

## Mitigation of Anoxia under Ice and Impermeable Covers on Annual Bluegrass Putting Greens

Darrell K. Tompkins, Philippe Rochette, and James B. Ross

### Summary

Winter damage to annual bluegrass putting greens caused by a lack of oxygen under ice or impermeable winter covers is an important problem in cold climates. The objective of this trial was to evaluate various covering systems that would maintain oxygen levels and, in turn, prevent damage associated with anoxia (lack of oxygen). White impermeable winter covers, some with an insulating air layer of either Enkamat or bubble wrap, were compared against ice cover and no ice (snow only) treatments. A ventilation system that could replenish oxygen under the impermeable covers was also evaluated. Following installation of the various covering systems, an ice layer was established in order to induce a condition of anoxia. Once the ice layer was established, gas samples were collected on 15 day intervals from under the covers in order to determine oxygen and carbon dioxide concentrations.

In this trial, oxygen concentrations under the various treatments remained constant for the first 75 days. However by day 90, there was a significant reduction in oxygen for the ice only and the ice, impermeable cover (no air layer) treatments. Values for the ice, impermeable cover treatments that had an air layer were better than the no air layer, but significantly lower than the no ice (snow only) treatment. Air replenishment did not appear to have an impact on oxygen concentration.

Carbon dioxide levels were lowest for the no ice (snow only) treatment. On day 90, the highest carbon dioxide levels were for the ice only and ice, impermeable cover (no air layer) treatments. There seemed to be some improvement in carbon dioxide levels that had the insulating materials.

With regards to survivability the ice only treatment was dead in both years of the study. Otherwise, there were no significant differences between the other treatments when considering relative hardness levels and these treatments survived completely.

### Introduction

Winter damage to golf greens can be caused by a number of different factors, but low temperature injury and injury related to ice cover are particular problems. Injury can be more serious if the predominant grass species is annual bluegrass (*Poa annua* L.), which often can infest older greens in cold climates (Tompkins *et al*, 2004).

In a previous study, it was determined that snow cover helped to maintain dormancy on putting greens (Tompkins *et al*, 2000). However, some years fluctuating temperatures can cause snow to melt and refreeze. Melted water can percolate through the snow to the soil surface where it refreezes, producing a layer of ice on the soil surface. This situation puts the turf at a higher risk of damage from freezing injury and injury associated with anaerobic conditions (Tompkins *et al*, 2004).

Anoxia is defined as a complete lack of oxygen. Previous research had determined that injury to annual bluegrass putting greens under impermeable winter covers was associated with anoxic conditions (Rochette *et al.*, 2006). It is assumed that similar anoxic conditions occur under ice cover.

Covering the greens with impermeable covers is becoming an accepted practice for preventing the more extreme problems associated with freeze-thaw cycles during the spring. The observed response of greens which are covered is generally better in the spring, but some greens respond poorly to winter cover (Dionne *et al.*, 1999; Rochette, *et al.*, 2006). The cause of this poor outcome does not seem to be related to weather conditions, but is an inherent problem with the specific problem green.

By providing a passive air layer under an impermeable winter cover, it is thought that conditions of anoxia might be delayed and the length of time before injury occurred would be increased. If the air could be replenished, it is thought that annual bluegrass plants might live indefinitely under these types of covering systems.

Plants require a free exchange of atmospheric gases for photosynthesis and respiration. Limitations to gas movement in the soil have been shown to be a problem for the maintenance of healthy greens. Generally, the most common impediment to gas diffusion is water in the root environment due to excessive rainfall or irrigation (McKersie and Lesham, 1994). During the winter, similar low oxygen conditions can occur if a solid ice layer forms above the turf. The use of impermeable covers can protect the greens from exposure to extreme weather and prevent ice formation, but may also aggravate conditions of anoxia (Rochette, *et al.*, 2006).

