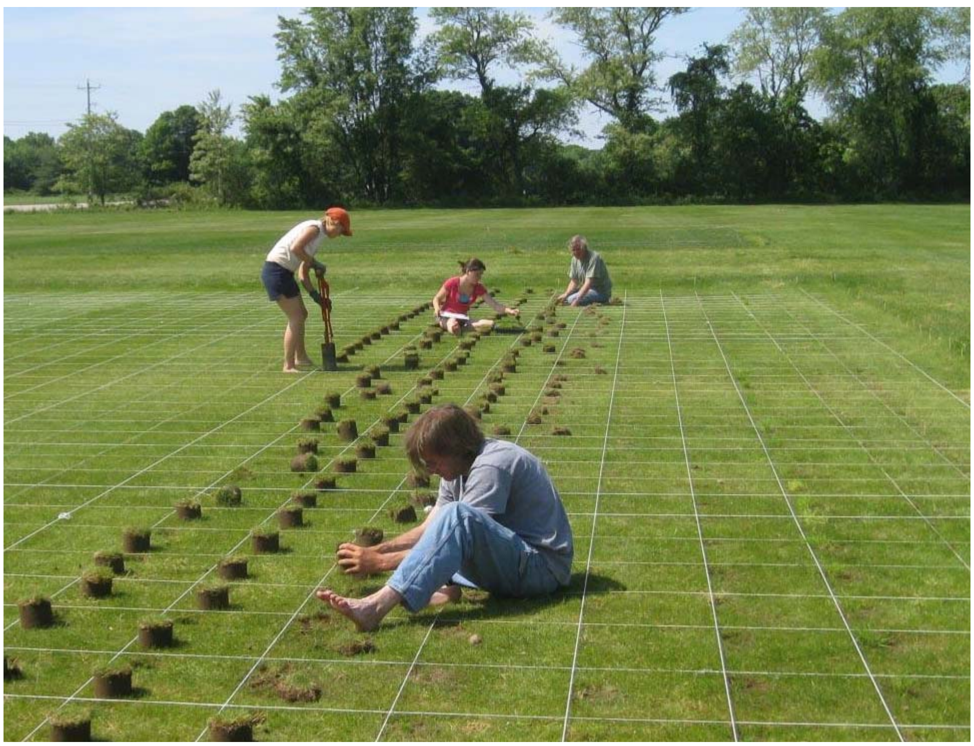
# Turfgrass and Environmental Research Online

Using Science to Benefit Golf



University of Rhode Island researchers collected vegetative plugs of velvet bentgrass (*Agrostis canina*) from golf courses in Rhode Island, Connecticut, and Massachusetts to create the New England Velvet Bentgrass Collection. Accessions are being tested for salt tolerance in hopes of improving the salt tolerance of future velvet bentgrass cultivars.

**Volume 10, Number 14**
July 15, 2011

## PURPOSE

The purpose of *USGA Turfgrass and Environmental Research Online* is to effectively communicate the results of research projects funded under USGA's Turfgrass and Environmental Research Program to all who can benefit from such knowledge. Since 1983, the USGA has funded more than 400 projects at a cost of $31 million. The private, non-profit research program provides funding opportunities to university faculty interested in working on environmental and turf management problems affecting golf courses. The outstanding playing conditions of today's golf courses are a direct result of ***using science to benefit golf***.


### Editor

Jeff Nus, Ph.D.
1032 Rogers Place
Lawrence, KS 66049
jnus@usga.org
(785) 832-2300
(785) 832-9265 (fax)

### Research Director

Michael P. Kenna, Ph.D.
P.O. Box 2227
Stillwater, OK 74076
mkenna@usga.org
(405) 743-3900
(405) 743-3910 (fax)

# Assessment of Salt Tolerance in Velvet Bentgrass

Rebecca Nelson-Brown and Geunhwa Jung

**SUMMARY**

University of Rhode Island researchers collected vegetative plugs of velvet bentgrass from putting greens on golf courses in southern New England to form the New England Velvet Bentgrass Collection. Ninety-eight accessions were confirmed as diploid velvet bentgrass using flow cytometry. The collection was screened for salt tolerance in the greenhouse.

