CHAPTER 3

EFFECTS OF ICE COVERAGE ON CREEPING BENTGRASS AND ANNUAL BLUEGRASS SURVIVAL

Introduction

The causes of ice-related damage to highly-maintained turf are not well understood. Moreover, ice-related damage can be very extensive, and current information does not provide satisfactory explanations or prevention techniques.

Ice damage is usually a result of the turf being covered with ice for a long period of time or of turf crowns partially or entirely encased in ice. It has been reported in small grains and alfalfa that ice coverage can decrease or stop gas diffusion (Andrews, 1977; Andrews and Poweroy, 1975; Olien and Smith, 1981; and Sprague and Graber, 1940). A gas diffusion barrier can create either anoxia and/or elevated levels of toxic by-products of biological reactions for the underlying turf.

Ice encasement of plants usually results in a physical alteration and damage of plant cells due to membrane disfunction (Olien and Smith, 1981). The depth to which the crowns are encased in ice is also a factor affecting plant survivability (Olien and Smith, 1981).

The amount of research on ice-related damage on turf is extremely limited. Beard (1965a, 1965b) reported that Kentucky bluegrasses and creeping bentgrasses had tolerances to ice coverage of 51 and 120 days, respectively. He also reported that there were differences in ice coverage tolerance between bentgrass selections.

The objectives of this research were to (1) determine the differences between creeping bentgrass and annual bluegrass tolerances to ice coverage; (2) determine if flushing the turf-ice air inter-space with air could affect survivability; and (3) determine if CO_2 accumulated under the ice.

Materials and Methods

Plant material in this experiment was grown in (24 mm diameter by 124 mm long) plastic root tubes in Hagerstown silt loam soil. The soil was steam sterilized at 82 C for four hours prior to use to decrease weed infestations. Tubes were planted with 4-5 tillers of annual bluegrass or creeping bentgrass.

The annual bluegrass was selected from a putting green at the Village Green Golf Course in Hickory, PA. Selections were made following a winter in which extensive ice damage occurred to putting greens. Annual bluegrass selections were cultivated from areas that exhibited either good (VG18A) or poor (VG18D) tolerance to the ice coverage. 'Penncross' and 'Penn A-4' creeping bentgrass selections were removed from 3-yr-old field plots maintained as a putting green at the Valentine Turfgrass Research Center, University Park, PA.

Turf was clipped with scissors as needed to maintain heights at approximately 4 to 5 mm. Soluble fertilizer (28-7-14) was sprayed at 97 kg N/ha at a delivery rate 374 l/ha. Fertilizing was applied to minimize growth and was approximately 291 kg N/ha/year. Fertilizer applications were lightly watered in after application to remove the spray solution from the foliage. Tubes were placed in shallow trays filled with water to provide ample moisture for growth and maintained in a greenhouse.

Tubes for the hardened treatment were placed outside in mid-October to allow for natural hardening. Tubes were brought inside on February 5 for experiment 1 and February 23 for experiment 2 and maintained in a –4 C freezer for a few hours prior to the initiation of the experiment. Tubes for the unhardened treatment were maintained in a greenhouse prior to treatment.

Ice Coverage of Root Tubes

Plastic beakers were used to create an ice layer over top of the root tubes. Twenty-four mm diameter holes were drilled in the bottom of 50-ml plastic beakers, and the root tubes were pushed (bottom first) through the holes until the beaker bottoms were snug with the top lip of the root tubes. The compression seal between the beaker and root tubes was air and water tight.

Tubes that received air injections had 3 mm holes drilled in the side of the beakers 5-mm from the bottom. Brass tubing fittings were screwed into the holes, and 2-mm venting holes were drilled in the opposite sides of the beakers. The vent holes allowed for the air spaces between the turf and ice layer to be vented when air was injected into the beakers.

Manifolds carrying the air were constructed from 12 mm diameter pvc pipe 30 cm long. The pvc manifolds had an inlet tee in the middle and caps on the end. Thirty 3-mm brass tubing fittings were inserted into each manifold. The tubing fittings were connected to the beakers receiving air injections with 60-cm long, 4-mm id Bev-a-line XX tubing. This tubing was used because it had low oxygen permeability.

