CHAPTER 4

EFFECTS OF TEMPERATURE DROP AND WATER PHASE ON CREEPING BENTGRASS AND ANNUAL BLUEGRASS

Introduction

Decreased plant survival under ice sheets on putting greens has often been considered to result from crown hydration damage which was first described by Beard and Olien (1963). Crown hydration damage typically results when hardened crown tissues hydrate under warm conditions followed by a period of below freezing temperatures and extensive intercellular ice formation occurs, causing membrane dysfunction and cellular collapse. Beard (1964) also reported that the injury of creeping bentgrass and annual bluegrass due to ice coverage may be a result of high plant moisture content. He reported that turf flooded with water and then frozen had much lower survival than turf under a snow cover capped with ice. Therefore, the presence of water and the phase that it is in may also affect the survivability of turf.

Other factors, such as ice temperature and the number of freeze-thaw cycles, have been associated with ice damage. Andrews and Pomeroy (1975) observed an increase in the survivability of wheat when ice temperature and the number of freeze-thaw cycles were increased. They reported that ice temperatures of –1 C did not cause intercellular ice formation, but decreasing the temperature to –3 C did initiate intercellular ice formation, and ice accumulation increased as the temperatures were lowered.

Carbohydrate levels have also been shown to affect the winter hardiness of warm season grasses. Rogers et al. (1975) reported an increase in the winter hardiness of Meyer zoysiagrass as total non-structural carbohydrates (TNC) increased. Similar findings were reported for bermudagrass by Fry et al. (1993).

Research in the role of carbohydrates in ice-related damage for cool-season turfgrasses is very limited. Dionne et al. (2000) reported a significant increase of fructan levels in cold acclimated annual bluegrasses. Even though the fructan levels increased, they did not observe a correlation between fructan or sucrose levels and freezing tolerances of the three annual bluegrass ecotypes evaluated.

Previous experiments (Chapters 1 and 3) indicated that creeping bentgrass and annual bluegrass are differentially affected by reduced irradiance and ice coverage, but relationships between those two conditions were not evaluated. The objectives of this research are to determine if the TNC concentrations induce by low irradiance and/or water phase (i.e. water or slush) affects the survivability of creeping bentgrass and annual bluegrass to cold temperatures and ice coverage.

Materials and Methods

Plant materials used for this study were removed from field plots that were established from seed on a USGA specification putting green in the spring of 2000. Plots were maintained as a putting green, and plugs were removed from the plots with an 18-mm diameter sampling tube to a depth of 27 mm.

To reduce crown TNC levels of the turf plugs to be used for the low carbohydrate treatments, plugs for experiment one were placed in a growth chamber maintained at

20 C. Illumination was supplied by incandescent bulbs for 16 hr daily to maintain phytochrome responses, but the irradiance levels were insufficient to support significant levels of photosynthesis. Plants were maintained in the growth chamber for 14 to 21 days prior to the beginning of an experiment to ensure TNC levels were reduced. For experiment two, turf plugs were removed from the field and maintained in a greenhouse. Plugs for the high carbohydrate treatments were maintained in full sun, and plugs for the low carbohydrate treatments were maintained under a shade cloth that reduce irradiance 80%.

TNC levels were measured at the start of the experiment. A scalpel was used to remove the shoots and roots from the crowns. The remaining crown tissue was approximately 1 to 2 mm in length. The samples were put in coin envelopes and dried at 100 C for 1 hr and 65 C for 24 hours.

Dried samples were reduced with an Udy Corporation (Fort Collins, CO) Cyclone mill using a 60-mesh screen and placed in 25 ml glass scintillation vials with screw-on caps. Samples were analyzed by the West Virginia University Rumen Profiling Laboratory for TNC contents using the method described by Smith (1964). Average TNC levels are in Table 4.1.

The experimental design of both experiments was a 2 x 2 x 3 x 2 factorial using species, carbohydrate level, water phase, and temperature drop as the factors, respectively. The species used were creeping bentgrass and annual bluegrass, and the carbohydrate levels were designated high or low. Water phase treatments were water, slush (50% water, 50% ice), and no water (i.e. air). The temperature drops were –1 C to –4 C at one degree C $hour^{-1}$, and –1 C to –4 C at 0.25 degree C $hour^{-1}$.

