

# TURFGRASS SCIENCE

## Exogenous Salicylic Acid Enhances Post-Transplant Success of Heated Kentucky Bluegrass and Tall Fescue Sod

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### ABSTRACT

A primary economic concern of sod producers is loss of sod quality during the transportation and storage phases of a sale. Previous research and field experience indicate that soil and plant respiration rates, and thus the rate of pallet heating, may be reduced by harvesting in the morning, lowering mowing heights and removing clippings, and minimizing tissue nitrogen and soil moisture before harvest. However, even when proper cultural guidelines are followed, excessive sod heating and tissue damage often occurs. Various pre- and post-harvest chemical treatments aimed at protecting leaf tissue integrity during and after supraoptimal heating have shown promise for increasing transplant success. One of these compounds is the natural plant growth regulator salicylic acid (SA). This study was conducted to investigate the influence of pre-harvest foliar application of SA on transplant injury and root strength of tall fescue (TF; *Festuca arundinacea* Schreb.) and Kentucky bluegrass (KBG; *Poa pratensis* L.) sod following supraoptimal heating. Salicylic acid was applied at 0.5 kg ha<sup>-1</sup> to the turfgrass 10 d before harvest and canopy photochemical efficiency was measured 1 d before harvest. Harvested and rolled sod was subjected to high temperature stress (38–40°C for 72 or 96 h), transplanted into the field, and injury and root strength were determined. Application of SA enhanced the pre-harvest canopy photochemical efficiency of KBG and TF sod in both years. Averaged over years and heat duration, SA increased canopy photochemical efficiency by 12% for KBG and 14% for TF. Salicylic acid reduced visual injury and enhanced post-harvest root strength in both years. Averaged over years and heat duration, SA increased transplant root strength by 26% for KBG and 9% for TF. These data suggest that pre-harvest foliar SA application may improve shelf life and transplant success of supraoptimally heated cool-season sod.

KENTUCKY BLUEGRASS AND TALL FESCUE are widely used cool-season turfgrass species for sod production in many parts of the world. Market forces often require cool-season sod to be harvested, transported, stored, and transplanted during hot summer periods resulting in damaged plant tissue because of supraoptimal heating (King et al., 1982; Heckman et al., 2001). Heat-damaged sod, especially when transplanted into a field environment characterized by high temperature and ultraviolet (UV) radiation, can result in poor transplantation success and loss of sod producer revenue (Zhang et al., 2003a, 2003b). High temperature stress damages plants through accumulation of oxy free radicals (Leshem, 1981) causing degradative events such as

lipid peroxidation and protein denaturation, resulting in a reduction of photosynthetic function and increased senescence (Salisbury and Ross, 1992; Jiang and Huang, 2001).

Recovery and maintenance of photosynthetic activity along with rapid root growth of sod after transplanting is important for survival and successful establishment (Goatley and Schmidt, 1991; Giese et al., 1997; Zhang et al., 2003a, 2003b). Natural and synthetic plant growth regulators have been used to enhance turfgrass tolerance to environmental stresses, including UV radiation and supraoptimal temperature (Ervin et al., 2004a; Schmidt and Zhang, 2001; Zhang et al., 2003a, 2003b).

Salicylic acid is a naturally occurring phenolic in many plants and has been shown to function as a signal compound initiating plant defense systems in response to stress, with some reporting responses such as reduced transpiration and enhanced adventitious root initiation (Malamy and Klessig, 1992). Bowler et al. (1989) reported that SA induces manganese superoxide dismutase (MnSOD) genes. Salicylic acid induced increases in MnSOD would serve to detoxify superoxide radicals and protect plants from damage from oxidative stress. Foliar application of SA before sustained UV-B stress resulted in increased antioxidant activity and higher pigment content which were correlated with less leaf injury and greater maintenance of canopy photochemical efficiency of KBG (Ervin et al., 2004b). Soil drenches and foliar applications of SA resulted in enhanced tolerance of bean (*Phaseolus vulgaris* L.) and tomato (*Lycopersicon esculentum* Mill.) to heat, chilling, and drought stresses (Senaratna et al., 2000). Foliar application of SA before 48 h of exposure to 40°C followed by 3 d of UV-B stress resulted in 30% greater maintenance of canopy photochemical activity of creeping bentgrass (*Agrostis palustris* Huds.) (Schmidt and Zhang, 2001).

