwall stainless steel tube (i.d. 2.54 cm) to a depth of 20 cm into roadside soils. The 20 cm sampling depth was chosen as representative depth to which the roots of many grass species penetrate the soil. Three samples were collected from the following places at each field site: 1 meter, 3 meters, and 10 meters from the edge of the pavement. Wherever possible, sites were chosen near intersections or along ramps to allow the 10 meter sample to be collected on an upslope of the roadside grade. Collections at Site 4 were done only at the 1 and 3 meter intervals because of the narrowness of the right-of-way and difficulty in parking.
along the roadway. Collecting sites were marked with flags and recorded to avoid duplicate sampling. The four corners of each intersection were sampled at least twice. Three samples were collected within a meter of each other at every interval and were pooled, thoroughly mixed, oven-dried at 60 C for two days, and submitted for ICP analysis by the Soil Testing Service at the University of Minnesota. In addition, a sample of the road salt used by Mn/DOT for salting operations was analyzed in duplicate by the Soil Testing Service.

Soil pH was determined by the standard method of making a slurry of 50:50 (v:v) of soil and double distilled water, shaking for 10 minutes, and reading the soil pH using a pH meter.

Data for soil and salt analysis is reported as ppm (mg/kg) of ions present in the samples. Since sodium was the only ion measured for the salt analysis (measurement of Cl is a separate and rather expensive analysis), all raw data for total sodium in roadsides soils was transformed by multiplying sodium concentrations by a factor of 2.542 (Scientific Tables, 1970) to find total NaCl concentrations. Other ions analyzed included P, K, Ca, Mg, Al, Fe, Mn, Zn, Cu, B, Pb, Ni, Cr, and Cd. These ions were pooled for the 1 and 3 meter intervals at each site and recorded as an average value for the entire year.

Task 2. Development of a selection scheme for increased salt tolerance in two species. The first goal of this task was to determine the ability of the six selected species of warm season grasses to germinate under saline conditions. Slightly more than four hundred seeds of each of the six species were surface sterilized by soaking seeds for 15 minutes in 10% sodium hypochlorite, dipping the seeds briefly in 95% ethanol, and
rinsing three times in sterile distilled water. The seeds were planted into several large petri dishes on three sheets of sterile filter paper and allowed to imbibe with solutions of 1/2 strength Hoagland's solution containing $0.0, 2.5 \times 10^3, 5.0 \times 10^3, 1.0 \times 10^4, 2.0 \times 10^4$ and $4.0 \times 10^4$ ppm salt as supplied by Mn/DOT. Dishes were incubated in a growth chamber at 26 C and maintained in a 12 hr light/dark cycle. The petri dishes were sealed with parafilm but occasionally opened to replenish salt solutions as water was imbibed by the seeds. Fungal contamination of some of the plates was observed but kept to a minimum by occasional removal of contaminated seeds from the plates. Seeds were scored as germinated when radicle and first foliage leaf appeared and the seedling grew to a height of 1 cm. The majority of seeds germinated within two weeks, however seeds were scored for germination for 30 days to ensure that seedlings survived and grew briefly. The experiment was replicated three times for a total of 1200 seeds per salt treatment.

The second goal of this task was to develop a recurrent scheme to select individual salt tolerant plants from a population as a basis for developing highly salt tolerate strains. The hardiness of such strains may then be compared with non-selected types in field demonstration plots. The three species most tolerant of highly saline conditions, buffalo grass, blue grama and little bluestem as a less salinity tolerant comparison, were chosen as species desirable for screening based on germination trials (see Fig. 2). Five % seedling survivability under highly saline conditions for 30 days was used as a target for this recurrent selection scheme.