Table 4.1. Average TNC concentrations of creeping bentgrass and annual bluegrass at the beginning of the experiment.

	Creeping Bentgrass	Annual Bluegrass
	TNC (%)	
High level	4.66	5.44
Low level	4.09	4.53
% reduction	12.2	16.7

The experiment was conducted in a Thermo Neslab programmable water bath (Model RT-221). The turf plugs were 25 mm in diameter and 25 mm long and were placed in a constant temperature room maintained at 4 C with no lights for 24 hours immediately before the treatment applications. This was done to get the turf plug temperature to 4 C.

Individual plugs were placed in 125 ml test tubes and placed in the bath set at 4 C. Twenty-five mls of distilled water was added to all water and slush treatments and to an empty test tube in the bath. The bath temperature was then dropped from 4 C to –1 C in two hours.

Temperature was maintained at –1 C for one hour and 200 g of ice was placed in an empty test tube in the bath at the beginning of the 1-hr period. This was done to equilibrate the temperature of the ice to –1 C. At the end of the hour, 25 g of the ice was added to all slush treatments, and 25 mls of the chilled water was added to all of the

water treatments. A few ice chips were added to the water treatments to serve as ice nucleators. Otherwise, the water would supercool and would not freeze, even with the submerged turf plug in the water. Thermocouple probes, monitored by a Hobo H08-006-04 dataloger (Onset Corp., Bourne, MA), were placed in one of the water, slush, and no water treatments. Temperatures were logged every minute of the experiment.

The temperature was lowered differentially, depending on the treatment, and then all treatments were raised to 4 C for two hours. Plugs were removed from the test tubes and separated into individual tillers. Percent survival was determined by planting 10 tillers of each treatment in cell trays filled with a 50-50 mixture (volume basis) of Promix potting soil and Hagerstown silt loam soil. The individual cells were 22 mm square by 35 mm deep. Planted cell trays were placed in shallow pans containing 5 mm of water to ensure saturation and to prevent the soil mix from drying out. Percent survival was determined 14 days after planting.

All data were analyzed as three replicates of a two species by two carbohydrate level by three water phases by two temperature drops complete factorial in a complete randomized block design with the trial being replicated. Variances were homogenous across trials (Levene, 1960), so the data of both trials were pooled and analyzed using mixed models analysis (Littell et al., 1996) with the mixed procedure of SAS (1999). Trial and replicates were considered random and percent survival was inverse sine transformed prior to analysis (Steele and Torrie, 1980).

Results and Discussion

Immediately upon removal from the low temperature bath, the color of the plants in the air treatment was not affected, but the plants in the water and slush treatments were dark green. During the tiller separation and planting to determine percent survivals, the tillers subjected to water and slush were very fragile and lacked turgor. Many of the tillers had the stems easily pulled out of the sheaths. The slush treated plants displayed more of these tissue damage characteristics than the water treated plants. The air treated plants did not appear to be structurally damaged in any way.

The water treatments created visually noticeable affects on the submerged turf plugs. The water and slush treatments, in all replications, caused the verdure tissue to lose structure; and the tissue was darker and appeared water soaked. The appearance was similar to tissue damage created by pressure being applied to a frosted turf. The plugs subjected to air only did not appear to be affected by the –4 C temperature.

The temperature drop of 3 degrees C from –1 to –4 at rates of 0.25 and 1.0-degree $hour^{-1}$ did not significantly affect survivability (Table 4.2). The fast rate of 1-degree $hour^{-1}$ did decrease survival rate more than the 0.25-degree $hour^{-1}$ rate (Fig. 4.1), but the difference was not statistically different.

There were also no significant differences in survival between creeping bentgrass and annual bluegrass either (Fig. 4.2). This is contrary to observations made by Beard (1964). A significant difference between this research and Beard's study was that Beard used field hardened plant material, and I used unhardened plant material. Using hardened plant material may have introduced other unmeasured variables into the experiment such

Table 4.2. Analysis of variance for the effects of TNC concentrations, temperature drop, water phase on the survival of creeping bentgrass and annual bluegrass (Data transformed using inverse arc-sine).