These results suggest that exogenous application of SA before summer harvests and supraoptimal heating of cool-season turfgrass sod may up-regulate defense systems before this stress event and result in greater post-transplant success. Therefore, the objectives of this field study were to investigate the influence of an exogenous SA application on pre-harvest canopy photochemical efficiency and post-transplant visual injury and root strength of Kentucky bluegrass and tall fescue sod subjected to 72 or 96 h of storage at 38 to 40°C.

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Published in Crop Sci. 45:240–244 (2005).  
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**Abbreviations:** Fv/Fm, photochemical efficiency; HD, heat duration; KBG, Kentucky bluegrass; LSD, least significance difference; SA, salicylic acid, TF, tall fescue.

## MATERIALS AND METHODS

### Plant Material and SA Treatment

Three-year-old stands of Kentucky bluegrass ('Georgetown') and tall fescue ('Rebel Jr.') grown on a Groseclose silt loam soil (clayey, kaolinitic, mesic Typic Hapludult, pH 6.2, OM 22 g kg<sup>-1</sup>, P 67 kg ha<sup>-1</sup>; K 280 kg ha<sup>-1</sup>) at the Virginia Tech Turfgrass Research Center, Blacksburg, VA, were used for this study. The area was irrigated to prevent wilting and mowed at 5 cm (KBG) or 6 cm (TF). Pesticides were applied as needed to control disease, insects and weeds, but no herbicides were used 30 d before sod treatment. Fertilizer was applied at 49 kg N ha<sup>-1</sup> in May 2000 with urea (46-0-0) and in October 2000 and May 2001 with a 10-4.4-8.3 granular product containing soluble N sources.

The grasses were treated with SA at 0.5 kg ha<sup>-1</sup> with non-treated grasses serving as controls. This rate was selected on the basis of previous research results (Schmidt and Zhang, 2001). Salicylic acid (100% pure), purchased from Research Organics (Cleveland, OH), was mixed with a wetting agent (Aqua-Gro, Aquatrols Corporation, Cherry Hill, NJ) at 0.05% (v/v) to promote a uniform SA suspension. The possible confounding effects of the wetting agent on Kentucky bluegrass and tall fescue under heat stress were examined in a separate trial and no significant effects on visual injury or canopy photochemical efficiency were observed (data not shown). Water (control) or the SA solution was applied to the turfgrass canopy with a CO<sub>2</sub> pressurized boom sprayer delivering 376 L ha<sup>-1</sup> at 290 KPa in 2000 and 2001. No irrigation was applied to the treated plots after SA application and before sod harvest. There was no precipitation in the 24 h after SA treatment in either year. Dates of SA application for KBG were 30 June 2000 and 05 June 2001. For TF, SA application occurred on 15 Aug. 2000 and 9 July 2001. Plots (1.8 by 1.8 m) were arranged in a randomized complete block design with four replications.

### Sod Heating Treatments

The sod was heated according to the procedure described by Zhang et al. (2003a). Briefly, two pieces of sod (0.3 by 1.8 m) were removed from each plot on 17 July 2000 and 15 June 2001 for KBG and 24 Aug. 2000 and 19 July 2001 for TF, rolled and placed in a heat controlled storage container set at 40°C. This building has been engineered to heat sod similar to that stored during summer months in the center of pallet stacks or rolls except that the temperature increases gradually from the outside to the inside of each sod roll. The sod was placed on an open metal screen bench (1 by 4 m). The bench is 0.5 m high. Two plastic pipes (15-cm diam.) are used to deliver heat and a box fan is used to enhance uniform heat circulation within the building. A metal bar (5-mm diam. by 35 cm long), connected to a thermocouple (Omega model HH23, Stamford, CT), was inserted into the center of each roll to measure and confirm that uniform temperature conditions for each roll at 48 h (39.3 ± 1.2°C) and 72 h (39.5 ± 0.9°C) had been reached and were being maintained.

A sod roll from each treated plot was removed from the building after being heated for 72 or 96 h and transplanted (30 cm apart) onto prepared soil. A piece of sod (0.3 by 0.3 m) was cut from the center of each roll and a 0.3 by 0.3 m open mesh metal sheet (3 mm thick) was inserted under this piece of sod for subsequent transplant root strength determination. The metal sheet has uniformly distributed diamond-shaped openings (3.8 cm long by 1.6 cm wide at middle) for roots to grow past (Goatley and Schmidt, 1991; Schmidt et al., 1986). There was a distance of 30 cm between each piece of sod.

