GROWTH CHARACTERISTICS AND FREEZING TOLERANCE OF ZOYSIAGRASS

CULTIVARS AND EXPERIMENTAL PROGENY

by

DAVID ODIWUOR OKEYO

B.S., Maseno University, Kenya, 2001
M.S., Maseno University, Kenya, 2005

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
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Abstract

'Meyer' zoysiagrass (*Zoysia japonica* Steud.) has been the predominant cultivar in the transition zone of the U.S. since its release in 1952, primarily because of its good freezing tolerance. However, it is slow to establish and recover after sod harvest, and has poor shade tolerance. I evaluated 'Meyer', some commonly used cultivars, and 18 progeny from crosses of 'Emerald' (*Z. japonica* × *Z. tenuifolia* Willd. ex Thiele) × *Z. japonica* or *Z. matrella* (L.) Merr. × *Z. japonica* for stolon growth characteristics; sod tensile strength and recovery after harvest; shade resistance; freezing tolerance and its relationship to autumn color retention; and the potential influence of dehydrin and chitinase gene expression in freezing tolerance. After planting vegetative plugs, rates of stolon initiation (r = 0.66 in 2007, r = 0.94 in 2008) and elongation (r = 0.66 in 2007, r = 0.53 in 2008) were positively correlated (*P* < 0.05) with zoysiagrass coverage. At 60 days after sod harvest, recovery growth coverage ranged from 17% to 97% and a progeny from *Z. matrella* × Meyer (97% coverage) demonstrated superior sod recovery growth to Meyer (38% coverage). Under 68% silver maple (*Acer saccharinum* L.) tree shade, stolon number was reduced 38 to 95% and stolon length 9 to 70% compared to turf in full sun. Several progeny from crosses between Emerald or a *Z. matrella* x *Z. japonica* produced more and/or longer stolons than Meyer in the shade, suggesting potential for increased shade tolerance. Autumn color in October and November, 2007 was positively correlated (r = 0.44 and r = 0.58, *P* < 0.01) with the lethal temperature killing 50% of tillers (LT$_{50}$) in December, 2007. All grasses except Cavalier and one progeny were equivalent to Meyer in freezing tolerance with LT$_{50}$s ranging from -0.2 to -12.2 $^{\circ}$C. Dehydrin-like (11.9, 23, 44.3, and 66.3 kDa) and chitinase (26.9 kDa) gene expression increased with cold acclimation and was similar among all grasses.

In general, some new zoysiagrass progeny exhibited superior growth and/or stress tolerances compared to Meyer, which bodes well for potential release of a new cultivar for use in the transition zone.

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Approved by:

Major Professor
Dr. Jack D. Fry

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'Meyer' zoysiagrass (*Zoysia japonica* Steud.) has been the predominant cultivar in the transition zone of the U.S. since its release in 1952, primarily because of its good freezing tolerance. However, it is slow to establish and recover after sod harvest, and has poor shade tolerance. I evaluated 'Meyer' and some other commonly used cultivars along with progeny from crosses of 'Emerald' (*Z. japonica* × *Z. tenuifolia* Willd. ex Thiele) × Z. japonica or *Z. matrella* (L.) Merr. × *Z. japonica* for stolon growth characteristics; sod tensile strength and recovery after harvest; shade resistance; freezing tolerance and its relationship to autumn color retention; and the potential influence of dehydrin and chitinase gene expression in freezing tolerance. After planting vegetative plugs, rates of stolon initiation (r = 0.66 in 2007, r = 0.94 in 2008) and elongation (r = 0.66 in 2007, r = 0.53 in 2008) were positively correlated ($P < 0.05$) with zoysiagrass coverage. At 60 days after sod harvest, recovery growth coverage ranged from 17% to 96.8% and a progeny from *Z. matrella* × Meyer (97% coverage) demonstrated superior sod recovery growth to Meyer (38% coverage). Under 68% silver maple (*Acer saccharinum* L.) tree shade, stolon number was reduced 38 to 95% and stolon length 9 to 70% compared to turf in full sun. Several progeny from crosses between Emerald or a *Z. matrella* × *Z. japonica* produced more and/or longer stolons than Meyer in the shade, suggesting potential for increased shade tolerance. Autumn color in October and November, 2007 was positively correlated (r = 0.44 and r = 0.58, $P < 0.01$) with the lethal temperature killing 50% of tillers (LT_{50}) in December, 2007. All grasses except Cavalier and one progeny were equivalent to Meyer in freezing tolerance with LT_{50}s ranging from -0.2 to -12.2 ^{o}C. Dehydrin-like (11.9, 23, 44.3, and 66.3 kDa) and chitinase (26.9 kDa) gene expression increased with cold acclimation and was similar among all grasses.

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Dedication

I dedicate this work to my late father, Shadrack Okeyo Adel, who insisted on my

university education even when I was reluctant after graduating from high school.

CHAPTER 1 - STOLON GROWTH CHARACTERISTICS AND ESTABLISHMENT RATES OF ZOYSIAGRASS

This paper is written in the format for *HortScience*

Abstract

Zoysiagrass (*Zoysia* spp.) is recognized for its low requirements for pesticide and fertilizer input, but 'Meyer' (*Z. japonica* Steud.), the cultivar commonly used in the transition zone of the U.S., is slow to establish. I evaluated new zoysiagrass progeny for stolon growth characteristics, rate of establishment, and determined the relationship between stolon growth characteristics and coverage. Meyer, DALZ 0102, and eighteen progeny from crosses of 'Emerald' (*Z. japonica* × *Z. tenuifolia* Willd. ex Thiele) or a *Z. matrella* (L.) Merr. × *Z. japonica*, were planted as 6-cm diam. plugs on 30.5 x 30.5 cm centers in 1.5 by 1.5 m plots in 2007, and as single 10-cm diam. plugs in 1.2 by 1.2 m plots in 2008 in Manhattan, KS. Data were collected weekly on stolon number per plug, stolon elongation, and number of stolon branches. Coverage was rated visually by two researchers near the end of each growing season. Rate of stolon initiation ranged from 2.2 wk^{-1} to 8.6 wk^{-1}. Elongation rate ranged from 18.8 to 65.1 mm wk^{-1}. At 11 weeks after planting in 2007, four of 18 progeny had superior coverage to 'Meyer'; while at 11 weeks after planting in 2008, 13 of 18 progeny had superior coverage to 'Meyer'. Rate of stolon initiation was positively correlated ($P < 0.01$) with zoysiagrass coverage (r = 0.66, in 2007; r = 0.94 in 2008); likewise, stolon elongation was positively correlated with coverage in 2007 (r = 0.52, $P < 0.01$) and 2008 (r = 0.53, $P < 0.05$). Greater stolon initiation or elongation of experimental zoysiagrass progeny make them promising for overcoming slow establishment rates commonly observed with Meyer.

INTRODUCTION

Zoysiagrass is a desirable turfgrass species for use in the transition zone due to its heat, drought, and wear tolerance, and resistance to pests (Fry and Huang, 2004). 'Meyer' zoysiagrass, a *Z. japonica* cultivar, has been the predominant cultivar used in the transition zone since its release in 1952, primarily because of its good freezing tolerance (Fry et al., 2008). Since the release of 'Meyer', researchers at Texas A&M University have developed several *Z. matrella* cultivars with high turf quality, including better density, finer texture, and better color compared to 'Meyer'. Among these cultivars are 'Diamond', 'Cavalier', and 'Zorro'. Unfortunately, these cultivars are not suitable for use in the transition zone due to lack of freezing tolerance (Fry and Huang, 2004).

Since 2004, turfgrass researchers at Kansas State University have evaluated over 600 new zoysiagrass progeny for winter survival and quality. These progeny were the result of genotypic crosses made at Texas A&M-Dallas, most of which involved one parent from *Z. japonica* and one from a *Z. matrella* cultivar or Emerald zoysiagrass. The ideal result would be a dense, fine textured zoysiagrass with quality similar to the aforementioned *Z. matrella*, but freezing tolerance as good or better than 'Meyer'.

One of the primary complaints regarding 'Meyer' from transition zone turf managers is its slow rate of vegetative establishment (Patton et al, 2007b; Patton et al, 2006; Zuk and Fry, 2005; Patton et al, 2004). This, according to Patton et al. (2004, 2006, and 2007), hinders widespread use of zoysiagrass. Although cultural practices have been evaluated for their effects on rate of zoysiagrass establishment, most of them have been shown to have little effect (Patton et al., 2007b; Richardson and Boyd, 2001; Fry and Dernoeden, 1987).

Researchers have shown that zoysiagrasses vary widely in establishment rate, depending on genotype. Cultivars of *Z. japonica* have been reported to have the fastest establishment rate,

followed by *Z. matrella* , and then *Z. tenuifolia* (Brosnan and Deputy, 2008; Patton et al., 2007b).

Fry (1984) compared the establishment rate and stolon growth characteristics of five *Z. japonicas*, 'Emerald', and a *Z. matrella* cultivar in Maryland. When planted as 5-cm diam. plugs on 30 cm centers, 'Midwest', a *Z. japonica*, and 'Bel-Zrt-1', an experimental *Z. japonica*, had the greatest coverage rate. Coverage of 'Meyer' was comparable to 'Belair' (*Z. japonica*), 'Emerald', and *Z. matrella*. The *Z. matrella* produced the greatest number of stolons, but all others were similar. 'Bel-Zrt-1' and 'Midwest' had the longest stolons, but 'Meyer' had the most nodes per stolon.

According to Patton et al. (2007a, b), *Z. japonica* cultivars that had been seeded or planted vegetatively had greater coverage 91 days after planting than vegetatively established *Z. matrella* cultivars in Indiana. In particular, the *Z. japonicas* DALZ 0102, 'El Toro', and 'Chinese common' were among those exhibiting the fastest rate of coverage. Conversely, the *Z. matrellas* 'Cavalier,' 'Diamond,' and 'Emerald,' were among the slowest cultivars both in terms of rate of coverage and stolon elongation rates. 'Zorro', another *Z. matrella*, had a faster establishment rate than 'Cavalier', 'Diamond', or 'Meyer' (Patton et al., 2007a, b).

In southern California, a comparison of establishment rates of 'El Toro' (*Z. japonica*), 'Emerald', and a selection from *Z. matrella*, indicated that 'El Toro' was fully established in three months, whereas 'Emerald' and the *Z. matrella* required four months for complete cover, whether established from plugs or sprigs (Gibeault and Cockerham, 1988).

More information is needed on growth characteristics and establishment rates of promising zoysiagrasses that could be used in the transition zone. Therefore, the objective was

to evaluate the stolon growth characteristics and establishment rates of zoysiagrasses and

determine the relationship among these characteristics and coverage.

MATERIALS AND METHODS

Two separate studies were conducted at the Rocky Ford Turfgrass Research Center, Manhattan, Kansas (39.128N longitude, 96.358W latitude) in 2007 (Study I) and 2008 (Study II). Soil was a Chase silt loam (fine, montmorillonitic, mesic, Aquic, Argiudolls). At the beginning of the study, soil pH was 7.3, P level was 123 mg kg^{-1} and K level was 475 mg kg^{-1}. Just after planting, oxadiazon [5-tert-butyl-3-(2, 4-dichloro-5-isopropoxyphenyl)-1, 3, 4-oxadiazol-2(3H)-one] at a rate of 3.4 kg a.i. ha^{-1} was applied to prevent emergence of annual grasses. Irrigation was applied three days weekly during each study period to provide approximately 2.5 cm water per week. No mowing was performed during either year. A weather station located within 100 m of the study area was used to monitor air temperature. In addition, a soil-encapsulated thermocouple assembled according to Ham and Senock (1992) was installed at 2.5 cm deep in one plot of each replicate to monitor soil temperature. Soil temperature was recorded hourly using a CR-10 (Campbell Scientific, Inc. Logan, UT) data logger. Average daily minimum and maximum air and soil temperatures at the study site at Manhattan, Kansas in 2007 and 2008 are presented in Fig. 1-8 and 1-9, respectively. Total monthly precipitation was obtained from the K-State Research and Extension weather data library (www.ksre.ksu.edu/wdl/).

Study I - 2007

Eighteen zoysiagrass progeny from crosses of 'Emerald' (*Z. japonica* × *Z. tenuifolia* Willd. ex Thiele) or a *Z. matrella* (L.) Merr. × *Z. japonica* along with 'Meyer' and DALZ 0102, another experimental *Z. japonica* that has been evaluated extensively, were propagated from 2-node stolon cuttings in 8-cm diam. pots in the greenhouse beginning in autumn, 2006. Another of the *Z. matrella* lines, listed as 8501 in Table 1-1, was never commercially released. Growing medium was a standard greenhouse potting mix (Metro-Mix® 510, SUNGRO Horticulture,

Bellevue, WA). The greenhouse was set to provide a 30 $^{\circ}$C/25 $^{\circ}$C day/night temperature, and a 14 h photo period was achieved using supplemental lighting of up to 580 µmol m^{-2} s^{-1} at the canopy level (Zhang, 2007). Grasses were watered twice weekly to thoroughly wet the root zone, and mowed using a scissor at 4 cm once weekly after grasses had grown to that height.

On 5 June 2007, sixteen 6-cm diameter plugs of each zoysiagrass were planted on 30.5 cm by 30.5 cm centers in 1.5 m by 1.5 m plots at the Rocky Ford Turfgrass Research Center, Manhattan, Kansas. The plots were arranged in a randomized complete block with three replications per progeny or cultivar. Urea was applied to provide N at 49 kg ha^{-1} on 12 July and 31 August 2007.

Data were collected on the number of stolons, stolon elongation, number of stolon branches, and percent plot coverage. The number of stolons, stolon elongation, and number of branches were determined weekly beginning on 18 June in 2007 using three randomly selected plugs from each plot. Stolon initiations were determined by counting the number of stolons originating from each plug. Stolon elongation and branching were evaluated on each of the first three stolons emerging from each of three randomly selected plugs in 2007 (Fig. 1-1). Each of the stolons used to evaluate elongation and branching was labeled with a loose knot of thread tied around the stolon to facilitate its identity. Elongation was determined by inserting a colored plastic toothpick in the ground at the tip of the stolon. The next week, after elongation had occurred, the distance was measured from the end of the stolon back to the location of the toothpick. Branching was determined by counting the number of new branches that were atleast 2-cm long on the same stolons used for elongation measurements. Percentage coverage was rated visually by two researchers on 24 August and 24 September 2007 using a 0 to 100% scale.

Study II - 2008

The same zoysiagrass progeny used in Study I, along with 'Meyer' and DALZ 0102, were sampled as single 10-cm diam. plugs from plots at the Olathe Horticulture Research center, Olathe, Kansas on 23 June 2008. Turf in Olathe had been planted on 5 June 2007 and maintained under home lawn conditions until sampling. Each of the individual 10-cm diam. plugs was planted in the center of a 1.2 m by 1.2 m plot at the Rocky Ford Research Center in Manhattan on 24 June 2008. Plots were arranged in a randomized complete block design with three replications. Urea was applied to provide N at 49 kg ha^{-1} on 14 July and 12 August 2008.

Data collection on stolon initiation, elongation, and branching began weekly starting on 17 July 2008. Stolon initiations were determined by counting all stolons from the single plug planted in each plot. Elongation and branching were determined as described previously for the first three stolons that emerged from each plug. Percent plot coverage was rated visually by two researchers on 4 September.

Data Analysis

Stolon initiation data were averaged over the three plugs per plot in 2007, as were stolon length and number of branches for nine stolons per plot in 2007 and three stolons per plot in 2008. Data on percent coverage taken by two researchers were also averaged to obtain a single value for each of plot. Both stolon growth data and coverage were square-root transformed before analysis. Stolon growth data were subjected to linear regression analysis to determine slopes of lines (rate) using PROC REG (SAS Institute, Cary, NC, 2003). Progeny and cultivars were then compared using Bonferroni's t test (Hochberg and Tamhane, 1987) at $P < 0.05$ (adjusted for multiple comparison). Coverage data were subjected to analysis of variance using SAS, and means were separated using the Ryan-Einot-Gabriel-Welsch (REGWQ) mean

separation test (Hochberg and Tamhane, 1987; Mickey et al., 2004) at $P < 0.05$ (SAS Institute, Cary, NC, 2003). Correlation analysis was done to determine the relationships among rates of stolon initiation, elongation, and branching in each year, and percentage coverage (24 Aug. and 24 Sept. 2007, and 24 Sept. 2008) using PROC CORR (SAS Institute, Cary, NC, 2003).

RESULTS AND DISCUSSION

Stolon growth characteristics

Rate of stolon initiation in Study I in 2007 ranged from 2.2 wk^{-1} (progeny 5321-48, Fig. 1-2D) to 6.3 wk^{-1} (5311-26, Fig. 1-2B) (Table 1-1). All grasses in the 'Cavalier' × 'Chinese Common' cross had higher stolon initiation rates than 'Meyer' (2.9 wk^{-1}), as did 'Cavalier' × 'Meyer' (5283-27), one progeny from 'Emerald' × 'Meyer' (5321-3), and two progeny from 8501 × 'Meyer' (5324-18 and 5324-27, Fig. 1-2E).

In Study II in 2008, stolon initiation rates were generally greater than in 2007 (Table 1-1, Fig. 1-3A-F). Differences in plug size, planting time, and growth period likely influenced stolon production. In addition, average monthly precipitation was generally higher at the peak growth period (June to August) in 2008 compared to 2007, which could have promoted vigorous growth (Table 1-3). The highest stolon initiation rate occurred with progeny 5321-3 (8.6 wk^{-1}) and the lowest rate with progeny 5321-48 (3.2 wk^{-1}). Only 5321-3 (Fig. 1-3D) and 5324-18 (Fig. 1-3E) had higher stolon initiation rates than 'Meyer' (3.4 wk^{-1}). Fry (1984) reported that *Z. matrella* produced twice as many stolons as other *Z. japonica* cultivars in Maryland at the end of a full season of growth from plugs.

Stolon elongation in 2007 ranged from 22 mm wk^{-1} (5321-24, Fig. 1-4D) to 62 mm wk^{-1} (5312-49, Fig. 1-4C) (Table 1-1). Only 5312-49 had a faster rate of stolon elongation than 'Meyer' (38 mm wk^{-1}). In 2008, stolon elongation rate ranged from 19 mm wk^{-1} (5321-48, Fig. 1-5D) to 65 mm wk^{-1} (5312-49, Fig. 1-5C) (Table 1-1). Only the progeny from 'Zorro' × 'Chinese common' (5312-36 and 5312-49) had a higher rate of elongation than 'Meyer' (26.8 wk^{-1}). In West Lafayette, IN, elongation rates observed across several *Z. japonica* and *Z. matrella* cultivars ranged from 11.9 mm wk^{-1} to 79.1 mm wk^{-1} (Patton et al., 2007a). I observed

elongation rates that were comparable to these (19 to 65 mm wk^{-1}). Earlier reports of stolon

elongation rates for 'Meyer' (35 mm wk^{-1}) by Patton et al. (2007a) were similar to what I

observed (38 mm wk^{-1} in 2007 and 26.8 mm wk^{-1} in 2008). Stolon elongation rates reported for

DALZ 0102 (53.9 mm wk^{-1}) by Patton et al. (2007a) were somewhat higher than what I observed

(26.4 mm wk^{-1} in 2007 and 36.3 mm wk^{-1} in 2008).

Stolon branching in 2007 ranged from 2.3 wk^{-1} (5324-27) to 7.1 wk^{-1} (5324-53) (Table 1-

1, Fig. 1-6E). 'Meyer' was not significantly different from any progeny in rate of stolon

branching. In 2008, rate of stolon branching ranged from 1.8 wk^{-1} (5311-3, Fig. 1-7B) to 7.7 wk^{-1} (5324-18, Fig. 1-7E) (Table 1-1). Similarly, none of the grasses was different from 'Meyer' in

rate of branching.

Coverage

In Study I on 24 Aug. 2007, coverage ranged from 42.5% (5321-24) to 78.3% (5311-22)

(Table 1-2). Progeny exhibiting higher levels of coverage than 'Meyer' (55.8%) were 5311-22,

5311-26, 5321-3, and 5324-18. On 24 Sept. 2007, coverage ranged from 73.3% (5321-48) to

99% (5321-3). In Study II, coverage of 'Meyer' (94.7%) was not significantly different from all

grasses except 5321-24 and 5321-48, which were lower. Coverage on 4 Sept. 2008 ranged from

50% (5321-48) to 95% (5321-3). All grasses were superior to 'Meyer' (50%) except 5311-26,

5321-45, 5321-48, 5324-52, and 5327-19.

Coverage at the end of 2008 was generally lower than that measured at the end of 2007

(Table 1-2). Such differences were likely the result of a later planting date and slower spread in

2008 when individual plugs were evaluated instead of numerous plugs (with more overlapping

stolons) as was the case in 2007. In 2007, initial coverage at planting was 0.045 m^2 (2%) of the

2.25 m^2 plot, indicating a change in coverage to between 0.956 m^2 (40.5% increase) and 1.762

m^2 (76.3% increase). In 2008, the plot area covered at planting was 0.008 m^2 (0.5% of the plot) compared to between 0.713 m^2 (49.5% increase) and 1.361 m^2 (a 94.5% increase). Average ground coverage of *Z. japonica* cultivars after a period of 91 days after planting in Indiana was 0.23 m^2; whereas, coverage of cultivars of *Z. matrella* was 0.13 m^2 (Patton et al, 2007a). In the same evaluation, 'Meyer' (0.04 m^2) also had greater coverage than 'Cavalier' (0.04 m^2) or 'Diamond' (0.02 m^2) at 59 days after planting. In Maryland (Fry, 1984), coverage of zoysiagrass planted using the same plot arrangements as in my evaluation in 2007 was lower (45 to 60%), which may have been the result of a sandier soil at that location. In that evaluation, 'Meyer' coverage was comparable to that of a *Z. matrella* and 'Emerald' zoysiagrass at the end of the first growing season. In the second growing year, significant winter injury had occurred to the *Z. matrella* and 'Emerald', resulting in low percentage coverage ratings.

My findings indicate that some new zoysiagrass progeny have faster establishment rates than 'Meyer', which should increase their acceptance by sod producers and homeowners who choose to establish these grasses from vegetative plugs. In particular, all six crosses of 'Cavalier', traditionally a relatively slow spreading cultivar, with 'Chinese Common', a fast-spreading type, resulted in progeny that had superior coverage compared to 'Meyer' on at least one of the three rating dates. Both crosses evaluated from 'Zorro' × 'Chinese Common' (5312-36 and 5312-49) were superior in coverage to 'Meyer' on the 4 Sept. 2008 rating date. Some progeny originating from parents not recognized for quick coverage also exhibited superior coverage to 'Meyer' on at least one rating date, including those from 'Emerald' × 'Meyer' (5321-3) and 8501 × 'Meyer' (5324-18 and 5324-27).

