

CHAPTER VII

**SUCROSE METABOLISM AND TOTAL NONSTRUCTURAL
CARBOHYDRATES IN ZOYSIAGRASS AND TALL FESCUE
IN RESPONSE TO DEFICIT IRRIGATION**

ABSTRACT

This study was conducted to investigate sucrose metabolism and carbohydrate metabolic responses to deficit irrigation in 'Meyer' zoysiagrass (*Zoysia japonica* Steud) and 'Falcon II' tall fescue (*Festuca arundinacea* Schreb.). Sods of both grasses were established in PVC tubes (10 cm in diam.x 50 cm long) filled with a mixture of sand and fritted clay (9:1, v:v) and then placed in a growth chamber. Deficit irrigation treatments were: (1) well-watered control; (2) mild drought stress (60% ET); (3) severe drought stress (20% ET). Reference evapotranspiration (ET) was determined by weighing containers containing well-watered turf every 3 days to determine water loss, and converting to ET. Deficit irrigation increased leaf sucrose content in both species on most sampling dates. Zoysiagrass and tall fescue receiving 60% and 20% ET exhibited increased activities of sucrose phosphate synthase (SPS) and sucrose synthase (SS), and decreased acid invertase activity. The root:shoot ratio of total nonstructural carbohydrates (TNC) increased compared to well-watered turf at 60% and 20% ET for zoysiagrass, and at 60% ET for tall fescue. Compared to zoysiagrass, tall fescue had a higher sucrose content and SPS, SS, and acid invertase activity. Sucrose in leaves of deficit-irrigated turf may serve as an osmoticum to reduce cell water loss. Increasing TNC in roots relative to shoots during deficit irrigation may help to encourage root growth and soil exploration during periods of drought stress

ABBREVIATIONS

ET, evapotranspiration; SPS, sucrose phosphate synthase; SS, sucrose synthase. TNC, total nonstructural carbohydrates.

INTRODUCTION

Maintenance of quality turf requires periodic irrigation, even in humid climates. Strategies to minimize turf water inputs include the use of drought resistant species and cultivars, and deficit irrigation, defined as applying water in amounts less than ET.

Improved responses of bean plants (*Phaseolus vulgaris* L.) to water deficits is associated with sucrose metabolism (Castrillo, 1992; Vassey et al., 1991). However, sucrose accumulation during deficit irrigation of turfgrasses, and associated enzyme dynamics have never been reported.

Sucrose is the dominant form of carbon transported to developing plant organs, and is an important form of stored sugar in higher plants (Kharat and Zieslin, 1987). Sucrose also serves as an osmotic solute (Premachandra et al., 1992; Tan et al., 1992; Zhang and Archbold, 1993; Rekika et al., 1998). Effect of water deficits on sucrose levels has been reported in some plants. McManus et al. (2000) found that after white clover (*Trifolium repens* L.) was exposed to a period of moderate drought stress, the proportion of sucrose content in water-sufficient leaf rose 2- to 3-fold. A 300% increase in sucrose level was detected at the end of a 8-day long drought period in sugarbeet plants (*Beta vulgaris* L.) (Harn and Daie, 1992).

Sucrose-phosphate synthase is a key enzyme in the regulation of sucrose synthesis (Huber and Huber, 1996; Huber and Huber, 1992; Stitt et al., 1987). In leaves, changes in SPS activity are often correlated with changes in the rate of sucrose synthesis and export (Huber and Israel, 1982; Rocher et al., 1989; Stitt et al., 1988). Water deficits led to an increase in SPS activity in potato (*Solanum tuberosum* L.) tubers (Geigenberger et al., 1997), soybean (*Glycine max* (L.) Merrill) leaves (Cheikh and Brenner, 1992), pigeonpea

(*Cajanus cajan* L. Millsp) (Keller, 1993). However, Castrillo (1992) reported that the values of total (substrate saturating conditions) and Pi-insensitive (substrate limiting conditions plus inorganic phosphate) SPS activity in bean were reduced by drought stress. These conflicting results implied that effects of drought stress on SPS activity depends upon experimental conditions.

Sucrose synthase is another enzyme involved in sucrose metabolism that affects sucrose synthesis, and has been reported in bean (Castrillo, 1992) and wheat (*Triticum aestivum* L.) grain (Yang et al., 2001). Yang et al. (2001) reported that water deficit enhanced SS assayed in the cleavage direction; but he did not measure SS in the synthesis direction. Castrillo (1992) reported that SS as a synthesis of sucrose was increased by water deficit.

Acid invertase is involved in supplying hexoses for respiration, the establishment of sucrose gradients, and vacuolar osmotic-turgor related to cell expansion (Hawker, 1985). Dorion (1996) observed that the activity of soluble acid invertase in wheat declined four fold during drought stress period and never recovered.

Reduced photosynthesis often limits plant growth when soil water availability declines. A negative whole plant carbon balance could occur as a result of reduced photosynthetic capacity during drought. Roots are major consumers of the carbon produced through photosynthesis, and use it mainly for respiration and tissue construction (Lambers, 1987; Lambers et al., 1996). Huang and Fu (2000) reported that carbon allocation to roots in tall fescue and Kentucky bluegrass increased when the upper 20 cm of soil was allowed to dry. Efficient carbon allocation to roots might help to encourage root growth and soil exploration during periods of drought stress (Sisson, 1989).

