Freezing Tolerance of Six Cultivars of Buffalograss

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ABSTRACT

Freezing tolerance is an important trait that determines geographical adaptation of a turfgrass. This study was conducted to assess the relative freezing tolerance, seasonal changes in freezing tolerance (LT_{50}), and winter survival of six cultivars of buffalograss [*Buchloë dactyloides* (Nutt.) Engelm.]. The cultivars 91-118, Tatanka, Texoka, Stampede, UCR-95, and 609 were grown in the field at Fort Collins, CO. From September 1998 to April 1999 and from October 1999 to May 2000, stolons were sampled monthly from each plot and subjected to laboratory freezing tests. Survival and recovery following the freezing test indicated that all cultivars had similar LT_{50} in September and gradually increased in winter hardiness during fall. However, the capacity to acclimate and the maximum freezing tolerance were significantly different among the cultivars. Ranking of grasses for mean LT_{50} (°C) was Tatanka (−18.1) = 91-118 (−18.0) ≤ Texoka (−17.1) < 609 (−14.4) < Stampede (−12.4) < UCR-95 (−9.2) during midwinter in 1998-1999 and Texoka (−21.7) = 91-118 (−21.6) = Tatanka (−21.0) < 609 (−15.8) = Stampede (−15.1) < UCR-95 (−14.0) during midwinter in 1999-2000. Following freezing treatments, Tatanka and 91-118 maintained a higher relative shoot and root regrowth than other cultivars. Root regrowth was reduced by freezing to a greater extent than shoot regrowth for all cultivars. Field winter survival, a measure of winter hardiness, in 1998, 1999, and 2000 was generally in agreement with laboratory test results, showing that Stampede, 609, and UCR-95 were more susceptible to winterkill than 91-118 and Tatanka. The differences in freezing tolerance among the cultivars tested indicates substantial intraspecific variation that may be used for breeding improvement.

Freezing tolerance is the primary limiting factor for the use of warm-season turfgrasses in the transition zone and northern climate conditions. Intraspecific differences in freezing tolerance have been studied in zoysiagrass (*Zoysia* spp.), bermudagrass (*Cynodon* spp.), St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze], centipedegrass [*Eremochloa ophiruoides* (Munro) Hackel], and seashore paspalum (*Paspalum vaginatum* Swartz) (Cardona et al., 1997; Fry et al., 1991; 1993; Philley et al., 1995). Zoysiagrass is reported to have good freezing tolerance with LT_{50} values ranging from −8 to −14°C (Dipaola and Beard, 1992; Dunn et al., 1999; Rogers et al., 1975, 1977). In bermudagrass, research has shown that LT_{50} ranged from −7°C for 'Tifgreen' to −11°C for 'Midiron' by electrolyte leakage and regrowth tests (Anderson et al., 1988, 1993). Ibitayo et al. (1981) reported that 'Brookings' bermudagrass survived −17°C at Fort Collins, CO.

Buffalograss, native to the short-grass prairie region of North America, is a low maintenance warm-season turfgrass with excellent drought and heat resistance (Beetle, 1950). With water becoming a more limited resource, and environmental issues of greater concern to the public, there is increased interest in the use of more resource-efficient turf, such as buffalograss (Riordan et al., 1993). Recently, a number of turf-type buffalograss cultivars have been commercialized. These cultivars provide significant improvements in turf quality over previously released buffalograss cultivars; including darker green color, greater shoot density, finer leaf texture, improved fall color retention, and early spring green-up. Since buffalograss genotypes used in breeding programs are selected from diverse latitudes, ranging from Mexico to southern Canada, large differences in freezing tolerance are expected (Engelke and Hickey, 1983). After being subjected to −12°C in a freezer, Texoka exhibited >85% growth recovery, whereas two selections from Mexico were completely killed (Wu and Harivandi, 1989).