Correlation between Stolon Characteristics and Coverage

In both years, rate of stolon initiation was positively correlated with stolon elongation and coverage (Table 1-4). Stolon elongation was positively correlated with stolon branching in 2007 and coverage in both years. Similarly, positive correlations were observed between coverage (24 Sept. 2007) and stolon initiation ($r = 0.58$, $P = 0.008$), stolon elongation ($r = 0.67$, $P = 0.001$), and stolon branching ($r = 0.48$, $P = 0.032$). Patton et al. (2007a, b) also found that the zoysiagrass cultivars which had faster rates of stolon elongation had faster rates of establishment. In Fayetteville, AR (Richardson and Bordelon, 2000), 'Cavalier' established faster from sprigs than 'El Toro' and 'Meyer'. Although measurements were not taken, they suggested this was because it had higher rates of stolon initiation and elongation, thus more stolon number per unit area with more growth points per unit area. In southern California, turfgrasses with higher stolon initiation had the fastest rates of plug growth and establishment among 'El Toro', 'Emerald', and a selection from *Z. matrella* (Gibeault and Cockerham, 1988). A correlation between both stolon initiation or elongation rates, and coverage, means the faster stolons are initiated or elongate, the faster the development of more vegetative growth points that will lead to faster establishment.

In summary, I found that zoysiagrass cultivars and progeny vary widely in the rates of stolon initiation and elongation, and both parameters were positively correlated with percentage coverage. The positive relationship between stolon initiation or elongation and coverage indicates that short-term evaluations of the former could be used to predict rate of zoysiagrass coverage from plugs. Several zoysiagrass progeny evaluated had superior rates of coverage compared to 'Meyer' zoysiagrass, which will make them more attractive to sod producers and homeowners.

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Figure 1-1. Measuring stolon growth.
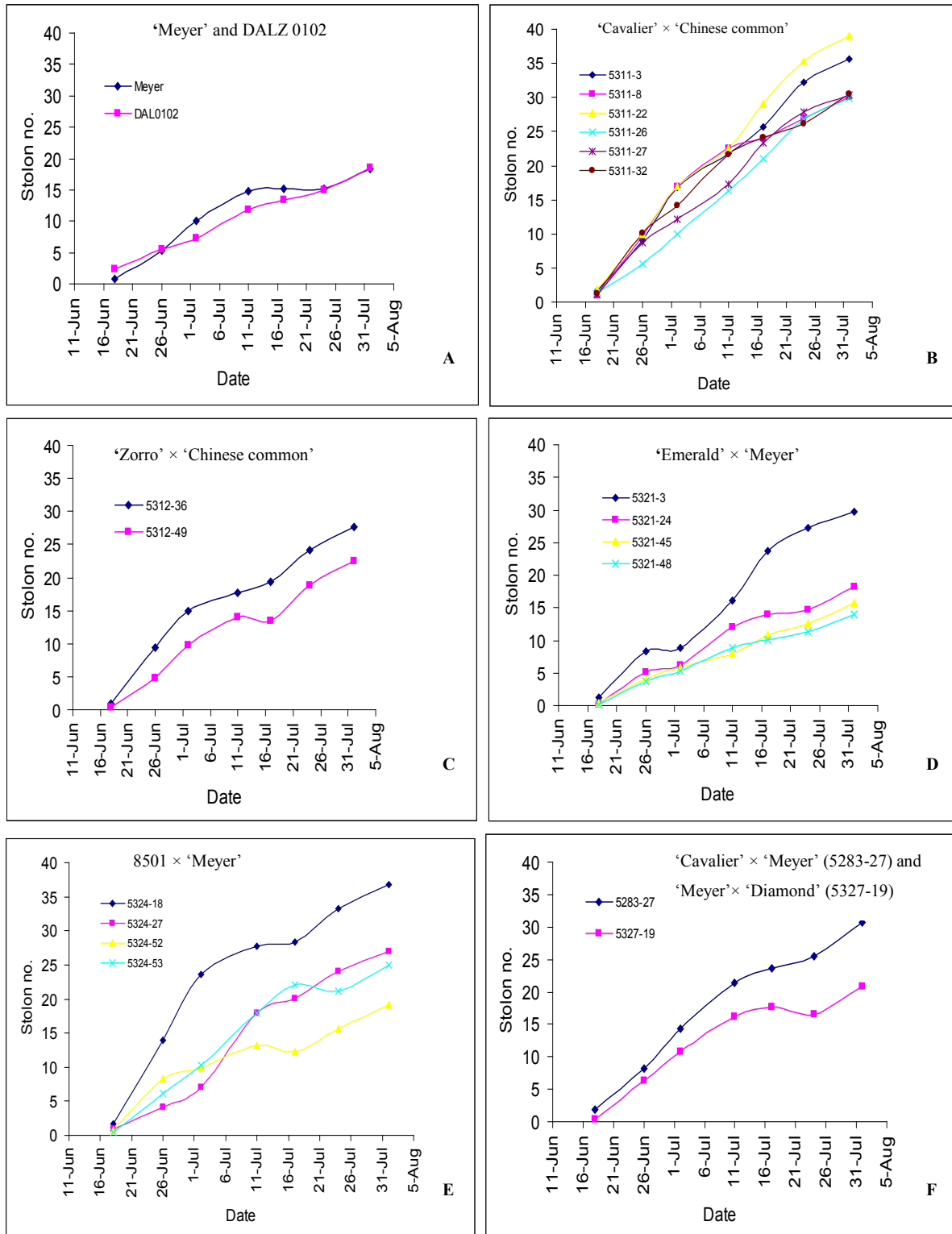
Figure 1-2. Cumulative stolon number of zoysiagrass cultivars and progeny planted as sixteen 6-cm diameter plugs on 30.5-cm centers in 1.5 by 1.5 m plots on 5 June, 2007. Headings at the top of each figure indicate parents of the progeny presented.

16
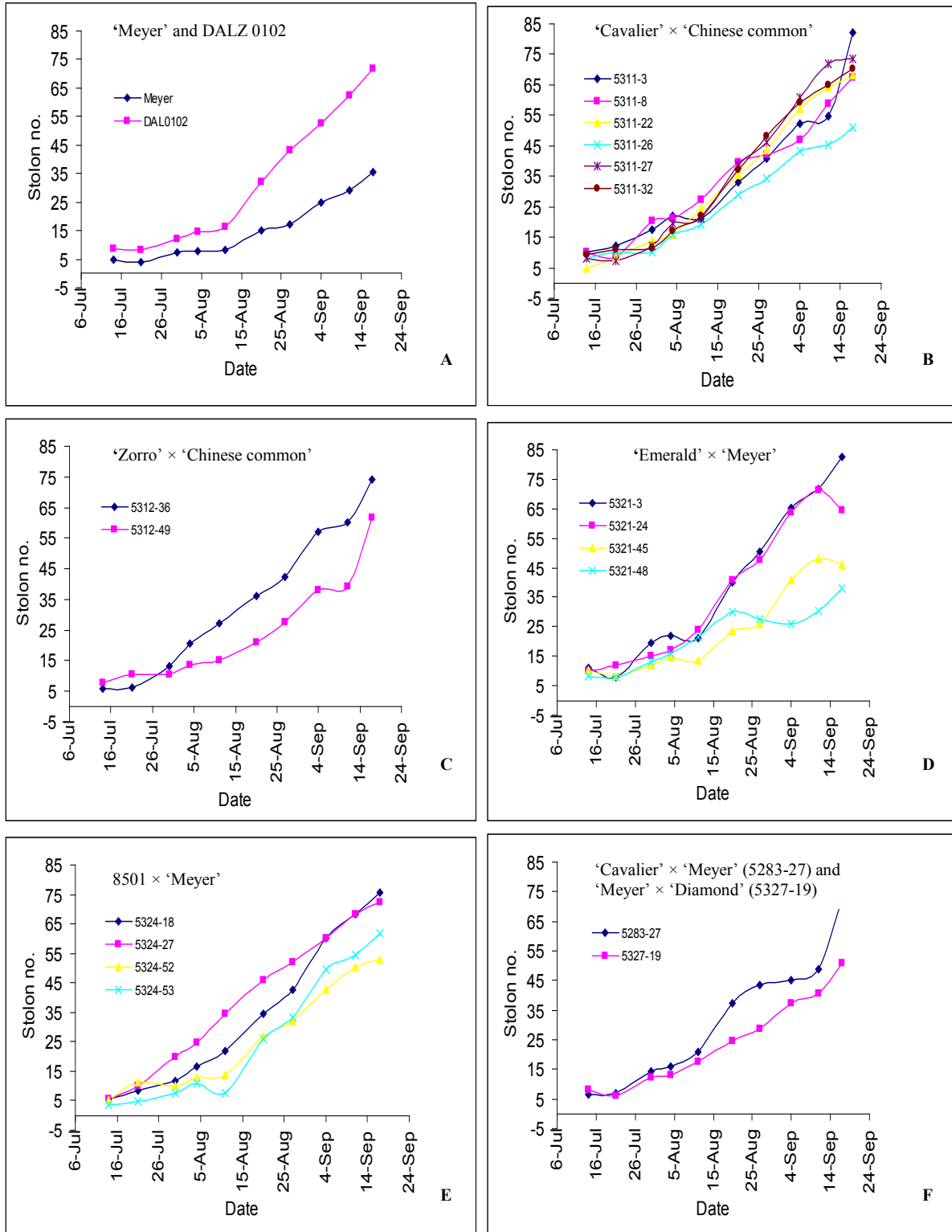
Figure 1-3. Cumulative stolon number of zoysiagrass cultivars and progeny planted as one 10-cm diameter plugs in the center of 1.2 by 1.2 m plots on 24 June, 2008.

Headings at the top of each figure indicate parents of the progeny presented.

17
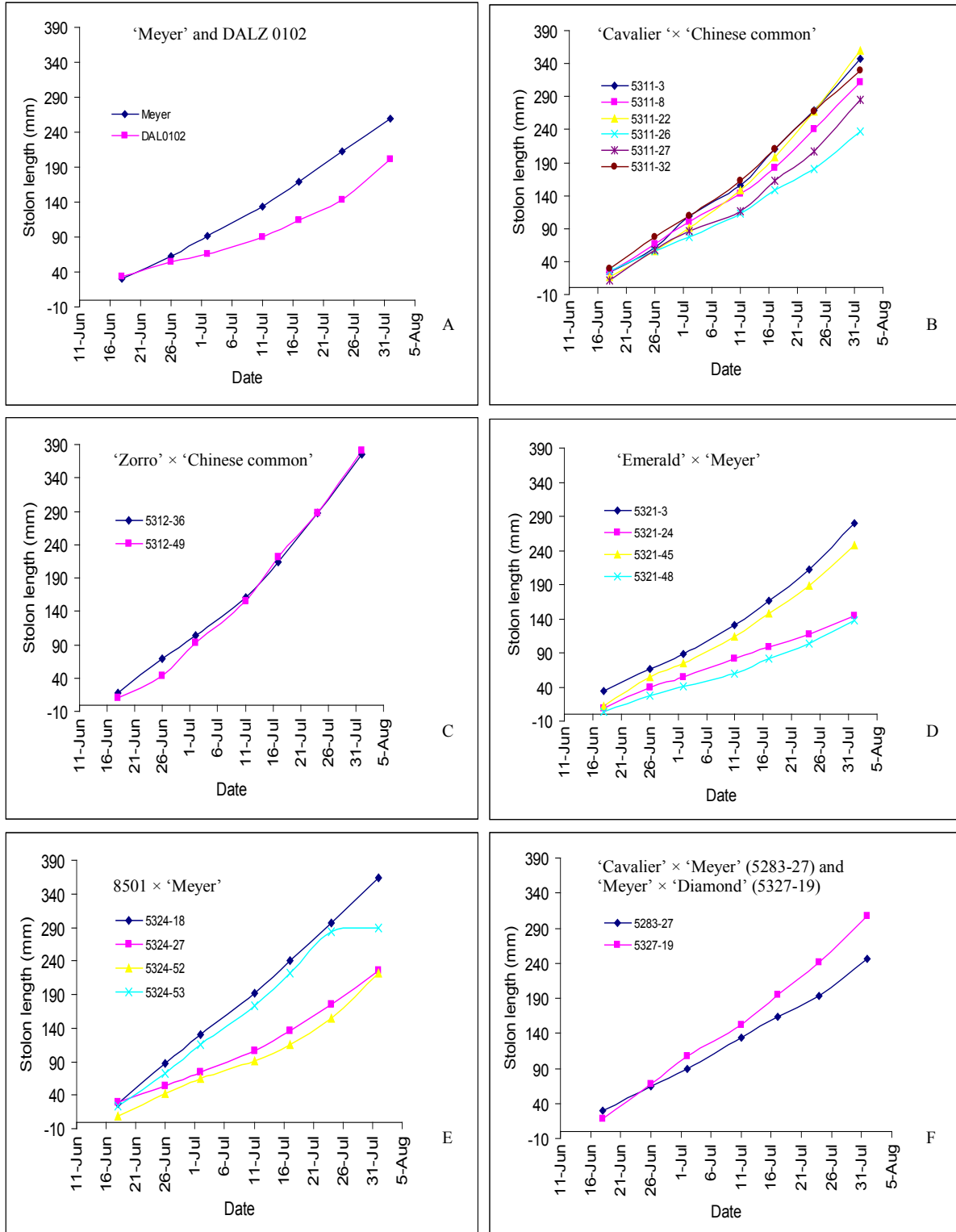
Figure 1-4. Cumulative stolon length of zoysiagrass cultivars and progeny planted as sixteen 6-cm diameter plugs on 30.5-cm centers in 1.5 by 1.5 m plots on 5 June, 2007. Headings at the top of each figure indicate parents of the progeny presented.

18
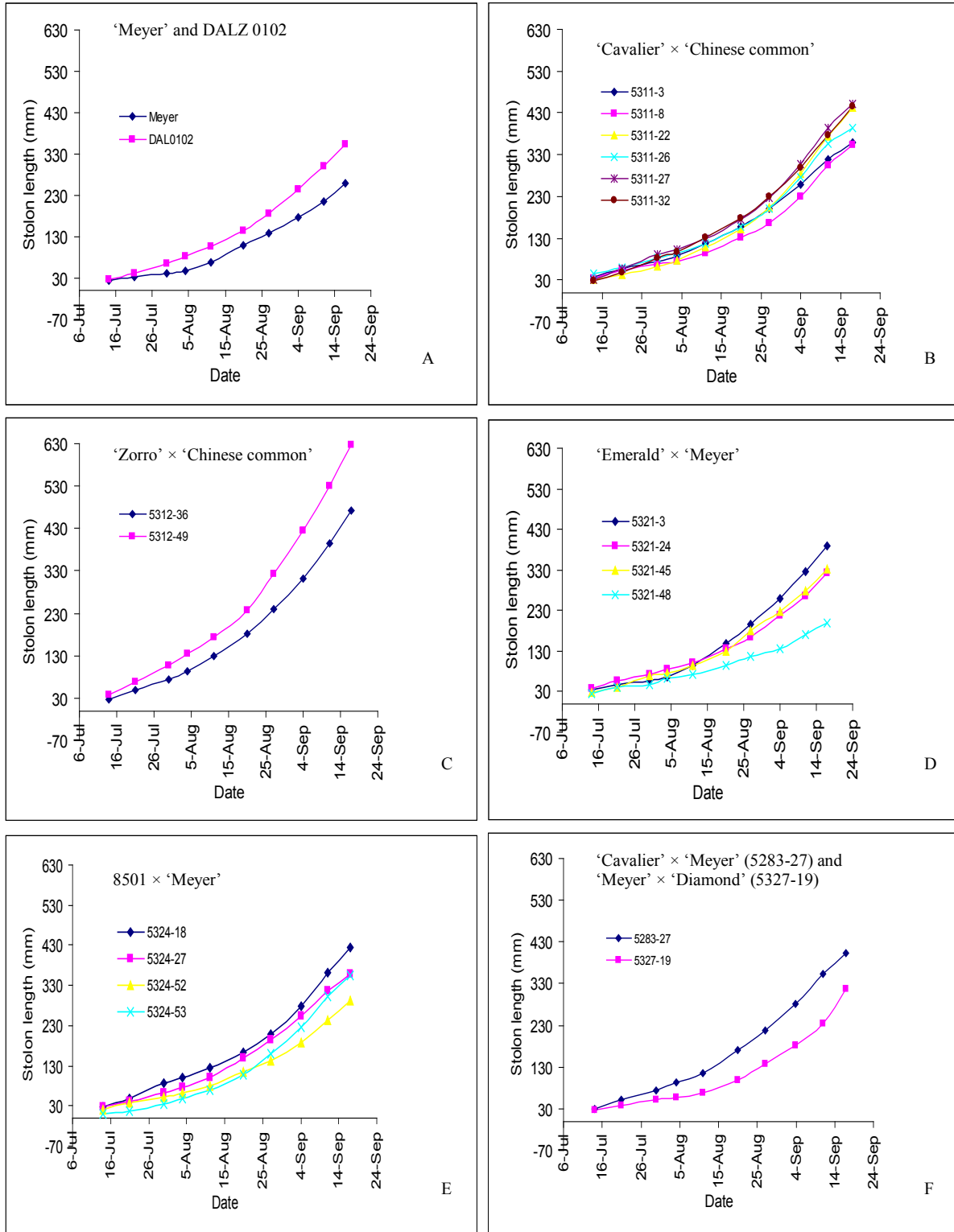
Figure 1-5. Cumulative stolon length of zoysiagrass cultivars and progeny planted as one 10-cm diameter plugs in the center of 1.2 by 1.2 m plots on 24 June, 2008.

Headings at the top of each figure indicate parents of the progeny presented.

19
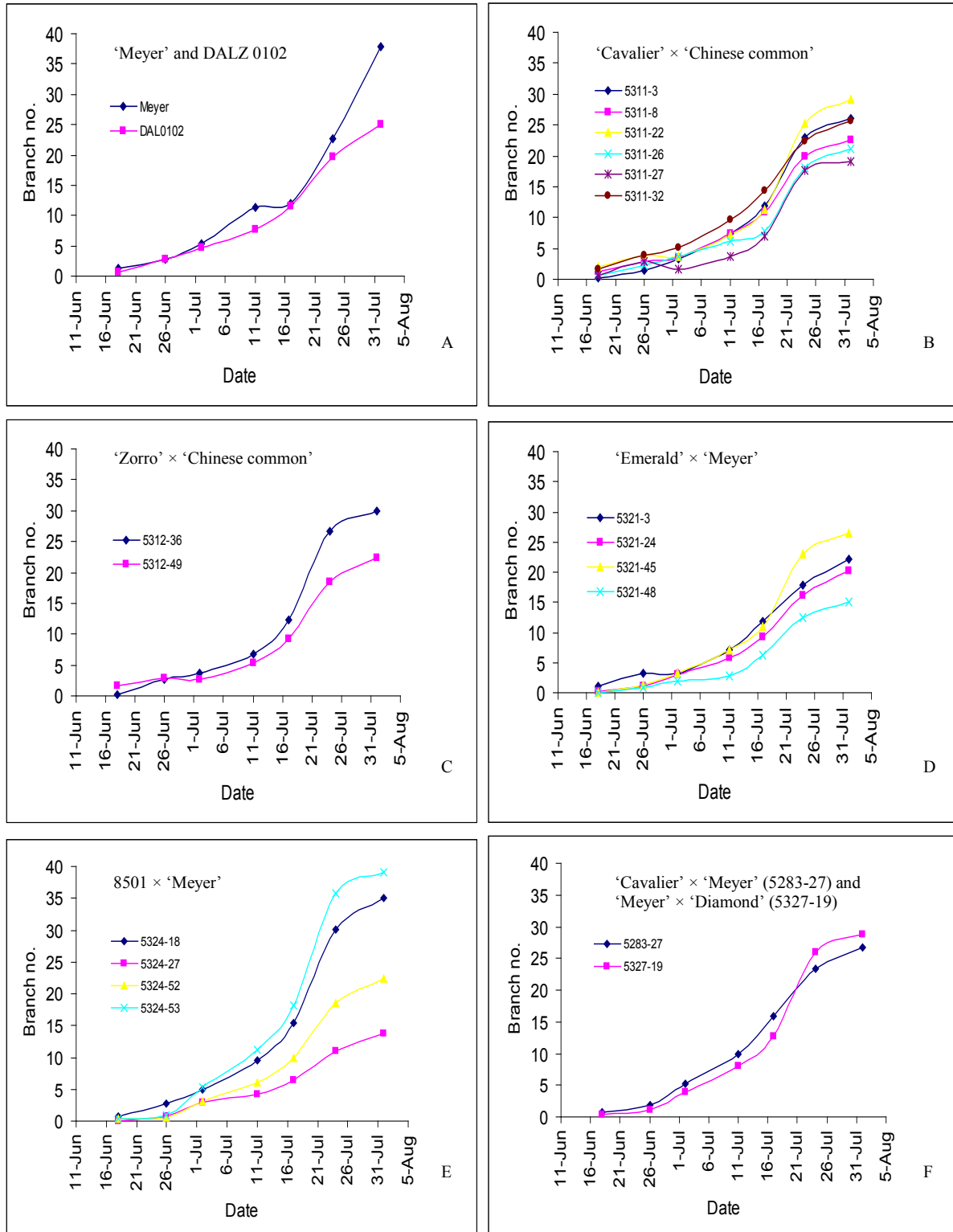
Figure 1-6. Cumulative stolon branch number of zoysiagrass cultivars and progeny planted as sixteen 6-cm diameter plugs on 30.5-cm centers in 1.5 by 1.5 m plots on 5 June, 2007. Headings at the top of each figure indicate parents of the progeny presented.