Source	df	F-Values	Pr > f
Species	1	0.50	0.4826
Carb. conc.	1	67.11	0.0001
Species * carb. conc.	1	1.01	0.3173
Temp. drop rate	1	1.57	0.2128
Species * temp. drop rate	1	0.71	0.4021
Carb. conc. * temp. drop rate	1	0.00	0.9822
Species * carb. conc. * temp. drop rate	1	0.06	0.8066
Water phase	2	29.44	0.0001
Species * water phase	2	0.73	0.4843
Carb. conc. * water phase	2	4.96	0.0085
Species * carb. conc. * water phase	2	0.16	0.8522
Temp. drop rate * water phase	2	0.53	0.5874
Species * temp. drop rate * water phase	2	0.30	0.7382
Carb. conc. * temp. drop rate * water phase	2	0.14	0.8704
Species * carb. conc. * temp. drop rate * water phase	2	0.08	0.9251

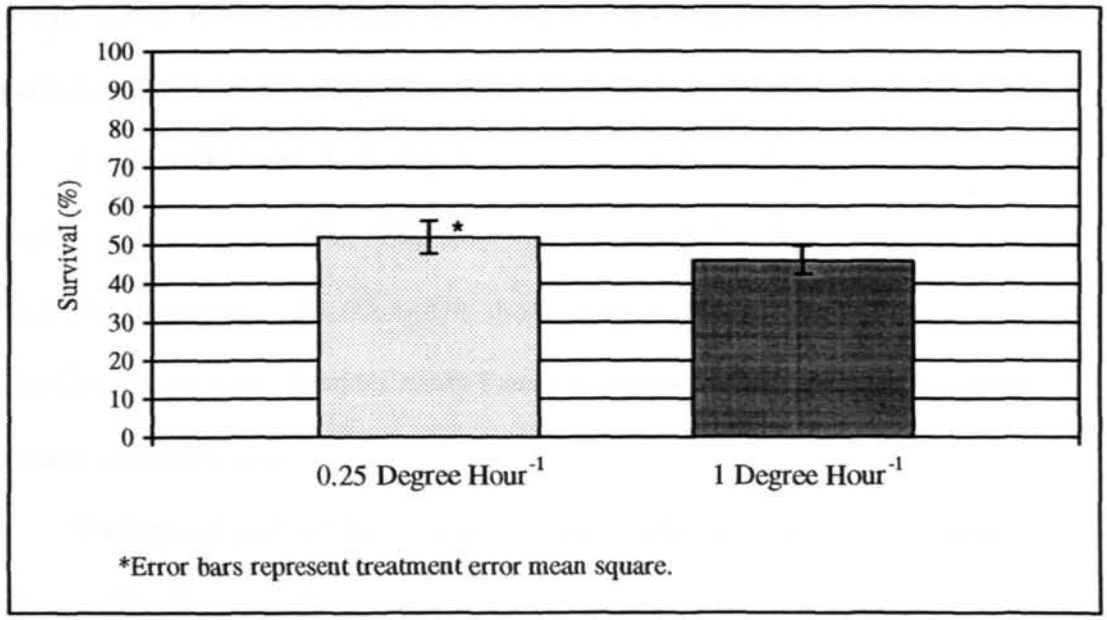

Fig. 4.1. Percent survival of plants subjected to two temperature drop rates averaged across species, carbohydrate concentration, and water phase.