The air source was a compressed air line in the laboratory. The outlet was fitted with a 12 mm brass solenoid valve controlled by an electronic timer. Four manifolds were connected to the solenoid valves with 20 mm id vinyl tubing. Several extra meters of the 20-mm tubing was placed inside the freezer to allow cooling of the incoming air to be equivalent to the freezer temperature and prevent the ice from melting. The system was evaluated and adjusted prior to the study to ensure uniform air flow was provided to all beakers.

Beaker and root tube assemblies were placed upside down in 20-mm deep distilled water contained in 250-ml plastic beakers. Tubes were placed in a –4 C freezer until all water was frozen. The ice layers were approximately 20 mm thick, 50 mm in diameter. The ice formed approximately 20 mm above the surface of the turf to leave a small air space so the ice did not contact the turf.

The tubes and ice were removed from the 250 ml beakers and randomly placed in tubing racks in a –4 C freezer. The tubes that received the air injection treatment were connected to the manifolds. The timer was set to open the solenoid valve and deliver air for one hour per day (an ample amount of air to flush the air spaces in all beakers).

Root tubes were removed 30, 60, and 90 days from the initiation of the study and placed in a 3 C refrigerator for 48 hours and then moved to a greenhouse for 24 hours. The turf plugs were removed from the tubes and separated to determine percent survival and TNC determinations.

Percent survival was determined by planting 10 tillers of each treatment in cell trays filled with a 50-50 (volume basis) mixture of Promix potting soil and Hagerstown silt loam soil. The individual cells were 25 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 seven days after planting.

Remaining plants from each replication were combined for TNC analysis. Verdure and roots were removed from the crowns with a scalpel, and verdure and crowns were placed in coin envelopes. Plant samples were placed in a 100 C-drying oven for 1 hour and then into a 65 C-drying oven for 24 hours. Samples were weighed again to obtain oven dry weights and reduced with an Udy Corporation Cyclone mill using a 60-mesh screen and placed in 25 cc 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 et al. (1964). Crown and shoot percent moisture content was calculated gravimetrically using the fresh and oven-dry plant weights.

In experiment two, prior to ice creation, 38 mm long #18 syringe needles were installed through the side of the root tube, up through the rootzone material so that the tips of the needles were positioned into the air spaces. A small amount of epoxy was used to seal the insertion area of the root tube. Needles were only installed in the 90-day removal tubes of the no-air injection treatments.

The connecting end of the needles was fitted with Luer Lock valves. The valves were placed in the closed positions at the beginning of the study to prevent air flow through the needles. Upon removal at 90 days, the needles were connected to 5 cc air-tight gas syringes, and 2 cc of gas was removed from the air spaces immediately upon removal from the freezer. Gas was sampled from the air injection treatments by inserting a syringe needle into the brass tubing connector and evacuating 2 cc of air from the air space.

Gas samples were analyzed using a Hewlett Packard 5890 Series II gas chromatograph equipped with a Porapak Q column. The detection was accomplished using a thermal conductivity detector using UHP helium gas for a carrier. The gas chromatograph was interfaced with a Windows-based computer running OMS Tech (Miami, FL) E-lab Chromatography software (Version 4).

Samples were injected through a magnesium perchlorate trap to remove water and into a 10 cc sample loop. Standard curves were developed using standard concentrations of 363 and 2001 ppm CO_2 (Fig. 3.1) supplied from Scotty Specialty Gases. Collected air samples were analyzed twice. The data from the two runs was averaged. Standards were run every three to four hours to ensure the system was stable. Sample "slopes" were calculated by dividing the area of the CO_2 peak by the sample loop pressure. Sample CO_2 concentrations were calculated by dividing the sample "slope" by the standard (363 ppm) "slope" and multiplying by the standard concentration (363 ppm).