20
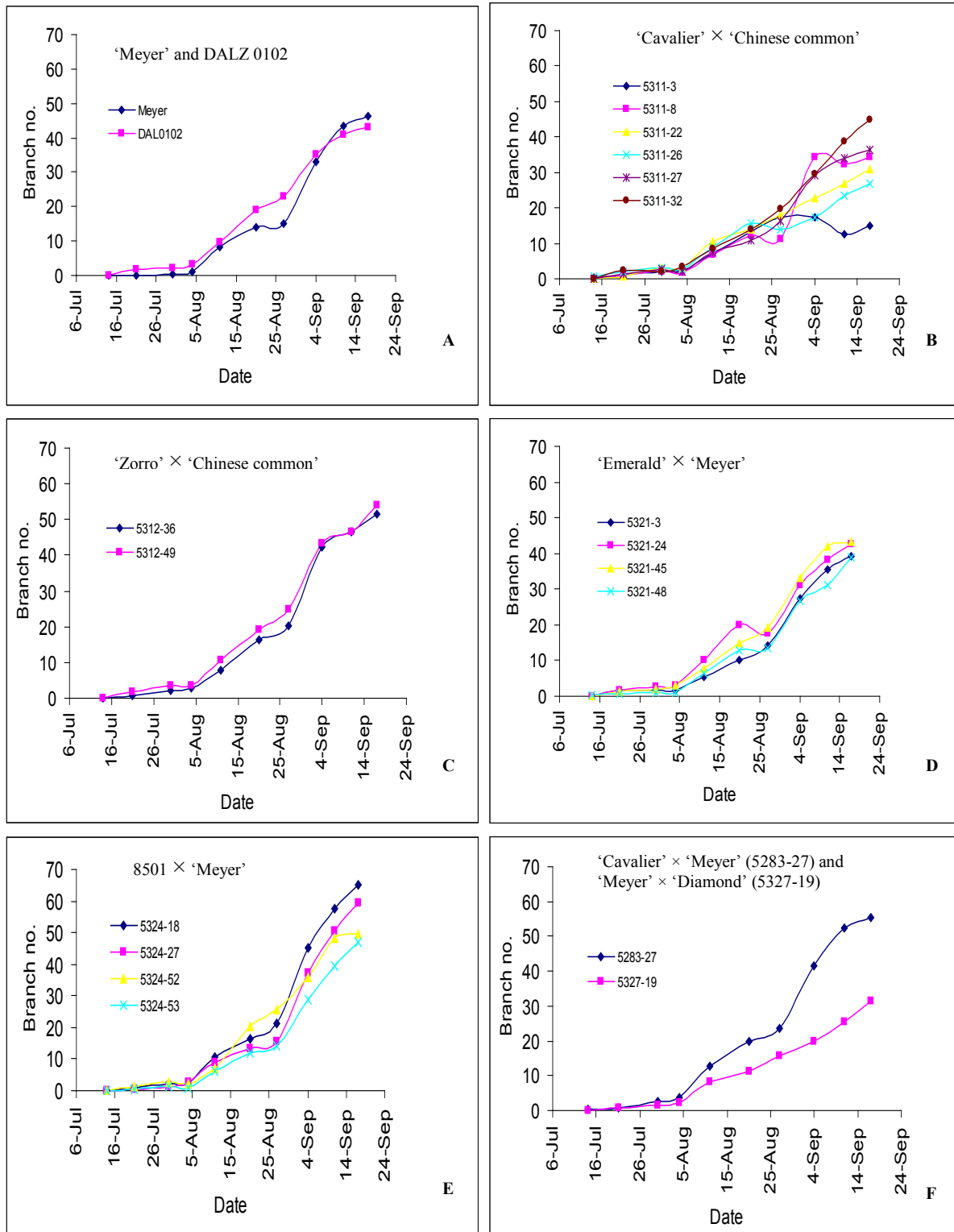
Figure 1-7. Cumulative stolon branch number of zoysiagrass cultivars and progeny planted as one 10-cm diameter plugs in the center of 1.2 by 1.2 m plots on 24 June, 2008. Headings at the top of each figure indicate parents of the progeny presented.

21
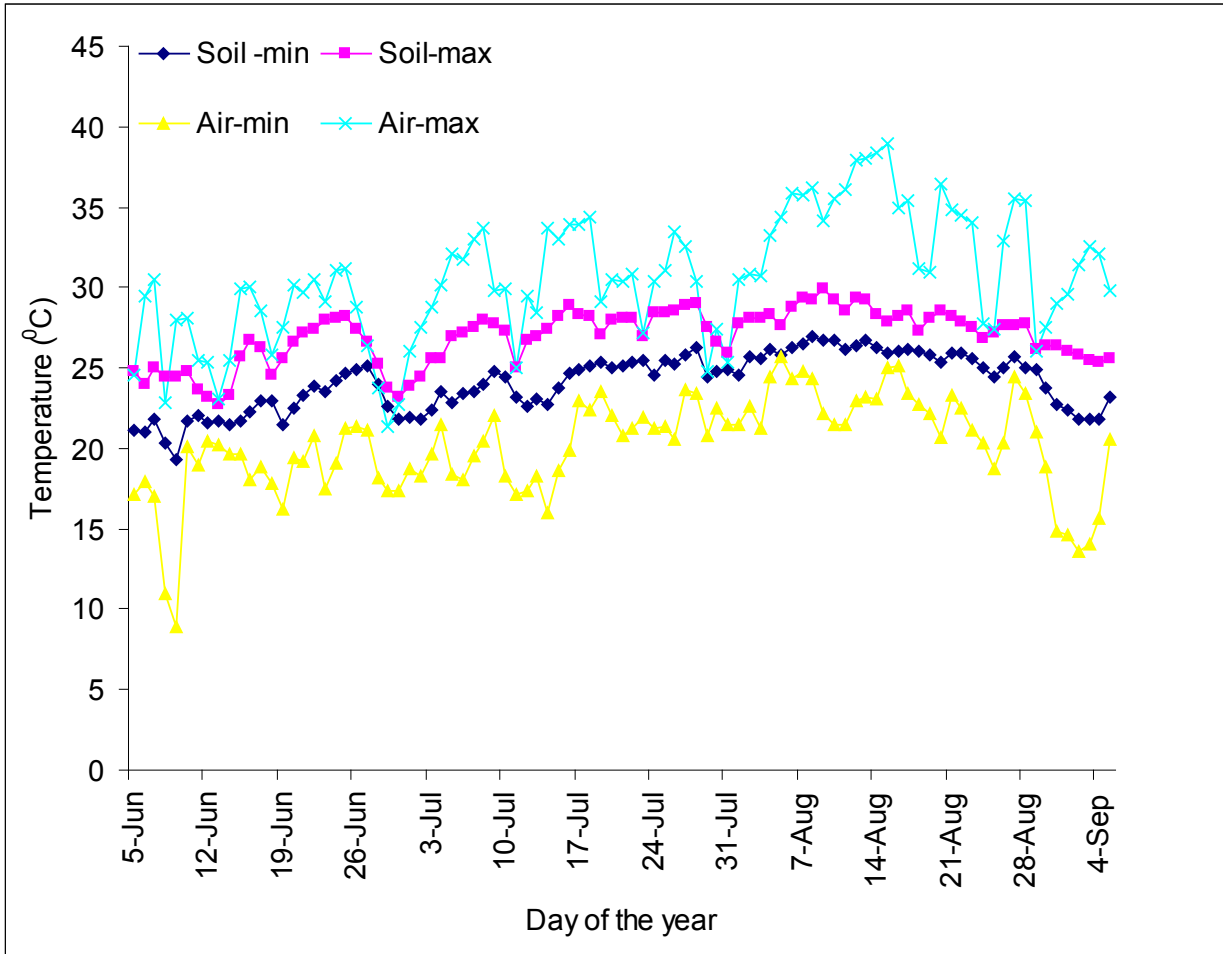
Figure 1-8. Average daily minimum and maximum air and soil temperatures at the study site at Manhattan, Kansas in 2007.
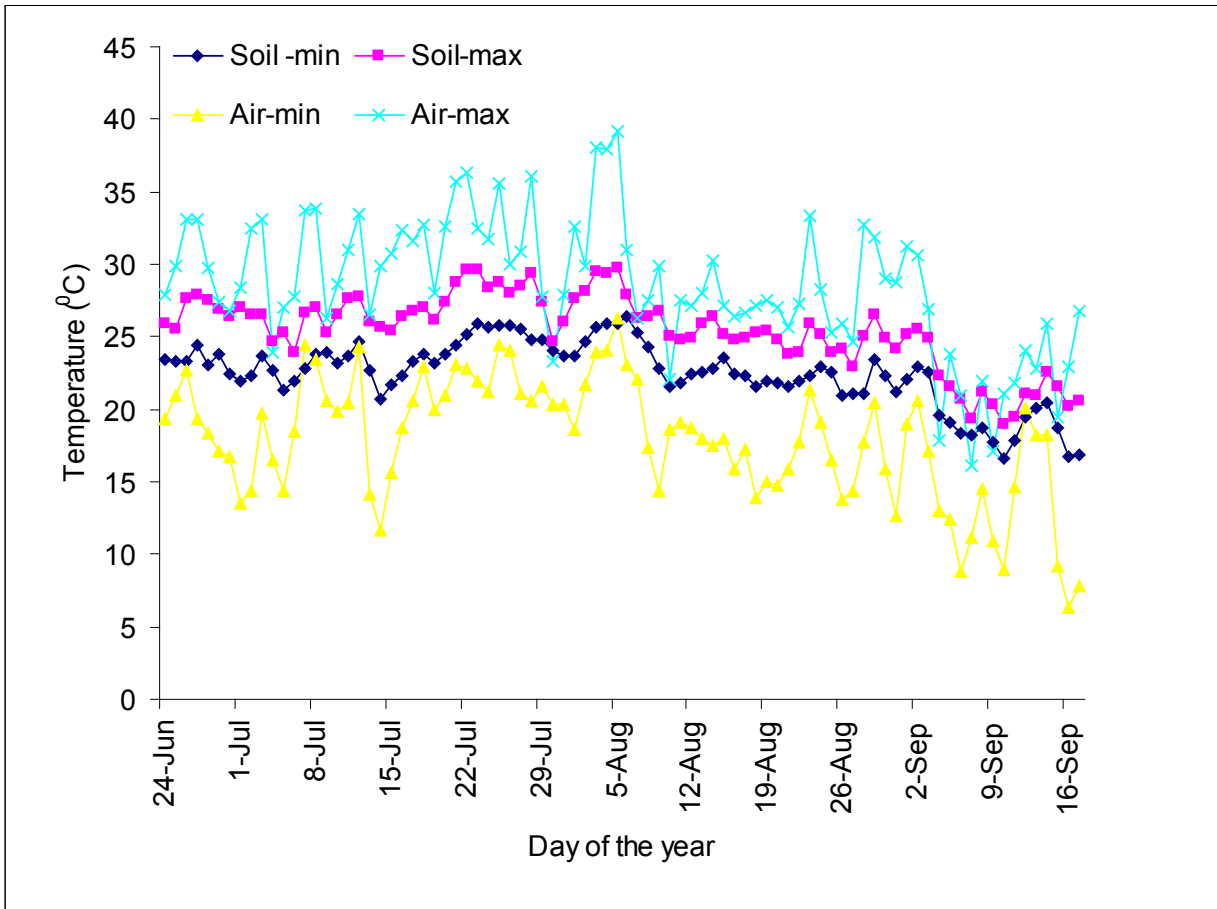
Figure 1-9. Average daily minimum and maximum air and soil temperatures at the study site at Manhattan, Kansas in 2008.

Table 1-0-1. Average weekly rates of stolon production, elongation, and branching of zoysiagrasses at Manhattan, KS in 2007 and 2008.[z]

Cultivar or Progeny	Stolons (no. wk^{-1}) [y]		Elongation (mm wk^{-1}) [x]		Branching (no. wk^{-1}) [w]	
	2007	2008	2007	2008	2007	2008
'Meyer'	2.9 c[v]	3.4 b	38.0 bc	26.8 cd	5.6 ab	5.6 ab
DALZ 0102	2.6 c	7.5 ab	26.4 c	36.3 bc	4.1 b	5.4 ab
'Cavalier' × 'Meyer' (5283-27)	4.7 b	6.9 ab	35.1 bc	41.9 bc	4.7 ab	6.8 ab
'Cavalier' × 'Chinese common'						
5311-3	5.6 ab	7.2 ab	53.3 ab	36.7 bc	4.6 ab	1.8 c
5311-8	4.6 b	6.5 ab	46.2 b	35.0 bc	3.7 b	4.4 bc
5311-22	6.3 a	7.6 ab	55.7 ab	46.0 bc	4.7 ab	3.7 bc
5311-26	5.0 ab	5.3 b	34.1 bc	39.6 bc	3.5 b	3.0 c
5311-27	4.8 ab	7.9 ab	43.6 b	46.3 bc	3.2 b	4.5 bc
5311-32	4.7 b	7.7 ab	49.3 ab	46.0 bc	4.2 b	5.1 bc
'Zorro' × 'Chinese common'						
5312-36	4.1 bc	7.8 ab	58.0 ab	49.1 b	5.2 ab	6.4 ab
5312-49	3.5 bc	5.3 b	61.9 a	65.1 a	3.6 b	6.5 ab
'Emerald' × 'Meyer'						
5321-3	4.9 ab	8.6 a	39.6 bc	40.3 bc	3.6 b	4.6 bc
5321-24	2.9 c	7.6 ab	21.6 c	30.6 cd	3.4 b	5.1 bc
5321-45	2.4 c	4.6 b	37.4 bc	33.5 c	4.7 ab	5.4 b

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5321-48	2.2 c	3.2 b	21.3 c	18.8 d	2.6 b	4.4 bc
8501 × 'Meyer'						
5324-18	5.3 ab	8.4 a	54.8 ab	43.2 bc	6.0 ab	7.7 a
5324-27	4.7 ab	7.8 ab	32.0 bc	37.8 bc	2.3 b	6.7 ab
5324-52	2.6 c	5.5 b	32.5 bc	29.1 cd	3.8 b	6.2 ab
5324-53	4.1 bc	7.1 ab	58.1 ab	39.1 bc	7.1 a	5.3 b
'Meyer' × 'Diamond' (5327-19)	3.2 bc	4.9 b	46.5 b	29.8 cd	5.1 ab	3.6 bc

[z]Grasses were planted as 6-cm diam. plugs on 30.5 cm centers in 1.5 m by 1.5 m plots on 5 June, 2007, and data were collected until 1 August. In 2008, individual 10 cm diam. plugs were planted on 24 June in plots measuring 1.2 m by 1.2 m, and data were collected until 17 September. Rates of stolon initiation, elongation, and branching of zoysiagrasses were calculated from 18 June to 24 Sept. in 2007, and from 1 July to 4 Sept. in 2008 using PROC REG (SAS Inst., Cary, NC, 2003) to obtain slope estimates.

[y]Stolon number per week is the average of three replicates from three randomly selected plugs per plot and an average of a single plug over three replicates in 2008.

[x]Stolon elongation per week is the average length of one stolon from three randomly selected plugs per plot, and over three replicates, in 2007. Stolon elongation per week in 2008 is the average of three randomly selected stolons per plug and three replicates.

<superscript>w</superscript>Stolon branching per week is the average number of branches on one stolon from three randomly selected plugs per plot and over replicates in 2007. In 2008, branching per week is the average of three selected stolons per plug over three replicates.

<superscript>v</superscript>Means followed by the same letter in a column are not significantly different according to Bonferroni's t test at $P \leq 0.05$ (corrected for multiple comparisons).

Table 1-0-2. Coverage of zoysiagrasses at Manhattan, KS in 2007 and 2008.[z]

Cultivar or progeny	Coverage (%)[y]		
	Study I		Study II
	24 Aug. 2007	24 Sept. 2007	4 Sept. 2008
'Meyer'	55.8 cd[x]	94.7 ab	50.0 d
DALZ 0102	66.7 bc	94.7 ab	90.0 ab
'Cavalier' × 'Meyer' (5283-27)	53.3 cd	85.0 abcd	85.0 ab
'Cavalier' × 'Chinese common'			
5311-3	54.2 cd	90.0 abc	85.0 ab
5311-8	63.3 bc	95.0 ab	73.3 abc
5311-22	78.3 a	97.7 a	75.0 abc
5311-26	67.5 b	96.0 ab	70.0 bcd
5311-27	65.4 bc	96.3 ab	85.0 ab
5311-32	64.2 bc	94.7 ab	90.0 ab
'Zorro' × 'Chinese common'			
5312-36	60.0 c	97.7 a	85.0 ab
5312-49	66.7 bc	94.7 ab	75.0 abc
'Emerald' × 'Meyer'			
5321-3	72.5 ab	99.0 a	95.0 a
5321-24	42.5 e	76.7 cd	75.0 abc
5321-45	51.7 d	85.0 abcd	56.7 cd
5321-48	43.3 e	73.3 d	50.0 d
8501 × 'Meyer'			
5324-18	74.2 ab	97.7 a	90.0 ab
5324-27	44.2 e	81.7 bcd	90.0 ab
5324-52	55.8 cd	86.7 abcd	70.0 bcd
5324-53	56.7 cd	93.3 ab	80.0 ab
'Meyer' × 'Diamond' (5327-19)	58.3 cd	88.3 abc	70.0 bcd

[z]Grasses were planted as 6-cm diam. plugs on 30.5 centers of 1.5 by 1.5 m plots on 5 June 2007, and as individual 10-cm diam. plugs in the center of 1.2 m by 1.2 m plots on 24 June 2008.

[y]Coverage (%) is the average visual estimate of two researchers and over three replicates.

[x]Means followed by the same letter in a column are not significantly different according to Ryan-Einot-Gabriel-Welsch test at *P < 0* .05.

Table 1-0-3. Total monthly precipitation at the study site in Manhattan, Kansas.

| Year | Precipitation (cm) [z] | | | | | |
	May	June	July	Aug.	Sept.	Total
2007	12.6	4.6	3.7	3.0	2.4	26.3
2008	3.5	8.1	3.8	6.0	4.9	26.3

[z]Total monthly precipitation are shown only for the months when zoysiagrasses were actively growing.

[x]Total monthly precipitation are the average monthly Manhattan, Riley county precipitation found at the K-State research and extension weather data library.

Table 1-0-4. Pearson correlation coefficients for average weekly rates of stolon initiation, elongation, branching, and coverage of zoysiagrass cultivars and progeny at Manhattan, Kansas in 2007 and 2008.[z]

Growth index	Stolon (no.wk^{-1})		Stolon elongation (wk^{-1})		Stolon branching (no. wk^{-1})		Coverage	
	2007	2008	2007	2008	2007	2008	2007	2008
Stolon (no.wk^{-1})	1.000	1.000	0.573**	0.533**	0.169	0.043	0.663**	0.938**
Stolon elongation (wk^{-1})	0.573**	0.533**	1.000	1.000	0.554**	0.211	0.524**	0.531*
Stolon branching (wk^{-1})	0.169	0.043	0.554**	0.211	1.000	1.000	0.317	0.188
Coverage	0.663**	0.938**	0.524**	0.531*	0.317	0.188	1.000	1.000

[z]In 2007, correlation analysis was performed on average weekly stolon initiation, elongation, and branching, and coverage on 24 Sept.

In 2008, correlation analysis was performed on average weekly stolon initiation, elongation, and branching and coverage on 4 Sept.

*, ** Significant at $P < 0.05$ or 0.01, respectively.

CHAPTER 2 - SOD TENSILE STRENGTH AND RECOVERY GROWTH OF ZOYSIAGRASSES

This paper is written in the format for *Applied Turfgrass Science*

Abstract

Information is limited on the tensile strength of zoysiagrass (*Zoysia* spp.) sod and recovery growth after harvest. 'Meyer' and DALZ 0102 (both *Z. japonica* Steud.) and eighteen zoysiagrass progeny originating from crosses between a *Z. matrella* (L.) Merr. × *Z. japonica* or 'Emerald' (*Z. matrella* × *Z. tenuifolia* Willd. ex Thiele) × *Z. japonica* were evaluated for sod tensile strength in Manhattan, KS in 2008 and for sod recovery growth after harvest at Manhattan in 2008 and 2009, and in Olathe, KS in 2009. Sod pieces (51-cm wide x 102-cm long and 5-cm thick) were harvested on 9 July 2008 at Manhattan and on 7 July 2009 at Olathe using a mechanical sod cutter. For each zoysiagrass, two 34 x 51 cm sections of sod, 5-cm thick, were placed on a sod stretcher to determine tensile strength. Tensile strength ranged from 1.268 to 6.571 kiloNewtons per meter (kN m^{-1}). Sod strength of Meyer (4.064 kN m^{-1}) was similar to other grasses evaluated. Sod recovery growth in areas of harvested strips was determined by taking an overhead digital image of a 40 by 35 cm area. At 60 days after harvest (DAH) in Manhattan, coverage ranged from 17% to 96.8%; a progeny from *Z. matrella* x Meyer (96.8% coverage) demonstrated superior sod recovery growth to Meyer (37.6% coverage). Recovery growth was slower in Olathe where irrigation was applied less frequently, and all progeny were similar to Meyer. At least one zoysiagrass progeny evaluated, 5324-27 (8501 × Meyer), recovered faster after sod harvest than all other grasses evaluated.

INTRODUCTION

Zoysiagrass (Zoysia spp) requires low levels of nitrogen fertilizer and pesticides, and also has lower evapotranspiration rates and better drought resistance than cool-season grasses (6). 'Meyer' zoysiagrass, a *Z. japonica,* has been the predominant cultivar used in the transition zone since its release in 1952, primarily because of its good freezing tolerance (7). Prior to the release of Meyer, and even since its release, there have been zoysiagrass cultivars with better quality that have interested transition zone turf managers. 'Emerald' zoysiagrass (*Z. japonica* × *Z. tenuifolia*) is an older, fine textured cultivar that has been used extensively in the southern U.S. Researchers at Texas A & M University have developed several *Z. matrella* cultivars with high turf quality, including better density, finer texture, and better color compared to Meyer. Among these cultivars are 'Diamond', 'Cavalier', and 'Zorro'. Unfortunately, these cultivars are not suitable for use in the transition zone due to lack of freezing tolerance (6).

Since 2004, turfgrass researchers at Kansas State University have evaluated over 600 new zoysiagrass progeny for quality and winter survival (6). These progeny were the result of genotypic crosses made at Texas A & M-Dallas, most of which involved one parent from *Z. japonica* and the other from Emerald or a *Z. matrella*. The goal of this work is to develop one or more cultivars that have good density, a fine leaf texture, and quality similar to the aforementioned *Z. matrella* cultivars, but freezing tolerance equal to or better than Meyer.

An ideal zoysiagrass sod should have good tensile strength after harvest to prevent it from tearing during handling, shipping, and laying (2, 14). Tensile strength is greatly enhanced when grasses form an interconnecting network of stolons and/or rhizomes, and is measured by the force (kN m⁻1) required to tear a section of sod apart (15). Significant research has been done with tensile strength of Kentucky bluegrass (*Poa pratensis* L.), with values ranging from

0.220 to 1.167 kiloNewtons per meter ($kN\ m^{-1}$) in Nebraska (18); and 2.499 to 29.488 kNm^{-1} in Rhode Island (11). Buffalograss [*Buchloe dactyloides* (Nutt.) Engelm.] tensile strength ranged from 0 to 0.3099 $kN\ m^{-1}$ in Nebraska and 0 to 0.2096 kNm^{-1} in Austin, TX (8).

In addition to tensile strength, rate of sod recovery after harvest is also important to sod producers. Because zoysiagrass has the capability to grow back from rhizomes after harvest, it has the potential to fully recover without additional effort in vegetative propagation, such as plugging or sprigging. In Maryland, ground from which Meyer was harvested in August required nearly two full growing seasons before complete coverage occurred from rhizome recovery growth (3). After sod harvest in south Alabama, it has been reported that 14 to 17 months is required for full recovery of Emerald or Meyer zoysiagrass (1). In Dallas, TX (4), Diamond zoysiagrass could be harvested from the same field three times in 24 months, and Diamond also had the greatest number of tillers generated from rhizomes compared to other *Z. matrella* cultivars at 42 days after harvest (4). Clearly, there are differences among zoysiagrass genotypes in sod recovery growth rates. My objective was to evaluate zoysiagrass cultivars and progeny for tensile strength and rate of recovery growth after harvest.