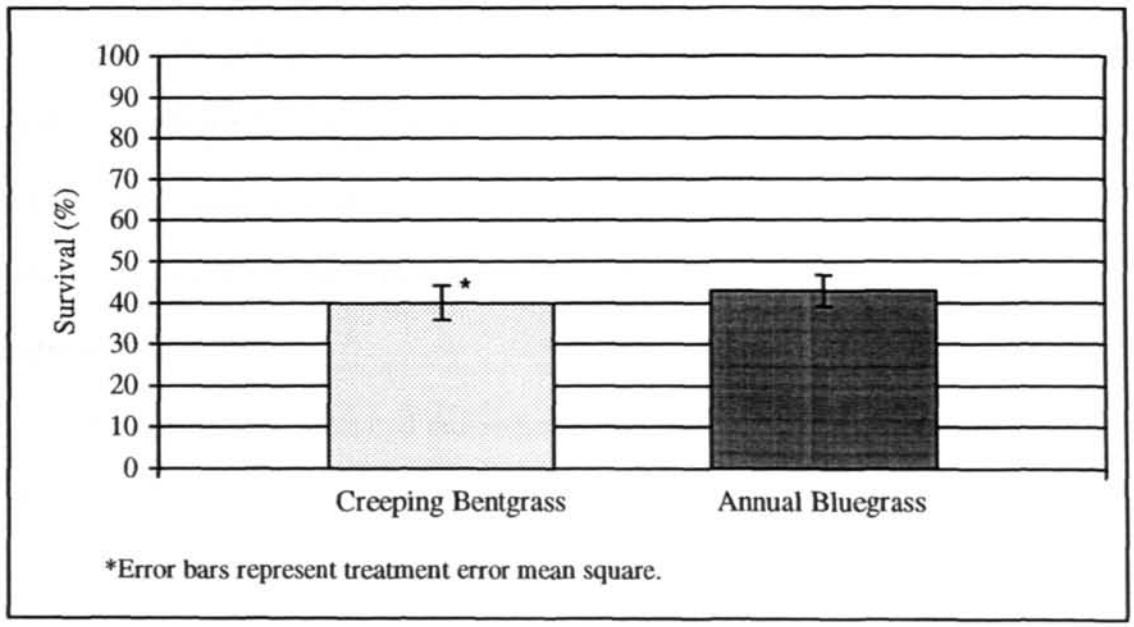

Fig. 4.2. Survival of creeping bentgrass and annual bluegrass averaged across species, carbohydrate concentration, and water phase.

as carbohydrate levels and state of hardening. Dehardening of creeping bentgrass and annual bluegrass can be highly variable (Tompkins et al., 2000).

Another difference between these studies and those of Beard was the duration of exposure to water treatments. This study used durations less than 24 hours, whereas Beard used durations of 30, 60, and 90 days. My study focused on immediate damage caused by freezing, and Beard's study focused on long term build up of metabolic by-products and suffocation.

The type of annual bluegrass used in the two studies may have also been different biotypes. The Beard study may have used the annual form of annual bluegrass (Hovin, 1957; Tutin, 1957), whereas this study used the perennial form (Koyama, 1987). Few studies have been conducted on perennial annual bluegrass and ice-related damage (Dionne et al., 2001; Tompkins et al., 2000).

The carbohydrate concentration by water phase interaction on plant survival was significant (Table 4.2). Percent survival was significantly lower for the water and slush treatments as compared to the air treatments and the low carbohydrate treatments were more affected by the water and slush treatments as compared to the high-carbohydrate treatment (Fig. 4.3).

This interaction indicated that reduced TNC concentrations increase susceptibility to freezing injury, especially when water was present. The relationship between carbohydrate status and freezing tolerance has been reported for warm-season grasses by Rogers et al. (1975) and Fry et al. (1993). They observed an increase in freezing tolerance as carbohydrate concentrations increased. McKersie et al. (1982) also reported