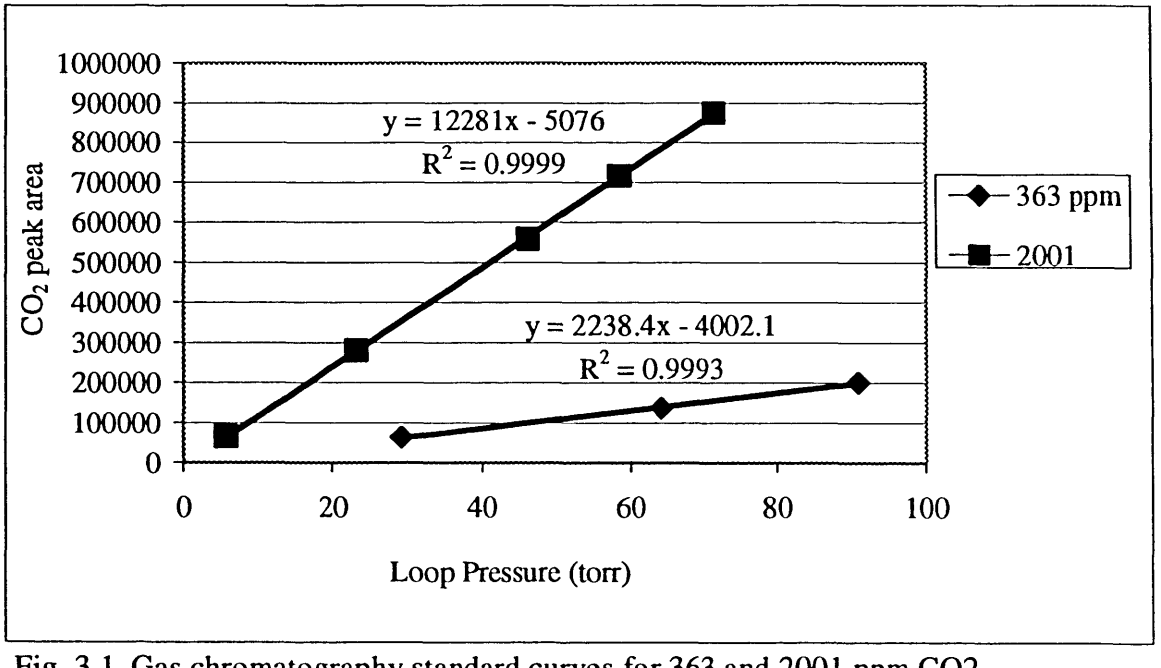


Fig. 3.1. Gas chromatography standard curves for 363 and 2001 ppm CO2.

All data, except for the CO_2 concentrations, were analyzed as three replicates of a two species by two hardiness by two treatments (air injection and no air injection) by three removal time complete factorial in a completely random design with the trial being repeated once. 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). The CO_2 concentration data was analyzed as a three replicate by two species by two hardiness by two treatment complete factorial in a completely randomized design, and the trial was not repeated.

Results and Discussion

Survival of Creeping Bentgrass and Annual Bluegrass Under Ice Coverage

After removal and thawing, the unhardened plants of both species appeared green and alive at all three removal times. Many of the unhardened bentgrass plants showed signs of growth after one or two days in the greenhouse. However, this early growth usually did not continue, and the majority of healthy-looking tillers turned brown and lost turgor after three or four days in the greenhouse.

The hardened plants of both species appeared light or dark brown, the same as when the experiment began. The verdure of most plants not receiving air injections was dry, and some plants receiving air injection treatments were wet. This may have been the result of a slight increase in temperature from the injection of air that was slightly above

0 C or an increase of humidity in the space between the ice and turf, also resulting from the air injections.

Averaged over hardiness and air injection treatment, creeping bentgrass demonstrated increased survivability under ice as compared to the annual bluegrass (Table 3.1; Fig. 3.2). Although bentgrass survivability decreased more than the annual bluegrass when comparing hardened versus unhardened treatments.

Table 3.1. Mean percent survivals of creeping bentgrass and annual bluegrass under ice coverage averaged across removal times (30, 60, and 90 days) and air injection treatments.