- Velvet bentgrass is considered to be extremely sensitive to salt in soil and irrigation water, but the salt $LD_{50}$ values for 183 clones screened ranged from <3,000 ppm to >14,500 ppm NaCl.
- Seventy-three accessions had $LD_{50}$ values of 6,000 ppm or less, similar to the standard varieties 'SR7200' and 'Greenwich'.
- Twenty-five accessions had salt tolerance superior to the standard varieties, with $LD_{50}$ values ranging from 7,000 ppm to over 14,000 ppm.
- Twenty-five percent of diploid accessions screened retained at least 50% green cover after two weeks at 6,000 ppm sodium chloride.
- Sixteen accessions retained at least 50% green cover after 2 weeks at 9,000 ppm sodium chloride.
- Two accessions retained more than 50% green cover after 2 weeks at 14,000 ppm sodium chloride.
- Genetic potential exists to develop velvet bentgrass varieties with moderate salt tolerance.

In the earliest days of golf, South German Bent was considered to be the very best seed for use on putting greens. South German Bent was a seed mixture which contained up to 50% velvet bentgrass (*Agrostis canina*) and little or no actual creeping bentgrass (*Agrostis stolonifera*) (2, 6). It was the velvet bentgrass which made South German Bent greens superior to greens seeded with Rhode Island bentgrass or colonial bentgrass (*Agrostis capillaris*)(10). Piper and Oakley recommended velvet bentgrass for use north of Philadelphia and creeping bentgrass for use in the transition zone (11, 12).

Until the 1950s, bentgrass for use on greens was often propagated vegetatively, as there were no sources of pure seed. However, changes in bentgrass seed availability in the 1930s and 1940s led to most courses using creeping bentgrass. These changes included the introduction of Oregon-grown certified creeping bentgrass seed into the market in the 1920s (6), the collapse of the German bentgrass seed industry, and the introduction of 'Penncross' creeping bentgrass in 1957 (3). At the same time, golf course management practices changed dramatically in ways that favored creeping bentgrass.

Velvet bentgrass is more tolerant of low nitrogen fertility than creeping bentgrass. The dense shoot growth of velvet bentgrass prevents incursions of annual bluegrass, crabgrass, and other weeds. Velvet bentgrass is also more drought tolerant and less susceptible to dollar spot (*Sclerotinia homoeocarpa*) than creeping bentgrass. All of these traits were advantageous when compost topdressings were a significant source of

Greenhouse studies were conducted to screen accessions of the New England Velvet Bentgrass Collection for salt tolerance at the University of Rhode Island.

REBECCA NELSON BROWN, Ph.D., Assistant Professor, Department of Plant Sciences and Entomology, University of Rhode Island, Kingston, RI; and GEUNHWA JUNG, Ph.D., Associate Professor, Department of Plants, Soil and Insect Sciences, University of Massachusetts, Amherst, MA.

*USGA Turfgrass and Environmental Research Online* 10(15):1-7.
TGIF Record Number: 185476

fertility for greens, irrigation systems were uncommon, and pesticide options were very limited.

With the advent of inexpensive nitrogen fertilizers, synthetic pesticides, and irrigation systems, superintendents could compensate for the weaknesses of creeping bentgrass. The faster damage repair, darker color, and greater heat tolerance of creeping bentgrass, combined with the tendency of velvet bentgrass to develop thatch under high fertility led to velvet bentgrass being considered difficult to manage (4). However, management practices are changing again in ways that make the strengths of velvet bentgrass more desirable and the corresponding weaknesses of creeping bentgrasses more problematical.

**Why Salt Tolerance?**

One area in which management practices are changing is irrigation. Water supplies have become limited in many areas, and golf courses are increasingly being required to use reclaimed water for irrigation. Reclaimed water is higher in salts than potable water, and use of reclaimed water can increase soil salt levels (13).

