

### CHAPTER THREE - ZOYSIAGRASS SPECIES AND CULTIVARS DIFFER IN THEIR WINTER INJURY AND FREEZE TOLERANCE

#### Abstract

Lack of cold hardiness may limit widespread use of newly released zoysiagrass (*Zoysia* spp.) cultivars in the transition zone. Our objective was to quantify differences in the winter injury of 35 zoysiagrass cultivars in field plots in West Lafayette, IN, and the freeze tolerance of 13 cultivars in a cold stress simulator. Winter injury varied among 35 zoysiagrass cultivars between years and cultivars. *Zoysia japonica* Steud. cultivars had less winter injury each year than *Z. matrella* (L.) Merr. cultivars. Cultivars of *Z. japonica* available commercially as seed had less winter injury (2% in both years) than cultivars of *Z. japonica* (41%, 2005; 54%, 2006) and *Z. matrella* (51%, 2005; 73%, 2006) sold commercially as vegetative propagules. ‘Meyer’, ‘Chinese Common’ and ‘Zenith’ were the commercially available cultivars exhibiting the least winter injury (<6%) in both years, whereas ‘Victoria’, ‘DeAnza’, ‘Diamond’, and ‘Empress’ had the most winter injury (>88%) both years. There was a relationship ( $r^2 = 0.48$ ,  $p = 0.0088$ ) between  $LT_{50}$  and winter injury in the field. Freeze tolerance ranged from -8.4°C (Diamond) to -11.5°C (Meyer and Zenith). Meyer has been the industry standard for zoysiagrass, but our research has identified other commercially available and experimental cultivars with winter injury similar to Meyer.

Zoysiagrass (*Zoysia* spp.) is a warm-season grass well-adapted for lawn and golf turfs in the transitional and warm climatic regions of the USA and requires minimal maintenance inputs. Thus, expanded use of zoysiagrass could play a key role in making transition zone golf courses and lawns more environmentally friendly and sustainable. However, one barrier to widespread zoysiagrass use is a relative lack of cold hardiness in the transition zone, especially compared to cool-season grasses. Winter injury of zoysiagrass was first reported in 1947 by Forbes and Ferguson. They found that both *Zoysia japonica* Steud. and *Zoysia matrella* (L.) Merr. survived winters in Maryland, but that *Z. japonica* had better spring green-up and less winter injury than *Z. matrella*.

Zoysiagrass was first used on golf courses in the 1950's when the cultivar 'Meyer' was released (Grau and Radko, 1951) and gained immediate popularity because of its heat, freezing, and drought tolerance in the transition zone (Grau, 1952). Rogers et al. (1977) evaluated differences in survival of zoysiagrass cultivars with an artificial freezing test using rhizomes and stolons and found that Meyer had superior cold tolerance compared to a selection of *Zoysia matrella*. Rogers et al. (1975) also evaluated the freezing tolerance of Meyer in Missouri and found that maximum survival occurred in early January with 50% survival occurring between -11.1 and -12.8°C. Warmund et al. (1998) evaluated the survival and recovery of Meyer rhizomes to extracellular freezing. Extracellular voids formed as a result of ice formation in Meyer rhizomes cooled to -7°C, but rhizomes survived temperatures as low as -11°C (Warmund et al., 1998).

Many new cultivars of zoysiagrass released after Meyer have improved texture, shade tolerance, drought tolerance, establishment rate, divot recovery and pest resistance compared to Meyer (White and Engelke, 1990; Reinert and Engelke, 2001; White et al., 2001; Patton and Reicher, 2005; Karcher et al., 2005). Dunn et al. (1999) evaluated the low temperature tolerance of newer cultivars of zoysiagrass using a programmable freezer and rhizomes harvested in

January. Although they did not report freezing tolerance ( $LT_{50}$ ) in their study, they found that ‘Meyer’ was among the most hardy entries, whereas ‘Emerald’, ‘El Toro’, ‘Cavalier’ and ‘Palisades’ were among the least hardy entries. Since other cultivars are less cold hardy than Meyer, extensive use of zoysiagrass in the transition zone is limited to Meyer (Dunn et al., 1999; Dunn and Diesburg, 2004).

Freeze tolerance of warm-season grasses is most often evaluated by measuring electrolyte leakage (EL) or regrowth of plant tissues after freezing. Lethal temperatures predicted by EL and regrowth are correlated (Gusta et al., 1980; Maier et al., 1994) and use of EL is more popular than regrowth measurements because freeze tolerance can be assessed rapidly. However, a drawback to EL is its questionable accuracy. Electrolyte leakage underestimated freezing tolerances of seashore paspalum (*Paspalum vaginatum* Swartz) (Cardona et al., 1997) and centipedegrass [*Eremochloa ophiuroides* (Munro) Hack](Fry et al., 1993) and overestimated freezing tolerance of St. Augustinegrass [*Stenotaphrum secundatum* (Walter) Kuntze](Maier et al., 1994). Inconsistency in the EL procedure may make regrowth tests a better option to determine freeze tolerance of zoysiagrass cultivars.

Researchers at Oklahoma State University developed a standardized, rapid, reproducible regrowth method for quantifying freeze tolerance of bermudagrass (*Cynodon* spp.) producing results that agree with field observations (Anderson et al., 1993). This method cold acclimates plants using a controlled-environment chamber set at 8/2°C day/night cycles with a short photoperiod (10-h) and low light ( $\sim 300 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) for 4 wks. Controlled-environment chambers provide an acclimation setting for plants that is independent of yearly fluctuations in the field (Cardona et al., 1997) and have been used successfully to acclimate and test freeze tolerance of other warm-season grasses such as buffelgrass (*Pennisetum ciliare* L.) and seashore paspalum (Cardona et al., 1997; Stair et al., 1998).

Although many newer zoysiagrass cultivars have either previously been evaluated or are currently being evaluated in field trials (Morris, 1998; 2004), conditions necessary for separating cold hardiness among cultivars may occur only once per decade (Dunn et al., 1999). As a result, cultivar trials lasting five years are usually insufficient to separate differences in cold hardiness among cultivars. In addition to testing for winter injury in field plots, it is necessary to use a standardized method to determine the relative freeze tolerance of zoysiagrass cultivars since winter injury can result in substantial reestablishment costs and loss of use. The objectives of our research were to: 1) determine the differences in winter injury of commercially available and experimental zoysiagrass cultivars in field plots; 2) determine if winter injury is related to growth rate or leaf width; and 3) evaluate the freeze tolerance of zoysiagrass cultivars using controlled environment acclimation and freezing procedures.