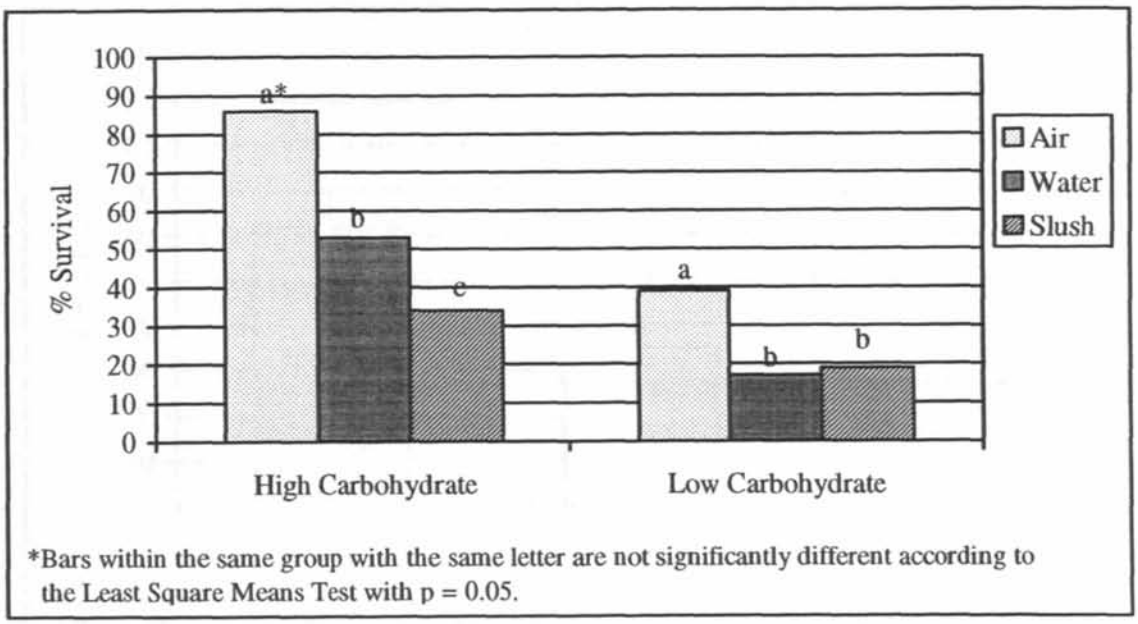

Fig. 4.3. Survival of high carbohydrate and low carbohydrate grass treatments subjected to air, water, and slush treatments averaged across species and temperature cooling rate.

a strong correlation between carbohydrate concentrations and ice coverage tolerance of several wheat selections.

The water and slush treatments caused significantly lower survival than the air treatment (Fig. 4.4), and although the slush provided the lowest survival, it was not statistically lower than water.

To create the slush, ice was added to water that had already super cooled to –1 C. The ice addition caused a very quick (within minutes) freeze of the super cooled water. This rapid freeze would not allow for the submerged plant's cells to make decreases in cellular water content or osmotic potentials to decreasing temperatures, which may have increased cellular damage (Steponkus, 1984). The use of unhardened plant material could have compounded this problem.