Study sites and field layout

The study was conducted at two locations: the Rocky Ford Turfgrass Research Center in Manhattan, Kansas (39.128 N longitude, 96.358 W latitude) and the Olathe Horticultural Research center in Olathe, Kansas (38.897 N longitude, 94.993 W latitude). At each location, eighteen zoysiagrass progeny, and Meyer and DALZ 0102 (*Z. japonica*) (Table 2-1), were planted in June, 2007 as vegetative plugs on 30-cm centers in 1.5 m x 1.5 m plots. Another of the *Z. matrella* lines, listed as 8501 in Table 2-1, was never commercially released. Plots were arranged in a randomized complete block design with three replicates. A weather station located

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at each location was used to monitor air temperature and precipitation. In addition, a soil-encapsulated thermocouple assembled according to Ham and Senock (9) was installed at 2.5 cm deep in one plot of each replicate to monitor soil temperature in Manhattan. Soil temperature was measured using a CR-10 (Campbell Scientific, Inc. Logan, UT) data logger. Weather data for Olathe during the study period were obtained from Kansas State University weather data library (http://www.ksre.ksu.edu/wdl).

Soil in Manhattan was a Chase silt loam (fine, montmorillonitic, mesic, Aquic, Argiudolls). Soil pH was 7.3, P level was 123 mg kg^{-1} and K level was 475 mg kg^{-1}. In 2007, turf received N from urea at 49 kg ha^{-1} on 12 July and 31 August. Plots were mowed at a 5 cm height with a rotary mower until midsummer, when the height was gradually reduced to 1.3 cm. In-ground irrigation was used to prevent stress and supplement rainfall to provide 2.5 cm of water weekly. The mean monthly air and soil temperatures, respectively, during the study period in Manhattan in 2008 were: 25.2 and 25.2 ^{o}C in July; 23.5 and 24.3 ^{o}C in August; and 19.2 and 20.4 ^{o}C in September.   In 2009, respective air and soil temperatures were: 18.8 and 21.2 ^{o}C in May; 24.0 and 25.2 ^{o}C in June; 23.2 and 26.2 ^{o}C in July; 22.1 and 24.8 ^{o}C in August; and 17.0 and 19.9 ^{o}C in September. Total monthly precipitation was 4.7 cm in July, 5.3 cm in August, and 5.4 cm in September 2008; 0.98 cm in May, 8.5 cm in June, 6.5 cm in July, 4.5 cm in August, and 2.0 cm in September 2009.

Sod was harvested on 10 July 2008 at Manhattan by cutting a 5-cm deep, 102-cm long, 51-cm wide swath through the center of each plot using a walk-behind mechanical sod cutter (Ryan, Johnson Creek, WI**).** After cutting, strips were separated into two pieces using a straight-edge shovel, each 34 cm long x 51 cm wide and 5 cm thick. Tensile strength of each of the two sections per plot was evaluated using a stretching mechanism described by Sharpe et al. (17).

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Briefly, each of the sod pieces was secured to a platform grate, half of which was fixed, and the other half mobile (Fig. 2-1). Stretching occurred by pulling on an S-beam load cell that was attached to the mobile end of the grate, and measuring peak resistance before the sod tore as described by Giese et al. (8).

Sod recovery growth from rhizomes, stolons, or both was determined by taking overhead photographs of a 40 by 35 cm area, for which permanent corners were established within the harvested area of each plot (Fig. 2-2) to determine percentage area covered by green growth. Photos were taken with a Nikon D100 AF LR 28:105 mm camera (Nikon Corp, Japan) mounted on a tripod at 1.2 m above the plots beginning on 12 July and continuing every two to three weeks until 23 September (Fig. 2-3). Although stolons from plants growing on the perimeter of the sod harvested area could have contributed to recovery, most of the recovery growth was occurring from rhizomes that had been left in the soil below the harvesting depth. Digital images were analyzed with Sigma Scan Pro 5 (v. 5.0, SPSS, Inc., Chicago, IL) under a color hue threshold of between 30 and 100 and saturation of between 0 and 100 (12).

Soil at Olathe was a Kennebec silt loam. Soil pH was 7.0, P level was 524 mg kg^{-1} and K level was 312 mg kg^{-1}. In 2008 and 2009, N from sulfur coated urea (39-0-0) was applied in June at 49 kg ha^{-1} and in August at 49 kg ha^{-1}. The plots were irrigated to prevent dormancy and mowed one or two times weekly at a 1.9 cm height in 2008. Mean monthly air and soil temperatures, respectively, for Olathe were 24.5 and 26.8 ^{o}C in July; 21.6 and 23.5 ^{o}C in August; and 18.6 and 20.7 ^{o}C in September 2009. Total monthly precipitation was 3.8 cm in July, 5.6 cm in August, and 1.7 cm in September 2009. Sod was harvested as described for Manhattan on 8 July 2009. A measurement of tensile strength was attempted, but the equipment used to measure sod tensile strength failed, presumably due to the high tensile strength of the

sod, and could not be repaired in time to allow tensile strength data collection. Recovery growth was evaluated as described for Manhattan beginning on 8 July and continuing every 2 weeks until 18 September.

Sod tensile strength and recovery growth data were subjected to analysis of variance using SAS procedures (16), and means were separated using the Ryan-Einot-Gabriel-Welsch (REGWQ) multiple comparison test (10, 13) at $P < 0.05$.

Sod tensile strength

Zoysiagrass sod tensile strength ranged from 1.274 kiloNewtons per meter (kN m^{-1}) for 5311-27 ('Cavalier' × 'Chinese common') to 6.589 kN m^{-1} for 5311-22 (Cavalier × Chinese common) (Table 2-1). Data were variable and all progeny were not significantly different from Meyer in tensile strength. The sod stretching mechanism that collapsed due to the strength of the zoysiagrass sod indicates how strong zoysiagrass sods were compared to other turfgrass sods. This strength was also evident when values I recorded are compared with those reported by researchers evaluating other grasses. Average tensile strength across all zoysiagrasses in my study was 4.401 kN m^{-1}. An evaluation of plant growth regulators for their effect on strength in Wisconsin indicated a range of tensile strengths between 1.199 kN m^{-1} and 0.6023 kN m^{-1} for Kentucky bluegrass (19). Based on my results in Manhattan, zoysiagrass produces a sod that is, on average, at least twice as strong as that produced by other turfgrass species that have been evaluated.

Sod recovery growth

Differences among grasses in recovery growth were observed on 3 of 6 dates in Manhattan in 2008 (Table 2-2). On 12 July, coverage of 5324-27 (8501 × Meyer) was greater than all other grasses except 5321-24 (Emerald × Meyer). On 9 September, recovery growth of

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5324-27 was over 95%, and greater than Meyer, 5327-19 (Meyer × Diamond), 5283-27 (Cavalier × Meyer), two progeny within Cavalier × Chinese Common and within 8501 × Meyer, and one within Zorro × Chinese Common and Emerald × Meyer. On 23 Sept., coverage of 5324-27 (Fig. 2-2) was superior to 5324-18, but similar to all other grasses.

In 2009 in Manhattan, differences were observed on 18 June and 2 July among the progeny; however, none of the progeny differed from Meyer on either date (Table 2-2). Coverage values were lower for some progeny on 2 July 2009 than on 23 September 2008, which was likely due to some winter damage.

In Olathe in 2009, irrigation was applied only to prevent severe stress, which resulted in a lower rate of recovery growth for all grasses compared to Manhattan. On 7 May 2010, coverage were lower than those recorded for all progeny in Sept. 2009, which is a similar scenario observed after winter period in Manhattan in 2009. However, coverage was generally higher on 15 July 2010 in Olathe, two weeks longer than on 2 July 2009 in Manhattan (Table 2-3). Other factors which could have contributed to the differences include the type of soil and weather patterns. Differences among grasses occurred on all dates, but Meyer coverage was as good as the top performing progeny on all dates except 8 July when coverage of DALZ 0102 was superior (Table 2-3).

I found that Meyer had about 66% coverage 2 months after harvest (Fig. 2-2) and 72% coverage by 2 July 2009 (12 months after harvest) in Manhattan (Table 2-2). In Olathe, where less irrigation was applied, Meyer had a coverage was 28% on 4 Sept. 2009 (2 months after harvest) and 48.3% on 15 July 2010 (12 months after harvest) (Table 2-3). In Silver Spring, MD (3), Meyer recovery growth after harvest was about 10% after 11 months and 95% coverage 26 months after harvest. Several factors could have influenced differences in rate of recovery

between the Maryland site and the one described herein. Their experiment was conducted on a

sandy loam soil and they also tilled the harvested area to a 2.5 cm depth at the beginning of the

study; contrasts in harvest depth and climatic conditions during the study period could also have

influenced recovery growth differences.

In southern Alabama, zoysiagrass (Meyer and Emerald) sod producers were told to

expect 17 months before complete recovery growth occurred after harvest (1). Recovery growth

of Diamond zoysiagrass (*Z. matrella*) had resulted in 940 plants m^{-2} at 42 DAH in Dallas, TX;

whereas, Emerald had 300 plants m^{-2} and Meyer 110 plants m^{-2} (5). Complete recovery growth

had occurred by 4 months after harvest. In my findings, I suspect that 5324-27 (8501 × Meyer)

is a prolific rhizome producer as well, as demonstrated by 97% coverage by 11 weeks after

harvest in Manhattan in 2008. It was the only progeny that had greater coverage than Meyer in

Manhattan or Olathe.   One of the parents of this particular progeny, 8501, is a *Z. matrella* and,

much like other *Z. matrella* cultivars such as Diamond and Zorro, produces an extensive network

of rhizomes when grown in the greenhouse. Color, texture, and density of 5324-27 more closely

resemble Meyer than a *Z. matrella* in the field, however (Fig. 2-2). Rate of stolon production for

5324-27 (4.7 wk^{-1}) was faster than Meyer (2.9 wk^{-1}) in 2007, but similar to Meyer in 2008

(Chapter I). Furthermore, coverage in the field (Chapter I) was lower than Meyer at 50 days

after planting (DAP) in 2007, but higher than Meyer at 60 DAP in 2008.   This shows that rate of

establishment is not indicative of recovery growth after sod harvest.

This study demonstrated that zoysiagrasses differ in sod tensile strength, and average

strength was over double that commonly reported for other grasses. However, data were quite

variable and none were stronger than Meyer. Rate of recovery growth was also quite variable

among grasses, but one experimental progeny (5324-27 from 8501 × Meyer) in particular which

resulted from the cross of a *Z. matrella* × Meyer exhibited rapid coverage and was superior to Meyer on two of five evaluation dates in Manhattan. Faster time between sod harvests resulting in more profit for the sod producers would likely be possible with a zoysiagrass with this characteristic.

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Figure 2-1. A piece of zoysiagrass sod on the stretching mechanism.

Figure 2-2. Zoysiagrass experimental plots after sod harvest on 10 July, 2008 to evaluate sod strength and recovery growth at Manhattan, Kansas.

(a) (b)

Figure 2-3. Photographs for recovery growth of zoysiagrasses, progeny 5324-27 (8501 × Meyer) at 89.8% coverage (a) and Meyer at 39.6% coverage (b) at 60 days after sod harvest at Manhattan, Kansas in 2008.

Table 2-1. Mean sod tensile strength of zoysiagrasses at Manhattan, Kansas in 2008.

Cultivar or progeny [x]	Tensile strength (kNm^{-1})[y]
Meyer (control)	4.058 abc[z]
DALZ0102	6.491 ab
Cavalier × Meyer (5283-27)	4.926 abc
Cavalier × Chinese common	
5311-3	5.350 abc
5311-8	5.776 ab
5311-22	6.589 a
5311-26	3.981 abc
5311-27	3.981 c
5311-32	5.301 abc
Zorro × Chinese common	
5312-36	6.292 ab
5312-49	3.024 abc
Emerald × Meyer	
5321-3	4.903 abc
5321-24	4.511 abc
5321-45	5.168 abc
5321-48	3.318 abc
8501 × Meyer	
5324-18	4.133 abc
5324-27	2.260 bc
5324-52	2.802 abc
5324-53	4.358 abc
Meyer × Diamond (5327-19)	3.491 abc

[x]Grasses were planted as sixteen 6-cm diam. plugs on 30.5 cm centers in 0.6 m by 0.6 m plots on

5 June, 2007.

[y]Tensile strength in kiloNewtons per meter was determined by placing a 34 cm long x 51 cm wide x 2 cm thick sod pieces on platform grate, half of which was fixed, and the other half mobile. Stretching occurred by creating pull on the mobile end of the grate and measuring peak resistance before the sod tore using an 'S' beam load cell.

[z]Means followed by the same letter in the means column are not significantly different according to Ryan-Einot-Gabriel-Welsch test ($P < 0.05$).

Table 2-2. Recovery growth of zoysiagrasses after sod harvest at Manhattan, Kansas.

| Cultivar or progeny[x] | Coverage (%)[y] | | | | |
| | 2008 | | | 2009 | |
	12 July	9 Sept.	23 Sept.	18 June	2 July
Meyer	0.5 b[z]	37.6 b	66.2 ab	77.0 ab	71.9 ab
DALZ0102	1.4 b	62.0 ab	69.3 ab	63.3 abcd	69.0 ab
Cavalier × Meyer (5283-27)	1.0 b	40.0 b	49.2 ab	53.3 abcde	61.7 abc
Cavalier × Chinese common					
5311-3	0.5 b	40.9 b	45.3 ab	47.4 bcde	66.9 abc
5311-8	1.5 b	47.4 ab	56.2 ab	40.6 cde	52.8 abc
5311-22	0.6 b	30.2 b	33.9 ab	31.1 e	46.7 bc
5311-26	0.8 b	51.0 ab	64.2 ab	70.3 abc	71.4 ab
5311-27	0.8 b	55.8 ab	69.9 ab	62.6 abcd	72.0 ab
5311-32	0.5 b	34.2 b	73.5 ab	52.0 abcde	63.2 abc
Zorro × Chinese common					
5312-36	0.5 b	29.7 b	41.8 ab	51.0 abcde	61.4 abc
5312-49	0.3 b	50.9 ab	58.6 ab	61.7 abcde	64.0 abc
Emerald × Meyer					
5321-3	1.1 b	40.9 b	57.0 ab	68.0 abc	70.7 ab
5321-24	2.5 ab	64.0 ab	73.1 ab	49.0 bcde	61.7 abc
5321-45	1.5 b	68.7 ab	69.1 ab	65.3 abc	72.2 ab
5321-48	1.8 b	66.3 ab	72.8 ab	70.1 abc	71.4 ab
8501 × Meyer					

5324-18	0.3 b	16.9 b	19.1 b	32.8 de	42.2 c
5324-27	4.5 a	96.8 a	96.6 a	80.6 a	77.0 a
5324-52	1.3 b	38.1 b	50.6 ab	52.8 abcde	67.8 abc
5324-53	1.5 b	49.7 ab	60.1 ab	46.3 bcde	52.4 abc
Meyer × Diamond (5327-19)	0.5 b	42.0 b	53.5 ab	47.2 bcde	59.3 abc

[x]Grasses were planted as sixteen 6-cm diam. plugs on 30.5 cm centers in 0.6 m by 0.6 m plots on 5 June, 2007, and sods were harvested on 10 July, 2008.

[y]Sod recovery growth was determined by percentage green color using digital analysis of overhead images from a 35 cm x 40 cm area within each harvested strip.

[z]Means followed by the same letter in the means column are not significantly different according to the Ryan-Einot-Gabriel-Welsch test ($P < 0.05$).

Table 2-3. Recovery growth of zoysiagrasses after sod harvest at Olathe, Kansas.

Progeny identification[x]	Coverage (%)[y]							
	2009						2010	
	8 July	21 July	4 Aug.	21 Aug.	4 Sept.	18 Sept.	7 May	15 July
Meyer	2.6 cd[z]	10.7 abc	20.1 abcd	27.4 abcd	27.8 ab	38.7 ab	26.3 ab	48.3 c
DALZ0102	7.5 ab	16.7 abc	32.9 ab	46.3 a	37.1 ab	57.1 ab	35.0 ab	78.3 abc
Cavalier × Meyer (5283-27)	4.5 abcd	6.8 abc	13.6 cd	19.3 bcd	22.8 ab	31.4 ab	31.0 ab	88.3 abc
Cavalier × Chinese common								
5311-3	3.2 cd	8.5 abc	14.5 bcd	20.0 bcd	32.4 ab	40.7 ab	33.3 ab	94.3 ab
5311-8	4.1 abcd	10.1 abc	16.7 abcd	30.2 abcd	33.6 ab	53.8 ab	31.0 ab	82.7 abc
5311-22	3.8 abcd	4.5 c	11.3 d	15.4 cd	25.8 ab	32.0 ab	32.3 ab	83.3 abc
5311-26	2.8 cd	9.3 abc	17.5 abcd	24.5 abcd	31.4 ab	41.9 ab	34.5 ab	90.7 abc
5311-27	2.5 cd	7.6 abc	11.6 d	14.9 cd	23.5 ab	28.5 b	20.0 ab	74.0 abc
5311-32	3.5 cd	11.7 abc	21.6 abcd	27.3 abcd	33.4 ab	41.9 ab	28.0 ab	88.3 abc
Zorro × Chinese common								
5312-36	3.2 cd	8.5 abc	12.6 cd	22.9 bcd	29.1 ab	44.6 ab	28.7 ab	77.7 abc
5312-49	2.1 d	7.2 abc	14.6 bcd	17.5 bcd	25.6 ab	29.8 b	19.3 ab	50.0 bc
Emerald × Meyer								
5321-3	4.1 abcd	13.2 abc	24.4 abcd	30.3 abcd	33.2 ab	42.0 ab	25.3 ab	45.0 c
5321-24	5.4 abcd	19.6 a	31.1 abc	34.9 abc	38.0 ab	43.6 ab	39.7 ab	78.3 abc
5321-45	3.8 abcd	13.2 abc	17.3 abcd	24.1 abcd	33.8 ab	43.9 ab	37.7 ab	90.7 abc
5321-48	4.5 abcd	11.9 abc	23.8 abcd	40.3 ab	46.4 a	71.1 a	54.3 a	96.7 a
8501 × Meyer								

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5324-18	4.4 abcd	6.6 abc	7.5 d	8.3 d	18.6 b	26.7 b	15.3 b	45.0 c
5324-27	7.7 a	18.0 ab	33.2 a	46.5 a	30.8 ab	50.7 ab	24.7 ab	55.0 abc
5324-52	3.7 bcd	5.9 bc	15.2 abcd	18.2 bcd	27.4 ab	32.0 ab	24.7 ab	46.7 c
5324-53	4.0 abcd	7.5 abc	17.1 abcd	22.3 bcd	30.1 ab	37.8 ab	25.7 ab	63.3 abc
Meyer × Diamond (5327-19)	6.2 abc	9.1 abc	24.1 abcd	29.9 abcd	35.5 ab	44.2 ab	31.3 ab	82.7 abc

[x]Grasses were planted as sixteen 6-cm diam. plugs on 30.5 cm centers in 0.6 m by 0.6 m plots on 13 June, 2007, and sods were harvested on 8 Jul., 2009.

[y]Sod recovery growth was determined by percentage green color using digital analysis of overhead images from a 35 cm x 40 cm area within each harvested strip.

[z]Means followed by the same letter in the means column are not significantly different according to the Ryan-Einot-Gabriel-Welsch test ($P < 0.05$).

CHAPTER 3 - ZOYSIAGRASS GROWTH UNDER TREE SHADE

This paper is written in the format of *Crop Science.*

Abstract

Zoysiagrass (*Zoysia* spp.) grown under shade on golf courses and in home lawns typically declines in quality over time. We evaluated growth of Meyer and Chinese common (both *Z. japonica* Steud.); Zorro, Diamond, Cavalier [all *Z. matrella* L. (Merr.)]; Emerald (*Z. matrella* × *Z. tenuifolia* Willd. ex Thiel) and 6 experimental progeny from Emerald × *Z. japonica* and *Z. japonica* × *Z. matrella*, under silver maple (*Acer saccharinum* L.) shade and in full sun in 2008 and 2009 in Manhattan, KS. A single 6-cm diam. plug was planted in the center of 1.2 m by 1.2 m plots, arranged in a randomized complete block design with six replicates. Data were collected weekly on the number of stolons, stolon elongation, number of stolon branches, and aboveground biomass at the end of the study. Tiller number was collected at the start and end of the study period. Zoysiagrasses under an average of 68% tree shade exhibited reductions of 38 to 95% in stolon number; 9 to 70% in stolon length; 10 to 93% in branching; and 56 to 98% in total aboveground biomass. Tillering declined for 6 or 7 of the 10 grasses from the study's beginning to end in 2008 and 2009; none of the grasses differed from Meyer in change in tiller number under shade. In general, several progeny from crosses between Emerald or a *Z. matrella* × *Z. japonica* produced more and/or longer stolons than Meyer in the shade, suggesting potential for increased shade tolerance.

INTRODUCTION

Zoysiagrass grown under shade on golf courses and in home lawns typically begins to decline in quality over time. Tree shade reduces photosynthetically active radiation (PAR), solar radiation in the range of 400 to 700 nanometers, resulting in altered spectral quality, reduced photosynthesis and altered photomorphogenesis (Bell et al., 1999, 2000). Shade induces leaf elongation and results in a substantial decrease in turf density and rooting (Fry and Huang, 2004; Bell, 1999). Beard (1997) also suggested that turfgrass exposure to shade may result in reduced tillering, density, stolon growth, and an increased number of lateral stems. Total non-structural carbohydrates also decline over time as turfgrasses grow under shaded conditions (Qian and Engelke, 2000; Qian et al., 1998; Stier, 2007). Zoysiagrass grown in shade is also more susceptible to freezing injury and diseases due to a weakened physiological condition (Qian and Engelke, 1998; Qian et al., 1998; Wayne and Bryan, 2007).

Zoysiagrass cultivars from *Z. japonica* have poorer shade tolerance compared to Emerald zoysiagrass or cultivars from *Z. matrella* (Engelke and Reinert, 2003). Under the shade of live oak [*Quercus virginiana* (Mill.)] trees that allowed 10% light penetration, Cavalier had greater coverage (72%) than Meyer (32%) on yearly evaluations during a three a three-year period of establishment (Engelke et al., 2002a). Under 90% live oak tree shade in Dallas, TX, coverage of Diamond (94%), Zorro (84%), and Cavalier (74%) was superior to Meyer (33%) and El Toro (*Z. japonica*, 65%) after three years of growth (Engelke and Reinert, 2003).