A modified hydroponic method was used to select for salt tolerant individuals. In May of 1992, enough seeds of buffalo grass, blue grama, and little bluestem were planted in perlite-filled flats in the greenhouse to obtain 1000 germinated seedlings. Three
replicates were performed for a total of 3000 treated seeds per species. The seeds were lightly covered with additional perlite and watered with 1/2 strength Hoagland's solution containing 10,000 ppm salt. The salt solution was made with salt used in normal road salting operations by Mn/DOT. Flats were placed in the greenhouse, watered to excess every three days and allowed to drain freely to maintain salt concentrations at 10,000 ppm. Surviving seedlings were rescued at the end of 30 days by thoroughly flushing flats with tap water and watering for an additional 2 weeks with 1/2 strength Hoagland's solution. Approximately fifty surviving seedlings of buffalo grass and blue grama were planted in flats in potting soil. After two weeks, 25 plants of each species were transplanted outdoors in isolation plots and 25 plants of each species were transplanted to clay plots and maintained in the greenhouse.

Task 3. Establishment of an outdoor garden plot to measure the ability of six native species of warm season grasses to tolerate elevated levels of soil salinity over one winter. A 22 m x 14 m plot using a randomized complete block design with three replications was set up in April of 1991 on the south side of the College of Biological Sciences greenhouse, University of Minnesota, St. Paul campus (Fig. 1). Five strips (2 m wide) corresponding to the five salt treatments were roto-tilled using a tractor mounted roto-tiller to a uniform depth of approximately 10 inches. East-west walkways were not roto-tilled and retained as original turf to lessen salt contamination from adjacent treatments. North-south walkways were roto-tilled, not planted, and covered with marsh straw. The east-west strips of roto-tilled soil were covered with 10 mm black plastic sheeting until planting.
Planting was accomplished between June 8-14, 1992, by hand broadcasting and hand-raking into each plot. Species planted and planting rate were: little bluestem \((Schizachyrium scoparium)\) @ 4 lbs/acre, buffalo grass \((Buchloe dactyloides)\) @ 8 lbs/acre,
blue grama (*Bouteloua gracilis*) @ 3 lbs/acre, sideoats grama (*Bouteloua curtipendula*) @ 3 lbs/acre, prairie dropseed (*Sporobolis heterolepis*) @ 2 lbs/acre, sand dropseed (*Sporobolis cryptandrus*) @ 2 lbs/acre, and Kentucky bluegrass (*Poa pratensis*) @ 3 lbs/acre. Kentucky bluegrass was used as a control species, i.e., as a representative cool season grass that Mn/DOT often plants as sod on rights-of-way. Plots were watered daily or as needed to maintain proper conditions for germination. Plots were lightly fertilized in the fall of 1992 with 10-10-10 fertilizer. Salt treatments were to be done during the winter of 1992-93 (see Results and Discussion).

**Task 4. Establishment of field sites for future monitoring.**

Field sites were established at the intersection of T.H. 100 and highway I-394 in May of 1993 and at the intersection of T.H. 55 and T.H. 62 in late September of 1993.
Results and Discussion

Many factors influence plant growth in the difficult environment of the roadside. These include accumulation of salt (NaCl) in roadside soils from winter salting operations; long period accumulation of toxic compounds in soils from vehicular exhaust; direct shorter-term exposures to toxic gases from vehicle emissions; compaction of soils along roadside edges; and smothering by both sanding operations and snow accumulation. These factors have not been explored to a great extent by researchers in the transportation field. In this report, we examined the role of road salt in limiting germination in selected warm season grasses, surveyed the amounts of salt present in the roadside environment over the course of a single year, and briefly studied the potential of selection and breeding for salt tolerance in several species.

Task 1. A survey of the concentrations of sodium and other metals along the rights-of way of several of the most heavily traveled and salted highways in the metropolitan area of Minneapolis/St. Paul. Soils were surveyed at five different sites along heavily travelled highways in the metropolitan area to a) collect baseline data on salinity for one year, and b) to allow selection of potential sites to test salt tolerant plants as roadside vegetation in the future.

Soil Salinity. Table 1 shows the elemental composition of the salt Mn/DOT used in road salting operations. As expected, sodium and chlorine are the major elements present in the sample. Other elements that are important nutritive elements for plant growth include calcium (at a relatively high concentrations), potassium, iron, and magnesium. Other elements, some of which are biologically toxic, are present in the samples but found generally in less than 10 ppm. Elemental nitrogen was not determined for this sample.
Table 1. Elemental composition of MNDOT road salt. Mean values in parts per million (ppm) of two samples.