In a comprehensive review, Dipaola and Beard (1992) listed a relative ranking of warm-season turfgrass freezing tolerance with LT_{50} values. However, the ranking for buffalograss was not given because information on buffalograss LT_{50} values were not available. Existing information about winter hardiness in buffalograss is limited to field observations of survival following winters. No research results are available concerning seasonal patterns of buffalograss freezing tolerance. In combination with field observation, LT_{50} data may be useful to assess winter hardiness of buffalograss cultivars more quickly and accurately and to provide recommendations for regional use. Winterkill of 609 buffalograss, a commonly used turf cultivar, occurs frequently along the front range of the Rocky Mountains in Colorado (Qian and Koski, personal observation), and justifies research to select freezing tolerant buffalograsses.

The objective of this study was to determine the relative freezing tolerance, seasonal changes in freezing tolerance, and winter survival of six buffalograss cultivars in northern Colorado.

MATERIALS AND METHODS

Cultivar Establishment

Buffalograss cultivars 609, 91-118, Tatanka (University of Nebraska, Lincoln, NE), Stampede (Crenshaw & Doguet Turfgrass, Bastrop, TX), UCR-95 (University of California, Riverside, CA), and Texoka (Oklahoma Agric. Exp. Sta., Stillwater, OK) were planted at the W.D. Holley Plant Environmental Research Center at Colorado State University, Fort Collins, CO, in July 1996. Tatanka and Texoka are seeded cultivars and the others are vegetatively propagated. Grasses were established in 3- by 3-m plots on a sandy clay loam (Aridic Argiustoll), and entries were replicated three times in a randomized complete block design. During the 1997 and 1998

Dep. of Horticulture and Landscape Architecture, Colorado State Univ., Fort Collins, CO 80523-1173. Received 7 July 2000. *Corresponding author (yaqian@lamar.colostate.edu).

Published in Crop Sci. 41:1174–1178 (2001).

Abbreviation: LT_{50}, Lethal temperature resulting in 50% mortality.

growing seasons, the plots received 98 kg N ha^{-1} yr^{-1} from a 40-0-0 N-P-K fertilizer applied in June and August.

Daily maximum and minimum air temperatures were recorded at a weather station within 2 km of the study site.

Laboratory Test

Field plots were sampled at approximate monthly intervals from September 1998 through April 1999 and from October 1999 through May 2000. On each sampling date, stolons were collected from each of the three replicates of each cultivar. After being washed with cold water to remove soil and plant debris, stolons from each plot were divided into 8 to 12 fractions. Each fraction, containing at least 10 stolon nodes, was individually wrapped in moist tissue paper, enclosed in aluminum foil to prevent moisture loss, and labeled for a targeted freezing temperature. Samples were subjected to low temperature treatments in a thermo-controlled freezer (Tenny Jr. Programmable Freezer, Tenny Inc., South Brunswick, NJ). The freezing chamber was programmed to cool linearly at 2°C h^{-1} after 16 h at 0°C. One fraction of stolons from each plot was removed as target temperatures were reached. Target temperatures (2°C intervals), varying with cultivars and sampling dates, spanned a range of anticipated LT$_{50}$. Samples were thawed overnight at 2°C following removal from the freezing chamber. Nonfrozen controls were held at 2°C during the freezing test.

Following thawing, individual nodes were planted in a plastic cone (3-cm-inside diam. by 8 cm deep) filled with commercial potting soil. All plants were maintained in the greenhouse at 25°C room temperature. Irrigation was applied every 2 h by a mist system to provide ~3 mm d^{-1}. Turf response to freezing temperature was evaluated on the basis of stolon survival, as well as shoot and root regrowth. Survival was recorded by observing regeneration of shoots 2 to 4 wk after planting. Shoot and root regrowth were harvested 8 wk after planting, and dried at 80°C for 24 h to determine dry mass. The relative shoot and root regrowth following low temperature treatment were calculated by expressing regrowth as a percentage of the regrowth of the control. The advantage of relative shoot and root regrowth rather than an absolute growth value as the measure of response is that it accounts for the genetic differences in growth rates among the cultivars. Shoot and root regrowth data were collected monthly for tests between November 1998 and February 1999.