Some varieties of creeping bentgrass have excellent salt tolerance, but velvet bentgrass has generally been considered to be extremely sensitive to salt. In a 2001 study of salt tolerance in 35 bentgrass cultivars, the velvet bentgrass cultivar 'Avalon' (SR7200) died after 5 weeks at a salinity of 8 dS/m while 29 of 33 creeping bentgrass cultivars survived 10 weeks of treatment (9).

In a multi-species study Brod and Pruesse (5) found that the velvet bengrass cultivar 'Novobent' had salinity tolerance similar to 'Bardot' colonial bentgrass and was more sensitive than the red fescue cultivars 'Dawson' and 'Ensylva', the Kentucky bluegrass cultivar 'Skofti', and the perennial ryegrass cultivars 'Loretta' and 'Pelo'. Colonial bentgrass has been shown to tolerate only low salinity levels of 2-3 dS/m (8). The classification of velvet bentgrass as extremely sensitivity to salt is based on testing of only two varieties in separate tests each of which utilized only a single level of salt stress.

The objective of the study reported here was to screen a large number of velvet bentgrass accessions for tolerance to salt in order to determine if there is variation within the species that can be utilized to develop cultivars with improved salt tolerance.

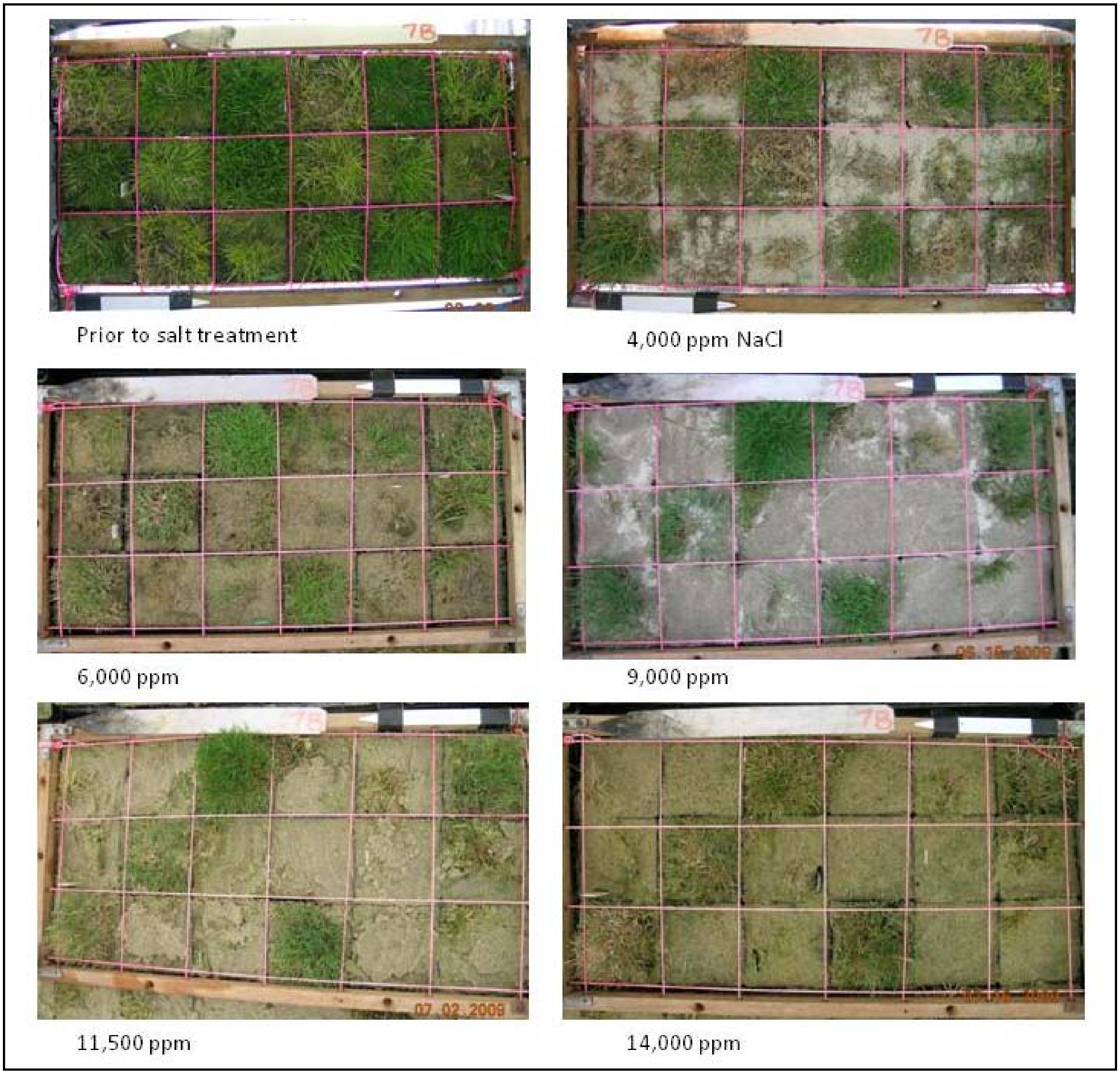
**The New England Velvet Bentgrass Collection**

