FREEZING TOLERANCE IN ZOYSIAGRASS

by

QI ZHANG

B.S., Northeast Agriculture University, China, 1999
M.S., Kansas State University, 2004

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Horticulture, Forestry and Recreation Resources
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2007

Abstract

‘Meyer’ zoysiagrass (*Zoysia japonica* Steud.) has been the predominant cultivar used in the transition zone since its release in 1952, primarily because of its excellent freezing tolerance. Six hundred and nineteen zoysiagrass progeny were evaluated over 3 years, and 31 were identified from which one or more cultivars may be released with a finer texture and/or faster establishment and recovery rate compared to Meyer, but with comparable freezing tolerance. DALZ 0102 (*Z. japonica*), a selection tested in the 2002 National Turfgrass Evaluation Program (NTEP) Zoysiagrass Study has exhibited a faster establishment and recovery rate than Meyer; however, a lower percentage of living rhizomes and nodes was observed in DALZ 0102 compared to Meyer at temperatures $\leq$ -15 C in a controlled freezing chamber experiment. Physiological contributors to freezing tolerance, including lipid and fatty acid composition, and endogenous abscisic acid (ABA) levels, were monitored in ‘Cavalier’ [*Z. matrella* (L.) Merrill] (cold sensitive, LT_{50} = -9.6 C) and Meyer (cold tolerant, LT_{50} = -16.2 C) rhizomes during cold acclimation over two years. The most abundant lipids in *Zoysia* rhizomes were digalactosyl diacylglycerol (DGDG), monogalactosyl diacylglycerol (MGDG), phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidic acid (PA). It has been suggested that DGDG and PC adopt bilayer structure; whereas MGDG, PE and PA have higher tendency to form a non-bilayer, hexagonal II (H_{II}) phase, which compromises bilayer structure and cell function. Greater fluctuations in PC, PA, and the ratio of PC to (PE + PA) were observed in *Zoysia* rhizomes during cold acclimation compared to the galactolipids (DGDG and MGDG). Changes in PC and PA levels and the ratio of PC to (PE + PA) were more gradual in Meyer than in Cavalier in one year of the two-year study. There was no clear relationship between double bond indices (DBI) and LT_{50} in Cavalier and Meyer; thus, DBI might not be a good indicator of freezing tolerance. abscisic acid (ABA) levels were higher in Meyer than in Cavalier on all sampling dates and were significantly correlated with LT_{50} ($r = -0.65$, $P = 0.01$).

FREEZING TOLERANCE IN ZOYSIAGRASS

by

QI ZHANG

B.S., Northeast Agriculture University, China, 1999
M.S., Kansas State University, 2004

A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Horticulture, Forestry and Recreation Resources
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2007

Approved by:

Major Professor
Dr. Jack D. Fry

Abstract

‘Meyer’ zoysiagrass (*Zoysia japonica* Steud.) has been the predominant cultivar used in the transition zone since its release in 1952, primarily because of its excellent freezing tolerance. Six hundred and nineteen zoysiagrass progeny were evaluated over 3 years, and 31 were identified from which one or more cultivars may be released with a finer texture and/or faster establishment and recovery rate compared to Meyer, but with comparable freezing tolerance. DALZ 0102 (*Z. japonica*), a selection tested in the 2002 National Turfgrass Evaluation Program (NTEP) Zoysiagrass Study has exhibited a faster establishment and recovery rate than Meyer; however, a lower percentage of living rhizomes and nodes was observed in DALZ 0102 compared to Meyer at temperatures ≤ -15 C in a controlled freezing chamber experiment. Physiological contributors to freezing tolerance, including lipid and fatty acid composition, and endogenous abscisic acid (ABA) levels, were monitored in ‘Cavalier’ [*Z. matrella* (L.) Merrill] (cold sensitive, LT_{50} = -9.6 C) and Meyer (cold tolerant, LT_{50} = -16.2 C) rhizomes during cold acclimation over two years. The most abundant lipids in *Zoysia* rhizomes were digalactosyl diacylglycerol (DGDG), monogalactosyl diacylglycerol (MGDG), phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidic acid (PA). It has been suggested that DGDG and PC adopt bilayer structure; whereas MGDG, PE and PA have higher tendency to form a non-bilayer, hexagonal II (H_{II}) phase, which compromises bilayer structure and cell function. Greater fluctuations in PC, PA, and the ratio of PC to (PE + PA) were observed in *Zoysia* rhizomes during cold acclimation compared to the galactolipids (DGDG and MGDG). Changes in PC and PA levels and the ratio of PC to (PE + PA) were more gradual in Meyer than in Cavalier in one year of the two-year study. There was no clear relationship between double bond indices (DBI) and LT_{50} in Cavalier and Meyer; thus, DBI might not be a good indicator of freezing tolerance. Abscisic acid (ABA) levels were higher in Meyer than in Cavalier on all sampling dates and were significantly correlated with LT_{50} (r = -0.65, P = 0.01).

Table of Contents

List of Figures

List of Tables

Acknowledgements

I am thankful for the great love, support, and encouragement from my father, Demo Zhang; mother, Xiuying Song; sister, Hong Zhang, and her family, without whom I could not accomplish my goals. I wish to dedicate my work to them.

I would like to thank my advisor, Dr. Jack Fry, for his patient guidance. His support, advice, and knowledge were an inspiration to me.

I also want to thank my graduate committee, Dr. Dale Bremer, Dr. Channa Rajashekar, and Dr. Milton Engelke for their guidance and counsel.

I appreciate the support from Dr. Ruth Welti, Mary Roth, and Richard Jeannotte from Kansas Lipidomics Research Center for cooperating on the project in membrane profiling; assistance from the Donald Donforth Plant Science Center for the help in abscisic acid analyses; and the financial support from the Heat of America Golf Course Superintendents Association, the Kansas Golf Course Superintendents Association, and Kansas Turfgrass Foundation. Many other professors, staff, and graduate colleagues also assisted in my research. I express my gratitude to Dr. George Liang, Dr. Harold Trick, Dr. Alan Zuk, Duane Wilson, Ward Upham, Lea Westervelt, Kemin Su, Jason Lewis, Anthony Goldsby, and Rose Ogutu.

I am thankful for this great experience and wonderful friendships in my life.

INTRODUCTION

Zoysiagrass (*Zoysia* spp.) is a common warm-season turfgrass used on golf course fairways, tees, and roughs in the turfgrass transition zone and southern U.S. because of its excellent heat tolerance, density, and low pesticide requirements. ‘Meyer’ zoysiagrass (*Zoysia japonica* Steud.) has been the predominant cultivar used in the transition zone since its release in 1952, primarily because it has excellent freezing tolerance. As good as Meyer is, it has a comparatively coarse leaf texture compared to cultivars of *Z. matrella* and slow establishment and recuperative rates (Fry and Dernoeden, 1987).

Researchers at Texas A & M University collected *Zoysia* germplasm in Asia in the 1980’s, and some of this material ultimately contributed to the development and release of several vegetative cultivars including ‘Cavalier’ (*Z. matrella*) (Engelke et al., 2002a), ‘Diamond’ (*Z. matrella*) (Engelke et al., 2002b) and ‘Palisades’ (*Z. japonica*) (Engelke et al., 2002c). All of these cultivars exhibited higher turf quality scores than Meyer in southern evaluations, but lacked the freezing tolerance necessary for long-term survival in the transition zone (Morris, 1996 and 2001). For example, in Indiana, Diamond and Cavalier zoysiagrass had 95% and 46% winter injury, respectively. In the same study, Meyer and ‘Chinese Common’ (*Z. japonica*) zoysiagrass had no freezing damage (Patton and Reicher, 2007). Dunn et al. (1999) observed shoot regrowth from rhizomes exposed to -18 C in Meyer in a controlled freezing chamber experiment; whereas, Cavalier and ‘Emerald’ (*Z. japonica* x *Z. pacifica*) were killed at -10 C. As part of my dissertation research, I have evaluated over 600 progeny mostly resulting from crosses of high quality *Z. matrella* from Texas A and M, and cold-hardy *Z. japonica* in field studies. My goal was to advance the effort to produce a cold-hardy cultivar with better quality and, potentially, a faster spread and recovery rate than Meyer.

DALZ 0102 zoysiagrass, a *Z. japonica,* was evaluated as an experimental selection at 14 locations across the U.S. in the 2002 National Turfgrass Evaluation Program (NTEP) Zoysiagrass Study. It consistently had higher quality and density ratings than Meyer in these evaluations (Morris, 2003, 2004, 2005, and 2006). Furthermore, it has a fast rate of lateral spread as indicated by a higher surface coverage than Meyer (3480 cm^2 vs. 1203 cm^2) and stolon growth rate (7.7 $cm\ d^{-1}$ vs. 5.0 $cm\ d^{-1}$) after 91 days of establishment in Indiana (Patton et al., 2007).

Similar or less winter injury was observed in DALZ 0102 compared to Meyer when evaluated nationally (Morris, 2004, 2005, 2006, and 2007). Further evaluation of freezing tolerance in DALZ 0102 is needed as it will be a limiting factor in its release as a commercial cultivar for use in the transition zone.

Freezing injury to zoysiagrass results primarily from dehydration stress that occurs when ice forms outside cells and causes water to move outward across membranes. This causes membrane destabilization and ultimately injures cells (Uemura and Steponkus, 1998). During cold acclimation, membrane chemical properties change, including composition of lipids and fatty acids (Alberdi and Corcuera, 1991). Membrane lipids undergo phases from bilayer to hexagonal II (H_{II}) that compromise membrane function and integrity (Cullis and De Kruijef, 1979; Uemura and Steponkus 1998 and 1999). Lipids, digalactosyl diacylglycerols (DGDG) and phosphatidylcholines (PC), are suggested to stabilize bilayer structures; while monogalactosyl diacy glycerols (MGDG), phosphatidylethanolamines (PE), and phosphatidic acids (PA) are more likely to form hexagonal II (H_{II}) phase. The level of membrane fatty acid saturation is an indicator of membrane fluidity, and is thus an adaptive response to cold temperature (Cyril et al., 2001). Accumulation of unsaturated fatty acids is often observed in various plants during cold acclimation (Alberdi and Corcuera, 1991; Cyril et al., 2001; Cyril et al., 2002). Changes in lipids and fatty acid composition and their relationship to freezing tolerance in turfgrasses, and specifically zoysiagrass, have not been measured.

Abscisic acid (ABA) has also been shown to influence freezing tolerance in plants in several ways. First, endogenous ABA content increased in tomato (*Lycopersicon esculentum* Mill), wheat, and pea after cold acclimation or low temperature treatments (Daie and Campbell, 1981; Dörffling et al., 1990; Welbaum et al., 1997). Second, freezing tolerance was increased following exogenous ABA application in *Arabidopsis* and strawberry (*Fragaria* X *ananassa* Duch.) (Lång et al., 1989; Rajashekar et al., 1999). Heino et al. (1990) showed that ABA application restored cold acclimation capability in an ABA-deficient *Arabidopsis* mutant. Abscisic acid may serve as a secondary messenger and trigger other events, such as the production of cold-related proteins and expression of cold-regulated (COR) genes in signaling transduction during cold stress. More effort is needed to determine changes in ABA levels during acclimation, and potential effects on freezing tolerance of zoysiagrass.

My objectives were to: 1) evaluate the freezing tolerance of new zoysiagrass progeny in field plantings; 2) to compare the freezing tolerance of DALZ 0102 to Meyer zoysiagrass; 3) compare differences in rhizome membrane lipid and fatty acid composition between Cavalier (cold-sensitive) and Meyer (cold-tolerant); and 4) compare differences in rhizome ABA content during acclimation in Cavalier and Meyer.

REFERENCES

Alberdi, M., and L. J. Corcuera. 1991. Cold acclimation in plants. Phytochemistry 30:3177-3184.

Chen, J., J.J. Burke, Z. Xin, C. Xu, and J. Velten. 2006. Characterization of the *Arabidopsis* thermosensitive mutant atts02 reveals an important role for galactolipids in thermotolerance. Plant, Cell and Environ. 29:1437-1448.

Cullis, P.R., and B. De Kruijff. 1979. Lipid polymorphism and the functional roles of lipids in biological membranes. Biochem. Biophys. Acta 559:399-420.

Cyril, J. G. L. Powell, and W. V. Baird. 2001. Changes in membrane fatty acid composition during cold acclimation and characterization of fatty acid desaturase genes in bermudagrass. International Turfgrass Soc. Res. J. 9:259-267.

Cyril, J., G. L. Powell, R. R. Duncan, and W. V. Baird, W. V. 2002. Changes in membrane polar lipid fatty acids of seashore paspalum in response to low temperature exposure. Crop Science 42:2031-2037.

Daie, J., and W. F. Campbell. 1981. Response of tomato plants to stressful temperature. Plant Physiol. 67:26-29.

Dörffling, K., S. Schulenburg, G. Lesselich, and H. Dörffling. 1990. Abscisic acid and proline levels in cold hardened winter wheat leaves in relation to variety-specific differences in freezing resistance. J. Agronomy & Crop Science 165:230-239.

Dunn, J. H., S. S. Bughrara, M. R. Warmund, and B. F. Fresenburg. 1999. Low temperature tolerance of zoysiagrass. HortSci. 34:96-99.

Engelke, M., J. Reinert, P. Colbaugh, R. White, B. Ruemmele, K. Marcum, and S. Anderson. 2002a. Registration of ‘Cavalier’ zoysiagrass. Crop Sci. 52:302-303.

__ 2002b. Registration of ‘Diamond’ zoysiagrass. Crop Sci. 52:304-305.

__ 2002c. Registration of ‘Palisades’ zoysiagrass. Crop Sci. 52:305-306.

Fry, J., and P. Dernoeden. 1987. Growth of zoysiagrass from vegetative plugs in response to fertilizers. J. Amer. Soc. Hort. Sci. 112:286-289.

Heino, P., G. Sandman, V. Lång, K. Nordin, and E.T. Palva. 1990. Abscisic acid deficiency prevents development of freezing tolerance in *Arabidopsis thaliana* (L.) Heynh. Theor. Appl. Genet. 79:801-806.

Lång, V. P. Heino, and E. T. Palva. 1989. Low temperature acclimation and treatment with exogenous abscisic acid induce common polypeptides in *Arabidopsis thaliana* (L.) Heynh. Theor. Appl. Genet. 77:729-734.

Morris, K. 1996. National zoysiagrass test: 1991. Final report. NTEP 96-15. USDA-ARS, Beltsville, MD.

Morris, K. 2001. National zoysiagrass test: 1996. Final report. NTEP 01-15. USDA-ARS, Beltsville, MD.

Morris, K. 2004. National zoysiagrass test: 2002. Progress Report. NTEP 04-2. USD-ARS, Beltsville, MD.

Morris, K. 2005. National zoysiagrass test: 2002. Progress Report. NTEP 05-6. USD-ARS, Beltsville, MD.

Morris, K. 2006. National zoysiagrass test: 2002. Progress Report. NTEP 06-7. USD-ARS, Beltsville, MD.

Morris, K. 2007. National zoysiagrass test: 2002. Progress Report. NTEP 07-8. USD-ARS, Beltsville, MD.

Patton, A.J., J.J. Volenec, and Z.J. Reicher. 2007. Stolon growth and dry matter partitioning explain differences in zoysiagrass establishment rates. Crop Sci. 47:1237-1245.

Patton, A. J., and Z. J. Reicher. 2007. Zoysiagrass species and genotypes differ in their winter injury and freeze tolerance. Crop Sci. 47:1619-1627.

Rajashekar, C., H. Zhou, K. Marcum, O. Prakash. 1999. Glycine betaine accumulation and induction of cold tolerance in strawberry (*Fragaria x ananassa* Duch.) plants. Plant Sci. 148:175-183.

Uemura, M., and P.L. Steponkus. 1998. Effect of cold acclimation on membrane lipid composition and freeze-induced membrane destabilization. p.171-179. *In* Li and Chen (ed.) Plant Cold Hardiness. Plenum Press, New York.

Uemura, M., and P.L. Steponkus. 1999. Cold acclimation in plants: relationship between lipid compositon and the cryostability of the plasma membrane. J. Plant Res. 112:245-254.

Verleij, A.J., R. DeMaagd, J. Leunissen-Bijvelt, and B. Dekruijff. 1982. Divalent cations and chlorpromazine can induce non-bilayer structures in phosphatidic acid-containing model membranes. Biochim. Biophys. Acta 684:255-262.

Welbaum, G. E., D. Bian, D. R. Hill, R. L. Grayson, and M. K. Gunatilaka. 1997. Freezing tolerance, protein composition, and abscisic acid localization and content of pea epicotyl, shoot and root tissue in response to temperature and water stress. Journal of Experimental Botany. 48:643-654.

Welti, R., Li, W., Li, M., Sang, Y., Biesiada, H., Zhou, H., Rajashekar, C., Williams, T., and Wang, X. 2002. Profiling membrane lipids in plant stress responses. J. Biol. Chem. 277:31994-32002.

Zhang, W., C. Wang, C. Qin, T. Wood, G. Olafsdottir, R. Welti, X. Wang. 2003. The oleate-stimulated phospholipase D, PLDδ, and phosphatidic acid decreased H_2O_2-induced cell death in *Arabidopsis*. Plant Cell 15:2285-2295.

CHAPTER 1 - Preliminary Evaluation of Freezing Tolerance of New Zoysiagrass Progeny

The northern border of the turfgrass transition zone in the United States is roughly Interstate 70 from Maryland through eastern Kansas, and the southern boundary is roughly the southern borders of North Carolina, Kentucky, and Tennessee (Dunn and Diesburg, 2004). Whether a particular warm-season turfgrass species or cultivar will perform well in the transition zone is usually determined by its low temperature tolerance. Bermudagrass [*Cynodon dactylon* (L.) Pers.], buffalograss [*Buchloe dactyloides* (Nutt.) Engelm.], and zoysiagrass (*Zoysia spp.*) are used throughout the transition zone and heralded for their heat and drought tolerance. Buffalograss is the most cold hardy among the three species with LT_{50}s (the temperature that is lethal to 50% of the population) ranging from -14 to -22 C (Qian et al., 2001), followed by zoysiagrass (-8.4 to -11.5 C) (Patton and Reicher, 2007), and bermudagrass (-7 to -11 C) (Anderson et al., 1988 and 1993).

Zoysiagrass is native to Asia and the South Pacific, along the eastern Pacific Rim to westward of the Indian Ocean (Engelke and Anderson, 2002). David Fairchild, son of George Fairchild who was KSU’s President from 1879 to 1897, introduced “Birodoshiba” (Plant Introduction 9299) (*Z. japonica* Steud.) into the United States in 1902 (Fairchild, 1938). Among the 11 zoysiagrass species identified, three have been grown as turfgrasses in the U.S. since the 1930’s (Halsey, 1956; Engelke and Anderson, 2002). *Zoysia pacifica* (Goudsw.) M. Hotta & Kuroi produces a very attractive turf with the finest leaf texture, but is only used in areas of Florida and southern California, as it does not tolerate cold weather. Grasses in the *Z. matrella* (L.) Merr. group form a thick and tough turf that is wear resistant, but generally lack cold hardiness for use in the transition zone. Those in the Z. *japonica* group are the most cold hardy among the three turf-type zoysiagrass species, but most have a coarse leaf texture and inferior turf quality (Halsey, 1956).

‘Meyer’ zoysiagrass is named after Frank N. Meyer who collected *zoysia* seed in Korea in 1905. It is an improved *Z. japonica*, selected based on an intermediate leaf width between typical Z. *japonica* and *Z. matrella,* and its freezing tolerance (Grau and Radko, 1951, Grau, 1952). Since the release of Meyer in 1952, it has been the principal commercial cultivar used in the transition zone, primarily because it has excellent freezing tolerance. As good as Meyer is, it does have limitations, including a comparatively coarse leaf texture compared to cultivars of *Z. matrella*, slow establishment and recuperative rates (Fry and Dernoeden, 1987), relatively shallow rooting depth and below-average drought avoidance capability (Marcum et al., 1995), and susceptibility to some pests, including the disease large patch (*Rhizoctonia solani*) (Green et al., 1993).

Researchers at Texas A & M University collected *Zoysia* germplasm in Asia in the 1980's, and some of this material ultimately contributed to the development and release of several vegetative zoysiagrass cultivars including 'Cavalier' (*Z. matrella*) (Engelke et al., 2002a), 'Crowne' (*Z. japonica*) (Engelke et al., 2002b), 'Diamond' (*Z. matrella*) (Engelke et al., 2002c), and 'Palisades' (*Z. japonica*) (Engelke et al., 2002d). All of these cultivars exhibited high turf quality scores in southern evaluations, but lacked the freezing tolerance necessary for long-term survival in the transition zone (Morris, 1996 and 2001). In a controlled freezing chamber experiment, 'Belair' (*Z. japonica*), 'Chinese Common' (*Z. japonica*), and Meyer exhibited regrowth from rhizomes exposed to -18 C, but no living tissue was observed in Cavalier, Crowne, 'Emerald' (*Z. japonica* x *Z. pacifica*), or Palisades after exposure to temperatures < -10 C (Dunn et al., 1999). Field observations in Indiana have supported this controlled environment research. There, Diamond had the highest winter injury (98 to 100 %), followed by Palisades (31 to 61%) and Cavalier (17 to 45%); whereas, Meyer and Chinese Common had no injury (Patton and Reicher, 2007). Recently, researchers at Texas A & M crossed some of the aforementioned high quality cultivars with cold-hardy *Z. japonica* in an effort to produce a cold-hardy cultivar with better quality and, potentially, a faster spread and recovery rate than Meyer. Some of the cultivars of *Z. japonica* included in these crosses were Meyer and two selections of Chinese Common that were seeded at Alvamar Country Club in Lawrence, Kansas in the 1950s (referred to as Anderson #1 and #2 after Mel Anderson, the golf course superintendent who originally seeded these common *Z. japonica* cultivars).

My objective was to evaluate the cold tolerance and quality of these new zoysia progeny in the field and narrow the number of progeny for further evaluation under golf course fairway conditions.

MATERIALS AND METHODS

Study I

Three hundred seventy-eight genetically unique zoysiagrass progeny resulting from crosses at Texas A & M were space-planted (1-meter centers) as 8 by 8 cm plugs at the Rocky Ford Turfgrass Research Center in Manhattan, Kansas on 16 Aug. 2004 for field evaluation (Table 1-1). In most cases, 18 off spring from a cross represented a "family" and were arranged in groups of six in a randomized complete block design with three replicates. Parental lines used to generate the crosses were planted and replicated three times in the same experiment, but only Meyer was used as control treatment for analysis. The plugs were watered twice weekly and mowed at 7.5 cm once each week with a rotary mower during the growing season in 2005 and 2006. Urea was applied at 49 kg ha^{-1} on 20 July 2005 and 28 July 2006.