Field Evaluation

To evaluate buffalograss response to seasonal climatic changes, leaf color was visually rated by a scale of 1 to 9 (9 = green and 1 = brown) in October and November 1998, and April 1999. In May or June 1998 to 2000, winter survival of buffalograss cultivars in the field were assessed by visually rating the percentage of turf area that exhibited green-up.

Data Analysis

The PROC PROBIT procedure of the Statistical Analysis System (SAS Institute, 1989) was used to predict LT$_{50}$, defined as the sub-freezing temperature that resulted in 50% survival. The lower LT$_{50}$ value indicates greater freezing tolerance. Since we had three replicated plots in the field, three lethal temperatures were generated from each cultivar on each testing date. The LT$_{50}$ of each replication was then treated as a response variable and subjected to the analysis of variance (ANOVA) procedure of SAS (SAS Institute, 1989). Since a significant cultivar × date interaction was found, means separations among cultivars were performed within sampling dates by the Fisher's LSD test at $P = 0.05$. Shoot and root regrowth, leaf color, and field survival were likewise subjected to ANOVA. All percentage data were arcsine transformed to meet the normality assumption of the ANOVA procedure.

RESULTS

Seasonal Lethal Low Temperature

September 1998 to April 1999

In September, all cultivars exhibited good survival at −6.0°C, but no survival was observed at −8.0°C (Fig. 1). Although all entries exhibited decreasing LT$_{50}$ values from September to November in response to field environments, cultivars varied in their capacity and rate of

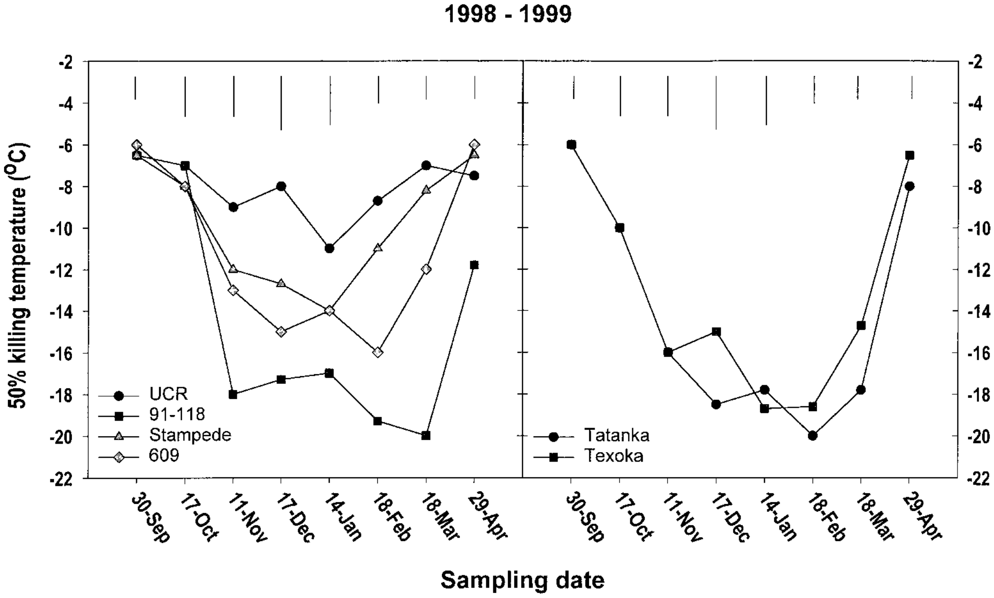

Fig. 1. Seasonal patterns of 50% lethal low temperature (LT$_{50}$) of six buffalograss cultivars sampled from field plots at Fort Collins, CO, in 1998–1999. Vertical bars on the top indicate LSD ($P = 0.05$) for cultivar comparison within each date.

Table 1. Winter survival and color of buffalograsses grown in the field.

