Received: 9 February 2021 | Revised: 30 March 2021 | Accepted: 8 April 2021

DOI: 10.1002/agg2.20170

Agrosystems, Geosciences & Environment OPEN ACCESS

ORIGINAL RESEARCH ARTICLE

Agrosystems

Evaluating the freeze tolerance of bermudagrass genotypes

Lakshmy Gopinath[1] | **Justin Quetone Moss**[1] | **Yanqi Wu**[2]

[1] Dep. of Horticulture and Landscape Architecture, Oklahoma State Univ., Stillwater, OK 74078, USA

[2] Dep. of Plant and Soil Sciences, Oklahoma State Univ., Stillwater, OK 74078, USA

Correspondence
Lakshmy Gopinath, Dep. of Horticulture and Landscape Architecture, Oklahoma State Univ., Stillwater, OK 74078, USA.
Email: lakshmy.gopinath@okstate.edu

Assigned to Associate Editor Larry Redmon.

Funding information
National Institute of Food and Agriculture, Grant/Award Numbers: 2019-51181-30472, OKL03146; Oklahoma Center for the Advancement of Science and Technology, Grant/Award Number: AR16-051-3

Abstract

Bermudagrasses (*Cynodon* spp.) are commonly used in golf courses, athletic fields, and home lawns in the transitional climatic region of the United States. Winterkill, however, is a major concern for bermudagrasses grown in this region. Controlled environment testing is a reliable method to evaluate freeze tolerance contributing to the selection of freeze tolerant genotypes. Therefore, this study was designed to test the freeze tolerance of two elite experimental genotypes, OKC1873 and OKC1406, along with two industry standards (‘Tifway’ and ‘Tahoma 31’), by exposing them to various freeze temperatures (−4 to −14 °C) in a controlled environment. The freezing test was replicated in time, and the mean lethal temperature to kill 50% of the population (LT_{50}) for each genotype was determined. Tifway (freeze-sensitive standard) had an LT_{50} value of −7.0 °C. The genotype OKC1873 (−7.2 °C), was in the same statistical group as Tifway. Tahoma 31 was the best-performing genotype with the lowest LT_{50} value of −9.1 °C. The genotype OKC1406 (−8.8 °C) was in the same statistical group as Tahoma 31. Top-performing experimental genotypes will move on for further screening in replicated field trials for future consideration for commercial release based on qualities such as improved freeze tolerance, desirable turfgrass quality, and sufficient disease resistance.

1 | INTRODUCTION

Winterkill is a general term used to define turfgrass loss during the winter (Beard, 1973). Winterkill or winter survivability depends on various factors, including crown hydration, desiccation, direct low temperatures, ice sheets, and snow mold (Beard, 1973). Restoring the turfgrass lost to winterkill is labor intensive and expensive. Intensively managed areas such as golf courses and athletic fields are predisposed to winterkill due to aggressive fertilization programs, low mowing heights, and vehicular and foot traffic (Hartwiger & Moeller, 2015; Richardson, 2002). Bermudagrasses (*Cynodon* spp.) are the most commonly used turfgrasses on home lawns, athletic fields, golf courses, and other utility turfgrass areas in the U.S. transition zone. Bermudagrass, a warm-season turfgrass, is regarded as having excellent tolerance to heat and drought but low tolerance to freezing temperature (Beard, 1973). Developing bermudagrasses with better freeze tolerance is a priority of bermudagrass germplasm improvement programs.

Past research has reported significant variation in freeze tolerance in bermudagrass cultivars, indicating that genetic improvement for freeze tolerance could be achieved (Anderson et al., 1993, 2002; Dunne et al., 2019). Many studies have been conducted to determine the freeze tolerance of bermudagrass in controlled environments by estimating the temperature to kill 50% of the population (LT_{50}) (Anderson

Abbreviations: LT_{50}, lethal temperature to kill 50% of the population.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
© 2021 The Authors. *Agrosystems, Geosciences & Environment* published by Wiley Periodicals LLC on behalf of Crop Science Society of America and American Society of Agronomy

et, al., 1993, 2002). The LT_{50} values obtained in controlled environment studies showed significant negative correlations to spring green-up and positive correlation to winterkill estimated in the field (Dunne et al., 2019; Patton & Reicher, 2007). Although field evaluations are common for plant breeders to assess winter survivability of a large number of genotypes, environmental conditions in the field are unpredictable and difficult to replicate (Anderson et al., 2002; Wu & Anderson, 2011). Controlled environment studies can quickly identify genotypic differences in freezing tolerance based on exposure to direct freezing temperatures. Therefore, the objective of this study was to determine the LT_{50} values of two experimental interspecific hybrid bermudagrass genotypes and two commercially available cultivars by exposing these to 11 target freezing temperatures (−4 to −14 °C) under controlled environment conditions.