	Hardened	Unhardened
	% Survival	
Creeping bentgrass	87.2	18.1
Annual bluegrass	17.2	10.8

The species by hardening interaction was significant (Table 3.2). Annual bluegrass had low survivability regardless of hardening, whereas hardened creeping bentgrass had greater than 87% survival. The tolerance of creeping bentgrass to ice coverage or ice encasement has been reported by Beard, 1965a and 1965b; Tompkins et al., 2000; and Cattani et al., 2000. Interestingly, in this study, unhardened creeping bentgrass exhibited tolerance equivalent to hardened annual bluegrass (Table 3.1). This indicates that annual bluegrass had poor tolerance to ice coverage regardless of hardened

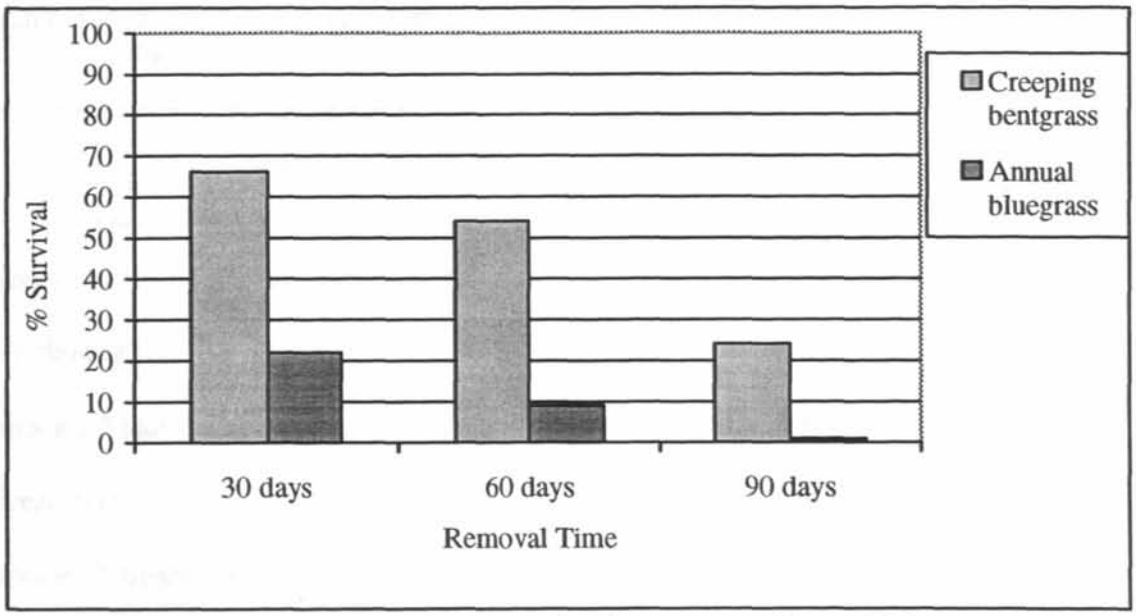


Fig. 3.2. Mean percent survivals of creeping bentgrass and annual bluegrass averaged over hardened condition and air injection treatments.

condition. Furthermore, the data indicated that the hardened condition of creeping bentgrass is critical to ice coverage tolerance (Tompkins et al., 2000).

The hardening by removal interaction was also significant (Table 3.2). The hardened plants maintained moderate survivability across all three removal times and unhardened plants survivability decreased over time (Fig. 3.3).

This data indicates that there is a factor besides freezing damage that decreased the survivability of unhardened plants over time. If cellular damage due to ice formation in the tissue was the only cause of plant freezing damage, then all of the damage should have occurred prior to the 30-day removal, and a large decrease in survivability should not have occurred beyond that time.

Table 3.2. Survival analysis of variance for species, hardening, treatment, and removal factors.