Zoysiagrass progeny were evaluated visually for quality, color, texture, rate of growth (diameter or % of coverage), winter injury, and spring green-up. Turfgrass visual quality was rated monthly on a 1 to 9 scale, where 1 = dead turf; 6 = acceptable quality for a home lawn; and 9 = optimum color, density and uniformity. Leaf color was used as an indicator for the rate at which each selection entered autumn dormancy (evaluated in Oct. and Nov.) and green-up in the spring (evaluated in May) from 2004 to 2006 using a 1 to 9 scale, where 1 = straw-brown and 9 = dark green. Lateral spread was determined by measuring the diameter of each selection on 18 May 2005. Percentage surface coverage in a 90 x 90 cm square of each selected progeny was measured using a First Growth digital camera (Decagon Devices, Inc. Pullman, WA) monthly from June to Oct. 2005 and using visual ratings in 2006. Leaf texture of each progeny was evaluated monthly on a 1 to 9 scale, where 1 = coarsest texture and 9= finest texture. Winter kill was evaluated visually using a 1 to 9 scale, where 1 = dead and 9 = no winter injury on 15 May 2005 and 10 May 2006. Percentage of green coverage was rated visually on 8 May, 2007.

Soil-encapsulated thermocouples (SET) sensors and dual probe heat capacity sensors, constructed following the method of Ham and Senock (1992) and Bremer (2003), respectively, were placed in contact with four randomly selected crowns and connected to a CR-10 datalogger (Campbell Scientific, Inc., Logan, UT) to monitor the soil temperatures at 0 and 2.5-cm depths. Soil temperatures were recorded daily with 1 h interval and downloaded from the datalogger once monthly. Air temperature was monitored using a weather station located within 10 m of the study area. Soil and air temperature data were collected from 1 Oct. 2004 to 15 May 2005 (Fig. 1-1), from 3 Oct. 2005 to 10 May 2006 (Fig.1-2), and from 1 Oct. 2006 to 8 May 2007 (Fig. 1-3).

Data were subjected to analysis of variance with PROC MIXED procedure (SAS Institute, Cary, NC) and means of families (grasses from the same cross) were separated with Tukey's protected LSD.

Study II

Another 241 zoysiagrass progeny from 13 crosses were planted at the Rocky Ford Turfgrass Research Center (10 m away from Study I) on 14 June 2005 with the number of off-spring varying from 2 to 41 per family (Table 1-2). Grasses were grown under identical conditions to Study I. The experiment was set up as a completely randomized design; each progeny was treated as a replication of the family. Data were collected on quality, color, texture, growth, and winter kill as described previously. Data were subjected to analysis of variance using PROC GLM (SAS Institute, Cary, NC) and means of families (grasses from the same crosses) were separated using a least significant difference (LSD) procedure at $P \leq 0.05$. As Meyer was not included in this field trial, mean values were compared among the progeny tested.

RESULTS AND DISCUSSION

Study I

Meyer and Cavalier x Meyer (TAES #5283) were the first to lose green color in October, 2004 (Table 1-3). By November, Meyer x Cavalier (TAES #5282), Cavalier x Meyer (TAES # 5283), Cavalier x Anderson # 1, Meyer x BMZ230, Meyer x Anderson #2, Companion x Diamond, and Emerald x Zenith had the same color ratings as Meyer (Table 1-3). Leaf texture ratings were variable, but some families had a finer mean texture than Meyer (Table 1-3).

Minimum air temperatures at or below freezing were first recorded on 6 Nov. 2004 and generally persisted from 25 Nov. 2004 to 20 March 2005 (Fig. 1-1A). The lowest air temperature was -18.1 C on 25 Dec. 2004, and the lowest soil surface temperature was -13.7 C on the same date (Fig. 1-1B). Less fluctuation was observed in the soil temperatures at 2.5- cm depth, compared to the surface temperatures, with minimum temperature at -11.5 C (Fig. 1-1B). The mid-August planting date likely lead to greater freezing injury than would have been observed had planting been done earlier the previous summer. Lack of deep rhizomes and a shallow root system have been associated with more winter injury when warm-season turfgrasses were sodded late in the year (Waltz, 2007). Progeny from Cavalier x Anderson #1, Cavalier x Anderson #2, Meyer x BMZ230, Meyer x Anderson #2, Palisades x Companion, and Emerald x Zenith had the lowest winter injury ratings on 15 May 2005, and were comparable to Meyer (Table 1-3). Seven parental lines, Diamond, Cavalier, '8501' (*Z. matrella*), 'Zorro' (*Z. matrella*), '8508' (*Z. japonica*), Palisades, and Emerald, did not survived when evaluated on the same date (data not shown).

Seventy-eight individual progeny exhibiting comparable freezing tolerance and quality characteristics to Meyer were selected on 15 May 2005 (Table 1-3). Half of the selected progeny were from crosses of Cavalier x Anderson #1 and Meyer x Anderson #2. No progeny were selected from crosses of Meyer x Cavalier (TAES #5282), Meyer x BMZ230, Meyer x Diamond, or 8501 x Zenith due to severe winter injury. Only the selected progeny were further evaluated in 2005 and 2006.

Little differences were observed in green-up among the selected families in May 2005 (Table 1-3). Meyer plugs had grown to a diameter as large as any of the average diameters of other families on 18 May 2005. By October, only the progeny selected from Crowne x Companion had greater coverage than Meyer. Most of the families had better fall color than Meyer in November, 2005, except cultivars selected from Cavalier x Meyer (TAES #5283), Palisades x Meyer, and Meyer x Anderson #2.

In the second year of evaluation, an air temperature below 0 C first occurred on 25 Oct. 2005 (Fig. 1-2A). The coldest air temperature was -20.4 C on 9 Dec. 2005; the lowest soil temperatures were -4.6 C at the surface on 15 Feb. 2006 and -3.0 C at 2.5-cm depth on 6 Dec. 2005 (Fig. 1-2B). Air temperatures were comparable in 2004 and 2005; therefore, higher minimum soil temperatures in the second winter were presumably because zoysiagrass had spread and was covering the area of ground where temperature was being measured (Fig. 1-1B and 1-2B). Possibly because soil temperatures were warmer during this winter, all progeny which survived the 2004 - 2005 also survived in 2005 - 2006. Turfgrass crowns are typically near the soil surface, although rhizomes can be sampled as deep as 12.5 cm (Ahring and Irving, 1969; Rogers et al., 1975; Dunn et al., 1999; Ebdon et al., 2002). Soil is an excellent buffer to cold temperatures, and our results also indicate that a turf cover on the surface also maintains a warmer soil temperature.

On 10 May 2006, progeny from the families Meyer x Anderson #2, Meyer x 8501, Companion x Diamond, and Meyer, had the least winter injury (Table 1-4). From these observations, 64 of the original 78 selected progeny were removed from further evaluation, but 11 were added that had not been selected in the previous year (Table 1-5). On 30 Aug. 2006, all selected progeny had 100% coverage and those from Cavalier x Meyer (TAES #5283) and 8501 x Meyer had the finest texture (Table 1-4). Crowne x Companion and 8501 x Meyer had the best color on 13 Oct. 2006 and all progeny were dormant by 18 Nov. 2006 (Table 1-4).

The first sub-zero air temperature was on 13 Oct. 2006 (Fig. 1-3A). Minimum air temperatures were below freezing from 29 Nov. to 9 Dec. 2006 and 5 Jan. to 19 Feb. 2007. The lowest air (-18.2 C) and soil temperatures (-4.1 and -4.4 C at 0 and 2.5-cm, respectively) were similar to that experienced in 2005 - 2006 (Fig. 1-3), except that the lowest temperatures were recorded between January to February 2007, one month later than the previous year. Soil temperatures at 0 and 2.5-cm depths were almost identical after 2-year of establishment (Fig. 1-3B). Green tissue was first observed on 2 April 2007; however, a rapid drop in air temperature (20 C) from 2 to 5 April resulted in a loss of green color. All families had comparable color to Meyer on 8 May 2007 (Table 1-4). However, there was a lot of variability in the progeny selected within the crosses. After rating for winter injury on 8 May, further changes in progeny selection were made, and a total of 23 progeny from Study I were chosen for further evaluation (Tables 1-5).

Study II

Progeny were planted in June 2005, two months earlier than Study I planted in 2004. This resulted in larger plug diameters relative to Study I; all families had 100% coverage by 30 Aug. 2006, except Companion x Diamond (75%), Emerald x Zenith (25%), and 8501 x Zenith (31%) (Table 1-6). Progeny from Meyer x 8508 and 8501 x Zenith had the most winter injury on 5 May 2006, and grasses from 8501 x Zenith were the first to lose color on 12 Nov. 2006. All progeny had similar color on 8 May 2007, except Emerald x Zenith and 8501 x Zenith; Companion x Diamond and 8501 x Zenith had the lowest green up rating (Table 1-6). Based on the field observation over two winters, 8 progeny from Study II were selected for further evaluation (Table 1-7).

Summary of Observations

Dramatic variation in freezing tolerance was observed within a family from a particular cross. For example, 18 progeny from the cross of Companion x Diamond had an average winter damage rating of 3.72, significantly lower than Meyer (7.67) when rated on 15 May 2005 (Table 1-3). However, one of the progeny selected within Companion x Diamond [ID = 5332-62] had a winter damage rating similar to Meyer on 10 May, 2006 (Tables 1-4).

By May, 2007, the list of prospective progeny to evaluate was further narrowed to 31 (Table 1-5 and 1-7). Progeny were identified based primarily upon spring recovery, and to a degree, narrow leaf texture, following the winters of 2005, 2006, and 2007 (Table 1-8). Of the 31 progeny, one is from Cavalier x Meyer (TAES #5283); seven are from Cavalier x Anderson #1; three are from Cavalier x Anderson #2; four are from Zorro x Meyer; six are from Emerald x Meyer; six are from 8501 x Meyer; one is from Meyer x 8508; two are from Meyer x Diamond, and 1 is from Emerald x Zenith. None were selected from Meyer x Cavalier (TAES #5282), Palisades x Meyer, Meyer x BMZ230, Meyer x Anderson #2, Crowne x Companion, Palisades x Companion, Companion x Diamond, 8501 x Zenith because of their poor freezing tolerance or coarse texture (Table 1-5 and 1-7). Twenty-three out of the thirty-one final selections were from crosses of *Z. matrella* x *Z. japonica*, indicating there is potential for a cultivar with comparable freezing tolerance, but higher quality, than Meyer. In 2007, these progeny were established in larger replicated plots for intensive evaluation under golf course fairway conditions in Manhattan and Olathe, KS.

References

Anderson, J., M. Kenna., and C. Taliaferro. 1988. Cold hardiness of ‘Midiron’ and ‘Tifgreen’ bermudagrass. HortSci. 23:748-750.

Anderson, J., C. Taliaferro, and D. Martin.1993. Evaluating freeze tolerance of bermudagrass in a controlled environment. HortSci. 28:955.

Ahring, R., and R. Irving. 1969. A laboratory method of determining cold hardiness in bermudagrass, *Cynodon dactylon* (L.) Pers. Crop Sci. 9:615-618.

Bremer, D.J. 2003. Evaluation of microlysimeters used in turfgrass evapotranspiration studies using the dual-probe heat-pulse technique. Agron. J. 95:1625-1632.

Dunn, J., and K. Diesburg. 2004. Turf Management in the Transition Zone. John Wiley & Sons, Inc. Hoboken, NJ.

Dunn, J., S. Bughrara, M. Warmund, and B. Fresenburg. 1999. Low temperature tolerance of zoysiagrass. HortSci. 34:96-99.

Edbon, J., R. Gagne, and R. Manley. 2002. Comparative cold tolerance in diverse turf quality genotypes of perennial ryegrass. HortSci. 37:826-830.

Engelke, M., and S. Anderson. 2003. Zoysiagrasses (*Zoysia* spp.). p. 271-286. *In* M.D. Casler and R.R. Cuncan (ed.) Turfgrass Biology, Genetics, and Breeding. John Wiley & Sons, Inc. Hoboken, NJ.

Engelke, M., J. Reinert, P. Colbaugh, R. White, B. Ruemmele, K. Marcum, and S. Anderson. 2002a. Registration of ‘Cavalier’ zoysiagrass. Crop Sci. 52:302-303.

__ 2002b. Registration of ‘Crowne’ zoysiagrass. Crop Sci. 52:303-304.

__ 2002c. Registration of ‘Diamond’ zoysiagrass. Crop Sci. 52:304-305.

__ 2002d. Registration of ‘Palisades’ zoysiagrass. Crop Sci. 52:305-306.

Fairchild, D. 1938. The World was My Garden: Travels of a Plant Explorer. Charles Scribner’s Sons, New York, NY.

Fry, J., and P. Dernoeden. 1987. Growth of zoysiagrass from vegetative plugs in response to fertilizers. J. Amer. Soc. HortSci. 112:286-289.

Grau, F.V., and A.M. Radko. 1951. Meyer (Z-52) zoysiagrass. USGA J. and Turf Manage. 4:30-31.

Grau, F.V. 1952. Report on two improved turf grasses. USGA J. and Turf Manage. 5:31-32.

Green, D., J. Fry, J. Pair, and N. Tisserat. 1993. Pathogenicity of *Rhizoctonia solani* AG-2-2 and *Ophiosphaerella herpotricha* on zoysigrass. Plant Dis. 77:1040-1044.

Halsey, H. 1956. The *Zoysia* lawn grasses. The National Horticultural Magazine 35:152-161.

Ham, J.M., and R.S. Senock. 1992. On the measurement of soil surface temperature. Soil Sci. Soc. of Am. J. 56:370-377.

Marcum, K., M. Engelke, S. Morton, and R. White. 1995. Rooting characteristics and associated drought resistance of zoysiagrass. Agron. J. 87:534-538.

Morris, K. 1996. National zoysiagrass test: 1991. Final report. NTEP 96-15. USDA-ARS, Beltsville, MD.

Morris, K. 2001. National zoysiagrass test: 1996. Final report. NTEP 01-15. USDA-ARS, Beltsville, MD.

Patton, A.J., and Z.J. Reicher. 2007. Zoysiagrass species and genotypes differ in their winter injury and freeze tolerance. Crop Sci. 47:1619-1627.

Portz. H. 1981. Zoysiagrass establishment from seed. USGA Green Section Record. 19:10-13.

Qian, Y., S. Ball, Z. Tan, A. Koshi, and S. Wilhelm. 2001. Freezing tolerance of six cultivars of buffalograss. Crop Sci. 41:1174-1178.

Rogers, R., J. Dunn, and C. Nelson. 1975. Cold hardening and carbohydrate composition of 'Meyer' zoysia. Agron. J. 67:836-838.

Waltz, C. 2007. Installation of dormant sod. Available at http://commodities.caes.uga.edu/turfgrass/georgiaturf/Articles/Misc/Off_Sea_Sod.pdf (accessed 22 Sept., 2007).

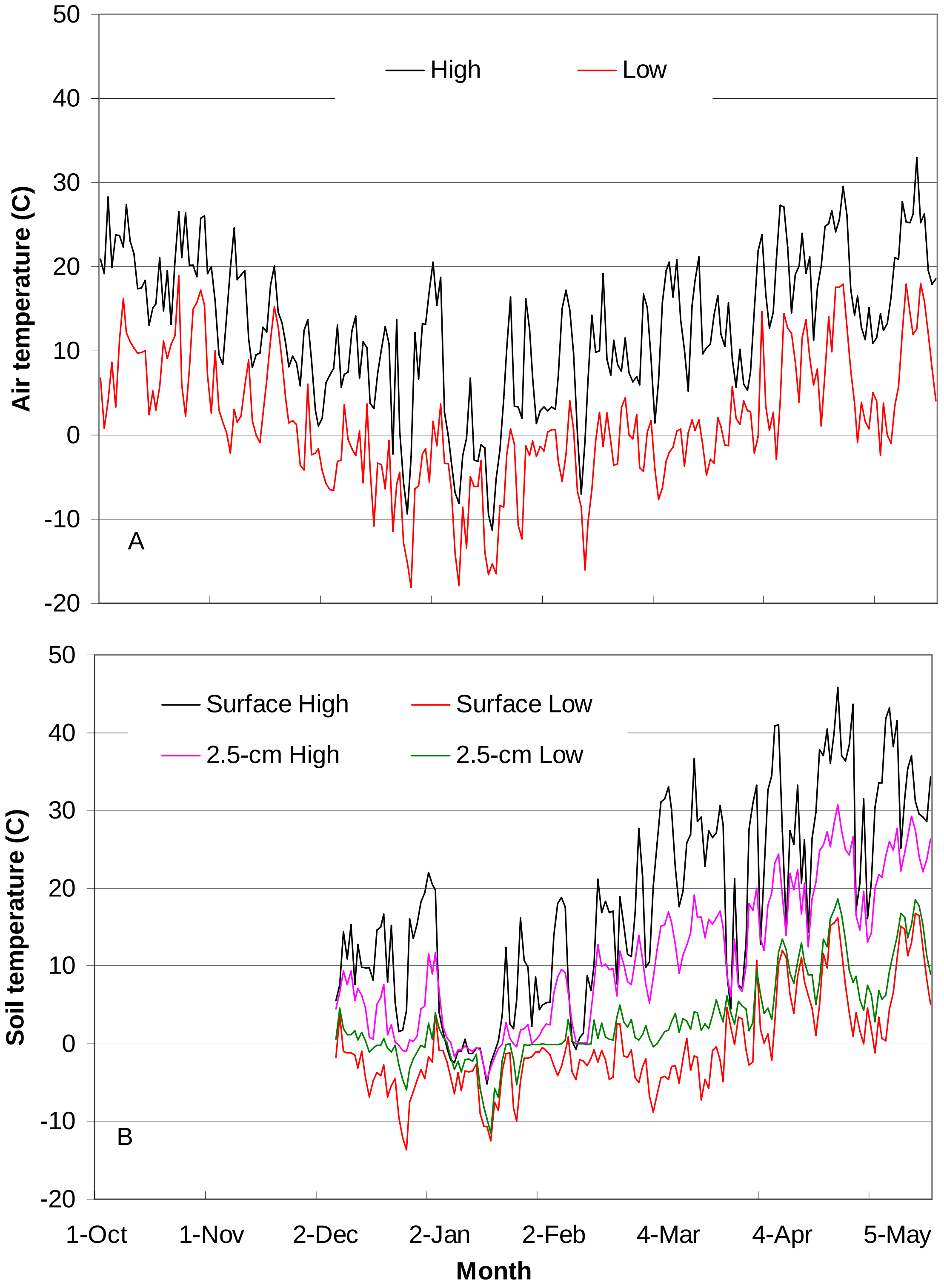


Figure 1-1. Daily air (A) and soil temperatures at 0 and 2.5-cm depths (B) from 1 Oct., 2004 to 15 May 2005.

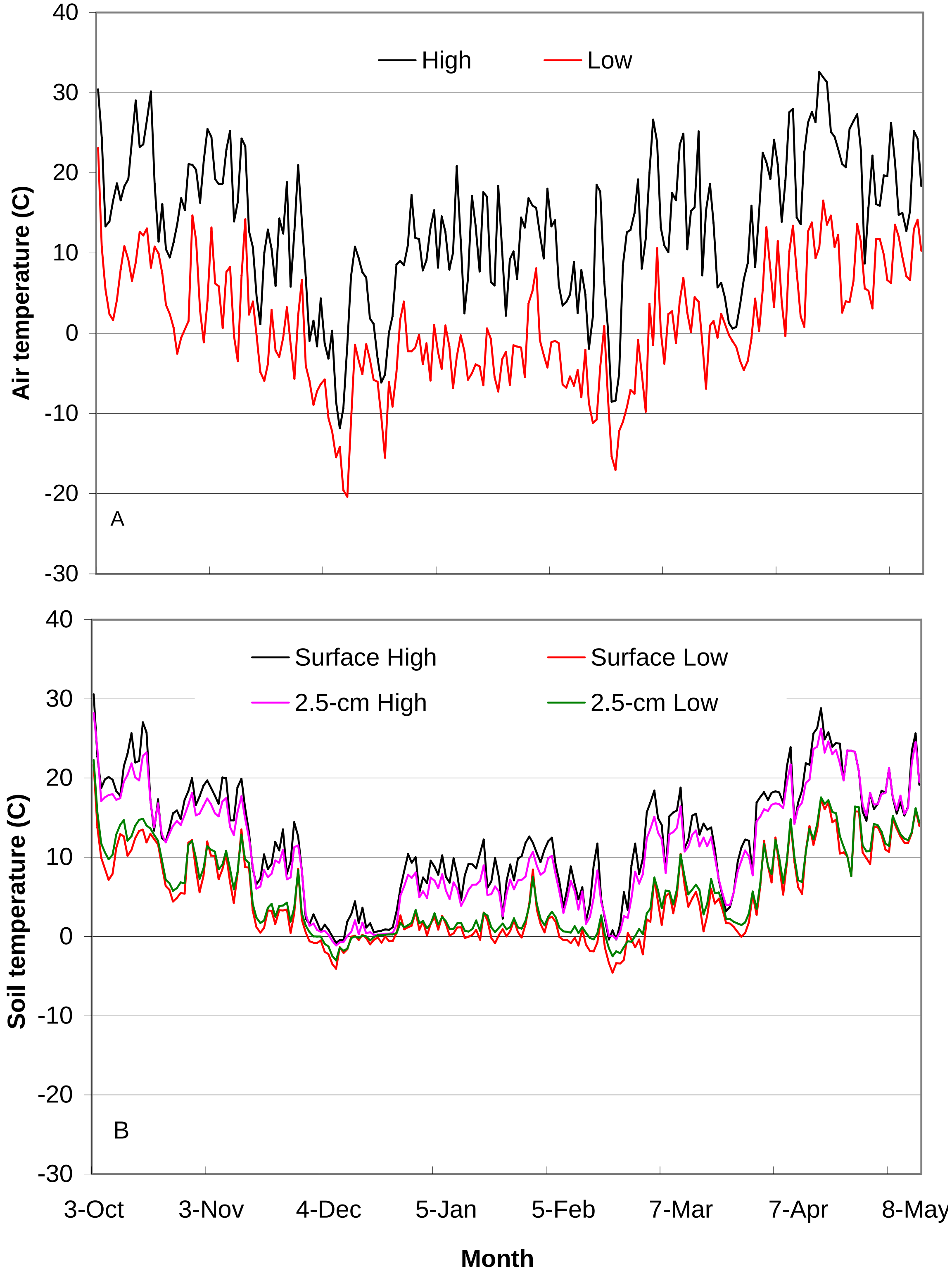


Figure 1-2. Daily air (A) and soil temperatures at 0 and 2.5-cm depths (B) from 3 Oct., 2005 to 10 May, 2006.

