CHAPTER 2

LOW OXYGEN CONCENTRATIONS ON CREEPING BENTGRASS AND ANNUAL BLUEGRASS SURVIVAL AND TOTAL NON-STRUCTURAL CARBOHYDRATE CONCENTRATION

Introduction

The reduction of oxygen under ice sheets is a theory that has been associated with turf damage caused by ice (Beard, 1996). The impermeable characteristics of ice have been shown to prevent the movement of O_2 and CO_2 by Rakitina (1965). This prevention of gaseous exchange could create hypoxic or even anoxic conditions under continuous ice sheets or allow toxic biological by-products, such as ethanol, to build up under the ice (Andrews, 1977).

Dionne et al. (2000) evaluated the atmospheric environments under permeable synthetic covers used to protect putting greens in the winter. They observed that some of the covers created anoxic conditions, which caused a decrease of the LT_{50} (lethal temperature required to kill 50% of a population) values for annual bluegrass to decline 7 C after exposure to the anoxia. They also reported a decrease in high molecular weight fructans in hardened annual bluegrass under anoxic conditions.

Rochette et al. (2000) also evaluated atmospheric conditions under winter covers. Under impermeable covers, anoxic conditions ($< 1\%$ O_2; $> 10\%$ CO_2) could develop in less than 60 days. They also observed that hardened annual bluegrass could tolerate these conditions for longer than 40 days.

Perhaps one reason for limited research on this topic is the difficulty in sampling gas from under ice and controlling all the variables associated with ice over-lying a turf area. My experiment did not include ice as a variable but evaluated the effect of low-oxygen exposure on two turfgrass species. The objectives of my research were to determine if low oxygen concentrations affected the survivability of creeping bentgrass and annual bluegrass and to determine if the tolerance to low oxygen concentration was dependent on the hardened condition of the plants. Another objective was to determine if low oxygen environments affected total non-structural carbohydrate (TNC) concentrations in the crowns and verdure of creeping bentgrass and annual bluegrass.

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. Plants were maintained in a greenhouse for several months prior to the initiation of the experiment.

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 that were filled with water to provide ample moisture for growth.

A second study was conducted using hardened plant material removed from field plots maintained as a putting green. The plots were constructed according to United States Golf Association Putting Green Construction Specifications. Creeping bentgrass selections were Penncross and Penn A-4 and the annual bluegrasses (WH, PA) were selections from golf courses located in the Mid-Atlantic region.

These studies were conducted in a controlled temperature room, and artificial light was provided with two 450-watt high-pressure sodium lamps mounted 11 cm above the experiment bench. The lamps were controlled with an electronic timer and set to provide a 16-hr photoperiod.

All tubes were treated for disease prevention with 0.6 kg ai/ha of azoxystrobin and 0.5 kg ai/ha of metenoxam delivered in a spray solution at 747 l/ha. Two tubes of each selection were placed in a support rack in a 5-liter polycarbonate carboy situated on its side. Two tubes were used to provide enough plant material for TNC analysis. Two liters of water were added to the carboy to provide a moist atmosphere for the tubes during the study. Irradiance was approximately 190 µmols m^{-2} sec^{-1} inside of the carboys, and temperatures were approximately 18 C (± 1 C) and 10 C (± 1 C) (day/night).

Four oxygen treatments—0.8, 2.5, 4.0, and 20.6% (20.6% was equivalent to atmospheric O_2 %)—were provided to the respective carboys with pre-mixed gases through MG Industry 158 regulators. The four treatment gases also contained 0.036% CO_2, and the balance was nitrogen. The regulators had a flow range of 0.1 L min^{-1} to 5.0 L min^{-1} and were set at 0.1 L hr^{-1} and at a pressure of 0.13 bars during the study.

Two 3 mm brass tubing connectors were installed in the carboy caps for gas input and output. The outputs of the carboys were connected to a Sable multiplexer for flow control. The multiplexer was controlled with a computer running Sable Datacan software and was connected to a Sable Systems FC-1B oxygen analyzer that monitored the air stream flowing from the multiplexer.

A Cole-Palmer (Chicago, IL) 7017 perastalic pump fitted with 7 mm id tubing (Nalgene Fuel and Lubricant) was used to pump air from the carboys, through the multiplexer, and into the oxygen analyzer. The pump moved 17 L of air per hour, and operation was controlled with a digital timer. All component air flow connections were made through Bev-a-line XX (Cole-Palmer, Chicago, IL) 4 mm id tubing, except for flow through the pump. Both types of tubing were used because of their low oxygen permeability characteristics.