Zoysiagrass (C₄) and tall fescue (C₃) are warm-season and cool-season grasses that are widely used in temperate climate, respectively. Carrow (1996) reported that grasses varied in drought resistance from medium-high (Rebel II tall fescue), medium (K-31 tall fescue), and medium low ('Meyer' zoysiagrass). Fu et al (2003) observed that zoysiagrass (Meyer) and tall fescue (Rebel II) required 80% and 60 % ET irrigation respectively and slightly different minimum irrigation amount to maintain acceptable quality. However, how sucrose metabolism and TNC contribute to the difference in drought resistance between both species and how deficit irrigation influences sucrose metabolism and associated enzymes is not well understood. Greater knowledge of these responses might provide insights into plant drought-resistance mechanisms.

The objectives of this study were to investigate the influence of deficit irrigation on sucrose and total nonstructural carbohydrate metabolism, and enzyme activity related to production of sucrose in tall fescue and zoysiagrass.

MATERIALS AND METHODS

Plant materials and growth conditions

Sods of 'Falcon II' tall fescue and 'Meyer' zoysiagrass were collected from the field and planted on a mixture of sand and fritted clay (9:1, v:v) retained in polyvinyl chloride (PVC) tubes (10 cm in diam. x 50 cm long). 3-cm thick growth medium soil (Scotts company, Marysville, OH) was placed on the top of the mixture to prevent excessive water evapor. Grasses were grown for about 90 d in green house with a temperature of 25/20 °C (day/night) for tall fescue and 35/30 °C for zoysiagrass and a 14 h photoperiod with a photosynthetically active radiation of 400 $\mu\text{mole m}^{-2} \text{s}^{-1}$. Before the experiment, plants were fertilized weekly with half-strength Hoagland's solution (Hoagland and Arnon, 1950). Turf was mowed every other day at a 5-6 cm height with an electric clipper. Once irrigation treatments began, PVC tubes were moved into growth chamber. Day night temperatures for both species were 27.5/22.5 °C based on average temperature (27.2/22.2 °C, day/night) from June 3 to September 14 in 2001.

The study was set up as a split-plot design with turfgrass species as the whole plots and irrigation levels of 20, 60, and 100% of ET as the sub-plots. Amounts of water to deliver respective deficit irrigation levels were determined by taking the fraction of turf ET measured in well-watered tubes using the water balance method (Qian and Fry, 1996). The day before the study began, tubes were watered until drainage occurred from the bottom of each tube. 24 h later, holes were blocked with stoppers and tubes were weighed to determine reference weights. To determine ET, tubes were weighed between 0900 h and 1000 h. The mass of water lost from each was recorded and converted to ET. Water was then added to return each to its reference weight.

Measurements

Grass leaves (about 1 gram) from each treatment were collected randomly every 5 to 9 days. To prepare leaves for analysis of leaf water content, sucrose content, and TNC, half of collected leaves (about 0.5 gram) were weighed, exposed to 105 °C for 30 minutes, and dried at 75 °C in a convection oven until sample weight remain constant.

Total nonstructural carbohydrate and sucrose levels were measured using the methods described by Chatterton et al. (1987).

To extract enzymes, half of collected leaves (about 0.5 gram) were weighed, and ground using a mortar and pestal in liquid N₂. The ground sample was combined with 5 ml of extraction medium containing 50 mM Hepes-NaOH buffer (pH 7.5), 0.5 mM MgCl₂.H₂O, 1 mM Na₂EDTA, 2 mM diethyldithiocarbamic acid (DIECA), 2.5 mM DTT, 1% BSA, and 2% PVPP, employing a modification of a method described previously by Schaffer (1986). The extract was centrifuged for 20 min at 20,000 g. The supernatant was assayed for SPS, SS, and acid invertase activity.

Assays for SPS and SS were performed by incubating aliquots of tissue extracts for 30 min at 37 °C with an equal volume of reaction mixture according to Khayat and Zieslin (1987). The SPS assay contained 15 mM NaF, 5 mM Na₂MoO₄.2H₂O, and 50 mM Hepes-NaOH buffer (pH 7.5). For the SS assay, fructose 6-P was replaced by fructose. The Reaction was terminated by the addition of 70 µl of 1 N NaOH, and sucrose formation was determined by the resorcinal colorimetric method (Rufty and Huber, 1983). Enzymatic activity was determined by reference to a blank reaction mixture from which UDP glucose was excluded.

Acid invertase activity was determined according to Khayat and Zieslin (1986).

Aliquots (200 μ l) of plant extracts were incubated for 30 min at 37 °C with an equal quantity of 1 M sucrose and 600 μ l phosphate citrate buffer (pH 5.0). The reaction was terminated by the addition of 1 ml of Sumner reagent. Enzyme blanks were incubated in Sumner reagent. Activity was expressed as the quantities of reducing sugars formed by sucrose hydrolysis (Khayat and Zieslin, 1986).

RESULTS

Compared to well-watered turf, irrigation at 60% ET increased sucrose content in zoysia at 8 and 14 DOT (Fig. 1). Zoysia irrigated at 20% ET had higher sucrose levels than well-watered turf at 14 DOT, but lower levels at 23 and 28 DOT. Sucrose content of tall fescue irrigated at 20% and 60% ET was higher than that of well-watered turf beginning at 8 DOT; however, levels were similar to well-watered turf beginning at 28 DOT at 20% ET and 23 DOT at 60% ET. Tall fescue had higher sucrose contents than zoysiagrass.