Siduron [1-(2-methylcyclohexyl)-3-phenylurea] was applied at 6.1 kg ha<sup>-1</sup> over and between the sod pieces to prevent summer annual weed germination between the sod strips during the 6-wk re-establishment period. Irrigation was applied as needed to prevent desiccation. Three weeks after transplanting, turfgrass injury was rated based on a visual scale of 1 to 9, with 1 indicating no injury (or 0% visible leaf chlorosis or necrosis and no noticeable loss of tiller density) and 9 indicating the most injury (or 100% visible leaf necrosis).

### Transplant Root Strength and Canopy Photochemical Efficiency Measurement

After 6 wk, transplant root strength was determined by ascertaining the energy required to vertically lift the expanded sheet of metal through which the sod roots had grown (Schmidt et al., 1986; Goatley and Schmidt, 1991; Zhang et al., 2003a).

Photochemical efficiency (Fv/Fm) was determined on pre-harvest sod by measuring chlorophyll fluorescence with a dual wavelength fluorometer (OS-50, Opti-Sciences, Inc., Tyngsboro, MA). The ratio of variable fluorescence to maximum fluorescence at 690 nm (Fv690nm/Fm690nm or Fv/Fm) is an indicator of the photochemical efficiency of photosystem II or relative photochemical efficiency (Bjorkman and Demmig, 1987; Miles, 1990; Zhang and Schmidt, 2000). Chlorophyll fluorescence was measured on the whole turfgrass canopy consisting of shoot material of various age and physiological status from newly emerged tillers to senescing tissue. The canopy area in each plot was selected randomly and covered for 15 min by a PVC ring (10-cm diam. by 5 cm high) filled with styrofoam (10 mm thick) for dark acclimation. An opening the size of the probe (10-mm diam.) is present in the styrofoam of each PVC ring and covered by a plastic plate. After the canopy is subjected to dark acclimation, the plastic plate is removed and the probe for the actinic light source inserted immediately into the opening. Next, the ring is rotated 90° three times after each reading and another fluorescence measurement is collected. The values of Fv/Fm were calculated on the basis of an average of the three measurements per experimental unit.

Since TF and KBG were not harvested on the same date, the two species were not compared directly. For each turfgrass species, the treatments were arranged in a randomized complete block design with four replications before harvest. For transplanted sod, a split plot design was used with heat duration (72 and 96 h) as main plots and SA treatment as subplots. All treatments were replicated four times. Data were analyzed with analysis of variance and mean separations were performed with a Fisher's protected LSD test at  $P = 0.05$ .

