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# GREENS-TYPE ANNUAL BLUEGRASS RESISTANCE TO ABIOTIC AND BIOTIC STRESSES DURING WINTER

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## ABSTRACT

Unseeded annual bluegrass (*Poa annua* L.) is an important component of golf greens in many regions of northern countries. Although this turfgrass species has desirable playing attributes, it suffers from susceptibility to environmental and biological stresses including subfreezing temperatures, anoxia, and snow molds. During the last decade (1999-2008), we undertook a series of experiments to better understand the mechanisms of adaptation and resistance of annual bluegrass to winter biotic and abiotic stresses. When assessing freezing tolerance of three genotypes originating from North Eastern Canada and United States, we observed that the genotypes differed significantly in their freezing tolerance with an unexpected lower level of tolerance for the genotype originating from the most northern latitude, in Québec, where the depth and duration of snow cover are important factors affecting winter survival. After assessing the level of organic reserves, the results showed that the genotype originating from Québec maintained higher levels of reserves than the two other genotypes. Our studies also showed that annual bluegrass is sensitive to anoxic conditions and that its susceptibility could be linked to the reduction of organic reserves under these conditions. We observed large genetic variability for pink snow mold resistance among 29 genotypes collected on golf greens located in Québec and Ontario and concluded that snow mold disease exerts major selection pressure for the generation of genetic diversity among annual bluegrass genotypes in northern climates. Our results suggest that high level of reserves is a characteristic of annual bluegrass genotypes adapted to deep, long lasting snow cover and could be more critical for winter survival than freezing tolerance to allow plants to survive long winter period without new carbohydrate synthesis.

**Keywords:** *Poa annua,* stress resistance, freezing tolerance, anoxia, pink snow mold, organic reserves

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## INTRODUCTION

The primary turf species used for golf course putting green establishment in Canada and the northern United States is creeping bentgrass (*Agrostis stolonifera* L.). However, unseeded annual bluegrass (*Poa annua* L.) thrives under intensive management and gains a competitive edge against creeping bentgrass until it ultimately dominates many golf green putting surfaces (Huff, 1996). Selection pressure provided by intensive management on golf greens has resulted in the development of high quality perennial genotypes of annual bluegrass (*Poa annua* var. *reptans* [Hauskn] Timm) with desirable morphological and aesthetic attributes such as high tiller density and dark green color (Huff, 1999; Carson et al., 2007). In spite of these attributes, annual bluegrass has been historically considered as a weed rather than as a usable turf-type grass with phenotypic variability to be exploited as a source of useful genes. However, recent efforts have been undertaken to develop commercial sources of greens-type annual bluegrass suitable for new putting green construction, overseeding or damage repair (Kind, 1997; Huff, 1999). Breeding programs will need to consider adaptation to environmental conditions in cultivar development to ensure the reliability and long term survival of the seeded material.

One perceived weakness of the greens-type annual bluegrass is its reported susceptibility to environmental stresses (Beard et al., 1978; Tompkins et al., 2004). Winter damage to putting greens is a major problem for golf courses in cold climates. Winter protective covers are very efficient in preventing excess water, desiccation and freezing temperatures at the turf crown level, thereby increasing annual bluegrass tolerance to winter stresses and improving turfgrass quality and surface conditions earlier in spring (Dionne et al., 1999). However, cool and humid conditions along with atmospheric modifications that occur under these long lasting insulating covers (Rochette et al., 2006) can lead to anoxic conditions and to the development of low temperature diseases that can severely affect winter survival. These winter stresses could also affect greens-type annual bluegrass under natural snow covers.

### Freezing tolerance

Temperature fluctuations and extreme freezing temperatures at crown level, occurring during winter and early spring, cause recurrent losses of annual bluegrass on golf greens (Dionne et al., 1999). Plant cold acclimation is a highly active process resulting from the expression of a number of physiological and metabolic adaptations to low temperature (Guy, 1990). Major metabolic changes have been documented during the acquisition of cold tolerance including changes in carbohydrates, amino acids, proteins, nucleic acids, growth regulators and fatty acids (Castonguay et al., 1997). Evidence suggest that soluble sugars, such as sucrose and fructan, in combination with cold-induced proteins play a determinant role in winter stress tolerance by protecting macromolecules and membranes from freeze-induced denaturation. A sufficient level of carbon (C) and nitrogen (N) reserves is also required for an optimal spring regrowth (Dhont et al., 2006).