## Materials and Methods

### Winter Injury in Field Plots

Plant material of commercially available and experimental cultivars of zoysiagrass (Table 3.1) was collected in the fall of 2003 and propagated in the greenhouse ( $23 \pm 5$  °C) in plug trays with 8 by 8 by 8 cm divisions filled with fritted clay (Turface, Profile Products LLC, Buffalo Grove, IL). Vegetatively established cultivars were planted into trays as plugs or stolons and seeded cultivars were seeded ( $49 \text{ kg ha}^{-1}$ ) into trays. Plants in the greenhouse were fertilized monthly with  $49 \text{ kg ha}^{-1}$  N,  $21 \text{ kg ha}^{-1}$  P, and  $40 \text{ kg ha}^{-1}$  K using a soluble fertilizer (18N-7.9P-17.4K) and mowed weekly at 4.0 cm. Plants were transplanted into field plots at the W.H. Daniel Turfgrass Research and Diagnostic Center, West Lafayette, IN. Experimental plots were 1.0 by 1.0 m arranged in a randomized complete-block design with four replications. One vegetative

plug (8 by 8 by 8 cm) of zoysiagrass was transplanted into the center of each plot for both seeded and vegetative cultivars on 7 June 2004 and 2005 and irrigated four times daily for the first month to encourage establishment and then irrigated as needed to prevent wilting. Soil type was a Stark silt loam (fine-silty mixed mesic Aeric Ochraqualfs) with a pH of 7.0, 224 kg ha<sup>-1</sup> P, 808 kg ha<sup>-1</sup> K and 84 g kg<sup>-1</sup> organic matter in 2004 and with a pH of 6.8, 224 kg ha<sup>-1</sup> P, 639 kg ha<sup>-1</sup> K and 29 g kg<sup>-1</sup> organic matter in 2005. Areas were fumigated with methyl bromide at 732 kg/ha prior to establishment each year to minimize weed competition. Plots received 49 kg ha<sup>-1</sup> N from urea (46N-0P-0K) on 1 July and 1 August of each year and weeds were manually removed during establishment. A weather station was located on the plots which recorded air temperatures and bare soil temperatures 2.5 cm deep.

Digital images were taken of each plot once weekly with a Nikon Coolpix 3200 (Nikon, Melville, NY) digital camera mounted on a monopod to insure a consistent height from the lens to the soil surface (1.05 m). Coverage of zoysiagrass was determined using digital image analysis (DIA) (SigmaScan Pro, Systat Software Inc., Richmond, CA)(Richardson et al., 2001). To selectively identify green leaves in the images, hue range was set from 47 to 107 and saturation was set from 10 to 100. Images were taken of a calibration disk and data were converted from selected green pixels to zoysiagrass coverage (cm<sup>2</sup>).

Coverage was determined from weekly DIA of plots and establishment rate was determined and reported previously (Patton, 2006). To determine the rate of establishment, coverage was transformed using the natural logarithm and applied to the linear model [Coverage = (K\*DAP) + I], where K is the rate of increase (establishment rate), DAP is days after plugging, and I was equal to the natural logarithm of 64 cm<sup>2</sup> which was the starting coverage for all plots. An autumn growth rating was determined as the increase in coverage between 6 September (91 DAP) and 28 September (113 DAP) each year. Leaf blade width was also measured with a Vernier caliper at 64 DAP each year.

The start of winter dormancy (leaf discoloration) occurred 112 and 113 DAP in 2004 and 2005, respectively. Images with the maximum green coverage before winter dormancy and images taken after winter dormancy (spring green-up) were used to calculate winter injury.

Winter injury was determined using the following formula:

$$\text{winter injury} = 100 - [(\text{coverage after winter dormancy} \div \text{coverage before winter dormancy}) * 100]$$

Maximum green coverage before winter was collected on 28 September in 2005 and 2006, and spring green-up data was collected on 23 May 2005 and 20 May 2006. Some cultivars with good cold tolerance and earlier spring green-up had winter injury <0% (minimum -24%) due to new growth prior to spring green-up data recording. Therefore, plots with <0% winter injury were set to 0% prior to analysis.

Winter injury data were arcsine transformed prior to analysis to improve homogeneity of the error variance and are presented as backtransformed means for easier interpretation (Steel et al., 1997). Data were analyzed using PROC ANOVA, PROC CORR and PROC TTEST (SAS Institute, Cary, NC). Error variances were tested for homogeneity between years and for year  $\times$  cultivar interactions before analysis of variance. When differences were examined between species, 'J-14' (*Zoysia sinica* Hance) was grouped with *Z. japonica* and 'Emerald' (*Z. japonica*  $\times$  *Z. pacifica* Goudsw.) was grouped with *Z. matrella* because of the similarities in leaf color, leaf texture, and plant density with respective species. Means were separated using Fisher's protected least significant difference when *F* tests were significant at  $\alpha \leq 0.05$ .

#### Freeze Tolerance

A cold stress simulator was constructed by modifying a 0.55 m<sup>3</sup> chest freezer similar to the method of Beard et al. (1980, 1991). The freezer was modified by adding an elevated rack

inside the chamber with attached 120 mm diameter computer cooling fans (Philmore Manufacturing Company, Inc., Rockford, IL) to circulate air within the chamber. A programmable controller (Watlow 981, Watlow Electric Manufacturing Co., St. Louis, MO) and a type T Teflon tipped thermocouple (ThermoWorks, Alpine, UT) connected to the controller were used to control temperature inside the chamber. Soil temperatures are more important than air temperatures when assessing freezing stress (Beard et al., 1991), so the thermocouple was inserted 2.5 cm into the potting-mix to control temperature.

Zoysiagrass plant material was collected in the fall of 2003 and propagated in the greenhouse in pots filled with fritted clay (Turface, Profile Products LLC, Buffalo Grove, IL). Vegetatively established cultivars were planted into trays as plugs or stolons and seeded cultivars were seeded ( $49 \text{ kg ha}^{-1}$ ) into trays. Plants were established in 2.5 cm diameter Ray Leach containers (Stuewe & Sons, Inc., Corvallis, OR) filled with potting-mix (BM-2, Berger Peat Moss Ltd., Canada) using a phytomere containing root, crown, and shoot material starting in the fall of 2005. One or more zoysiagrass cultivars with low, medium, and high winter injury were selected from each group (*Z. japonica* cultivars established vegetatively, *Z. japonica* cultivars established from seed, and *Z. matrella* cultivars established vegetatively) of commonly used zoysiagrass cultivars based on winter injury results from the spring of 2005. ‘Midlawn’ bermudagrass (*Cynodon dactylon* L. (Pers)  $\times$  *C. transvaalensis* Burt-Davy) and ‘Pennncross’ creeping bentgrass (*Agrostis stolonifera* L.) were used as controls in this experiment because their freeze tolerances were known. After 10 wks of establishment in the greenhouse ( $25 \pm 5^\circ\text{C}$ ), plants were cold acclimated for 4 wks using a controlled-environment chamber (PGR15, Controlled Environments Inc., Pembina, ND) set at  $8/2^\circ\text{C}$  day/night cycles  $\text{s}^{-1}$  and a 10-h photoperiod of  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$  photosynthetically active radiation based upon the procedure of Anderson et al. (1988).