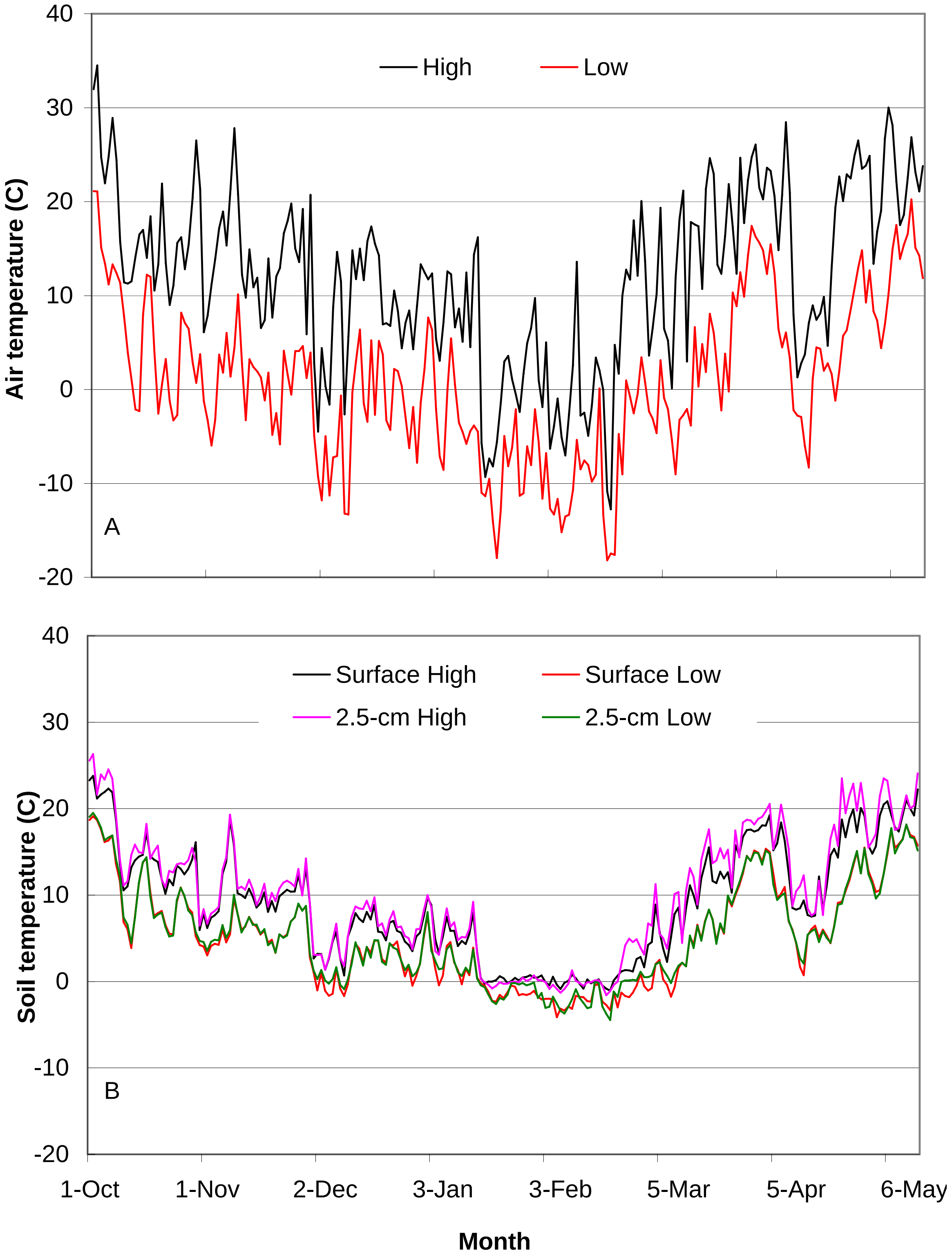


Figure 1-3. Daily air (A) and soil surface temperatures at 0 and 2.5-cm depths (B) from 1 Oct., 2006 to 8 May, 2007.

Table 1-1. Zoysiagrass progeny planted on 16 Aug., 2004 (Study I) at Manhattan, KS.

TAES (#)	Pedigree (Female x Male)	# of progeny to start
5282	Meyer (*Z. japonica*) x Cavalier (*Z. matrella*)	8
5283	Cavalier (*Z. matrella*) x Meyer (*Z. japonica*)	10
5311	Cavalier (*Z. matrella*) x Anderson #1 (*Z. japonica*)	36
5312	Cavalier (*Z. matrella*) x Anderson #2 (*Z. japonica*)	36
5313	Zorro (*Z. matrella*) x Meyer (*Z. japonica*)	36
5320	Palisades (*Z. japonica*) x Meyer (*Z. japonica*)	18
5321	Emerald (*Z. japonica x Z. pacifica*) x Meyer (*Z. japonica*)	18
5322	Meyer (*Z. japonica*) x BMZ230 (*Z. japonica*)	18
5323	Meyer (*Z. japonica*) x Anderson #2 (*Z. japonica*)	36
5324	8501 (*Z. matrella*) x Meyer (*Z. japonica*)	36
5325	Meyer (*Z. japonica*) x 8508 (*Z. pacifica*)	18
5327	Meyer (*Z. japonica*) x Diamond (*Z. matrella*)	18
5330	Crowne (*Z.japonica*) x Companion (*Z. japonica*)	18
5331	Palisades (*Z. japonica*) x Companion (*Z. japonica*)	18
5332	Companion (*Z. japonica*) x Diamond (*Z. matrella*)	18
5334	Emerald (*Z. japonica x Z. pacifica*) x Zenith (*Z. japonica*)	18
5343	8501 (*Z. matrella*)x Zenith (*Z. japonica*)	18
Meyer	*Z. japonica*	3

Table 1-2. Zoysiagrass progeny planted on 14 June, 2005 (Study II) at Manhattan, KS.

TAES (#)	Pedigree (Female x Male)	# of progeny to start
5311	Cavalier (*Z. matrella*) x Anderson #1 (*Z. japonica*)	24
5312	Cavalier (*Z. matrella*) x Anderson #2 (*Z. japonica*)	16
5313	Zorro (*Z. matrella*) x Meyer (*Z. japonica*)	18
5320	Palisades (*Z. japonica*) x Meyer (*Z. japonica*)	26
5321	Emerald (*Z. japonica x Z. pacifica*) x Meyer (*Z. japonica*)	27
5322	Meyer (*Z. japonica*) x BMZ230 (*Z. japonica*)	10
5323	Meyer (*Z. japonica*) x Anderson #2 (*Z. japonica*)	15
5324	8501 (*Z. matrella*) x Meyer (*Z. japonica*)	25
5325	Meyer (*Z. japonica*) x 8508 (*Z. pacifica*)	29
5327	Meyer (*Z. japonica*) x Diamond (*Z. matrella*)	41
5332	Companion (*Z. japonica*) x Diamond (*Z. matrella*)	2
5334	Emerald (*Z. japonica x Z. pacifica*) x Zenith (*Z. japonica*)	2
5343	8501 (*Z. matrella*) x Zenith (*Z. japonica*)	6

Table 1-3. Field performance of zoysiagrass progeny in Study I from autumn 2004 to 2005 at the Rocky Ford Turfgrass Research Center, Manhattan, KS.

	# of progeny to start	2004			2005	# of progeny after selection†	2005†				
		16 Oct.		11 Nov.	15 May		18 May		19 Oct.		12 Nov.
Cross (Female x Male)		Color‡	Texture‡	Color	Winter injury‡		Color	Diameter (cm)	Color	Coverage§ (%)	Color
Meyer x Cavalier	8	5.50 g[*]	7.63 abcd	5.15 bcd	2.56 ef	0	--------	--------	--------	--------	--------
Cavalier x Meyer	10	5.10 gh	7.30 de	4.88 cd	2.26 ef	2	6.92 abc	19.2 bc	7.07 a	39.0 ab	1.84 abc
Cavalier x Anderson #1	36	6.33 de	7.19 e	5.28 bcd	7.39 ab	22	7.57 a	23.2 bc	6.23 a	28.3 bc	2.92 a
Cavalier x Anderson #2	36	6.28 e	7.44 cd	5.22 ab	6.31 b	10	7.59 a	20.9 bc	6.23 a	34.2 b	2.76 a
Zorro x Meyer	36	6.64 abc	7.92 a	5.81 ab	1.89 f	2	7.04 abc	19.9 bc	7.00 a	31.0 bc	3.58 a
Palisades x Meyer	18	6.41 bcde	6.77 ef	5.64 abc	2.28 ef	1	7.12 abc	20.6 bc	5.86 a	25.0 bc	1.03 bc
Emerald x Meyer	18	6.61 abcd	7.72 abc	5.44 abc	4.11 cde	4	7.25 ab	16.9 c	5.82 a	27.0 bc	3.10 a
Meyer x BMZ230	18	6.18 ef	6.82 ef	3.83 ef	4.89 bc	0	--------	--------	--------	--------	--------
Meyer x Anderson #2	36	6.00 f	6.58 fg	3.11 f	8.06 a	19	7.03 abc	28.7 ab	6.23 a	27.0 bc	1.20 bc
8501 x Meyer	36	6.72 ab	7.92 a	5.86 ab	3.33 de	6	7.13 abc	14.9 c	6.50 a	22.8 c	3.42 a
Meyer x 8508	18	6.67 abc	7.50 bcd	6.17 a	1.61 f	1	7.12 abc	23.6 abc	6.86 a	17.0 c	4.04 a
Meyer x Diamond	18	6.88 a	7.75 ab	5.54 abc	1.06 f	0	--------	--------	--------	--------	--------
Crowne x Companion	18	6.33 cde	6.39 g	5.67 abc	3.67 cde	2	6.96 abc	41.1 a	7.14 a	49.0 a	3.63 a
Palisades x Companion	18	6.39 cde	6.06 h	5.72 abc	6.56 b	4	6.68 bc	23.6 bc	7.03 a	33.8 bc	2.91 a
Companion x Diamond	18	6.47 bcde	6.77 ef	4.54 de	3.72 cde	1	7.92 a	35.7 ab	6.00 a	42.0 ab	3.84 a
Emerald x Zenith	18	6.22 ef	7.50 bcd	5.28 bcd	4.72 bcd	4	6.49 c	28.6 ab	6.53 a	28.0 bc	2.28 ab
8501 x Zenith	18	6.39cde	7.72 ab	5.67 abc	0.50 f	0	--------	--------	---------	--------	--------
Meyer	3	4.67 h	6.67 efg	4.00 def	7.67 ab	3	7.33 ab	31.2 ab	6.33 a	32.7 bc	1.00 c

[*] Means of each family before and after selection. Numbers in a column followed by the same letter are not significantly different according to Tukey's LSD ($P \leq 0.05$).

[†] Zoysiagrasses with promising quality characteristics and freezing tolerance were selected on 15 May, 2005. Selection was based upon performance of the group and individuals. Data were collected and analyzed only from selected individuals within each hybrid line after 15 May, 2005.

[‡] Color, texture, and winter injury were rated on a 1 to 9 scale, where 1 = straw-brown color, coarsest texture, or winter-killed turf and 9 = dark green color, finest texture, and no winter damage. Color and texture were rated monthly and winter injury was rated on 15 May, 2005.

[§] Coverage was measured using a First Growth digital camera (Decagon Devices, Inc. Pullman, WA) monthly from June to October, 2005.

Table 1-4. Field performance of zoysiagrass progeny in Study I in 2006 and 2007 at the Rocky Ford Turfgrass Research Center, Manhattan, KS.

Cross (Female x Male)	# of progeny after selection[†]	2006[†]					2007[†]	
		10 May		30 Aug.		13 Oct.	8 May	
		Color[‡]	Winter Injury[‡]	Color	Texture[‡]	Color	Color	Green-up[§] (%)
Meyer x Cavalier	0	---------------	---------------	---------------	--------------	------------	---------------	---------------
Cavalier x Meyer	2	3.91 a[*]	4.57 d	7.00 a	7.50 a	6.00 b	5.50 ab	23.4 a
Cavalier x Anderson #1	22	5.12 a	7.34 b	6.73 a	7.05 ab	6.09 b	5.82 ab	31.7 a
Cavalier x Anderson #2	10	4.84 a	6.48 bc	6.90 a	7.00 ab	5.80 b	5.47 b	19.3 a
Zorro x Meyer	2	5.13 a	5.63 cd	7.00 a	7.00 ab	7.00 ab	7.00 ab	33.2 a
Palisades x Meyer	1	5.18 a	2.87 d	7.00 a	6.00 bc	7.00 ab	6.00 ab	23.2 a
Emerald x Meyer	4	5.36 a	6.01 bc	7.00 a	7.00 ab	6.50 ab	7.11 a	24.4 a
Meyer x BMZ230	0	---------------	----------------	---------------	--------------	------------	---------------	---------------
Meyer x Anderson #2	19	4.91 a	8.53 a	7.00 a	6.68 b	5.63 b	5.26 b	27.8 a
8501 x Meyer	6	5.37 a	7.04 bc	7.00 a	7.50 a	7.17 a	7.00 ab	26.0 a
Meyer x 8508	1	5.18 a	7.87 ab	7.00 a	7.00 ab	7.00 ab	7.00 ab	43.2 a
Meyer x Diamond	0	----------------	----------------	---------------	--------------	--------------	---------------	---------------
Crowne x Companion	2	4.59 a	6.38 bc	7.00 a	6.00 c	7.50 a	4.00 b	15.6 a
Palisades x Companion	4	4.57 a	5.66 cd	6.50 a	6.00 c	6.75 ab	4.78 b	9.8 a
Companion x Diamond	1	3.73 a	7.75 ab	6.00 a	7.00 ab	7.00 ab	4.00 b	3.7 a
Emerald x Zenith	4	5.02 a	6.35 bc	7.00 a	6.75 b	7.00 ab	6.06 ab	32.0 a
8501 x Zenith	0	----------------	----------------	---------------	--------------	--------------	---------------	---------------
Meyer	3	5.67 a	9.00 a	7.00 a	7.00 ab	6.00 b	6.00 ab	41.7 a

[*] Means of each family before and after selection. Numbers in a column followed by the same letter are not significantly different according to Tukey's LSD ($P \leq 0.05$).

[†] Zoysiagrasses with promising quality characteristics and freezing tolerance were selected on 15 May, 2005. Selection was based upon performance of the group and individuals. Data were collected and analyzed only from selected individuals within each hybrid line after 15 May, 2005.

[‡] Color, texture, and winter injury were rated on a 1 to 9 scale, where 1 = straw-brown color, coarsest texture, or winter damaged turf and 9 = dark green color, finest texture, and no winter damage. Color and texture were rated monthly and winter injury was rated on 10 May, 2006.

[§] Green-up was rated visually as percentage of green plot area on 8 May 2007.

Table 1-5. Selection process for individual zoysiagrass progeny in Study I.

15 May, 2005		10 May, 2006		8 May, 2007	
ID	Pedigree (Female x Male)	Retained*	Added	Retained*	Added
5283-11	Cavalier x Meyer	N			
5283-13		N			
			5283-27	Y	
5311-2	Cavalier x Anderson #1	N			
			5311-3	Y	
5311-5		N			
5311-6		N			
5311-7		Y		N	
5311-8		Y		Y	
5311-13		N			
5311-16		N			5311-16
5311-18		N			
			5311-22	Y	
5311-26		Y		Y	
5311-27		Y		Y	
5311-29		N			
5311-31		N			
5311-32		Y		Y	

Table 1-5. Selection process for individual zoysiagrass progeny in Study I (Cont').

15 May, 2005		10 May, 2006		8 May, 2007	
ID	Pedigree (Female x Male)	Retained*	Added	Retained*	Added
5311-38		N			
5311-45		N			
5311-50		N			
5311-51		N			
5311-54		N			
5311-55		N			
5311-58		N			
5311-59		N			
5312-8	Cavalier x Anderson #2	N			
5312-15		N			
5312-17		N			
5312-20		N			
5312-25		N			
			5312-36	Y	
5312-37		N			
5312-38		N			
5312-40		N			
5312-45		N			
5312-46		N			

Table 1-5. Selection process for individual zoysiagrass progeny in Study I (Cont').

15 May, 2005		10 May, 2006		8 May, 2007	
ID	Pedigree (Female x Male)	Retained*	Added	Retained*	Added
5313-10	Zorro x Meyer	Y		N	
5313-19		N			
					5313-23
					5313-34
					5313-46
5320-48	Palisades x Meyer	N			
5321-1	Emerald x Meyer	N			
5321-2		N			
5321-3		Y		Y	
					5321-9
			5321-16	N	
			5321-17	N	
					5321-18
5321-24		Y		Y	
5323-6	Meyer x Anderson #2	N			
5323-7		N			

Table 1-5. Selection process for individual zoysiagrass progeny in Study I (Cont').

15 May, 2005		10 May, 2006		8 May, 2007	
ID	Pedigree (Female x Male)	Retained*	Added	Retained*	Added
5323-10		N			
5323-13		N			
5323-15		N			
5323-17		N			
5323-18		N			
5323-19		N			
5323-26		N			
5323-28		N			
5323-34		N			
5323-37		N			
5323-38		N			
5323-40		N			
5323-41		N			
5323-44		N			
5324-4	8501 x Meyer	N			
5324-12		Y		N	
			5324-18	Y	
5324-22		Y		N	

Table 1-5. Selection process for individual zoysiagrass progeny in Study I (Cont').

15 May 2005		10 May 2006		8 May 2007	
ID	Pedigree (Female x Male)	Retained*	Added	Retained*	Added
					5324-26
5324-27		Y		Y	
5324-32		Y		Y	
5324-39		N			
5325-23	Meyer x 8508	N			5325-11
			5327-19 (Meyer x Diamond)	Y	
5330-40	Crowne x Companion	N			
5330-55		N			
5331-59	Palisades x Companion	N			
5331-63		N			
5331-64		N			
5331-65		N			
5332-62	Companion x Diamond	N			
5334-40	Emerald x Zenith	Y		N	
5334-41		N			

Table 1-5. Selection process for individual zoysiagrass progeny in Study I (Cont').

15 May, 2005		10 May, 2006		8 May, 2007	
ID	Pedigree (Female x Male)	Retained*	Added	Retained*	Added
5334-58		Y		N	
					5334-59
			5334-63	N	
			5334-69	N	
5334-71		N			
Total number of selected progeny					
78			24		23

*Y = Yes; N = No.

Table 1-6. Field performance of zoysiagrass progeny in Study II in 2005, 2006, and 2007 at the Rocky Ford Turfgrass Research Center, Manhattan, KS.

Cross (Female x Male)	# of Progeny to start	2005				2006						2007	
		7 Sept.			12 Nov.	10 May			30 Aug.		13 Oct.	8 May	
		Color[†]	Texture[†]	Diameter (cm)	Color	Color	Winter Injury[†]	Coverage[†] (%)	Color	Texture	Color	Color	Green up[§] (%)
Cavalier x Anderson #1	24	8.6 b*	7.3 de	91.6 a	3.3 a	5.0 d	7.0 a	25.8 b	6.8 b	6.9 ab	5.5 bcd	6.3 abc	28.3 ab
Cavalier x Anderson #2	16	8.8 ab	7.1 ef	100.5 a	3.0 ab	5.1 cd	7.4 a	36.3 a	6.9 b	7.0 ab	5.9 ab	6.6 abc	29.7 ab
Zorro x Meyer	18	8.9 a	8.2 ab	65.1 cd	4.3 a	5.9 a	5.2 a	22.6 bc	6.8 b	7.1 a	5.6 bcd	6.8 ab	28.9 ab
Palisades x Meyer	26	8.9 a	6.3 g	79.0 b	3.9 a	4.8 d	5.3 a	24.0 bc	7.4 a	6.2 b	6.0 ab	6.2 bc	22.3 ab
Emerald x Meyer	27	9.0 a	7.8 bc	52.0 ef	3.6 a	5.6 ab	6.6 a	20.9 bc	7.0 ab	7.1 a	5.7 bc	7.1 a	31.9 a
Meyer x BMZ230	10	8.6 b	6.6 fg	63.8 cd	3.2 a	4.7 d	4.8 a	13.2 d	6.8 b	6.6 b	5.1 cd	6.2 bc	21.0 ab
Meyer x Anderson #2	15	8.8 ab	6.5 fg	73.6 bc	2.5 ab	5.0 cd	7.1 a	36.7 a	6.5 b	6.5 b	4.9 d	6.0 c	28.0 ab
8501 x Meyer	25	9.0 a	7.7 cd	64.5 cd	4.1 a	5.6 ab	5.0 a	17.4 cd	6.9 b	7.2 a	6.1 ab	6.6 abc	26.0 ab
Meyer x 8508	29	8.9 a	7.0 ef	63.6 cd	4.0 a	5.4 bc	2.6 b	10.1 d	6.9 b	6.9 ab	5.7 bc	6.7 abc	19.7 b
Meyer x Diamond	41	9.0 a	8.0 ab	54.7 de	4.4 a	5.9 a	5.0 a	13.9 d	6.9 b	7.2 a	6.2 a	7.0 ab	31.1 a
Companion x Diamond	2	7.5 c	6.0 g	61.6 cde	4.0 a	4.5 d	4.5 ab	6.5 d	7.0 ab	6.5 b	5.0 cd	6.5 abc	5.0 c
Emerald x Zenith	2	N/A[‡]	N/A	N/A	5.0 a	5.0 cd	9.0 a	5.0 d	5.0 c	8.0 a	5.0 cd	4.0 d	25.0 ab
8501 x Zenith	6	8.5 b	8.5 a	36.0 f	1.8 b	2.8 e	1.7 b	9.7 d	4.8 c	5.0 c	4.8 d	2.7 e	1.8 c

[*] Means of each family. Numbers in a column followed by the same letter are not significantly different ($P \leq 0.05$).

[†] Color, texture, and winter injury were rated with a 1 to 9 scale, where 1 = straw-brown color, coarsest texture, or winter damaged turf and 9 = dark green color, finest texture, and no winter damage; coverage was rated visually with a 0 to 100 scale. Color, texture, and coverage were rated monthly and winter injury was rated on 5 May, 2006.

[‡] N/A = rating not available as progeny were damaged by glyphosate when applied to control weeds.

[§] Green-up was rated visually as % of green tissue on 8 May, 2007.

Table 1-7. Selection process for individual zoysiagrass progeny in Study II.

	10 May 2006		8 May 2007
ID	Pedigree (Female x Male)	Retained*	Added
5312-49	Cavalier x Anderson #2	Y	
5312-53		N	
			5312-55
5313	Zorro x Meyer	N	
			5313-71
5321-28	Emerald x Meyer	N	
5321-44		N	
5321-45		Y	
5321-48		Y	
5324-52	8501 x Meyer	Y	
5324-53		Y	
5324-59		N	
	Meyer x Diamond		5327-67
Total number of selected progeny			
10			8

*Y = Yes; N = No.