Since 2004, turfgrass researchers at Kansas State University have evaluated over 600 new zoysiagrass progeny for quality and winter survival (Fry et al., 2008). These progeny were the result of genotypic crosses made at Texas A & M-Dallas, most of which involved one parent from *Z. japonica* and one from 'Emerald' or a *Z. matrella*. The goal of this work is to develop

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one or more cultivars that has good density, a fine leaf texture, and quality similar to the aforementioned *Z. matrella*s, but freezing tolerance as good or better than Meyer. Additionally, zoysiagrasses grown in shade is also more susceptible to freezing injury and diseases due to a weakened physiological condition (Qian and Engelke, 1998; Qian et al., 1998; Wayne and Bryan, 2007). Thus, developing new cultivars with shade better shade tolerance than Meyer will be desirable.

I hypothesized that crosses between Emerald × *Z. japonica* or *Z. matrella* × *Z. japonica* have the potential to perform better than Meyer in shade because one of the parents has superior shade tolerance to Meyer. The objective of this study was to evaluate growth of zoysiagrass cultivars and experimental progeny, including those from Emerald x *Z. japonica* and *Z. japonica* x *Z. matrella*, for shade tolerance.

MATERIALS AND METHODS

Six experimental zoysiagrass progeny from Emerald × *Z. japonica* and *Z. japonica* × *Z. matrella*; Meyer and Chinese common (both *Z. japonica* Steud.); Zorro, Diamond, Cavalier [all *Z. matrella* L. (Merr.)]; and Emerald (*Z. matrella* × *Z. tenuifolia* Willd. ex Thiel) were evaluated under tree shade and in full sun at the Rocky Ford Turfgrass Research Center, Manhattan, KS (39.128N longitude, 96.358W latitude) in 2008 and 2009 (Table 3-1). Shaded plots measuring 1.2 m by 1.2 were arranged in a randomized complete block design with six replicates between 1.5 and 7.5 m to the north side of a line of silver maple (*Acer saccharinum* L.) trees, most between 8 and 15 m tall (Fig. 3-1). Soil was a Chase silt loam (fine, montmorillonitic, mesic, Aquic, Argiudolls). The soil in the full sun study area had a pH of 7.8 and P level was 111 mg kg^{-1} and K level was 451 mg kg^{-1}; whereas, soil test results for soil under tree shade indicated a pH of 5.5 , P level of 73 mg kg^{-1} , and K level of 368 mg kg^{-1}. A weather station located within 100 m of the study area was used to monitor air temperature and precipitation.

The study was conducted in 2008, the turf was removed, and a second study conducted in 2009. A single 6-cm diam. plug was planted in the center of each plot on 30 June 2008 and on 26 June 2009. A separate, identical study area was planted on the same day in full sun approximately 100 m from the shaded area. Turf was not mowed. Nitrogen from urea was applied on 14 July and 12 Aug. 2008 and 21 July and 18 Aug. 2009 to provide 49 kg ha^{-1}. To minimize differences in soil water content between study areas, irrigation was used to supplement natural rainfall and maintain the soil moisture level at 25 to 35% v/v at 0 to 15 cm as measured twice weekly using a time-domain reflectometer (Soilmoisture Equipment Corp., CA).

Photosynthetically active radiation was measured once monthly during each study period using a ceptometer (AccuPAR model LP-80, Decagon, Pullman, WA) at 0730 h, 1030h, 1330 h,

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1630 h, and 1930h. The ceptometer was held at the grass canopy level in the center of each plot, a reading taken and stored, and the average over the study area determined (Table 3-2). Measurements of replicates in the shaded area were alternated with those within the full sun area to minimize the effects of time on PAR measurements. Root zone temperatures in each study area were measured using tough soil bimetallic digital thermometer (Model 6300, Spectrum Tech., Plainfield, IL) at a depth between 0 to 12 cm (Table 3-1).

Data were collected on the number of stolons, stolon elongation, number of stolon branches, and aboveground biomass. The number of stolons, stolon elongation, and number of branches were determined weekly beginning on 14 July in 2008 and 15 July in 2009. Stolon numbers were determined by counting the number of stolons originating from each plug. Stolon elongation and branching were evaluated on each of the first three stolons that emerged. Each of the stolons used to evaluate elongation and branching was labeled with a loose knot of thread tied around the stolon to facilitate its identity. Elongation was determined by inserting a colored plastic toothpick in the ground at the tip of the stolon. The next week, after elongation had occurred, the distance was measured from the end of the stolon back to the location of the toothpick. Branching was determined by counting the number of branches on the stolon. Aboveground biomass was determined at the end of study period by excavating all green tissue; air drying for 4 weeks at 25 to 30 $^{\circ}$C, removing any remaining soil; and weighing. To simplify data presentation, means for stolon number, elongation, and branching are presented for the last rating date of each year (29 Sept., 2008 and 24 Sept., 2009). Data from the full sun area (Appendix 3A) was used to calculate the growth reduction that occurred in shaded turf. Stolon growth data and biomass were subjected to square root transformation before analysis. Data were analyzed using the general linear models procedure (SAS, 2003) and means were

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separated using the Ryan-Einot-Gabriel-Welsch (REGWQ) multiple comparison test (Hochberg

and Tamhane, 1987; Mickey et al., 2004) at $P < 0.05$.

RESULTS AND DISCUSSION

Photosynthetically active radiation in the shaded plots was reduced between 64% to 76% across months and years evaluated (Table 3-2). It is common for zoysiagrass managers to note the difficulty of getting turf to recover from injury, even under moderate shade. My results showed that zoysiagrasses growing under tree shade exhibited reductions of 38 to 95% in stolon number; 9 to 70% in stolon length; 10 to 93% in branching; and 56 to 98% in total aboveground biomass compared to turf in full sun (Tables 3-3 and 3-4). In addition, tillering declined from beginning to end of the study for 7 of the 10 grasses in 2008 and 6 of the 10 grasses in 2009 (Table 3-5).

Progeny from crosses of *Z. matrella* cultivars with *Z. japonica* have demonstrated good winter survival in the field and good freezing tolerance under controlled conditions (Chapter 4). Grasses with higher stolon numbers in the shade compared to Meyer in one or both years included 5311-22 and 5311-27 (Cavalier × Chinese Common), 5312-49 (Zorro × Chinese Common) and 5327-19 (Meyer × Diamond) (Tables 3-3 and 3-4). Several of these grasses also exhibited a lower reduction in stolon number in shade vs. full sun compared to Meyer. The reductions in growth in response to shade demonstrate how zoysiagrass recovery in moderate shade is inhibited, and quality often declines. However, the overall reduction in stolon number among all grasses under shade in the current study seems to contradict a suggestion by Beard (1997) that turfgrasses produce more lateral stems as a mechanism to cope with shade stress. A number of factors such as variable soil temperature and PAR due to a heterogeneous canopy, tree roots competing for nutrients and water, and other forms of interactions (synergistic or antagonistic) with the roots might have influenced the stolon production by the zoysiagrasses.

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Under silver maple tree shade in this study, 'Emerald' was superior to Meyer in stolon numbers in each year, and the reduction in stolon numbers in shade compared to full sun was less than in Meyer in 2008 (Tables 3-3 and 3-4). Emerald also increased tiller numbers under shade in each year (Table 3-5), and its ability to maintain or increase density under shade is likely a strong contributor to its reputation for good shade tolerance. In Dallas, TX, 'Emerald' was among the best-performing turfgrasses under 90% oak tree shade (Engelke and Reinert, 2003). It is slow to establish from plugs, however, even in full sun (Patton and Zeicher, 2007). Although Emerald does not have good freezing tolerance (Fry and Huang, 2004), progeny from Emerald × Meyer have exhibited good winter survival in the field and good freezing tolerance under controlled conditions (Chapter 4). In this study, under shade, 5321-3 (Emerald × Meyer) had an increase in tiller number in shade in both years, but neither it nor 5321-18 (Emerald × Meyer) was different from Meyer.

Z. matrella lines are generally considered to have better shade tolerance than *Z. japonica* cultivars. In four years of testing in Dallas, TX, Meyer zoysiagrass had an average of 25% coverage under 90% Live Oak tree (*Quercus virginiana* Mill) shade, whereas *Z. matrella* genotypes had between 50 and 75% in coverage (Morton et al., 1994). In Arkansas, subjecting established zoysiagrass plots to 50% artificial shade for 12 weeks resulted in >97% coverage for Cavalier, Diamond, and Zorro, and 90% coverage for Meyer (Trappe et al., 2009). In my study, Zorro had longer and more highly branched stolons in the shade compared to Meyer in 2008, and produced more stolons than Meyer in 2009 (Tables 3-3 and 3-4). In addition, the reduction in stolon numbers in Diamond and Zorro in shade compared to full sun was less than that observed for Meyer.

Z. matrella lines are also known for their high tiller density (Morris 2006; 2000; Morris and Shearman, 1995). Higher tiller densities in Cavalier and Zorro compared to Meyer occurred at the end of the study in 2009 (Table 3-5). Changes in tiller density across all *Z. matrella*s from the beginning to end of the studies were similar to Meyer in both years. The superior shade tolerance of *Z. matrella*s relative to Meyer that has been reported by others could be due, in part, to the high tiller densities of these cultivars. All *Z. matrella* cultivars except Diamond (2009) declined in tiller density from the beginning to the end of the studies. Despite declining tiller densities, however, numbers were higher than in Meyer at the end of the studies for Diamond in 2008, and for Cavalier and Zorro in 2009. As such, these cultivars can decline in tiller number in shade, yet maintain better density than other grasses because they have a larger initial number of tillers.

Chinese common, a *Z. japonica*, itself is not considered shade tolerant (Engelke and Reinert, 2003), although production of stolons in shade compared to full sun was less affected than Meyer in 2008 in this study (Table 3-3). Chinese common exhibited a 21% increase in tillers in 2008 and an 18% increase in 2009. If evaluated solely based on its stolon growth characteristics and tiller density under the conditions I imposed, Chinese common could be considered to have relatively good shade tolerance.

Growth under shade would indicate that the plant has received enough light to produce carbohydrates for maintenance respiration, plus additional carbohydrates are available to be used for growth, i.e. stolons in this case (Fry and Huang, 2004). A 85% decrease in total nonstructural carbohydrates in Diamond zoysiagrass occurred under 86% shade in the field (Qian and Engelke, 1999); under 88% shade in a polyhouse, carbohydrate levels declined by 65% a similar study, 10 weeks after shading (Qian et al., 1998;). Although stolon production and elongation indicates

that the zoysiagrass had produced carbohydrates above a maintenance level, stolon growth per se does not necessarily equate to good shade tolerance. For example, grasses which maintain tiller density and color in shade may have good tolerance because they are addressing maintenance respiration needs. Furthermore, some zoysiagrasses inherently produce rhizomes more readily than stolons, such as Diamond (Engelke et al., 2002a,b).

In summary, based upon stolon growth characteristics and absolute tiller density, I found that several zoysiagrasses exhibited better shade tolerance than Meyer, including Emerald, Cavalier, Diamond, and Zorro. In addition, progeny of crosses between these cultivars and a *Z. japonica* (Meyer or Chinese common), also demonstrated superior stolon growth and tillering under shade compared to Meyer. An important next step will be to evaluate these grasses in longer-term studies using larger, established swards of turf under shaded conditions, so that turf quality and physiological responses can be evaluated.

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Figure 3-1. Site of the shade study on the northern side of a line of silver maple (Acer saccharinum L.) at Manhattan, KS in 2008.

Table 3-1. Mean soil temperature ranges during the study period at the study sites in Manhattan, Kansas*.

Year	Shade (^{o}C)		Full Sun (^{o}C)	
	Max.	Min.	Max.	Min.
2008	18.6[**]	17.9	25.0	21.1
2009	20.7	14.1	26.1	23.9

*Temperatures were measured using soil thermometers inserted at a depth of between 0 to 10 cm.

**Mean soil temperatures are the averages of three values obtained from three randomly selected plots per replicate over six replications.

Table 3-2. Photosynthetically active radiation (PAR) in the shade and full sun study areas at the study site at Manhattan, Kansas in 2008 and 2009.

	PAR (μmol m^{-2} s^{-1})**											
	July				August				September			
	Shade		Full sun		Shade		Full sun		Shade		Full sun	
Time*	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
0730 hr	95.3[†]	110.9	612.1	663.2	58.5	51.4	137.1	215.1	56.7	47.4	199.5	169.6
1030hr	144.5	203.4	1533.9	1521.0	105.7	156.4	1317.8	1317.6	73.8	62.3	1196.6	1017.1
1330hr	407.9	364.0	2045.9	2012.5	367.1	379.7	1909.4	1020.3	269.0	171.9	1687.6	1624.5
1630hr	898.3	688.1	1580.2	1481.4	602.6	524.3	1275.7	1353.9	426.7	363.1	1027.8	873.6
1930h	107.2	220.5	612.1	254.2	83.3	112.4	156.8	171.4	6.3	13.3	11.7	18.0
% Daily	23.8	36.3			34.0	35.5			29.2	32.0		

*Time was based on US Naval central daylight time.

**PAR is the average of PAR from 8-sensors AccuPAR LP-80 ceptometer, across six replicates and 12 plots in 2008, and 13 plots in 2009.

[†] PAR was measured on 7 July, 18 Aug., and 9 Sept., 2008 and 9 July, 12 Aug., and 9 Sept., 2009.

Table 3-3. Stolon number, length, branching, and total biomass of shade-grown zoysiagrasses and reduction in growth compared to grasses in full sun at Manhattan, Kansas in 2008.

| Cultivar or progeny[*] | Stolon | | | | | | Total biomass(g)[§] | |
| | Number** | | Length (mm)[†] | | Branches (no./ stolon) [‡] | | | |
	Shade	Reduction in shade compared to sun (%)	Shade	Reduction in shade compared to sun (%)	Shade	Reduction in shade compared to sun (%)	Shade	Reduction in shade compared to sun (%)
Emerald (*Z. japonica* × *Z. tenuifolia*)	12.3 abcd[#]	78.4 b	184 de	18.6 a	16.5 abc	66.1 bcd	33.0 abc	67.0a
Z. japonica								
Meyer	3.8 f	95.0 a	162 de	30.6 a	7.8 cd	82.9 ab	26.7 abcd	73.4 a
Chinese Common	6.8 ef	78.0 b	319 abc	20.8 a	15.1 abc	59.5 bcd	29.3 abcd	70.7 a
Z. matrella								
Diamond	3.2 f	94.5 a	95 e	31.4 a	3.1 d	92.5 a	34.4 ab	65.6 a
Cavalier	10.7 bcde	85.1 ab	243 bcd	18.0 a	11.6 bcd	68.2 abcd	17.8 bcd	82.2 a
Zorro	14.8 ab	86.0 ab	303 abc	29.3 a	19.6 ab	72.2 abcd	6.1 d	93.9 a
Cavalier × C. Common								
5311-22	17.5 a	79.5 b	309 abc	28.7 a	13.3 abc	61.1 bcd	15.4 bcd	84.6 a
5311-27	12.0 bcde	75.0 b	213 cde	44.3 a	10.9 bcd	73.8 abc	43.9 a	56.2 a
Zorro × C. Common								
5312-49	9.2 cde	81.9 ab	336 ab	37.7 a	15.2 abc	52.8 cd	17.2 bcd	82.8 a
Emerald × Meyer								

5321-3	13.0 abc	86.9 ab	383 a	8.7 a	19.9 ab	57.1 bcd	9.98 cd	90.0 a
5321-18	-		-	-	-	-	-	-
Meyer × Diamond								
5327-19	9.7 bcde	80.2 b	348 ab	13.6 a	21.3 a	46.6 d	18.8 bcd	81.2 a

*Grasses were planted in silver maple tree shade and in full sun as 6-cm diameter plugs with six replicates on 30 June 2008.

 **The average number of stolons per plug over six replicates on 29 Sept. 2008.

[†]The average total length of three stolons per plug over six replicates on 29 Sept. 2008.

[‡]The average number of branches on three stolons per plug over six replicates on 29 Sept. 2008.

[§]The average dry weight of all plant part except roots, over six replicates after harvest on 29 Sept. 2008.

[#]Means followed by the same letter in a column are not significantly different according Ryan-Einot-Gabriel-Welsch test at $P < 0.05$.

Table 3-4. Stolon number, length, branching, and total biomass of shade-grown Zoysiagrass cultivars and experimental progeny relative to those grown in full sun as percentage of that observed in full sun at Manhattan, Kansas in 2009.

| | Stolon | | | | | | Total biomass(g) [§] | |
| | Number** | | Length (mm) [†] | | Branches (no./ stolon) [‡] | | | |
Cultivar or progeny [*]	Shade	Reduction in shade compared to sun (%)	Shade	Reduction in shade compared to sun (%)	Shade	Reduction in shade compared to sun (%)	Shade	Reduction in shade compared to sun (%)
Emerald (*Z.japonica* × *Z.tenuifolia*)	25.8 abc[#]	76.0 ab	407 bc	70.1 a	12.1 c	58.1 a	32.0 ab	89.1 a
Z. japonica								
Meyer	5.8 d	85.3 a	336 c	56.9 ab	14.2 bc	50.0 a	13.2 b	97.2 a
Chinese Common	9.7 cd	89.2 a	545 abc	44.4 bc	16.4 bc	24.3 a	29.3 ab	97.9 a
Z. matrella								
Diamond	14.3 abcd	57.4 bc	379 bc	65.1 ab	15.8 bc	20.4 a	14.0 b	90.5 a
Cavalier	21.2 abcd	76.0 ab	454 bc	64.6 ab	15.4 bc	48.7 a	17.7 ab	97.0 a
Zorro	28.2 ab	38.0 c	520 abc	68.6 a	20.5 abc	47.5 a	26.2 ab	96.8 a
Cavalier × C. Common								
5311-22	30.2 a	65.3 abc	770 a	63.9 ab	31.9 a	10.4 a	41.3 ab	97.9 a
5311-27	20.7 abcd	74.0 ab	482 bc	67.9 a	23.1 abc	51.5 a	34.2 ab	96.2 a
Zorro × C. Common								
5312-49	22.5 abc	72.8 ab	638 ab	59.4 ab	25.8 ab	8.5 a	54.7 a	92.8 a
Emerald × Meyer								
5321-3	21.8 abcd	80.2 a	601 abc	56.8 ab	24.9 ab	19.1 a	25.3 ab	96.0 a

5321-18	22.5 abcd	59.3 abc	470 bc	63.0 ab	16.8 bc	33.7 a	29.0 ab	91.2 a
Meyer × Diamond								
5327-19	19.5 abcd	43.9 bc	515 abc	52.7 abc	16.1 bc	44.1 a	20.7 ab	85.7 a

*Grasses were planted in tree shade and in full sun as 6-cm diameter plugs with six replicates on 26 June 2009.

**The average number of stolons per plug over six replicates on 24 Sept. 2009.

[†]The average total length of three stolons per plug over six replicates on 24 Sept. 2009.

[‡]The average number of branches on three stolons per plug over six replicates on 24 Sept. 2009.

[§]The average dry weight of all plant part except roots, over six replicates after harvest on 24 Sept. 2009.

[#]Means followed by the same letter in a column are not significantly different according to the Ryan-Einot-Gabriel-Welsch test at *P*<0.05.

Table 3-5. Changes in tiller number of zoysiagrass cultivars and experimental progeny in shade from 14 July to 27 Sept. 2008, and 1 July to 23 Sept. 2009 at Manhattan, Kansas.

| Cultivar or Experimental progeny* | Tillers (no. 20 cm^{-2})** | | | | | |
| | 2008 | | | 2009 | | |
	14 July	17 Sept.	Change (%)	1 July	23 Sept.	Change (%)
Emerald (*Z. japonica* × *Z. tenuifolia*)	68.3 bc[†]	76.2 b	+ 22.4 a	78.8 bcd	83.8 b	+ 17.4 a
Z. japonica						
Meyer	72.2 bc	56.3 bcde	- 18.7 abc	53.3 cde	50.7 de	+0.7 ab
Chinese common	29.3 c	34.5 e	+ 21.1 ab	33.2 e	36.2 f	+18.3 a
Z. matrella						
Diamond	189.7 a	114.2 a	- 39.2 bc	131.3 a	139.5 a	+19.4 a
Cavalier	89.5 b	65.8 bcd	- 8.4 abc	82.8 bcd	70.8 bc	- 1.9 ab
Zorro	72.2 bc	69.2 bc	- 0.9 abc	91.3 bc	77.0 b	- 13.6 ab
Cavalier × Chinese common						
5311-22	47.5 bc	36.2 de	- 22.8 abc	55.8 cde	43.8 ef	- 20.6 ab
5311-27	87.3 b	45.2 cde	- 43.3 c	44.0 de	45.3 ef	+ 4.7 ab
Zorro × Chinese common						
5312-49	41.7 c	39.8 cde	- 4.4 abc	49.5 de	56.8 cde	+20.8 a
Emerald × Meyer						
5321-3	42.0 c	45.0 cde	+9.9 abc	50.7 de	50.3 de	+6.9 ab
5321-18	-	-	-	96.7 b	65.7 bcd	-29.8 b

Meyer × Diamond						
5327-19	31.8 c	32.3 e	+6.3 abc	63.7 bcde	48.0 de	-19.6 ab

*Grasses were planted in tree shade and full sun as 6-cm diameter plugs with six replicates on 30 June 2008.

**The average number of tillers is the number of tillers counted within 20 cm^2 center of the planted plug over six replicates on 14 Jul. and 27 Sept. 2008.

[†]Means followed by the same letter in a column are not significantly different according to Ryan-Einot-Gabriel-Welsch test at $P <$ 0.05.

CHAPTER 4 - FREEZING TOLERANCE AND SEASONAL COLOR RETENTION OF ZOYSIAGRASSES

This paper is written in the format of *Applied Turfgrass Science*

Abstract

'Meyer' zoysiagrass (*Zoysia japonica* Steud.) is the most popular cultivar used in the transition zone of the U.S., primarily because it has an excellent freezing tolerance. My objectives were to: 1) evaluate 10 zoysiagrass progeny arising from crosses between *Z. matrella* (L.) Merr. × *Z. japonica* or 'Emerald' [*Z. japonica* × *Z. tenuifolia* Willd. ex Thiel] × Meyer, 'Cavalier' (*Z. matrella*), and DALZ 0102 (*Z. japonica*) for freezing tolerance; and 2) evaluate autumn and spring green color of the grasses, and determine the potential relationship between autumn color and freezing tolerance. Grasses were managed under golf course fairway conditions. Cores of each grass were sampled from the field in December 2007 and 2008; October, 2008, and February 2008 and 2009 and exposed to temperatures from -6 to -22 $^{\circ}$C in the laboratory. Lethal temperatures resulting in 50% death of tillers (LT_{50}) were determined by counting live tillers after 6 weeks of recovery in the greenhouse. Across sampling dates, LT_{50}s ranged from -0.2 C to -12.2 C. All grasses were equivalent to Meyer in freezing tolerance, with exception of Cavalier on 3 of 5 dates, and one *Z. matrella* x *Z. japonica* progeny in December 2007 performing worse than Meyer. Digital images were analyzed to determine fall and spring color. Five progeny were superior to Meyer in autumn color retention, but none were superior to Meyer in spring color. Autumn color in October and November, 2007 was positively correlated (r=0.40 to 0.58, *P* < 0.05) with LT_{50} in December, 2007, suggesting that a greener color corresponds to a higher LT_{50}. Progeny originating from the aforementioned crosses

demonstrated good freezing tolerance, and in some cases, superior autumn color compared to Meyer.