Plants were placed in the cold stress simulator to test for relative freeze tolerance of each cultivar after 4 wks of acclimation. The cold stress simulator was programmed to cool  $1^\circ\text{C h}^{-1}$

after 15 h at -3°C similar to Anderson et al. (1993). Target soil temperatures (1°C intervals, -6 to -15°C) covered a range anticipated to span the limits from complete survival to complete mortality based upon preliminary testing. To increase sample size near the anticipated freeze tolerance, six cone-tainers were removed at each test temperature from -8 to -12°C for each cultivar, and only three cone-tainers were removed for each cultivar for test temperature -6 and -7 where complete survival was expected and at -13, -14, and -15°C where complete mortality was expected. Plants were thawed in a walk-in refrigerator (5°C) overnight after freezing. Plants were then transferred to a greenhouse (26 ±6°C) and evaluated for regrowth for a period of 4 wks after freezing. The temperature resulting in no regrowth from 50% of the plants (LT<sub>50</sub>, freeze tolerance) was determined by nonlinear regression using an equation from Ingram and Buchanan (1984) which was also used by Anderson et al. (2002), Cardona et al. (1997), Stair et al. (1998) and Väinölä et al. (1999) to predict survivability of bermudagrass, seashore paspalum, buffelgrass and *Rhododendron* spp., respectively. Freeze tolerance was determined by the following equation:

$$\text{Survival} = a + (b-a)/(1 + e^{c(T_m-T)})$$

where a is the base line of survival, b is the maximum survival, c is a function of the slope of the line at the inflection point, T<sub>m</sub> is the temperature at the inflection point which is also the LT<sub>50</sub>, and T is the treatment temperature. This experiment was conducted six times. Data were fit to sigmoidal curves using PROC NLIN (Gauss-Newton method)(Fig. 3.1) and LT<sub>50</sub> values were analyzed using PROC ANOVA (SAS Institute, Cary, NC). Means were separated using Fisher's protected least significant difference when *F* tests were significant at  $\alpha \leq 0.05$ .

## Results and Discussion

### Winter Injury in Field Plots

Winter injury was greater ( $p=0.09$ ) in 2006 (36%) than 2005 (26%) (Table 3.2) when averaged across cultivars even though the minimum soil temperature was lower in 2004-2005 (-7.4°C, 28 Jan. 2005) than in 2005-2006 (-3.4°C, 19 Feb. 2006)(Fig. 3.2). Differences in winter injury between years could be attributed to differences in plant cold acclimation status, snow cover, or soil moisture. Zoysiagrass is not as susceptible to air temperature fluctuations in winter as stoloniferous warm-season grasses such as centipedegrass and St. Augustinegrass since it also has rhizomes that lie protected below the soil surface (DiPaola and Beard, 1992).

There were significant year by cultivar interactions for winter injury and thus results from 2004-2005 and 2005-2006 are presented separately (Table 3.2). ‘BMZ 230’, ‘DALZ 0102’, ‘Chinese common’, ‘J-36’, ‘PST-R7LT’, ‘Meyer’, ‘VJ’, ‘J-37’, ‘PZB33’, ‘Emerald’, ‘J-14’, and ‘PZA 32’ had the least amount of winter injury in the spring of 2005. With the exception of PZB 33, VJ, DALZ0102, and Emerald, these same cultivars along with ‘PST-R7MA’, ‘PST-R7ZM’, ‘PST-R7TH’, and ‘Zenith’ had the least amount of winter injury in the spring of 2006. ‘Diamond’, ‘DALZ 0105’, ‘6186’, ‘DALZ0104’, ‘Empress’, ‘Victoria’, and ‘DeAnza’ had the highest winter injury in the spring of 2005. With the exception of Victoria, these same cultivars along with ‘Empire’ had the highest winter injury in the spring of 2006. ‘Companion’, ‘DALZ0101’, ‘Himeno’, ‘Zorro’, ‘Cavalier’, ‘Zeon’, ‘El Toro’, ‘6136’, ‘Palisades’, ‘GNZ’, and ‘Royal’ were among the cultivars whose winter injury was intermediate (14 to 79%) both years. Winter injury of cultivars in our study are comparable to previous reports of winter survival across the nation (Morris, 1996, 2001; Dunn et al., 1999; Fry, 2002).

Overall, cultivars with low winter injury in 2005 also had low winter injury in 2006. Additionally, cultivars with high winter injury in 2005 also had high winter injury in 2006. However, cultivars with intermediate winter injury in 2005 usually had more winter injury in 2006. For instance, Emerald and Empire exhibited dramatically more winter injury in 2006 than 2005. Emerald also had significant winterkill in Kansas in 2001 and Kentucky in 1997 (Morris, 2001) and is not considered well-adapted to the transition zone compared to other cultivars (Dunn and Diesburg, 2004). However, Himeno exhibited the opposite effect with more winter injury in 2005 (35%) than 2006 (14%). This demonstrates the inconsistency typical when assessing winter injury in the field (Anderson et al., 1988; Dunn et al., 1999).

Chinese common, Meyer, and Zenith were the only commercially available cultivars with less than 6% winter injury in both years (Table 3.2). Among these cultivars, Meyer is the only one commercially available as vegetative propagules. Several improved cultivars commercially available as vegetative propagules were tested in our study, but winter injury of these cultivars was higher than Meyer. Meyer is the most widely-used cultivar in the transition zone (Dunn and Diesburg, 2004), and our studies indicate that apart from Zenith and Chinese common, there are few additional commercially available options for use in the transition zone based upon the criteria of winter injury. However, many experimental cultivars exhibited levels of winter injury similar to Meyer in both years including BMZ 230, PST-R7LT, PST-R7MA, PST-R7ZM, PST-R7TH, J-14, J-36, J-37, and PZA 32.

Overall, *Z. japonica* cultivars had less winter injury than *Z. matrella* cultivars (Table 3.3) which is consistent with earlier reports (Forbes and Ferguson, 1947; Daniel, 1955; Turgeon, 1991; Duple, 1996; McCarty, 2001). Zorro and Cavalier had the least amount of winter injury among commercially available *Z. matrella* cultivars over both years, but their winter injury was greater than Meyer. *Zoysia japonica* is sold commercially both as seed or as vegetatively propagules, but *Z. matrella* is only available as vegetative propagules. All cultivars were

established by vegetative plugs in the field for this study, but it was found that *Z. japonica* cultivars with commercial seed availability had winter injury lower than cultivars of *Z. japonica* and *Z. matrella* available as vegetative propagules (Table 3.3). This is consistent with winter injury observations in Kansas where seeded cultivars had the lowest winter injury (Fry, 2002) and indicates that cultivar with seed availability are likely genetically similar.

We explored the relationship between winter injury and leaf width (Table 3.4). *Zoysia japonica* cultivars have wider leaves (2 to 4 mm) than *Z. matrella* (< 2 mm) (Anderson, 2000). Winter injury decreased with increasing leaf width ( $r = -0.57, p < 0.001, 2005$ ;  $r = -0.60, p < 0.001, 2006$ ) when analyzed across species (Table 5). However, when each species was analyzed separately, this correlation only occurred in *Z. japonica* ( $r = -0.70, p < 0.001, 2005$ ;  $r = -0.53, p = 0.005, 2006$ ) (Table 3.5). This correlation was strongly influenced by a group of *Z. japonica* cultivars sold commercially as seed that exhibit low winter injury and have wide leaf blades. Therefore, species not leaf width likely influences winter injury.