Source	df	F-Values	Pr > F
Species	1	134.67	< .0001
Hardening	1	138.82	< .0001
Species * hardening	1	88.77	< .001
Treatment	1	7.4	0.0075
Species * treatment	1	2.04	0.1505
Hardening * treatment	1	0.23	0.6349
Species * hardening * treatment	1	2.75	0.1002
Removal	2	13.88	< .0001
Species * removal	2	3.03	0.0520
Hardened * removal	2	4.67	0.0112
Species * hardening * removal	2	1.34	0.2650
Treatment * removal	2	2.22	0.1132
Species * treatment * removal	2	1.66	0.1943
Hardening * treatment * removal	2	0.19	0.8294
Species * hardening * treatment * removal	2	0.38	0.6834

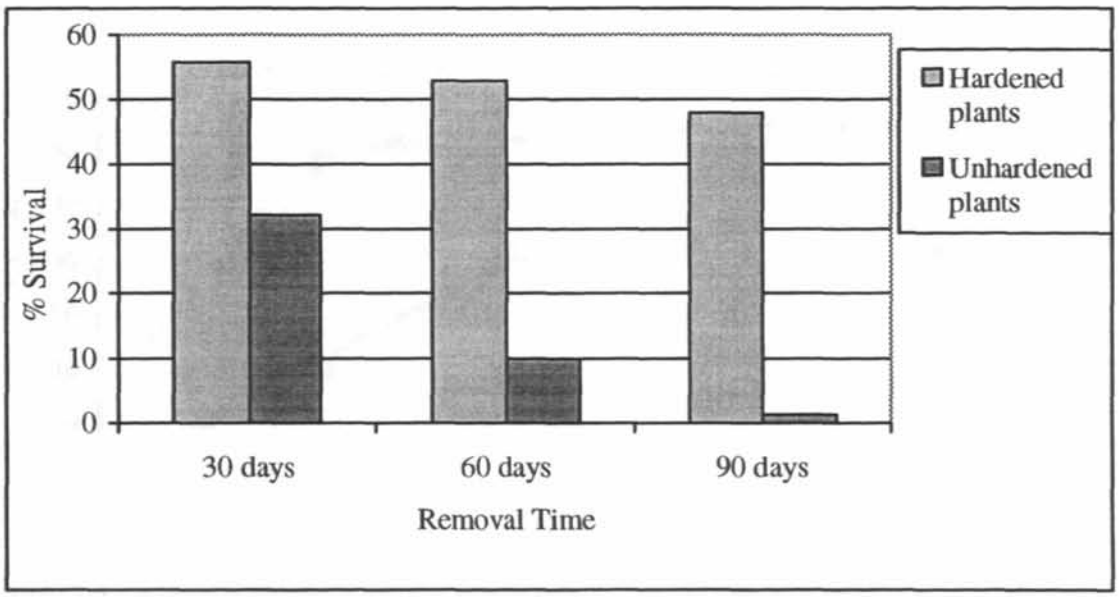


Fig. 3.3. Mean percent survivals of hardened and unhardened plants following 30, 60, and 90 d of ice coverage.

Crown TNC Concentrations

The total non-structural carbohydrate (TNC) levels of the crowns for both the unhardened and hardened plants decreased over the three removal times (Fig. 3.4). The unhardened plants started with a lower TNC concentration than the hardened plants and may have reached a critically low concentration early in the experiment. This may explain the faster decline in survival for the unhardened plants as compared to the hardened plants. Continued depletion of reserves of the hardened plants may have decreased survival, especially since photosynthesis was stopped.