<table>
<thead>
<tr>
<th>Element</th>
<th>Amount (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (Na)</td>
<td>349,714.0</td>
</tr>
<tr>
<td>Chlorine (Cl)</td>
<td>539,259.0*</td>
</tr>
<tr>
<td>Calcium (Ca)</td>
<td>4,573.5</td>
</tr>
<tr>
<td>Potassium (K)</td>
<td>187.5</td>
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<tr>
<td>Iron (Fe)</td>
<td>73.9</td>
</tr>
<tr>
<td>Magnesium (Mg)</td>
<td>55.7</td>
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<td>Aluminum (Al)</td>
<td>27.7</td>
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<td>Lead (Pb)</td>
<td>6.7</td>
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<td>Phosphorus</td>
<td>4.6</td>
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<tr>
<td>Manganese (Mn)</td>
<td>3.1</td>
</tr>
<tr>
<td>Copper (Cu)</td>
<td>2.0</td>
</tr>
<tr>
<td>Zinc (Zn)</td>
<td>1.9</td>
</tr>
<tr>
<td>Nickel (Ni)</td>
<td>1.7</td>
</tr>
<tr>
<td>Chromium (Cr)</td>
<td>1.1</td>
</tr>
<tr>
<td>Cadmium (Cd)</td>
<td>0.4</td>
</tr>
</tbody>
</table>

* Chlorine was not analyzed directly using ICP analysis. It is calculated from the following standard conversion: 349,714 ppm Na x 2.542 to determine total ppm of NaCl then subtracting ppm of Na (Scientific Tables. 1972. Ciba-Geigy Limited, Ardsley, NY. p. 251).

Figs. 2 (intersection of highways I-35W and I-694), 3 (intersection of T.H. 7 and I-494), 4 (intersection of highways I-494 and I-35W), 5 (I-35W right-of-way between T.H. 62 and I-94) and 6 (I-94 right-of-way from the intersection with I-35W to Lexington Parkway) illustrate the salt found in soils at our five study sites over the course of one calendar year. Some general trends can be noted for each of these 5 graphs.
Fig. 2. NaCl Concentrations in Soils vs. Time:
Site 1: Intersection of Highways I-35W and I-694

Fig. 3. NaCl Concentrations in Soils vs. Time.
Site 2: Intersection of T.H. 7 and I-494
Fig. 4. NaCl Concentrations in Soils vs. Time:
Site 3. Intersection of highways I-494 and I-35W

- 1 meter
- 3 meters
- 10 meters

Mean NaCl Concentration (ppm)

Time (months)

Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec Jan
Fig. 5. NaCl Concentrations in Soils vs Time:
a) As might be anticipated, salt levels are highest during the winter and spring months of October to May and probably correlate well with snowfall events in the Twin Cities. Concentrations generally range from a low of ca. 1000 ppm to extremely high concentrations of up to $2.2 \times 10^4$ (Figs. 4 and 5). Highest concentrations of salt are found in the 1 meter interval adjacent to the roadway and decrease rapidly at the 3 and 10 meter sampling intervals. Site 4 (Highway I-35W right-of-way between T.H. 62 and I-94 generally appears to have the highest persistent yearly salt load of our sample sites.

b) In the summer and early fall, salt concentrations tend to fall below $2.5 \times 10^3$ ppm at all of the sampled intervals. NaCl is a very water soluble compound and we suggest that most salt from salting operations remains close to the surface where it is dissolved.
by spring rains and flushed into culverts and storm sewers. This observation suggests
that most warm season grasses will be capable of germination during the early
summer when salt concentrations are at their lowest annual levels.

Salt concentrations are generally quite low at the 10 meter sampling interval. Most
grasses, both warm and cool season will be able to germinate and grow 3 or more
meters from the roadway without experiencing salt stress.