Table 1-8. Performance in 2005, 2006, and 2007 of 31 progeny that were ultimately selected based upon individual performance.

				2005				2006				2007
Selection time	ID	Pedigree (Female x Male)	Study	May Winter injury†	Aug. Quality†	Aug. Texture†	Nov. Color†	May Winter injury	Aug. Quality	Aug. Texture	Oct. Color	May Green-up‡ (%)
May, 2005	5311-8	Cavalier x Anderson #1	I	8	8	8	4	6	5	7	5	25
	5311-16		I	8	7	8	2	8	5	7	6	70
	5311-26		I	8	8	8	3	8	6	7	6	30
	5311-27		I	7	8	8	4	8	7	7	6	30
	5311-32		I	9	8	8	2	7	7	7	7	40
	5321-3	Emerald x Meyer	I	7	8	8	3	7	6	7	6	30
	5321-24		I	9	8	8	3	7	7	7	7	30
	5324-27	8501 x Meyer	I	7	8	8	4	7	7	7	7	30
	5324-32		I	6	8	8	3	5	5	8	8	20
	Meyer		I	8	8	7	1	9	7	7	6	42
May, 2006§	5283-27	Cavalier x Meyer	I					9	6	7	7	35
	5311-3	Cavalier x Anderson #1	I					8	7	7	6	40
	5311-22		I					7	5	8	5	40
	5312-36	Cavalier x Anderson #2	I					7	7	7	5	30
	5324-18	8501 x Meyer	I					5	6	7	7	35
	5327-19	Meyer x Diamond	I					8	4	7	6	25
	5312-49	Cavalier x Anderson #2	II					9	5	7	7	40
	5321-45	Emerald x Meyer	II					8	6	7	6	40
	5321-48		II					9	6	7	5	50
	5324-52	8501 x Meyer	II					9	7	7	5	40
	5324-53		II					6	7	7	7	40

Table 1-8. Performance in 2005, 2006, and 2007 of 31 progeny that were ultimately selected based upon individual performance (Cont').

				2005				2006				2007
Selection time	ID	Pedigree (Female x Male)	Study	May	Aug.		Nov.	May	Aug.		Oct.	May
				Winter injury[†]	Quality[†]	Texture[†]	Color[†]	Winter injury	Quality	Texture	Color	Green-up[‡] (%)
May, 2007[§]	5313-23	Zorro x Meyer	I					6	7	7	7	70
	5313-34		I					5	6	7	7	60
	5313-46		I					6	7	8	7	70
	5321-9	Emerald x Meyer	I					8	7	7	5	80
	5321-18		I					7	7	7	7	70
	5324-26	8501 x Meyer	I					7	6	8	7	70
	5325-11	Meyer x 8508	I					7	4	7	5	80
	5334-59	Emerald x Zenith	I					6	4	7	5	80
	5312-55	Zorro x Meyer	II					7	6	7	5	50
	5313-71	Zorro x Meyer	II					5	7	7	6	50
	5327-67	Meyer x Diamond	II					7	7	7	7	50

[†] Quality, color, texture, and winter injury were rated with a 1 to 9 scale, where 1 = dead turf, straw-brown color, coarsest texture, or winter-killed turf and 9 = best quality, dark green color, finest texture, and no winter damage. Quality, color, and texture were rated monthly and winter injury was rated on 15 May 2005 and 10 May 2006.

[‡] Green-up was rated visually as percentage of green plot area on 8 May, 2007.

[§] Data from these progeny were missing in 2005 because they were excluded from the first selection.

CHAPTER 2 - Freezing Tolerance in DALZ 0102 Zoysiagrass

The turfgrass transition zone is a region where neither cool-season nor warm-season grasses are well adapted because of high and low temperature extremes (Fry and Dernoeden, 1987). Golf course superintendents in the transition zone have been long challenged to maintain good, reliable, and playable turf. Cool-season turfgrasses may be fraught with disease problems during mid-summer and have relatively high water requirements. Zoysiagrass (*Zoysia* spp.), a warm-season species, has been used effectively on transition-zone golf courses for over 50 years, and some have suggested that there is no better turf for use on fairways (Zontek, 1983). Zoysiagrass is more tolerant to heat and drought conditions, requires less fertilizer and pesticide input than cool-season turfgrasses, and is more cold hardy and greens up earlier than bermudagrass [*Cynodon dactylon* (L.) Pers.] (Zontek, 1983).

Zoysiagrass was first introduced to the U.S. in the early 1900s and was grown as a lawn grass in Florida, Georgia, and Alabama (Halsey, 1956). In 1952, ‘Meyer’ zoysiagrass (*Z. japonica* Steud.), named after Frank N. Meyer who made the first zoysia seed collection in Korea in 1905, was jointly released by the United States Department of Agriculture and United States Golf Association based on its intermediate leaf width between typical Z. *japonica* (coarse texture) and *Z. matrella* (L.) Merr. (fine texture), and its resistance to high and low temperature stresses (Grau and Radko, 1951; Grau, 1952). Since its release, Meyer has been the principal commercial cultivar in the transition. As good as Meyer is, it is slow to establish compared to most other *Z. japonica* cultivars, including ‘El Toro’, ‘Chinese Common’, and ‘Palisades’ (Patton et al., 2007).

‘DALZ 0102’ zoysiagrass is an experimental cultivar developed by Texas A & M turfgrass breeding group and included in the 2002 National Turfgrass Evaluation Program (NTEP) (Morris. 2004, 2005, 2006, and 2007). DALZ 0102 had better visual quality and density, similar or less winter injury, and the same rate of spring green-up as Meyer in the NTEP evaluations (Morris, 2004, 2005, 2006, and 2007). It is also faster to spread than Meyer, exhibiting 96.1% cover at the end of the first growing season compared to 71.3% for Meyer when averaged over all 14 evaluation locations (Morris, 2004). Unfortunately, temperatures over the past four winters in Kansas have not been low enough to allow a good comparison of freezing tolerance between Meyer and DALZ 0102, and this is the main limiting factor to introducing new warm-season grasses into the transition zone (Fry and Huang, 2004).

Patton and Reicher (2007) used a growth chamber to artificially acclimate several zoysiagrasses, including Meyer and DALZ 0102, using methods described by Anderson et al. (1993) for bermudagrass. They reported an LT_{50} of -10.2 C for DAL Z0102 compared to -11.5 C for Meyer. This artificial acclimation procedure does not always result in accurate freezing tolerance, however. For example, Patton and Reicher reported a similar LT_{50} for Palisades and Meyer, and earlier field and freezing chamber evaluations have demonstrated that Meyer is hardier than Palisades (Morris, 1996; Dunn et al., 1999; Patton and Reicher, 2007). This led to the desire to sample DALZ 0102 and Meyer that had been naturally acclimated, from the field, to compare freezing tolerance.

My objective was to determine the relative freezing tolerance of naturally acclimated field-grown Meyer and DALZ 0102 by sampling rhizomes during winter and exposing to a low temperature regime in a controlled freezing chamber.

MATERIALS AND METHODS

Meyer and DALZ 0102 were maintained as golf course fairways at the Rocky Ford Turfgrass Research Center, Manhattan, KS. Grasses were mowed at 1.5 cm three times a week and watered as needed to prevent drought stress. Urea was applied at 49 kg ha^{-1} on 21 July 2005 and 27 July 2006.

Freezing tolerance was determined using the recovery growth procedure described by Fry et al. (1993). One hundred and ninety-two rhizomes from Meyer and DALZ 0102 were sampled on 24 Feb. and 1 March 2006. Sixteen rhizomes of each cultivar (four replications and four subsamples) were exposed to non-freezing (control), or freezing temperatures ranging from -3 to -23 C in a circulating ethylene glycol bath (Forma Scientific, Marietta, OH). Rhizomes measured about 7-cm long and averaged 4 nodes each. Each group of four rhizomes was wrapped in a wet paper towel and then in aluminum foil. Rhizome bundles were placed in test tubes with a lead weight in the bottom of each to prevent floating, and then set in a rack for submersion in the circulating bath. Rhizomes in test tubes were first exposed to -3 C for 3 hours, and ice pieces were added to the top of each tube to ensure ice formation. The temperature in the bath was reduced 2 C/h; one group of rhizomes was taken out each hour. After freezing, rhizomes were slowly thawed in a growth chamber at 4 C overnight, planted in small pots containing Metro-Mix 510 (Sungro Horticulture, Bellevue, WA), and placed in a greenhouse maintained at a 30/25 C day/night temperature with a 14 h photoperiod and supplemental lighting to provide 580 $\text{umol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ at the canopy height. The experiment was repeated on 7 Dec. 2006 and 15 Feb. 2007 with the number of rhizome subsamples increased from four to ten, and freezing temperatures ranging from -3 to -19 C.

The experiment was set up as a complete randomized design with four replications. Data were collected on the percentage of living rhizomes and nodes, number of shoots, and leaf and root dry weights (dried at 80 C overnight) 8 weeks after exposure to the freezing temperatures. Analysis of variance was determined with the PROC GLM procedure (SAS Institute, Cary, NC) and means were separated by least significant difference (LSD) at $P \leq 0.05$. PROC CORR was applied to evaluate the relationships among all the parameters.

RESULTS AND DISCUSSION

This study was complicated by a relatively low percentage (< 65%) of surviving rhizomes in control plants that were not exposed to freezing temperatures. When a rhizome is washed in the field after sampling, it is unknown if it is alive or dead. As such, not all of the Meyer and DALZ 0102 rhizomes sampled were living at the time of sampling. The LT_{50}, the temperature killing 50% of the grasses, was not calculated because the percentage of living rhizomes and nodes from several sampling dates was low.

Data are presented on individual sampling dates, as there was a sampling time x cultivar interaction. On 24 Feb. 2006, the greatest decrease in rhizome survival occurred between -13 and -15 C in both Meyer and DALZ 0102 (Table 2-1). Meyer exhibited better rhizome survival at -7 and -9 C, and had more living nodes than DALZ0102 at -9 C. Meyer exhibited some rhizome survival to -17 C, whereas no survival occurred below -13 C in DALZ 0102. Both grasses seemed to have lost some hardiness when sampled 1 week later on 1 March 2006 (Table 2-2). Meyer was superior to DALZ 0102 for all measured variables except root weight at -7 C; otherwise, responses were similar between cultivars. No survival occurred < -15 C in either cultivar.

DALZ 0102 had more shoots and biomass following freezing at -3 and -7 C when sampled on 7 Dec. 2006 (Table 2-3). As temperatures dropped, Meyer and DALZ 0102 responded similarly, and both cultivars survived at -19 C.

Meyer performed better following freezing treatments after sampling on 15 Feb. 2007, with a higher percentage of living rhizomes and nodes, especially when the temperature dropped below -13 C (Table 2-4). Meyer had 47.5% of living rhizomes at -19 C, whereas 12.5% of rhizomes survived in DALZ 0102. The number of shoots in Meyer was nine times higher than DALZ 0102 after freezing at -15 C; leaf and root dry weight were also higher in Meyer than in DALZ 0102. Better freezing tolerance of Meyer than DALZ 0102 on 15 Feb. 2007 may have been because of the lower temperatures occurring between 12 Jan. to 3 Feb. 2007 compared to the same period in 2006 (Fig. 2-1).

Frost was first observed on 14 Oct. 2006. The coldest period was between 3 Jan. to 19 Feb. 2007, with the lowest maximum air temperature of -12.8 C on 14 Feb. 2007 and lowest minimum of -18.2 C on 15 Feb. 2007 (Fig. 2-2). A quick decrease in temperature occurred from 5 to 15 April 2007 (Fig. 2-2). On 9 May 2007, spring green-up ratings taken in the NTEP zoysiagrass study in Manhattan indicated that Meyer had a significantly ($P = 0.01$) higher green up rating (7.3 on a 1 to 9 scale) than DALZ 0102 (5.0). This supports the results from 15 Feb. 2007 indicating that the freezing tolerance of DALZ 0102 is inferior to that of Meyer.

My results are supportive of those reported by Patton and Reciher (2007) who found that artificially acclimated Meyer was slightly hardier than DALZ 0102. The lowest temperature that Meyer had living tissue was -17 to -19 C in our experiment, consistent with results from a Missouri study in which shoot regrowth occurred at -18 C (Dunn et al., 1999). In contrast to Dunn et al. (1999), I observed a correlation between shoot numbers and shoot and root biomass on all sampling dates ($P \leq 0.0001$). Temperature was positively related to percentage of living rhizomes and nodes ($r = 0.44$ to 0.72, $P \leq 0.0001$) on all sampling dates except 15 Feb. 2007. Temperature was also positively correlated with all parameters in recovery growth on the four sampling dates ($r = 0.25$ to 0.72, $P = 0.0001$ to 0.04).

Should it be released, DALZ 0102 zoysiagrass would provide an alternative to Meyer that has a rapid establishment rate. Its primary areas of use would be home lawns, and golf course fairways, tees and roughs. However, the use of DALZ 0102 in the northernmost portion of the transition zone may have to be cautioned due to a slightly inferior freezing tolerance compared to Meyer zoysiagrass.

References

Anderson, J.A., M.P. Kenna, and C.M. Taliaferro. 1988. Cold hardiness of ‘Midiron’ and ‘Tifgreen’ bermudagrass. HortSci. 23:748-750.

Anderson, J.A., C.M. Taliaferro, and D.L. Martin. 1993. Evaluating freeze tolerance of bermudagrass in a controlled environment. HortSci. 28:955.

Chalmers, D.R., and R.E. Schmidt.1979. Bermudagrass survival as influenced by deacclimation, low temperatures, and dormancy. Agron. J. 71:947-949.

Dunn, J. H., S. S. Bughara, M. R. Warmund, and B. F. Fresenburg. 1999. Low temperature tolerance of zoysiagrass. HortSci. 34:96-99.

Dunn, J.H., and C.J. Nelson. 1974. Chemical changes occurring in three bermudagrass turf cultivars in relation to cold hardiness. Agron. J. 66:28-31.

Ebdon, J.S., R.A. Gagne, and R.C. Manley. 2002. Comparative cold tolerance in diverse turf quality genotypes of perennial ryegrass. HortSci. 37:826-830.

Fry, J., and P. Dernoeden. 1987. Growth of zoysiagrass from vegetative plugs in response to fertilizers. J. Amer. Soc. Hort. Sci. 112:286-289.

Fry, J., and B. Huang. 2004. Applied Turfgrass Science and Physiology. John Wiley & Sons, Hoboken, NJ.

Fry, J., N.S. Lang, R.G.P. Clifton, and F.P. Maier. 1993. Freezing tolerance and carbohydrate content of low-temperature acclimated and nonacclimated centipedegrass. Crop Sci. 33:1051-1055.

Grau, F.V., and A.M. Radko. 1951. Meyer (Z-52) zoysiagrass. USGA Journal and Turf Management 4:30-31.

Grau, F.V. 1952. Report on two improved turf grasses. USGA Journal and Turf Management 5:31-32.

Halsey, H. R. 1956. The zoysia lawn grasses. The National Horticultural Magazine 5:152-161.

Maier, F.P., N.S. Lang, and J.D. Fry. 1994. Freezing tolerance of three St. Augustinegrass cultivars as affected by stolon carbohydrate and water content. J. Amer. Soc. Hort. Sci. 119:473-476.

Morris, K. 1996. National zoysiagrass test: 1991. Final report. NTEP 96-15. USDA-ARS, Beltsville, MD.

Morris, K. 2004. National zoysiagrass test: 2002. Progress Report. NTEP 04-2. USD-ARS, Beltsville, MD.

Morris, K. 2005. National zoysiagrass test: 2002. Progress Report. NTEP 05-6. USD-ARS, Beltsville, MD.

Morris, K. 2006. National zoysiagrass test: 2002. Progress Report. NTEP 06-7. USD-ARS, Beltsville, MD.

Morris, K. 2007. National zoysiagrass test: 2002. Progress Report. NTEP 07-8. USD-ARS, Beltsville, MD.

Patton, A.J., J.J. Volenec, and Z.J. Reicher. 2007. Stolon growth and dry matter partitioning explain differences in zoysiagrass establishment rates. Crop Sci. 47:1237-1245.

Patton, A.J., and Z.J. Reicher. 2007. Zoysiagrass species and genotypes differ in their winter injury and freeze tolerance. Crop Sci. 47:1619-1627.

Qian, Y., J. Fry, and W. Upham. 1997. Rooting and drought avoidance of warm-season turfgrasses and tall fescue in Kansas. Crop Sci. 37:905-910.

Rajashekar, C., D. Tao, and P.H. Li. 1983. Freezing resistance and cold acclimation in turfgrass. HortSci. 18:91-93.

Richardson, M., and J. Boyd. 2001. Establishing *Zoysia japonica* from sprigs: effects of topdressing and nitrogen fertility. HortSci. 36:377-379.

Warmund, M.R., R. Fuller, and J.H. Dunn. 1998. Survival and recovery of ‘Meyer’ zoysiagrass rhizomes after extracellular freezing. J. Amer. Soc. Hort. Scci. 123:821-825.

Zontek, S. 1983. The St. Louis Solution – zoysiagrass for fairways. USGA Green Section Record. 21: 1-5.

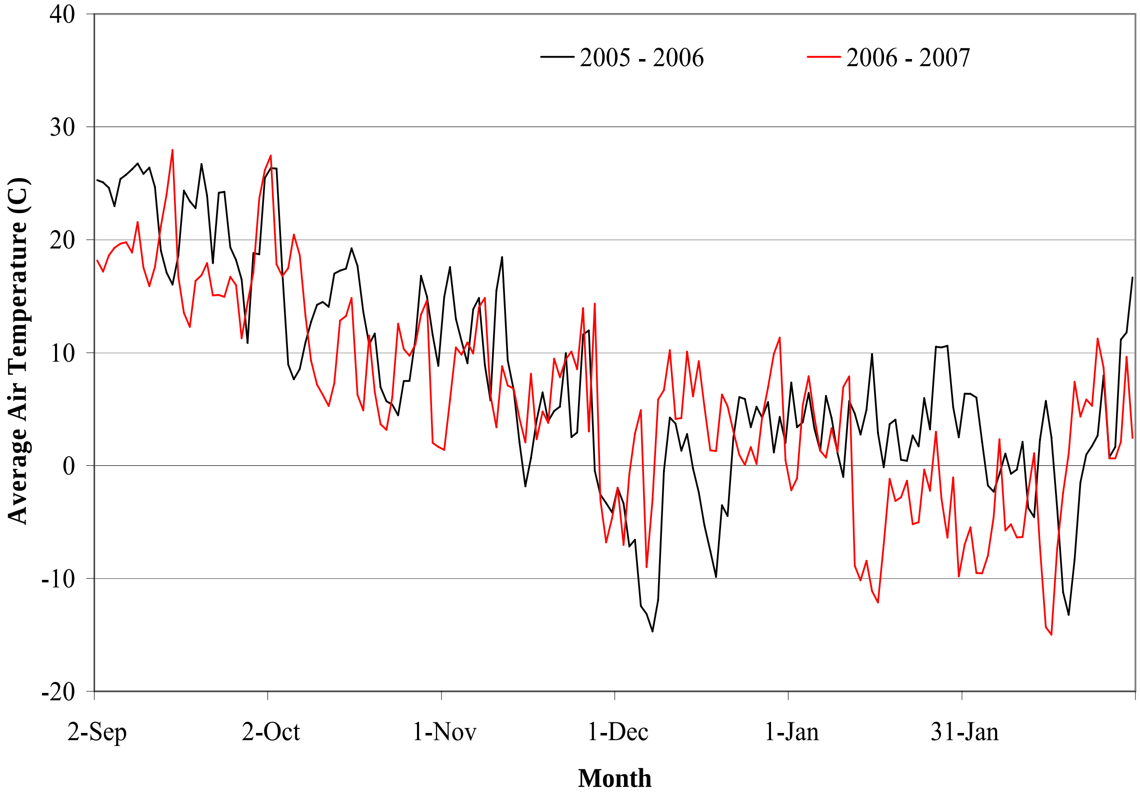


Figure 2-1. Daily average air temperatures from 2 Sept. to 1 March during study periods in 2005 - 2006 and 2006 - 2007.

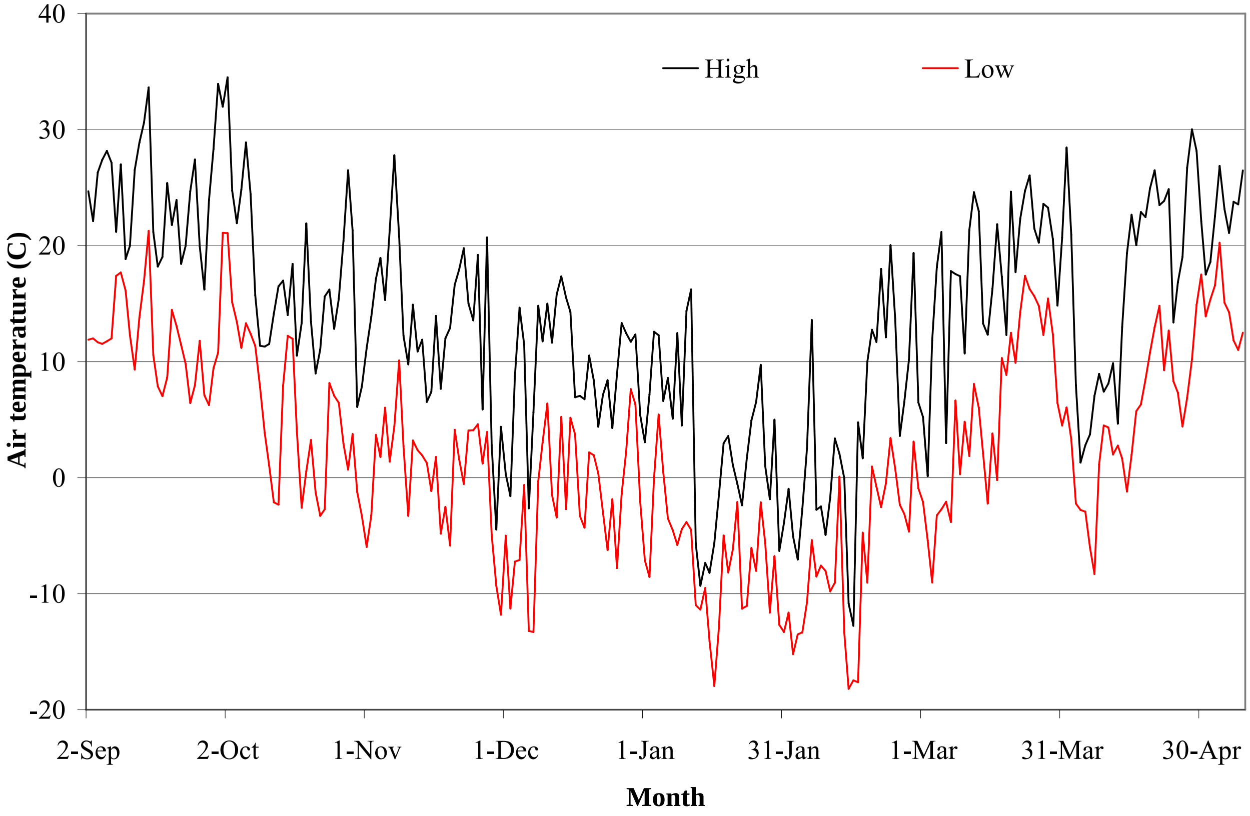


Figure 2-2. Daily air temperature from 2 Sept. to 9 May during study periods in 2006 - 2007.