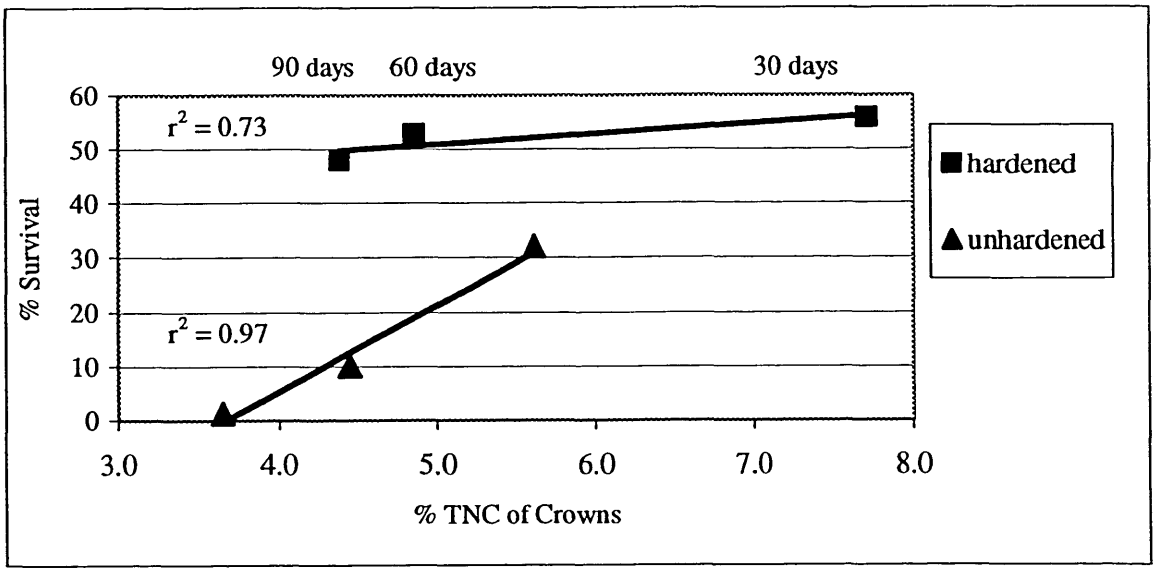


Fig. 3.4. Mean percent survivals of hardened and unhardened plant crowns versus %TNC for 30, 60, and 90 d of ice coverage averaged across species and air injection treatments.

There was a positive association between survival and TNC level for hardened and unhardened plants (Fig. 3.4). The r^2 value for the linear regression was 0.73 and 0.97 for hardened and unhardened plants, respectively, indicating that there is a strong relationship between survival and TNC combination. This relationship suggests that species that have the capacity to store large amounts of TNC or utilize them at a reduced rate, would have increased survivability when ice or snow cover limits photosynthesis for extended periods.

The species by removal interaction was also significant (Table 3.3).

Table 3.3. Mean average percent survivals of creeping bentgrass and annual bluegrass following 30, 60, and 90 days of ice coverage averaged across hardened condition and air injection treatments.

Duration of Ice Coverage (days)	Creeping Bentgrass	Annual Bluegrass
	% survival	
30	a* 65.4 x	a 22.1 y
60	a 53.8 x	b 10.7 y
90	c 38.6 x	b 13.0 y

*Means within the same column prefaced with the same letter and means within the same row followed by the same letter are not statistically different according to the Least Square Means Test with p = 0.05.

Annual bluegrass had significantly better survival at 30 days versus 60 and 90 days. Creeping bentgrass also had significantly better survival at 60 days compared to 90 days, whereas, there was no difference in survivability of annual bluegrass for those same removal times. Comparing species, creeping bentgrass had significantly better survivability than annual bluegrass at all three removal times.

The treatments of air injection and no air injection were significant (Table 3.2). Plants receiving air had lower survival percentages than the enclosed plants (Fig. 3.5).

The tissue of the plants receiving air appeared to be very flaccid. The hardened plants appeared to have an increased tissue moisture content and did not appear to retain hardiness. The unhardened plants had a significant loss of tissue structure, as compared to the hardened plants. However, the treatment by hardening interaction for survival was not significant (Fig. 3.2).