Core Ideas

- Controlled environment evaluation of freeze tolerance provides valuable information for breeders to gauge the genetic gain in breeding winter-hardy bermudagrasses.
- 'Tahoma 31' was the top-performing cultivar in this study.
- OKC1406 had an LT_{50} value significantly lower than 'Tifway' and similar to Tahoma 31, indicating superior freeze tolerance.

2 | MATERIALS AND METHODS

2.1 | Plant materials and growing conditions

The study consisted of two experimental genotypes, OKC1873 and OKC1406, developed by the bermudagrass breeding program at Oklahoma State University (OSU) and two industry standards, 'Tifway' (a freeze-sensitive standard) and 'Tahoma 31' (a freeze-tolerant standard). The study was replicated in time, with staggered planting to allow uniform establishment periods (Anderson et al., 2007). All genotypes were clonally propagated in potting mix (Berger BM 2 propagation mix) in cone-tainers (RayLeach Cone-tainer Nursery) of 21-cm depth and 3.8-cm diameter. A single phytomer consisting of a root, crown, and shoot material was used as the propagation material for each cone-tainer. The bermudagrasses were established in a plant growth chamber (PGC Flex growth chamber) at the OSU Controlled Environment Research Laboratory, Stillwater, OK. The chamber was maintained at 32/28 °C day/night temperatures for 13 wk with a photoperiod of 14 h and a photosynthetically active radiation (PAR) of 900 µmol m^{-2} s^{-1}. The cone-tainers were fertilized weekly with a general-purpose 20–10–20 N–P–K fertilizer (J.R Peters) at 0.6 g L^{-1} and trimmed to maintain a height of 2.5 cm. As a precautionary measure during the establishment phase, the cone-tainers were treated every 14 d with bifenthrin (Talstar insecticide, FMC Corporation Agricultural Products Group). At the end of 13 wk, the temperatures were lowered to 24/20 °C day/night for a week to pre-acclimate the cone-tainers prior to cold acclimation. The cone-tainers were then subjected to cold acclimation by lowering the temperature to 8/2 °C day/night for 4 wk with a photoperiod of 10 h and a PAR of 400 µmol m^{-2} s^{-1}.

2.2 | Freeze treatment

At the end of cold acclimation, the cone-tainers were placed into a freeze chamber (E8 plant growth chamber, Conviron). Ten thermocouple sensors were inserted 2.5 cm into the potting medium at the center of randomly selected cone-tainers to monitor the soil temperature. Ice chips were placed on all cone-tainers to prevent supercooling and induce freezing. The freeze chamber was programmed to stay at −3 °C for 18 h for the dissipation of latent heat and then cool linearly at the rate of 1 °C h^{-1}. The 11 target temperatures (1 ˚C interval, −4 to −14 ˚C) covered a range anticipated to span the limits from complete survival to complete mortality. When each target temperature was reached, four cone-tainers of each genotype (16 cone-tainers in total) were removed immediately. These cone-tainers were moved to a plant growth chamber set at 4 °C overnight to induce thawing. The temperature was increased to 24/20 °C for a week and then to 32/28 °C to encourage recovery. The regrowth based on shoot emergence was visually evaluated after 5 wk using binary values (1 = alive, 0 = dead).

2.3 | Experimental design and statistical analysis

The LT_{50} values for each genotype were determined by logistic regression using the PROC PROBIT procedure (SAS version 9.4, SAS Institute) (Qian et al., 2001; Shahba et al., 2003). The probit procedure generated a table of predicted percentage survival at each temperature, and the temperatures corresponding to 50% survival were used as the estimates of LT_{50} for each genotype. Since the freeze test was repeated three times, there were three LT_{50} values for each genotype. The LT_{50} of each replication was treated as a response variable and subjected to the ANOVA procedure. Means were separated using Fisher's protected LSD when F tests were significant at $P \leq .05$.

TABLE 1 Mean lethal temperatures resulting in 50% survival (LT_{50}) of four bermudagrass genotypes when exposed to temperatures ranging from −4 to −14 °C under controlled environment conditions

Genotype/cultivar[a]	LT_{50}
	°C [b]
Tifway	−7.0a[c]
OKC1873	−7.2a
OKC1406	−8.8b
Tahoma 31	−9.1b
LSD (.05)	0.5
CV, %	3.3

[a]The two standard freeze tests were conducted on three dates for this batch, constituting replications in time.
[b]Lethal temperature to kill 50% of the population (LT_{50}) values were calculated using the PROC PROBIT procedure (SAS version 9.4; SAS Institute) based on regrowth that was visually evaluated after five weeks using binary values (1 = alive, 0 = dead).
[c]Mean separation in column by Fisher's protected LSD test at $P \leq .05$.