Table 2-1. Evaluation of freezing tolerance and recovery growth of Meyer and DALZ 0102 zoysiagrass eight weeks after sampling from the field and exposing to freezing temperatures on 24 Feb. 2006.

Treatment	Living Rhizomes (%)		Living Nodes (%)		Shoots (no. pot)		Leaf Weight (mg/pot)		Root Weight (mg/pot)	
	Meyer	DALZ	Meyer	DALZ	Meyer	DALZ	Meyer	DALZ	Meyer	DALZ
Control[†]	62.5 a*	43.8 a	28.0 a	26.3 a	5.0 a	6.8 a	19.6 a	34.1 a	11.6 a	19.8 a
-3 C	68.8 a	37.5 a	26.5 a	22.8 a	5.0 a	7.5 a	25.0 a	46.2 a	10.0 a	25.1 a
-5 C	87.5 a	37.5 a	35.8 a	16.5 a	8.0 a	6.3 a	50.7 a	35.7 a	20.1 a	16.3 a
-7 C	81.3 a	43.8 b	33.5 a	27.5 a	8.5 a	6.0 a	59.9 a	30.0 a	25.6 a	15.3 a
-9 C	56.3 a	18.8 b	35.0 a	14.3 b	6.8 a	2.8 a	42.2 a	17.8 a	33.4 a	22.5 a
-11 C	31.3 a	31.3 a	11.8 a	10.3 a	3.0 a	1.8 a	16.2 a	9.2 a	9.1 a	4.7 a
-13 C	50.0 a	31.3 a	9.8 a	10.8 a	4.5 a	3.0 a	18.0 a	16.2 a	10.8 a	5.1 a
-15 C	6.3 a	0.0 a	1.8 a	0.0 a	0.3 a	0.0 a	0.5 a	0.0 a	0.2 a	0.0 a
-17 C	12.5 a	0.0 a	3.8 a	0.0 a	0.5 a	0.0 a	2.1 a	0.0 a	0.0 a	0.0 a
-19 C	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
-21 C	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
-23 C	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a

*Means followed by the same letter treated at the same temperature on each measurement between Meyer and DALZ 0102 are not significantly different at $P \leq 0.05$.

[†] Rhizomes exposed to no freezing temperatures.

[‡] Four rhizomes, each with ~ 4 nodes, were treated as one replication in each temperature.

Table 2-2. Evaluation of freezing tolerance and recovery growth of Meyer and DALZ 0102 zoysiagrass eight weeks after sampling from the field and exposing to freezing temperatures on 1 March 2006.

Treatment	Living Rhizomes (%)		Living Nodes (%)		Shoots (no. pot)		Leaf Weight (mg/pot)		Root Weight (mg/pot)	
	Meyer	DALZ	Meyer	DALZ	Meyer	DALZ	Meyer	DALZ	Meyer	DALZ
Control[†]	60.0 a*	37.5 a	41.5 a	24.5 a	8.5 a	8.3 a	49.0 a	42.7 a	25.2 a	19.8 a
-3 C	68.8 a	81.3 a	35.0 a	47.3 a	9.0 a	8.8 a	47.1 a	40.2 a	25.8 a	19.5 a
-5 C	68.8 a	50.0 a	45.3 a	34.8 a	8.8 a	5.8 a	29.4 a	21.5 a	19.1 a	9.8 a
-7 C	37.5 a	12.5 b	30.0 a	5.0 b	7.0 a	0.8 b	26.7 a	3.0 b	12.2 a	1.1 a
-9 C	81.3 a	41.7 a	38.5 a	19.3 a	8.0 a	4.7 a	40.5 a	19.0 a	20.4 a	13.3 a
-11 C	33.3 a	8.3 a	18.3 a	2.7 a	4.0 a	0.3 a	11.0 a	0.7 a	4.6 a	0.4 a
-13 C	50.0 a	8.3 b	23.3 a	4.3 a	4.7 a	0.7 a	15.8 a	0.7 a	4.9 a	0.4 a
-15 C	8.3 a	6.7 a	2.0 a	1.7 a	0.7 a	0.3 a	1.7 a	0.7 a	0.0 a	0.3 a
-17 C	0.0 a	0.0 a	0.0 a	0.0 a	0.3 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
-19 C	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
-21 C	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
-23 C	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a

*Means followed by the same letter treated at the same temperature on each measurement between Meyer and DALZ 0102 are not significantly different at $P \leq 0.05$.

[†] Rhizomes exposed to no freezing temperatures.

[‡] Four rhizomes, each with ~ 4 nodes, were treated as one replication in each temperature.

Table 2-3. Evaluation of freezing tolerance and recovery growth of Meyer and DALZ 0102 zoysiagrass eight weeks after sampling from the field and exposing to freezing temperatures on 7 Dec. 2006.

Treatment	Living Rhizomes (%)		Living Nodes (%)		Shoots (no. pot)		Leaf Weight (mg/pot)		Root Weight (mg/pot)	
	Meyer	DALZ	Meyer	DALZ	Meyer	DALZ	Meyer	DALZ	Meyer	DALZ
Control[†]	45.0 a*	50.0 a	16.2 a	22.5 a	11.0 b	22.8 a	56.1 b	192.2 a	21.2 a	97.6 a
-3 C	32.5 a	52.5 a	12.9 a	25.7 a	8.0 b	23.8 a	48.2 b	200.0 a	23.8 b	109.7 a
-5 C	12.5 a	40.0 a	5.3 a	16.6 a	3.0 a	14.3 a	11.1 a	98.8 a	11.1 a	61.0 a
-7 C	20.0 a	40.0 a	6.1 a	17.4 a	2.5 b	11.5 a	5.3 a	75.8 a	1.3 b	46.5 a
-9 C	27.5 a	37.5 a	11.8 a	18.0 a	8.0 a	18.5 a	34.1 a	124.5 a	18.2 a	78.5 a
-11 C	35.0 a	50.0 a	14.7 a	17.6 a	9.0 a	13.3 a	46.1 a	78.2 a	18.6 a	47.9 a
-13 C	27.5 a	23.3 a	10.0 a	9.7 a	5.8 a	5.0 a	17.8 a	24.5 a	12.1 a	14.9 a
-15 C	20.0 a	17.5 a	7.7 a	7.5 a	10.5 a	5.3 a	34.4 a	36.0 a	18.5 a	25.5 a
-17 C	20.0 a	2.5 a	5.4 a	0.7 a	2.5 a	0.3 a	5.4 a	0.9 a	2.5 a	0.1 a
-19 C	15.0 a	15.0 a	5.2 a	3.0 a	3.0 a	1.8 a	12.0 a	7.63 a	6.1 a	5.1 a

*Means followed by the same letter treated at the same temperature on each measurement between Meyer and DALZ 0102 are not significantly different at $P \leq 0.05$.

[†] Rhizomes exposed to no freezing temperatures.

[‡] Ten rhizomes, each with ~ 4 nodes, were treated as one replication in each temperature.

Table 2-4. Evaluation of freezing tolerance and recovery growth of Meyer and DALZ 0102 zoysiagrass eight weeks after sampling from the field and exposing to freezing temperatures on 15 Feb. 2007.

Treatment	Living Rhizomes (%)		Living Nodes (%)		Shoots (no. pot)		Leaf Weight (mg/pot)		Root Weight (mg/pot)	
	Meyer	DALZ	Meyer	DALZ	Meyer	DALZ	Meyer	DALZ	Meyer	DALZ
Control†	35.0 a*	17.5 a	14.8 a	12.5 a	16.8 a	13.3 a	144.7 a	91.8 a	26.2 a	27.1 a
-3 C	62.5 a	15.0 b	28.5 a	8.5 b	28.3 a	14.3 a	194.8 a	131.3 a	53.2 a	27.4 a
-5 C	52.5 a	20.0 b	24.3 a	7.5 b	19.5 a	10.8 a	156.6 a	85.2 a	51.4 a	30.1 a
-7 C	62.5 a	17.5 b	32.0 a	6.0 b	26.5 a	11.0 b	240.7 a	117.0 a	72.7 a	27.3 a
-9 C	40.0 a	20.0 a	19.0 a	9.0 a	15.0 a	11.3 a	110.2 a	118.5 a	36.9 a	33.1 a
-11 C	40.0 a	17.5 b	18.0 a	10.5 a	15.5 a	12.0 a	116.0 a	111.3 a	40.0 a	25.3 a
-13 C	52.5 a	12.5 b	21.0 a	7.5 a	14.0 a	6.8 a	100.5 a	49.5 a	28.7 a	16.3 a
-15 C	57.5 a	17.5 b	26.0 a	5.5 b	24.3 a	8.8 b	150.6 a	93.7 a	38.7 a	33.9 a
-17 C	52.5 a	7.5 b	19.0 a	2.8 b	18.8 a	2.0 b	107.2 a	8.0 b	33.5 a	3.6 b
-19 C	47.5 a	12.5 b	18.3 a	5.3 b	19.0 a	7.3 b	147.6 a	55.2 a	40.0 a	10.5 a

*Means followed by the same letter treated at the same temperature on each measurement between Meyer and DALZ 0102 are not significantly different at $P \leq 0.05$.

†Rhizomes exposed to no freezing temperatures.

‡Ten rhizomes, each with ~ 4 nodes, were treated as one replication in each temperature.

CHAPTER 3 - Changes in Membrane Polar Lipids in Meyer and Cavalier Zoysiagrass during Cold Acclimation

Zoysiagrass (*Zoysia spp.*) is a common turfgrass for golf fairways and tees in southern and transition-zone climates because of its good heat and drought tolerance and low requirement for maintenance. ‘Meyer’ (*Z. japonica* Steud.) has been the most common commercial cultivar used in the transition zone since its release in 1952 because of its relatively fine leaf texture compared to common *Z. japonica* and excellent freezing tolerance. As good as Meyer is, it does have limitations, including a comparatively coarse leaf texture compared to cultivars of *Z. matrella* (L.) Merr. and slow establishment and recuperative rates (Fry and Dernoeden, 1987). Several high quality cultivars, including ‘Cavalier’ (*Z. matrella*) (Engelke et al., 2002a), ‘Diamond’ (*Z. matrella*) (Engelke et al., 2002b), and ‘Palisades’ (*Z. japonica*) (Engelke et al., 2002c), have been developed and released by researchers at Texas A & M; however, poor freezing tolerance has limited their use in the transition zone (Morris, 1996 and 2001; Patton and Reicher, 2007).

Freezing injury results from ice crystals forming inside cells (intracellular freezing) or between cells (extracellular freezing). Intracellular freezing commonly occurs during a rapid drop in temperature in turf that is not properly hardened. During extracellular freezing, ice crystals draw water from the cytoplasm, causing cell dehydration. Thus, extracellular freezing is a type of dehydration, similar to that caused by drought or salinity. More information is needed regarding physiological contributors to freezing tolerance in *Zoysia* spp. to assist in selection and development of high quality, cold-tolerant cultivars.

Membranes are the primary sites of freezing injury in plants (Steponkus and Wiest, 1978). Phospholipids are the principal lipids in most cellular membranes, with fatty acids esterified to two of the carbons of the glycerol and the third carbon occupied by a phosphate group (Fig. 3-1). Based on the type of phosphate group, phospholipids can be classified into phosphatidylcholines (PC), phosphatidylethanolamines (PE), phosphatidylglycerols (PG), phosphatidylinositols (PI), phosphatidylserines (PS), and phosphatidic acids (PA). Galactolipids

(Fig. 3-2), monogalactosyl diacylglycerol (MGDG) and digalactosyl diacylglycerol (DGDG), are the major lipids (80%) in chloroplasts. Galactolipids have similar structure as phospholipids with the phosphate group replaced by the sugar galactose. Membrane fatty acids can be classified as saturated and unsaturated. Unsaturated fatty acids have bonds between adjacent carbon atoms that help maintain membrane flexibility. Phospholipids and galactolipids are polar lipids that have both hydrophilic (phosphate group or galactose) and hydrophobic (fatty acids) properties.

Under normal conditions, membrane lipids form a bilayer structure, referred to as the Lα phase (Fig. 3-3). During freeze-induced dehydration (freezing injury), plasma membrane and chloroplast may form the hexagonal II phase (H_{II}), which compromises membrane integrity and cell function (Fig. 3-3) (Cullis and De Kruijef, 1979; Uemura and Steponkus 1998 and 1999). Thus, during cold acclimation, changes in the lipid composition and fatty acid saturation take place to preserve the membrane bilayer structure, fluidity, and function (Alberdi and Corcuera, 1991). Research has shown polar lipids change under stress conditions, such as drought, oxidative assaults, and extreme temperatures (Selstam and Öquist, 1985; Toivonen et al., 1992; Styer et al., 1996; Li et al., 2004; Wang, 2005). Higher concentrations of polar lipids were observed in woody evergreen leaves [*Nothofagus dombeyi* (Mirb.) Oerst.] grown in higher altitude (LT_{50} = -10.2 C) than in lowland (LT_{50} = -5.0 C) (Alberdi et al., 1990). Furthermore, dominant lipids in *N. dombeyi*, PG, DGDG, and MGDG, increased 200 – 400% during cold acclimation. Welti et al. (2002) observed higher PC and lower PA levels in *Arabidopsis* with enhanced freezing tolerance after cold acclimation at 4 C for 3 days or exposure to -8 C. Cyril et al. (2001) and Cyril et al. (2002) reported higher content of linolenic acid (C18:3), a tri-unsaturated fatty acid, in cold-resistant bermudagrass (*Cynodon dactylon* L.) and seashore paspalum (*Paspalum vaginatum* Swartz.) cultivars than in cold-sensitive cultivars after cold acclimation. However, relationships between membrane alteration and freezing tolerance were not observed in some plants (Senser and Beck, 1984; Uemura and Steponkus, 1994). Changes in lipids and their association with freezing tolerance in turfgrass, and specifically zoysiagrass, have not been measured.

My objective was to compare differences in levels of polar lipids classes during acclimation in rhizomes of Cavalier (less hardy *Z. matrella*) and Meyer (more hardy *Z. japonica*).

MATERIALS AND METHODS

Meyer zoysiagrass, sampled from Rocky Ford Turfgrass Research Center at Manhattan, KS, and Cavalier, kindly provided by Dr. Milt Engelke from Texas A & M, were propagated as plugs in containers (8 cm-diam., 20 cm-deep) holding a root zone mix of Metro-Mix 510 (Sungro Horticulture, Bellevue, WA). Grasses were established in a greenhouse with an average day and night temperature of 30/20 C in April, 2005 and 2006. Grasses were mowed once weekly at 2 cm and watered every 2 day and fertilized with Peat-Lite Special (20-10-20) (Scotts, Marysville, OH) to provide N at 10 kg ha^{-1} once every other week until the containers were transferred outside to a sand-filled tank (2.5-m diam., 60-cm deep) on 15 Sept. 2005 and 17 Aug. 2006, respectively, at the Rocky Ford Turfgrass Research Center, Manhattan, KS. Containers were set in the tank so that the soil level in containers was level with that of the surrounding sand. The sand-filled tank provided a buffer against soil temperature fluctuations and helped to create an environment more like grasses would experience when growing in the field. Air temperature was recorded using a weather station located 3 m north of the sand-filled tank from 1 Sept. to 9 Jan. in 2005 - 2006 and 2006 - 2007.

Grasses were sampled once monthly from October to January 2005 and 2006 to determine freezing tolerance and changes in lipid levels and composition in rhizomes. For freezing tolerance evaluation, 16 containers from each cultivar were placed in a freezing chamber at -3 C, ice crystals were spread on each canopy to prevent supercooling, and thermal sensors were installed at 2 cm below the soil surface in two randomly selected containers to monitor temperature. The next day, temperature was reduced 2 C/h. Four containers of Cavalier were removed from the freezing chamber at -3, -6, -9, and -12 C; four containers of Meyer were removed at -5, -9, -13, and -17 C; as Cavalier is less cold hardy than Meyer. After thawing slowly at 4 C overnight, grasses were placed in a greenhouse maintained at a 30/25 C day/night temperature with a 14 h photoperiod and supplemental lighting to provide 580 umol·m^{-2}·s^{-1} at the canopy level. Four containers of each cultivar served as controls and were not subjected to freezing. Percentage of regrowth in each container was evaluated visually after 6-weeks of recovery in the greenhouse. A non-linear regression of recovery growth vs. freezing temperature was generated with Microsoft Excel, from which the LT_{50} (the temperature killing 50% of grasses) was calculated.

Four containers of each cultivar were sampled at the same time as those evaluated for freezing tolerance to profile membrane lipids in rhizomes. The root zone mix was washed from rhizomes with water cooled at 4 C overnight, and roots and leaves were removed from rhizomes. Rhizomes were then immersed in liquid N_2 and stored in a -80 C freezer. Lipids were extracted from rhizomes following the method described by Welti et al. (2002). The frozen rhizomes were heated for 15 minutes at 75 C in a preheated test tube with 3 ml of isopropanol with 0.01% butylated hydroxytoluene. Tissue was then ground in a mortar with 1.5 ml of chloroform, and transferred back to the tube followed by adding 0.6 ml of water. The tube was shaken for 1 h before the lipid layer (upper layer) was transferred to a new tube. Tissue was re-extracted 5 times with chloroform/methanol (2:1) with 0.01% butylated hydroxytoluen with 30 min agitation each time. All the extractions were combined and washed with 1 ml of 1M KCl and 2 ml of H_2O after the final extraction. The remaining tissue was dried (105 C overnight) and weighted. A polar lipid profile was generated with electrospray ionization tandem mass spectrometry (ESI-ES/MS) at the Kansas Lipidomics Research Center, Manhattan, KS.

Lipid species within a class were separated by the number of carbon atoms and double bonds. The double bond indices (DBI) of each lipid class was calculated as DBI = sum of [(mol% of each lipid specie x N) / mol% of the class], where N is the number of double bond of the specie (Chen et al., 2006).

The experiment was set up as a completely randomized design with four replications. Lipid contents and LT_{50} were determined by analysis of variance with the PROC GLM procedure (SAS Institute, Cary, NC) and means were separated by least significant difference (LSD) at $P \leq 0.05$.

RESULTS AND DISCUSSION

Interactions between year and recovery growth and year and lipid contents occurred. Therefore, data were analyzed separately in each year.

Freezing Tolerance

Meyer had a lower LT_{50} than Cavalier on all sampling dates in both years (Fig. 3-4A and 3-5A). Cavalier had its lowest LT_{50} in November during its acclimation, one month earlier than that in Meyer. Cavalier that was not subjected to freezing treatment exhibited almost no regrowth by 2 Dec. 2005 in the first year and 9 Jan. 2007 in the second year. As such, an LT_{50} for Cavalier could not be calculated for these sampling dates. No LT_{50} was calculated for Meyer on 4 Jan.

2006 due to the severe freezing damage (data not shown). Higher freezing injury on Cavalier and Meyer in 2005 was likely due to longer freezing conditions in December, 2005 comparing to 2006 (Fig. 3-6). The poorer freezing tolerance in Cavalier relative to Meyer is consistent with previous reports. Patton and Reicher (2007) observed 17 to 45% of winter injury Cavalier in a field evaluation, significantly higher than Meyer (0%). In a controlled freezing experiment, the lowest temperature with shoot regrowth was -18 C in Meyer, but Cavalier was killed at -10 C (Dunn et al., 1999).

Changes in Membrane Lipids

The most abundant lipid groups in zoysiagrass rhizomes were DGDG, MGDG, PC, PE, and PA, which comprised 90% of the polar lipids (Fig. 3-4 and 3-5). No differences were observed in DGDG between Meyer and Cavalier on any sampling date in 2005 (Fig. 3-4B). Cavalier had a lower MGDG level than Meyer on 2 Dec. 2005, which resulted in a higher ratio of DGDG to MGDG (Fig. 3-4C and D). Digalactosyl diacylglycerol (DGDG) was higher in Meyer than in Cavalier on 2 Oct. 2006, but had no effect on the ratio of DGDG to MGDG (Fig. 3-5B and D).

Overall, the galactolipids, DGDG and MGDG, comprised 20 to 40% of the polar lipids in zoysiagrass rhizomes. Galactolipids are unique to chloroplast membranes. Detection of DGDG and MGDG in zoysiagrass rhizomes indicates that the rhizomes contained chloroplasts. It is common for stolons to contain chloroplasts, and in zoysiagrass, stolons often penetrate the soil surface, in effect becoming rhizomes. This likely explains the presence of these lipids in rhizome samples. Comparing to *Arabidopsis* leaves, zoysiagrass rhizomes had lower galactolipids (about 20%) and higher DGDG than MGDG (Welti et al., 2002; Chen et al., 2006) that is likely due to the organ difference. Higher DGDG levels were observed in frost-hardened pine seedlings (*Pinus silvestris*) than non-hardened (Selstam and Õquist, 1990). However, galactolipid levels were not correlated with LT_{50} for either zoysiagrass cultivar. It is possible that chloroplasts in the rhizomes were less active than those in leaves.

Phosphatidylcholine (PC) is known to stabilize the membrane bilayer; whereas, PE and PA have been associated with membrane injury resulting from the H_{II} phase (Culli and DeKrukjff, 1979; Verleij et al. 1982; Welti et al., 2002). Levels of PC in *Arabidopsis* leaves were highest when freezing tolerance was best (Welti et al., 2002). Accordingly, a higher PC level in

Meyer and a lower corresponding LT_{50} was observed on 2 Dec. 2005 (Fig. 3-4E). However, on 5 Nov., PC content was higher in Cavalier than in Meyer. It could be that a higher PC levels were measured when each of the grasses reached their maximum hardiness: November for Cavalier and December for Meyer. Therefore, it might be more critical to evaluate seasonal patterns of lipid composition, rather than composition on a particular sampling date. In 2006, however, no differences in PC occurred between cultivars (Fig. 3-5E).