Zoysia irrigated at 20% and 60% ET had higher SPS activity than well-watered turf beginning at 14 and 23 DOT, respectively (Fig. 2). Tall fescue had higher SPS activity than well-watered turf, beginning at 14 DOT. Tall fescue had significantly higher SPS activity than zoysiagrass at any given irrigation level.

Zoysia and tall fescue receiving 20% and 60% ET had higher SS activity than well-watered turf beginning at 14 DOT (Fig. 3). However, no difference in SS activity of zoysia was observed between 60% and 100% ET at the end of experiment (28 DOT). Tall fescue had significantly higher SS activity than zoysiagrass.

Irrigation at 20% and 60% ET reduced acid invertase activity in zoysia compared to well-watered turf, beginning at 14 and 23 DOT, respectively (Fig. 4). At 20% and 60% ET, irrigation reduced acid invertase activity of tall fescue compared to well-watered turf, beginning at 8 DOT and 14 DOT, respectively. Acid invertase activity was significantly higher for tall fescue than zoysiagrass.

No differences in TNC were observed among irrigation levels for either species (Fig. 5). Tall fescue had significantly higher TNC than zoysiagrass at 8, 23, and 28 DOT.

The ratio of root to shoot TNC for both tall fescue and zoysiagrass receiving 60% ET was higher than well-watered turf (Fig. 6). Irrigation at 20% ET reduced the root to shoot TNC ratio in zoysiagrass but had no effect in tall fescue.

DISCUSSION

I found that deficit irrigation levels of 60 or 20% ET increased sucrose content in zoysia and tall fescue during most of the experiment. This increase could imply that sucrose played an important role in plant adaptation to drought stress. My results were consistent with those of Spollen and Nelson (1994) who also found that sucrose content increased 258% in the leaf base of tall fescue. The low carbon supply led to the allocation of some carbon to osmotic adjustment under water deficits (Daie, 1988). Carbohydrates, including sucrose, are considered to be important solutes in osmoregulation (Spyropoulos, 1982; Premachandra et al., 1992; Tan et al., 1992; Zhang and Archbold, 1993; Rekika et al., 1998) and as protectants in maintaining integrity and function of membranes (Koster, 1991). Under drought conditions, the low carbon supply could induce a shift in the partitioning of carbon in favor of sucrose synthesis (Daie, 1988; Castrillo, 1992; Vassef and Sharkey, 1989).

The measured increase in sucrose formation was associated with markedly increased activities of SPS and SS and decreased activities of acid invertase. This indicated that activities of enzymes involved in sucrose biosynthesis were clearly affected during deficit irrigation. Rufty and Huber (1983) reported that the increase in SPS activity might be related to its suitability as a central control point in sucrose. Involvement in sucrose biosynthesis apparently is the only metabolic function of SPS, and the equilibrium constant of the SPS reaction heavily favors formation of sucrose phosphate (Mendicino, 1960). The changes in SPS activity was consistent with the demand for sucrose in soybean (Rufty and Huber, 1983). Other and my research implied that the demand for sucrose activates SPS activity under drought stress conditions.

Vassey et al. (1991) reported that the activity of SS in their extracts from water stressed bean was negligible. I observed an increase in SS activity in tall fescue and zoysiagrass under deficit irrigation. Similar to my work, at anthesis of wheat, acid invertase activity in anthers from water stressed plants was half that observed in well-watered controls (Vassey et al. 1991). This implied that the increase in SS activity and the decrease in acid invertase activity could contribute to the sucrose increase.

Total nonstructural carbohydrate concentrations in shoots and roots of zoysiagrass and tall fescue were unaffected by deficit irrigation. Huang and Fu (2000) reported that TNC in both shoots and roots of Kentucky bluegrass and tall fescue increased or were unaffected during progressive drought. Similar increases in TNC of drought-stressed tall fescue was observed by Huang and Gao (2000). The lack of effects herein suggests that carbohydrate availability was not a limiting factor for shoot and root growth in zoysiagrass and tall fescue.

I did observe an increase in the ratio of root to shoot TNC. In order to adapt to conditions of limited water availability, plants may require a considerable amount of carbon investment below ground. Several studies have reported that the amount of carbon allocated to roots in proportion to the total of newly fixed carbon increased during drought stress conditions (Huang and Fu, 2000; Huang and Gao, 2000; Nicolas et al., 1985).

The difference in sucrose metabolism might contribute that in drought resistance between zoysiagrass and tall fescue. Our results showed that tall fescue had much higher sucrose content and activity of SPS, SS, and acid invertase than zoysiagrass. However, tall fescue had slightly higher or the same drought resistance as zoysiagrass (Carrow,

1996; Fu et al., 2003). This implied that other factors affect drought resistance of turfgrass besides sucrose metabolism. Higher sucrose content may help tall fescue grow faster than zoysiagrass, because sucrose is an important form of stored sugar in higher plants (Kharat and Zieslin, 1987).

In summary, the increase in activity of SPS and SS, and the decrease in acid invertase activity, play an important role in sucrose metabolism. The enhancement in sucrose content and carbon allocation to roots may help zoysiagrass and tall fescue tolerate deficit irrigation.

