Freeze tolerance of Zoysiagrass Cultivars - Pilot Study

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Objectives

Determine whether a low-cost cold stress simulator can be used to accurately determine the freeze tolerance of zoysiagrasses. Additionally, we hope to determine the effectiveness of an acclimation procedure used for bermudagrass with the cold acclimation of zoysiagrass.

Rationale

Zoysiagrass (*Zoysia* spp.) is a popular warm-season grass in the transitional and warm climatic regions of the USA because it creates an excellent golfing surface with minimal maintenance inputs and costs. Because zoysiagrass requires minimal inputs, it could play a key role in making transition zone golf courses more environmentally friendly and sustainable.

One of the roadblocks to widespread zoysiagrass use is its winter hardiness. Many new cultivars of zoysiagrass were released in the last ten years. These cultivars exhibit improved texture, shade tolerance, drought tolerance, establishment rate, divot recovery and pest resistance compared to Meyer (White and Engelke, 1990; White et al., 2001; Patton and Reicher, 2005; Karcher et al., 2005; Reinert and Engelke, 2001). However, the winter hardiness of these cultivars is yet to be proven and may restrict extensive use of zoysiagrass in the transition zone to only Meyer (Dunn et al., 1999; Dunn and Diesburg, 2004). Although many newer cultivars have either previously appeared or are currently in NTEP trials, conditions necessary for separating winter hardiness among cultivars often occurs only once per decade. As a result, a five-year NTEP trial is usually insufficient to separate differences in cold tolerance among cultivars. Identifying a standardized method to determine the relative freeze tolerance of zoysiagrass cultivars is important since winterkill results in substantial reestablishment costs and loss of use.

Researchers at the University of Oklahoma developed a standardized, rapid and reproducible method to quantify the freeze-tolerance of bermudagrass (*Cynodon* spp.) cultivars that produces results in agreement with field observations (Anderson et al., 1993). This method has helped to generate information that is important for helping golf course superintendents to select proper cultivars for their climatic area. We are proposing to use similar techniques to those at the University of Oklahoma to screen cultivars of zoysiagrass for their freeze-tolerance.

As a starting point for this project, we screened 39 cultivars for winter survival in Indiana. First year data indicates dramatic differences in winter survival between cultivars (data not shown). However, until the freeze-tolerance of these cultivars and others is quantified, it will not be known whether these cultivars could dependably be used in the transition zone.

We hope to identify cultivars with good freeze tolerance in order to increase the number of cultivars available to superintendents in the transition zone with the ultimate goal of this research being to expand the use of zoysiagrass, thereby creating more sustainable and affordable golf courses.

Materials and methods

A cold stress simulation chamber was constructed by modifying a 0.55 m^3 chest freezer similar to the method of Beard et al. (1980; 1991). The freezer was modified by adding an elevated rack inside the chamber with attached 120 mm diam. AC computer cooling fans (Philmore Manufacturing Company, Inc., Rockford, IL) to uniformly blend the air within the chamber. A programmable controller (Watlow 981, Watlow Electric Manufacturing Co., St. Louis, MO) and a type T Teflon tipped thermocouple (ThermoWorks, Alpine, UT) connected to the controller were used to control the temperature inside the chamber. The thermocouple was inserted 2.5 cm into the potting medium so that the controller would control temperature based upon soil temperatures since they are the most critical when assessing low temperature stress (Beard et al., 1980; 1991). The controller was programmed to reduce the temperature linearly by 1 °C h^{-1} similar to Anderson et al. (1993).

Plants were established in cone-tainers filled with potting-mix using a phytomere containing root, crown, and shoot material. At least one zoysiagrass cultivar with poor, medium, and good winter survival were selected based on results from field trials (Table 1). Additionally, ‘A-1’ creeping bentgrass (*Agrostis stolonifera* L.) and ‘Midlawn’ bermudagrass [*Cynodon dactylon* (L.) Pers. × *C. transvaalensis* Burtt Davy] were used as controls with known freeze tolerances (Gusta et al, 1980; Anderson et al., 1993; 2003). After 8 weeks of establishment in the greenhouse, plants were acclimated for 4 wks using a controlled-environment chamber set at 8/2 ˚C day/night cycles and a 10 h day length at 300 µmol m^{-2} s^{-1} (photosynthetically active radiation) (Anderson et al., 1988). Additional plants of Meyer were acclimated for 0 or 2 wks to determine the effectiveness of the acclimation procedure with zoysiagrass (Table 2).

After acclimation, plants were placed in a cold stress simulator to test for the relative freeze tolerance of each cultivar. Ice was added over the cone-tainers to prevent supercooling. The cold stress simulator was programmed to cool 1 ˚C per hour after 15 hours at -3 ˚C. Target temperatures (1˚C intervals, -4 ˚C to -19˚C) were programmed to cover a range anticipated to span the limits from complete survival to complete mortality. For each cultivar three cone-tainers were removed at each test temperature. Temperatures were monitored using a thermocouple inserted into the soil medium. After freezing tests plants were evaluated for regrowth in the greenhouse and the temperature resulting in no regrowth from 50% of the plants (LT_{50}) was be determined by nonlinear sigmoidal regression. This pilot experiment was not repeated.