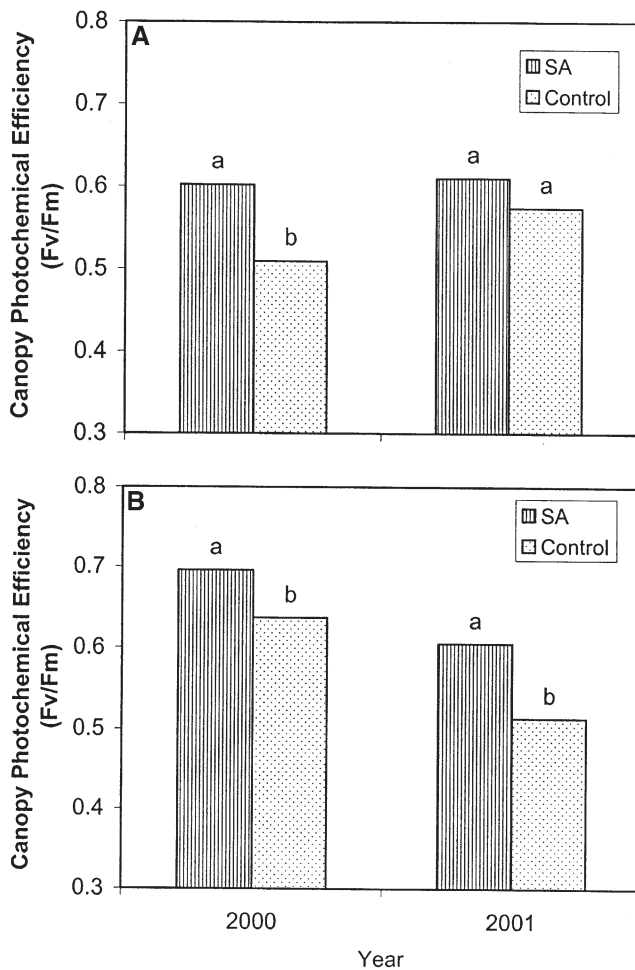
## RESULTS

### Canopy Photochemical Efficiency (Fv/Fm)

Application of SA significantly enhanced pre-harvest canopy photochemical efficiency of KBG in 2000, but not in 2001 (Fig. 1A). Tall fescue canopy photochemical efficiency was greater before harvest because of SA treatment in both years (Fig. 1B). On average, SA increased canopy photochemical efficiency by 12% for KBG and 14% for TF.

### Visual Turf Injury

Visual turf injury was rated 3 wk after transplanting for each trial. Extending heat duration from 72 to 96 h



**Fig. 1A.** Day-of-harvest Kentucky bluegrass (KBG) canopy photochemical efficiency (Fv/Fm) as influenced by salicylic acid (SA) application in 2000 and 2001. Bars with different letter designations within years indicate a significant difference at  $P = 0.05$ . **1B.** Day-of-harvest tall fescue (TF) canopy photochemical efficiency (Fv/Fm) as influenced by salicylic acid (SA) application in 2000 and 2001. Bars with different letter designations within years indicate a significant difference at  $P = 0.05$ .

caused greater injury to the KBG sod in 2000 and 2001 and greater injury to the TF sod in 2000 (Table 1). No SA  $\times$  heat duration interaction was found for KBG or TF injury in either year. However, when averaged over both heat duration treatments, SA application reduced visual turf injury for KBG and TF in both years (Table 1). Averaged over both years and heat durations, SA reduced transplant turf injury by 18% for KBG and 17% for TF.

### Transplant Root Strength

Extending heat duration from 72 to 96 h reduced transplant root strength for TF, but not for KBG in either year (Table 2). When averaged over heat duration treatments, SA application enhanced transplant root strength of KBG in both years and of TF only in 2000. On average, SA increased transplant root strength by 26% for KBG and 9% for TF. No significant heat duration  $\times$  SA interaction for transplant root strength was found for KBG or TF in either year.

## DISCUSSION

Although our goal was to measure Fv/Fm on unstressed KBG and TF canopies consisting of tillers of mixed age, the range of readings (0.51–0.70) indicate this may not have been the case at every sample date. Liu et al. (2002) reported growth chamber readings of about 0.76 for creeping bentgrass growing at constant leaf and soil temperatures of 20/20°C. However, these Fv/Fm readings were likely taken on most recently expanded single leaf blades. Using our canopy Fv/Fm method, Zhang et al. (2003b) reported August field readings on a mixed age sward of irrigated tall fescue of between 0.62 and 0.69. In a 20-to-25°C greenhouse environment, Zhang and Ervin (2004) reported well-watered creeping bentgrass canopy photochemical efficiency readings of 0.67 to 0.69; however, under drought stress, visual quality fell below acceptable levels ( $<6$ ), when Fv/Fm was 0.50 or less. Given this information our data appear to be a realistic representation of the summer conditions that may be present in a sod production field and, everything else being equal, an accurate assessment of the effects on canopy photochemical efficiency of SA relative to the control.

Increased superoxide dismutase activity with exogenous application of SA has been shown with *Nicotiana plumbaginifolia* Viv. (Bowler et al., 1989). Small, but significant (10–20%), increases in pre-harvest photochemical efficiency (Fig. 1) may have been an indication that SA treatment increased antioxidant activity before heating. Thus, our results indirectly support the statement that SA protects photosystem function under various environmental stresses via activation of antioxidant defense systems as a signaling molecule (Dat et al., 1998; Senaratna et al., 2000). Further evidence may be found in Schmidt and Zhang (2001) who reported that KBG sustained greater photochemical efficiency at three and five days of UV irradiation because of SA treatment. Ervin et al. (2004b) also found that SA-treated KBG resulted in greater maintenance of photochemical efficiency during UV-B irradiation and greater recovery of photochemical efficiency and visual quality once UV-B was removed. They also found that SA-treated KBG had greater pigment (chlorophyll, carotenoid, and anthocyanin) concentrations and greater catalase activity at Day 1 of UV-B exposure. At the end of the UV-B stress period (10 d), pigment levels of the SA-treated KBG remained higher than the control and catalase activity was positively correlated ( $r = 0.78^{**}$ ) with reduced visual turf injury. Up-regulation of antioxidant activity due to exogenously applied SA before stress events may function to reduce free radical damage and reduce the rate of pigment degradation during the stress period. If exogenous SA before heat-treatment of sod boosts the antioxidant defense system, protecting pigment and photosystem integrity, then it may be reasonable to expect less damage when sod is transplanted into a full sun (high UV) summer field environment. To more fully support this line of reasoning further field research is required where the UV environment is fully characterized and a time-series of photochemical effi-

