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Heat Tolerance of Kentucky Bluegrass as Affected by Trinexapac-ethyl

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Abstract. **Heat accumulation during storage of sod may reach lethal temperatures within 4 days, decreasing sod quality. Treatment with trinexapac-ethyl reduces heat accumulation during sod storage. However, heat tolerance of grasses treated with trinexapac-ethyl has not been documented. Our objectives were to: 1) determine the lethal temperatures for Kentucky bluegrass (*Poa pratensis* L.); and 2) identify the effect of a single application of trinexapac-ethyl on heat tolerance. Experimental design was a randomized complete block with three replications and a two (trinexapac-ethyl vs. control) × two (cultivars) factorial arrangement of treatments. Ten days after chemical treatment, Kentucky bluegrass sprigs were exposed to heat stress for 4 days in a temperature gradient block under low vapor pressure deficit. Treatment with trinexapac-ethyl at 0.23 kg·ha^{-1} reduced heat tolerance. Temperature needed to kill 50% of the population was 35.5 °C for treated vs. 36.1 °C for nontreated grass. Trinexapac-ethyl is in the same chemical family as the cyclohexanedione herbicides that interfere with lipid syntheses in grasses. This may be a reason for the slight decrease in heat tolerance. The practical value of trinexapac-ethyl treatment in reducing heat accumulation during storage of sod may be partially negated by a decrease in heat tolerance. Chemical name used: [(4-cyclopropyl-α-hydroxy-methylene)-3,5-dioxocyclohexanecarboxylic acid methyl ester] (trinexapac-ethyl).**

Cool-season grass stands are often thinned or lost due to heat damage in the summer season. Heat tolerance has become a major problem in areas of the southern United States, where cool-season grasses are under extremely high maintenance (Wehner and Watschke, 1981). The optimum temperature range for cool-season turfgrass shoot growth is 15 to 24 °C (Beard, 1973); however, many of these grasses are being utilized in the transition zone and farther south where temperatures are higher.

One method used to measure heat tolerance is to expose plants to relatively high temperature over a long period of time and to record tissue damage or plant death (Marcum, 1998; Wehner et al., 1985). Howard and Watschke (1991) exposed whole grass plants to high temperatures for a short time by submerging them in water ranging in temperature from 41 to 49 °C for 30 min. They immediately replanted the grasses and rated the survival over several weeks. Others have evaluated heat damage by exposing plants to a constant high temperature for varied periods of time (Saadalla et al., 1990).

Sullivan (1972) developed a method to evaluate heat tolerance in sorghum [*Sorghum bicolor* (L.) Moench] by measuring the thermostability of cellular membranes. This procedure involves exposing sections of leaves in vials containing distilled water to high temperatures for a fixed time. An initial electrolyte leakage is evaluated by measuring the conductivity of the solution in the vials after the heat treatment. The plant tissues are then autoclaved and assumed to have a complete release of electrolytes. A second conductivity measurement is taken and the ratio of electrolyte leakage before and after autoclaving can be calculated. This efficient means of measuring cell membrane thermostability has been used on soybeans [*Glycine max* (L.) Merr.] (Martineau et al., 1979), wheat (*Triticum aestivum* L.) (Saadalla et al., 1990), potatoes (*Solanum tuberosum* L.), tomatoes (*Lycopersicon esculentum* Mill.) (Chen et al., 1982), and Kentucky bluegrass (Marcum, 1998).

In another system used to measure heat tolerance, plants are subjected to prolonged periods of heat stress. Using this method, Watschke et al. (1972) and Howard and Watschke (1991) showed the importance of photosynthetic efficiency on heat tolerance among different cultivars of Kentucky bluegrass. Perdomo et al. (1996) documented a lower basal osmotic potential, which was correlated with greater heat tolerance, in 'Midnight' Kentucky bluegrass than in 'Nugget'. This may have led to greater transpirational cooling in 'Midnight' by allowing larger stomatal openings.