Results

Midlawn bermudagrass and A-1 creeping bentgrass both had freeze tolerances consistent with those reported (Table 3). Meyer zoysiagrass LT_{50} values ranged from -8.1 to -11.5 depending upon acclimation treatment. This is consistent with Rogers et al. (1975) who found that Meyer freeze tolerance increased from September to January indicating that plants were not fully acclimated in early autumn and that acclimation does occur in autumn. Although Rogers et al. (1975) does not report LT_{50} values for Meyer, Beard and DiPaola (1992) extrapolated that the LT_{50} value is near -11.1 which is consistent with our findings of -11.0 (2 weeks acclimation) and -11.5 (4 weeks acclimation). Freeze tolerances of other cultivars tested in this pilot study do not appear in the literature, but relative rankings seem to be consistent with field trials (Table 1).

Most C_4 forage grasses have maximum hardening when night temperatures range from 5 to 10 ˚C for one week, but more frost-resistant species may require lower temperature for maximum hardening (Jones, 1985). This is consistent with Chang et al. (2000) who found that one week of acclimation 22/-1 ˚C day/night cycles and an 8 h day length 10 µmol m^{-2} s^{-1} improved regrowth of zoysiagrass compared to non-acclimated plants. Additionally, they found that zoysiagrass acclimated for four weeks resisted dehydration better than non-acclimated plants and that 8 weeks of cold acclimation resulted in better cryopreservation. Differing acclimation treatments with Meyer in our study confirm that acclimation is necessary when testing freeze tolerance. We did not test acclimation timings of longer than 4 weeks, but our testing shows that 2 weeks acclimation may be adequate to cold acclimate zoysiagrass but four weeks would be preferred. We used the acclimation method (4 wks of acclimation using a controlled-environment chamber set at 8/2 ˚C day/night cycles and a 10 h day length at 300 µmol m^{-2} s^{-1}) successfully used by Anderson et al. (1993) for bermudagrass. Bermudagrass and zoysiagrass both discolor and growth halts with air temperatures less than 10 ºC (Youngner, 1961) and soil temperatures less than 16 ºC (Baltensperger, 1962). There is some evidence that hardening occurs differently between bermudagrass and zoysiagrass. Zoysiagrass is more photosynthetically active (Rogers et al., 1977) and retains fall color longer than bermudagrass (Kopec, 1998; Fagerness, 2001), which may be a result of increased frost resistance and a longer hardening period prior to winter. However, this pilot study indicates that 8/2 ˚C day/night cycles are useful for cold acclimating and testing for freeze tolerance of zoysiagrass since the LT_{50} of Meyer is consistent with other reports. We plan on screening more zoysiagrass cultivars for their freeze tolerance in the future.

Table 1. Zoysiagrass cultivars used in pilot test and their field survival.

Cultivar	Species	Type†	Winter survival‡
			%
Meyer	*Z. japonica*	Vegetative	98.3
El Toro	*Z. japonica*	Vegetative	66.3
Victoria	*Z. japonica*	Vegetative	4.4
$LSD_{0.05}$			14.0

Experimental controls

Cultivar	Species	LT_{50}	Reference
Midlawn	*Cynodon dactylon*	-9.5 to –10.3 ºC	Anderson et al., 1993; 2003
Penncross	*Agrostis stolonifera*	-35 ºC	Gusta et al., 1980

† type of plant material commercially available
‡ winter survival = (after winter dormancy coverage ÷ before winter dormancy coverage) * 100

Table 2. Treatments tested with cold stress simulator.

Cultivar	acclimation duration
	---------wks----------
'Meyer' zoysiagrass	0, 2, or 4
'El Toro' zoysiagrass	4
'Victoria' zoysiagrass	4
'Midlawn' bermudagrass	4
'A-1' creeping bentgrass	4

Table 3. Freeze tolerance of zoysiagrass cultivars – pilot study

Cultivar	Acclimation duration	Freeze tolerance	Value reported in literature
	----------wk-----------	--------ºC--------	--------------ºC--------------
A-1 [a]	4	> -19.0	-35.0[b]
Meyer	4	-11.5	-11.1[c]
Meyer	2	-11.0	
El Toro	4	-11.0	
Victoria	4	-10.5	
Midlawn [a]	4	-10.0	-9.5 to -10.3[d]
Meyer	0	-8.1	

[a] 'Midlawn' bermudagrass and 'A-1' creeping bentgrass were used as checks with known LT_{50} values.
[b] Gusta et al., 1980
[c] Beard and DiPaola, 1992; Rogers et al., 1975
[d] Anderson et al., 1993; 2003

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