Velvet bentgrass faded from popularity in the mid twentieth century as turfgrass breeding was in its infancy. As a result, the species has received little attention from breeders, particularly compared to creeping bentgrass. There are six velvet bentgrass varieties currently on the market. Prior to the release of 'SR7200' in 1993 and 'Greenwich' in 2000, the only velvet bentgrass seed variety commercially available in the United States was 'Kingstown', which was released in 1963 and based on a single inbred selection made in 1929 (14). In comparison, there are over 55 varieties of creeping bentgrass currently available in the United States. Like most turfgrasses, velvet bentgrass is not well represented in public germplasm repositories, which tend to focus their resources on important food crops.

In order to develop improved velvet bentgrass varieties or use traits from velvet bentgrass to improve the stress and disease tolerance of creeping bentgrass, breeders must have access to velvet bentgrass germplasm. One source is wild velvet bentgrass populations, most of which are found in Europe. However, these populations may not be well adapted to golf course conditions or to the soils and climates of the United States.

Fortunately there is another source of velvet bentgrass germplasm: the many golf courses in New England that date back to the earliest years of golf. The greens at these courses were built in the first decades of the twentieth century when South German Bent was the seed mix of choice. Over the past 80-100 years, the strongest and best-adapted velvet bentgrasses from the original mix have come to dominate the greens.

Another source of velvet bentgrass germplasm is the golf courses in southern New England that were constructed in the 1960s using




Accession of velvet bentgrass prior to salt treatment, 4,000, 6,000, 9,000, 11500, and 14000 ppm NaCl. Photos were taken after two weeks treatment at each level. The pink grid was used to divide the larger image into individual pots using SigmaScan.

'Kingstown' velvet bentgrass. While 'Kingstown' was never popular nationally, it was released by the Rhode Island Agricultural Experiment Station and was widely used in Rhode Island. As with the South German Bent, the strongest individuals from the original 'Kingstown' seed have come to dominate the greens.

The New England velvet bentgrass collection is a set of over 250 accessions that were collected from eleven golf courses in Rhode Island, Connecticut, and Massachusetts and from naturally occurring velvet bentgrass patches on the research farms at the University of Rhode Island and the University of Massachusetts. The collection is maintained at both University of Rhode Island and University of Massachusetts where the accessions have been evaluated for a variety of traits and are being converted to seed populations for long-term preservation and distribution through the USDA Germplasm Repository System.

## Materials and Methods

The New England Velvet Bentgrass Collection was screened for salt tolerance in the greenhouse at the University of Rhode Island. Each accession was vegetatively propagated from stolons to produce three genetically identical plugs. The plugs were transplanted into 3-inch pots filled with #50 sand and placed in an ebb-and-flow hydroponics system.