	Winter survival†			Color‡		
Cultivars	**1998**	**1999**	**2000**	**Oct. 1998**	**Nov. 1998**	**April 1999**
	%			1–9		
Stampede	25c§	45b	50c	7.6a	4.6a	1.5a
Tatanka	73a	82a	82a	1.6c	1.1c	1.4a
91-118	71a	87a	84a	2.4c	1.1c	1.3a
Texoka	58ab	80a	85a	1.8c	1.1c	1.4a
609	20c	50b	73b	7.5a	4.7a	1.5a
UCR-95	32bc	51b	53c	6.2b	4.1b	1.4a

† Winter survival was assessed by visually rating the percentage of turf area that exhibited green-up in the late spring.

‡ Turf color was rated visually on a 1 to 9 scale where 1 = brown and 9 = green turf.

§ Means within columns followed by the same letter are not significantly different at $P \leq 0.05$ using Fisher's LSD test.

lowering LT_{50}. The amount of decrease in LT_{50} from September to November was negatively correlated with fall color retention ($r = -0.72$, $P < 0.001$). Tatanka, 91-118, and Texoka stopped growth, turned brown, and became dormant in early to mid-October, whereas Stampede, 609, and UCR-95 maintained green color until late November (Table 1).

Cultivars differed markedly in their LT_{50} during midwinter (Fig. 1). The mean LT_{50} values between November and February were Tatanka (−18.1°C) = 91-118 (−18.0°C) ≤ Texoka (−17.1°C) < 609 (−14.4°C) < Stampede (−12.4°C) < UCR-95 (−9.2°C).

On 18 February, 91-118, Tatanka, 609, and Texoka maintained lower or equal values of LT_{50} as in December and January, whereas the LT_{50} of Stampede and UCR-95 elevated ~3°C. On 18 March, 609, Texoka, and Tatanka showed signs of deacclimation with a 2.5 to 4°C increase in LT_{50}, but 91-118 maintained an LT_{50} of −20°C. All cultivars deacclimated in April when turf exhibited initial green-up. Except for 91-118, which had an LT_{50} = −11.8°C, all other cultivars only survived −6 to −8°C in April.

October 1999 to May 2000

In October, LT_{50} values of Tatanka, Texoka, and 91-118 were between −11.7 and −14.0°C, which were 4 to 6°C lower than those of Stampede, UCR-95, and 609 (Fig. 2). It appears that some acclimation had taken place before the October sampling for Texoka, 91-118, and Tatanka. In November 1999, the LT_{50} of 91-118, Tatanka, and Texoka was lowest, reaching −21°C. Cultivar 609 had an LT_{50} of −14.7°C, which was lower than that of Stampede (−12.0°C). The LT_{50} of UCR-95 was not different from that of either 609 or Stampede. The cultivars 91-118, Tatanka, and UCR-95 exhibited little additional acclimation after November, whereas Stampede and 609 were 3.3 to 5.3°C hardier in January compared to November. Ranking of grasses for midwinter LT_{50} in 1999–2000 was Texoka (−21.7°C) = 91-118 (−21.6°C) = Tatanka (−21.0°C) < 609 (−15.8°C) = Stampede (−15.1°C) < UCR-95 (−14.0°C). In March, 91-118, Tatanka, and UCR-95 maintained a similar LT_{50} as in December and January, whereas the LT_{50} of Stampede, 609, and Texoka increased 2 to 4°C. Except for 91-118 which had an LT_{50} of −8.7°C, all other cultivars only survived to −7°C in early May.

Shoot and Root Regrowth

As the freezing test temperature was lowered, all cultivars exhibited a linear decrease in shoot and root regrowth (Table 2). Compared with other cultivars, 91-118 and Tatanka exhibited higher relative shoot and root regrowth after being subjected to temperatures below −10°C. At most test temperatures, the relative shoot and root regrowth of Texoka was less than that of 91-118 and Tatanka but higher than 609, Stampede, and UCR-95.