### Anoxia

Atmospheric composition under impermeable covers can become progressively anaerobic reaching anoxic levels within 80 d on some golf greens (Rochette et al., 2006). Under an ice cover, anoxic conditions build up rapidly and ice encased winter cereals are killed in less than 20 d (Andrews, 1988). The exposure to anaerobic conditions leads to metabolic changes in the plant since oxygen is

involved in many biosynthetic and degradative processes (Van Der Werf et al., 1991). Partial or irreversible damage to the plants has been attributed to the accumulation of toxic metabolites, low energy production, and to a lack of substrates for respiration (Drew, 1997). This could lead to the induction of the fermentation metabolism and to an increase in the production of potentially phytotoxic metabolites such as ethanol, lactic acid and carbon dioxide (Bertrand et al., 2001). In addition to its direct damage to the plant, an anaerobic period can also interfere with plant acclimation to low temperatures.

**Snow mold**

Snow molds are the most prevalent and destructive winter diseases affecting cool-season turf in northern locations in the United States and Canada (Nelson, 2004). Snow mold diseases often result in extensive damage to golf greens that significantly decrease the quality of putting surfaces for many weeks in the spring and cause major economic losses associated with green repairs and lost revenues. The psychrophilic fungus *Microdochium nivale* (Fr.) Samuels & I.C. Hallett is the most widespread snow mold pathogen, and it causes pink snow mold (SM) on turf and forage grasses (Tronsmo et al., 2001). *Microdochium nivale* is also favored by high humidity and ambient temperatures between 0-7°C (Tani and Beard, 1997) that occur underneath persistent snow covers or impermeable tarps applied onto golf greens as protection against freezing damage.

Alternatives to current preventive applications of fungicides in the fall to control SM are required in order to implement low pesticide maintenance programs. The development of seed sources that are more resistant to freezing temperatures and SM is one of the most effective and sustainable approaches to improve winter survival and quality of spring regrowth of turfgrass in a low input system. Significant genetic variations in SM resistance in genotypes of creeping bentgrass were used in breeding new bentgrass varieties (Casler et al., 2006). This indicates that improvement of SM resistance through selection is a valuable approach that could be extended to annual bluegrass.

**Integrated perspective on annual bluegrass tolerance to winter stresses**

During the last decade we undertook a series of experiments to better understand the mechanisms of adaptation and resistance of annual bluegrass to various abiotic and biotic winter stresses. We assessed the resistance of annual bluegrass to three major stresses: freezing, anoxia, and SM. Furthermore, we characterized the physiological and biochemical changes occurring in annual bluegrass exposed to cold and anoxia to better understand the mechanism of resistance of this species. We also characterized genotypes of greens-type annual bluegrass with contrasting levels of resistance to SM. Our results showed that annual bluegrass possess a large genetic variability for many desirable traits that can be exploited to select best-fit genotypes to ultimately provide golf course managers with genetic material better able to survive winter under northern latitudes. Knowledge of the molecular and genetic basis of resistance to biotic and abiotic stresses of annual bluegrass will also contribute to the development of best management practices (BMP) to optimize winter survival of turfgrass while reducing pesticide use.

## MATERIALS AND METHODS

**Freezing tolerance**

One annual bluegrass genotype originating from Western Pennsylvania (OK) and one genotype from Coastal Maryland (CO), obtained by courtesy of Dr. David Huff from

his collection of annual bluegrass at The Pennsylvania State University, and one local genotype from Central Québec (CR) were selected from a preliminary screening including more than 40 genotypes tested for their freezing tolerance. Annual bluegrass tillers were transplanted in late summer in tubes filled with 4:1 (v/v) sand and peat moss and grown 5 to 6 wk in a greenhouse. Conditions were as follows: photoperiod 12 h, light temperature, 22°C; dark temperature 18°C; natural irradiance was supplemented by artificial lighting provided by high pressure sodium lamps (400 W, Philips Lighting Co., Somerset, NJ). Plants were watered daily and fertilized once a week with a 1 g $L^{-1}$ of a commercial fertilizer (20-20-20 plus micronutrients, Plant-Prod, Brampton, Canada). Plants were subsequently transferred to an unheated greenhouse near the City of Québec to acclimate to low temperature under natural conditions as described in Dionne et al. 2001a. Throughout winter, air temperature was monitored with copper-constantan thermocouples (Omega Engineering, Stanford, CA) connected to a data acquisition system (CR10, Campbell scientific, Logan, UT), and soil temperature was monitored with thermocouples and recorded at 40-min intervals with a temperature logger (RD-TEMP-XT; Omega Engineering, Stanford, CA). When air temperatures started to fall below -10ºC, plants were covered with a layer of insulating fiberglass wool to simulate snow cover. Samples were collected every 2 to 4 wk for carbohydrate and amino acid analysis and freezing tolerance was assessed on five occasions. Freezing tests were performed in programmed freezers according to a procedure described previously (Castonguay and Nadeau, 1998) and the 50% killing temperature ($LT_{50}$) was estimated with the SAS Probit procedure (SAS Corp., Cary, NC). Water soluble carbohydrates and amino acids were extracted from a pooled sample (0.5 to 1.0 g fresh weight) of crown tissues (approximately 1 cm above and below the zone of transition between the foliage and the roots) from three to five plants ground in liquid nitrogen. The procedure for extraction and quantification of water soluble carbohydrates and amino acids by high performance liquid chromatography (HPLC, Waters, Milford, MA) is described in detail in Dionne et al. (2001a and b).