Some crops such as alfalfa (*Medicago sativa* L.) are prone to winter injury if late fall growth is high (Schwab et al., 1996). Using data on the establishment rate of zoysiagrass cultivars from our earlier study (Patton, 2006) and data on autumn growth (Table 3.4), we found no relationship between winter injury and autumn growth in either year of the study and a weak correlation between winter injury and establishment rate ( $r = -0.37, p = 0.03$ ) in 2006 (Table 3.5). This weak relationship is likely an artifact of the use of two species of zoysiagrass in our study. *Zoysia japonica* cultivars have a faster establishment rate (Patton and Reicher, 2005) and lower winter injury than *Z. matrella* cultivars. As a result, two groups are formed where *Z. japonica* is fast establishing with low winter injury and *Z. matrella* is slow establishing with high winter injury. Within species there was no relationship between winter injury and establishment rate (Table 3.5). Therefore, species is the cause of the weak correlation between winter injury and establishment rate in 2006.

Zoysiagrass cold hardiness is likely genetically controlled (Engelke and Anderson, 2003). Although *Z. japonica* cultivars as a whole had less winter injury than *Z. matrella* cultivars, some cultivars of *Z. japonica* such as Victoria and DeAnza had more winter injury while others such as Palisades and El Toro exhibited intermediate winter injury. Engelke and Anderson (2003) proposed that many commercially available *Z. japonica* cultivars are not a single species as classified by their morphological characteristics, but instead are interspecific hybrids. This is supported by Anderson (2000) who found probable interspecific hybrids based on restriction fragment length polymorphism fingerprint characterization and by Yaneshita et al. (1997) who found interspecific hybridization of *Zoysia* spp. among natural populations. Cultivars such as Victoria, DeAnza, El Toro and Palisades are likely crosses of *Z. japonica* with *Z. matrella* and/or *Z. pacifica* (Goudswaard) Hotta & Kuroki (formerly *Z. tenuifolia* Thiele) (Anderson, 2000; Engelke et al., 2002; Gibeault, 2003), but are classified as *Z. japonica* because of their predominant morphological characteristics. *Zoysia pacifica* and *Z. matrella* have poor cold hardiness compared to *Z. japonica* (Daniel, 1955; Turgeon, 1991; McCarty, 2001). Therefore, the genetic makeup of Victoria, DeAnza, El Toro and Palisades may help explain why they had more winter injury than most other *Z. japonica* cultivars.

#### Freeze Tolerance

Freeze tolerance ( $LT_{50}$ ) of zoysiagrass cultivars ranged from  $-8.4^{\circ}\text{C}$  (Diamond) to  $-11.5^{\circ}\text{C}$  (Meyer and Zenith) with a mean of  $-10.2^{\circ}\text{C}$  (Table 3.6). *Zoysia matrella* cultivars Diamond, Royal, and Zorro and the *Z. japonica* cultivar Victoria had poor tolerance to freezing. *Zoysia japonica* cultivars Meyer, Zenith, Palisades, El Toro, Companion, and J-36 were all similar and had good freeze tolerance. The  $LT_{50}$  of the experimental control Midlawn bermudagrass was  $-8.6^{\circ}\text{C}$  which is similar to previous reports ranging from  $-8.4$  to  $-10.3^{\circ}\text{C}$  (Anderson et al., 1993, 2002, 2003). Additionally, Penncross creeping bentgrass had a  $LT_{50}$  outside of the range of

temperatures we tested ( $< -15^{\circ}\text{C}$ ), which is consistent with its reported  $\text{LT}_{50}$  of  $-35^{\circ}\text{C}$  (Gusta et al., 1980). Freeze tolerance of Meyer ( $\text{LT}_{50} = -11.5^{\circ}\text{C}$ ) in our study was similar to that in a previous report of  $-11.1$  to  $-12.8^{\circ}\text{C}$  (Rogers et al., 1975) and consistent with studies showing rhizome survival at  $-11^{\circ}\text{C}$  (Warmund et al., 1998; Dunn et al., 1999; Zhang and Fry, 2006).

There was a relationship ( $r^2 = 0.48$ ,  $p = 0.0088$ ) between  $\text{LT}_{50}$  and winter injury in the field (Fig. 3.3). However, El Toro and Palisades were more freeze tolerant than anticipated based upon our winter injury data and a previous report (Dunn et al., 1999), whereas Zorro was less freeze tolerance than anticipated. Freeze tolerance does not always reflect absolute cold hardiness (Anderson et al., 2002) due to additional environmental factors in the field such as snow cover, soil moisture, and temperature fluctuations. Additionally, environmental conditions in the field may induce a greater level of acclimation compared to the induced growth chamber acclimation (Anderson et al., 2002).

Mechanisms involved in the cold hardiness of zoysiagrass are not well understood. Membrane stability (Samala et al., 1998; Cyril et al., 2002; Munshaw et al., 2006), carbohydrate composition (Ball et al., 2002; Shahba et al., 2003; Cai et al., 2004), proline concentration (Cai et al., 2004; Lu et al., 2005; Munshaw et al., 2006) and cold-regulated proteins (Gatschet et al., 1996) are known to be important in the freezing tolerance of other warm-season turfgrasses. Additional research is needed to determine physiological basis for differences in zoysiagrass cold hardiness.

In summary, our results show large differences between species and cultivars of zoysiagrass. *Zoysia japonica* cultivars exhibited less winter injury than *Z. matrella* cultivars. Additionally, *Z. japonica* cultivars that can be propagated by seed exhibit less winter injury and have wider leaves than other cultivars, which may indicate that this group is more genetically similar than vegetatively established *Z. japonica* cultivars. Our results indicate that there are a limited number of currently available cultivars (Meyer, Chinese common, and Zenith) well-

adapted to the transition zone, but that experimental cultivars with low winter injury are being developed. Relative rankings of the winter injury of zoysiagrass in West Lafayette, Indiana, which is north of the transition zone will be useful for practitioners selecting zoysiagrass cultivars for use in the transition zone. Selecting and establishing cultivars well-adapted to the transition zone will help reduce winter injury and ensuing reestablishment costs, thus increasing golf course revenues and sustainability of turfs.

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Table 3.1. Zoysiagrass cultivar and experimental names, species, typical type of establishment method and source of plant material.