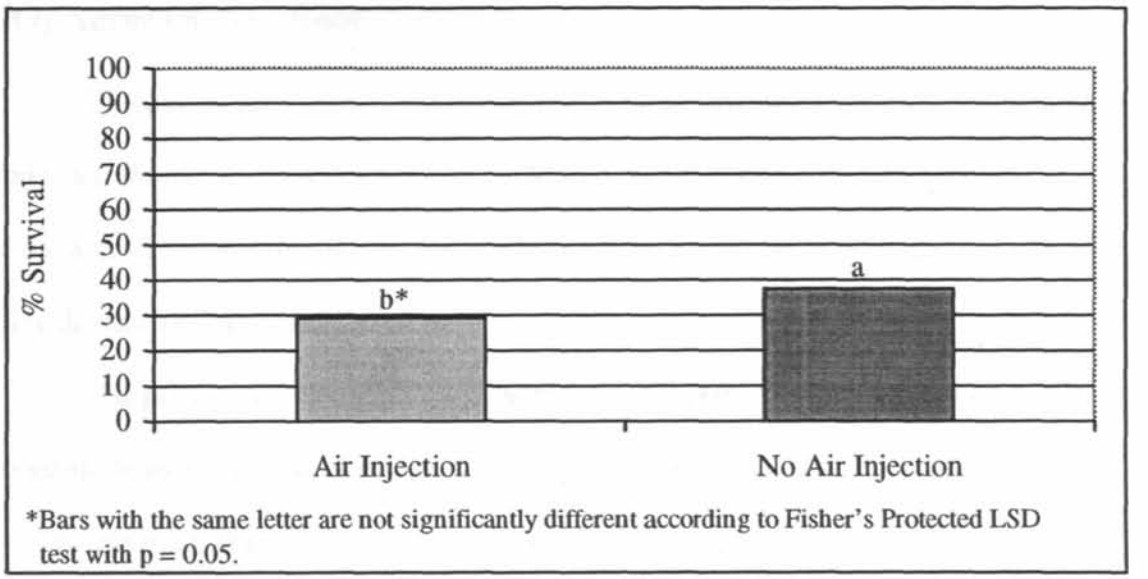


Fig 3.5. Mean percent survivals averaged across hardened condition, removal times, and species.

The ventilated air channel between the turf and ice layer of the air injection treatments had ample amounts of O_2 for respiration to continue, and the daily air injection should have been enough to flush or dilute any buildup of toxic substances. The daily air injection, albeit a small volume of air, may have increased desiccation loss of leaf and meristematic tissue. Another cause for the significant difference could have been a slight increase in temperature from the injected air, which could have increased respiration or decreased hardness. Attempts were made to cool the air sufficiently by looping several meters of tubing in the bottom of the freezer between the solenoid valve and manifolds. Air temperature was only measured at the beginning of the experiment, and it was –4 C (same as the freezer).

CO_2 Accumulation Under Ice

CO_2 and other products of biological reactions have been shown to accumulate under ice sheets (Andrews, 1977). In the 90-day removal of the second experiment, 2 cc of air was removed from the air space between the ice and turf for CO_2 analysis 12 hours after the last air injection.

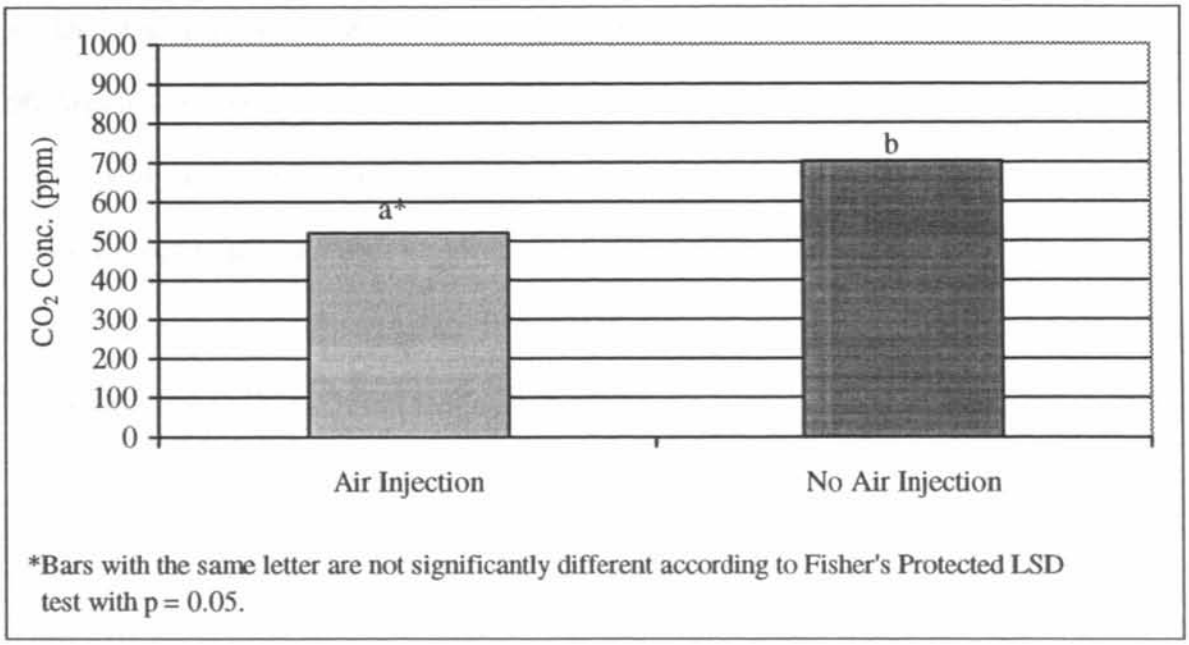
The plants receiving air injections had significantly (Table 3.4) lower CO_2 concentrations (521 ppm) than the enclosed plants (702 ppm) (Fig. 3.6). However, the system was not tested for leaks, and it cannot be determined if some CO_2 had leaked out of the beakers during the experiment.