Analysis of variance was done using the General Linear Model procedure of SAS statistical software. The experiment was a completely randomized design with five replications and when significant main treatment effects were found ($P < 0.05$), the Fisher Least Significant Difference test (LSD) was used for comparisons of $LT_{50}$, carbohydrate and amino acids concentrations.

**Anoxia**

The experimental procedure is described in more details in Castonguay et al. (2009). Samples of annual bluegrass were collected on a native soil golf green located near the City of Québec (46º47’15’’, 71º12’00’’W; elev. ≈45m) after plants had been exposed to natural fall hardening conditions. Samples were collected with a cup cutter (5 cm O.D. by 8 cm depth). Fungicide treatment (iprodione) was applied at the recommended rate before collecting the samples. Polyvinyl tubes containing annual bluegrass samples were incubated in Mason jars, hermetically sealed with silicone II (GE, Canada Inc., Pickering, Canada). Two, 2-way luer-type stoplock valves (Cole Palmer, Vernon Hills, IL) fitted to plastic tubing (Bar-o-line IV, Ryan Herco, Industrial plastic, Seattle, WA) were used as openings to modify and to sample the atmosphere inside the jars.

The Mason jars with plants were incubated in the dark in a cold chamber set at 1°C for a period of 147 d simulating long overwintering conditions. Plants were exposed to four different atmospheric composition treatments at 1°C: 1) LL: Low $O_2$ (< 1%) and low $CO_2$ (~0 %); 2) LH: Low $O_2$ (< 1%) and high $CO_2$ (15-17%); 3) HH: High $O_2$ (≅20%) and high $CO_2$ (15-17%); and 4) AT: Control treatment at normal atmospheric value of 21% oxygen and ≅0.04% $CO_2$. Gas concentrations in Mason jars were determined as described by Chantigny et al. (2002).

Three pots of each treatment were removed from the cold chamber every two wk for a total of 9 sampling dates. At the time of the opening of the jars, each sample was split into two portions. One half of the sample was used to assess the visual quality of regrowth and the remaining was used for analyses of the biochemical composition of the crown tissues. Plant samples retained for regrowth assessment were transplanted individually in multi-cellular trays filled with a 4:1 (v/v) sand and peat moss medium and grown for 3 wk in a greenhouse under the greenhouse growing conditions described previously. Plants were kept well watered and fertilized. After 3 wk, the visual quality of the regrowth was assessed using a 1-9 scale (1= no growth, 6= medium growth and 9= maximum growth).

At each sampling date, the plants were washed free of soil under a stream of cold water. A sample from 0.5 to 1.0 g (fresh weight) of the crown tissue was collected and immediately used for extraction of soluble sugars, including fructan, and of amino acids, according to the procedure described by Dionne et al. (2001a and b ).

The experimental design was a randomized complete block with 4 atmospheric composition treatments, 9 sampling dates and 3 replications. The analysis of variance was carried out by using SAS Proc GLM. Fisher's Least Significant Difference (LSD) test was used for comparison of visual quality of regrowth, soluble carbohydrate, amino acid and volatile fatty acid concentrations between treatments when the F-test for main effects was significant ($P < 0.05$) at a given sampling date.

**Snow mold**

Twenty-nine genotypes of greens-type annual bluegrass from different geoclimatic environments were collected during the summer of 2005 on 27 greens located on different golf courses across Québec (45°N to 50°N, 65°W to 80°W) and on two greens from the same golf course in southern Ontario (43°28'N, 80°33'W). On each green, a single 5-cm diameter core was collected in a zone covered with annual bluegrass. Genotypes were maintained and vegetatively propagated at the Agriculture and Agri-Food Canada Research Centre in Québec, as described in Bertrand et al. (2008).