The oxygen analyzer determined oxygen concentration by use of a fuel cell. The fuel cell utilized an acid electrolyte, heavy metal anode and thin gas-permeable membrane. Oxygen diffused through the membrane and liberated electrons by oxidizing the electrode. Thus, the fuel cell created an electric current linearly proportional to the partial pressure of O_2 at the membrane outer surface. The oxygen analyzer recorded measurements daily on the computer in ASCII formatted files.

Oxygen measurements of the carboys were recorded daily for one hour per carboy. Since this was a pressure positive (0.14 bars) system up to the pump, minor leaks that could cause varying oxygen concentrations would not affect the concentrations in the carboys. Minor gaseous changes due to respiration, photorespiration, and photosynthesis were corrected by pumping out 4 L of gas per day during analysis. Chambers were also vented for 15 to 30 seconds per day to ensure oxygen concentrations remained stable. Oxygen concentrations varied somewhat throughout the duration of the study as confirmed by the oxygen analyzer data (Table 2.1).

Table 2.1. Oxygen concentration minimums, maximums, and averages for the duration of the study as determined by an oxygen analyzer.

Minimum	Maximum	Average
---------- % O_2 ----------		
0.5	1.5	0.8
1.2	2.8	2.5
3.3	4.3	4.0
21.03	21.69	20.6

Each treatment was replicated three times over time. The oxygen concentration treatments were randomized among the carboys and bench locations for each replication.

After 35 days of exposure to the treatments, the tubes were removed from the carboys. The turf plugs were removed from the tubes and washed in water to remove the root zone material and then separated into component tissues. A scalpel was used to

remove the shoots and roots from the crowns. The remaining crowns were approximately 5 mm in length and included a short section of shoots and roots.

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 20 mm of water to ensure saturation and to prevent the soil mix from drying out. Plant counts were made seven days after planting to determine percent survival.

The 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 (1964).

The treatments were arranged in a randomized complete block design with a two species by two selection by four oxygen concentration complete factorial design. The SAS (1999) Univariate procedure was used for all exploratory data analysis (Tukey, 1977), and percent survival data was inverse sine transformed (Steele and Torrie, 1980) before analysis. Means were separated using Fisher's Protected LSD Test with $p = 0.05$.

Results and Discussion

Survival of Unhardened Plants

There was almost 100% survival of all plants for all treatments in the first replication. The plants in the 20.6% O_2 (control) treatment in replications two and three were severely infected with disease. Preventative fungicides were used to limit pythium (*Pythium* spp.) and brown patch (*Rhizoctonia* spp.) infestation. Unfortunately, the humidity and temperature inside of the carboys created a very favorable environment for these diseases, and the preventative applications did not suppress disease development for the entire duration of the study. Maintaining the selected oxygen treatment levels made it impractical to retreat the plants during the experiment. The lower oxygen concentrations may have inhibited severe disease development in the other treatments.

The plants had excellent survival (> 80%) at both 0.8 and 2.5% O_2 concentrations (Fig. 2.1). Percent survival at 4.0% O_2 was above 60% and equivalent to the 0.8% O_2 survival rate. The decreased survival rate at a higher oxygen concentration may have been a result of slight disease activity on the plants in the second and third reps. Although the disease was not obvious as it was in the 20.6% O_2 treatment, a few blighted blades were observed. Data from the 20.6% O_2 treatment were not used in the analysis of variance.

The 4.0% O_2 concentration may also have allowed some level of photorespiration to continue. This would decrease the efficiency of the limited photosynthesis, which would cause a decrease in stored carbohydrates. Although there was no significant treatment effect for crown or verdure TNC concentrations, they exhibited a numerical