Other metals in roadside soils...Figs 7 and 8 illustrate the average concentrations of
other elements present in roadside soils at our study sites. Fig. 7 shows annual
average concentrations of aluminum, iron, magnesium, calcium and potassium in
sampled soils.
Potassium, magnesium, iron and calcium are important higher plant nutrients. They occur in relatively high concentrations (along with aluminum which is not known to be mineral nutrient for plants) and probably place additional stress on plants in the roadside environment. The source of these elements in roadside soils is not clear except to suggest that they have accumulated over long periods of time from salting and sanding operations along rights-of-ways and from vehicular debris and exhaust. Calcium may come principally from Mn/DOT salting operations. Our sample contained an average of 4573 ppm calcium (Table 1). This calcium input into the roadside soils from salting operations is probably beneficial. Calcareous soils tend to have a high pH, remain rather rich in nutrients, keep solubility of toxic heavy metal
ions low, and aid in nitrogen cycling via soil bacteria. Soils at all of our study sites were alkaline with pH in the range of 7.1 - 8.2.

Fig. 8 shows mean concentrations for cadmium, boron, nickel, chromium, copper, zinc, lead, manganese and phosphorus at our study sites. While several of these metals are important micro-nutrients for plants (e.g., boron, copper and manganese and phosphorus which is a major plant nutrient), many of these metals are toxic to plants if found in high concentrations.

Heavy metal contamination of roadside soils, especially by lead, cadmium, nickel and zinc, occurs from atmospheric deposition of these trace metals from exhaust gases (e.g., see Muskett and Jones, 1980). A first inspection of Fig. 8 might suggest that the soils in the metro area are heavily contaminated but levels appear to reflect observations garnered from analysis of roadside soils in various parts of the world. For example, Collins (1984) found lead levels in the range of 141-321 ppm up to 10 meters from a roadside in New Zealand. Reinbold and Rolfe (1976) detected lead concentrations of up to 1000 ppm near a high traffic volume highway in Illinois. Fytianos et.al., (1985) found lead and zinc in roadside soils to be in the range of 10 to 190 ppm in Greece. Wheeler and Rolfe (1979) observed lead levels in the range of 55 ppm to 1225 ppm in soils within 10 meters of highly traveled roads in central Illinois. And, Motto et al., (1970) found lead levels in soils to average 159.5 ppm within 8 meters of a heavily travelled highway in New Jersey. In any case, our data suggests that heavy metal contamination of soils in the metro area is not very dissimilar, and in many cases carries a lesser load of lead and other heavy metals than soils in other parts of the U.S and the world.
Fig. 8. Mean concentrations of B, Cd, Cr, Cu, Mn, Ni, Pb, P, and Zn in Soils at 5 Sites

![Bar chart showing mean concentrations of various elements in soils at 5 sites](chart.png)

Collection Site

Task 2. Development of a selection scheme for increased salt tolerance in two species.

Fig. 9 illustrates the results of germination trials for our six non-halophytic species under saline conditions.
Data are reported as the means of our three replicates and the standard deviation. Fig. 9 shows that salt tolerance occurs in approximately the following order (from most tolerant to least tolerant):

blue grama > buffalo grass > side oats grama > little bluestem > prairie dropseed > sand dropseed

Increasing salt concentrations generally inhibit germination in all tested species, even at the lowest tested concentration of $2.5 \times 10^3$ ppm. Blue grama and buffalo grass appeared to be the most capable of germinating under highly saline conditions. If compared to the 0.0 ppm control, ca. 10% of buffalo grass seeds could germinate at salt concentrations of $2.0 \times 10^4$ and $4.0 \times 10^4$ ppm; ca. 46% of blue grama seeds could germinate at salt concentrations of $4.0 \times 10^4$ ppm; and ca. 24% of side oats grama seeds
could germinate at salt concentrations of $2.0 \times 10^4$ ppm. Since salt concentrations rarely approach $2.5 \times 10^3$ ppm in soils adjacent to the right-of-ways in the month of June (see next section), most of the tested species should be able to germinate in roadside soils.