Snow mold resistance of the 29 genotypes was assessed under environmentally-controlled conditions. The steps of the screening method for SM resistance are detailed in Bertrand et al. (2009). Briefly, cold acclimated genotypes were inoculated with a 0.01 g $cm^{-2}$ mixture of four isolates of *Microdochium nivale* var. *nivale* from diverse host origin (one from British Columbia *Poa annua*, two from Ontario *Poa pratensis*, and one from Ontario *Agrostis stolonifera*), and incubated 7 wk at 2°C and 98% relative humidity in the dark. Autoclaved inoculum was similarly applied to control plants. After 7 wk of incubation, control and inoculated plants were removed

from the cold room and placed in a growth chamber for one week under regrowth conditions, and plant injury was evaluated visually after one week of regrowth using the Horsfall and Barratt visual scale (Couture, 1980). The experiments were conducted using a completely randomized block design with 10 replicates. Analysis of variance with nested factors using the MIXED procedure of SAS was applied to the percentage of injury of each genotype. LSD means comparisons were used to statistically compare the susceptibility of the genotypes to SM.

To assess the relationship between winter climatic conditions at the site of collection and the genetic potential for SM resistance, we used climatic records (1971-2000) from the meteorological station nearest to each site of collection. The relationship between climatic variables and SM percentage of injury was estimated using Pearson's correlation coefficients as implemented in SAS Proc CORR.

## RESULTS

### Freezing tolerance

To assess the level of freezing tolerance of annual bluegrass genotypes, plants were kept in an unheated greenhouse from November to March for cold acclimation and overwintering under natural conditions. Soil temperature generally decreased gradually to 0ºC in early December, remained below freezing until mid-March and progressively rose above freezing afterwards (illustrated in Dionne et al. 2001a). In response to these hardening conditions, freezing tolerance of annual bluegrass increased from fall (30 October) until midwinter (2 February) and subsequently decreased in spring when air and soil temperatures rose above freezing (Figure 1).

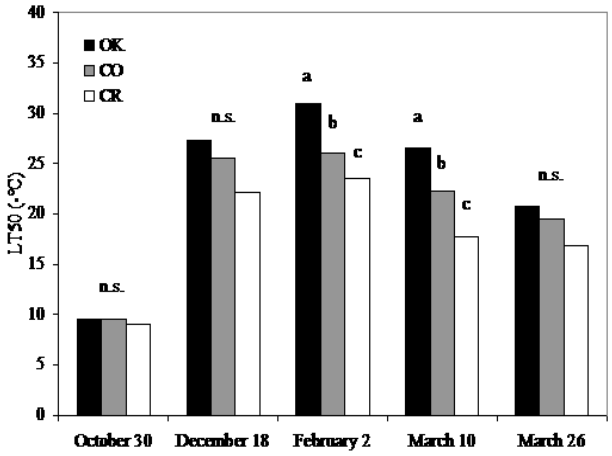


Figure 1. Freezing tolerance, expressed as the lethal temperature for 50% of the plants ($LT_{50}$), of three annual bluegrass genotypes from Western Pennsylvania (OK), Coastal Maryland (CO), and Central Québec (CR), acclimated in an unheated greenhouse during fall and winter. Within each sampling date, bars with different letters are significantly different at *P=0.05* (Source: Dionne et al. 2001 a).

Freezing tolerance increased from an $LT_{50}$ of -9ºC in October to a maximum in early February of -30.9ºC, -26.1ºC and -23.5ºC for OK, CO, and CR genotypes, respectively. Cold acclimated OK was significantly more cold tolerant than CO and CR in February and March.

In all genotypes, fructan (Figure 2A) and sucrose (Figure 2B) were the major carbohydrates found in cold-hardened crown tissues. Sucrose increased until mid-winter and reached its highest level concomitantly with the highest level of freezing tolerance of all genotypes. Fructan concentrations were slightly higher in the most tolerant genotype at the end of the experiment. The concentration of total free amino acids (Figure 2C) was significantly higher in the less freezing-tolerant CR genotype compared to CO and OK genotypes.