Higher PA contents were reported in wild-type *Arabidopsis* with poorer freezing tolerance compared to a freezing-tolerant mutant (Welti et al., 2002). In this study, PA levels were higher in Cavalier compared to Meyer when it had suffered freezing injury on 2 Dec. 2005 (Fig. 3-4G). In 2006, Cavalier had a higher PA level than Meyer on 2 Oct. (Fig. 3-5G). This was surprising, for the average air temperature was >10 C prior to the 2 Oct. sampling date (Fig. 3-6). There were no differences in PA between the cultivars after this sampling date, including 9 Jan. 2007 when Cavalier again had suffered significant freezing injury (Fig. 3-5G). There was a general trend that the PA levels were higher in Cavalier than in Meyer on all sampling dates, except 5 Nov. 2005.

Greater temporal fluctuations in PA and PC were also observed in Cavalier compared to Meyer. For example, from 5 Nov. 2005, when Cavalier had reached its peak hardiness, to 2 December, when freezing injury was substantial, PC content decreased 33% compared to an 11% increase in Meyer (Fig. 3-4E). Phosphatidic acid (PA) levels in Cavalier on 2 Dec. 2005 were 280% higher than on 5 Nov.; whereas, PA increased only 60% in Meyer. Similarly, the ratio of PC to (PE + PA) decreased 60% in Cavalier during the same period, compared to a 21% reduction in Meyer. However, no similar trend was observed in 2006.

The number of lipid species varied from 10 to 64 within the 5 major lipid classes, with the highest number in the PC group (data not shown). Double bond indices were calculated individually for each class having unique functionalities. On 5 Nov. 2005, Meyer had a higher DBI in DGDG, MGDG, and the ratio of DGDG to MGDG (Table 3-1). Although Cavalier had a lower DBI in PC on the same sampling date, the ratio of PC to (PE + PA) was not affected (Table 3-1). More variations were observed in the DBIs in 2006 - 2007; however, no consistent trend was observed. Higher levels of di-unsaturated fatty acids in PC and PE were observed in winter rye (*Secale cereale* L. cv Ogle) with a LT_{50} of - 6 C than in spring oat (*Avena sativa* L. cv

Ogle) with an LT_{50} of -3 C (Uemura and Steponkus, 1994). There was no clear relationship between DBI and cold acclimation or the zoysiagrass cultivars.

To summarize, five major polar lipid groups were identified in Meyer and Cavalier zoysiagrass rhizomes. More alterations were observed in PC, PE, and PA contents during acclimation than galactolipids (DGDG and MGDG) in both cultivars. Higher numerical levels of PA were observed in Cavalier compared Meyer, which is more freezing tolerant than Cavalier. Changes in lipid composition in these cultivars during cold acclimation were variable during the two-year study period. Comparing the two cultivars, phospholipids changed more gradually in Meyer than in Cavalier. Freezing tolerance was not related to DBI in either cultivar. It is clear that we do not fully understand the specific roles of lipid composition in acclimation and response to freezing stress.

References

Alberdi, M., J. Fernandez, R. Cristi, M. Romero, and D. Rios. 1990. Lipid changes in cold hardened leaves of *Nothofagus dombeyi*. Phytochemistry 29:2467-2471.

Alberdi, M., and L.J. Corcuera. 1991. Cold acclimation in plants. Phytochem. 10:3177-3184.

Anonym. 2007. Brain phospholipid composition in schizophrenia. Available at www.uams.edu/radiology/info/research/phospholipid/. (accessed 7 September, 2007).

Chen, J., J.J. Burke, Z. Xin, C. Xu, and J. Velten. 2006. Characterization of the *Arabidopsis* thermosensitive mutant atts02 reveals an important role for galactolipids in thermotolerance. Plant, Cell and Environ. 29:1437-1448.

Cyril, J. G. L. Powell, and W. V. Baird. 2001. Changes in membrane fatty acid composition during cold acclimation and characterization of fatty acid desaturase genes in bermudagrass. International Turfgrass Soc. Res. J. 9:259-267.

Cyril, J., G. L. Powell, R. R. Duncan, and W. V. Baird, W. V. 2002. Changes in membrane polar lipid fatty acids of seashore paspalum in response to low temperature exposure. Crop Science 42:2031-2037.

Cullis, P.R., and B. De Kruijff. 1979. Lipid polymorphism and the functional roles of lipids in biological membranes. Biochem. Biophys. Acta 559:399-420.

Dunn, J. H., S. S. Bughrara, M. R. Warmund, and B. F. Fresenburg. 1999. Low temperature tolerance of zoysiagrass. HortSci. 34:96-99.

Engelke, M., J. Reinert, P. Colbaugh, R. White, B. Ruemmele, K. Marcum, and S. Anderson. 2002a. Registration of 'Cavalier' zoysiagrass. Crop Sci. 52:302-303.

_ 2002b. Registration of 'Diamond' zoysiagrass. Crop Sci. 52:304-305.

_ 2002c. Registration of 'Palisades' zoysiagrass. Crop Sci. 52:305-306.

Fry, J., and P. Dernoeden. 1987. Growth of zoysigrass from vegetative plugs in response to fertilizers. J. Amer. Soc. HortSci. 112:286-289.

Li, W., M. Li, W. Zhang, R. Welti, and X. Wang. 2004. The plasma membrane-bound phospholipase-Dδ enhanceds freezing tolerance in *Arabidopsis thaliana*. Nat. Biotechnol. 22:427-433.

Morris, K. 1996. National zoysiagrass test: 1991. Final report. NTEP 96-15. USDA-ARS, Beltsville, MD.

Morris, K. 2001. National zoysiagrass test: 1996. Final report. NTEP 01-15. USDA-ARS, Beltsville, MD.

Patton, A. J., and Z. J. Reicher. 2007. Zoysiagrass species and genotypes differ in their winter injury and freeze tolerance. Crop Sci. 47:1619-1627.

Samala, S., J. Yan, and W. V. Baird. 1998. Changes in polar lipid fatty acid composition during cold acclimation in 'Midiron' and 'U3' bermudagrass. Crop Sci. 38:188-195.

Seddon, J.M., and R.H. Templer. 1995. Polymorphism of lipid-water systems. p. 97-160. *In* R. Lipowsky and E. Sackmann (ed.) Structure and Dynamics of membranes (Vol. 1). Elsevier Science & Technology Books. North-Holland.

Selstam, E., and G. Õouist. 1990. Galactolipid biosynthesis in chloroplasts of non-hardened and frost-hardened seedlings of Scots pine. Plant Physiol. Biochem. 28:477-483.

Senser, M., and E. Beck. 1984. Correlation of chloroplast ultrastructure and membrane lipid composition to the different degrees of frost resistance achieved in leaves of spinach, ivy, and spruce. J. Plant Physiol. 117:41-55.

Smith, H (ed.). 1977. The molecular biology of plant cells. Berkeley: University of California Press.

Steponkus, P.L., and S. C. Wiest. 1978. Plasma membrane alternations following cold acclimation and freezing. *In* Li and Sakai (ed.) Plant Cold Hardiness and Freezing Stress. Academic Press. Inc. Oval Road, London.

Styer, E.H., B.D. Whitake, S.L. Kitto, and D.G. Hoover. 1996. Lipid composition of microsomes from heat-stressed cell suspension cultures. Phytochemistry 41:187-191.

Toivonen, L., S. Laakso, and H. Rosenqvist. 1992. The effect of temperature on growth, indole alkaloid accumulation, and lipid compostion of *Catharanthus roseus* cell suspension cultures. Plant Cell Reports 11:390-394.

Uemua, M., and P.L. Steponkus. 1994. A contrast of the plasma membrane lipid composition of oat and rye leaves in relation to freezing tolerance. Plant Physiol. 104:479-496.

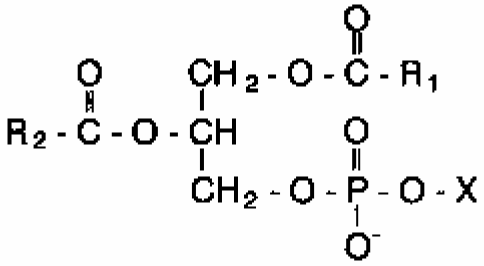
Uemura, M., and P.L. Steponkus. 1998. Effect of cold acclimation on membrane lipid composition and freeze-induced membrane destabilization. p.171-179. *In* Li and Chen (ed.) Plant Cold Hardiness. Plenum Press, New York.

Uemura, M., and P.L. Steponkus. 1999. Cold acclimation in plants: relationship between lipid composition and the cryostability of the plasma membrane. J. Plant Res. 112:245-254.

Verleij, A.J., R. DeMaagd, J. Leunissen-Bijvelt, and B. Dekruijff. 1982. Divalent cations and chloropromazine can induce non-bilayer structures in phosphatidic acid-containing model membranes. Biochim. Biophys. Acta 684:255-262.

Wang, X. 2005. Regulatory functions of phospholipase D and phosphatidic acid in plant growth, development, and stress responses. Plant Physiol. 139:566-573.

Welti, R., Li, W., Li, M., Sang, Y., Biesiada, H., Zhou, H., Rajashekar, C., Williams, T., and Wang, X. 2002. Profiling membrane lipids in plant stress responses. J. Biol. Chem. 277: 31994-32002.



$-X$	PL
$-H$	PA
$-CH_2CH_2NH_3^+$	PE
$-CH_2CH_2N(CH_3)_3^+$	PC
$-CH_2CH(OH)CH_2OH$	PG

Figure 3-1. Sample structure of phospholipids. R1 and R2 are fatty acids (saturated or unsaturated); X is the head-group for the phospholipids (Anonymous, 2007).

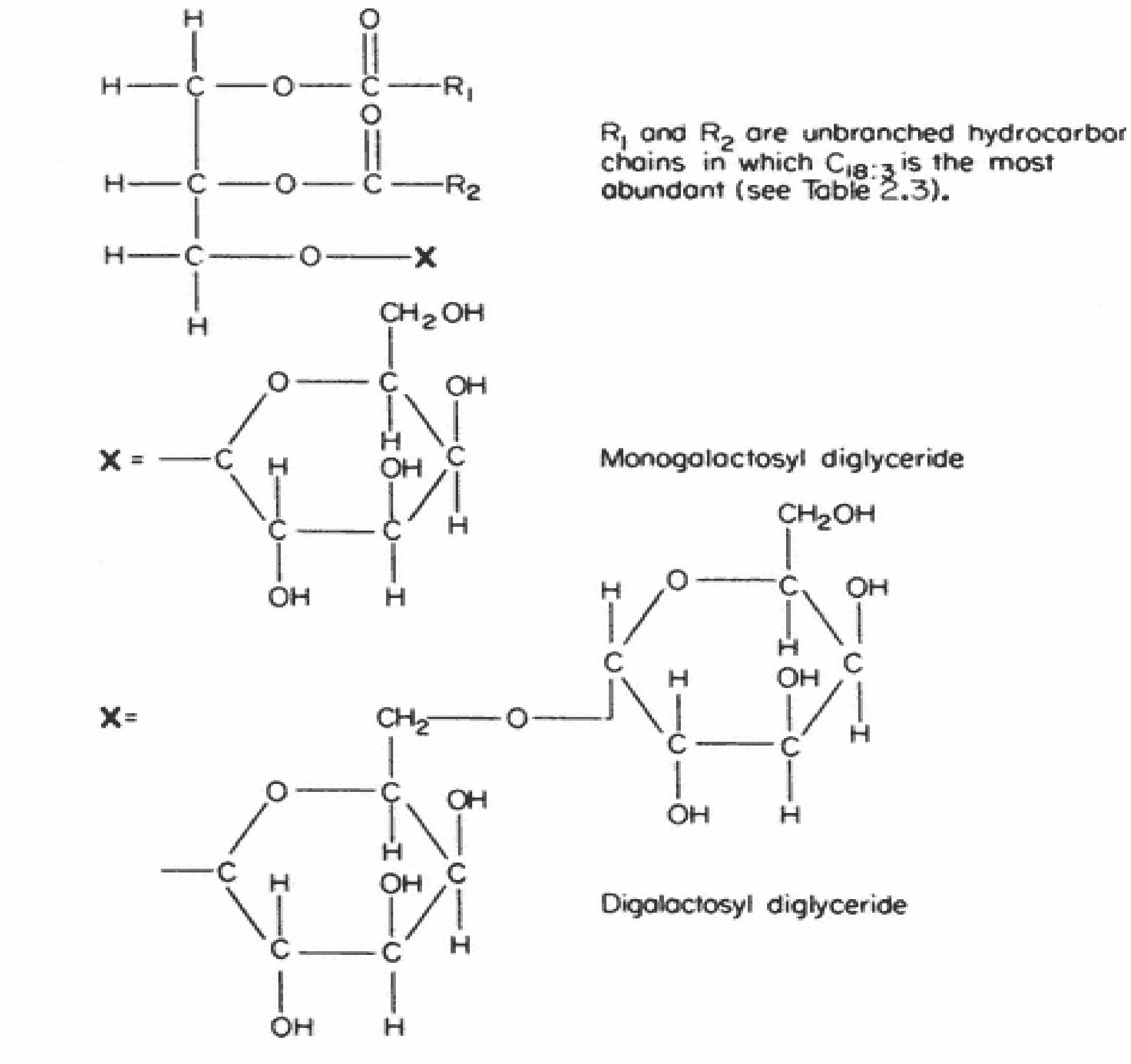


Figure 3-2**.** Structure of galactolipids. R1 and R2 are fatty acids (saturated or unsaturated); X is the head-group of galactose (Smith, 1977).

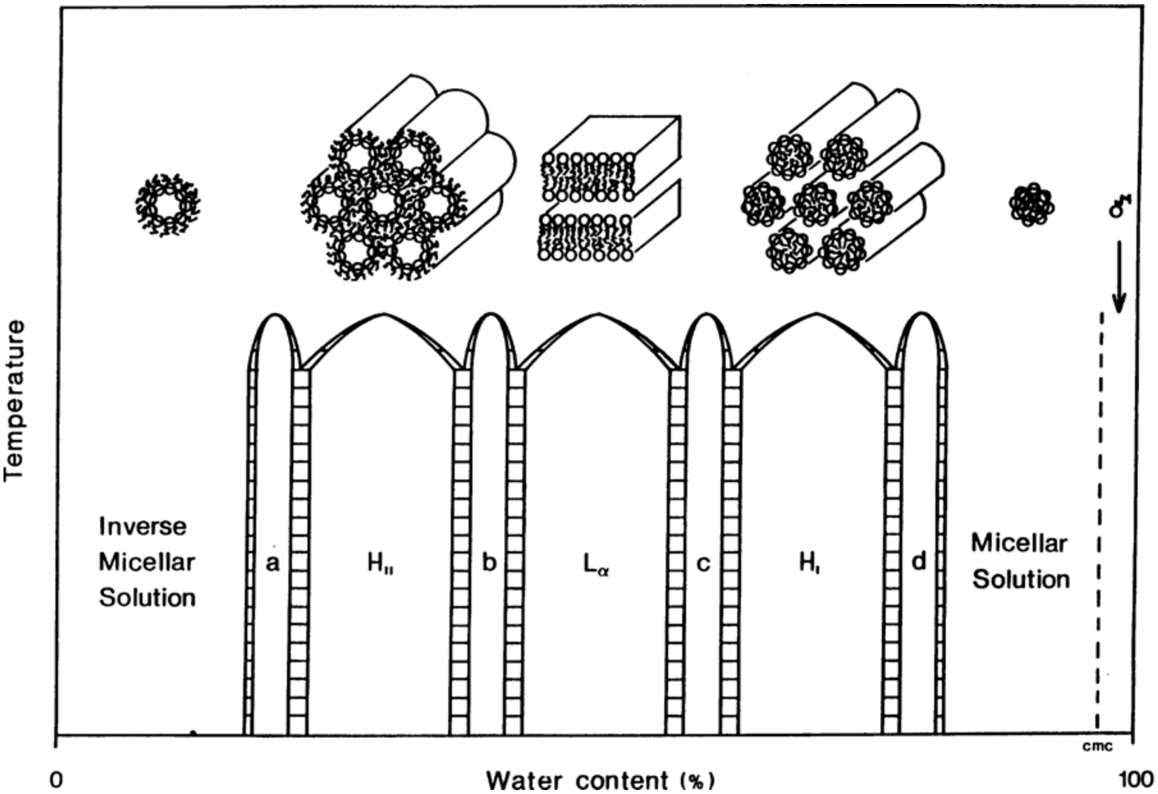


Figure 3-3. Hypothetical lipid/water binary phase diagram, where the transitions are driven by varying the water content (Seddon and Templer, 1995). Lα, normal fluid lamella phase; H$_I$, hexagonal phase I; H$_{II}$, hexagonal phase II. Regions denoted a, b, c and d contain intermediate phases, many of which are cubic.

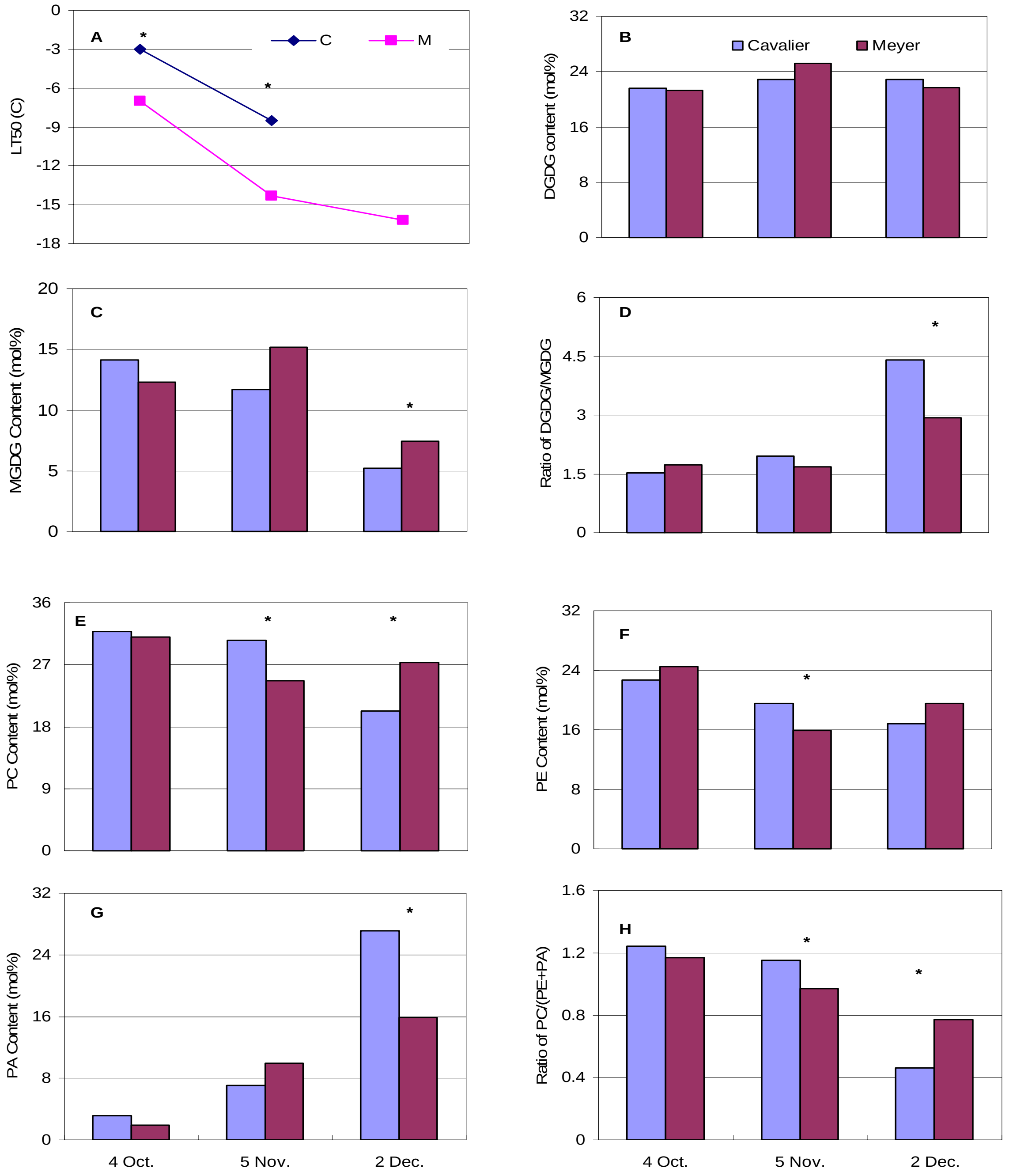


Figure 3-4. Changes in LT$_{50}$ (A), digalactosyl diacylglycerol (DGDG, B); monogalactosyl diacylglycerol (MGDG, C); phosphatidylcholine (PC, D); phosphatidylethanolamine (PE, F); phosphatidic acid (PA, G); and the ratio of PC/(PE + PA) in Cavalier and Meyer rhizomes in 2005. A * above means of LT$_{50}$ and lipid groups indicates that cultivars were different ($P \leq$ 0.05). The LT$_{50}$ was not determined for Cavalier on 2 Dec. due to significant winter injury.

