

Longer Exposure Durations Increase Freeze Damage to Turf Bermudagrasses

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ABSTRACT

Bermudagrasses, *Cynodon sp.*, are susceptible to winter injury in the transition zone for warm- and cool-season turfgrasses. Our objectives were to determine relative freeze tolerance of recently released and standard cultivars using laboratory-based methodology, and to determine the effect of extended exposure duration on survival. Plants were clonally propagated, then established and acclimated in growth chambers before exposure to a range of temperatures in a freeze chamber. 'Tifway' and 'TifSport' (-7.9°C) were significantly hardier than 'Princess' (-6.9°C), but less freeze tolerant than 'U-3' (-8.9°C), 'Patriot' (-9.7°C), and 'Midlawn' (-10.3°C). Riviera (-8.3°C) was significantly hardier than Princess, but less freeze tolerant than Patriot and Midlawn. In a second set of experiments, acclimated plants were held at constant, subfreezing temperatures for various periods of time in a refrigerated bath. Survival of U-3 and Riviera decreased to 25% or less when exposed to -7.0°C for 2 or 5 d, compared with 100 and 83% survival, respectively, when plants were removed from the bath immediately after equilibrating at -7.0°C . Princess exhibited 89% survival when removed immediately after equilibrating at -5.4°C , but survival after 2, 24, and 72 h was 67, 30, and 11%, respectively. Although minimum exposure temperature is a primary determinant of survival, freeze damage to turf bermudagrasses increased as exposure duration increased.

BERMUDAGRASSES GROWN in the transition zone for warm- and cool-season grasses are subject to freeze damage (Fry, 1990; Hiscock, 1996). Information on relative freeze tolerance is vital to turfgrass managers selecting cultivars to be planted in the transition zone. Winter survival is frequently reported as percentage area of live aboveground shoots in the spring (Martin et al., 2001). Often, several years of exposure are required to experience temperatures causing significant freeze damage. To overcome the unpredictable occurrence of test winters and to expand evaluations year-round, laboratory-based approaches to measure freeze tolerance have been developed. Plant material has been acclimated in growth chambers, followed by exposure to a range of temperatures spanning the presumptive killing temperature in a freeze chamber (Anderson et al., 1993). The combined approach with plant material acclimating in the field, followed by laboratory-based exposure to subfreezing temperatures also has been employed (Maier et al., 1994). Laboratory-based evaluations generally correspond well with field observations (Qian et al., 2001), and have provided useful information on relative freeze tolerance of turfgrasses.

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One of the difficulties in evaluating freeze tolerance data from field studies is the variability that may occur. Different years may have contrasting temperature patterns during the acclimation period that can contribute to year-to-year differences in hardiness estimates. Similarly, differences in freezing rate or duration, even with the same minimum exposure temperature, could result in different plant responses. Although most laboratory studies have standardized procedures, several have explored variables such as exposure duration. Gusta and Fowler (1977) reported that freeze tolerance of wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.) crowns was 4 to 5°C less when the exposure duration was 24 h, compared with removing samples at the moment of equilibration with the test temperature. Similarly, survival of onion (*Allium cepa* L.) bulbs was affected by the length of time held frozen at -11°C (Palta et al., 1977). Limited information is available on the effects of prolonged exposure on survival of turfgrasses at subfreezing temperatures. The objective of the study reported here was to determine the effect of exposure duration on freeze tolerance of bermudagrass by holding plants at a constant, subfreezing temperature for various periods of time, then observing regrowth. Initial studies were performed to characterize freeze tolerance of selected cultivars and to establish appropriate targets for constant temperature studies.