INTRODUCTION

Zoysiagrass (*Zoysia* spp.) is a warm-season turfgrass commonly used on lawns and golf courses in the transition zone and the southern parts of the U.S. mainly because of its high quality but low management requirements. 'Meyer' (*Z. japonica*) has been the cultivar used predominantly in the transition zone since its release in 1952, in part because it has an excellent freezing tolerance. However, Meyer is relatively slow to establish, is coarser in texture than *Z. matrella* or *Z. tenuifolia* cultivars, and has relatively slow recuperative rates (6, 18).

Since the release of Meyer, researchers have developed and released numerous zoysiagrass cultivars, including the *Z. matrella* cultivars 'Cavalier', Diamond, and 'Zorro' (2, 3, 4). Furthermore, 'Emerald' has been used in the southern U.S. since its release in 1955 (25). These cultivars have finer leaf textures and better quality characteristics compared to Meyer; however, poor freezing tolerance has limited their use in the transition zone. For example, in a field study in Indiana, Meyer exhibited no freezing injury, whereas other cultivars and their respective percentage of plots damaged by freezing temperatures were: Zorro and Cavalier, 28%; Emerald, 78%; and Diamond, 98% (18). Among 13 vegetatively propagated zoysiagrass cultivars exposed to an artificial freeze in a cold stress simulator, cultivars and respective lethal temperature resulting to death of 50% plants (LT_{50}s) were Meyer, -11.5 $^{\circ}$C ; Zorro, -9 $^{\circ}$C; Diamond, -8.4 $^{\circ}$C; and Cavalier, -10.3 $^{\circ}$C (18).

Recently, researchers at Texas A&M University crossed Emerald (*Z. matrella* × *Z. tenuifolia*) or *Z. matrella* cultivars with *Z. japonica* in an effort to develop one or more cultivars that have good density, a fine leaf texture, and quality similar to the aforementioned *Z. matrellas*,

but freezing tolerance as good or better than Meyer. In 2004, 600 zoysiagrass progeny were brought to Manhattan, Kansas, from Texas A & M, and planted as single 10-cm diameter plugs on 1 m centers in a large nursery. Since then, turfgrass researchers at Kansas State University have evaluated the new zoysiagrass progeny for quality and winter survival (26).

Among characteristics of interest to zoysiagrass managers are extended fall color and early spring green up (7, 24). Cessation of active growth of zoysiagrass in autumn is characterized by a dormant straw-brown color (15, 22). Some consider this objectionable and would prefer retention of green color longer into the autumn, but longer color retention has been associated with poorer freezing tolerance in warm-season turfgrasses. For example, Qian et al. (19) evaluated six buffalograss [*Buchloe dactyloides*(Nutt.) Engelm.] cultivars for freezing tolerance and reported that the extent of decrease in LT_{50} was negatively correlated to fall color retention (r = -0.72, *P* < 0.001) as grasses progressed into dormancy in Fort Collins, CO. However, enhancing autumn bermudagrass color with late-season N applications in the southern U.S. had no adverse effects on bermudagrass spring recovery (5, 8, 16, 21). In general, *Z. matrella* cultivars retain color longer in the autumn, which could contribute to their higher sensitivity to freezing. For example, fall color retention was 10% for Meyer, compared to Emerald (20%) and Zorro (46%) in a southern California National Turfgrass Evaluation Program trial (13). Average November green color of 'Meyer' was 43%, whereas that of Zorro was 57% across data collected in Riverside, CA, Manhattan, KS, and Raleigh, NC (14).   I hypothesized that new zoysiagrass progeny which exhibited longer color retention were less freezing tolerant than Meyer.

My objective was to evaluate the freezing tolerance, and fall and spring color of new zoysiagrass progeny, and determine if there is a relationship between autumn color and freezing tolerance.

MATERIALS AND METHODS

Freezing tolerance

Ten experimental zoysiagrass progeny from crosses of *Z. matrella* × *Z. japonica* or Emerald × *Z. japonica*, along with 'Meyer', 'Cavalier', and 'DALZ0102'(another experimental *Z. japonica* that has been evaluated extensively), Meyer, and DALZ 0102 (a *Z. japonica* that has undergone evaluation in the National Turfgrass Evaluation Program) were planted as 5-cm diam. plugs on 30-cm centers on 5 June 2007, in 1.5 m by 1.5 m plots arranged in a randomized complete block design with three replications at the Rocky Ford Turfgrass Research Center, Manhattan, Kansas (Table 4-1). Another of the *Z. matrella*s, listed as 8501, was never commercially released. Cavalier was growing approximately 100 m away from the study area in a nonreplicated plot that measured 1 by 3 m, but was maintained the same as turf within the study area. Soil was a Chase silt loam (fine, montmorillonitic, mesic, Aquic, Argiudolls) with pH 7.3, P level of 123 mg kg^{-1} and K level of 475 mg kg^{-1} at the start of the study. Just after planting, oxadiazon (5-tert-butyl-3-(2,4-dichloro-5-isopropoxyphenyl)-1,3,4-oxadiazol-2(3H)-one) at 3.4 kg ha^{-1} was applied to prevent emergence of annual grasses. Urea was applied to provide N at 49 kg ha^{-1} on 12 July and 31 August 2007, and 14 July and 12 August 2008. Plots were mowed 2 or 3 times weekly at a 5 cm height with a rotary mower until midsummer 2007, when the height was gradually reduced to 1.3 cm. A weather station at the site was used to monitor precipitation and air temperature. In-ground irrigation was used to prevent stress and supplement rainfall to provide 2.5 cm of water weekly. In addition, a soil-encapsulated thermocouple assembled according to Ham and Senock (9) was installed at 2.5 cm deep in one plot of each replicate to monitor soil temperature. Soil temperature was measured using a CR-10 (Campbell Scientific, Inc. Logan, UT) data logger.

Three (2008 and 2009) or four (2007) replicate cores, 10-cm diam. by 10-cm deep of each zoysiagrass were sampled randomly from the respective field plots starting on 15 Dec., 2007; 15 Feb., 15 Oct., and 15 Dec., 2008; and 15 Feb., 2009. DALZ 0102 was included in 2008 and 2009 evaluations only (Fig. 4-3a). Replicate one (with 6 cores of each progeny and cultivar) was sampled and exposed to artificial freezing on the aforementioned dates, and other replicates were sampled on consecutive days. Soil on each core was wrapped with aluminum foil to keep it intact. With the exception of the cores designated as controls and placed directly in a 4 ^{o}C growth chamber, plugs were placed in a freezer overnight at -3 ^{o}C and the canopy was covered with crushed ice to prevent supercooling (1, 26). On two randomly selected cores in each replicate, one thermocouple was inserted 2 cm below the soil surface and another at canopy level. Wires ran outside the chamber and allowed inside temperatures to be monitored (Fig. 4-3b).

The following day (approximately 20 h later), the temperature in the chamber was reduced 2 ^{o}C per hour and one core of each representative grass was removed at -6, -10, -14, -16, -18, and -22 ^{o}C. After removal, plugs were placed in a chamber set at 4 ^{o}C to slowly thaw overnight.

After thawing, grasses were planted in 8–cm diam. containers containing Metro-Mix$^®$ 510 (SUNGRO Horticulture, WA), and grown in a greenhouse maintained at a 30 ^{o}C/25 ^{o}C day/night temperature with a 14 h photo period under supplemental lighting to provide 580 umol·m^{-2}·s^{-1} at canopy level (Fig. 4-3c). Water was applied daily to maintain moist soil conditions. Recovery growth was evaluated after 6 weeks by counting the number of living tillers in each plug at each temperature. The number of surviving tillers for each plug at each

temperature was converted to a percentage of surviving tillers compared to the same grass exposed only to the 4 $^{\circ}$C treatment.

Color evaluations

Digital images of the plots for the aforementioned zoysiagrasses (except Cavalier) were taken using a Nikon D100 AF LR 28:105 mm camera (Nikon Corp, Japan) mounted on a tripod at a 1.2 m height, perpendicular to the surface of the plots, and facing the same direction. In 2007, photos were taken on 19 and 25 Oct.; and 1, 14, 20, and 27 Nov. In 2008, photos were taken on 17, 22, and 28 April; 6 May; 8, 16, and 28 Oct.; 7 and 19 Nov; and 2 Dec. Dates for photos in 2009 were 17 and 21 April; and 1, 8, and 18 May. Digital images were analyzed to obtain percent green color using Sigma Scan Pro (v. 5.0, SPSS, Inc., Chicago, IL) under a color hue threshold between 30 and 100 and saturation between 0 and 100 (11).

Data analysis

Data for tiller recovery and color were subjected to analysis of variance using SAS, and means were separated using the Ryan-Einot-Gabriel-Welsch (REGWQ) mean separation test (10, 12) at $P < 0.05$. All the percentage tiller survival values were adjusted by addition of 0.0001, a minimum possible value to eliminate the infinite natural $\log_{10}$ value which could result from zero percentage survival. Regression analysis was performed using PROC CORR in SAS using $\log_{10}$ percentage tiller survival plus 0.0001(y axis) vs. temperature (x axis) for each of the treatment temperatures. After generation of the regression equation, the value 50% was entered for tiller survival, and the corresponding LT_{50} was calculated. A correlation analysis was done to determine the relationship between percentage fall color and LT_{50}s using PROC CORR (23).

RESULTS AND DISCUSSION

Freezing tolerance

No sub-zero soil temperatures were recorded during the 2007/2008 evaluation period (Fig. 4-1). Air temperatures reached below 0 ^{o}C from 21 Nov. 2007 and continued intermittently until 9 Mar. 2008. In 2008/2009, soil temperatures occasionally dropped to < 0 C from 16 December through 3 March (Fig. 4-2). Sub-zero air temperatures were recorded from 7 Nov. and lasted intermittently until 10 April 2009. The 2008/2009 study period experienced earlier and longer period of air temperatures below zero compared to 2007/2008 winter.

Differences in freezing tolerance were observed on 15 Dec. 2007, 15 Feb. 2008, and 15 Dec. 2008 (Table 4-1). Cavalier had higher LT_{50}s than Meyer on each of these dates. The only grass exhibiting inferior freezing tolerance to Meyer was 5324-53 (8501 × Meyer) on 15 Dec. 2007; otherwise, all were not significantly different from Meyer. Emerald and cultivars from *Z. matrella*, such as Cavalier, have been shown to have inferior freezing tolerance to Meyer. For example, Meyer had an LT_{50} of -16.2 ^{o}C, whereas Cavalier had an LT_{50} of -9.6 ^{o}C when they were exposed to artificial freezing after sampling from the field in Manhattan, KS (26). All of the grasses evaluated herein that involved crosses between either Emerald or a *Z. matrella* and a *Z. japonica* resulted in progeny with freezing tolerance equivalent to Meyer, with the exception of 5324-53 as noted above. Several of these grasses have demonstrated faster establishment rates (Chapter 1), better shade tolerance (Chapter 3), and improved quality compared to Meyer, which bodes well for potential release of a freezing tolerant cultivar in the near future.

Color

Meyer was always in the lowest statistical group regarding fall color retention and the highest statistical group regarding spring green up (Table 4-2). Grasses that had superior fall color compared to Meyer in one or both years were: 5283-27 (Cavalier × Meyer) on all dates in 2007 (Fig. 4-4); 5311-3 and 5311-8 (Cavalier × Chinese Common) on 14 Nov. and 1 Nov. 2007, respectively; 5321-3 (Emerald × Meyer) and 5324-53 (8501 × Meyer) on 14 and 27 Nov. 2007 and 19 Nov. 2008.

A positive correlation between LT_{50} and fall green color would indicate that green color retention corresponds to poor freezing tolerance (i.e., the LT_{50} was increasing along with green color). This relationship was observed when LT_{50} on 15 Dec. 2007 was compared to green color on 25 Oct., and 14, 20, and 27 Nov. 2007 (Table 4-3, Fig. 4-4). As previously mentioned, 5324-53 (8501 × Meyer) was the only zoysiagrass which had inferior freezing tolerance to Meyer, and this occurred on 15 Dec. 2007. As noted above, it also sustained green color longer into the fall than Meyer. The relatively high LT_{50} for 5324-53 on 15 Dec. likely contributed to the positive correlation observed. The lack of additional positive relationships between fall green color and LT_{50} was likely due to the generally good freezing tolerance that occurred among the zoysiagrass progeny evaluated. Curiously, the LT_{50} on 15 Oct. 2008 was negatively correlated to fall green color on 19 Nov. and 2 Dec. (Table 4-3). On 15 October, however, grasses did not yet appear dormant, and the correlation, although statistically significant, may not be horticulturally significant. My results on 15 Dec. 2007 are in agreement with those of Qian et al. (19) who evaluated six buffalograss cultivars for freezing tolerance and reported that the amount of decrease in LT_{50} between September and November was negatively correlated to fall color retention (r = -0.72, $P < 0.001$) as grasses progressed into dormancy in Fort Collins, CO.

Differences among selected progeny and Meyer in spring green color occurred only on the first evaluation dates in 2008 and 2009. Furthermore, it was the same progeny in each year (with the exception of 5321-3, Emerald × Meyer, on 17 April 2008) which exhibited lower spring color than Meyer. These grasses were all from Cavalier x Chinese Common, and included 5311-3, 5311-8, 5311-26, 5311-27, and 5311-32. Slower spring green up of these progeny could be considered a negative attribute relative to Meyer. In mid-April in Indiana, spring color of Chinese common (41% green) was higher than Meyer (30%), which likewise was higher than Emerald and Zorro (22%) (20). Spring green-up evaluations in 2008 across seven locations including Riverside, CA, Gainesville, FL, West Lafayette, IN, Manhattan, KS, Las Cruces, NM, Raleigh, NC, and Dallas, TX revealed that Meyer was ranked first among nine zoysiagrass cultivars for spring green up (61%) compared to nine other zoysiagrass cultivars evaluated (15). Evaluation of early spring green up over three years in Fayettville, AR, revealed that Meyer averaged 63% , Cavalier 56%, Chinese common 41%, DALZ 0102 56% , Emerald 50%, and Zorro 46% (17).

In summary, I found that zoysiagrass progeny originating from *Z. matrella* × *Z. japonica* or Emerald × *Z. japonica* were as freezing tolerant as Meyer. The one exception was a progeny from 5324-53 (8501 × Meyer), which was inferior to Meyer on 15 Dec. 2007. Some of the progeny exhibited more green color than Meyer in autumn and this resulted in a positive correlation between LT_{50} on 15 Dec. 2007 and green color in October and November 2007. Spring color of Meyer was equivalent or superior to all progeny evaluated. Autumn color provides a means of gauging the extent of freezing tolerance of zoysiagrasses and would be a better to for selecting freezing tolerant zoysiagrass cultivars.

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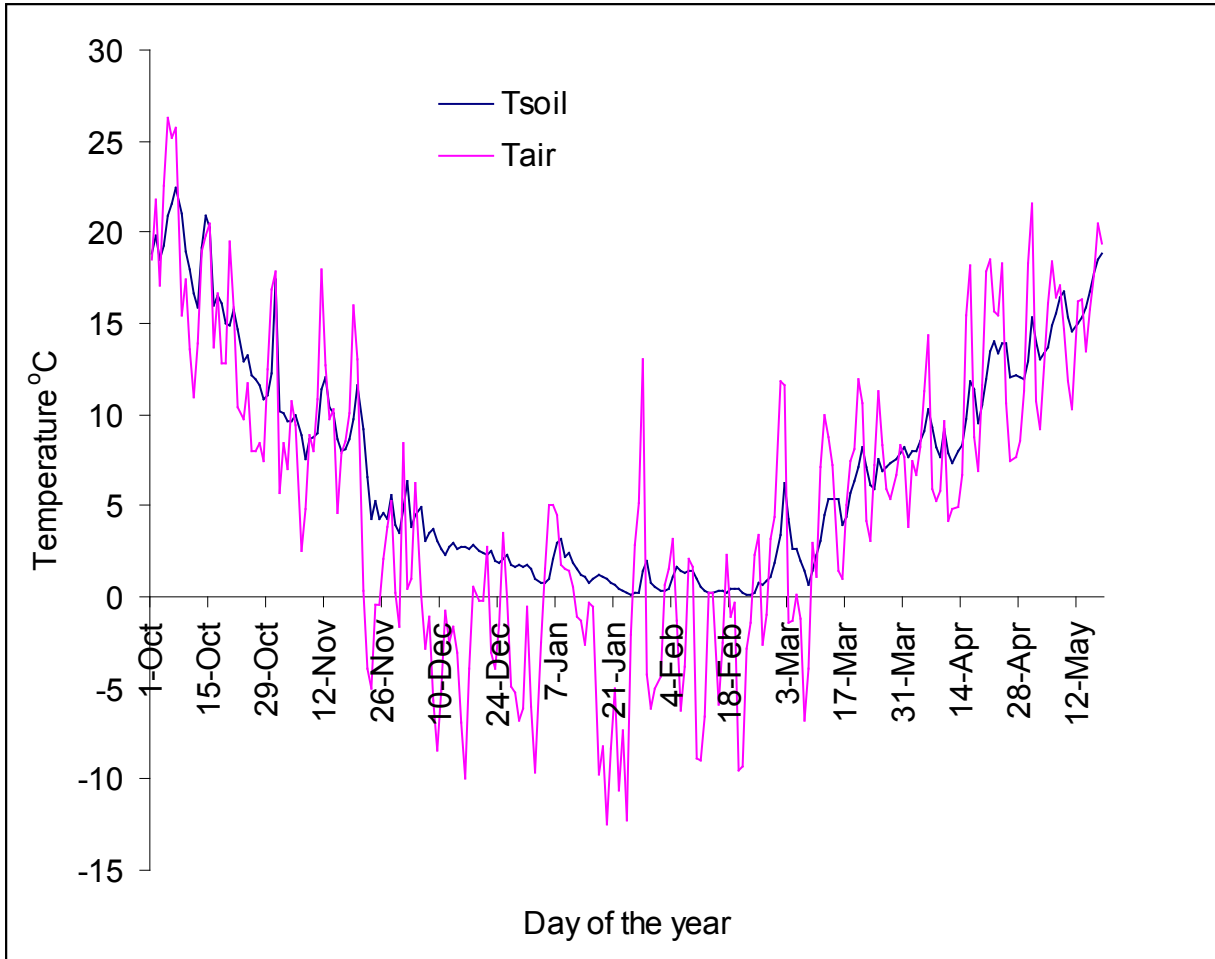
Figure 4-1. Average daily air and soil temperatures at the study site in Manhattan, Kansas in 2007 and 2008. Temperature was measured using a thermocouple installed at 5 cm deep in one plot of each replicate to monitor soil temperature and data downloaded using a CR-10 data logger.
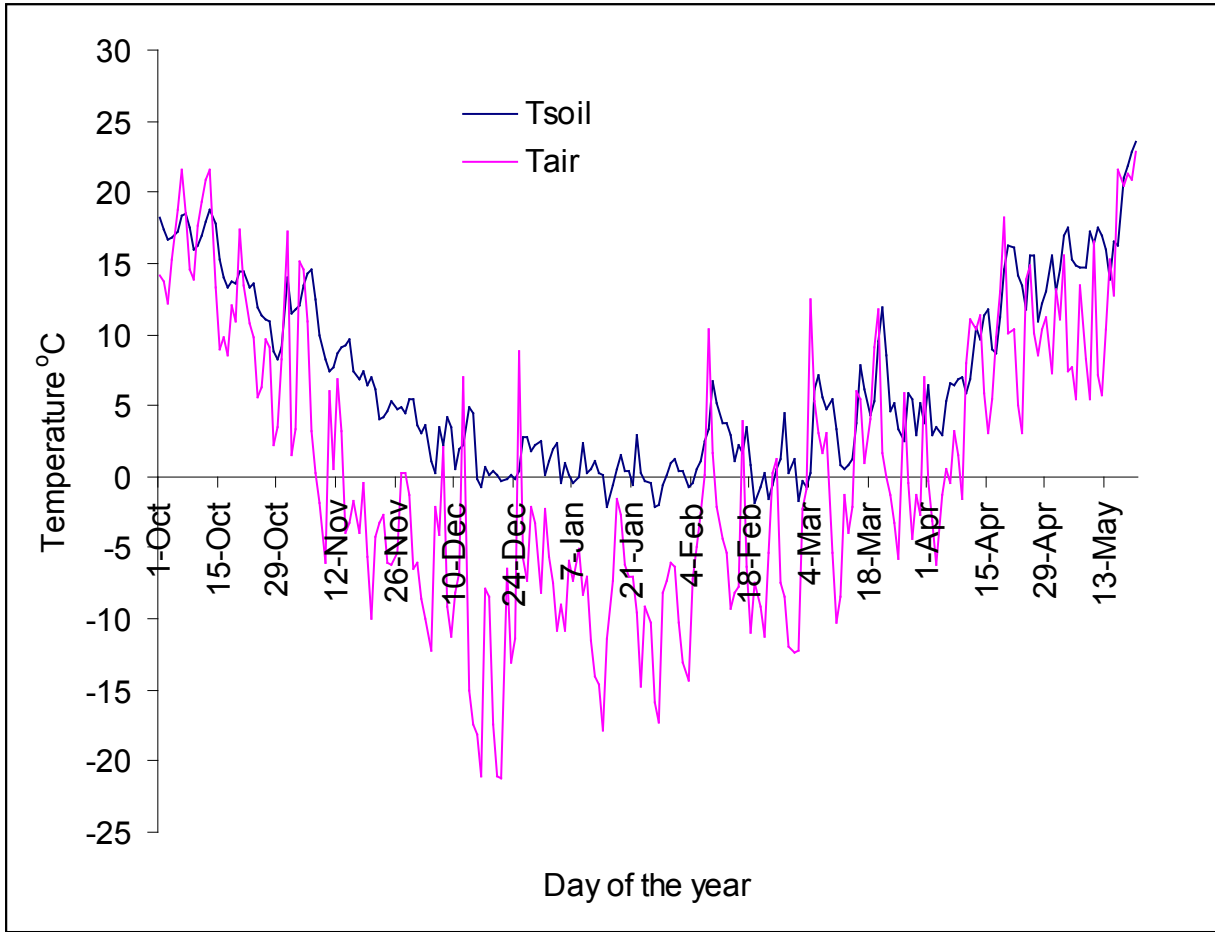
Figure 4-2. Average daily air and soil temperatures at the study site in Manhattan, Kansas in 2008 and 2009.

Temperature was measured using a thermocouple installed at 5 cm deep in one plot of each replicate to monitor soil temperature and data downloaded using a CR-10 data logger.