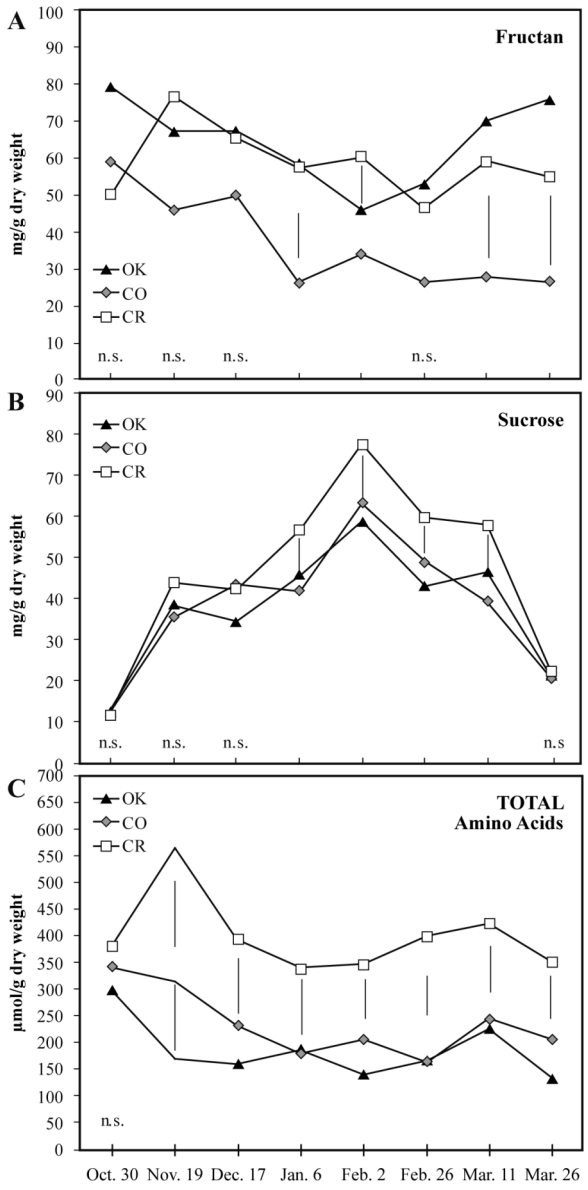


Figure 2. Concentration of fructan (A), sucrose (B) and total amino acids (C) in three annual bluegrass genotypes from Western Pennsylvania (OK), Coastal Maryland (CO), and Central Québec (CR), acclimated in an unheated greenhouse during fall and winter. Vertical bars represent LSD values *(P=0.05)* indicating statistically significant differences among genotypes at a given day of treatment. (Source: Dionne et al. 2001 a and b).

## Anoxia

The visual quality of regrowth of annual bluegrass started to be significantly affected after 42 d of incubation for the LH treatment and after 63 d for the LL treatment with the most damaging treatment being clearly the combination of low $O_2$ and high $CO_2$ (Figure 3). In most cases, exposure to high $CO_2$ alone (HH) did not affect the quality of re-growth. Complete plant mortality was observed in low $O_2$ treatments (LL and LH) after 133 d of incubation.

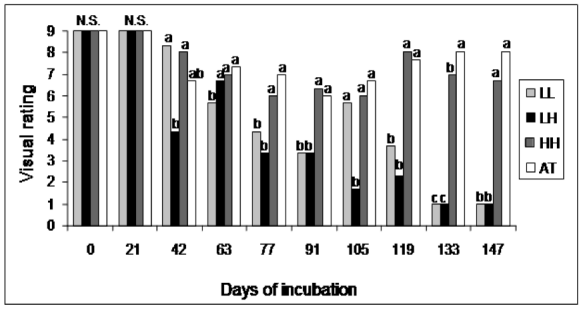


Figure 3. Visual quality of the regrowth of annual bluegrass assessed using a 1-9 scale (1= no growth, 6= medium growth and 9= maximum growth). Annual bluegrass was exposed to either LL: Low $O_2$ (< 1%) and low $CO_2$ (~0 %); LH: Low $O_2$ (< 1%) and high $CO_2$ (15-17%); HH: High $O_2$ (≅20%) and high $CO_2$ (15-17%); or AT: Control treatment at normal atmospheric value of 21% oxygen and ≅0.04% $CO_2$. Within the same day of incubation, bars with different letters are significantly different at *P=0.05* (Source: Castonguay et al., 2009).

Changes in carbohydrates composition in crowns of annual bluegrass were monitored during plant incubation at low temperature (1ºC) under the four different atmospheric treatments. There was a decrease in sucrose concentration at all atmospheric composition treatments during the initial three month period of incubation (Figure 4A). However, after 90 d, sucrose levels remained stable in plants maintained under high concentration of $O_2$ (HH and AT) whereas it continued to decline to reach nearly 0 mg $g^{-1}$ DW in the experiment with low $O_2$-treated plants LL and LH (Figure 4A). In all treatments, fructan concentrations decreased during incubation (Figure 4B). Glutamine accumulation remained at low levels throughout the incubation period in anoxia-treated plants (LL and LH) but increased markedly