**Table 1. Visual turf injury of Kentucky bluegrass (KBG) and tall fescue (TF) sod after heating (3 wk post-transplant) as influenced by salicylic acid (SA).**

Turfgrass	SA (kg ha <sup>-1</sup> )	2000			2001		
		72 h	96 h	Mean	72 h	96 h	Mean
Visual turf injury rating (1–9 with 9 = the most)†							
KBG	0.5	4.8	6.5	5.6b‡	4.3	6.6	5.4b
	0	5.3	7.0	6.1a	5.5	7.3	6.4a
Mean		5.0B	6.8A		4.8B	6.9A	
Source of variation							
Heat duration (HD)		**			**		
SA		*			*		
HD × SA		NS			NS		
TF	0.5	4.0	6.0	5.0b	4.2	5.0	4.6b
	0	4.4	7.2	5.8a	5.5	6.3	5.9a
Mean		4.2B	6.6A		4.9A	5.7A	
Source of variation							
Heat duration (HD)		**			NS		
SA		*			**		
HD × SA		NS			NS		

\* Significant at 0.05 probability level.

\*\* Significant at 0.01 probability level.

NS, not significant at *P* = 0.05.

† Visual turf injury was rated 3 wk after transplanting on the basis of a visual scale of 1–9 with 9 indicating the most injury.

‡ Values within same column for each data set followed by same letter are not different significantly at *P* = 0.05; mean within same row for each data set followed by same capital letters are not different significantly at *P* = 0.05.

**Table 2. Transplant root strength of Kentucky bluegrass (KBG) and tall fescue (TF) sod after heating as influenced by salicylic acid (SA).**

Turfgrass	SA (kg ha <sup>-1</sup> )	2000			2001		
		72 h	96 h	Mean	72 h	96 h	Mean
Transplant root strength (kg m <sup>-2</sup> )†							
KBG	0.5	290.0	281.7	285.9a‡	233.7	275.7	254.7a
	0	220.4	215.1	217.8b	218.3	202.2	210.3b
Mean		255.2A	248.4A		226.0A	239.0A	
Source of variation							
Heat duration (HD)			NS			NS	
SA			*			*	
HD × SA			NS			NS	
TF	0.5	691.4	613.9	652.7a	659.1	548.4	603.8a
	0	618.3	564.5	591.4b	584.9	537.6	561.3a
Mean		654.9A	589.2B		622.0A	543.0B	
Source of variation							
Heat duration (HD)		*			*		
SA		*			NS		
HD × SA		NS			NS		

\* Significant at 0.05 probability level.

\*\* Significant at 0.01 probability level.

NS, not significant at *P* = 0.05.

† Transplant root strength was measured 6 wk after transplanting.

‡ Values within same column for each data set followed by same letter are not different significantly at *P* = 0.05; mean within same row for each data set followed by same capital letter are not different significantly at *P* = 0.05.

ciency readings and leaf antioxidant activity and pigment concentration levels are quantified in relation to SA treatment.

Post-transplant rooting and turfgrass quality recovery are primary indices for evaluating sod establishment success. Salicylic acid pre-conditioning of KBG and TF before sod harvest and heat stress during storage and transportation at an expense of approximately \$100 dollars ha<sup>-1</sup> resulted in less visual leaf tissue damage and increased root development following transplantation. Further research is needed to more specifically quantify the level of leaf tissue damage and recovery over the establishment period. For example, time-series measurements of leaf electrolyte leakage, antioxidant activity, photochemical efficiency, pigment levels, and stem carbohydrate concentrations would provide a clearer understanding of post-transplant establishment phenomena because of pre-harvest SA application. While our

results are not definitive, it appears that SA application before harvest may be a convenient method of inducing tolerance to supraoptimal heating and increasing summer transplant success of cool-season turfgrass sod.

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