The specific cause of problems under ice cover or artificial cover has been attributed to CO<sub>2</sub> buildup (Andrews and Pomeroy, 1975), ethanol accumulation (Andrews, 1977), and secondary metabolites produced by soil microorganisms (Brandsaeter, *et al.*, 2005). The changes in gases observed can be traced to microbial activity occurring in the soil and respiration in the plant roots during the winter period. The temperature below snow cover or ice cover rarely drops below -5° C, and temperature is therefore maintained at a level which is capable of sustaining growth for a number of soil organisms. Snow covered greens are able to exchange gases with the surface air, and do not accumulate high levels of CO<sub>2</sub> and toxic byproducts. However, under ice cover or artificial covers, no air exchange occurs and the accumulation of potentially dangerous metabolic waste products can be substantial. It is thought that conditions under impermeable winter covers would produce the same type of anaerobic environment that would be produced under a covering of ice.

The metabolic activity of the soil can be highly variable, with the soil microflora likely contributing more of the metabolic activity than the plant roots (Bertrand, *et al.*, 2001). The activity will depend on the amount of available food sources and the type of microorganisms present. High levels of organic matter for consumption will increase the metabolic activity, as will higher numbers of cold-tolerant microbes. Most of the soil microbes will use oxygen if it is available, and under closed conditions, can exhaust the oxygen present under cover relatively quickly. The amount of metabolic activity in the soil therefore becomes an important factor in predicting the rate of oxygen depletion and subsequent buildup of toxic byproducts of anaerobic activity.

The key indicator of turf survival under ice cover would therefore be the rate of oxygen depletion for that green. Greens with low levels of metabolic activity would have a slower rate of oxygen loss which leads to a slower accumulation of toxic compounds and thus an increased likelihood of winter survival. This situation has been observed (Rochette, *et al.*, 2006) where the ability of a number of greens to overwinter under cover was directly linked to a lower observed oxygen consumption, while greens with a high rate of oxygen consumption did very poorly over the same time period. This suggests that maintaining the levels of oxygen under the cover or reducing the metabolic rate of the soil may be a viable way to minimize the winter damage to problem greens. The simplest intervention would be to maintain the oxygen levels and prevent anaerobic metabolism from occurring.

From previous work conducted at the PTRC (Tompkins *et al.*, 2004) it was determined that annual bluegrass rapidly lost tolerance to cold temperatures between 45 and 60 days after being covered with ice. Possible mechanisms of injury may be that as oxygen decreases, anaerobic byproducts accumulate in the plant and eventually reach toxic levels; or, these anaerobic byproducts cause the plants to rapidly lose their hardiness levels and make them more susceptible to injury from freezing.

The provision of additional oxygen on an ongoing basis could prevent the anaerobic conditions from occurring. We will test a series of treatments that could alleviate these anoxic conditions.

This study is intended to evaluate the benefits of specific treatments to increase oxygen levels and prevent anoxia-associated damage to ice covered annual bluegrass putting greens. If a system to cover annual bluegrass putting greens can prevent ice cover injury, this may solve the difficult problem of over wintering this troublesome species. As this system of covering will also provide an air layer between the turf and the environment, cold temperature injury should also be reduced due to the insulation effects of the air layer.

This experiment was designed to test the hypothesis posited by Rochette *et al.*, 2006 that the damage observed on some covered greens is due to increased rates of oxygen consumption that lead to damaging anaerobic conditions. This suggests that the loss of oxygen creates a situation that is potentially toxic to the plant, and that subsequent anaerobic metabolism generates that damage. This hypothesis leads to the experimental question: is it possible to adjust conditions under ice cover and impermeable covers to maintain oxygen levels and prevent damage?

Rochette concludes that, 'golf course superintendents face a difficult situation when selecting the best management practices to ensure winter survival of recurrently damaged greens: either they protect the greens with impermeable covers and expose the turfgrass to anoxia problems or they do not use covers and increase the risk of damage related to extreme subfreezing temperatures, excess water, and ice formation. Future research should aim at developing passive or active ventilation methods that could efficiently supply O<sub>2</sub> under the cover and withdraw CO<sub>2</sub> and other metabolic gases.'