Cultivar†	Experimental	Species	Type‡	Source
Chinese Common§*		<i>Zoysia japonica</i> Steud.	Seeded	Natl. Turfgrass Evaluation Program
J-36	J-36	<i>Zoysia japonica</i> Steud.	Seeded	Jacklin Seed Division J.R. Simplot
Meyer*	Z-52	<i>Zoysia japonica</i> Steud.	Vegetative	Natl. Turfgrass Evaluation Program
PST-R7LT	PST-R7LT	<i>Zoysia japonica</i> Steud.	Seeded	Pure-Seed Testing, Inc.
BMZ 230	BMZ 230	<i>Zoysia japonica</i> Steud.	Vegetative	Turfgrass America
J-14	J-14	<i>Zoysia sinica</i> Hance	Seeded	Jacklin Seed Division J.R. Simplot
J-37	J-37	<i>Zoysia japonica</i> Steud.	Seeded	Jacklin Seed Division J.R. Simplot
PZA 32	PZA 32	<i>Zoysia japonica</i> Steud.	Seeded	Patten Seed Co.
PST-R7TH	PST-R7TH	<i>Zoysia japonica</i> Steud.	Seeded	Pure-Seed Testing, Inc.
PST-R7MA	PST-R7MA	<i>Zoysia japonica</i> Steud.	Seeded	Pure-Seed Testing, Inc.
DALZ0102	DALZ 0102	<i>Zoysia japonica</i> Steud.	Vegetative	Turfgrass America
Zenith*	ZNW-1	<i>Zoysia japonica</i> Steud.	Seeded	Patten Seed Co.
PST-R7ZM	PST-R7ZM	<i>Zoysia japonica</i> Steud.	Seeded	Pure-Seed Testing, Inc.
VJ		<i>Zoysia japonica</i> Steud.	Vegetative	Bladerunner Farms, Inc.
PZB 33	PZB 33	<i>Zoysia japonica</i> Steud.	Seeded	Patten Seed Co.
Companion*	ZMB-2	<i>Zoysia japonica</i> Steud.	Seeded	Seed Research of Oregon, Inc.
DALZ0101	DALZ 0101	<i>Zoysia matrella</i> (L.) Merr.	Vegetative	Turfgrass America
Himeno		<i>Zoysia japonica</i> Steud.	Vegetative	Zoysian Japan Co.
Zorro*	DALZ 9601	<i>Zoysia matrella</i> (L.) Merr.	Vegetative	Natl. Turfgrass Evaluation Program
Cavalier*	DALZ8507	<i>Zoysia matrella</i> (L.) Merr.	Vegetative	M.C. Engelke, Texas A&M Univ.
Emerald*	34-35	<i>Z. japonica</i> x. <i>Z. pacifica</i> ¶	Vegetative	Natl. Turfgrass Evaluation Program
Zeon*		<i>Zoysia matrella</i> (L.) Merr.	Vegetative	Bladerunner Farms, Inc.
6136	6136	<i>Zoysia japonica</i> Steud.	Vegetative	Bladerunner Farms, Inc.
El Toro*	UCR#1	<i>Zoysia japonica</i> Steud.	Vegetative	Seedland, Inc.
Palisades*	DALZ 8514	<i>Zoysia japonica</i> Steud.	Vegetative	M.C. Engelke, Texas A&M Univ.
Royal*	DALZ 9006	<i>Zoysia matrella</i> (L.) Merr.	Vegetative	M.C. Engelke, Texas A&M Univ.
Empire*	SS-500	<i>Zoysia japonica</i> Steud.	Vegetative	Sod Solutions
GNZ*	ZT-11	<i>Zoysia japonica</i> Steud.	Vegetative	Greg Norman Turf
Victoria*	Z88-14	<i>Zoysia japonica</i> Steud.	Vegetative	West Coast Turf
DeAnza*	Z88-8	<i>Zoysia japonica</i> Steud.	Vegetative	West Coast Turf
6186	6186	<i>Zoysia japonica</i> Steud.	Vegetative	Bladerunner Farms, Inc.
Diamond*	DALZ 8502	<i>Zoysia matrella</i> (L.) Merr.	Vegetative	M.C. Engelke, Texas A&M Univ.
Empress*	SS-300	<i>Zoysia japonica</i> Steud.	Vegetative	Sod Solutions
DALZ0104	DALZ 0104	<i>Zoysia matrella</i> (L.) Merr.	Vegetative	Turfgrass America
DALZ0105	DALZ 0105	<i>Zoysia matrella</i> (L.) Merr.	Vegetative	Turfgrass America

† Cultivars sorted according to winter injury (Table 3.2).

‡ Type of establishment (propagation) method typically used by practitioners for each cultivar. Cultivars available by seed are typically seeded, with other cultivars typically established vegetatively by sprigs, plugs or sod. All cultivars were established by vegetative plugs into field plots for this study.

§ Cultivar names followed by an asterisk (\*) are commercially available in 2006.

¶ Formerly *Zoysia japonica* Steud. x. *Zoysia tenuifolia* Willd. ex Thiele (Forbes, 1962), now *Z. japonica* x. *Z. pacifica* Goudsw. (Anderson, 2000).

Table 3.2. Zoysiagrass mean winter injury by cultivar from a field trial established in June 2004 and 2005 in West Lafayette, Indiana.

Cultivar‡	Species§	Type¶	Winter injury†	
			2005	2006
---backtransformed %---				
Chinese Common	j	Seed	0 a#	0 a
J-36	j	Seed	0 a	0 a
Meyer	j	Vegetative	0 abc	0 a
PST-R7LT	j	Seed	0 ab	1 ab
BMZ 230	j	Vegetative	0 a	3 abc
J-14	s	Seed	3 abc	0 a
J-37	j	Seed	1 abc	4 abcd
PZA 32	j	Seed	3 abc	1 ab
PST-R7TH	j	Seed	5 cde	0 a
PST-R7MA	j	Seed	4 bcd	2 ab
DALZ0102	j	Vegetative	0 a	9 bcde
Zenith	j	Seed	5 bcd	6 abcd
PST-R7ZM	j	Seed	6 cdef	5 abcd
VJ	j	Vegetative	1 abc	14 cdef
PZB 33	j	Seed	1 abc	17 def
Companion	j	Seed	15 defg	16 cdef
DALZ0101	m	Vegetative	15 defg	22 efg
Himeno	j	Vegetative	36 hi	14 cdef
Zorro	m	Vegetative	18 fgh	28 fg
Cavalier	m	Vegetative	17 efg	45 gh
Emerald	jp	Vegetative	3 abc	78 jkl
Zeon	m	Vegetative	35 hi	53 fg
6136	j	Vegetative	31 ghi	58 hij
El Toro	j	Vegetative	37 i	54 hi
Palisades	j	Vegetative	31 ghi	61 hij
Royal	m	Vegetative	36 hi	79 jkl
Empire	j	Vegetative	37 hi	93 lmn
GNZ	j	Vegetative	59 j	73 ijk
Victoria	j	Vegetative	97 kl	88 klm
DeAnza	j	Vegetative	92 kl	94 lmn
6186	j	Vegetative	99 kl	97 mn
Diamond	m	Vegetative	100 l	98 mn
Empress	j	Vegetative	99 kl	99 mn
DALZ0104	m	Vegetative	99 kl	99 n
DALZ0105	m	Vegetative	99 kl	100 n
Mean			26	36

† Winter injury was determined using the following formula: winter injury = 100 – [(after winter dormancy coverage ÷ before winter dormancy coverage) \* 100]. Some cultivars with good cold tolerance had low winter injury <0% because of new growth prior to spring green-up data recording. Plots with <0% winter injury were set to 0% prior to analysis. Data were arcsin transformed prior to analysis and are displayed as backtransformed winter injury for easier interpretation.

‡ Cultivars sorted in ascending order of winter injury (mean of both years).