As treatment temperature decreased, shoot regrowth declined less than that of root regrowth for all cultivars ($P < 0.01$), suggesting that root regrowth was more

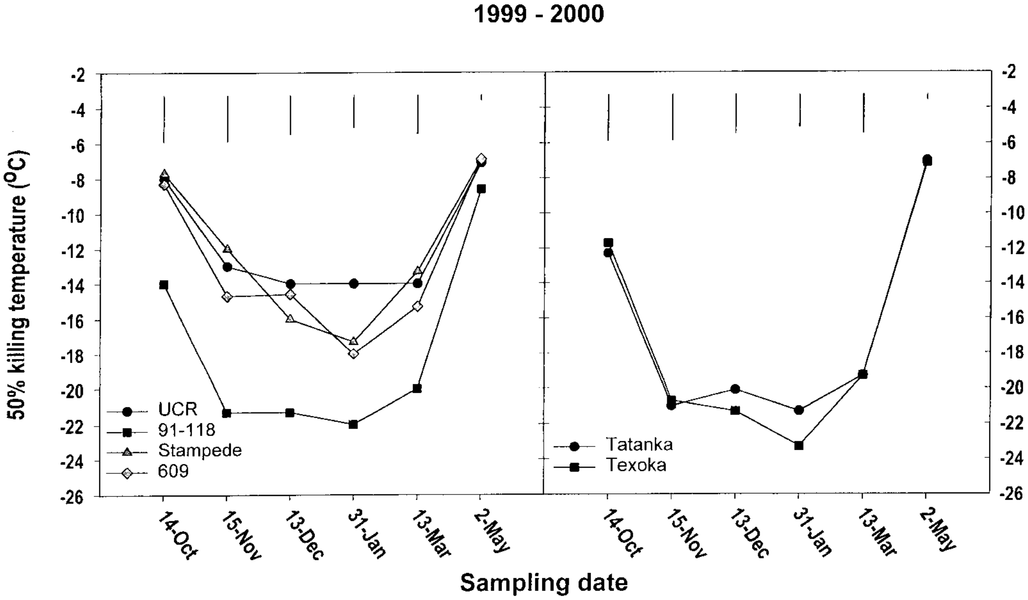

Fig. 2. Seasonal patterns of 50% lethal low temperature (LT_{50}) of six buffalograss cultivars sampled from field plots at Fort Collins, CO, in 1999–2000. Vertical bars on the top indicate LSD ($P = 0.05$) for cultivar comparison within each date.

Table 2. Relative shoot and root growth from stolons of six buffalograsses following freezing treatment.

Cultivar	Control	−8	−10	−12	−14	−16	−18	−20	−22	Linear temperature effect
	Temperature treatment (°C)									
				Relative shoot regrowth (%)						
91-118	100	113	110a	106a	94a	76b	77a	52b	25a	**
Tatanka	100	103	98ab	105a	81b	89a	81a	80a	27a	**
Texoka	100	105	90b	83b	61c	52c	47b	–	–	****
609	100	96	71c	70c	57c	40d	38b	–	–	****
Stampede	100	94	90b	70c	63c	38d	–	–	–	****
UCR-95	100	97	41d	37d	–	–	–	–	–	****
				Relative root regrowth (%)						
91-118	100	105a	98a	94a	89a	56a	53a	26	–	****
Tatanka	100	95ab	93a	83a	68b	65a	42a	13	–	****
Texoka	100	106a	91a	67b	46c	40b	13b	–	–	****
609	100	77b	56b	44c	28d	15c	8b	–	–	****
Stampede	100	96ab	92a	66b	47c	18c	–	–	–	****
UCR-95	100	66b	25c	10d	–	–	–	–	–	****

† Relative shoot and root growth, calculated as % of 2°C control. Data presented were the mean between November 1998 and February 1999.

‡ Means within parameters and columns followed by the same letter are not significantly different at $P \leq 0.05$ using Fisher's LSD test.