The objective of this study was to test the use of winter covers with active and passive ventilation to prevent winter damage as a result of prolonged ice cover on annual bluegrass putting greens.

### **Specific Objectives:**

- Evaluate the potential of various covering systems to maintain oxygen levels and prevent anoxia-associated damage on ice covered annual bluegrass putting greens.
- Compare gas concentrations following active ventilation with treatments that are not ventilated.
- Assess the suitability of insulating materials for the prevention of low temperature injury.
- Assess cold hardiness levels and correlate these with gas concentration changes.

### **Methodology**

A two year field study was initiated in November 2007 at the Prairie Turfgrass Research Centre on a soil based annual bluegrass test area that was maintained at putting green height 4.75mm (0.187"). The test area was initially seeded in 2001 to Petersen's creeping bluegrass on a black chernozemic soil.

Prior to the initiation of the study, the area was left exposed in order for plants to reach maximum hardiness. The following individual treatments were then established.

1. No ice (snow only, untreated control)
2. Ice only
3. Ice, impermeable cover
4. Ice, impermeable cover, bubble wrap
5. Ice, impermeable cover, bubble wrap, and ventilation
6. Ice, impermeable cover, Enkamat
7. Ice, impermeable cover, Enkamat, and ventilation

Plots that measured 1 by 1 meter were laid out with 0.5 meter buffers around each plot. Trenches that were 10cm wide and 15 cm deep were then excavated and the soil was removed. Winter covers, which measured 1.5 by 1.5 meters, were laid over the plots and down into the trenches. A wooden frame, which was slightly larger than the plots, was constructed of 5 by 20 cm wood and then was placed over the covers and pushed down into the trenches. In order to construct the various treatments, insulating materials were laid on the corresponding plots prior to the installation of the covers. Soil temperature probes were loosely inserted into the turf and temperatures were recorded for each treatment using a data logger (CR10X, Campbell Scientific, Edmonton, Alberta) running thermocouples through a multi-plexer (AM25T, Campbell Scientific).

As white is most reflective, the chosen winter cover was a white impermeable reinforced polyethylene material (RPE4, Layfield Geosynthetics, Edmonton, Alberta). Two materials, 1.2 cm thick bubble wrap and 1.0 cm thick Enkamat (7010, Colbond bv, The Netherlands) were used in order to provide an air layer between the turf and the cover. The bubble wrap material was approximately 20mm in thickness with open bubbles on the bottom which provided an approximate 50% air space. Enkamat, which was approximately 1 cm thick, is an erosion control material that is very rigid in nature and has 95% air space. It was thought that these two materials would allow for an air space that should delay the occurrence of anoxic conditions even when there was significant weight on top of the covers from snow or ice.

Prior to installation, holes were cut in the covers for ventilation and gas sampling purposes. For the gas sampling holes, bulkhead unions that were 2.0 cm were used. Polyethylene tubing, with a three-way valve, was inserted through the gas sampling port and sealed. Two ventilation holes were established, one for inflow and one for outflow, and were sealed with bulkhead unions, that were 5 cm in diameter. The ventilation ports had a threaded PVC nipple attached to the bulkhead union, with a ball valve on the outflow and a threaded cap on the inflow. On the ice cover only treatment a small sealed sampling area was established using a 5 cm PVC pipe cap with a hole drilled in the top. The polyethylene tubing, with the three-way valve, was then attached to the cap. The cap was forced into the soil so that the size of the air reservoir was maintained as small as possible. This was done in order to simulate conditions where the ice adhered tightly to the turf surface while still allowing for gas sample collection.

Once the covers and the insulating materials were installed, the process of ice formation began. Ice was built in layers until it reached 5 cm. Ice was also developed in the trenches and around the covers in order to completely seal the individual treatments. In year one, ice development began on 23 Nov and was completed by 3 Dec. In year two, ice was formed between 2 Dec and 14 Dec. The treatments that were covered with ice were compared with an untreated control, that had no ice and allowed snow cover to accumulate and melt naturally.