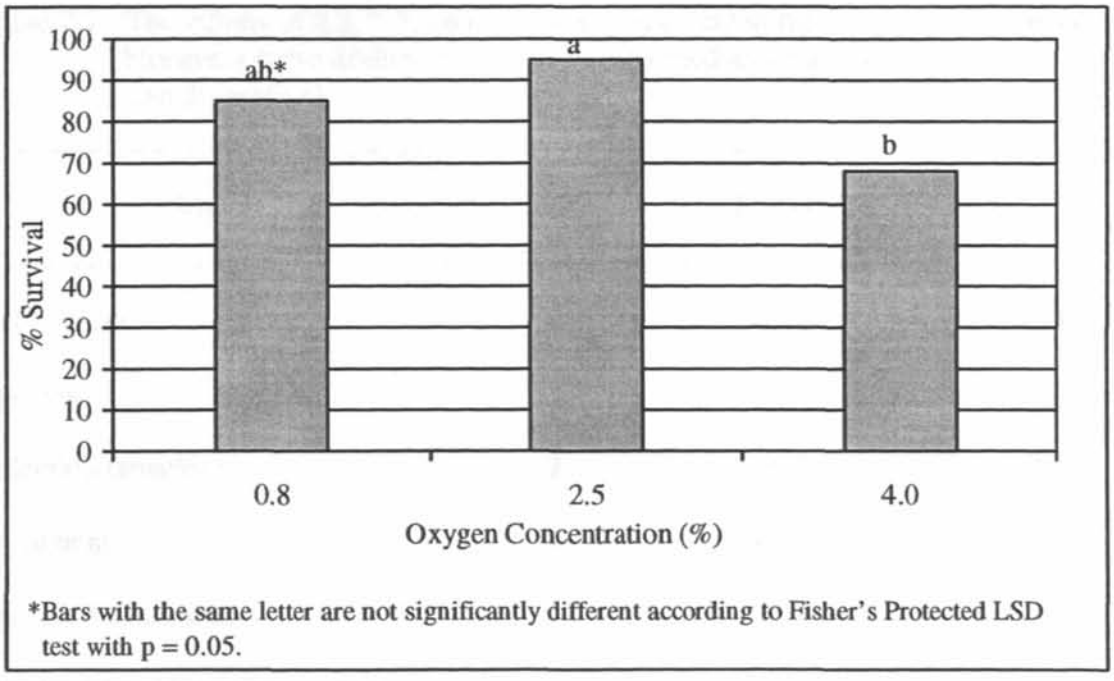

Fig. 2.1. Percent survival averaged over species following 35 d exposure to selected oxygen concentrations.

increasing trend as O_2 concentration decreased (Fig. 2.3). There may have been an even greater reduction in carbohydrate reserves if disease(s) were active during the experiment.

The selected levels of oxygen did have a significant affect on the survivability of creeping bentgrass and annual bluegrass (Table 2.2; Fig. 2.2). The annual bluegrass plants showed the first signs of discoloration caused by the low oxygen concentrations. The annual bluegrass in the 0.8% O_2 concentrations showed some discoloration seven days after the start of the treatments. A few days later, the annual bluegrass also began to lose green color in the 2.5 and 4.0% O_2 treatments. Two weeks after the beginning of treatments, the bentgrass in the 0.8% O_2 developed an increased prostrate growth; and the

Table 2.2. The effects of 0.8, 2.5, and 4.0% oxygen on creeping bentgrass and annual bluegrass survivability (Data was transformed using arc-sine transformation).

Source	df	F-Value	Pr > F
Replication	2	6.78	0.0051
Species	1	4.4	0.0476
Selection (species)	2	0.52	0.6030
Treatment	2	4.15	0.0296
Species * treatment	2	1.10	0.3501
Treatment * selection (species)	4	0.55	0.7027
Error mean square = 0.079 df = 22			