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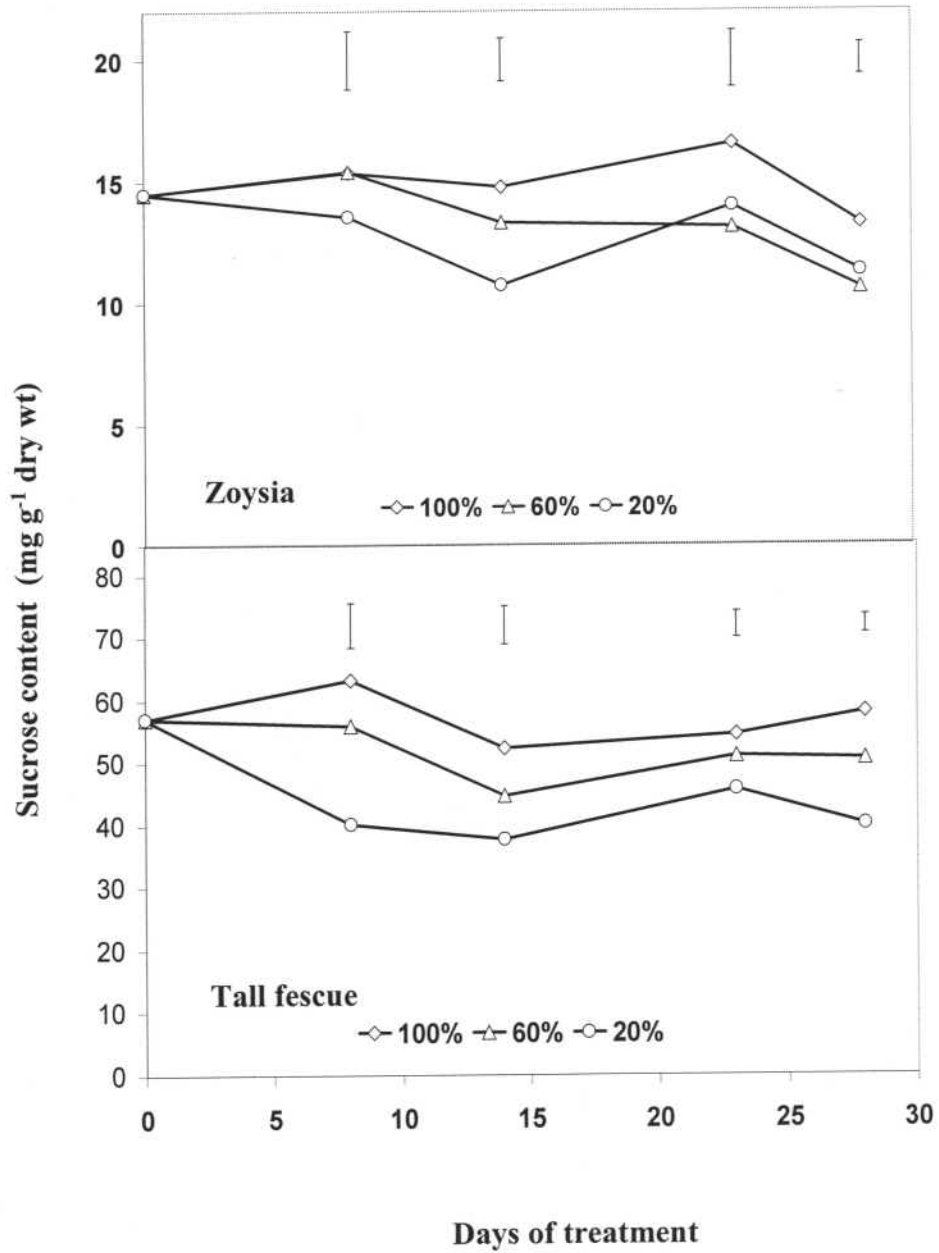


Fig. 1. Sucrose content of zoysiagrass and tall fescue in responses to deficit irrigation. Vertical bars on the top of figure are LSD values (P=0.05) for treatment comparison at a given day.