Ventilation was conducted at 30 day intervals and followed gas sampling. This was accomplished by forcing air under the cover for one minute using a portable pressure tank attached to the inflow port. The displaced air was vented through the outflow port.

Gases were sampled and analyzed on a bi-monthly basis beginning 30 days after ice cover was complete. Gas samples were collected by drawing gases out by means of a syringe and then expelling the gas into an evacuated collection vial. Gases were then quantified by liquid gas chromatography. This method was established in a previous study (Rochette *et al*, 2006).



---

Downloading temperatures data from the logger

Following the test period covers were removed and a single sample was collected from each of the plots in order to test plants for relative hardiness. Relative hardiness levels were determined using the method established by Tompkins *et al*, 2000. Plots were evaluated for survival and turf quality until mid June.

## **Results**

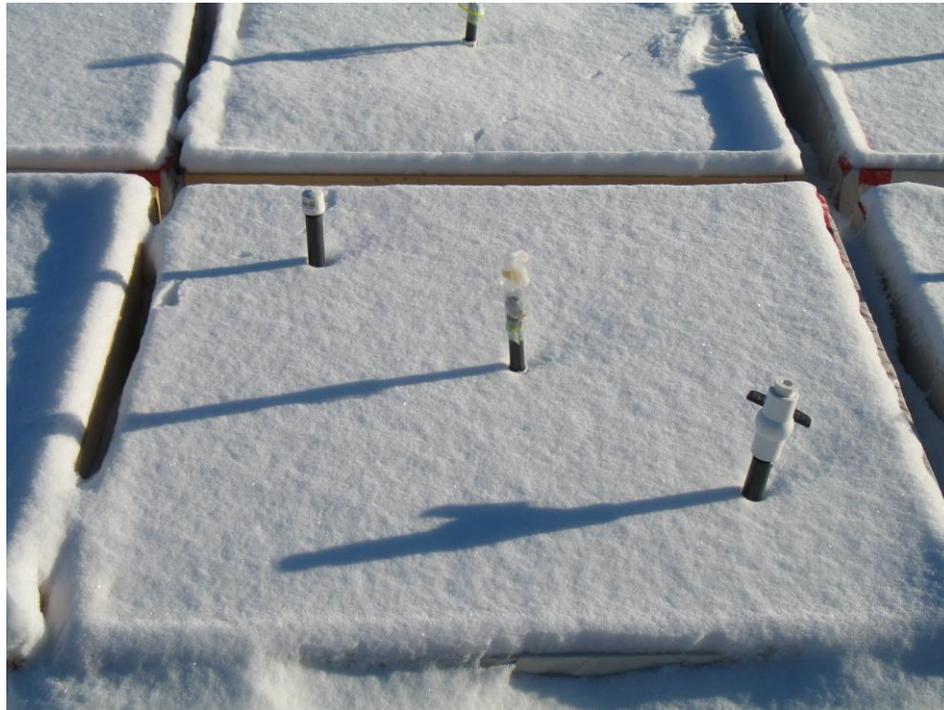
### *Weather and Temperatures under the Covers*

In year one, conditions were unseasonably warm prior to ice development which commenced on 23 Nov. Ambient temperatures were cold during the period of ice formation and plots were not covered with snow. The coldest soil temperature recorded during that time was  $-12.4^{\circ}\text{C}$ . Soon after ice development was complete a 5 cm snowfall occurred and temperatures under the covers ranged from  $-2$  to  $-8^{\circ}\text{C}$  until 75 d (days after ice formation complete). Following this, temperatures began to increase slightly and by 90 d temperatures were near  $0^{\circ}\text{C}$ , with one treatment slightly above freezing for the first time during the trial. The snow cover was minimal throughout the test period and ranged from 4.5-7.5 cm.

For year two, the coldest temperature recorded under the covers during ice formation period was  $-3.5^{\circ}\text{C}$ . Several snowfalls occurred shortly after ice formation and snow cover was maintained at 15-20cm for the duration of the winter. Snow melt was very late and the ice covered plots became exposed at 117 d. Soil temperatures ranged from  $-0.6$  to  $-5^{\circ}\text{C}$ .