MATERIALS AND METHODS

Midlawn, Patriot (OKC 18-4), Princess, Riviera (OKS 95-1), Tifway, TifSport, and U-3 (obtained from Ken Diesburg, Southern Illinois Univ.) bermudagrass plants were clonally established from phytomers in Cone-tainers (Ray Leach Cone-tainer Nursery, Canby, OR). Midlawn, Tifway, and TifSport are triploid hybrids of *C. dactylon* (L.) Pers. \times *C. transvaalensis* Burt Davy. Princess, Riviera, and U-3 are *C. dactylon* tetraploids, and Patriot is a tetraploid hybrid of *C. dactylon* \times *C. transvaalensis*. Phytomers of Princess and Riviera (seeded cultivars) used for freeze tolerance characterization and extended exposure duration studies were obtained from greenhouse pots established by planting seed. Accordingly, the propagules of these cultivars represented a sampling of genotypes comprising each cultivar. The other cultivars tested constituted clonally propagated single plants (genotypes). Planting dates were staggered to allow uniform establishment periods. The potting medium (Universal Mix, Strong-lite, Pine Bluff, AR) was supplemented with dolomite (2.97 g L^{-1}), superphosphate (0.74 g L^{-1}), micromax (The Scotts Co., Marysville, OH) (0.45 g L^{-1}), KNO_3 (0.59 g L^{-1}), and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.24 g L^{-1}). Plants were watered daily with soluble fertilizer (Peters 20 N-8.6 P-16.6 K, The Scotts Co.) at 0.7 g L^{-1} and trimmed with scissors as needed. After establishment for ≈ 10 wk in a growth chamber (model PGW36, Conviron, Ashville, NC) at $28^{\circ}/24^{\circ}\text{C}$ (day/night) temperatures with a 14-h photoperiod, plants were acclimated for 4 wk at $8^{\circ}/2^{\circ}\text{C}$ (day/

Abbreviations: T_{mid} , midpoint of survival-temperature response curve.

night) temperatures with a 10-h photoperiod as previously described (Anderson et al., 1993). Photosynthetic photon flux densities were $\approx 400 \mu\text{mol m}^{-2} \text{s}^{-1}$ during establishment and $\approx 350 \mu\text{mol m}^{-2} \text{s}^{-1}$ during acclimation. Experiments characterizing freeze tolerance were conducted in a convection freeze chamber (model CEC23, Rheem Scientific, Asheville, NC). Chamber temperature was held overnight at -2.5°C after loading samples, then cooled linearly at 1°C per hour. Four Cone-tainers from each genotype were removed when they reached the target temperature. Target temperatures (1°C intervals) covered a range anticipated to span from complete survival to complete mortality. Plant response was based on regrowth potential (Anderson and Taliaferro, 2002). The mid-points (T_{mid}) of survival vs. temperature curves were determined by nonlinear regression (Ingram, 1985) using Proc NLIN (SAS Institute, Cary, NC).

Experiments with extended subfreezing exposure durations were conducted in a 190-L refrigerated bath (model 2425, Forma Scientific, Marietta, OH) adapted for use with plants in Cone-tainers. Bath circulation was increased by additional submersible pumps. Holes were cut in a plywood sheet to support 6-cm-diam. plastic tubes (Fig. 1A). Cone-tainers were placed inside the tubes that extended 32 cm into the ethylene glycol. The tops of the Cone-tainers were about 12 cm below the liquid surface (Fig. 1B). When tubes were capped, Cone-tainers were held at nearly constant temperature without desiccation associated with evaporative demand from cold refrigeration coils in convection chambers. Notches were cut in the tops of the tubes to allow the thermocouple wires, used to measure sample temperature, to exit the tubes when capped.



Fig. 1. Refrigerated bath modified for use with Cone-tainers. Plastic tubes were glued to a plywood frame (A). A mock tube was supported next to the bath to show Cone-tainer depth after placement inside the tube (B).

Measuring junctions were inserted to a 2-cm depth in the potting medium. Cone-tainers were weighed before and after being placed into the tubes. Some samples required nucleation with ice chips to limit supercooling (Fig. 2), and their weight changes during freezing were not included in analyses.