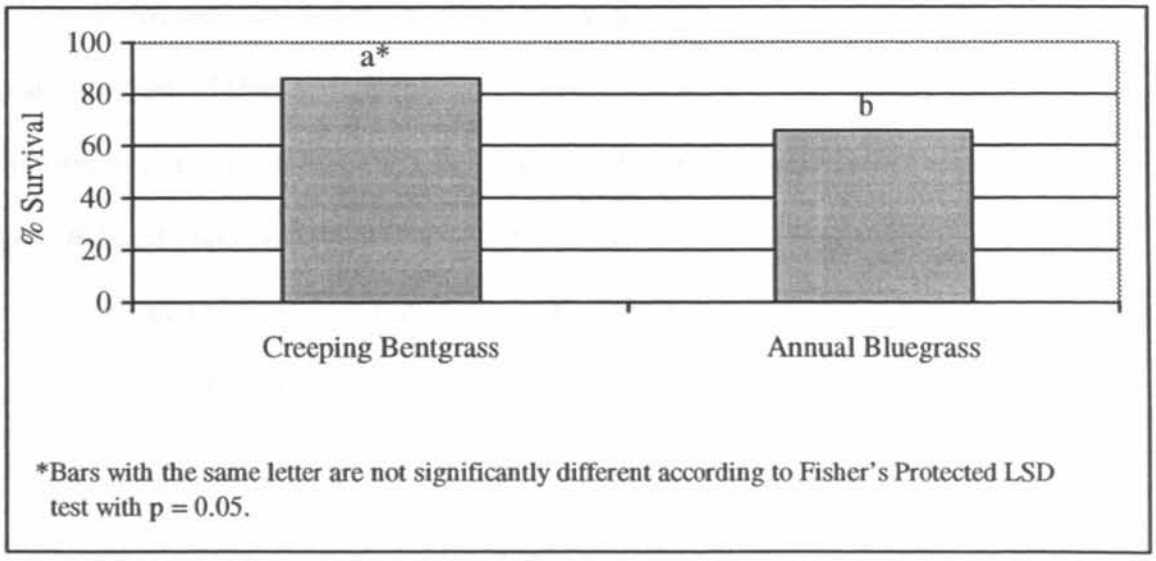

Fig. 2.2. Percent survivals averaged over oxygen 0.8, 2.5, and 4.0% concentrations for creeping bentgrass and annual bluegrass following 35 d exposure.

stems appeared elongated. These visual differences were most apparent between creeping bentgrass and annual bluegrass at the 2.5% O_2 level about three weeks after treatments began. At 35 days after treatment initiation, many of the leaves of both species appeared to lose chlorophyll content and turned white, especially in the 0.8 and 2.5% O_2 treatments.

Both species showed good survival under low oxygen concentrations even at practically anoxic conditions (i.e. 0.8% and 2.5% O_2). Creeping bentgrass had significantly better survival than annual bluegrass, although annual bluegrass averaged 66% survival over all oxygen concentrations (Fig. 2.2). Considering these were unhardened plants actively growing at 18 and 10 C (day/night), their survivability at low oxygen concentrations was not expected.

TNC Concentrations of Unhardened Plants

There were no significant effects of oxygen treatments on crown TNC concentrations (Table 2.3). The decrease in oxygen did not significantly affect TNC concentrations in the crowns of either species. The low oxygen concentrations should have reduced plant respiration enough, especially the lowest oxygen treatment, to cause an increase in TNC concentrations. However, irradiance intensities inside the carboys were very low (190 μmols m^{-2} sec^{-1}) and photosynthesis was probably limited, thus decreasing TNC concentrations.

Table 2.3. Analysis of variance of TNC concentrations of creeping bentgrass and annual bluegrass crowns exposed to 0.8, 2.5, and 4.0% oxygen concentrations for 35 days.

Source	df	F-Value	Pr > f
Treatment	2	0.25	0.8137
Rep (treatment)	6	112.65	< 0.0001
Species	1	0.06	0.8137
Species by treatment	2	0.45	0.6420
Treatment by selection (species)	6	2.51	0.0600
Error mean square = 4.95 df = 18			

There were no treatment effects on the verdure TNC concentrations as well (Table 2.4). Although statistically there were no effects by treatments, TNC concentrations tended to increase numerically in the crowns and verdure as O_2 concentrations decreased (Fig 2.3). Again, the limited irradiance may have restricted photosynthesis, and therefore, carbohydrate production and storage.

Conducting this experiment with higher irradiance (to increase photosynthesis) or lower temperatures (to decrease respiration) might yield different TNC concentration results. The lower temperatures would also decrease the incidence of diseases such as brown patch and pythium.

Table 2.4. Analysis of variance of TNC concentrations of creeping bentgrass and annual bluegrass verdure exposed to 0.8, 2.5, and 4.0% oxygen concentrations for 35 days.