Management also may have an influence on the stress tolerance of grasses. Trinexapac-ethyl is a plant growth regulator commonly used on high maintenance turf to reduce vegetative growth. Golf course superintendents have observed that multiple applications of trinexapac-ethyl may increase the stress tolerance of grasses. Jiang and Fry (1998) observed no reduction of root length or root density but an increase in visual quality of perennial ryegrass (*Lolium perenne* L.) after treatment with trinexapac-ethyl under drought conditions. Drought is often linked to heat stress, but little is known about the direct influence of trinexapac-ethyl on heat tolerance.

Heat accumulation occurs during sod storage and lethal temperatures may be reached within 4 d (King, 1970; King et al., 1982a, 1082b; Mitchell and Dickens, 1979). Kentucky bluegrass sod accumulated less heat when treated with a single application of trinexapac-ethyl at 0.23 kg·ha^{-1} (Heckman et al. 2001); however, the heat tolerance of treated sod was not examined. Most heat tolerance studies do not simulate stress supplied during sod storage, and common measurements give relative comparisons, but not actual temperatures. The objectives of this study were to: 1) determine the lethal temperature for two Kentucky bluegrass cultivars exposed to high temperatures for 4 d and; 2) identify the effect of a single application of 0.23 kg·ha^{-1} trinexapac-ethyl on Kentucky bluegrass heat tolerance.

Materials and Methods

Kentucky bluegrass cvs. Midnight and Huntsville were vegetatively propagated from established stands at the John Seaton Anderson Turfgrass and Ornamental Research Facility near Mead, Nebr., in Oct. 1998. Plants were grown in 15-cm pots in the greenhouse, where temperatures fluctuated between 15 and 30 °C. The growth medium was medium sand, and plants were irrigated daily. Supplemental lighting was supplied by metal halide lamps giving a 14-h photoperiod with an average photosynthetic photon flux (*PPF*) of 200 μmol·s^{-1}·m^{-2}. Weekly applications of 6.1N–1.3P–5.1K (kg·ha^{-1}) (Peters Professional; Scotts-Sierra Horticultural Products Co., Marysville, Ohio) were made.

In June through Sept. 1999, sprigs with three leaves were transplanted at a depth of 5 mm into 1.75-cm-diameter, 4.00-cm^3 pots 17 d before heat treatments were applied, and irrigated daily with distilled water. Growth medium was 3 sphagnum peat : 3 sand : 2 sandy clay loam : 1 vermiculite (by volume). Sprigs were grown in a controlled-environment growth chamber with a 14-h photoperiod supplied by fluorescent lights with an average *PPF* of 170 μmol·s^{-1}·m^{-2} and temperatures of 22 °C day/19 °C night. Sprigs

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were treated with trinexapac-ethyl at 0.23 kg·ha^{-1} 7 d after being placed in the growth chamber, while others remained as nontreated controls. Trinexapac-ethyl treatment was applied with a conveyer belt sprayer calibrated to deliver 374 L·ha^{-1}. Ten days after trinexapac-ethyl application, plants were placed in an aluminum temperature gradient block similar to the one used by Hensley et al. (1982). The block was insulated with 5.1 cm of styrofoam on the bottom and sides and 1.3 cm on the top to avoid excessive energy exchange. Each end of the block was connected to a circulating water bath to produce a gradient of temperatures ranging from 33 to 40 °C throughout the length of the structure. The block contained 55 chamber wells arranged as 11 rows at different temperatures with five samples per row. The temperature gradient was measured using 18 thermocouples with precision to 0.01 °C. Three thermocouples were placed at the soil surface in individual cells of every other row. Temperatures were recorded using a CR7 data logger (Campbell Scientific, Logan, Utah) programmed to read hourly. Cellophane was placed over the samples to keep the water vapor pressure deficit of the air low and prevent drought stress. Small holes in the cellophane (≈20) allowed for gas exchange. Supplemental lighting from florescent lights created a photoperiod of 14 h with an average *PPF* of 100 µmol·s^{-1}·m^{-2}. Plants underwent the chronic heat treatment for 4 d and were placed back into the same growth chamber with the environment as described earlier. Sprigs were allowed to regrow for 14 d and evaluated for survival after the regrowth period. Sprigs showing regrowth were classified as alive and sprigs that did not regrow were considered dead.