In an ebb-and-flow system, the nutrient solution is stored in tanks and periodically pumped into the bench containing the plants to saturate the sand. After 30 minutes, the nutrient solution is drained back into the tanks, permitting the sand to return to field capacity. The frequency of flooding is adjusted depending on the plants' transpiration rate so that the sand remains wet. The bentgrass accessions were flooded with a solution containing complete nutrients but no sodium chloride until the plugs had grown to cover the surface of the pots. All plugs were mowed at 0.5 inches with a reel mower modified for use on potted plants. The commercial varieties 'Greenwich' and 'SR7200' were included as standards comparisons.

When most of the accessions had reached 100 percent cover, the hydroponics tanks were drained and refilled with a solution containing complete nutrients plus 1,000 ppm sodium chloride. The plants were grown in this solution for two weeks. After two weeks, sodium chloride was added to increase the salinity to 2,000 ppm. The salinity continued to be increased by 1,000 ppm every two weeks until the solution reached 7,000 ppm sodium chloride. After two weeks at 7000 ppm, the salinity was increased to 9,000 ppm. Two weeks later the salinity was increased by 2,500 ppm to 11,500 ppm. The final increase brought the solution salinity to a level of 14,000 ppm sodium chloride.

The bentgrass accessions continued to be mowed as needed to maintain a height of 0.5 inch. Prior to the initiation of salt treatment and again at the end of each two week period, plants were lightly topdressed with dry sand and each flat of 18 plants was photographed using a digital camera and a portable light box. By photographing the plants every two weeks, we were able to record the loss of green tissue as salinity increased. The computer program SigmaScan was used to separate the image of each flat into images of the 18 individual pots and to calculate the percent green cover for each pot.