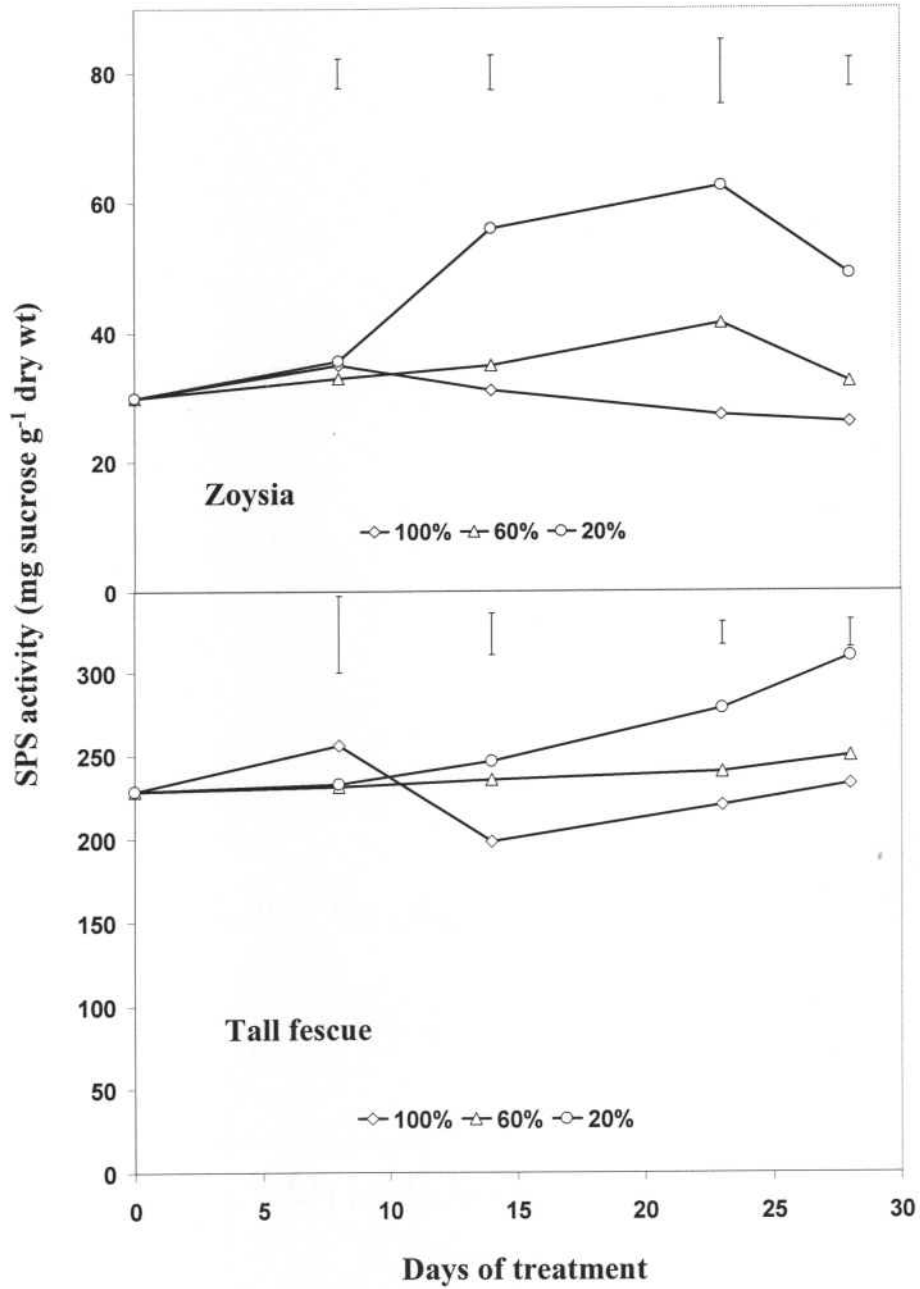


Fig. 2. SPS activity of zoysiagrass and tall fescue in responses to deficit irrigation. Vertical bars on the top of figure are LSD values ($P=0.05$) for treatment comparison at a given day.