§ j = *Zoysia japonica*, m = *Zoysia matrella*, s = *Zoysia sinica*, jp = *Z. japonica* x *Z. pacifica*

¶ Type of establishment (propagation) method typically used by practitioners for each cultivar. Cultivars with seed availability are seeded, with other cultivars typically established vegetatively by sprigs, plugs or sod. All cultivars were established by vegetative plugs into field plots for this study.

# Within columns, means followed by the same letter are not significantly different according to Fisher's protected LSD ( $\alpha=0.05$ ).

Table 3.3. Differences in zoysiagrass winter injury by species and by typical type of plant material available for establishment.

Species	Winter injury†		
	2005	2006	
	-backtransformed %-		
<i>Z. japonica</i>	19 a‡	24 a	
<i>Z. matrella</i>	51 b	73 b	
	Type§		
<i>Z. japonica</i>	Seeded	2 a	2 a
<i>Z. japonica</i>	Vegetative	41 b	54 b
<i>Z. matrella</i>	Vegetative	51 b	73 c

† Winter injury was determined using the following formula: winter injury =  $100 - [(after\ winter\ dormancy\ coverage \div before\ winter\ dormancy\ coverage) * 100]$ . Coverage of zoysiagrass was determined using digital image analysis.

‡ Within columns, means followed by the same letter are not significantly different according to Fisher's protected LSD ( $\alpha = 0.05$ ).

§ Type of establishment (propagation) method typically used by practitioners for each cultivar. Cultivars available by seed are typically seeded, with other cultivars typically established vegetatively by sprigs, plugs or sod. All cultivars were established by vegetative plugs into field plots for this study.

Table 3.4. Zoysiagrass leaf width, establishment rate and autumn growth from a field trial established in June 2004 and 2005 in West Lafayette, Indiana.

Cultivar§	Species¶	Leaf width		Establishment rate†		Autumn growth‡	
		2004	2005	2004	2005	2004	2005
		-----mm-----		----log <sub>e</sub> (coverage) d <sup>-1</sup> ----		-----cm <sup>2</sup> -----	
Chinese Common	j	4.8 a#	4.8 a	0.0375 c-f	0.0445 ab	656 f-k	1279 a-e
J-36	j	4.5 abc	4.7 ab	0.0365 c-h	0.0361 f-k	1530 a	821 f-l
Meyer	j	3.3 f-i	2.9 kl	0.0278 opq	0.0337 h-l	357 j-l	808 f-l
PST-R7LT	j	3.4 e-h	3.4 h-k	0.0363 d-h	0.0395 b-g	862 c-g	965 e-j
BMZ 230	j	4.9 a	4.8 a	0.0405 bcd	0.0390 b-h	865 c-g	875 f-k
J-14	s	4.6 abc	4.1 b-g	0.0343 f-j	0.0329 i-m	800 d-h	997 e-i
PST-R7TH	j	3.8 def	3.6 f-j	0.0297 k-q	0.0276 m-q	445 h-l	584 j-n
J-37	j	4.7 ab	4.6 abc	0.0352 f-i	0.0377 c-i	687 e-k	1197 b-f
DALZ0102	j	4.7 ab	4.1 b-f	0.0427 ab	0.0439 ab	851 c-g	1411 abc
PZA 32	j	4.1 b-e	3.8 e-i	0.0365 c-h	0.0308 k-o	1185 abc	448 lmn
PST-R7MA	j	3.4 efg	3.2 i-k	0.0287 n-q	0.0359 f-k	571 g-k	763 g-m
Zenith	j	4.1 b-e	3.9 c-h	0.0330 g-m	0.0408 a-f	1017 b-f	830 f-l
PST-R7ZM	j	4.0 b-e	3.9 d-h	0.0324 h-n	0.0371 d-j	936 b-g	1069 c-h
VJ	j	3.5 efg	3.5 g-k	0.0276 opq	0.0337 h-l	667 f-k	1283 a-e
PZB 33	j	4.0 c-f	4.2 a-f	0.0407 bc	0.0429 abc	832 d-h	1130 c-g
Companion	j	4.4 a-d	4.2 b-f	0.0376 c-f	0.0422 a-e	759 d-i	1000 d-i
DALZ0101	m	1.5 mn	1.8 o	0.0304 j-o	0.0327 i-m	737 d-j	807 f-l
Himeno	j	4.0 b-e	4.0 c-h	0.0338 l-q	0.0256 opq	1071 b-e	680 h-m
Zorro	m	1.9 lm	2.0 o	0.0317 i-o	0.0372 c-j	950 b-g	1390 a-d
Cavalier	m	1.5 mn	1.7 op	0.0259 qr	0.0335 h-l	843 d-h	1149 b-g
Emerald	jp	2.4 jkl	2.4 no	0.0220 r	0.0349 g-k	307 k-l	1073 a
Zeon	m	1.9 lm	1.9 o	0.0273 pq	0.0264 n-q	926 b-g	783 g-m
6136	j	2.6 i-l	2.3 mno	0.0371 c-g	0.0427 a-d	1015 b-f	1526 ab
El Toro	j	4.1 b-e	4.5 a-d	0.0464 a	0.0381 c-i	734 d-j	1300 a-e
Palisades	j	4.1 b-e	3.9 d-h	0.0395 b-e	0.0366 e-j	1275 ab	1449 abc
Royal	m	1.7 mn	1.7 op	0.0288 m-q	0.0237 pq	918 b-g	482 lmn
Empire	j	4.5 a-d	4.3 a-e	0.0360 e-h	0.0146 r	943 b-g	234 n
GNZ	j	2.9 g-j	3.1 jkl	0.0338 f-k	0.0371 d-j	959 b-g	812 f-l
Victoria	j	2.5 jkl	2.7 lmn	0.0273 pq	0.0315 j-n	754 d-j	810 f-l
DeAnza	j	2.7 h-k	2.9 klm	0.0368 c-g	0.0368 e-j	559 g-k	660 i-m
6186	j	3.3 f-i	3.2 mno	0.0436 ab	0.0459 a	1108 bcd	1623 a
Diamond	m	1.1 n	1.2 p	0.0163 s	0.0220 q	131 l	409 mn
Empress	j	2.1 klm	2.1 no	0.0270 pq	0.0291 l-o	367 i-l	557 k-n
DALZ0104	m	1.7 mn	1.7 op	0.0286 n-q	0.0363 f-k	702 e-k	1269 a-e
DALZ0105	m	2.0 lm	2.2 no	0.0335 f-l	0.0231 q	1296 ab	523 k-n
Mean		3.3	3.2	0.0331	0.0344	818	943

† Establishment rate was determined by fitting coverage data across time to the model [ $\log_e(\text{Coverage}) = (K \cdot \text{DAP}) + \log_e(64)$ ], where K is the rate of increase (establishment rate), DAP is days after plugging, and 64 cm<sup>2</sup> is the initial plug coverage (Patton, 2006).

‡ Autumn growth was determined as the increase in coverage (cm<sup>2</sup>) between 6 Sept (91 DAP) and 28 Sept (113 DAP) each year.