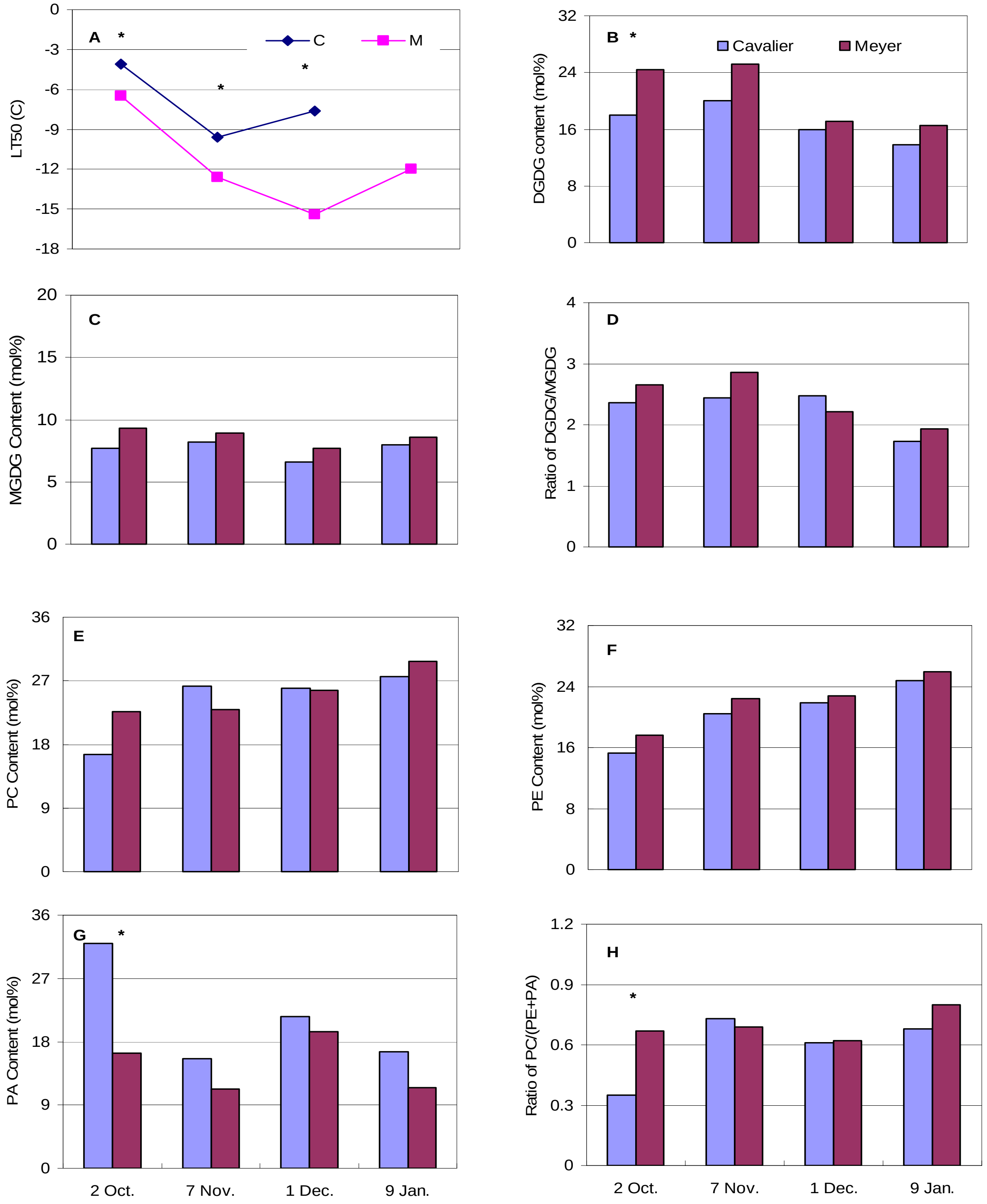


Figure 3-5. Changes in LT$_{50}$ (A), digalactosyl diacylglycerol (DGDG, B); monogalactosyl diacylglycerol (MGDG, C); phosphatidylcholine (PC, D); phosphatidylethanolamine (PE, F); phosphatidic acid (PA, G); and the ratio of PC/(PE + PA) in Cavalier and Meyer rhizomes in 2006-2007. A * above means of LT$_{50}$ and lipid groups indicates that cultivars were different ($P \leq 0.05$). The LT$_{50}$ was not determined for Cavalier on 9 Jan. 2007 due to significant winter injury.

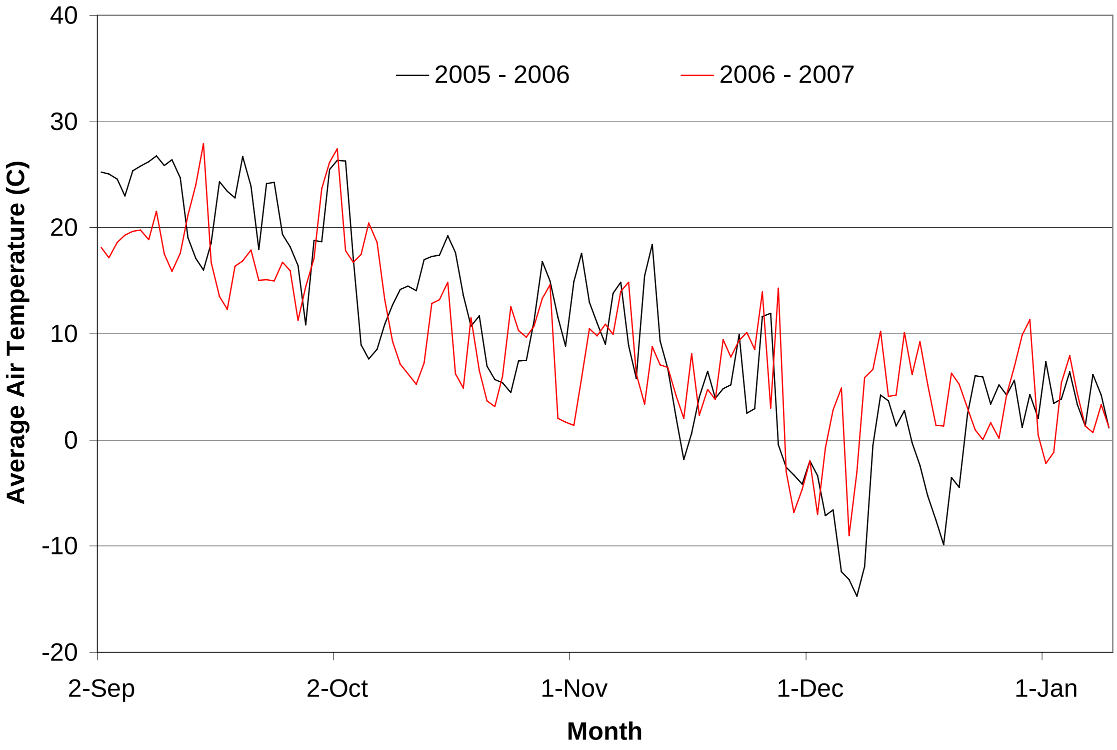


Figure 3-6. Average air temperatures (C) from 2 Sept. to 9 Jan. in the winter 2005 and 2006.

Table 3-1. Changes in the double bond indices (DBI) in major lipid groups in Meyer and Cavalier zoysiagrass rhizomes from 4 Oct. to 2 Dec. 2005.

Double bond indices (DBI)	4 Oct.		5 Nov.		2 Dec.	
	Cavalier	Meyer	Cavalier	Meyer	Cavalier	Meyer
Galactolipids[†]						
DGDG	3.34 a	3.35 a	3.18 b	3.73 a	3.24 a	3.40 a
MGDG	4.70 a	4.61 a	4.56 b	4.96 a	4.46 a	4.74 a
Ratio: DGDG to MGDG	0.71 a	0.73 a	0.70 b	0.75 a	0.73 a	0.72 a
Phospholipids[†]						
PC	2.83 a	2.92 a	2.83 b	2.95 a	2.87 a	2.90 a
PE	2.77 a	2.75 a	2.95 a	3.07 a	2.84 a	2.98 a
PA	3.52 a	3.11 a	2.83 a	3.06 a	2.95 a	2.92 a
Ratio: PC to (PE + PA)	0.46 a	0.51 a	0.49 a	0.48 a	0.50 a	0.49 a

* Means with the same letter following the cultivars in a row in a month are not significantly different at $P \leq 0.05$.

[†] DGDG, digalactosyl diacylglycerol; MGDG, monogalactosyl diacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PA, phosphatidic acid, PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine.

Table 3-2. Changes in the double bond indices (DBI) in major lipid groups in Meyer and Cavalier zoysiagrass rhizomes from 2 Oct. 2006 to 9 Jan. 2007.

Double bond indices (DBI)	2 Oct.		7 Nov.		1 Dec.		9 Jan.	
	Cavalier	Meyer	Cavalier	Meyer	Cavalier	Meyer	Cavalier	Meyer
Galactolipids								
DGDG	3.30 a	3.19 a	2.96 a	3.03 a	2.98 b	3.30 a	3.22 a	3.19 a
MGDG	4.86 a	4.52 b	4.29 a	4.55 a	4.27 b	4.83 a	4.66 b	4.94 a
Ratio: DGDG to MGDG	0.68 a	0.71 a	0.69 a	0.67 a	0.70 a	0.68 a	0.69 a	0.65 b
Phospholipids								
PC	2.62 a	2.67 a	2.73 a	2.87 a	2.73 a	2.92 a	2.93 a	3.01 a
PE	2.93 a	2.95 a	2.96 a	2.93 a	2.96 a	3.01 a	3.04 a	3.07 a
PA	2.76 a	2.83 a	2.82 b	2.98 a	2.81 a	3.05 a	2.97 a	3.04 a
Ratio: PC to (PE + PA)	0.46 a	0.46 a	0.47 a	0.49 a	0.47 a	0.48 a	0.49 a	0.50 a

* Means with the same letter following the cultivars in a row in a month are not significantly different at $P \leq 0.05$.

[†] DGDG, digalactosyl diacylglycerol; MGDG, monogalactosyl diacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PA, phosphatidic acid.

CHAPTER 4 - Changes in Abscisic Acid Content in Meyer and Cavalier Zoysiagrass during Cold Acclimation

Zoysiagrass (*Zoysia spp.*) is commonly used for golf course fairways and tees in southern and transition-zone climates because of its good heat and drought tolerance, and low maintenance requirement. Three zoysia species, *Z. japonica* Steud., *Z. matrella* (L.) Merr., and *Z. pacifica* (Goudsw.) M. Hotta & Kuroi (previously stated as *Z. tenuifolia*), have been used as turf among the 11 species identified (Engelke and Anderson, 2002), and grasses in the *Z. japonica* group are the most cold hardy (Halsey, 1956). 'Meyer' zoysia is an improved *Z. japonica* selected based on its intermediate leaf width between common *Z. japonica* and *Z. matrella,* and its excellent freezing tolerance (Grau and Radko, 1951; Grau, 1952). It has been the most commonly used cultivar in the transition zone since its release in 1952.

Cultivars of *Z. matrella*, including 'Cavalier' (Engelke et al., 2002a) and 'Diamond' (*Z. matrella*) (Engelke et al., 2002b) commonly receive higher turf quality scores than Meyer in National Turfgrass Evaluation Program (NTEP) evaluations, but they lack freezing resistance necessary for long-term survival in the transition zone (Morris, 1996 and 2001; Dunn et al., 1999). Currently, over 600 new zoysia progeny, most resulting from crosses between high quality *Z. matrella* cultivars and cold-hardy *Z. japonica* types, are under evaluation at the Rocky Ford Turfgrass Research Center at Manhattan, KS in an effort to produce a cold-hardy cultivar with better quality and, potentially, a faster spread and recovery rate than Meyer.

More information is needed regarding physiological contributors to freezing tolerance in *Zoysia* spp. to assist in development of high quality, cold-tolerant cultivars. Freezing injury can be caused by ice crystals forming inside cells (intracellular freezing) or between cells (extracellular freezing). Intracellular freezing commonly occurs during a rapid drop in temperature in turf that is not adequately hardened. During extracellular freezing, ice crystals draw water from the surface of the plasma membrane and from the cytoplasm. In another words, extracellular freezing is a type of dehydration, similar to that caused by drought or salinity.

Carbohydrates, lipids, proteins, and proline have been evaluated in various turfgrass species during acclimation, but their involvement in mitigating freezing stress is still not well understood (Fry et al., 1993; Thomas and James, 1993; Dionne et al., 2001; Cyril et al., 2002; Cai et al., 2004; Patton et al., 2007). Abscisic acid (ABA) is an important, naturally occurring plant hormone that has been shown to fluctuate in plants during exposure to drought, salinity, or temperature extremes. It is involved in plant growth regulation and also influences cell osmotic potential, stomata closure, protein synthesis, and gene expression (Lång et al., 1994; Ishitani et al., 1997; Welbaum et al., 1997; Li et al., 1998; Xiong et al., 2002; Wang and Huang, 2003; Khadri et al., 2007; Xu and Huang, 2007).

Rapid increases in ABA were observed in Kentucky bluegrass (*Poa pratensis* L.) and bentgrass (*Agrostis* spp.) during drought and heat stress, with less accumulation in tolerant cultivars compared to sensitive cultivars (Wang and Huang, 2003; DaCosta and Huang, 2007; Xu and Huang, 2007). Chen et al. (1982) reported a rapid increase in endogenous ABA level in cold-hardy potato leaves (*Solanum commersonii* Dun.) after the third day of acclimation at 2 C, which was followed by a peak in protein content; however, no such change was observed in cold-sensitive *S. tuberosum* L. Abscisic acid levels were correlated with chilling tolerance in maize seedlings (*Zea mays* L.), and chilling-sensitive genotypes had lower ABA contents (Janowiak et al., 2002; Janowiak et al., 2003).

My objective was to compare differences in rhizome levels of ABA during acclimation of Cavalier (cold-sensitive, *Z. matrella*) and Meyer (cold tolerant, *Z. japonica*) zoysiagrass.

MATERIALS AND METHODS

Meyer (sampled from Rocky Ford Turfgrass Research Center) and Cavalier (kindly provided by Dr. Milt Engelke from Texas A & M) were propagated as plugs in containers (8-cm diam., 20-cm deep) holding a root zone mix of Metro-Mix 510 (Sungro Horticulture, Bellevue, WA). Grasses were established in a greenhouse with an average day and night temperature at 30/20 C in April, 2005 and 2006. Grasses were mowed once weekly at 2 cm and watered every 2 days and fertilized with Peat-Lite Special (20-10-20) (Scotts, Marysville, OH) to provide N at 10 kg ha^{-1} once every other week until the containers were transferred outside to a sand-filled tank (2.5-m diam., 60-cm deep) on 15 Sept. 2005 and 17 Aug. 2006, respectively, at the Rocky Ford Turfgrass Research Center, Manhattan, KS. Containers were set in the tank so that the soil level in containers was level with that of the surrounding sand. The sand-filled tank provided a buffer against soil temperature fluctuations and helped to create an environment more like grasses would experience when growing in the field. Soil-encapsulated thermocouples (SET) sensors, constructed following the method of Ham and Senock (1992), were placed in contact with 2 randomly selected crowns and connected to a CR-10 datalogger (Campbell Scientific, Inc., Logan, UT) to monitor the soil surface temperatures. The sensors were used to record daily soil temperature at 1 h and downloaded from the datalogger once monthly from 29 Oct. to 9 Jan. in both winters.

Grasses were sampled once monthly from October to January 2005 and 2006 to determine freezing tolerance and changes in ABA levels in rhizomes. For freezing tolerance evaluation, 16 containers from each cultivar were placed in a freezing chamber at -3 C, ice crystals were spread on each canopy to prevent supercooling, and thermal sensors were installed at 2 cm below the soil surface in two randomly selected containers to monitor temperature. The next day, temperature was reduced 2 C/h. Four containers of Cavalier were removed from the freezing chamber at -3, -6, -9, and -12 C; four containers of Meyer were removed at -5, -9, -13, and -17 C; as Cavalier is more freezing sensitive compared to Meyer. After thawing slowly at 4 C overnight, grasses were placed in a greenhouse maintained at a 30/25 C day/night temperature with a 14 h photoperiod and supplemental lighting to provide 580 umol$\cdot$m$^{-2}\cdot$s^{-1} at the canopy level. Four containers of each cultivar served as controls and were not subjected to freezing. Percentage of regrowth in each container was evaluated visually after 6 weeks of recovery in the greenhouse. A non-linear regression of recovery growth vs. freezing temperature was generated with Microsoft Excel, from which the LT_{50} (the temperature killing 50% of grasses) was calculated.

Four containers of each cultivar were sampled at the same time as those evaluated for freezing tolerance to determine ABA levels in rhizomes. The root zone mix was washed from rhizomes with water cooled at 4 C overnight, and roots and leaves were removed from rhizomes. Rhizomes were then immersed in liquid N_2 immediately and stored in a -80 C freezer. Weight of frozen tissue was recorded before the extraction. Rhizomes were transferred to 2 mL centrifuge tubes with 0.5 ml of the solvent 1-propanol:H_2O:HCL (2:1:0.002 in volume) and 50 ng d_6-ABA (internal standard) and ground (Fast Prep, MP Biomedicals, Solon, OH) for 20 sec. After cooling on dry ice for 5 min, tissue was ground for another 20 sec. Following addition of 1 ml of dichloromethane, tubes were ground again for 20 sec. Two layers were generated after centrifuging the tubes at 13,000 g for 5 min. One ml of the bottom layer (mainly dichloromethane and propanol) was transferred to a glass vial and evaporated under a nitrogen flush. Abscisic acid content was analyzed with a HPLC-ESI-MS/MS system at the Donal Danforth Plant Science Center, St. Louis, Missouri.

The experiment was set up as a completely randomized design with four replications. Abscisic acid content and LT_{50} were determined by analysis of variance using PROC GLM (SAS Institute, Cary, NC) and means were separated by least significant difference (LSD) at $P \leq 0.05$. PROC CORR was applied to evaluate the relationship between ABA levels and LT_{50} in rhizomes.

RESULTS AND DISCUSSION

Freezing Tolerance

The minimum soil surface temperatures were similar in the winter 2005 and 2006, -13 C to -15 C (Fig. 4-1 and 4-2). Meyer had a lower LT_{50} than Cavalier on all sampling dates in both years (Fig. 4-3 and 4-4). Even Cavalier that was not subjected to freezing in the cold chamber exhibited severe freezing injury by 2 Dec. 2005 in the first year and by 9 Jan. 2007 in the second year (data not shown). As such, an LT_{50} for Cavalier could not be calculated for these sampling dates. Similarly, no LT_{50} was calculated for Meyer on 4 Jan. 2006 due to the severe freezing damage (data not shown). Cavalier and Meyer had higher freezing injury in 2005 - 2006 than in 2006 - 2007 due to longer freezing conditions in December, 2005 (Fig. 4-1 and 4-2). The poorer freezing tolerance in Cavalier relative to Meyer is consistent with previous reports. For example, Cavalier exhibited 35.7% winter kill when evaluated in the 1991 National Turfgrass Evaluation Program; whereas, Meyer had only 8.6% injury (Morris, 1996). In controlled freezing chamber tests evaluating field-sampled turf in Missouri, critical temperatures below which no shoot regrowth occurred were -18 C for Meyer and -10 C for Cavalier (Dunn et al., 1999).

Changes in ABA Content

Meyer had a higher level of ABA in rhizomes than Cavalier on 2 of 3 dates in 2005 - 2006 and 1 of 4 dates in 2006 - 2007 (Fig. 4-3 and 4-4). Numerical levels of ABA were higher in Meyer than in Cavalier on all sampling dates, and a negative correlation was observed between LT_{50} and ABA level in rhizomes (r = -0.65; $P = 0.01$) (Fig. 4-5).

Researchers working with other crops have substantiated relationships between ABA levels and freezing resistance. Abscisic acid levels in leaves of nine wheat (*Triticum aestivum* L.) cultivars were correlated (r = -0.89) with freezing tolerance (Dörffling et al., 1990). Exogenous ABA application also enhanced freezing tolerance in winter wheat, winter rye (*Secale cereal* L. cv Cougar), bromegrass (*Bromus inermis* Leyss) (Chen and Gusta, 1983), birdsfoot trefoil (*Lotus corniculatus* L.) (Keith and McKersie, 1985), and arabidopsis [*Arabidopsis thaliana* (L.) Heynh.] (Lång et al., 1989). Freezing tolerance was restored in ABA-deficient *Arabidopsis* after exogenous application of ABA, further supporting the evidence that ABA is involved in freezing resistance (Heino et al., 1990).

Gatschet et al. (1994) reported greater synthesis of low (20 ~26 kDa) and intermediate molecular weight (32 ~ 37 kDa) cold-regulated (COR) proteins in the cold-tolerant bermudagrass [*Cynodon dactylon* (L.) Pers.] cultivar, 'Midiron', than a cold-sensitive cultivar, 'Tifgreen', and the low molecular weight protein was later identified as a chitinase (Gatschet et al., 1996). Later, it was determined that exogenous ABA application and cold or drought induced expression of a chitinase gene, *CynCHT1* in bermudagrass (de los Reyes, 2001). A dehydrin-like polypeptide (23 kDa) has been reported to be positively associated with freezing tolerance in zoysiagrass genotypes (Patton et al., 2007). Its relationship with ABA needs to be further evaluated.

This is the first report indicating an increase in endogenous ABA level in zoysiagrass during acclimation. Based upon the significance ABA has had in freezing resistance of other crops, it likely contributes to the differences observed in freezing tolerance of Meyer and Cavalier zoysiagrass. The role that ABA may serve in freezing resistance of zoysiagrass is not known, but may related to its influence in osmotic potential and production of proteins important in freezing resistance (de los Reyes et al., 2001; Robertson et al., 1987 and 1988).

In summary, ABA levels increased during cold acclimation in cold-hardy Meyer, and less hardy Cavalier zoysiagrass, but to a higher and longer extent in Meyer. Elevated ABA levels in zoysiagrass rhizomes during acclimation may serve as indicator of freezing resistance. Relationships between ABA and other solutes, such as amino acids, proteins, and proline, should be further investigated for their roles in freezing resistance, as should the potential role of exogenous ABA application for enhancing resistance.

References

Cai, Q., S. Wang, Z. Cui, J. Sun, and Y. Ishii. 2004. Changes in freezing tolerance and its relationship with the contents of carbohydrates and proline in overwintering centipedegrass [*Eremochloa ophiuroides* (Munro) Hack.). Plant Prod. Sci. 7:421-426.

Chen, H. and P.H. Li. 1982. Potato cold acclimation. p. 5-22 *In* Li and Sakai (ed.) Plant cold hardiness and freezing stress: mechanisms and crop implications. Vol. 2. Academic Press, Inc. New York.

Chen, T.H., and L.V. Gusta. 1983. Abscisic acid-induced freezing resistance in cultured plant cells. Plant Physiol. 73:71-74.

Cyril, J., G. L. Powell, R. R. Dunn, and W. V. Baird. 2002. Changes in membrane polar lipid fatty acids of seashore paspalum in response to low temperature exposure. Crop Sci. 42:2031-2037.

DaCosta, M., and B. Huang. 2007. Drought survival and recuperative ability of bentgrass species associated with changes in abscisic acid and cytokinin production. J. Amer. Soc. Hort. Sci. 132:60-66.

DaCosta, M., Z. Wang, and B. Huang. 2004. Physiological adaptation of Kentucky bluegrass to localized soil drying. Crop Sci. 44:1307-1314.

de los Reyes, B.G., C.M. Taliaferro, M.P. Anderson, U. Melcher, and S. McMaugh. 2001. Induced expression of the class II chitinase gene during cold acclimation and dehydration of bermudagrass (*Cynodon* sp.). Theor. App. Genet. 103:297-306.

Dionne, J., Y. Castonguay, P. Nadeau, and Y. Desjardins. 2001. Amino acid and protein changes during cold acclimation of green-type annual bluegrass (*Poa annual* L.) ecotypes. Crop Sci. 41:1862-1870.

Dörffling, K., S. Schulenburg, G. Lesselich, and H. Dörffling. 1990. Abscisic acid and proline levels in cold hardened winter wheat leaves in relation to variety-specific differences in freezing resistance. J. Agron. Crop Sci. 165: 230-239.

Dunn, J.H., S.S. Bughrara, M.R. Warmund, and B.F. Fresenburg. 1999. Low temperature tolerance of zoysiagrasses. HortSci. 34:96-99.

Engelke, M. and S. Anderson. 2002. Zoysiagrasses (*Zoysia* spp.). p. 271-286. *In* Casler and Duncan (ed.) Turfgrass Biology, Genetics, and Breeding. Hoboken, NJ: John Wiley &

Sons, Inc.