Similar to continuous cooling experiments described above, Cone-tainers used in extended duration experiments were held overnight at -2.5°C , followed by cooling at 1°C h^{-1} . However, temperature was decreased stepwise in extended duration experiments, compared with linear cooling in continuous cooling experiments. Also, a single exposure temperature, $\approx 1.5^\circ\text{C}$ warmer than T_{mid} , was selected for each genotype in extended duration experiments. In the first set of experiments, four Cone-tainers each of U-3 and Riviera were removed when they reached -7.0°C . An additional four Cone-tainers were removed after 48 and 120 h at -7.0°C . Subsequent experiments with Princess and Tifway used target temperatures of -5.4°C and -6.4°C , respectively, and were conducted with a single cultivar on each date. In these studies, nine Cone-tainers were removed when they reached the target temperature. An additional nine Cone-tainers were removed after 2, 24, and 72 h, focusing on intervals expected to span nearly complete survival to nearly complete mortality, based on results from the previous experiment using U-3 and Riviera. Slight temperature adjustments when the bath approached the target temperature were used to overcome difficulties associated with a time-course experiment involving samples asymptotically approaching bath temperature. If necessary, bath temperature was lowered an additional 0.1 to 0.2°C to ensure that samples could be removed at the target temperature during the first 2 h. Bath temperature was then raised 0.2°C after the 2-h samples were removed to ensure that random temperature fluctuations did not result in sample temperatures below the target temperature during extended exposure (Fig. 2). After samples were removed from the bath they were thawed overnight at 4°C , then placed in a growth chamber for 6 wk to observe regrowth from any meristem.

All experiments were conducted at least three times, with dates constituting blocks. Significant differences in T_{mid} means for the continuous cooling experiments and the number of survivors in extended cooling experiments were determined by Duncan's New Multiple Range Test at $P \leq 0.05$ following ANOVA. Cultivars were analyzed separately in extended duration studies.

RESULTS

Bermudagrasses ranged in freeze tolerance from -6.9°C (Princess) to -10.3°C (Midlawn) based on continuous cooling experiments (Table 1). Tifway and TifSport (-7.9°C) were significantly hardier than Princess, but had less freeze tolerance than U-3 (-8.9°C), Patriot (-9.7°C) and Midlawn. Riviera (-8.3°C) was significantly hardier than Princess, but less freeze tolerant than Patriot and Midlawn. Midlawn was significantly hardier than all cultivars except Patriot. Although we have not previously examined this combination of bermudagrass cultivars, freeze tolerance estimates generally corresponded well with previous experience (Anderson et al., 1993, 2002). An exception occurred with Patriot, which was more freeze tolerant than expected based on field observations (NTEP, 2001). Additional studies will be required to determine the factors contributing to the discrepancy between laboratory and field estimates of the freeze tolerance of Patriot.

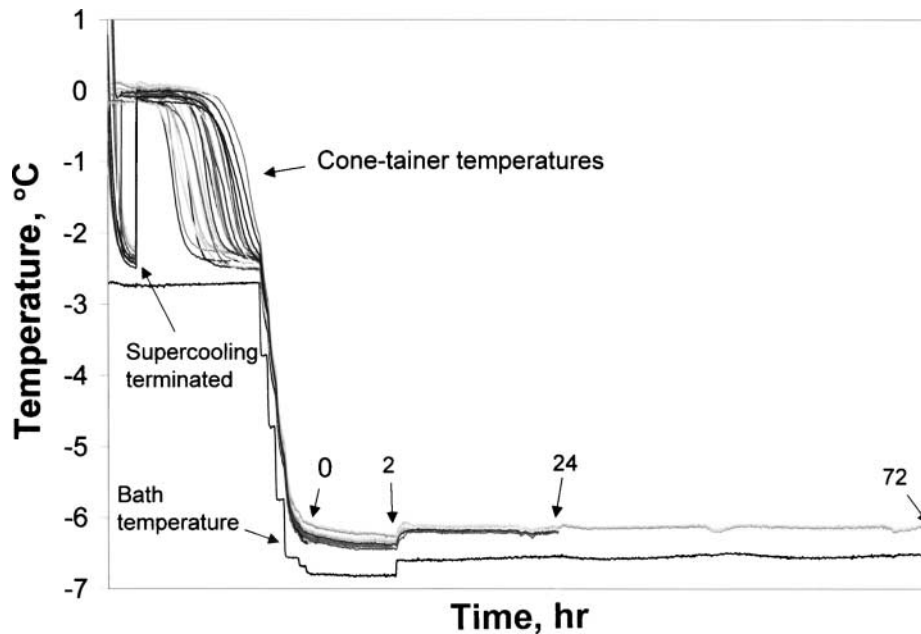


Fig. 2. Bath and Cone-tainer temperatures during an extended exposure experiment with a target temperature of -6.4°C . Time intervals (marked 0, 2, 24, and 72 h) are not evenly spaced due to changes in datalogger scan interval from 5 to 1 min to maximize precision when samples were being removed. Ice chips were added to some samples, indicated by “supercooling terminated”.