In another experiment, a recurrent selection scheme was used to select for salt tolerant individuals. Fig. 10 illustrates the percentage of surviving seedlings of two species we determined to be highly salt tolerant, buffalo grass and blue grama, and one less tolerant species as a comparison, little blue stem, after exposure for 30 days to a solution containing $1.0 \times 10^4$ ppm salt.

Fig. 10. Germination vs. Time: 10,000 ppm NaCl

The data is reported as the mean percentage of surviving seedlings in our three replicates and the standard deviation. As noted previously, these seedlings were germinated in flats and grown under ambient greenhouse conditions. Germination
was generally complete for the three species after approximately 15 days. Our selection intensity goal was 5%. At 30 days we rescued surviving seedlings at a selection intensity of 5% for blue grama and 14% for buffalo grass. These rescued seedlings were split into two groups and planted both in outdoor isolation plots and in potted plants in the greenhouse for interbreeding of the selected populations.

Growing conditions both in the greenhouse and outdoors were quite poor in 1992 because of the cold, cloudy weather. Both indoor and outdoor populations grew well but flowered very poorly. A very small amount of seed was collected from all populations (Cycle 0 seed) and has been retained for further testing and development of salt tolerance in these two species.

Both buffalo grass and blue grama appear to have promising potential for improvement of their tolerance to salinity, however, this type of approach must be expanded in scale and experiments continued for several years to develop salt tolerant strains (see recommendations below).

Task 3. Establishment of an outdoor garden plot to measure the ability of six native species of warm season grasses to tolerate elevated levels of soil salinity over one winter. Task 3 was not successfully accomplished during the scope of this project and will be re-done pending additional funding. Upon evaluation of the methods used to establish these plots, it has been determined that seeding rates must be increased substantially and more rigorous site preparation must be used to eradicate existing vegetation such as quack grass (Agropyron repens) and other undesirable species.
Task 4. Establishment of field sites for future monitoring.

Task 4 was completed by the fall of 1993. We have established two field sites for further experimentation. The first is located at I-394 and T.H. 100. It was hydroseeded following road construction in late May of 1993 and has grown for one full field season. The species seeded were; blue grama (*Bouteloua gracilis*), sideoats grama (*Bouteloua curtipendula*), little bluestem (*Schizachyrium scoparium*), sand dropseed (*Sporobolis cryptandrus*), slender wheat-grass (*Agropyron trachycaulum*), Canada bluegrass (*Poa compressa*) and alkali grass (*Puccinella distans*). A cover crop of annual rye-grass (*Lolium italicum*) was included with this mixture. The second is located at T.H. 62 and 55 and is called the Crosstown Prairie Restoration Project. It was seeded in late September 1993 using a Truax flex-type interseeder drill. The species seeded were: blue grama (*Bouteloua gracilis*), sideoats grama (*Bouteloua curtipendula*), little bluestem (*Schizachyrium scoparium*), slender wheat-grass (*Agropyron trachycaulum*), and June grass (*Koeleria macrantha*). An experimental cover crop called Regreen (HybriTech Inc.), was installed with this seeding. Regreen is a hybrid cross between winter wheat and slender wheat-grass. See the Appendix B for a tabulation of the seed mixtures and rates.
Recommendations

- Continue field trials to measure the ability of short stature warm season grasses to tolerate highly saline soils. A field experiment similar to the one described in this report should be performed as a longer-term study of the effects on increased salinity on grasses. A field site must be chosen that can be highly controlled and monitored, that has access to water to ensure that seedlings germinate when planted, will not experience competition from introduced warm season or cool season grasses, and that can be observed for several years.

- Begin field testing of various short stature native grass mixtures adjacent to roadways in the metro area. As indicated in Task 4, several sites have already been established and a number of additional sites should be established as well.