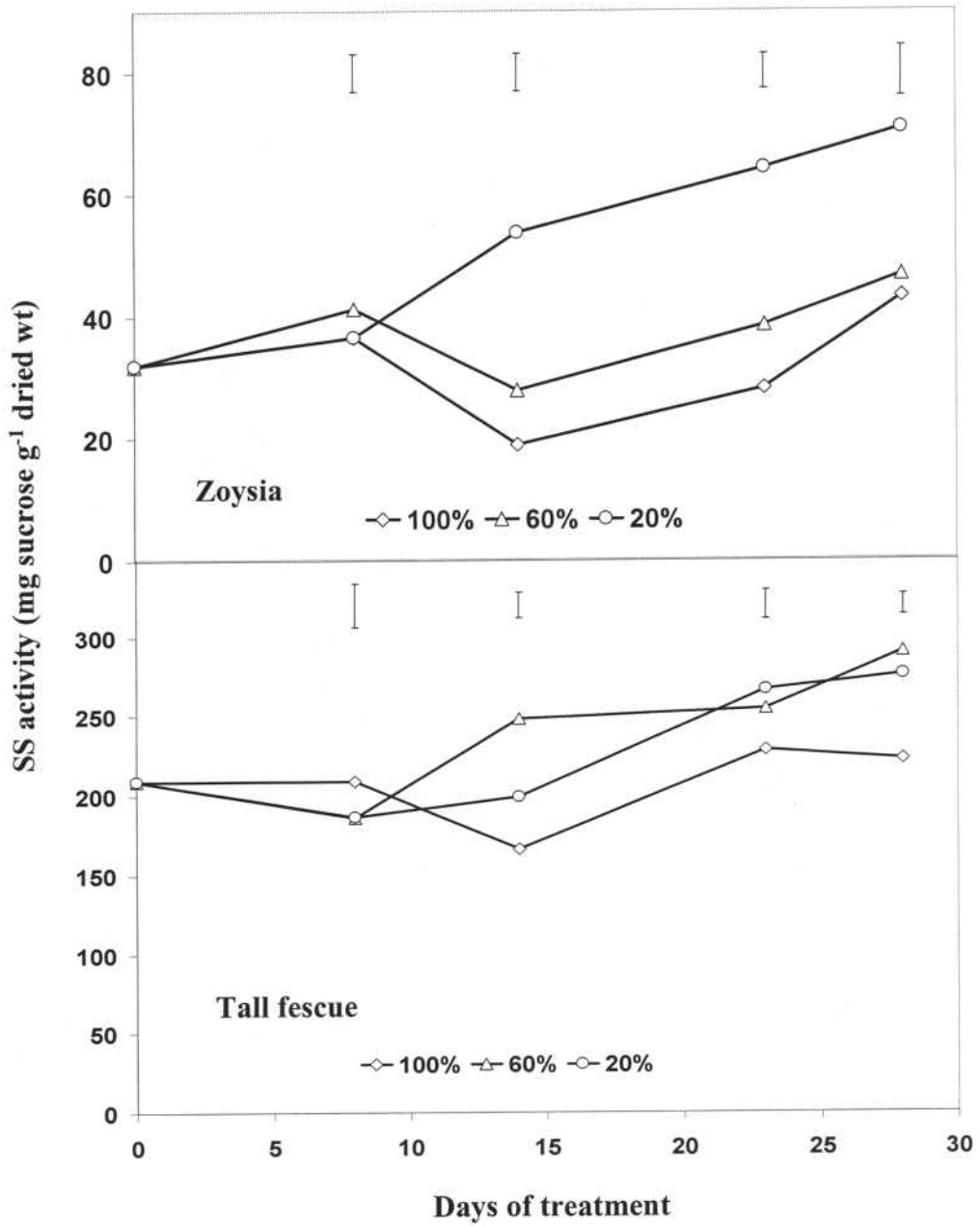


Fig. 3. SS activity of zoysiagrass and tall fescue in responses to deficit irrigation. Vertical bars on the top of figure are LSD values (P=0.05) for treatment comparison at a given day.

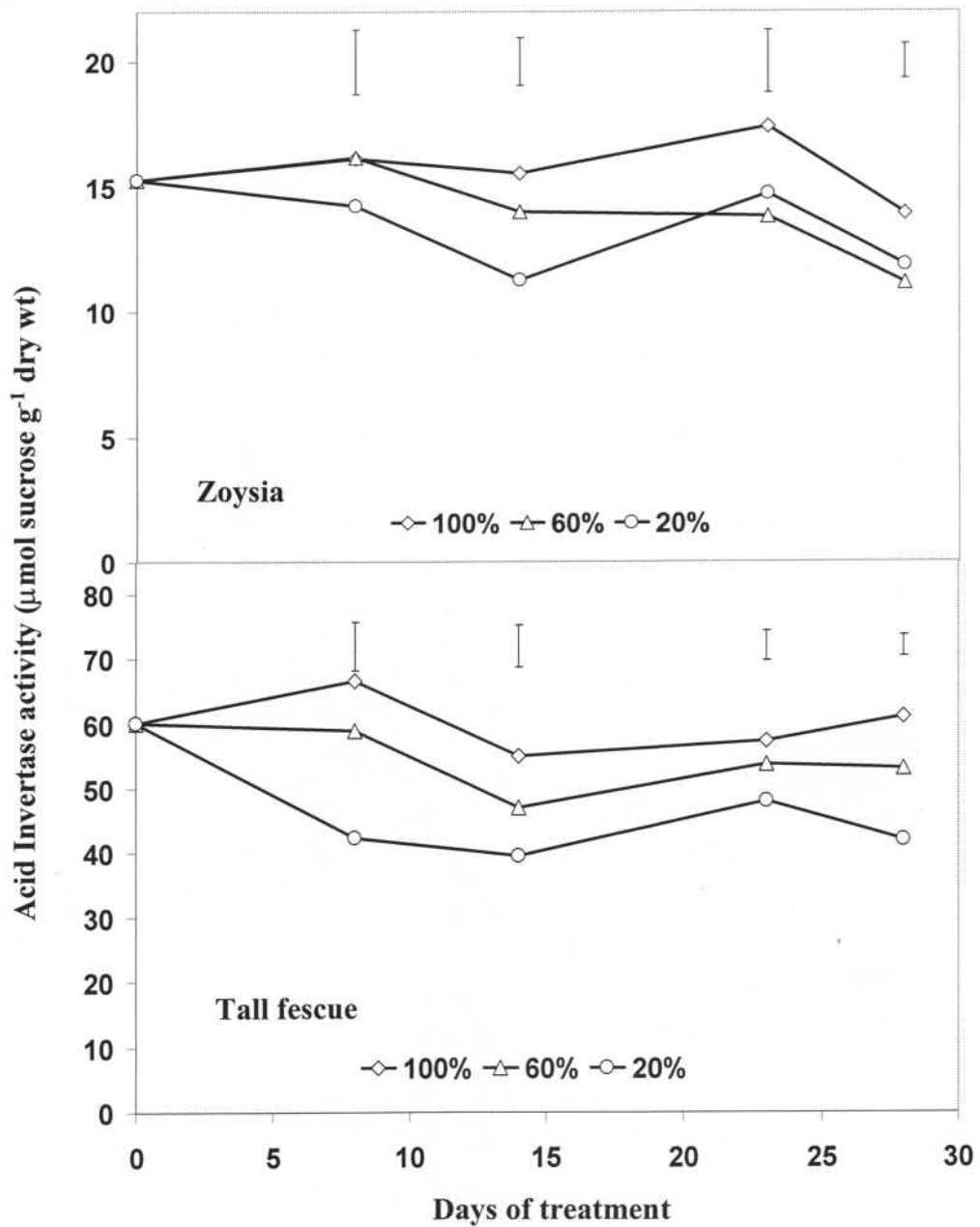


Fig. 4. Acid invertase activity of zoysiagrass and tall fescue in responses to deficit irrigation. Vertical bars on the top of figure are LSD values (P=0.05) for treatment comparison at a given day.