§ Cultivars sorted in ascending order of winter injury (Table 3.2).

¶ j = *Zoysia japonica*, m = *Zoysia matrella*, s = *Zoysia sinica*, jp = *Z. japonica* x. *Z. pacifica*

# Within columns, means followed by the same letter are not significantly different according to Fisher's protected LSD ( $\alpha = 0.05$ ).

Table 3.5. Correlation coefficients between winter injury and growth and leaf width for 2005 and 2006.

Growth parameter	Winter injury†	
	2005	2006
Leaf width (mm)		
All cultivars	-0.57 <sup>***</sup>	-0.60 <sup>***</sup>
<i>Z. japonica</i>	-0.70 <sup>***</sup>	-0.53 <sup>**</sup>
<i>Z. matrella</i>	-0.41 <sup>NS</sup>	-0.20 <sup>NS</sup>
Growth		
Establishment rate‡	-0.19 <sup>NS</sup>	-0.37 <sup>*</sup>
<i>Z. japonica</i>	-0.06 <sup>NS</sup>	-0.20 <sup>NS</sup>
<i>Z. matrella</i>	-0.09 <sup>NS</sup>	-0.48 <sup>NS</sup>
Autumn growth§	-0.09 <sup>NS</sup>	-0.15 <sup>NS</sup>
<i>Z. japonica</i>	-0.09 <sup>NS</sup>	-0.03 <sup>NS</sup>
<i>Z. matrella</i>	-0.01 <sup>NS</sup>	-0.42 <sup>NS</sup>

\*, \*\*, \*\*\* Significant at the 0.05, 0.01 and 0.001 probability levels, respectively. NS, non-significant.

† Winter injury was determined using the following formula: winter injury = 100 – [(after winter dormancy coverage ÷ before winter dormancy coverage) \* 100]. Coverage of zoysiagrass was determined using digital image analysis. Backtransformed means were used to calculate correlation coefficients.

‡ Establishment rate was determined by fitting coverage data across time to the model  $[\log_e(\text{Coverage}) = (K * \text{DAP}) + \log_e(64)]$ , where K is the rate of increase (establishment rate), DAP is days after plugging, and 64 cm<sup>2</sup> is the initial plug coverage.

§ Autumn growth was determined as the increase in coverage between 6 September and 28 September each year.

Table 3.6. Freeze tolerance ( $LT_{50}$ , temperature resulting in no regrowth from 50% of the plants) of zoysiagrass plants as determined by nonlinear regression. Plants were grown in a greenhouse for at least 10 wks, cold acclimated in a growth chamber at 8/2°C day/night cycles and a 10-h photoperiod of 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for 4 wks and then placed in a cold stress simulator and subjected to temperatures ranging from -6 to -15°C.

Cultivar	Species and Type†	$LT_{50}$ (°C)‡
Diamond	ZMV	-8.4 ± 0.3§ a¶
Royal	ZMV	-8.5 ± 0.3 a
Zorro	ZMV	-9.0 ± 0.4 ab
Victoria	ZJV	-9.3 ± 0.5 abc
DALZ0101	ZMV	-10.0 ± 0.3 bcd
DALZ0102	ZJV	-10.2 ± 0.5 cd
Cavalier	ZMV	-10.3 ± 0.4 cd
Companion	ZJS	-10.8 ± 0.7 de
J36	ZJS	-10.8 ± 0.8 de
El Toro	ZJV	-10.8 ± 0.7 de
Palisades	ZJV	-11.0 ± 0.5 de
Zenith	ZJS	-11.5 ± 0.5 e
Meyer	ZJV	-11.5 ± 0.8 e

† ZJV, *Zoysia japonica* commercially/experimentally available as vegetative propagules; ZJS, *Zoysia japonica* commercially/experimentally available as seed; ZMV, *Zoysia matrella* commercially/experimentally available as vegetative propagules.

‡  $LT_{50}$  was calculated with the following equation: survival =  $a + (b-a)/(1 + e^{c(T_m-T)})$ , where a is the base line of survival, b is the maximum survival, c is a function of the slope of the line at the inflection point,  $T_m$  is the temperature at the inflection point which is also the  $LT_{50}$ , and T is the treatment temperature.

§ Means of six values ± standard error.

¶ Within columns, means followed by the same letter are not significantly different according to Fisher's protected LSD ( $\alpha = 0.05$ ).

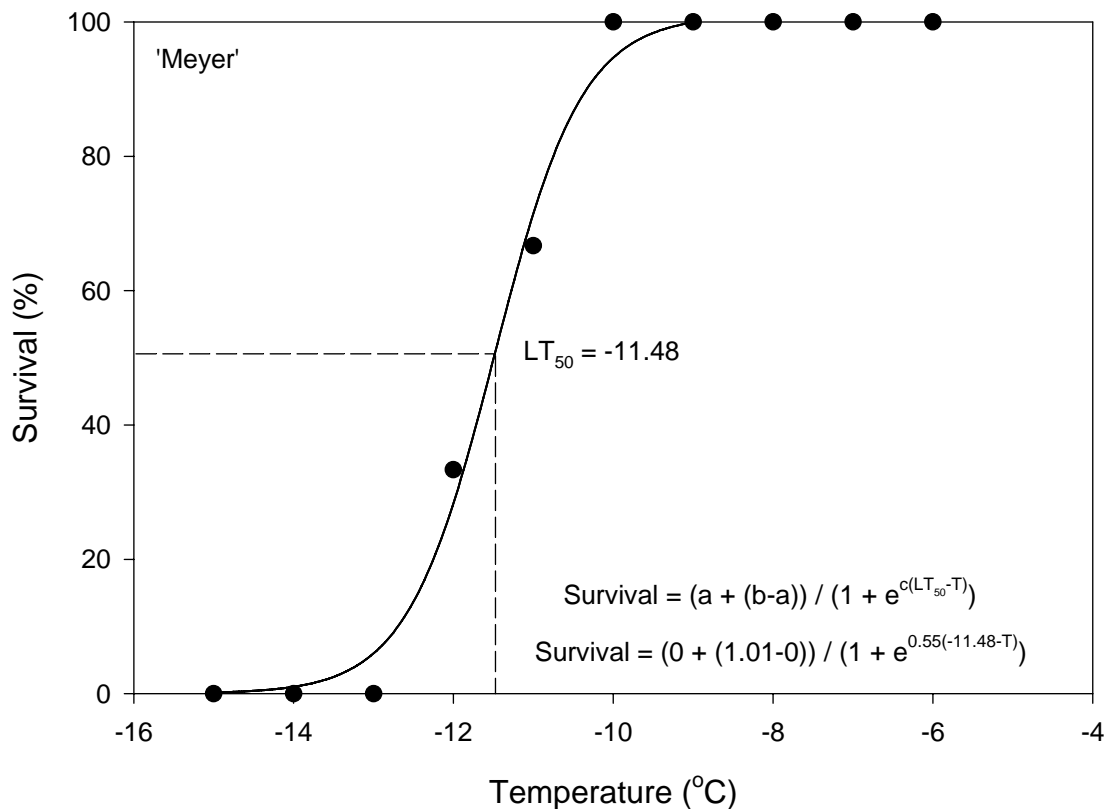


Figure 3.1. Sample sigmoidal curve fit to regrowth data from a pilot study using 'Meyer' zoysiagrass. Freeze tolerance ( $LT_{50}$ , temperature resulting in no regrowth from 50% of the plants) was determined by nonlinear regression [PROC NLIN (Gauss-Newton method)] using the following equation:  $Survival = a + (b-a)/(1 + e^{c(T_m-T)})$  where  $a$  is the base line of survival,  $b$  is the maximum survival,  $c$  is a function of the slope of the line at the inflection point,  $LT_{50}$  is the temperature at the inflection point, and  $T$  is the treatment temperature.