Engelke, M., J. Reinert, P. Colbaugh, R. White, B. Ruemmele, K. Marcum, and S. Anderson. 2002a. Registration of 'Cavalier' zoysiagrass. Crop Sci. 52:302-303.

__ 2002b. Registration of 'Diamond' zoysiagrass. Crop Sci. 52:304-305.

Fry, J. D., N. S. Lang, R. G. P. Clifton, and F. P. Maier. 1993. Freezing tolerance and carbohydrate content of low-temperature acclimated and nonacclimated centipedegrass. Crop Sci. 33:1051-1055.

Gatschet, M.J., C.M. Taliaferro, J.A. Anderson, D.R. Porter, and M.P. Anderson. 1994. Cold acclimation and alterations in protein synthesis in bermudagrass crowns. J. Amer. Soc. Hort. Sci. 119:477-480.

Gatschet, M.J., C.M. Taliaferro, D.R. Porter, M.P. Anderson, J.A. Anderson, and K.W. Jackson. 1996. A cold-regulated protein from bermudagrass crowns is a chitinase. Crop Sci. 36:712-718.

Grau, F.V., and A.M. Radko. 1951. Meyer (Z-52) zoysiagrass. USGA J. and Turf Manage. 4:30-31.

Grau, F.V. 1952. Report on two improved turf grasses. USGA J. and Turf Manage. 5:31-32.

Halsey, H. 1956. The *Zoysia* lawn grasses. The National Horticultural Magazine 35:152-161.

Heino, P., G. Sandman, V. Lång, K. Nordin, and E. T. Palva. 1990. Abscisic acid deficiency prevents development of freezing tolerance in *Arabidopsis thaliana* (L.) Heynh. Theor. Appl. Genet. 79: 801-806.

Ishitani, M., L. Siong, B. Stevenson, and J.K. Zhu. 1997. Genetic analysis of osmotic and cold stress signal transduction in *Arabidopsis*: interactions and convergence of abscisic acid-dependent and abscisic acid-independent pathways. Plant Cell 9:1935-1949.

Janowiak, F., E. Luck, and K. Dörffling. 2003. Chilling tolerance of maize seedlings in the field during cold periods in spring is related to chilling-induced increase in abscisic acid level. J. Agron. Crop Sci. 189:156-161.

Janowiak, F., B. Maas, and K. Dörffling. 2002. Importance of abscisic acid for chilling tolerance of maize seedlings. J. Plant Physiol. 159:635-643.

Jiang, Y., and H. Huang. 2002. Protein alterations in tall fescue in response to drought stress and abscisic acid. Crop Sci. 42:202-207.

Keith, C. N., and B. D. McKersie. 1986. The effect of abscisic acid on the freezing tolerance of callus cultures of *Lotus corniculatus* L. Plant Physiol. 80:766-770.

Khadri, M., N.A. Tejera, and C. Lluch. 2007. Sodium chloride-ABA interaction in two common bean (*Phaseolus vulgaris*) cultivars differing in salinity tolerance. Environ. Exper. Bot. 60:211-218.

Lång, V., P. Heino, and E.T. Palva. 1989. Low temperature acclimation and treatment with exogenous abscisic acid induce common polypeptides in *Arabidopsis thaliana* (L.) Heynh. Theor. Appl. Genet. 77:729-734.

Lång, V., E. Mäntylä, E., B. Welin, B. Sundberg, and E. T. Palva. 1994. Alterations in water status, endogenous abscisic acid content, and expression of *rab18* gene during the development of freezing tolerance in *Arabidopsis thaliana*. Plant Physiol. 104:1341-1349.

Li, P.H., W.P. Chen, L.C. Jiang, and Z. Xin. 1998. Abscisic acid-induced chilling tolerance in maize. p. 215-223. *In* Li and Chen (ed.) Plant Cold Hardiness. Plenum Press, New York.

Morris, K. 1996. National zoysiagrass test: 1991. Final report. NTEP 96-15. USDA-ARS, Beltsville, MD.

Morris, K. 2001. National zoysiagrass test: 1996. Final report. NTEP 01-15. USDA-ARS, Beltsville, MD.

Patton, A.J., S.M. Cunningham, J.J. Volenec, and Z.J. Reicher. 2007. Differences in freeze tolerance of zoysiagrasses: I. Role of Proteins. Crop Sci. 47:2162-2169.

Reaney, M.J.T., L.V. Gusta, S.R. Abrama, and A.J. Robertson. 1989. The effects of abscisic acid, kinetin, and gibberellin on freezing tolerance in smooth bromegrass (*Bromus inermis*) cell suspensions. Can. J. Bot. 67:3640-3646.

Robertson, A. J., L. V. Gusta, M. J. T. Reaney, and M. Ishikawa. 1987. Protein synthesis in bromegrass (*Bromus inermis* Leyss) cultured cells during the induction of frost tolerance by abscisic acid or low temperature. Plant Physiol. 84:1331-1336.

Robertson, A. J., L. V. Gusta, M. J. T. Reaney, and M. Ishikawa. 1988. Identification of proteins correlated with increased freezing tolerance in bromegrass (*Bromus inermis* Leyss. cv Manchar) cell cultures. Plant Physiol. 86:344-347.

Thomas, H., and A.R. James. 1993. Freezing tolerance and solute changes in contrasting genotypes of *Lolium perenne* L. acclimated to cold and drought. Ann. Botany 72:249-254.

Wang, Z., and B. Huang. 2003. Genotypic variation in abscisic acid accumulation, water relations, and gas exchange for Kentucky bluegrass exposed to drought stress. J. Amer. Soc. Hort. Sci. 128:349-355.

Welbaum, G. E., D. Bian, D. R. Hill, R. L. Grayson, and M. K. Gunatilaka. 1997. Freezing tolerance, protein composition, and abscisic acid localization and content of pea epicotyl, shoot, and root tissue in response to temperature and water stress. J. Exp. Bot. 48:643-654.

Xiong, L.M., K.S. Schumaker, and J.K. Zhu. 2002. Cell signaling during cold, drought, and salt stress. Plant Cell 14:S165-S183.

Xu, Y., and B. Huang. 2007. Heat-induced leaf senescence and hormonal changes for thermal bentgrass and turf-type bentgrass species differing in heat tolerance. J. Amer. Soc. Hort. Sci. 132:185-192.

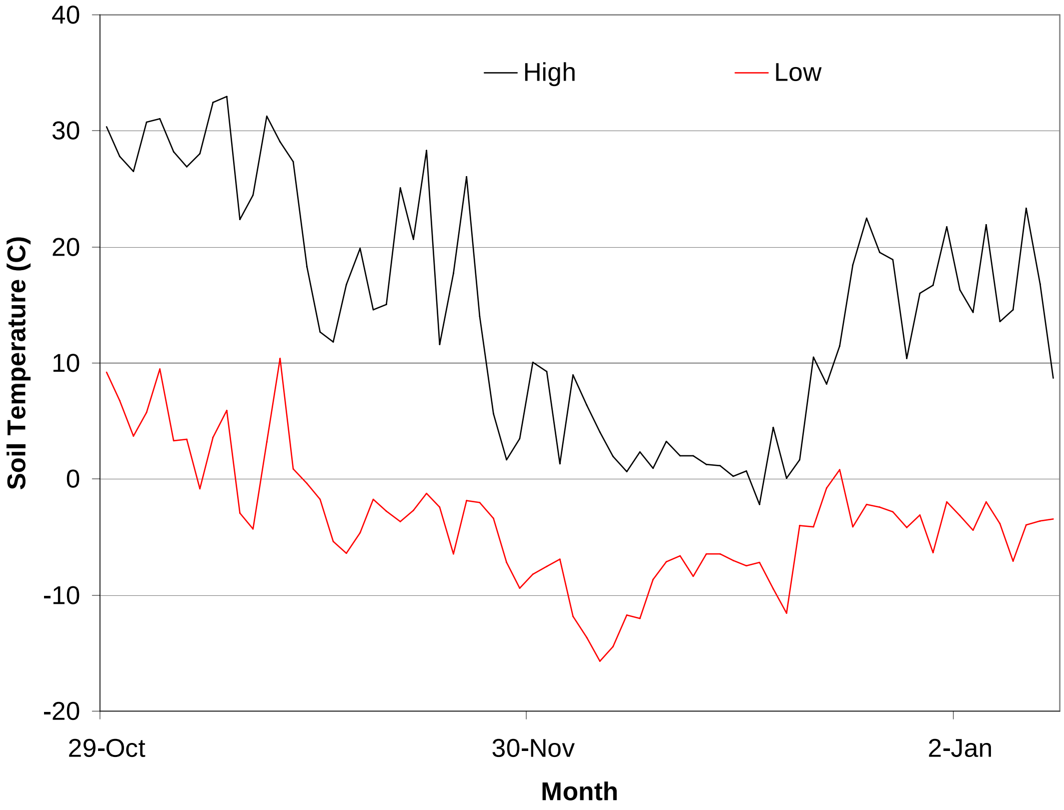


Figure 4-1. Daily soil surface temperatures (C) from 29 Oct. 2005 to 9 Jan. 2006.

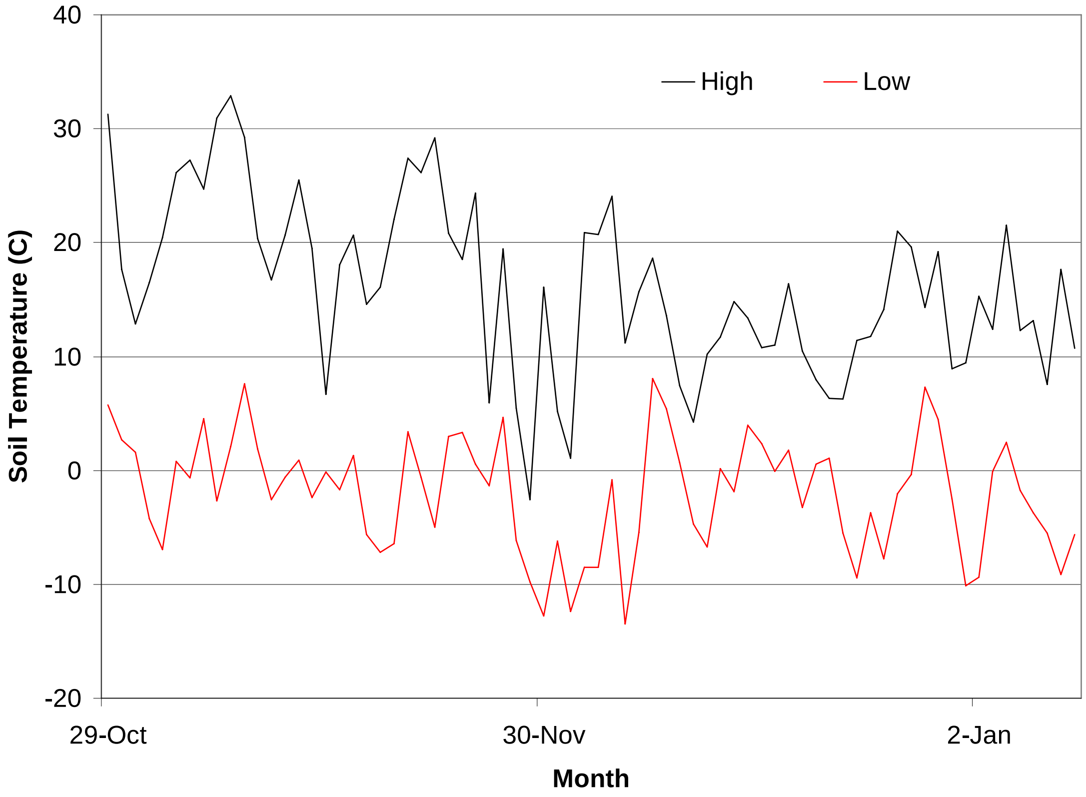


Figure 4-2. Daily soil surface temperatures (C) from 29 Oct. 2006 to 9 Jan. 2007.

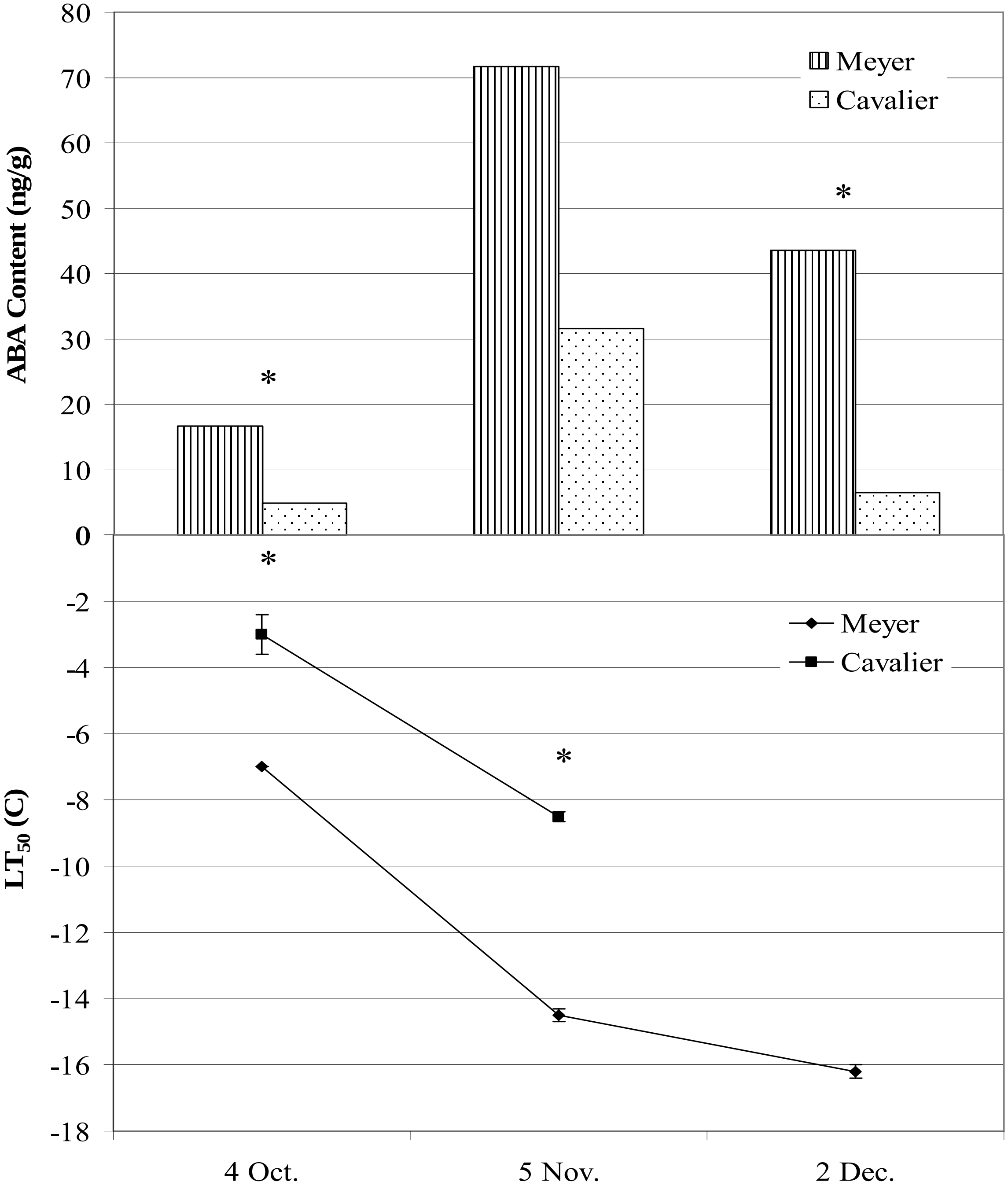


Figure 4-3. Abscisic acid (ABA) content (ng/g frozen weight) and LT_{50} (the temperature killing 50% of grasses) in 'Meyer' and 'Cavalier' rhizomes in 2005. A * above ABA or LT_{50} means indicates that cultivars were different ($P \leq 0.05$). Vertical bars at LT_{50} means indicate the standard error on each sampling date. The LT_{50} was not available for 'Cavalier' on 2 Dec. due to occurrence of severe freezing injury.

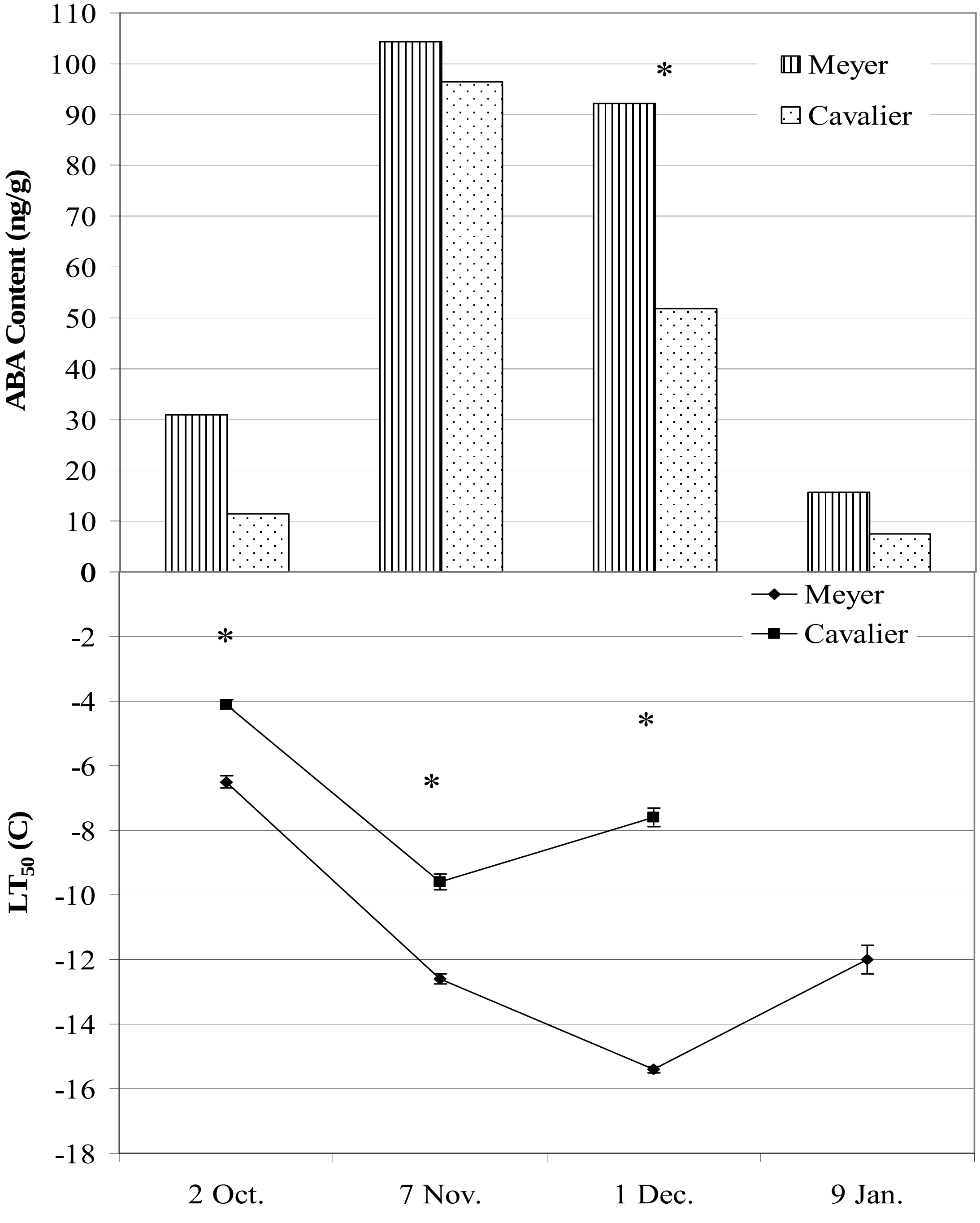


Figure 4-4. Abscisic acid (ABA) content (ng/g frozen weight) and LT_{50} (the temperature killing 50% of grasses) in 'Meyer' and 'Cavalier' rhizomes in 2006. A * above ABA or LT_{50} means indicates that cultivars were different ($P \leq 0.05$). Vertical bars at LT_{50} means indicate the standard error on each sampling date. The LT_{50} was not available for 'Cavalier' on 9 Jan. due to occurrence of severe freezing injury.

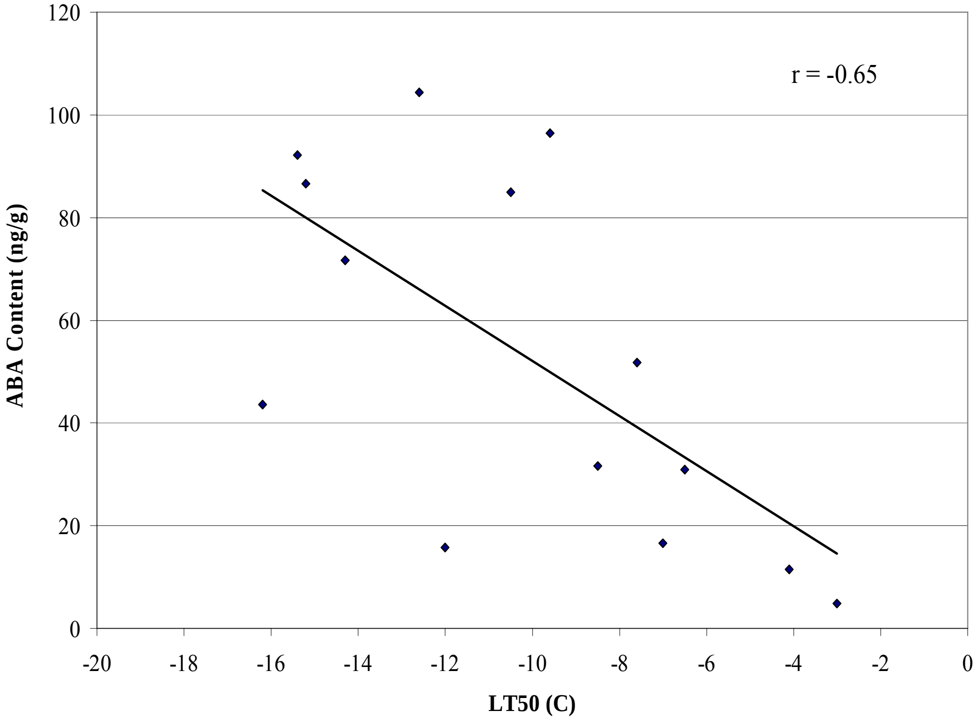


Figure 4-5. Relationship between LT_{50} (the temperature killing 50% of grasses) and abscisic acid (ABA) content in 'Meyer' and 'Cavalier' zoysiagrass rhizomes. Each point represents the mean of ABA and LT_{50} of 'Meyer' or 'Cavalier' on each sampling date.