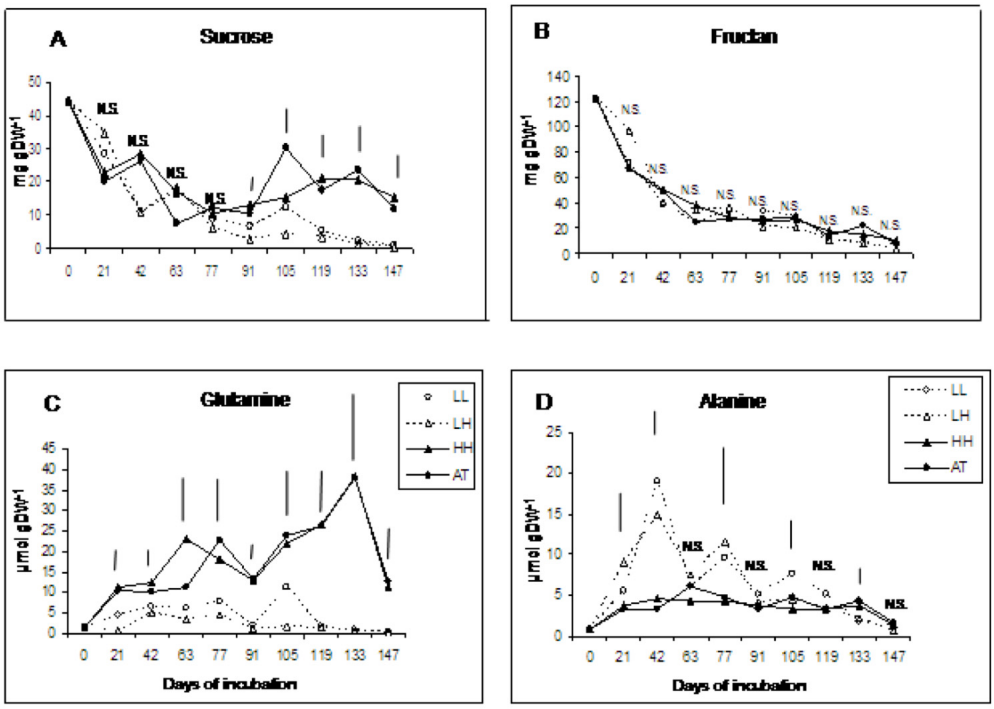


Figure 4. Concentration of sucrose (A), fructan (B), glutamine (C) and alanine (D) measured in crowns of annual bluegrass kept at 1˚C under either LL: Low $O_2$ (< 1%) and low $CO_2$ (~0 %); LH: Low $O_2$ (< 1%) and high $CO_2$ (15-17%); HH: High $O_2$ (≅20%) and high $CO_2$ (15-17%); or AT: Control treatment at normal atmospheric value of 21% oxygen and ≅0.04% $CO_2$. Vertical bars represent LSD values *(P=0.05)* indicating statistically significant differences among genotypes at a given day of treatment. (Source: Thibault, 2003).

throughout the incubation period in plants maintained under high $O_2$ (Figure 4C). In contrast, exposure of annual bluegrass to low concentrations of oxygen promoted the accumulation of alanine, particularly after 42 and 77 d of incubation (Figure 4D).

**Snow mold**

We measured the percentage of injury of 29 genotypes after a 7 wk incubation period with the pink snow mold fungus, *M. nivale*. The results showed that there is a large variability in SM resistance among these genotypes distributed through a large territory (45°N to 50°N, 65°W to 80°W) in Québec and Ontario (Table 1). Our screening approach was able to discriminate between levels of SM resistance within our collection of annual bluegrass with percentage of SM injury ranging from 17.5% in the more resistant up to 87.8% in the more susceptible genotypes.

Correlation analysis between the percentage of injury of the genotypes and winter conditions at the site of collection revealed that the relative susceptibility of the genotypes to SM was negatively correlated with the number of days with snow cover greater than 10 cm during the winter (Table 2). This indicated that genotypes that evolved in regions experiencing deep long lasting snow cover acquired unique mechanisms allowing SM resistance. Conversely, SM susceptibility was positively correlated with the maximum average daily maximum temperature in winter months. Higher temperature in winter are often linked with shallower snow covers, confirming that genotypes exposed to conditions prone to the development of SM are more likely to be resistant to SM.

## DISCUSSION

The assessment of freezing tolerance of three perennial genotypes of annual bluegrass from various origins revealed significant differences in genetic potential for this characteristic within the species. The unexpected lower level of freezing tolerance for the genotype originating from the most northern latitude (CR from Québec) and experiencing the most severe winter conditions suggests that additional traits must be considered to fully assess the winter survival capacity of annual bluegrass including anoxia tolerance, duration of snow cover, and susceptibility to low temperature pathogens like snow molds.