- Continue to select for salt tolerance in native species. This report represents a short, preliminary investigation of the salt tolerance exhibited by several native grasses; with both buffalo grass and blue grama showing some very good ability to tolerate highly saline environments and excellent survivability when grown for up to 30 days in a 1.0 x 10^4 ppm salt solution. A recurrent selection scheme should be continued to select for salt tolerance for the two species identified in this research and for other warm season grasses. For the next step we recommend selecting a much larger population of seeds (e.g., 10,000), using selection intensities of <1%, and select plants for several generations to determine realized heritability and narrow-sense heritability for the tested plants. Other native midwestern plant species that might be suitable for testing include: *Distichlis stricta* (Inland saltgrass) for alkaline soils that are poorly drained; *Eragrostis intermedia* (Plains lovegrass); *Hilaria jamesii*; and *Sporobolis airoides* (alkali sacaton).
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Appendix A. Map of Minneapolis-St. Paul metropolitan area study sites
MINNEAPOLIS-ST. PAUL
METROPOLITAN AREA
STUDY SITES
Appendix B. Special seed mixture used to establish the I-394/T.H. 100 field site

Short description of the Crosstown Prairie restoration project
**I-394/T.H. 100 Special Seed Mixture**

<table>
<thead>
<tr>
<th>Mixture</th>
<th>Plant Species</th>
<th>*Rate lbs/acre</th>
<th>% of Total</th>
<th>Suggested Vendor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Special</td>
<td>Alkali grass</td>
<td>8.0</td>
<td>16.0</td>
<td>Agassiz</td>
</tr>
<tr>
<td></td>
<td>Bluegrass, Canada</td>
<td>10.0</td>
<td>20.0</td>
<td>Agassiz</td>
</tr>
<tr>
<td></td>
<td>Bluestem, little</td>
<td>4.0</td>
<td>8.0</td>
<td>Mohn/Prairie Resto</td>
</tr>
<tr>
<td></td>
<td>Dropseed, sand</td>
<td>1.0</td>
<td>2.0</td>
<td>Mohn</td>
</tr>
<tr>
<td></td>
<td>Grama, blue</td>
<td>10.0</td>
<td>20.0</td>
<td>Mohn</td>
</tr>
<tr>
<td></td>
<td>Grama, sideoats</td>
<td>5.0</td>
<td>10.0</td>
<td>Mohn</td>
</tr>
<tr>
<td></td>
<td>Rye-grass, annual</td>
<td>10.0</td>
<td>20.0</td>
<td>Agassiz</td>
</tr>
<tr>
<td></td>
<td>Wheat-grass, slender</td>
<td>2.0</td>
<td>4.0</td>
<td>Mohn</td>
</tr>
<tr>
<td>Wildflowers</td>
<td>Aster, sky-blue</td>
<td>0.125</td>
<td>0.25</td>
<td>Mohn/Prairie Resto</td>
</tr>
<tr>
<td></td>
<td>Black-eyed susan</td>
<td>0.125</td>
<td>0.25</td>
<td>Mohn/Prairie Resto</td>
</tr>
<tr>
<td></td>
<td>Goldenrod, stiff</td>
<td>0.125</td>
<td>0.25</td>
<td>Mohn/Prairie Resto</td>
</tr>
<tr>
<td></td>
<td>Ox-eye, common</td>
<td>0.125</td>
<td>0.25</td>
<td>Mohn/Prairie Resto</td>
</tr>
<tr>
<td></td>
<td>Partridge pea</td>
<td>0.125</td>
<td>0.25</td>
<td>Mohn/Prairie Resto</td>
</tr>
<tr>
<td></td>
<td>Prairie clover, purple</td>
<td>0.125</td>
<td>0.25</td>
<td>Mohn/Prairie Resto</td>
</tr>
<tr>
<td></td>
<td>Tic-seed, stiff</td>
<td>0.125</td>
<td>0.25</td>
<td>Mohn/Prairie Resto</td>
</tr>
<tr>
<td></td>
<td>Vervain, blue</td>
<td>0.125</td>
<td>0.25</td>
<td>Mohn/Prairie Resto</td>
</tr>
<tr>
<td><strong>SUB-TOTALS:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GRASSES:</td>
<td>50.0</td>
<td>98.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>WILDFLOWERS</td>
<td>1.0</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>FINAL TOTAL:</strong></td>
<td>51.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

*Seeding Rates: Seeding rates for grasses are Pure Live Seed (PLS); seeding rates for wildflowers are by bulk weight.