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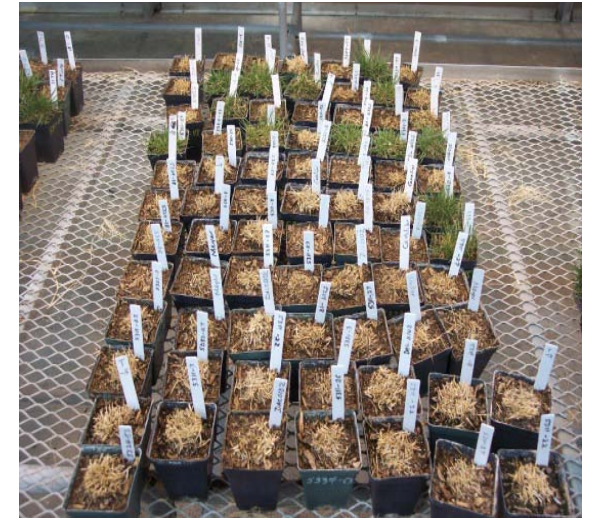
(a)   (b)   (c)

Figure 4-3. Sampling cores from the field (a), subjecting to freezing (b), and evaluating recovery in the greenhouse (c).

(a)

(b)

Figure 4-4. Digital overhead images of Meyer (a) and 5283-27 (Cavalier × Meyer) (b) field plots used for fall color analysis on 14 Nov. at Manhattan, Kansas in 2007.

Table 4-1. Lethal temperatures resulting in death of 50% of zoysiagrass tillers (LT50) after sampling from the field at Manhattan, KS in 2007, 2008, and 2009.

Cultivar or Progeny[x]	LT_{50} (C)[w]				
	15 Dec. 2007	15 Feb. 2008	15 Oct. 2008	15 Dec. 2008	15 Feb. 2009
Meyer	-10.7 c[z]	-12.9 b	-5.8 a	-12.0 b	-4.8 a
Cavalier	-0.2 a	-5.2 a	-4.8 a	-5.0 a	-4.8 a
DALZ 0102[y]	-------	-10.9 b	-7.3 a	-10.9 ab	-4.8 a
Cavalier × Meyer					
5283-27	-8.6 bc	-8.4 ab	-4.8 a	-9.0 ab	-4.8 a
Cavalier ×Chinese common					
5311-3	-9.0 bc	-11.8 b	-4.8 a	-11.6 b	-4.8 a
5311-8	-8.8 bc	-10.0 b	-4.8 a	-6.2 ab	-4.8 a
5311-22	-9.3 c	-10.8 b	-4.8 a	-9.5 ab	-4.8 a
5311-26	-10.3 c	-11.8 b	-4.8 a	-8.4 ab	-4.8 a
5311-27	-10.4 c	-12.2 b	-2.7 a	-8.5 ab	-4.8 a
5311-32	-9.0 bc	-10.9 b	-5.5 a	-11.0 ab	-4.8 a
Emerald × Meyer (5321-3)	-10.4 c	-10.8 b	-6.0 a	-9.6 ab	-4.8 a
8501 × Meyer					
5324-18	-8.4 bc	-11.3 b	-7.2 a	-8.8 ab	-4.8 a
5324-53	-3.4 ab	-10.9 b	-7.3 a	-8.2 ab	-4.8 a

[w]LT_{50}s were determined by fitting a linear regression of $\log_{10}$ (percent tiller number plus 0.0001) vs temperature, then substituting $\log_{10}$ 50% in the generated equation to obtain the corresponding temperature.

[x]Grasses were randomly sampled as sixteen 6-cm diam. cores from plots maintained under culture similar to the golf course fairway.

[y]DAL 0102 was not included in 2007 sampling.

[z]Means with the same letters along the column are not significantly different according to Ryan-Einot-Gabriel-Welsch (REGWQ) multiple range test at $P < 0.05$.

Table 4-2. Percent green color of zoysiagrasses at Manhattan, KS in 2007, 2008, and 2009.

Cultivar or progeny	Green color (%)[y]											
	2008			2009			2007			2008		
	17 Apr.	22 Apr.	6 May	17 Apr.	1 May	18 May	1 Nov.	14 Nov.	27 Nov.	8 Oct.	28 Oct.	19 Nov.
Meyer	36.0 a[z]	38.4 abc	73.0 a	30.3 a	31.4 ab	58.2 abc	79.5 cde	37.7 c	25.1 d	49.0 ab	45.3 a	5.7 c
DALZ 0102	41.3 a	65.1 a	70.9 a	27.2 a	46.2 a	91.5 ab	80.1 bcd	40.7 bc	26.0 d	58.5 ab	47.3 a	22.8 a
Cavalier × Meyer												
5283-27	39.9 a	43.9 abc	69.3 a	16.6 abc	27.9 ab	50.4 bc	85.5 a	55.4 a	34.9 ab	66.8 ab	47.3 a	15.2 abc
Cavalier × Chinese common												
5311-3	13.4 b	30.2 bc	73.7 a	2.7 c	25.1 b	44.1 c	83.0 abc	50.0 ab	28.8 cd	70.7 ab	42.9 a	16.1 abc
5311-8	8.3 b	16.7 c	87.3 a	3.8 bc	19.9 b	32.2 c	84.8 ab	41.2 bc	29.5 bcd	74.3 ab	45.5 a	8.5 abc
5311-22	29.0 ab	55.2 ab	89.8 a	20.4 a	30.6 ab	56.4 abc	79.7 bcde	40.4 bc	29.3 cd	79.4 a	41.2 a	7.8 bc
5311-26	14.7 b	55.0 ab	95.7 a	6.9 bc	22.0 b	37.0 c	74.9 e	37.5 c	29.9 bcd	45.5 b	44.7 a	5.3 c
5311-27	12.8 b	45.4 abc	62.1 a	6.1 bc	22.2 b	37.5 c	75.9 de	41.2 bc	28.2 d	44.7 b	42.9 a	4.2 c
5311-32	11.8 b	40.9 abc	80.7 a	6.6 bc	22.4 b	38.0 c	79.9 bcde	45.2 abc	29.1 cd	59.0 ab	61.0 a	9.4 abc
Emerald × Meyer												
5321-3	7.9 b	18.0 c	68.5 a	24.2 a	29.6 ab	54.0 abc	79.7 bcde	50.7 ab	33.3 abc	43.8 b	45.3 a	20.7 ab
8501 × Meyer												
5324-18	22.0 ab	57.9 ab	79.9 a	16.9 ab	39.8 ab	73.8 abc	83.1 abc	46.2 abc	27.7 d	67.5 ab	51.0 a	14.6 abc
5324-53	41.4 a	51.5 ab	60.3 a	22.7 a	47.7 a	94.8 a	83.0 abc	52.9 a	35.3 a	62.7 ab	41.8 a	21.0 ab

[y]Percent green color is an average percent color of four digital images taken using a Nikon Kodak Camera mounted 1.2 m from the ground, and analyzed using Sigma Scan Pro 5. .

[z]Means represent the average of three replicates. Numbers followed by the same letter in a column are not significantly different according to Ryan-Einot-Gabriel-Welsch multiple comparison test at $P < 0.05$.

Table 4-3. Correlation coefficients for LT50 vs fall color of zoysiagrasses at Manhattan, KS in 2007 and 2008.

| | Fall color (%)[z] | | | | | | | | | | | |
| | 2007 | | | | | | 2008 | | | | | |
LT_{50}[y]	19 Oct.	25 Oct.	1 Nov.	14 Nov.	20 Nov.	27 Nov.	8 Oct.	16 Oct.	28 Oct.	7 Nov.	19 Nov.	2 Dec.
15 Dec. 2007	-0.20	0.44**	0.30	0.40*	0.42*	0.58***	0.10	-0.12	0.02	0.17	0.28	0.23
15 Feb. 2008	0.07	0.22	0.25	0.27	0.23	0.31	0.14	-0.10	0.09	0.09	0.16	0.02
15 Oct. 2008	0.11	0.00	-0.14	-0.06	-0.06	0.01	-0.05	0.02	-0.06	-0.17	-0.35*	-0.43**
15 Dec. 2008	0.21	0.20	0.02	0.08	0.11	0.26	0.23	-0.10	0.09	0.06	-0.22	-0.21

*, **, ***Significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

[y]LT_{50}s were determined by fitting a linear regression of log_{10} (percent tiller number plus 0.0001) vs. temperature, then substituting log_{10} 50% in the generated equation to obtain corresponding temperature.

[z]Fall color is the percent of selected green pixels to the total pixels of digital images of plots established on 26 June 2007.

CHAPTER 5 - FREEZING TOLERANCE AND DEHYDRIN AND CHITINASE GENE EXPRESSION IN ZOYSIAGRASSES

This paper is written in the format of *Crop Science*

Abstract

'Meyer' (*Zoysia japonica* Steud.) is a popular zoysiagrass cultivar used in the transition zone of the U.S. primarily because it has an excellent freezing tolerance. My objectives were to: 1) identify and determine the extent of expression of dehydrin-like and chitinase genes in the rhizome and stolon tissues among 10 zoysiagrass progeny arising from crosses between a *Z. matrella* (L.) Merr. × *Z. japonica*, or 'Emerald' [*Z. japonica* × *Z. tenuifolia* Willd. ex Thiel] x Meyer, cultivars Meyer and 'Cavalier' (*Z. matrella*), and DALZ 0102 (a *Z. japonica* not yet released) during cold acclimation in the field; and 2) determine relationships between gene expression and freezing tolerance. Grasses were managed under golf course fairway conditions. Cores of each grass were sampled from the field in December 2007 and 2008; October, 2008, and February 2008 and 2009 and exposed to temperatures between -6 and -22 $^{\circ}$C in the laboratory. Lethal temperatures resulting in 50% loss of tillers (LT_{50}) were determined by counting tillers after 6 weeks of recovery in the greenhouse. Protein levels in rhizomes and stolons collected on the same dates was determined by immunoblotting. Across sampling dates, LT_{50}s ranged from -0.2 C to -12.2 C. All grasses except Cavalier and one progeny were similar to Meyer in freezing tolerance. Dehydrin and chitinase gene expression increased during cold acclimation and protein levels (dehydrin-like-11.9, 23, 44.3, and 66.3 kDa, and chitinase-26.9 kDa) were similar among all grasses, but were not correlated with freezing tolerance.

INTRODUCTION

Zoysiagrass (*Zoysia* spp.) is a warm-season turfgrass used in lawns and golf courses in southern U.S. and in the transition zone in the central region of the U.S. where high summer and low winter temperatures make it difficult to maintain high quality turf. Among the preferred characteristics of zoysiagrass are its good density, resistance to pests, and low-input management (Fry and Huang, 2004).

Freezing tolerance of zoysiagrass varies among species (Patton et al., 2007a & b). Cultivars from *Z. japonica* generally have better freezing tolerance than those from *Z. matrella* (Patton et al., 2007 a & b; Zhang et al., 2006). Since its release in 1952, 'Meyer' (*Z. japonica*) has been the predominant cultivar used in the transition zone because of its excellent freezing tolerance. However, it is slow to establish, has a relatively coarse leaf texture, and is slow to recover from injury (Patton and Reicher, 2007; Fry and Dernoeden, 1987). Researchers released several zoysiagrass cultivars after 'Meyer', including the *Z. matrella* cultivars 'Cavalier' (1996), 'Diamond' (1996), and 'Zorro' (*Z. matrella*) (2002); 'Emerald' (*Z. japonica* × *Z. tenuifolia*) (1955); and *Z. japonica* cultivars El Toro (2000), Crowne (1996), and Palisades (1996) (Goudswaard, 1980; Engelke et al., 1996; 2002a, b,c; Engelke and Reinert, 2003). Although some of these (Cavalier and Zorro) have been used successfully in southern portions of the transition zone, use at more northern locations is risky due to freezing tolerance that is inferior to Meyer. For example, in a field study in Indiana, Meyer exhibited no freezing injury, whereas other cultivars showed considerable freezing injury [Zorro and Cavalier, 28%; Emerald, 78%; and Diamond, 98% (Patton et al., 2007a)]. Among 13 vegetatively propagated zoysiagrass cultivars subjected to an artificial freezing in a cold chamber, Meyer was the most freezing

tolerant, exhibiting LT_{50} (the temperature that kills 50% of living tillers) of -11.5 $^{\circ}$C while

Diamond (LT_{50} of -8.4 $^{\circ}$C) was the least tolerant (Patton et al., 2007a).

Plant tissues freeze extracellularly and tolerate the presence of ice in the extracellular

spaces to varying degrees (Patton et al., 2007a & b; Guy, 1990). Extracellular freezing can cause

mechanical injury and dehydration, which may disrupt the integrity and functions of the plasma

membrane (Thomashow, 1999; Monroy 1993; Guy, 1990). As freezing continues, changes in

plasma membranes result to retardation of biochemical and chemical reactions, damage from

enzymes and toxic substances leaking from vacuoles, and loss of water (dehydration). This

results in a phenomenon referred to as freezing injury, characterized by membrane lesions with a

water soaked appearance, solute leakage, oxidative browning, loss of turgor, desiccation, and

crown injury (Thomashow, 1999). Warmund et al. (1998) and Patton et al. (2007) suggested that

'Meyer' zoysiagrass rhizomes sustain substantial dehydration stress whenever cells and tissues

were exposed to extracellular ice formation during freezing.

 In response to cold acclimation, freezing tolerant turfgrasses cease active growth and

accumulate sugars, unsaturated lipids, organic acids, proline, and soluble proteins (Patton et al.,

2007a & b; Zhang et al., 2006; Shahba et al., 2003; Fry et al., 1993). In addition, plants can

activate genes, resulting in the production of proteins that may have a role in enhancing their

freezing tolerance. These include a number of late embyogenesis (LEA) proteins such as

dehydrins (Close et al., 1996) and cold responsive (COR) chitinases (Yeh et al., 2000; Gaschet et

al., 1996).

Dehydrin accumulation has been associated with cell membrane stability, cryoprotection,

antifreeze activity, and antioxidation (Battaglia, 2008; Close et al, 1996). Previously, dehydrin-

like proteins (65, 60, and 14 kDa) had been reported to accumulate in floral buds of cold-tolerant

blueberry (*Vaccinium myrtillus* L.) cultivars and vegetative tissues of barley (*Hordeum vulgare* L.) during cold acclimation (Battaglia et al., 2008; Rowland et al., 2008; Close, 1996). A correlation between dehydrin accumulation and freezing tolerance in barley (*Hordeum vulgare* L.) (Choi et al., 1999), rhododendron species (Marian et al, 2003), and wheat (*Triticum aestivum* L.) (Danyluk et al., 1998) has been reported. Patton et al. (2007a and 2007b) suggested that differences in freezing tolerance among cultivars of zoysiagrass were due, in part, to the differences in accumulation of dehydrin–like proteins, carbohydrates, and proline in rhizomes and stolons. Likewise, several groups of cold responsive (COR) proteins (20-28 kDa) have been associated with bermudagrass freezing tolerance, and one of them was identified as a chitinase (Gatschet et al., 1996). Chitinases are widely known as pathogenesis-related (PR) proteins (Tamo et al, 2003; de los Reyes et al, 2001); however, PR proteins identified as class I and II chitinases have been reported to accumulate during cold acclimation, adsorb on ice surfaces, and inhibit their growth and recrystallization during freezing (Anderson et al., 2002; de los Reyes et al., 2001; Hoshino et al., 1999; Gaschet et al., 1996).

It is important to identify any relationship that may exist between the accumulation of these cold/dehydration-responsive proteins and freezing tolerance in zoysiagrass progeny. In addition, ability to detect proteins associated with cold hardiness could be an important tool for selecting freezing-tolerant zoysiagrasses for use in the transition zone. My objectives were to identify and determine the extent of expression of dehydrin-like and chitinase genes during cold acclimation under field conditions, and any possible association between the expression of these genes and freezing tolerance of zoysiagrass progeny.

MATERIALS AND METHODS

Ten zoysiagrass progeny from crosses of *Z. matrella* × *Z. japonica* or Emerald × *Z. japonica*, along with 'Meyer', 'Cavalier', and 'DALZ0102'(another experimental *Z. japonica* that has been evaluated extensively), were planted on 5 June 2007 in 1.5 m by 1.5 m plots at the Rocky Ford Turfgrass Research Center, Manhattan, Kansas. Plots were arranged in a randomized complete block design with three replications. The experiment was conducted on a chase silt loam soil (fine, montmorillonitic, mesic, Aquic, Argiudolls). Soil pH was 7.3, P level was 123 mg kg^{-1}, and K level was 475 mg kg^{-1} at the start of the study. Just after planting, oxadiazon (5-tert-butyl-3-(2,4-dichloro-5-isopropoxyphenyl)-1,3,4-oxadiazol-2(3H)-one) at a rate of 3.4 kg ha^{-1} was applied to prevent emergence of annual grasses. Irrigation was applied three days weekly to provide approximately 2.5 cm water per week. Plots were mowed at a 5 cm height with a rotary mower until midsummer, when the height was gradually reduced to 1.3 cm. A weather station located within 100 m of the study area was used to monitor air temperature and precipitation. In addition, a soil-encapsulated thermocouple assembled according to Ham and Senock (1992) was installed at 2.5 cm deep in one plot of each replicate to monitor soil temperature. Urea was applied to provide N at 49 kg ha^{-1} on 12 July and 31 August 2007; 14 July and 12 Aug. 2008; and 21 July and 18 Aug. 2009. Soil temperature data were downloaded biweekly using a CR-10 data logger (Campbell Scientific, Inc. Logan, UT). Soil and air temperature data can be found in Fig. 4-1 and 4-2.

Freezing tolerance

Freezing tolerance was determined as described in Chapter 4 (p. 83 to 84).

Protein analysis

Plugs measuring 15-cm diameter and 5-cm deep of ten zoysiagrass progeny, 'Meyer', 'Cavalier' (2007), and 'DALZ0102' (2008 and 2009) were collected from the field on 12 Dec. 2007; 15 Feb., 16 Sept., and 15 Dec., 2008; and 16 Jan., 2009. A single plug was sampled from each of the three field replications established in June 2007. Leaves were removed, soil was washed away, and rhizomes and stolons were cut into 10-cm lengths and frozen in liquid nitrogen. The frozen stolon and rhizome mixture was placed in 15 ml polypropylene corning tubes (Corning Inc., NY) and stored at -80 ^{o}C until protein extraction.

Protein extraction:

One gram of each sample of each replication was separately weighed and ground to a fine powder in liquid nitrogen using a mortar and a pestle. A 1.5 ml extraction buffer was measured from a stock solution consisting of 3.9 ml distilled, deionized water, 1 ml phosphorous buffer, 0.05 PVP, 5 μL β-mercaptoethanol, 100 uL protease inhibitor cocktail (Sigma, St. Louis, MO) was added onto the fine powder and stirred to thaw completely. The mixture was centrifuged at a speed of 13,200 rpm at 4 ^{0}C for 15 minutes in an Epperndorf centrifuge (5415D). The supernatant was transferred using a pipette into another tube and centrifuged again for 10 minutes at the same speed and temperature. The resulting supernatant was transferred using a pipette into new microfuge tubes, labeled, and placed on ice for analysis. Just for convenience, half of the extract was used to determine total concentration, and the rest used for sodium dodesulfate polyacrylamide gel electrophesis (SDS PAGE). Buffer-soluble protein concentrations were determined using a microplate reader (Biotek Instruments, Winooski, VT). A modified Bradford method was used in Dec. 2007 and Feb. 2008, while in Sept. and Dec. 2008, and Feb. 2009, the Lowry method was used following a trichloroacetic acid (TCA)

precipitation of the samples (Bio-Rad, Hercules, CA). The procedure was repeated for the three

replicates and on all sampling dates, however, because of smaller quantities of rhizomes and

stolons sampled, the protein analysis was not replicated in 2007 while in 2008 and 2009, enough

material was collected to allow for three replications.

SDS-PAGE:

Ten μg of protein was measured for each sample, adjusted to a total of 18 μL volume

using sample buffer, heated at 95 $^{\circ}$C for 10 minutes before loading onto a gel [1mm NuPAGE 4-

12% Bis-Tris Gel with MES gels) (Invitrogen Corp., Carlsbad, CA)] for electrophoresis. A

standard [5 μL of Mark 12^{TM} (Invitrogen corp., Carlsbad, CA, 1997-2002)] and a positive

control were used. Purified dehydrin protein from 'White Icicle' radish (*Raphanus sativus* L.)

seeds (Close et al., 1993, Patton et al., 2007a) and chitinase protein from malted barley

(*Hordeum vulgare* L.) extract were used as positive dehydrin and chitinase controls, respectively.

After electrophoresis, the gels were rinsed in water for 5 minutes and then stained using

Coomassie brilliant blue (Pierce Biotechnology, Rockford, IL) overnight (Patton et al, 2007).

Digital images for analysis were captured (Appendix 5-1), and then gels were destained in 50%

methanol (v/v) for western blot transfer. The 1 mm thick SDS-PAGE gels were equilibrated in

the transfer buffer consisting of 48 mM Tris, 39 mM glycine, 20% (v/v) methanol, 0.0375%

(w/v) SDS twice for 10 minutes each, before protein transfer.

Western blotting:

Proteins from SDS-PAGE gels (15% w/v) were transferred for one hour to a 7 cm by 8

cm nitrocellulose membrane (Westboro, MA) using 150 volts, and 143 milliamps**.** The

membranes were temporarily stained using 0.5% (w/v) ponceau S containing 1% (v/v) acetic

acid to confirm proper transfer had taken place onto the membranes. Membranes were rinsed

three times in tris-buffer saline (TBS) for 5 minutes. The membranes were blocked with dry milk

[(3 g in 100 ml TBS plus polyethylene glycol sorbitan monolaurate (Tween 20)] or TBST for 30

minutes and were then incubated with either a 1:250 dilution of class I and II binding anti-

chitinase from barley (prepared by Dr. Muthukrishnan et al., Dept. Biochemistry, Kansas State

University) or 1:250 anti-dehydrin (Stressgen, PLA-100) from rabbit (polyclonal antibody) in

TBST for 1 hour. After primary antibody incubation, membranes were washed in TBST 3 times

for 5 minutes and then immersed in TBST containing the secondary goat anti-rabbit IgG

antibody (dilution 1:3000) conjugated to alkaline phosphatase (170-6518, Bio-Rad Laboratories,

Hercules, CA) for 1.5 h. The membranes were then rinsed in TBST 3 times for 5 minutes. The

membranes were then incubated for color development in a buffer (400 μL AP conjugate

solution, 96 mL H_2O, 100 μL reagent A, 100 μL reagent B) for 15 minutes on a shaker. The

membranes were then rinsed with ddH_2O 3 times for 10 minutes and allowed to air-dry

overnight.