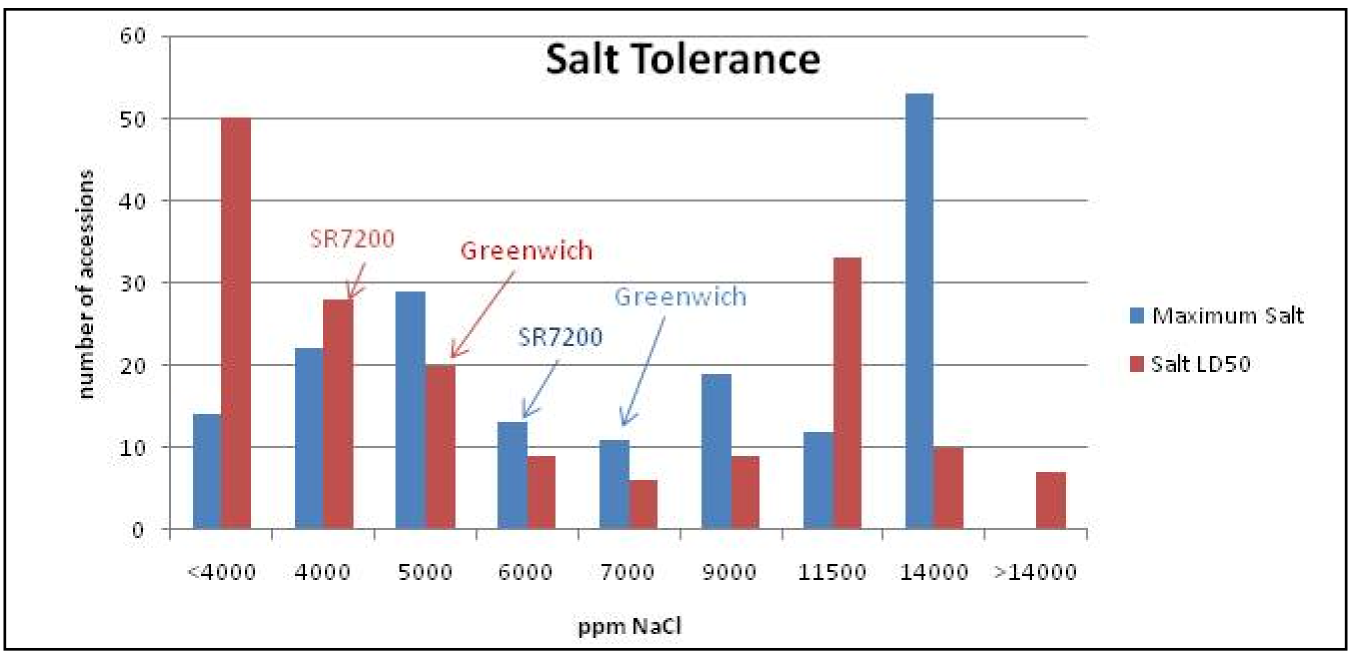



**Figure 1.** Distribution of salt LD50 and maximum tolerance values within the New England Velvet Bentgrass collection. Thirty-nine percent of the accessions were more tolerant than the standard varieties 'SR 7200' and 'Greenwich', indicating that salt tolerance of velvet bentgrass could be improved by selection.

Topdressing with dry sand prior to taking pictures prevented any algae growing in the pots from inflating the percent green cover. Green cover values were adjusted for each pot by the cover in the image taken just prior to the beginning of the salt treatment. This permitted us to compensate for differences in initial plant size.

**Salt Tolerance in the New England Collection**

We screened 183 accessions. For each accession we determined two salt tolerance values: the $LD_{50}$ and the maximum tolerance level. The $LD_{50}$ was the salinity level at which green cover dropped below 50%; the maximum tolerance level was the point at which green cover dropped below 10%. It is expected that the bentgrass accessions would survive long-term exposure to salinity up to the $LD_{50}$, and that they would recover from brief exposure to salinity levels between the $LD_{50}$ and the maximum tolerance level.

$LD_{50}$ values for the collection as a whole ranged from less than 3,000 ppm to greater than 14,000 ppm with a mean of 6,700 ppm (Figure 1). Maximum tolerance levels ranged from 3,000 ppm to greater than 14,000 ppm with a mean of 8,400 ppm. The standard varieties 'SR7200' and 'Greenwich' had $LD_{50}$ values of 4,000 ppm and 5,000 ppm, respectively. Their maximum tolerance levels were 6,000 and 7,000 ppm, respectively. These values are below the means for the collection as a whole, so it should be possible to develop varieties with improved salt tolerance.

