Chapter 3

PHOTOSYNTHESIS OF SUPINA BLUEGRASS (*POA SUPINA* SCHRAD.) AND KENTUCKY BLUEGRASS (*P. PRATENSIS* L.) IN REDUCED LIGHT CONDITIONS AS AFFECTED BY NITROGEN AND TRINEXAPAC-ETHYL

INTRODUCTION

Turfgrass performance in reduced light conditions (RLC; < 30% sunlight) is often poor due to insufficient light for photosynthesis and normal turf growth. Turfgrass species and cultivars may vary widely in their tolerance to RLC although all may exhibit reduced tillering, reduced rooting, and an upright spindly growth (Beard, 1973). Supina bluegrass (*Poa supina* Schrad.), a stoloniferous grass native to the sub-alpine regions of Europe, has been developed in Germany as a turfgrass with purportedly good to excellent shade and traffic tolerance (Berner, 1984; Nonn, 1994; Pietsch, 1989; Skirde, 1971). Preliminary research supports the hypothesis that Supina bluegrass is more tolerant of RLC than Kentucky bluegrass (*P. pratensis* L.) (Stier and Rogers, 1995) which is commonly used in the United States but has poor shade tolerance (Beard, 1973). The mechanism(s) for the apparent shade tolerance of Supina bluegrass is/are unknown.

In addition to the use of shade-tolerant turfgrasses proper management techniques are also important for turf performance in RLC. Previous research has indicated the potential for plant growth regulators (PGRs) which inhibit gibberellic acid (GA) biosynthesis to improve turf quality in reduced light conditions (Rogers et al., 1996; Stier and Rogers,

1995). Turf treated with GA-inhibitors in RLC was more uniform with darker color and increased density compared to untreated turf. While the GA-inhibitors effectively suppressed shoot elongation, other mechanisms by which the GA-inhibitors improved turf quality were unknown. Possibilities range from enhanced photosynthetic rates (Gausman et al., 1991), increased carbohydrate production or partitioning (Hanson and Branham, 1987; Wang et al. 1985), increased chlorophyll levels (Wang et al 1985, Archbold and Houtz, 1988), increased protein/enzyme levels and/or activity (Wang et al. 1985), altered hormonal levels affecting foliar production (Gausman et al., 1991), to gene expression (Gausman et al., 1991). Conversely, Archbold and Houtz (1988) reported flurprimidol and paclobutrazol decreased photosynthetic rates and Rubisco activities in strawberry plants. DeJong and Doyle (1984) found paclobutrazol reduced shoot growth of nectarine trees but did not affect photosynthesis. Mefluidide, generally considered a mitotic inhibitor which also may inhibit GA-biosynthesis (Wilkenson, 1982), consistently reduced photosynthetic rates of 'Baron' Kentucky bluegrass while amidochlor occasionally enhanced photosynthesis (Spokas and Cooper, 1991).

In the early 1990's a new GA-inhibitior, trinexapac-ethyl (TE), was labeled for use on turfgrasses, primarily to decrease mowing requirements by suppressing shoot growth (Vitolo et al., 1990). The potential side effects of TE on plant physiology are relatively unknown due to its recent release but may be different than other turf GA-inhibitors. TE apparently blocks 3-β oxidation of the biologically inactive GA_{20} to form the biologically active GA_1 as opposed to flurprimidol and paclobutrazol which inhibit ent-kaurene

oxidation oxidative steps earlier in the biosynthetic pathway (Coolbaugh et al., 1982; Rademacher, 1991).

In normal (full sun) conditions GA-inhibitor effects on turfgrass can vary with nitrogen (N) rate and turf species or cultivars. Watschke (1981) found differences in responses of two Kentucky bluegrass culitivars ('Merion' and 'Pennstar') to paclobutrazol and flurprimidol. Other studies showed high N rates reduced the effects of flurprimidol on common bermudagrass [*Cynodon dactylon* (L.) Pers.] (Devitt and Morris, 1988) but not on 'Tifway' hybrid bermudagrass [*Cynodon transvaalensis* Burtt-Davy x *C. dactylon* (L.) Pers.] (Johnson, 1988). Johnson (1994) corroborated the differences in response to trinexapac-ethyl between common bermudagrass and 'Tifway' hybrid bermudagrass. In RLC of approximately 5-6 mol photosynthetically active radiation (PAR) day^{-1}, medium to high N rates (48 and 96 kg ha^{-1} at four to six week intervals) resulted in significantly better quality Kentucky bluegrass compared to low N rates (24 kg ha^{-1} at four to six week intervals) when flurprimidol was applied, although low and medium N rates provided superior turf in the absence of flurprimidol (Chapter 1).