Data analysis

Intensities of bands were determined using Carestream Molecular Imaging Software 5.0

(Carestream Health, Inc., New Haven, CT, 2009). The data for both chitinase and dehydrin

intensities were standardized and then normalized to obtain z-scores using the values for Meyer

(control). The data were analyzed using the general linear models procedure to determine

differences among the samples (SAS, 2003). Mean intensity of protein bands among grasses

were separated using the Ryan-Einot-Gabriel-Welsch (REGWQ) multiple comparison test

(Hochberg and Tamhane, 1987; Mickey et al., 2004) at $P < 0.05$. A correlation analysis was done

to determine any relationship between the LT_{50}s and the protein levels.

RESULTS AND DISCUSSION

Freezing tolerance ranged from -0.2 $^{\circ}$C (Cavalier) to -12.9 $^{\circ}$C (Meyer) (Table 4-1).

Across the sampling dates, soluble protein concentrations ranged from 0.968 μg μl^{-1} to 1.462 μg

μl^{-1} on 15 September and 15 October, 0.957 to 1.878 μg μl^{-1} on 15 December, and 0.606 to 1.478

μg μl^{-1} on 15 February.

The dehydrin antibody usually reacts to the lysine-rich consensus sequence of dehydrin

proteins thus identifying these as dehydrins or dehydrin-like proteins (Close, 1996). In my

findings, a polyclonal dehydrin antibody bound to dehydrin-like polypeptides of molecular

weight of approximately 11.9, 23 (Appendix 5-2), 43.4, and 66.3 kDa were observed in samples

collected on 15 Sept. 2007 (Fig. 5-1), but only 43.4 and 66.3 kDa were evident on all dates,

except on 15 Sept. 2008 (Fig. 5-1 and 5-2). The reasons for this variable response in the grasses

are not clear. However, it is possible that it may be due to a procedural artifact as the proteins

were extracted and precipitated during 2008 analysis using trichloroacetic acid in acetone

whereas no precipitation was done in 2007. With regard to chitinase, the gene was activated

during cold acclimation and the gene product (26.9 kDa protein) was found in most grass

accessions during both 2007 and 2008 (Fig. 5-3 and 5-4).

Dehydrin-like and chitinase gene expression increased slightly from either 15 September

or 15 October to December in both years (Table 5-1 and 5-2). However, the expression of these

genes did not appear to be consistent between the two years. Cavalier was the least freezing

tolerant zoysiagrass while Meyer, known to be superior in freezing tolerance, was similar in its

ability to tolerate mid-winter temperatures as the other accessions except progeny 5324-53 (8501

× Meyer) on 15 Dec. 2007 (Table 4-1). Previous studies have reported that Cavalier, among

other *Z. matrella* cultivars, and Emerald, had higher LT$_{50}$s and sustained more winter injury

compared to those cultivars from *Z. japonica,* such as Meyer (Patton et al., 2007; Zhang et al., 2007). Crossing a cold-hardy Z. japonica with Emerald or a *Z. matrella* resulted in progenies which have improved freezing tolerance compared to progenies from parents other than Z. *japonica* (Cavalier or Emerald). However, level of dehydrin-like polypeptides in Cavalier was not significantly different from Meyer on all dates. In addition, there was no correlation between the level of expression of either dehydrin or chitinase and LT_{50}. There were, however, differences among zoysiagrasses in levels of dehydrin-like proteins during cold acclimation and deacclimation, on 15 Oct. and 15 Dec. 2008, and 15 Feb. 2009 (Table 5-1). Likewise, there were differences in levels of chitinase during cold acclimation and deacclimation on 15 Sept. 2007, 15 Dec. 2007, 15 Dec. 2008 and 15 Feb. 2009 (Table 5-2).

It is not uncommon to find fluctuations in the accumulation of cold responsive proteins in the plant tissues as they acclimate. Patton et al. (2007a) reported that dehydrin-like polypeptides were not detected in all zoysiagrass cultivars before acclimation. In the same study, cultivars which had dehydrin-like gene expression before acclimation had a decrease in expression of 41 and 75 kDa and an increase in expression of 12, 14, 18, 21, 23, and 25 kDa dehydrin polypeptides when rhizomes and stolons were cold-acclimated in controlled-environment chamber. In addition, an SDS-PAGE analysis showed that despite many dehydrin-like polypeptides observed, only five changed in level of accumulation as the zoysiagrasses became cold acclimated (Patton et al., 2007a). According to Patton et al. (2007a), only two (23 and 25 kDa) dehydrins were predominant in cold-acclimated zoysiagrass plants. Furthermore, variation in expression of the 25 kDa dehydrin was found among zoysiagrass cultivars, whether from *Z. matrella* or *Z. japonica* (Patton et al., 2007a). In my findings, there was an increase in expression of dehydrin-like polypeptides expression in samples collected on 15 Dec. 2007 and 15 Dec. 2008

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(43.4 and 66.3 kDa). However, 43.4 and 66.3 kDa polypeptides increased in some samples and decreased in others on 15 Feb. 2009, when grasses were considered more hardy compared to 15 Sept. 2007 or 15 Oct. 2008 (Fig. 5-1 and 5-2). Although none of the polypeptides I observed were correlated with freezing tolerance, I observed inconsistency in dehydrin-like gene expression, which is similar to a trend reported for most polypetides detected by Patton et al. (2007a).

Accumulation of chitinase proteins has been associated reduced growth and solidification of melting cellular ice crystals which may be injurious during freezing (Anderson et al., 2005; Hoshino et al., 1999). Gaschet et al. (1996) identified one of the groups of COR proteins (20 to 28 kDa) expressed in crowns of 'Midiron' and 'Tifgreen' bermudagrass (*Cynodon dactylon* L. Pers. × *C. transvaalensis* Burtt-Davy) cultivars, (COR27) as a chitinase, following 26 days of 10 hours of 8/2°C (day/night) acclimation in a controlled chamber. However, they observed that the correlation between increased accumulation of protein during cold acclimation and freezing tolerance was greater for cold-hardy 'Midiron' than cold-sensitive'Tifgreen'. Yeh et al. (2000) also reported expression of class II chitinase genes with an antifreeze property during cold acclimation in winter rye (*Secale cereale* L.). When exposed to cold acclimation, de-acclimation, and dehydration conditions, de los Reyes et al. (2001) found that bermudagrass (*Cynodon* spp.) crown tissues resulted in different levels of expression of chitinase genes (*CynCHT1*). Similar to the dehydrin-like polypeptides, a northern-blot analysis in bermudagrass indicated very low levels of chitinase genes in non-acclimated crown tissues of 'Midiron' and 'MSU', and none in'Uganda'; however, the chitinase mRNA increased in all cultivars from day 2 and 28 of cold acclimation (de los Reyes et al., 2001). The level of enhanced chitinase gene expression was inconsistent among the three cultivars , with levels about 3x higher in acclimated compared to

nonacclimated Uganda, and 6x higher in acclimated vs. nonacclimated MSU (de los Reyes et al., 2001). Hoshino et al. (1999) suggested that five antifreeze proteins similar to chitinases of 19, 26, 32, 34, and 36 kDa that accumulate during cold acclimation exhibit significant levels of antifreeze activity that increase freezing tolerance in winter rye. Swegle et al., (1992) reported an accumulation of chitinases of classes I (27 kDa), II (34 kDa) and III (35 kDa) in barley seeds in early stages of imbibition. In my results, the only chitinase protein band detected (molecular weight approximated at 26.9 kDa) using anti-chitinase antibody that specifically binds to chitinase of class I (27 kDa) and II (34 kDa) was likely chitinase class I.   The expression increased as the turfgrasses underwent cold acclimation from 15 September to 15 December 2007. However, an increase in chitinase protein accumulation from 15 Oct. 2008 through 15 Feb. 2009 was observed in only some accessions, a similar pattern of increase noted in the aforementioned findings with bermudagrass. Pathogenic-related proteins identified as class I and II chitinases have been reported to accumulate during cold acclimation, adsorb on ice surfaces, and inhibit their growth and recrystallization during freezing (Anderson et al., 2002; de los Reyes et al., 2001; Hoshino et al., 1999; Gaschet et al., 1996). Seemingly, increased accumulation of chitinase proteins during acclimation which I observed in this study, plays an important role in survival of zoysiagrasses. However, it is not clear whether 26.9 kDa polypetide plays a pathogen-defense role (induced by mechanical injury by ice) or it inhibits ice growth and recrystallization (survival strategy) in zoysiagrass.

Lack of a correlation between either dehydrin-like or chitinase gene expression and LT_{50}s does not necessarily indicate that the presence of these proteins may not be important in freezing tolerance. For example, Patton et al. (2007a) observed changes in accumulation of six polypeptides, but only the level of 23-kDa dehydrin-like protein was positively associated ($r^2 =$

0.41, $P = 0.018$) with freezing tolerance based on immunoblot analysis. In addition, Patton et al. (2007a and 2007b) suggested that many factors, including accumulation of sugars, proline, and proteins also influence cold hardiness of zoysiagrasses. Researchers have also suggested that inconsistency in the expression of cold responsive genes among turfgrass cultivars could be a result of interspecific hybridization among natural populations even though a standard cultivar may be considered solely as either a *Z. matrella* or a *Z. japonica* (Patton et al., 2007a). In the current study, differences in freezing tolerance of Cavalier and Meyer, yet similarities in dehydrin and chitinase expression, could indicate that other physiological and biochemical processes are involved, as also suggested by Qian et al. (2001). Additionally, acclimation under heterogeneous field conditions, as opposed to artificial acclimation procedures (a more homogeneous condition) in previous studies could lead to inconsistent gene expression and the results may be different from those reported on other zoysiagrass hybrids by others (Patton et al. (2007a). Evidently, more work is needed to understand the roles of chitinase and dehydrin-like gene expression and the dynamics of protein accumulation during turfgrass acclimation in relation to cold tolerance, a potential tool for selecting freeze tolerant lines among new zoysiagrasses.

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Polypeptide (kDa)	Date	Cultivars and progeny											
		Meyer	Cavalier	5283-27	5311-3	5311-8	5311-22	5311-26	5311-27	5311-32	5321-3	5324-18	5324-53
66.3	15 Sept.												
	15 Dec.												
43.4	15 Sept.												
	15 Dec.												
23	15 Sept.												
	15 Dec.												
11.9	15 Sept.												
	15 Dec.												

Figure 5-1. Western blot visualization of dehydrin-like gene expression in selected zoysiagrass progeny and cultivars in 2007.

Date	Polypeptide (kDa	Cultivars and progeny												
		5283-27	5311-3	5311-8	5311-22	5311-26	5311-27	5311-32	5321-3	5324-18	5324-53	Meyer	Cavalier	DAL0102
15 Oct. 2008	43.4 and 66.3													
15 Dec. 2008	66.3													
	43.4													
15 Feb. 2009	66.3													
	43.4													

Figure 5-2. Western blot visualization of dehydrin-like gene expression during acclimation on 15 October, 15 December 2008, and 15 February 2009.

Date	Meyer	Cavalier	5283-27	5311-3	5311-8	5311-22	5311-26	5311-27	5311-32	5321-3	5324-18	5324-53
				Cultivars and progeny								
15 Sept.												
15 Dec.												

Figure 5-3. Western blot visualization of chitinase gene (26.9 kDa) expression in selected zoysiagrass progeny and cultivars in 2007.

Date	Cultivars and progeny												
	5283-27	5311-3	5311-8	5311-22	5311-26	5311-27	5311-32	5321-3	5324-18	5324-53	Meyer	Cavalier	DAL0102
15 Oct. 2008													
15 Dec. 2008													
15 Feb. 2009													

Figure 5-4. Western blot visualization of Chitinase gene (26.9 kDa) expression during acclimation on 15 October, 15 December 2008, and 15 February 2009.

Table 5-1. Intensities of expression of dehydrin-like genes in stolons and rhizomes of zoysiagrasses*.

Progeny or Cultivar	Intensity [†]									
	43.4 kDa					66.3 kDa				
	2007		2008		2009	2007		2008		2009
	15 Sept.	15 Dec.	15 Oct.	15 Dec.	15 Feb.	15 Sept.	15 Dec	15 Oct.	15 Dec	15 Feb.
Meyer	0.0**	8.2	0.0 a[‡]	1.0 b	8.6 ab	0.0	2.4	0.0 b	3.3 bc	1.8 b
Cavalier	10.6	0.8	0.0 a	1.0 b	10.6 ab	0.0	1.5	0.0 b	4.7 bc	7.8 ab
DAL 0102	-	-	0.0 a	4.4 ab	3.6 b	-	-	33.3a	21.2 abc	3.4 b
8507 × Meyer (5283-27)	8.3	0.0	0.0 a	10.9 a	5.0 b	0.0	9.1	0.0 b	26.6 ab	10.1 ab
Cavalier × C. Common										
5311-3	0.0	0.0	0.0 a	16.4 a	33.0 a	0.0	0.4	0.0 b	29.4 a	27.7 a
5311-8	0.0	0.0	0.0 a	4.9 ab	18.7 ab	0.0	0.1	0.0 b	16.9 abc	7.4 ab
5311-22	7.1	13.7	0.0 a	8.7 ab	23.5 ab	2.9	0.1	0.0 b	10.6 abc	23.4 a
5311-26	32.6	0.0	0.0 a	3.7 ab	2.2 b	2.2	9.8	0.0 b	3.3 bc	8.8 ab
5311-27	21.0	0.0	0.0 a	6.0 ab	10.3 ab	1.9	9.5	0.0 b	22.1 ab	7.5 ab
5311-32	16.3	0.0	0.0 a	5.8 ab	0.8 b	2.8	17.3	0.0 b	15.5 abc	2.6 b
Emerald × Meyer (5321-3)	5.6	0.0	0.0 a	1.0 b	0.0 b	35.3	8.5	0.0 b	0.0 c	5.7 b
8501 × Meyer										
5324-18	0.0	7.5	0.0 a	1.0 b	13.3 ab	7.6	26.2	0.0 b	0.0 c	28.2 a
5324-53	10.1	33.6	0.0 a	1.0 b	0.9 b	0.0	29.1	0.0 b	5.4 bc	23.7 ab

* Stolons and rhizomes were randomly sampled from plots planted on 5 June, 2007.

**Means for protein expression among the grasses were not separated because of less replication of protein extracts.

[†]Approximate band intensities were intensities of expression determined using Carestream molecular imaging software 5.0, standardized using the control

(Meyer), and then normalized to obtain z-scores.

[‡]Means with the same letters along the column are not significantly different according to Ryan-Einot-Gabriel-Welsch multiple range test at $P < 0.05$.

Table 5-2. Intensities of expression of a 26.9 kDa chitinase gene in stolons and rhizomes of zoysiagrasses.*

Progeny	Intensity[†]				
	2007		2008		2009
	15 Sept.	15 Dec.	15 Oct.	15 Dec.	15 Feb.
Meyer	23.7**	10.4	0.0 a[‡]	0.0 c	0.4 b
Cavalier	28.7	35.7	0.0 a	0.0 c	0.5 b
DALZ 0102			0.0 a	1.0 c	0.4 b
8507 × Meyer (5283-27)	0.0	0.0	0.0 a	19.1 b	18.9 ab
Cavalier × C. Common					
5311-3	0.0	0.3	0.0 a	0.0 c	1.1 b
5311-8	2.8	4.5	0.0 a	33.1 a	33.4 a
5311-22	1.3	4.2	0.0 a	1.3 c	0.0 b
5311-26	1.4	2.7	0.0 a	2.5 c	6.8 b
5311-27	1.0	3.7	0.0 a	8.2 bc	7.6 b
5311-32	0.0	0.0	0.0 a	0.0 c	0.2 b
Emerald × Meyer (5321-3)	0.0	0.0	0.0 a	0.0 c	0.3 b
8501 × Meyer					
5324-18	1.3	4.3	0.0 a	0.0 c	0.2 b
5324-53	0.0	0.0	0.0 a	0.0 c	0.3 b

* Stolons and rhizomes were randomly sampled from plots which were planted on 5 June, 2007.

**Means for protein expression among the grasses were not separated because of fewer replications of protein extracts.

[†] Approximate band intensities were intensities of expression determined using Carestream molecular imaging software 5.0, standardized using the control (Meyer), and then normalized to obtain z-scores.

‡Means with the same letters along the column are not significantly different according to Ryan-Einot-Gabriel-Welsch (REGWQ) multiple range test at $P < 0.05$.

‡Means with the same letters along the column are not significantly different according to Ryan-Einot-Gabriel-Welsch multiple range test at $P < 0.05$.

Appendix 3A. Stolon number, length, branching, and total biomass of zoysiagrasses in full sun at Manhattan, KS.

Cultivar or progeny[*]	Stolon							
	Number**		Length(cm)[†]		Branches (no./ stolon)[‡]		Total biomass(g)[§]	
	2008	2009	2008	2009	2008	2009	2008	2009
Emerald (*Z .japonica* × *Z. tenuifolia*)	62.5 cd[z]	156.5 a	22.6 ef	138.3 ab	47.2 b	32.2 c	51.4 e	583.7ab
Z. japonica								
Meyer	171.2 a	61.5 bc	24.2 ef	85.3 b	47.3 b	37.0 c	55.1 e	617.3ab
Chinese common	47.8 d	119.7 abc	61.7 a	128.3 ab	55.6 ab	46.5 bc	140.1 ab	1317.8a
Z. matrella								
Diamond	55.3 d	45.7 c	14.4 f	114.9 ab	37.8 b	25.5 c	48.2 e	267.2 b
Cavalier	72.0 bcd	124.8 abc	31.7 de	136.1 ab	38.4 b	45.3 bc	95.1 bcde	989.7 ab
Zorro	115.8 b	94.3 abc	45.2 bc	193.6 ab	71.7 a	87.2 a	178.8 a	1107.8 ab
Cavalier × C. Common								
5311-22	86.5 bcd	154.7 a	44.9 bc	217.4 a	39.0 b	76.8 ab	112.3 bcd	1511.8 a
5311-27	61.3 cd	105.3 abc	38.9 cd	173.9 ab	41.6 b	50.5 abc	77.3 de	1392.3 a
Zorro × C. Common								
5312-49	57.3 cd	124.8 abc	53.1 ab	164.4 ab	41.4 b	51.3 abc	104.6 bcd	1008.7 ab
Emerald × Meyer								
5321-3	105.5 bc	105.5 bc	43.4 bcd	140.2 ab	51.0 ab	50.8 abc	136.5 abc	762.3 ab
5321-18	-	93.7 abc	-	155.6 ab	-	35.3 c	-	654.2 ab

Meyer × Diamond								
5327-19	61.3 cd	56.7 bc	41.8 bcd	113.3 ab	42.8 b	47.3 bc	70.1 de	626.5 ab

*Grasses were planted in tree shade and in full sun as 6-cm diameter plugs with six replicates on 30 June 2008, and on 26 June 2009.

**The average number of stolons per plug over six replicates on 29 Sept. 2008 and on 24 Sept. 2009.

[†]The average total length of three stolons per plug over six replicates on 29 Sept. 2008 and on 24 Sept. 2009.

[‡]The average number of branches on three stolons per plug over six replicates on 29 Sept. 2008 and on 24 Sept. 2009.

[§]The average dry weight of all plant part except roots, over six replicates after harvest on 29 Sept. 2008 and on 24 Sept. 2009.

[#]Means followed by the same letter in a column are not significantly different according Ryan-Einot-Gabriel-Welsch test at $P < 0.05$.

Appendix 5A. Sodium dodecylsulphate gel electrophoresis (SDS-PAGE) profiles of buffer-soluble protein from the selected zoysiagrass progeny and cultivars in 2007.
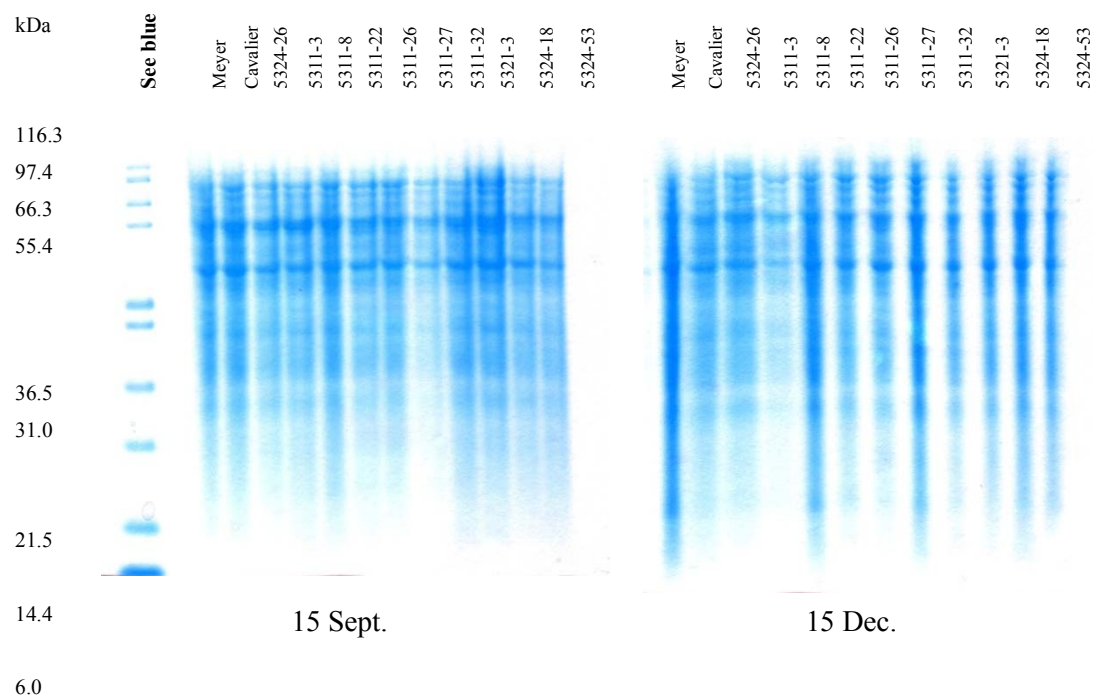
Appendix 5B. Intensities of expression of dehydrin-like gene expression in 2007.

Progeny or Cultivar	Approximated net intensity for different band sizes (kDa)†		
	15 Oct.	15 Dec.	
	11.9	11.9	23
Meyer	16.4**	7.3	7.0
Cavalier	0.0	10.8	0.4
8507 × Meyer (5283-27)	1.0	32.0	0.0
Cavalier × C. Common			
5311-3	12.6	24.7	0.0
5311-8	0.0	0.0	0.0
5311-22	7.1	4.3	3.8
5311-26	0.1	8.1	3.7
5311-27	0.0	10.1	7.6
5311-32	0.0	1.2	0.0
Emerald × Meyer (5321-3)	32.2	0.0	16.2
8501 × Meyer			
5324-18	1.4	4.7	33.7
5324-53	0.1	16.9	0.0

* Stolons and rhizomes were randomly sampled from plots which were planted on 5 June, 2007.

**Means for band intensities among the grasses were not separated because of fewer replications of protein extracts.

†Approximate band intensities were intensities of expression determined using Carestream Molecular Imaging Software 5.0, standardized using the control (Meyer), and then normalized to obtain z-scores.