** Significant linear relationship between relative regrowth and temperature at the $P \leq 0.01$ level.

**** Significant linear relationship between relative regrowth and temperature at the $P \leq 0.0001$ level.

sensitive to freezing temperature (Table 2). The temperatures that caused ≥50% reduction in root regrowth (compared with the respective control) were −10°C for UCR-95, −12°C for 609, −14°C for Stampede and Texoka, −18°C for Tatanka, and −20°C for 91-118. The temperatures that caused ≥ 50% reduction in shoot growth were −10°C (UCR-95), −16°C (609, Stampede), −18°C (Texoka), and −22°C (91-118, Tatanka).

Winter Survival in the Field

In 1998, Tatanka and 91-118 had 40, 52, and 47% higher winter survival than UCR-95, 609 and Stampede (Table 1). Texoka had an intermediate winter survival rating but was not statistically different from 91-118 and Tatanka or UCR-95. In 1999, Tatanka, 91-118, and Texoka had 29 to 42% higher winter survival than UCR-95, 609, and Stampede. In the spring of 2000, Texoka, 91-118, and Tatanka exhibited at least 9% higher winter survival than 609, and 609 had 20% higher winter survival than Stampede and UCR-95.

DISCUSSION

Little difference in LT_{50} among grasses in September suggested that cultivars had similar freezing tolerance when tissues were nonacclimated (Fig. 1). The differing ability to survive winter is likely dependent on the rate of acclimation, the ultimate level of hardiness, and the stability of hardiness. Changes in temperature and daylength in the fall likely trigger the transition of buffalograss from a cold tender state to a cold hardy state (Larsen, 1994). Tatanka, 91-118, and Texoka exhibited dormancy earlier (Table 1), acclimated faster, and possessed a higher capacity to acclimate than other cultivars (Fig. 1). The high negative correlation between cold acclimation and color retention suggested that the good fall color retention of UCR-95, Stampede, and 609 was maintained by a physiological condition that is not compatible with cold acclimation.

Though no significant differences among cultivars in breaking dormancy and green-up were observed in the spring (Table 1), our laboratory freezing test indicated

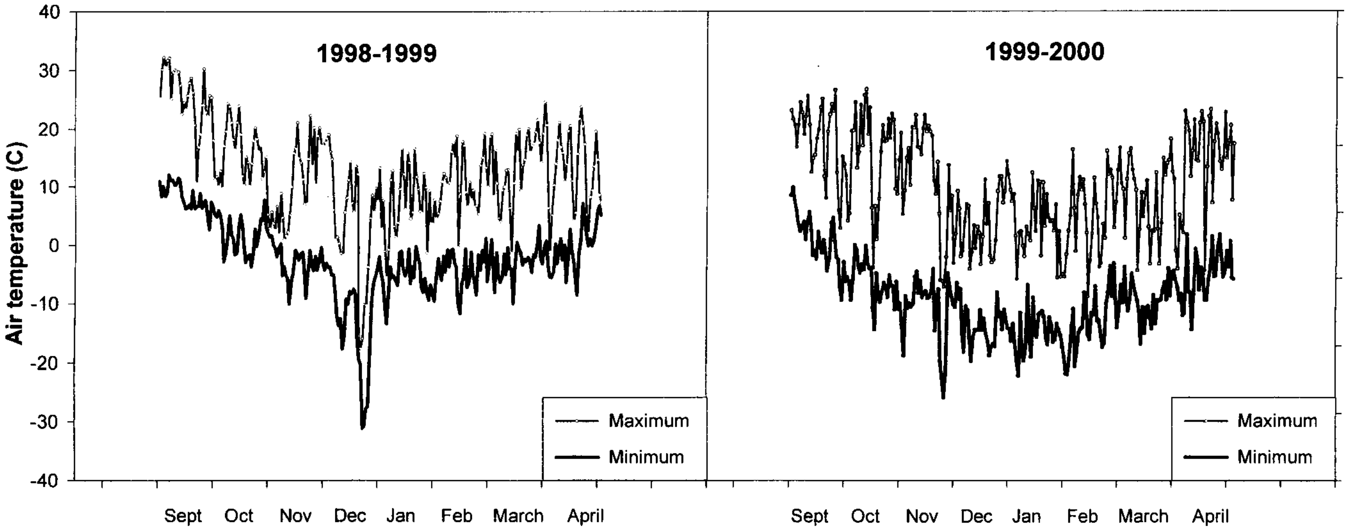

Fig. 3. The minimum and maximum air temperatures at Fort Collins, CO, from September to April in 1998–1999 and 1999–2000.

that cultivar 91-118 had the lowest LT_{50} in April and May (Fig. 1 and 2). This suggests that 91-118 possesses the highest stability of hardiness in the spring.

