



Length of trial: 4 years

Seeding rate: 6 lbs./1000 sq. ft.

Amount of seed needed of each entry: 18 lbs.

Deadline for receipt of seed by NTEP at Beltsville, MD: **August 23<sup>rd</sup>**.

Entry fee structure has been changed to the following:

1. **Named, commercially produced and/or marketed entry: \$2,000 per year, \$8,000 total.**
2. **Entry that has been previously entered in an NTEP trial: \$1,000 per year, \$4,000 total.**
3. **Experimental entry that has not been named, commercially produced and/or marketed: \$1,000 per year, \$4,000 total\*.**

**\* If the experimental entry is named, commercially produced or marketed during the testing period, NTEP will be notified within 30 days by the variety sponsor. At that time, the entry fee will be \$8,000 total for the trial. NTEP will immediately invoice the sponsor or licensee for any past entry fees. Current and future NTEP entry fees will be invoiced at \$2,000 per year. This will expire the date of the last trial data publication.**

#### STANDARD TRIAL LOCATIONS

*Number of standard trial locations: 14*

Schedule A (fairway)

- E. Lansing, MI - fairway
- Urbana, IL – fairway
- Lincoln, NE - fairway
- Amherst, MA – fairway
- College Park, MD – fairway

Schedule B (athletic field)

University Park, PA – athletic field  
Madison, WI – athletic field  
Blacksburg, VA – athletic field  
Riverside, CA – athletic field

Schedule C (home lawn)

New Brunswick, NJ – home lawn  
Ames, IA – home lawn  
Corvallis, OR – home lawn  
Columbia, MO – home lawn  
St. Paul, MN – home lawn

*Standard trial maintenance regimes*

SCHEDULE A (High Maintenance/Fairway)

1. Mowing height: 0.5" to 0.75"
2. Nitrogen rate (lbs. N/1000 sq. ft./growing month): 0.5 - 0.75
3. Irrigation: To prevent visual drought stress or dormancy
4. Fungicide use only if severe stand loss is possible
5. Weed and insect control to prevent stand loss

SCHEDULE B (Medium Maintenance/Athletic Field)

1. Mowing height: 1.0" to 2.0"
2. Nitrogen rate (lbs. N/1000 sq. ft./growing month): 0.5 - 1.0  
(high rate used during establishment only)
3. Irrigation: To prevent dormancy, only during severe stress or none
4. Fungicide use only if severe stand loss is possible
5. Weed and insect control to prevent stand loss

SCHEDULE C (Home Lawn)

1. Mowing height: 1.5" to 2.5"
2. Nitrogen rate (lbs. N/1000 sq. ft./growing month): 0.3 - 0.5
3. Irrigation: None, only during severe stress or to prevent dormancy
4. No fungicides or insecticides used
5. Weed control to prevent significant stand loss

*Standard trial data collection protocols*

Required for the standard trial locations:

- a. Establishment rate (% ground cover 2-4 weeks after seeding)
- b. Turfgrass quality ratings (taken monthly during each growing season of the test)
- c. Spring greenup ratings in second full year through last year of the test (2012-2014); not required in first year after seeding (2011)
- d. Genetic color ratings once in each full year of the test.

ANCILLARY TRIAL LOCATIONS

*Number of ancillary trial locations:* 13

Types of ancillary trials:

*Grey leaf spot* (two locations)

**New Brunswick, NJ**  
**Lexington, KY**

Protocol:

1. Seed in mid to late summer 2011 at both locations.
2. Protect one half of plots with fungicides, inoculate the other half of each plot to encourage seedling infection.
3. In summer 2012, inoculate or encourage infection on one-half of each plot that was protected in 2011, at both locations.
4. Run trial through 2014. This should provide one year of data on both seedling turf (at two locations) as well as potentially three years of data (from two locations) on mature turf tolerance.

*Salinity* (two locations)

**Ft. Collins, CO** – this is a field trial, irrigation water is 6 mmhos, soil salinity is probably much higher. Use the same water for germination, establishment and long-term maintenance.

**Kingston, RI** - greenhouse study (see Rebecca Brown's see protocol attached) – salt levels will be increased such that we can determine a threshold of salinity performance, trial will be repeated

*Drought* (two locations - one chronic and one acute)

**Puyallup, WA** – (chronic – deficit irrigation)

The trial will be seeded in fall 2010 on sand, using an old sport field research site on their research farm. Drought treatments will start the next spring/summer.