The experiment consisted of 12 individual cycles (1 cycle = 1 replicate), which included one cultivar and one PGR treatment. Each cycle consisted of transplanting, growth for 7 d, trinexapac-ethyl treatment, growth for 10 d, temperature gradient treatment for 4 d, and a 14-d regrowth period. Cycles were scheduled to minimize the time interval between temperature gradient block treatments. Cycles were administered in a randomized complete block with three replications, and a two (trinexapac-ethyl vs. control) × two (cultivars) factorial arrangement of treatments.

A second experiment was conducted from Sept. through Dec. 1999. Design and environmental conditions were the same as described for the first experiment.

The temperatures to which plants were subject were calculated by fitting a second order polynomial to the temperature vs. row of the gradient block data using PlotIt software (Scientific Programming Enterprises, Haslett, Mich.). Temperatures of individual rows were then derived from these equations. A probit model (analysis typically used to determine LD_{50} toxicology results) was used to develop lethal temperature curves (SAS Institute, 1988). The temperatures needed to kill 20% (LT_{20}), 50% (LT_{50}), and 80% (LT_{80}) of the plants were estimated from the temperature curves and separated using standard errors (P = 0.05).

Results and Discussion

Heterogeneity of lethal temperature curves was tested as described by Bates and Watts (1998). An overall test indicated that a single curve could not be used. Cultivars did not differ in response to temperature. However, responses of plants treated with trinexapac-ethyl treatment were significantly different from those of the controls. Thus, separate temperature curves for trinexapac-ethyl treatment and control were averaged across cultivars. Plant response did not differ significantly between the two experiments; thus, data from both were combined for analyses.

The LT_{50} for Kentucky bluegrass exposed to chronic heat stress for 4 d was between 35.5 and 36.1 °C (Table 1). Sprigs treated with a single application of trinexapac-ethyl were less heat tolerant than untreated sprigs ($P \leq 0.05$). The LT_{20}, LT_{50}, and LT_{80} of sprigs treated with trinexapac-ethyl at 0.23 kg·ha^{-1} were significantly lower than those of controls (Fig. 1). Trinexapac-ethyl is a cyclohexanedione chemical that is in the same chemical family as the herbicide sethoxydim {2-[1-(ethoxyimino) butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one}. Sethoxydim is a herbicide used to kill grasses by inhibiting acetyl coenzyme A activity, restricting the ability to synthesize lipids (Golz et al., 1994). The similar structures of trinexapac-ethyl and sethoxydim could allow some partial inhibition of acetyl coenzyme A. Lipids are a major component of cell membranes, which play a role in the heat tolerance of plants (Levitt, 1980).

Greater carbohydrate reserves can lead to improved heat tolerance (Levitt, 1980). Han et al. (1998) showed that the content of nonstructural carbohydrates in creeping bentgrass (*Agrostis stolonifera* L.) increased during the first 2 weeks after application of trinexapac-ethyl. The theory of increased carbohydrates leading to greater heat tolerance is not supported in this case if the nonstructural carbohydrates in the Kentucky bluegrass sprigs underwent a similar response to that of creeping bentgrass (Han et al., 1998). Other metabolic responses seem to be responsible for the difference in heat tolerance.

Levitt (1980) identified the major effect of proteins on heat tolerance. Since both lipid and proteins are components of cell membranes, proteins tend to have a major influence on the heat tolerance of cellular membranes. Proteins are extremely sensitive to acute exposure to temperatures above 50 °C and chronic exposure to lower temperatures dependent upon the length of heat treatment (Levitt, 1980). This could be a reason for the steep slope of the lethal temperature curves.