Both of these levels were higher than the 510 ppm level of laboratory air, and the injected air concentration of 230 ppm. Even though air (with a low CO_2 concentration) was being injected into tubes with vent holes, CO_2 accumulated in the airspaces. Therefore, in situations that are basically air-tight (e.g., large ice sheet coverage), the amount of CO_2 entrapment could be very large.

Additionally, ice sheets in the field may have single cracks or a series of cracks throughout the ice. If the cracks create an opening from the turf to the atmosphere, gas exchange of areas in close proximity to the cracks would be greatly improved.

Table 3.4. Analysis of variance for CO_2 concentrations of air removed from a turf-ice interface following 90 days of ice coverage.

Source	df	F-Value	Pr > F
Species	1	1.06	0.3176
Hardness	1	0.03	0.8679
Species * hardness	1	0.00	0.9759
Treatment	1	5.74	0.0292
Species * treatment	1	0.28	0.6026
Hardness * treatment	1	0.25	0.6251
Species * hardness * treatment	1	0.15	0.7011



*Bars with the same letter are not significantly different according to Fisher's Protected LSD test with p = 0.05.

Fig 3.6. CO_2 concentrations of air removed from turf-ice interface following 90 d of ice coverage.

Conclusions

Creeping bentgrass demonstrated an increased tolerance to ice coverage compared to annual bluegrass. The status of hardening affected creeping bentgrass more than annual bluegrass, although this may be because annual bluegrass had less than 20% survival regardless of hardening.

There was a strong linear relationship between percent survival and TNC levels. This relationship may indicate that plants that have increased carbohydrate storage capacity (e.g., stoloniferous and rhizomatous species) and/or lower respiration rates during dormancy have an increased chance of survival during long periods of reduced photosynthesis.

Injecting air to prevent anoxia or toxin build-up actually decreased survivability. The plants were affected by something other than O_2 deficiencies or toxin accumulations. Hence, anoxia or excessive toxin build-up did not decrease survivability, even after 90 days of ice cover.

CO_2 accumulated significantly more in the no air injection treatment as compared to the air injection treatment. The impermeability characteristics of ice can limit gas exchange and entrap gasses emitted from plants and soil organisms (Rakitina, 1965). Although the CO_2 levels measured in this experiment could not be correlated with plant survival, this data shows that the ice restricted gas movement.

Future research is needed to determine whether carbohydrate storage and utilization efficiencies of hardened and unhardened creeping bentgrass and annual bluegrass would be important to enhance the understanding of ice coverage damage.

Determining the biological reaction by-products and their toxic concentration thresholds would also be useful information in finding solutions to the problem of turf ice coverage.