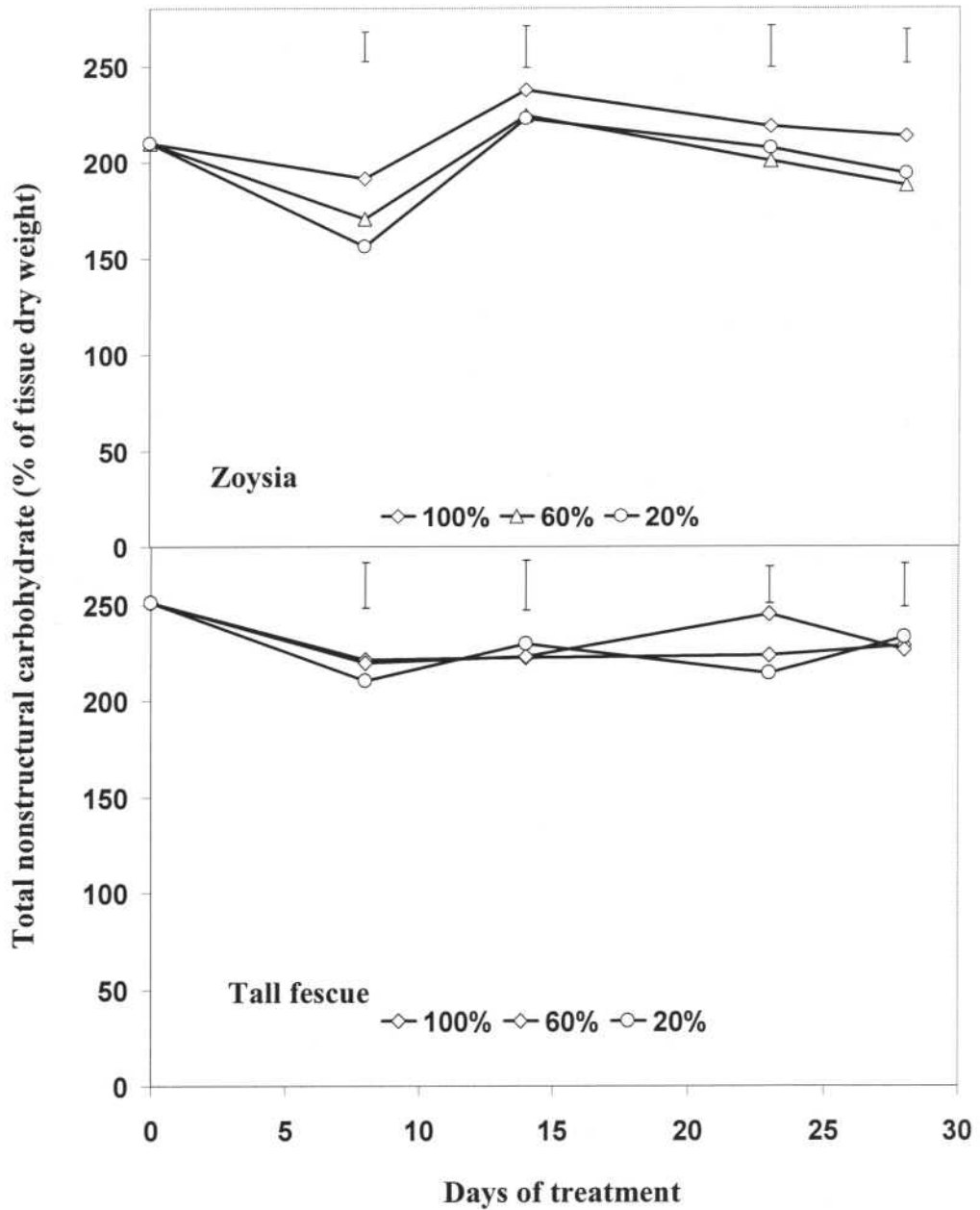


Fig. 5. Total nonstructural carbohydrate content of zoysiagrass and tall fescue in responses to deficit irrigation. Vertical bars on the top of figure are LSD values ($P=0.05$) for treatment comparison at a given day.