3 | RESULTS AND DISCUSSION

There were significant differences among the genotypes in the LT_{50} values. Tifway had the highest LT_{50} (low freeze tolerance) numerically (Table 1), which was similar to the values reported for this cultivar by Anderson et al. (2002, 2003) (−7.9 °C) and Anderson et al. (2007) (−7.7, −7.8, and −8.0 °C). However, the LT_{50} value was notably lower than that obtained by Dunne et al. (2019) (−5.4 °C). This discrepancy could be attributed to the differences in acclimation temperatures and recovery periods between the two studies. The samples in their study had a shorter establishment period and were acclimated at a higher temperature. The lower acclimation temperatures in our study could have induced a greater level of acclimation. In the same study, multiyear (2011–2015) field testing results indicated that Tifway had the highest winter survival among four commercial standards ('Patriot', Tifsport', 'Quickstand', and Tifway) in 2013, 2014, and 2015 (Dunne et al., 2019). This conflicts with other national reports, which showed that Tifway had high winterkill percentages in Indiana and Kentucky (NTEP, 2014). The inconsistency in the winter survival of Tifway could be due to the differences in environmental conditions and genotype × environment interactions during the acclimation period and the winter.

The low LT_{50} value of Tahoma 31 is consistent with field observations, exhibiting the least winterkill percentage of 4 and 25% in Indiana and Kentucky, respectively, with superior post-dormancy regrowth (NTEP, 2014). Tahoma 31 quickly recovered and reached 75% green coverage within 22 d after chilling stress was removed (Fontanier et al., 2020), indicating high recovery potential after freeze temperatures. The LT_{50} values of Tahoma 31 in this study were similar to the values obtained by Gopinath et al. (2021) (−7.8, −8.8, and −9.0 °C). Tahoma 31 had a turfgrass quality rating above 6 in five out of seven locations in the preliminary data of the National Turfgrass Evaluation Program's warm-season putting trial (NTEP, 2020), indicating its ability to tolerate lower mowing heights. With low LT_{50} reported in this study and the ability to tolerate a low mowing height, Tahoma 31 will serve as an ideal cultivar for fairways and/or putting greens in the U.S. transition zone.

OKC1873 was not significantly different from Tifway, suggesting its range of use should be similar to Tifway (Table 1). OKC1406 was in the same statistical group as Tahoma 31. The result is consistent with a previous report in which OKC1406 was ranked sixth among 53 experimental genotypes for winter survival tested in Kansas (Xiang et al., 2019). OKC 1406 had a higher winter survival percentage (88.3%) than industry standards Tifway (0%), 'Latitude 36'(20%), 'NorthBridge' (25%), Patriot (30%), and 'TifTuf' (23%). The higher winter survival percentage of OKC1406 than some of the current industry standards and the LT_{50} value similar to Tahoma 31 in this study indicate its high freeze tolerance. However, multilocation and multiyear testing of the experimental genotypes are required to evaluate turfgrass quality, mowing tolerance, and pest and disease resistance.

4 | CONCLUSION

The controlled-environment investigation revealed that OKC1406 was as freeze tolerant as Tahoma 31, whereas OKC1873 had freeze tolerance similar to Tifway. These evaluations provide valuable information for plant breeders to decide whether the experimental bermudagrass genotypes tested should be subjected to further evaluation. Using freeze-tolerant bermudagrass genotypes will help golf courses or athletic facilities decrease costs associated with the reestablishment of turfgrass lost to winter injury.

ACKNOWLEDGMENTS
This research was supported by the Oklahoma Center for the Advancement of Science and Technology (OCAST), the United States Golf Association Oklahoma Agricultural Experiment Station, and the USDA NIFA, Hatch Project OKL03146.