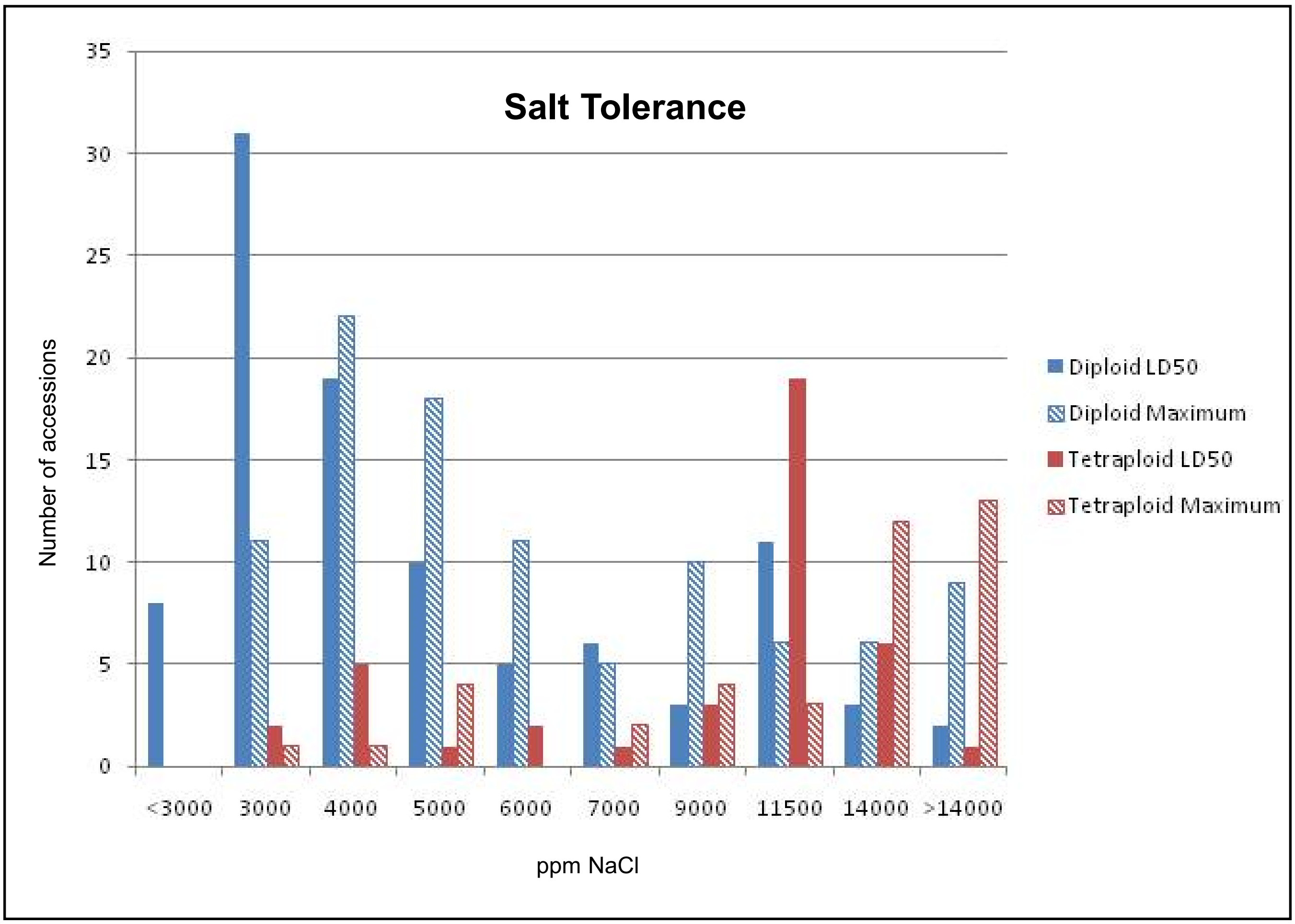



**Figure 2.** Differences in salt tolerance between the diploid and tetraploid accessions in the New England Velvet Bentgrass collection. As a group the tetraploids were more salt tolerant, but the range of tolerances were very similar for the two ploidy levels. None of the accessions had been specifically bred for salt tolerance.

### Is It Really Velvet Bentgrass?

Given velvet bentgrass' reputation as salt sensitive, we were surprised to find accessions that retained greater than 50% green cover after two weeks at 14,500 ppm sodium chloride. South German mixed bent contained creeping bentgrass as well as velvet bentgrass, and in 80-100 years, it is likely that most of the greens sampled had occasionally been overseeded with creeping bentgrass. Similarly, 'Kingstown' velvet bentgrass seed supplies were known to be contaminated with creeping bentgrass. When we collected the New England accessions, we selected individual bentgrass patches which had the fine texture associated with velvet bentgrass, but some creeping bentgrasses are very fine when mowed at greens height. Thus it was possible that some of the accessions in the collection were creeping bentgrass rather than velvet bentgrass.

The most reliable way to distinguish between creeping bentgrass and velvet bentgrass is to measure the DNA content of individual cells. Creeping bentgrass is a tetraploid, with four copies of each chromosome, while velvet bentgrass is diploid, with only two copies. Thus each cell from a creeping bentgrass plant contains twice as much DNA as a cell from a velvet bentgrass plant. The DNA content of individual cells is easily measured using a process called flow cytometry. In flow cytometry, leaf tissue is macerated to release the cell nuclei, and the nuclei are suspended in a solution containing a fluorescent dye which stains DNA. The nuclei are then injected into a computerized cell sorter which passes the nuclei one at a time past a laser beam, causing the dye to fluoresce, and records the fluorescence level. Tetraploid nuclei fluoresce twice as much as diploid nuclei.

We were able to obtain reliable ploidy data from three-quarters of the accessions that were screened for salt tolerance. Forty accessions were in fact tetraploids, and thus are likely creeping bentgrass. The other 98 accessions were confirmed as velvet bentgrass. The tetraploid accessions as a group were more salt tolerant than the diploid accessions, with an $LD_{50}$ range of 3,000 ppm to more than 14,000 ppm, a mean of 8,400 ppm, and a median of 11,500 ppm (Figure 2).