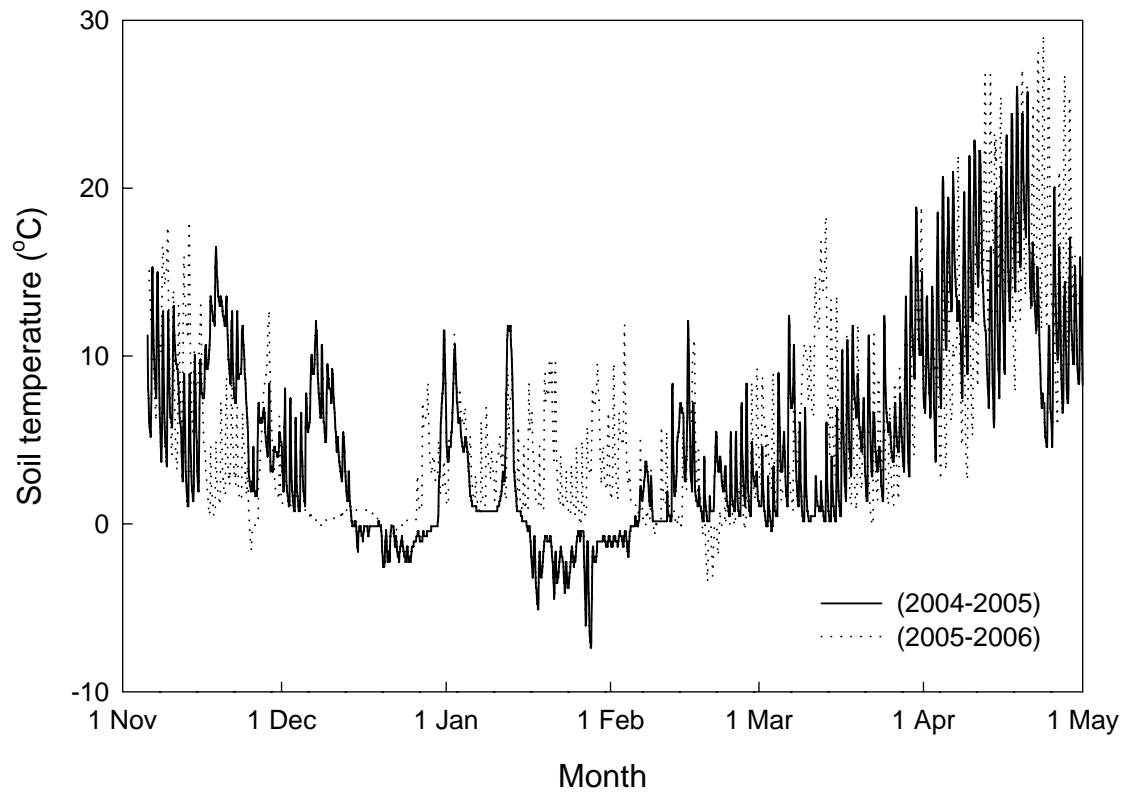


Figure 3.2. Soil temperatures at a 2.5 cm depth during the winter and spring of 2004-2005 and 2005-2006.

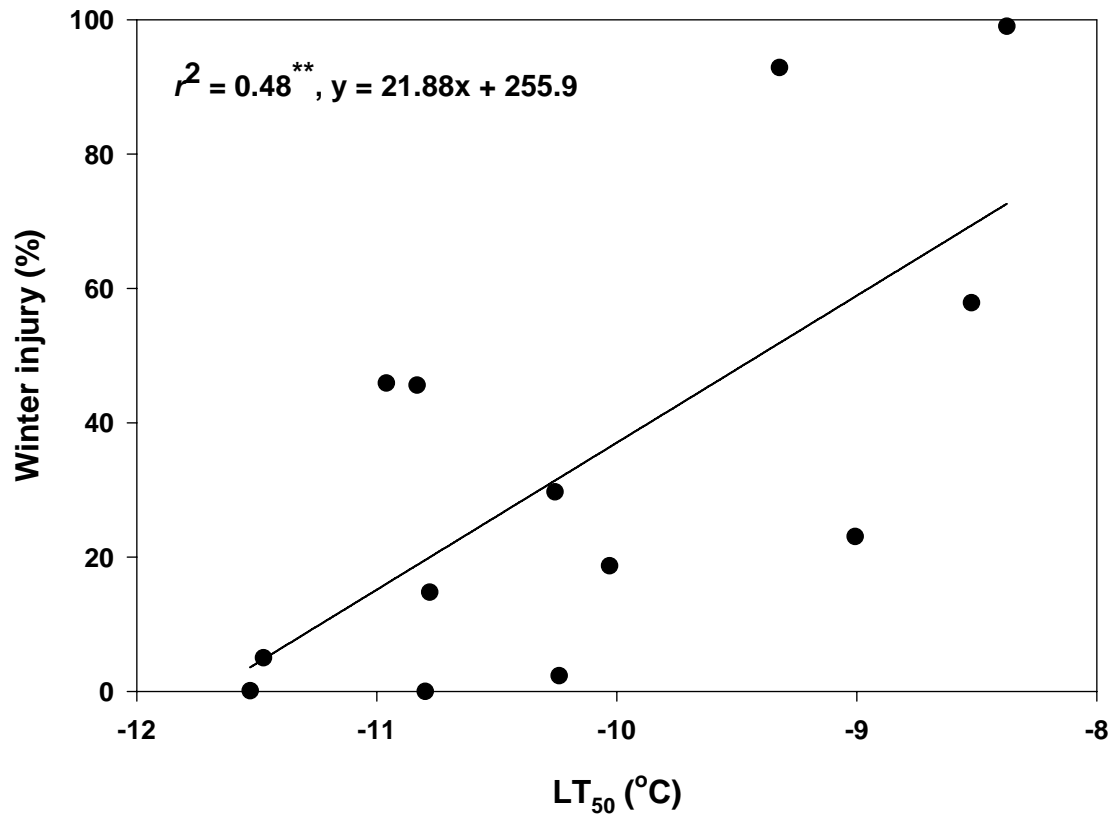


Figure 3.3. Relationship ( $r^2 = 0.48$ ,  $p = 0.0088$ ) between  $LT_{50}$  (growth chamber cold-acclimated and frozen in a cold stress simulator) and winter injury in the field.