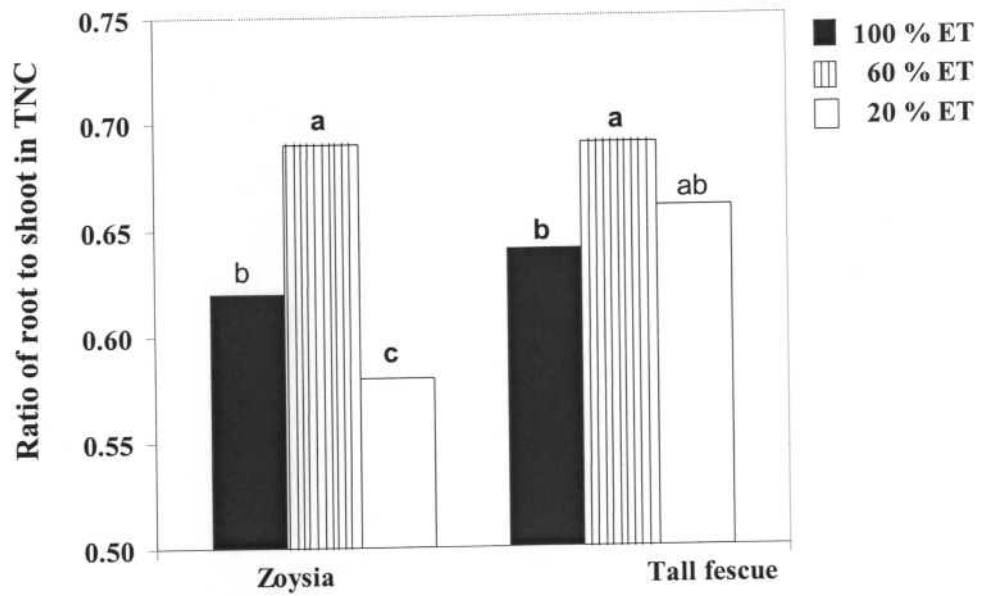
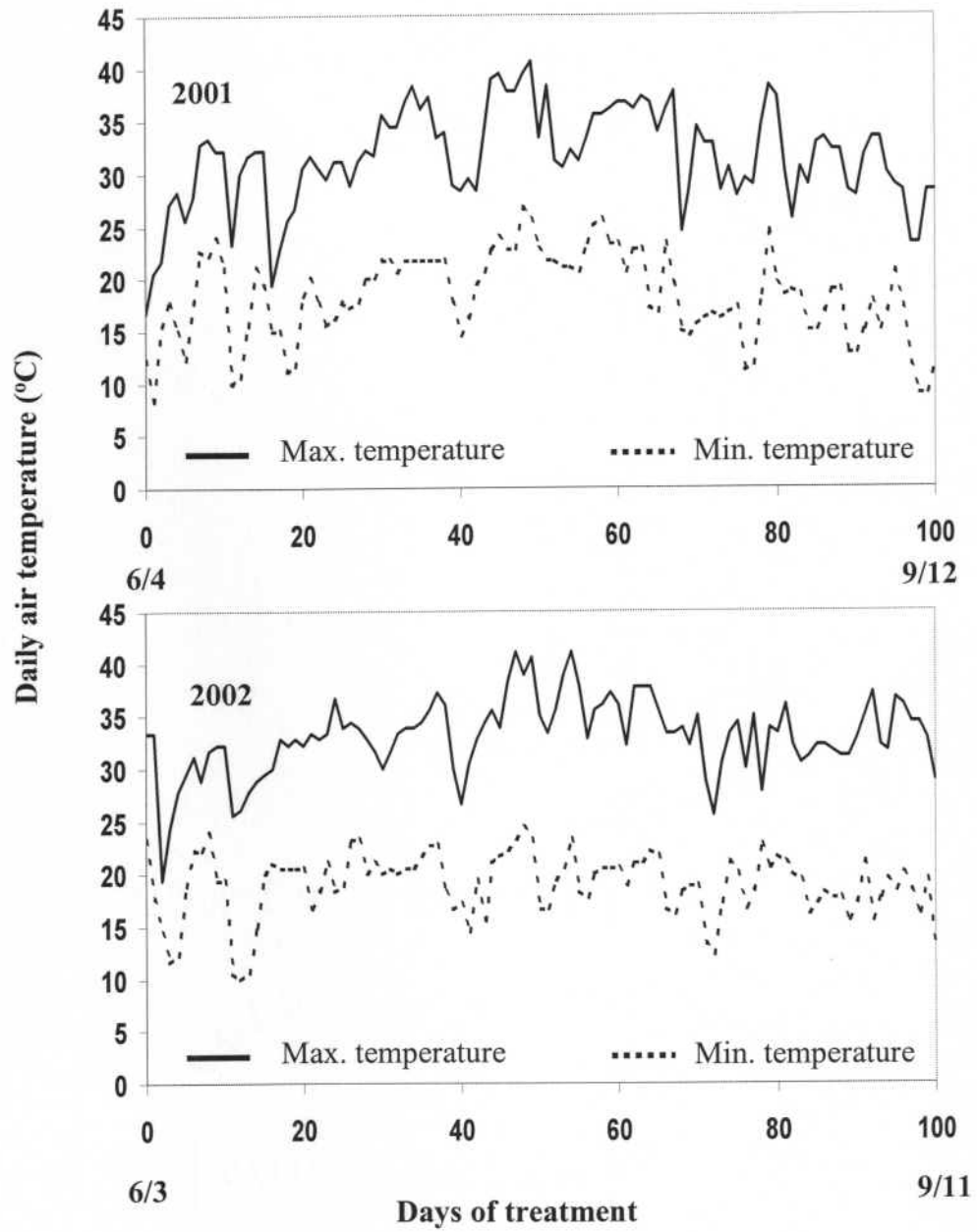


Fig. 6. The ratio of root to shoot in TNC of zoysiagrass and tall fescue in responses to deficit irrigation. Vertical bars on the top of figure are LSD values ($P=0.05$) for treatment comparison at a given day.



Appendix I. Daily maximum and minimum air temperature from June to September in 2001 and 2002.