Source	df	F-Value	Pr > f
Treatment	2	0.25	< 0.0001
Rep (treatment)	6	112.65	< 0.0001
Species	1	0.06	0.8137
Species by treatment	2	0.45	0.6420
Treatment by selection (species)	6	2.51	0.0609
Error mean square = 74.0 df - 35			

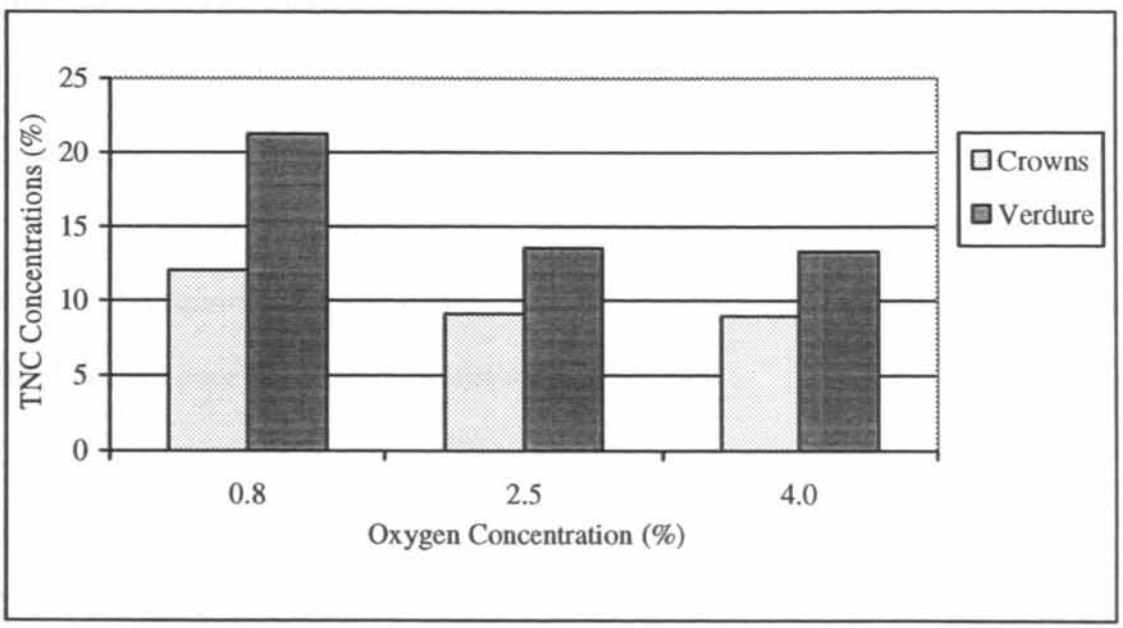

Fig. 2.3. TNC concentrations of crowns and verdure averaged over species after 35 d exposure to reduced oxygen concentrations.

Further research on the tolerances of creeping bentgrass and annual bluegrass to low oxygen or increased levels of CO_2 for long durations (i.e., several months) would be very valuable. Also, research on the difference between treatment responses of hardened and unhardened plants would be important as well.

Survival of Hardened Plants

The hardened plants had been removed from the field in early January and stored at –4 C for over four months. A few weeks after the start of this experiment using hardened plants, some of the unused creeping bentgrass and annual bluegrass hardened material was regrown in the greenhouse. All but one selection had practically no regrowth.

At the end of the experiment, all treatments were regrown in the greenhouse, and all of the selections, regardless of treatment, had practically more growth. It was obvious that most of the hardened plant material was dead prior to the beginning of the experiment.

It would be important in duplicating this experiment to maintain untreated controls throughout the duration of the study. This would avoid misinterpretation of data that indicates poor survival due to treatments.

Conclusions

The average survival rates of unhardened creeping bentgrass and annual bluegrass were above 60% following 35 days of exposure to 0.8, 2.5, or 4.0% oxygen. This high survival percentage may indicate that even unhardened plants may be able to survive

several weeks of hypoxic or anoxic conditions created under ice sheets. Creeping bentgrass had a significantly better tolerance to low oxygen concentrations than annual bluegrass. Considering that hardened, dormant plants have significantly lower respiration rates, and therefore a lower demand for oxygen, the survival rate and critical length of exposure should be even greater for hardened plants than observed in the present study (Rochette et al., 2000).

Decreasing oxygen concentrations to 4.0% or lower did not affect the TNC levels of creeping bentgrass or annual bluegrass crowns or verdure, although a trend of increasing TNC levels with decreasing oxygen concentrations was observed. Stress due to low carbohydrate levels created by hypoxic or anoxic conditions over a period of 35 days did not appear to be significant.