The diploid accession mean was only 5,400 ppm and the median was 3,000 ppm. However, there were two diploid accessions which retained more than 50% green cover at 14,000 ppm, and 16 diploid accessions with $LD_{50}$ values of 11,500 ppm or higher. Thus while velvet bentgrass as a species does appear to be more sensitive to salt than creeping bentgrass as a species, it should be possible to develop velvet bentgrass varieties which can tolerate at least moderate salinity levels. Development of such varieties would facilitate use of drought-tolerant velvet bentgrass on greens irrigated with reclaimed water.

### Acknowledgements

URI undergraduate students Amanda Zweigler and Rebecca Little assisted with maintenance and data collection for the salt screening study. This research was funded by a grant from the USGA Turfgrass and Environmental Research Program. Additional funds were provided by the University of Rhode Island and the University of Massachusetts.

### Literature Cited

1. Alderson J. S., and W. C. Sharp. 1995. Grass varieties in the United States. CRC Press, Boca Raton, FL. (TGIF Record 39828)

2. Anonymous. 1922. South German mixed bent seed. *Bulletin of the Green Section of the USGA* 2(2):31. (TGIF Record 47921)

3. Beard, J. B., and H. J. Beard. 2005. Beard's turfgrass encyclopedia for golf courses, grounds, lawns, sports fields. Michigan State University Press, East Lansing, MI. (TGIF Record 93886)

4. Brilman, L. A., and W. A. Meyer. 2000. Velvet bentgrass: Rediscovering a misunderstood turf-

grass. *Golf Course Management* 68:70-75. (TGIF Record 68883)

5. Brod, H. G., and H. U. Preusse. 1980. The influence of deicing salts on soil and turf cover. Pages 461-468. *In* J. B. Beard (ed). Proc. 3rd Int. Turfgrass Res. Conf., 11-13 July, 1977, Munich, Germany. ASA, CSSA, SSSA, and ITS, Madison, WI. (TGIF Record 17)

6. Hillman, F. H. 1921. South German mixed bent seed described. *Bulletin of the Green Section of the USGA* 1(3):37-39. (TGIF Record 47553)

7. Hyslop, G. R. 1930. Oregon bentgrass seed certification. *Bulletin of the Green Section of the USGA* 10(11):195-200. (TGIF Record 36572)

8. Marcum, K. 2008. Relative salinity tolerance of turfgrass species and cultivars. Pages 389-406. *In* M. Pessarakli (ed.). Handbook of Turfgrass Management and Physiology. CRC Press, Boca Raton, FL. (TGIF Record 128346)

9. Marcum K. B. 2001. Salinity tolerance of 35 bentgrass cultivars. *HortSci.* 36:374-376. (TGIF Record 73304)

10. Piper, C. V., and R. A. Oakley. 1921. The turfgrass seeds to buy and how to buy them. *Bulletin of the Green Section of the USGA* 1:14-17. (TGIF Record 47534)

11. Piper, C.V., and R.A. Oakley. 1921. Vegetative propagation of putting green grasses. *Bulletin of the Green Section of the USGA* 1(7):124-126. (TGIF Record 47596)

12. Piper, C. V., and R. A. Oakley. 1922. Some observations on velvet bent. *Bulletin of the Green Section of the USGA* 2(6):171-173. (TGIF Record 47963)

13. Qian Y., and A. Harivandi. 2009. Salinity issues associated with recycled wastewater irrigation of turfgrass landscapes. Pages 419-429. *In* M. Pessarakli (ed.). Handbook of Turfgrass Management and Physiology. CRC Press, Boca Raton, FL. (TGIF Record 128348)

14. Rose-Fricker, C. A., M. L. Fraser, W. A. Meyer, and S. A. Bonos 2004. Registration of ‘Greenwich’ velvet bentgrass. *Crop Sci.* 44:349-350. (TGIF Record 92567)