AUTHOR CONTRIBUTIONS
Lakshmy Gopinath: Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing-original draft; Writing-review & editing. Justin Quetone Moss: Funding acquisition; Project administration; Resources; Supervision; Validation; Writing-review

& editing. Yanqi Wu: Funding acquisition; Resources; Validation; Writing-review & editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Lakshmy Gopinath https://orcid.org/0000-0002-3115-2694

REFERENCES

Anderson, J. A., Taliaferro, C. M., & Martin, D. L. (1993). Evaluating freeze tolerance of bermudagrass in a controlled environment. *HortScience*, *28*, 955–955 https://doi.org/10.21273/HORTSCI.28.9.955

Anderson, J. A., Taliaferro, C. M., & Martin, D. L. (2002). Freeze tolerance of bermudagrasses: Vegetatively propagated cultivars intended for fairway and putting green use, and seed-propagated cultivars. *Crop Science*, *42*, 975–977. https://doi.org/10.2135/cropsci2002.9750

Anderson, J. A., Taliaferro, C. M., & Martin, D. L. (2003). Longer exposure durations increase freeze damage to turf bermudagrasses. *Crop Science*, *43*, 973–977. https://doi.org/10.2135/cropsci2003.9730

Anderson, J. A., Taliaferro, C. M., & Wu, Y. Q. (2007). Freeze tolerance of seed- and vegetatively-propagated bermudagrasses compared with standard cultivars. *Applied Turfgrass Science*, *4*, 1–7. https://doi.org/10.1094/ATS-2007-0508-01-RS

Beard, J. B. (1973). *Turfgrass science and culture*. Prentice-Hall.

Dunne, J. C., Tuong, T. D., Livingston, D. P., Reynolds, W. C., & Milla-Lewis, S. R. (2019). Field and laboratory evaluation of bermudagrass germplasm for cold hardiness and freezing tolerance. *Crop Science*, *59*, 392–399. https://doi.org/10.2135/cropsci2017.11.0667

Fontanier, C., Moss, J. Q., Gopinath, L., Goad, C., Su, K., & Wu, Y. (2020). Lipid composition of three bermudagrasses in response to chilling stress. *Journal of the American Society for Horticultural Science*, *145*, 95–103. https://doi.org/10.21273/JASHS04815-19

Gopinath, L., Moss, J., & Wu, Y. (2021). Quantifying freeze tolerance of hybrid bermudagrasses adapted for golf course putting greens. *HortScience*, *56*, 478–480. https://journals.ashs.org/hortsci/view/journals/hortsci/56/4/article-p478.xml

Hartwiger, C., & Moeller, A. (2015). Winter play: When to go and when to say no. *Green Section Record*, *53*, 1–9.

NTEP. (2014). *2013 national bermudagrass test*. (Progress Report NTEP No. 15-2). National Turfgrass Evaluation Program. http://ntep.org/reports/bg13/bg13_15-2/bg13_15-2.htm

NTEP. (2020). *National warm-season grass (putting green) test preliminary report*. National Turfgrass Evaluation Program. https://ntep.org/data/preliminary/2020/ws19g20tqloc.txt

Patton, A. J., & Reicher, Z. J. (2007). Zoysiagrass species and genotypes differ in their winter injury and freeze tolerance. *Crop Science*, *47*, 1619–1627. https://doi.org/10.2135/cropsci2006.11.0737

Qian, Y. L., Ball, S., Tan, Z., Koski, A. J., & Wilhelm, S. J. (2001). Freeze tolerance of six cultivars of buffalograss. *Crop Science*, *41*, 1174–1178. https://doi.org/10.2135/cropsci2001.4141174x

Richardson, M. (2002). Turf quality and freezing tolerance of 'Tifway' bermudagrass as affected by late-season nitrogen and trinexapac-ethyl. *Crop Science*, *42*, 1621–1626. https://doi.org/10.2135/cropsci2002.1621

Shahba, M. A., Qian, Y. L., Hughes, H. G., Koski, A. J., & Christensen, D. (2003). Relationships of soluble carbohydrates and freeze tolerance in saltgrass. *Crop Science*, *43*, 2148–2153. https://doi.org/10.2135/cropsci2003.2148

Wu, Y. Q., & Anderson, J. A. (2011). Genetic improvement of cold hardiness in bermudagrass. In M. Pessarakli (ed.) *Handbook of plant and crop stress* (3rd ed., pp. 851–865). CRC Press of Taylor & Francis Group.

Xiang, M. Y., Fry, J., & Wu, Y. Q. (2019). Winter survival of experimental bermudagrasses in the upper transition zone. *Kansas Agricultural Experiment Station Research Reports*, *5*(5). https://doi.org/10.4148/2378-5977.7770

How to cite this article: Gopinath L, Moss JQ, Wu Y. Evaluating the freeze tolerance of bermudagrass genotypes. *Agrosyst Geosci Environ*. 2021;*4*:1–4. https://doi.org/10.1002/agg2.20170