The number of Cone-tainers with plants exhibiting regrowth decreased as exposure duration increased. When Cone-tainers of U-3 and Riviera were removed from the bath immediately after reaching -7.0°C , 100 and 83% survived, respectively (Table 2). Survival after 48 and 120 h at -7.0°C was significantly lower, with 25% or less of the grass samples exhibiting regrowth. Similar results were obtained with Princess and Tifway using target temperatures 1.5°C warmer than T_{mid} values determined in continuous cooling experiments. Survival of Princess was 89% when Cone-tainers were removed from the bath immediately after reaching -5.4°C (Table 3). Survival decreased to 67% after 2 h, 30% after 24 h, and to 11% when samples were removed 72 h after reaching -5.4°C . Tifway had 78% regrowth following removal immediately after reaching -6.4°C . The percentage of survivors was not changed after 2 h, but no survivors were observed following 24 or 72 h of exposure (Table 3).

The mean minimum temperature recorded for Princess was -5.4°C for 0- and 2-h exposure, and -5.3°C for 24- and 72-h treatments (Table 3). The mean minimum temperature for all duration treatments matched the target temperature of -6.4°C for Tifway. Removal tem-

peratures tended to increase with increasing exposure duration, primarily due to raising bath temperature 0.2°C after 2-h samples were removed. No significant differences in weight loss were observed among the exposure durations in experiments with Princess or Tifway.

DISCUSSION

Although freeze tolerance evaluations were conducted with clonally propagated plants to permit comparisons, Princess and Riviera can be planted from seed. Propagules of Princess and Riviera were derived from a population of genotypes and were assumed to be representative of the populations. Riviera will be less likely to experience winterkill than Princess based on 1.4°C greater freeze tolerance (Table 1). Both cultivars may exhibit greater susceptibility to freeze damage the first year after seeding, compared with subsequent years (Philly and Krans, 1998). A greater range in freeze tolerance was observed among the five vegetatively propagated cultivars. On the basis of T_{mid} values, Midlawn and Patriot will be more likely to survive cold

Table 1. Freeze tolerance of turf bermudagrasses. T_{mid} values represent the midpoints of survival-temperature response curves.

Cultivar	T_{mid} °C
Princess	-6.9a^{\dagger}
Tifway	-7.9b
TifSport	-7.9b
Riviera	-8.3bc
U-3	-8.9cd
Patriot	-9.7de
Midlawn	-10.3e

† Means of four repetitions are separated by Duncan's New Multiple Range Test at $P \leq 0.05$.

Table 2. Percentage of Cone-tainers with surviving plants following exposure to -7.0°C for 0, 48, or 120 h. Plants exposed for 0 h were removed as soon as they reached the target temperature. Four Cone-tainers of each variety were removed at each time on each of three dates.

Cultivar	Survivors		
	Hours at -7.0°C		
	0	48	120
		%	
U-3	100a †	25b	8b
Riviera	83a	25b	0b

† Means within a row followed by the same letter are not significantly different at $P \leq 0.05$ using Duncan's New Multiple Range Test.

Table 3. Percentage of Cone-tainers with surviving plants following exposure to a constant, subfreezing temperature for 0, 2, 24, or 72 h. Plants exposed for 0 h were removed as soon as they reached the target temperature (−5.4°C for ‘Princess’, −6.4°C for ‘Tifway’). Sample temperature when removed and the minimum temperature reached during the entire exposure are reported. Samples were weighed before and after being placed into the bath. Nine Cone-tainers were removed at each time on each of three dates. Princess and Tifway were treated on separate dates and analyzed separately.