**Acceptable Varieties:**

**Native grasses and wildflowers** - Wild-types only are acceptable, except for sideoats grama, Kildeere or wild-type is acceptable.

**Non-native grasses** - Common varieties acceptable, Reubens Canada bluegrass is also acceptable.

**Approved Vendors:**

- Agassiz Seeds
  4121 1/2 South University Drive
  Fargo, ND 58104
  701-241-9760

- Mohn Seed Company
  Route 1, Box 152
  Cottonwood, MN 56229
  507-423-6482

- Prairie Restorations, INC
  Box 327
  Princeton, MN 55371
  612-389-4342
The intent of this project is to allow the prairie within the fence at T.H. 62 and 55 to expand outwards. In order to do this it was necessary to eliminate the existing vegetation that was seeded there in 1991. In addition, we observed that trefoil and crown vetch were beginning to invade the prairie remnant perimeters. We are planting mostly sideoats and blue grama, slender wheat grass and Canada wild-rye at the site with the idea that the big bluestem, Indian grass, switch grass, and forbs will be able to invade outwards into the gramas and cool-season natives because they are not dominant species at this site. We collected a small amount of seed from the remnant for installation at the time of seeding, however it is anticipated that it will take many years for the remnant to expand completely over the site.

The site was mowed in late July to prevent crown vetch and trefoil from setting seed. The site was then sprayed with Transline herbicide in mid-August to eliminate crown vetch, birds-foot trefoil and Canadian thistle. The site was sprayed again on 9/17/93 with a mixture of glyphosate and 2,4,D to eliminate existing exotic grasses and any broad-leaved species that survived the previous herbicide treatment.

The following seed mixture was installed at the crosstown prairie remnant site outside the chain-link fence on 9/24/93. Total rate for the mixture was 20 lbs PLS per acre.

<table>
<thead>
<tr>
<th>Species</th>
<th>Origin</th>
<th>Percent of Mix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue grama</td>
<td>Duel Co. SD wild-type</td>
<td>22%</td>
</tr>
<tr>
<td>Canada wild-rye</td>
<td>Isanti Co. wild-type</td>
<td>7%</td>
</tr>
<tr>
<td>June grass</td>
<td>Southern MN wild-type</td>
<td>5%</td>
</tr>
<tr>
<td>Little bluestem</td>
<td>Benton Co. wild-type</td>
<td>20%</td>
</tr>
<tr>
<td>Sideoats grama</td>
<td>Southern MN wild-type</td>
<td>22%</td>
</tr>
<tr>
<td>Slender wheat grass</td>
<td>Southern MN wild-type</td>
<td>8%</td>
</tr>
<tr>
<td>Regreen (cover crop)</td>
<td>Slender wheat x winter wheat</td>
<td>20%</td>
</tr>
</tbody>
</table>

The following species were collected off crosstown prairie remnant Fall 1993 and installed with the above native grasses. The amount of flower seed collected amounted to a few ounces for each species, with trace amounts for all native grasses except for big bluestem and Indian grass, for which we collected 2-3 lbs.

**Flowers**
- Alexanders (golden or heart-leaved)
- Bedstraw
- Bergamot
- Black-eyed susans
- Flowering spurge
- Grey-headed coneflower
- Mountain mint
- Rough blazingstar
- Stiff sunflower
- Thimbleweed

**Grasses**
- Big bluestem
- Indian grass
- Little bluestem
- Prairie/northern dropseed
- Sideoats grama
- Switch grass

- Zizia sp.
- Gallium sp.
- Monarda fistulosa
- Rudbeckia hirta
- Euphorbia corollata
- Ratibida pinnata
- Pycnanthemum virginianum
- Liatris aspera
- Helianthus rigidus
- Anemone cylindrica
- Andropogon gerardi
- Sorghastrum nutans
- Schizachyrium scoparium
- Sporobolus heterolepis
- Bouteloua curtipendula
- Panicum virgatum