We observed major metabolic changes in annual bluegrass during the acquisition of cold tolerance and overwintering. The coincidence between peak sucrose concentration and maximum freezing tolerance was particularly striking. It has been suggested that sucrose plays an important cryoprotective role by stabilizing membranes and proteins (Hoekstra et al. 1989). Sucrose concentration, however, was not related to the differential tolerance among the three genotypes tested. This could indicate that the lowest levels of sucrose were adequate to exert a cryoprotective role at each date, or that an interaction of sucrose with cold-induced proteins is required to achieve maximum protection of membranes against stress-induced denaturation (Gusta et al. 1996). Fructan are primarily considered a storage carbohydrate and their adaptive role in plant winter survival could be as a source of organic reserves available for spring regrowth. The lack of relationship between fructan concentrations and freezing tolerance among genotypes indicated that fructan do not likely play a direct role as a cryoprotectant as suggested by Livingston and Henson (1998). Alternatively, this could suggest that fructan were not a limiting factor for the acquisition of freezing tolerance under

Table 1. Percentage of injury of genotypes assessed using a Horsfall-Barrat modified scale after one week regrowth following incubation at 2°C under 98% humidity with Microdochium nivale. (Source: Bertrand et al. 2009).

| Genotype | Genotype origin | | % injury | *P=0.05* |
|---|---|---|---|---|
| | Latitude | Longitude | | |
| 27 | 48°34' N | 78°7' W | 17.5 | a |
| 20 | 46°7' N | 74°36' W | 18.9 | ab |
| 19 | 46°7' N | 74°29' W | 21.5 | abc |
| 32 | 43°25' N | 80°28' W | 23.1 | abcd |
| 5 | 48°28' N | 67°26' W | 23.9 | abcde |
| 7 | 46°58' N | 69°47' W | 24.0 | abcde |
| 15 | 45°53' N | 72°29' W | 28.2 | bcdef |
| 22 | 45°39' N | 74°56' W | 30.1 | cdefg |
| 30 | 48°6' N | 77°47' W | 30.7 | cdefg |
| 11 | 45°24' N | 71°54' W | 32.7 | cdefgh |
| 14 | 45°19' N | 73°16' W | 33.5 | defgh |
| 3 | 50°11' N | 66°38' W | 34.1 | defghi |
| 8 | 46°15' N | 72°57' W | 35.7 | efghij |
| 6 | 46°48' N | 71°11' W | 40.5 | fghijk |
| 9 | 45°17' N | 71°58' W | 41.4 | ghijk |
| 17 | 45°31' N | 73°39' W | 41.5 | ghijk |
| 18 | 45°28' N | 74°9' W | 45.8 | hijkl |
| 31 | 43°25' N | 80°28' W | 46.7 | hijkl |
| 21 | 45°54' N | 74°08' W | 48.1 | ijkl |
| 25 | 45°30' N | 75°47' W | 48.8 | jkl |
| 28 | 48°14' N | 79°1' W | 49.3 | jklm |
| 12 | 45°16' N | 71°54' W | 54.1 | klmn |
| 4 | 49°8' N | 66°30' W | 54.1 | klmn |
| 16 | 45°34' N | 73°12' W | 59.4 | lmn |
| 23 | 45°29' N | 75°39' W | 59.7 | lmn |
| 26 | 45°35' N | 75°25' W | 60.9 | lmn |
| 13 | 45°1' N | 72°6' W | 66.3 | mn |
| 24 | 45°29' N | 75°39' W | 68.6 | no |
| 10 | 45°24' N | 71°54' W | 87.8 | o |

Table 2. Spearman correlation coefficients of various winter climatic factors from the original site of collection against the percentage of injury assessed after one week regrowth of 27 annual bluegrass genotypes screened in the snow mold test. n=27, **P*=0.05, n.s = not significant. (Source: Bertrand et al. 2009).

| Factor / Period | Days with snow cover (> 10 cm) | Mean of max. daily temperature | Mean of min. daily temperature | Total rain precipitation (mm) | Total snow precipitation (cm) |
|---|---|---|---|---|---|
| December through | -0.714 | 0.738 | 0.643 | 0.619 | -0.333 |
| March | * | * | n.s | n.s. | n.s. |

our experimental conditions. Cold hardening induced major changes in amino acid levels in overwintering crowns of annual bluegrass. The concentration of free amino acids was significantly higher in the less freezing-tolerant genotype, CR, indicating a larger pool of nitrogen reserves in this genotype. Taken together, our results indicate that the less freezing-tolerant genotype, CR, has higher levels of organic reserves than the two other genotypes. CR originates from central Québec, where the depth and duration of snow cover are important factors affecting winter survival of turfgrasses. The accumulation of organic reserves has been previously linked to tolerance to both anoxia (Bertrand et al. 2003) and snow mold (Gaudet 1994) that typically occur under long lasting snow cover. Therefore, maintenance of high level of organic reserves could be an important feature of genotypes adapted to deep, long lasting snow cover. A high level of reserves may be more critical for winter survival than freezing tolerance per se when plants have to survive long winter periods without new carbohydrate synthesis, and an insulating snow cover protects the plants from extreme cold temperatures throughout winter.