In midwinter, considerable differences in freezing tolerance existed among cultivars. On the basis of the LT_{50} values collected between November 1998 and February 1999 and between November 1999 and January 2000, the ranking of freezing tolerance is 91-118 = Tatanka $\geq$ Texoka $>$ 609 $>$ Stampede $\geq$ UCR-95. Cultivars 91-118 and Tatanka had the greatest capacity to survive the winter under the prevailing conditions at Fort Collins, CO. UCR-95, 609, and Stampede were only alive in patches in the field. UCR-95 exhibited the poorest LT_{50} in the controlled environmental tests, but exhibited similar or even slightly lower winterkill than 609 and Stampede in the field. These results suggest that UCR-95 may possess other low temperature survival mechanisms in the field. Deeply buried crowns and rhizomes may promote avoidance of low temperature.

The differences in freezing tolerance observed among buffalograss cultivars may in part be related to ploidy levels. Four ploidy levels, including diploid, tetraploid, pentaploid, and hexaploid were reported in buffalograss (Huff et al., 1993; Johnson et al., 1998). UCR-95 and Stampede are diploids; 609 and 91-118 are tetraploids; while Texoka is a hexaploid. Tatanka is grouped as an intermediate between tetraploid and hexaploid (Johnson et al., 1998). This study indicated that diploid cultivars exhibited higher LT_{50} than hexaploid/tetraploid, supporting Huff et al. (1993) who suggested that diploid buffalograss is adapted to the southern parts of the Great Plains of North America, specifically Mexico and south Texas. Tetraploids, prevalent in the Great Plains region and the western boundary of the plains, appeared to be more diverse in their freezing tolerance.

Although the cultivar ranking for freezing tolerance was similar in 1998–1999 and 1999–2000 cycles, LT_{50} measured in 1999–2000 was 1 to 4°C lower than in 1998–1999 for each cultivar (Fig. 1 and 2). This may have been because the turf stand was more mature or due to differences in the climate conditions (Fig. 3).

Root regrowth was reduced by freezing temperatures to a greater extent than shoot regrowth. Therefore, in some situations, buffalograss stolons may survive freezing temperatures, but their root development could be severely reduced. Reduced rooting may negate buffalograss's drought resistance.

In summary, our results suggest that 91-118, Tatanka, and Texoka have better freezing tolerance than other tested cultivars. The LT_{50} values for 91-118, Tatanka, and Texoka buffalograsses (Fig. 1 and 2) are among the lowest values reported thus far for warm-season grasses. Outdoor minimum temperatures in northern Colorado could drop low enough to injure the stolons of UCR-95, Stampede, and 609, indicating that these three cultivars should not be recommended for turf uses in northern Colorado. This study did not provide information on physiological and biochemical mechanisms involved in freezing tolerance of buffalograss. A number of biochemical changes, including conversions of membrane stability (Cyril et al., 1998; Samala et al., 1998), carbohydrate composition and accumulation (Fry et al., 1993, 1991), and cold-regulated protein synthesis (Gatschet et al., 1996) have been reported to be important in freezing tolerance of turfgrasses. Additional research is needed to elucidate the nature of buffalograss freezing tolerance, which should be helpful for improving the cold hardiness of buffalograss through management and breeding.

ACKNOWLEDGMENTS

Funding was provided by the Rocky Mountain Turfgrass Research Foundation and Colorado Agricultural Experimental Station (Project 780). We thank Dr. Cecil Stushnoff and Ann McSay for discussion and help with the instrumentation.

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