Cultivar		Hours at target temperature			
		0	2	24	72
Princess	Survivors, %	89a†	67b	30c	11d
	Removal temperature, °C	−5.4a	−5.3b	−5.2c	−5.1d
	Minimum temperature, °C	−5.4a	−5.4a	−5.3b	−5.3b
	Weight change, g	−0.12a	−0.17a	−0.10a	−0.18a
Tifway	Survivors, %	78a	56a	0b	0b
	Removal temperature, °C	−6.4a	−6.3b	−6.2c	−6.1d
	Minimum temperature, °C	−6.4a	−6.4a	−6.4a	−6.4a
	Weight change, g	−0.12a	−0.09a	−0.12a	−0.13a

† Means within a row followed by the same letter are not significantly different at $P \leq 0.05$ using Duncan's New Multiple Range Test.

winters in the northern boundary of the bermudagrass adaptation zone than Tifway and TifSport. U-3, which exhibited freeze tolerance similar to Riviera and Patriot, was among three of 28 vegetatively propagated bermudagrasses that survived the 1989-1990 winter in Illinois (Diesburg and Bertauski, 1993). Although estimates of relative freeze tolerance levels are expected to be in general agreement for laboratory and field studies (Qian et al., 2001), T_{mid} values we reported for grasses acclimated in a growth chamber may differ from values obtained from plants acclimated in the field. This may be due, in part, to differences in environmental conditions during the acclimation period.

Even though the strong temperature dependence of freezing injury has been readily apparent, the effects of freeze duration have not been as well defined. This may be due to the limited number of studies conducted and difficulties interpreting data from experiments with temperature varying during extended exposures. Relatively large magnitude temperature cycling, as may be expected in a convection chamber, can lead to deleterious partial thawing and refreezing (Levitt, 1980). In the present study, bermudagrass plants in Cone-tainers were held at nearly constant temperature for up to 5 d in a modified refrigerated bath. Temperature records indicated that the decrease in survival with increasing exposure duration was unlikely to be attributed to sample temperatures falling below the target temperature (Fig. 2). Similarly, significant temperature cycling did not occur, and thus would not contribute to significant partial thawing and refreezing.

Discussions of the role of exposure duration on freeze damage have focused on plant tissues at thermal equilibrium with the stress. Under field situations, factors that increase the time period to reach the minimum temperature would add a freeze avoidance component. Freeze avoidance is a strategy distinct from freeze tolerance, although the two could be additive with respect to time.

At the cellular level, freezing stress has a large dehydrative component due to the formation of extracellular ice (Chen et al., 1995). Additional sublimational water loss from samples exposed to the atmosphere in a convection chamber may exacerbate a secondary dehydration stress. In the present study, loss in sample mass of 0.18 g or less after 72 h at the target temperature indicated little moisture was lost from the Cone-tainers

(Table 3). On the basis of water contents from oven-dried samples, ≈ 0.2 to 0.4% of the moisture associated with the Cone-tainer was removed to the enclosing plastic tube (data not presented). It is unlikely that time-dependent water loss from Cone-tainers contributed to increased mortality with increasing exposure duration since there was not a significant difference in weight change among the exposure durations. Similarly, very little water redistribution between plant tissue and soil was expected once thermal equilibrium was reached. However, the cellular effects of freeze-induced dehydration could have resulted in time-dependent injury to the bermudagrass plants held at constant temperature.

Levitt (1980) divided plant responses to freezing into three categories based on cooling and warming rates. At relatively warm temperatures, all samples survived exposure regardless of cooling or warming rates. At low temperatures, all plants were killed regardless of the rate of temperature change. In between, there was a critical zone of temperatures in which there was an exposure temperature-cooling/warming rate interaction on survival. Levitt (1980) also presented data from Sakai suggesting that the model could be extended to exposure duration. At temperatures above or below a critical zone, all plants survive or are killed, respectively, regardless of the length of exposure. In the critical zone of temperatures, shorter exposure durations would favor survival. Although we did not conduct a factorial experiment addressing all combinations of exposure temperature and duration, our collective data from continuous cooling and extended duration experiments support a strong temperature dependency for freeze damage and the role of exposure duration, at least within a critical zone of temperatures. It is unclear how extended durations result in greater freeze damage in the time-dependent zone of temperatures.

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