To protect golf greens against extreme freezing temperatures, winter protective covers are increasingly used by golf course superintendents in northern climates (Dionne et al., 1999). Rochette et al. (2006) observed that injury that occurs under impermeable covers was associated to a gradual diminution of $O_2$ and an increase in $CO_2$. The assessment of the impact of different atmospheric compositions on regrowth of annual bluegrass allowed us to reveal that the combination of low $O_2$ and high $CO_2$ concentrations at low temperature was more damaging than high $CO_2$ alone as was observed by Boru et al. (2003) at normal growth temperature. Annual bluegrass was not sensitive to high $CO_2$ alone indicating that the main cause of damage is likely attributable to anaerobic metabolism. The reduced energy available from fermentation compared to aerobic respiration, lack of substrate for respiration and the presence of phytotoxic products such as ethanol or volatile fatty acids are the principal factors suggested for the damage connected to the lack of oxygen (Drew, 1997). In low $O_2$ treatments (LL and LH) sucrose levels were totally depleted at the end of the incubation period. Sucrose was likely hydrolyzed to sustain the production of energy through acceleration of glycolysis and of the fermentative metabolism. Higher sucrose levels under the high $O_2$ treatments could be a significant advantage for the winter survival of annual bluegrass through the impact on freezing tolerance. Furthermore, the maintenance of higher carbohydrate reserves can sustain a more vigorous regrowth in the spring.

Our results showed that in all treatments, fructan were readily hydrolyzed during incubation at low temperature. Based on observations made in the current study, it seems unlikely that low $O_2$ or high $CO_2$ interfere with the catabolism of fructan. Our results showed a progressive accumulation of the amino acid glutamine in plants exposed to low temperature under high $O_2$. This cold-induced accumulation was inhibited under low $O_2$ (LL and LH treatments). This inhibition could be explained by the arrest of the Krebs cycle when fermentation occurred. In fact, glutamate is an important amino acid produced during aerobic respiration by the acetic acid cycle, and is a precursor in the synthesis of glutamine. It has been shown in *Arabidopsis* that the carbon levels in the plant positively affects the expression of the glutamine synthase gene and results in an increase of the enzyme activity (Oliveira and Coruzzi, 1999). A very low carbon level in plants subjected to anaerobic conditions,

could thus have the opposite effect: a reduction in the activity of the glutamine synthase which is necessary to the synthesis of glutamine.

Levels of alanine significantly increased in the absence of $O_2$. Alanine is a known product of the fermentative pathway, synthesized directly from pyruvate outside the mitochondria (Ricard et al., 1994; Rawyler et al., 1999). The physiological significance of the accumulation of alanine may be as a temporary store of carbon and nitrogen when the plant is in a state of anoxia (Sato et al., 2002). Taken together, the changes of metabolite concentrations suggest that the sensitivity of annual bluegrass to anoxic conditions is mainly due to the depletion of reserves under low $O_2$.

Diseases caused by the fungus *M. nivale* are considered the most prevalent and destructive winter disease on cool-season turfgrass in northern temperate zones (Tronsmo et al., 2001) and pink snow mold is enhanced by longer durations of snow cover (Johnston and Golob, 2004). Our results showed that although annual bluegrass is considered very susceptible to SM, genotypes highly resistant to SM can be identified. Other studies with grasses and cereals also observed large genetic variability for SM resistance and successfully integrated this feature into breeding programs for the development of resistant cultivars (Tronsmo, 1992; Zhao et al., 2005; Casler et al., 2006). Analyses of the relationships between SM resistance and climatic factors revealed that duration of snow cover and temperature in winter play important roles in the evolution of genetic resistance of annual bluegrass.

Our observations on adaptation to freezing stress, anoxia and snow mold reveal that resistance to several stresses that occur under deep, long lasting snow cover is required for winter survival of annual bluegrass in Eastern Canada. The capacity to survive winter seems to be linked with the potential of the genotypes to retain sufficient organic reserves for spring regrowth under adverse conditions.

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