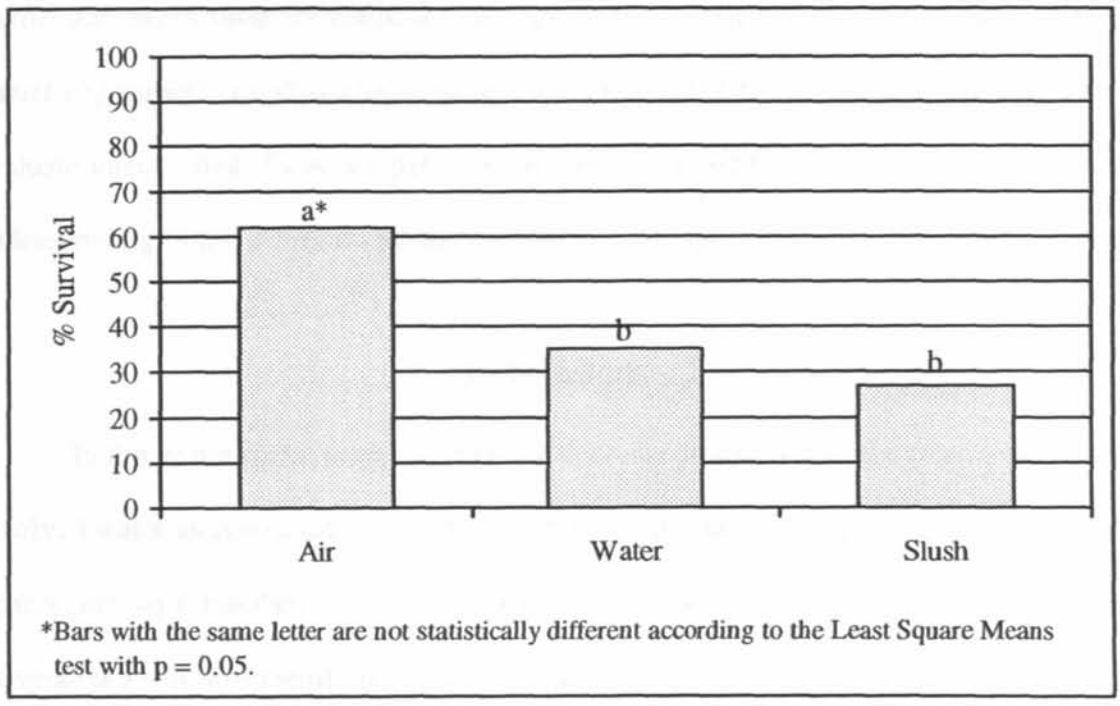

Fig. 4.4. Percent survivals of plants subjected to selected water phase treatments averaged over species, temperature drop, and TNC concentration.

Similar results related to water phase were reported by Beard and Olien (1963) and McKersie et al. (1982). Beard and Olien reported that ice encasement was more lethal to creeping bentgrass and annual bluegrass than surface ice or snow covered with ice. They suggested that ice encasement damage was more severe due to the ice's physical forces exerted on the plants, which would cause cellular damage.

McKersie et al. (1982) showed that ice encasement damage was more severe than flooding damage of winter wheat. They reported the flooding treatment increased ethanol production more than the ice encasement treatment. Also, tissue-reducing sugars were significantly reduced in the ice encasement treatment, but not in the flooding treatment.

The difference in freezing and flooding damage is just one example of the many complexities involved in diagnosing environmental stress injury. There are usually several

factors and interactions involved, and attempting to identify a single cause may be futile. Future experiments need to include several factors and use factorials and other designs that evaluate interactions. Complex problems will require complex research to develop a better understanding of the problem and thus generate solutions.

Conclusions

In this experiment, it appears that the decrease in survival of the treatments that involved water as compared to the air-only treatments was due to cellular damage caused by freezing since the damage occurred in less than 24 hours. The significant decrease in survival as a result of the turf being in contact with water or slush as the temperature drops below freezing indicates that ice encasement damage can occur at the time of initial freezing and may not be dependent on the duration of the ice coverage. Even though non-hardened plant material was used in this experiment, this type of damage could happen in the field since these two grasses (especially annual bluegrass) can quickly break dormancy (Cattani et al., 2000; Tompkins et al., 2000).

The TNC concentration appeared to affect the freezing tolerance of creeping bentgrass and annual bluegrass. This would imply that late winter or early spring ice events may be more damaging due to lower carbohydrate levels, as compared to late fall or early winter events. Cultural practices to increase carbohydrate levels, such as increasing mowing heights and minimizing late-season nitrogen applications, may increase tolerance to ice damage. Applications of plant growth regulators that increase carbohydrates may also be beneficial (Rossi and Buelow, 1997).

The relationship between carbohydrate levels and freezing tolerance may also indicate that the predisposition of turf to ice encasement freezing damage may be a more important factor than ice quality or duration. Previous theories of ice encasement freezing damage usually focused on ice density, suffocation, and toxic build-up, when in fact, a majority of damage might occur at the time of initial freezing, especially if it occurs in late winter or early spring.

Severe ice damage may not be caused by just a break in dormancy or crown tissue hydration. The presence of unbound water appeared to significantly increase freezing damage. Even though the plants examined were unhardened and fully hydrated, water and slush were found to decrease survival. This indicated that plants submerged in water, even for short periods of time (i.e., a few hours), may become over-hydrated and much more susceptible to freeze-related stress.

Further research needs to be conducted to better understand the role that carbohydrates play in ice coverage damage. It would also be beneficial to know more about the interactions of water and plant tissue, especially crown tissue. Both of these areas appear to be directly related to ice-related stresses.