These sprigs were not acclimated prior to implementation of constant high temperature stress for 4 d. Also, they received low levels of *PPF* during heat stress. This technique does not exactly simulate field conditions of heat stress during sod storage on pallets. However,

Table 1. Effects of treatment with trinexapac-ethyl (0.23 kg·ha^{-1}) on lethal temperature values[z] for two Kentucky bluegrasses.

	Lethal temp. (°C)		
Treatment	LT_{20}[y]	LT_{50}	LT_{80}
Treated	34.6*	35.5*	36.4*
Control	35.1	36.1	37.0

[z]Lethal temperature values were calculated from Fig. 1.
[y]Data represent the mean LT_{20}, LT_{50}, and LT_{80} for two experiments.
*Significantly different from control at $P \leq 0.05$.

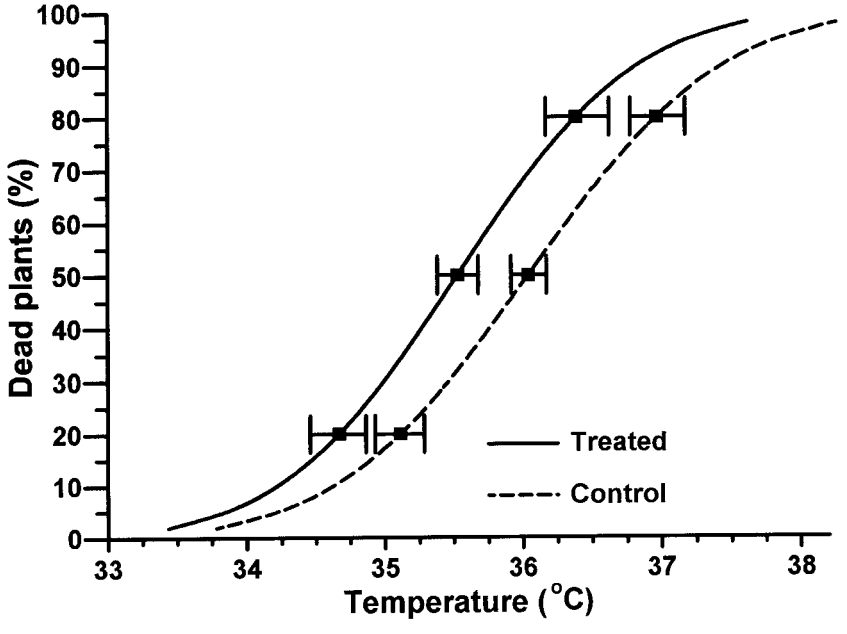

Fig. 1. Lethal temperature curves for Kentucky bluegrass treated with trinexapac-ethyl at 0.23 kg·ha^{-1} and control plants. Predicted curves based on probit analysis (n = 132). Data points (■) represent the mean LT_{20}, LT_{50}, and LT_{80} ± SE for two experiments.

no night relief from sod heating occurs during sod storage (King, 1970; King et al., 1982a, 1982b; Maw et al., 1998; Mitchell and Dickens, 1979), which was simulated in this experiment. The value of the dramatic reduction of heat accumulation following trinexapac-ethyl treatment of Kentucky bluegrass may be partially negated by the reduction in heat tolerance of treated turf. Even though there may be less heat accumulation within the sod, the treated grass might have a slightly lower lethal temperature.

A single application of trinexapac-ethyl at 0.23 $kg \cdot ha^{-1}$ significantly reduced the calculated lethal temperatures of Kentucky bluegrass following chronic heat stress. This reduction in heat tolerance needs to be considered when using a single application of trinexapac-ethyl to increase sod storage life. In the practice of attempting to reduce turfgrass growth and improve stress tolerance, turf managers are using multiple applications of trinexapac-ethyl. The effects of multiple applications at various rates of this PGR needs to be evaluated for their effects on heat tolerance.

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