Protocol:

1. Chronic drought stress will be imposed during the first full growing season after all entries are fully established.
2. Prior to the initiation of drought stress, irrigation will be maintained at 100% of  $ET_0$ .
3. To impose chronic stress, during the 2011 growing season, irrigation levels will be lowered to the appropriate levels needed to induce sufficient drought stress.
4. Using a light box and camera, digital images will be collected weekly to document plot color, percent greenness and ground cover. During year two (2012), irrigation levels will again be set as noted above, unless it is determined by NTEP and the evaluator that the level needs to be adjusted.

NTEP will have access to reference ET values, also the soil water fraction available to the plants for both drought sites. Soil water fractions can be determined by testing the soil at each site.

**Blacksburg, VA** – acute (rainout shelter)

Trial will be seeded in fall 2010 and drought treatments will be initiated the next summer or fall. To evaluate acute drought tolerance, rainout shelters will be used. Rainout shelters repel natural rainfall when it appears, but are either removed from the plot area during dry periods or are built in such a way that rainfall is restricted. Therefore, the rainout shelters will be used to simulate drought periods by restricting rainfall for a 60-75 day period in summer. One drought period will be simulated in each of 2011 and 2012, in either summer or early fall.

Protocol:

1. Procedures for the acute testing are similar to those used by Karcher et al. in 2008. To initiate the simulated drought period, first the experimental area will be saturated with 2.5 cm of irrigation per day for three consecutive days to produce uniform wet conditions.
2. Drought conditions will be initiated in summer or fall 2011, and imposed for a period of time based on stress levels. The drought period will continue until all three plots of one entry are <5% green, then continue for an additional 10-20 days.
3. Using a light box and camera, digital images will be collected every 3-5 days to document drought response and cultivar greenness. These images will be collected during the entire drought period on every entry that has green tissue visible.

4. After the drought period, plots will be irrigated with 5 cm of irrigation to simulate drought recovery. Thereafter, the experimental area will be irrigated with 2.5 cm of irrigation weekly, to further evaluate drought recovery. Digital images will be collected weekly until all entries are 100% recovered.
5. Fungicides will be used on the plots to protect from disease (contact fungicides only will be used, no systemics that have a strong growth regulation affect, specifically DMIs and strobilins).
6. Drought will be imposed again in summer or fall 2012, using the above procedures.

*Traffic* (four locations)

**Madison, WI**  
**University Park, PA**  
**Blacksburg, VA**  
**Riverside, CA**

Traffic protocols:

1. Plots will be split with traffic vs. no traffic, there are effectively two tests in one physical location (standard and ancillary). Maintenance schedule B will be used.
2. Brinkmann is the traffic simulator to be used at all sites.
3. Larger plots (5' x 10') will be established.
4. Traffic protocol - seed in fall 2010, start the first traffic 'season' in late summer 2011, simulate 60 games over a ten week period, this is equivalent to six games per week split into two traffic applications per week.
5. Also conduct traffic 'seasons' in late summer 2012 and late summer 2013.
6. The use of herbicides to control poa annua will be allowed.
7. Visual cover ratings will be collected at the beginning of the traffic season and then every two weeks through the season (rate two of three: percent green ground cover, percent straw or bare soil).
8. A digital cover assessment will be done every 30 days to validate the visual ratings, also we will collect quality monthly throughout the growing season.
9. Visual ground cover ratings will be collected every two weeks during recovery period (until dormancy) and then upon spring growth until recovery.

NTEP reporting will rank entries by the ground cover percentage at the end of *traffic* seasons, not percent cover at end of *recovery* periods.

*Overseeding* (three locations)

**Tucson, AZ**

**College Station, TX**

**Gainesville, FL**

Overseeding protocols:

1. This is an on-site fairway trial.
  2. Start out at ¾” mowing height....go down to heights of ½” or less.
  3. Pick a spot(s) for rep(s) that receive the same level of traffic within the rep itself (all entries get more or less exposed to the same conditions within that rep. Reps can separate from each other within a fairway or even occur on more than one fairway.
  4. Seeding rates are regional. At the Texas and Florida locations, we will use 400 lbs./acre. In Arizona, we will seed at 600 lbs./acre.
  5. Plot size = width should be six feet (2 widths of 3 foot Gandy drop spreader).
  6. Total plot size should be around 36 sq. ft.
  7. Description of overseed preparation (water, scalp vertical mow, Primo, turflon ester, etc) will be available.
  8. Ratings: Once every 10 days after seeding for four ratings (% cover of overseed, % Bermuda if visible)
  9. Monthly ratings: color, quality, density, uniformity (because bermuda may be present and cause discontinuity).
  10. Rate factors above twice monthly when NON-OVERSEEDED BERMUDA CHECK is at 50% green. Take for 6-8 weeks (4 ratings).
  11. Repeat trial in year two.
5. Standard entries
- 1) Brightstar SLT (salt tolerant and grey spot susceptible)
  - 2) Top ranked cultivar in current NTEP trial over four years
  - 3) A top ranked cultivar for grey leaf spot in current NTEP trial
  - 4) A top ranked cultivar in current NTEP trial traffic studies
  - 5) A top ranked cultivar under Schedule C (no or low irrigation – for drought)
  - 6) Linn
  - 7) Mach I or Manhattan II (for PVP and National Grass Variety Review Board)
  - 8) Pinnacle (for PVP and National Grass Variety Review Board)