### *Oxygen concentrations under the covers*

Oxygen concentration in the earth's atmosphere is 20.95%. There was little difference in oxygen concentrations under the no ice treatment in comparison to atmospheric conditions, as values ranged from 20.8-20.9% (Table 1). Oxygen concentrations were significantly lower for all treatments when compared with the no ice treatment on almost all rating dates. On all rating dates except one, oxygen concentrations were statistically similar for all white impermeable cover treatments. That one exception was for the white impermeable cover only treatment and occurred on the final rating date. This appeared to be temperature related. On two rating dates, the ice, impermeable cover had significantly higher oxygen concentrations than did the ice, no cover treatment. The no ice and ice, impermeable cover, and Enkamat treatments were similar on four of the five rating dates with the 90 d period being the exception. At 75 d, all ice and impermeable cover treatments that had either Enkamat or bubble wrap were similar to the no ice treatment for oxygen content. Otherwise, all treatments had lower oxygen concentrations than did the no ice treatment.



Plot with sampling tubes extending up through covers

Oxygen concentrations under the various treatments remained constant for the first 75 d. However by 90 d, there was a significant reduction in oxygen levels for the ice only and the ice, impermeable cover treatments in comparison to the other treatments. Oxygen content in samples from the ice only treatment was significantly lower than the ice, impermeable cover treatment. When considering the two insulating materials, there were no differences in oxygen concentrations. Ventilation did not significantly increase oxygen concentration under either the Enkamat or bubble wrap.

Table 1 – Oxygen levels under various treatments, combined years 2007 and 2008.

Treatments	30 d <sup>1</sup>	45 d	60 d	75 d	90 d
	%				
No ice	20.9a <sup>2</sup>	20.9a	20.9a	20.8a	20.8a
Ice only	20.3c	20.0c	20.2c	20.1c	18.7d
Ice, impermeable cover	20.5bc	20.3bc	20.5b	20.3bc	19.7c
Ice, impermeable cover, Enkamat	20.7ab	20.6ab	20.7ab	20.5ab	20.3b
Ice, impermeable cover, Enkamat, ventilation	20.6b	20.5b	20.6b	20.5ab	20.2b
Ice, impermeable cover, bubble wrap	20.6b	20.5b	20.6b	20.5ab	20.1b
Ice, impermeable cover, bubble wrap, ventilation	20.6b	20.5b	20.6b	20.5ab	20.3b
	LSD <sub>0.05</sub> =	0.2	0.3	0.2	0.3

<sup>1</sup> Days after ice formation complete

<sup>2</sup> Values that have the same letter as a suffix are not significantly different.

### *Carbon dioxide concentrations under the covers*

Carbon dioxide forms about 0.04% of the earth's atmosphere. For the no ice treatment carbon dioxide levels were slightly above atmospheric concentration and ranged between 0.05-0.06% (Table 2). This would indicate that snow cover has little effect on the accumulation of carbon dioxide at the turf surface.

Carbon dioxide concentrations were consistently higher for all treatments when compared with the no ice treatment although the differences were not always significant. On almost all rating dates, carbon dioxide concentrations were consistently higher for the ice only treatment when compared to the impermeable cover treatments. The one exception to this was the impermeable cover only treatment, which had differences but they were not considered to be significant. When comparing all impermeable cover treatments, carbon dioxide concentrations were similar. Carbon dioxide concentrations under the insulating materials were generally lower than the either the ice only or the ice, impermeable cover.

When comparing carbon dioxide accumulations under the impermeable cover treatments, there was only one sampling period when there were statistical differences. At 90 d, the ice only, the ice, impermeable cover and the ice, impermeable cover, bubble wrap, ventilation treatments had a significantly higher accumulation of carbon dioxide than did the other cover treatments. The ice only, the ice, impermeable cover treatment has a much smaller air space, which could account for differences. However, the ice, impermeable cover, bubble wrap, ventilation treatment had an air space and was ventilated, so this difference could not be explained.