# URI Greenhouse Salt Screening Protocol

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Dr. Rebecca Brown February 2010

## *Initial Growing Conditions:*

Grass is grown in #50 sand. Nutrients are provided by modified Hoaglands solution, which is half-strength for the macronutrients and full strength for the micronutrients. Entries are seeded directly into the sand, at a rate approximating typical turf seeding rates for the species. We normally use 3” square pots, but have also used 5.5” azalea pots for testing experimental varieties. Grass is grown without salt stress until the plants are fully established and have tillered. This is usually 2-3 months from seeding under winter greenhouse conditions. Plants are kept clipped to an appropriate height for the species, either by hand or with a modified reel mower.

## *Salt Stress Conditions:*

The entries are randomly assigned to flats of 18 pots (6 with azalea pots) which are randomly arranged in hydroponics bays in the greenhouse. We usually use 6 replications, with 4 replications exposed to salt treatment and 2 retained as controls (hydroponic solution but no salt). Sometimes when all entries are of the same species we eliminate the controls and either use 6 replications to increase statistical power or 3 replications to double the number of possible entries. The greenhouse is maintained with the heat set at 45 degrees F and the fans at 60 degrees F. No supplemental light is used except when testing C4 grasses. We normally run tests from February to May or June.

The hydroponic bays work on an ebb-and-flow system, where salt water is pumped in to fill the bays and then allowed to drain out into storage tanks. The pumps are on timers set to run for 30 minutes at a time; they normally run twice a day but frequency can be increased if needed to keep the sand moist. The goal is to try and maintain the sand as close as possible to field capacity so that the salt level in the root zone is comparable to the salt concentration in the tanks. 30 minutes is sufficient to fill the hydroponic bays and saturate the sand for long enough that it reaches equilibrium with the solution.

The salt solution is modified Hoagland’s solution augmented with NaCl. We use NaCl because we are mostly interested in the effects of road salt. The target salt level and rate of salinity increase depends on the species. For perennial ryegrass we have increased salinity by 2500 ppm every week from 0-7500 ppm, and then every two weeks from 7500 ppm to 22500 ppm. Two weeks is sufficient time for salt stress symptoms to develop; we have not seen symptoms on PR below 7500 ppm. Data is collected at the end of each 2-week treatment period. Salt concentration is monitored with a NaCl meter and water added to replace that lost to transpiration.

## *Data Collection:*

We use digital photography to collect the data. We have a controlled lighting box which can be set over flats in the greenhouse; each flat is photographed individually. Prior to photographing the plants are topdressed with dry sand so that the surface is uniform and free of algae, and the only source of green is the healthy leaf tissue. The camera uses a fixed white balance and exposure, so that conditions are identical across all images. Photographs are taken before the start of the salt treatment and at the end of every 2 weeks.

Lines are added in Photoshop to demarcate the individual pots within each flat, and the images are cropped to remove anything outside the flat. We then use a macro in SigmaScan to split the images of the flats into individual pot images labeled by their flat and position in the flat. A separate macro is used to measure the total pixels and number of green pixels in each image and calculate the % green cover. If all pots have 100% turf cover at the start of the experiment we can use the SigmaScan results without transformation. Otherwise we use the pre-stress images as maximum cover, and transform the percent cover at each salt level by converting it to a percentage of the pre-stress cover. This allows us to compare plants of different sizes. When comparing across species we transform the experimental data using the mean percent cover for the control pots of each entry photographed on the same day. This allows us to compensate for differences in growth habit, response to changes in daylength, and response to the hydroponic conditions among species. Data analysis is done using repeated measures general linear model ANOVA with the salt level as the repeated variable. We can of course also compare among entries at a specific salt level. The system is not sufficient to guarantee performance of a genotype at a specific salt level, but it is effective at determining relative tolerance and would provide a starting point for field testing.

#### *System Capacity:*

We currently have 4 hydroponics bays for a total of ~170 square feet, and can accommodate 100 flats, which is equivalent to 1800 3” pots or 600 5.5” pots.

#### *Trial Duration and Costs:*

The trial will be conducted in winter/spring 2011 and then repeated the next winter/spring.