Table 2 – Combined year analysis of carbon dioxide levels at various sampling dates.

Treatments	30 d <sup>1</sup>	45 d	60 d	75 d	90 d	
	%					
No ice	0.05c <sup>2</sup>	0.06c	0.05c	0.05c	0.06d	
Ice only	0.73a	0.97a	0.77a	0.80a	0.98a	
Ice, impermeable cover	0.49ab	0.63ab	0.47ab	0.63ab	0.98a	
Ice, impermeable cover, Enkamat	0.26bc	0.31bc	0.24bc	0.33bc	0.51bc	
Ice, impermeable cover, Enkamat, ventilation	0.32bc	0.43bc	0.28bc	0.40b	0.61bc	
Ice, impermeable cover, bubble wrap	0.39abc	0.41bc	0.35bc	0.46b	0.40cd	
Ice, impermeable cover, bubble wrap, ventilation	0.34bc	0.39bc	0.30bc	0.41b	0.80ab	
	LSD <sub>0.05</sub> =	0.37	0.45	0.34	0.33	0.34

<sup>1</sup> Days after ice formation complete

<sup>2</sup> Values that have the same letter as a suffix are not significantly different.

### *Relative Hardiness Levels*

A significant difference between relative hardiness levels, as measured by LT<sub>50</sub> values, occurred between years in this study (Table 3). Sample collection for relative hardiness determination occurred 90 d after ice formation in year one, while in year two sample collection did not occur until 117 d after ice formation. This was due to a late spring that prevented ice melt.

Plants in the ice only treatment were all dead for both years of the study. In comparison, plants within the treatments that had an impermeable cover between the ice layer and the turf were alive and had relative hardiness levels that were similar to the treatment that

had no ice cover. However, there were no significant differences between the treatments that utilized the impermeable cover. Creating an air layer with either the bubble wrap or the Enkamat did not improve the relative hardiness when compared with the ice, impermeable cover only treatment. Those treatments that were ventilated were no better than those treatments that were not ventilated.

Table 3 – Combined year analysis of relative hardiness (LT<sub>50</sub>) and percent living cover.

Treatments	LT <sub>50</sub> °C	% Living Cover Early Spring	% Living cover Late Spring
No ice	-10a <sup>1</sup>	43.7a	75.0a
Ice only	Dead	18.7b	13.1b
Ice, impermeable cover	-12a	45.0a	72.5a
Ice, impermeable cover, Enkamat	-10a	47.5a	70.0a
Ice, impermeable cover, Enkamat, ventilation	-12a	43.7a	78.1a
Ice, impermeable cover, bubble wrap	-12a	45.0a	73.7a
Ice, impermeable cover, bubble wrap, ventilation	-11a	40.0a	73.7a
	LSD <sub>0.05</sub> =	n/s	9.8
			15.1

<sup>1</sup> Values that have the same letter as a suffix are not significantly different.

#### *Percent Living Cover*

Ice only treatments showed significantly less living cover when evaluated in early and late spring (Table 3). All other treatments were similar to each other. When relative hardiness levels were determined, no plants were alive for the ice only treatment. The fact that there was some living cover for this treatment on both rating dates would indicate that there had been recovery from seed that lay dormant in the soil.

#### **Discussion**

Insulation of annual bluegrass is necessary throughout the winter months in order to prevent injury from cold temperatures. Typically, maximum hardiness levels for annual bluegrass are -20°C and air temperatures often drop below this during winter months in Alberta. Without some type of insulation, these temperatures can be lethal. Insulating materials vary in their ability to resist the transfer of heat. Generally, the insulation value (r-value) is based on the type of material and its thickness. The two insulating materials used in this experiment were Enkamat and bubble wrap. When the snow was removed during the time of ice development, there appeared to be an insulation effect from both materials in comparison to the ice only treatment and the ice impermeable cover treatment. The two insulation materials appeared to be quite similar. However, it is questionable whether either of these two materials provided enough insulation, as the coldest soil temperature was -12.4°C. If plants have reached maximum hardiness levels, these insulation materials would provide sufficient insulation. If plants had lost hardiness for any reason, these insulating materials might not be sufficient.

From this experiment, it could not be determined if this insulating system provided sufficient air reserve to ensure survivability. For example, the ice impermeable cover only treatment survived similarly to those that had an insulating layer. However, at 90 d there had been a decrease in oxygen and an increase in carbon dioxide concentration and the differences were considered significant. A longer period of warm temperatures may

have been necessary in order to clearly evaluate differences between the insulation and the no insulation treatments.

One of the objectives of this trial was to determine if active ventilation would be sufficient to prevent lethal gas concentrations from developing. In this trial, this question was not answered, as there were no differences between the active and the passive ventilation systems. If temperatures were warmer during the test period, gas concentration changes would be greater and differences between the passive and active system may have occurred.

In this study, simply putting an impermeable cover between the ice layer and the turf prevented mortality. When water was being applied during the ice formation stage greater quantities were required to form the ice on the ice only treatment. It appeared that the water would freeze in the root zone and then encase the plants in comparison to forming on the top of the cover. This may have allowed for a larger air space between the cover and the turf, which may have prevented lethal gas buildup.

The experimental design for determining gas concentrations on the ice only treatment was questioned. As there was a larger air space with the sampling chamber than would be with ice only, it was thought that there was less change in gas concentrations than there would be if the ice encased the turf. Therefore, the gas concentrations for the ice only treatment may have been much greater than actually reported.

Another objective of this trial was to determine the affect of temperature on changes in gas concentrations. Weather data showed that temperatures were consistently below -2°C, except for the final period between 75 and 90 days. This period corresponded to the greatest gas concentration changes. This would indicate that temperatures above -2°C were required for gas concentration changes to occur.

---

## REFERENCES

- Andrews, C.J. 1977. Accumulation of ethanol in ice-encased winter cereals. *Crop Science* 17:157.
- Andrews, C.J. and M.K. Pomeroy. 1975. Survival and cold hardiness of winter wheat during partial and total ice immersion. *Crop Sci.* 15: 561-566.
- Bertrand, A., Y. Castonguay, P. Nadeau, S. Laberge, P. Rochette, R. Michaud, G. Bélanger and M. Benmoussa. 2001. Molecular and biochemical responses of perennial forage crops to oxygen deprivation at low temperature. *Plant Cell Environ.* 24:1085-1093.
- Brandsæter, L. O., E. Haugland, M. Helgheim, B. E. Gudleifsson, and A. M. Tronsmo. 2005. Identification of phytotoxic substances in soils following winter injury of grasses as estimated by a bioassay. *Canadian Journal of Plant Science* 85:115-123.
- Dionne J., P.-A. Dube, M. Laganier, Y. Desjardins. 1999. Golf green soil and crown-

- level temperatures under winter protective covers. *Agronomy Journal* 91:227-233.
- McKersie, B.D. and Y.Y. Lesham. 1994. *Stress and Stress Coping in Cultivated Plants*. Kluwer Academ. Publ., Dordrecht, Netherlands.
- Rochette, P., J. Dionne, Y. Castonguay and Y. Desjardins. 2006. Atmospheric composition under impermeable winter golf green protections. *Crop Sci.* 46:1644-1655.
- Tompkins, D.K., J.B. Ross and D.L. Moroz. 2000. Dehardening of annual bluegrass and creeping bentgrass during late winter and early spring. *Agron. J.* 92:5-9.
- Tompkins D.K., J.B. Ross and D.L. Moroz. 2004. Effects of Ice Cover on Annual Bluegrass and Creeping Bentgrass Putting Greens. *Crop Sci.* 44: 2175-2179.
- The Alberta and Canadian Turfgrass Research Foundations are gratefully acknowledged for their financial support of this trial.*