Due to the demand for improved turfgrasses and management schemes for turf in RLC, studies were initiated to examine the effects of N rate, trinexapac-ethyl, and species on turf photosynthesis in RLC. Two hypotheses were tested: 1) Supina bluegrass was more tolerant of RLC compared to Kentucky bluegrass due to a greater carbon exchange rate (CER), i.e., enhanced photosynthetic rate, and 2) Trinexapac-ethyl improved turfgrass quality in RLC by enhancing CER. The objectives of this research were to determine if differences in CER existed between Supina bluegrass and Kentucky

bluegrass and to determine the effects of trinexapac-ethyl on CER of the two species. A second set of objectives were to determine the influence of nitrogen rate and trinexapac-ethyl on the CER of Supina bluegrass.

MATERIALS AND METHODS

Plot establishment and testing

Experiment I: Species x PGR study

Portable plots were established outside in full sun conditions. Wooden boxes (1.2 x 1.2 x 0.15 m depth) were filled with a sand:peat mixture (80:20 v/v) (Table 78, Appendix). The pH was 7.8 with initial P and K levels of 85 kg ha^{-1} and 90 kg ha^{-1}, respectively. Sixteen holes (0.6 cm diam) were drilled on approximately 23 cm spacings in the bottom of each box to provide drainage. Starter fertilizer (13-25-12) was applied to the soil and supplied 76 kg N ha^{-1}, 64 kg P ha^{-1}, and 58 kg K ha^{-1}. Ten plots each were sodded in Sept. 1995 with Supina bluegrass 'Supra' or Kentucky bluegrass 'Blacksburg'. The sod had been grown in a composted wood mulch on polyethylene sheeting during the summer of 1995 (Cairol and Chevallier, 1981). Plots were mowed two to three times weekly to 3 cm height and irrigated as necessary to prevent moisture stress. Plots were fertilized bimonthly with 48 kg ha^{-1} N, 3 kg ha^{-1} P, and 40 kg ha^{-1} K. To prepare plots for testing in reduced light conditions (RLC), plots were fertilized with 48 kg N ha^{-1}, 41 kg P ha^{-1}, and 38 kg K ha^{-1} on 26 Aug. 1996. Plots were fertilized thereafter on a biweekly basis with 37 kg N ha^{-1}, 3 kg P ha^{-1}, and 30 kg K ha^{-1}.

On 18 Sept. 1996 trinexapac-ethyl (0.19 kg ha^{-1}) was applied to five plots each of Supina bluegrass and Kentucky bluegrass. Plots were moved into the Covered Stadium Simulator Facility (CSSF) on 4 October 1996 and arranged in a randomized complete block (RCB) design with five replications. Air temperature was maintained at 15.9 C ± 2.9 C (range was 10-20 C). Relative humidity was 55.4 ± 8.7%.

High pressure sodium lamps (400 W), suspended 2.7 m above the turf surface, provided a steady but reduced light condition of approximately 100 ± 9 μmol m^{-2} s^{-1} on a 12 h photoperiod (ppd) and provided approximately 4.3 mol PAR m^{-2} day^{-1}, not including ambient light (Table 62). Iprodione (3-(3,5-dichlorophenyl)-N-(1-methethyl)-2,4-dioxo-1-imidazolidinecarboximide), 5.93 kg ha^{-1}, was applied with a CO_2-powered backpack sprayer on 2 November 1996 to control Microdochium patch (*Microdochium nivale*). An open gas exchange system was used to determine photosynthetic rates (Sams and Flore, 1982) using a polycarbonate chamber (4.9 cm^2, approximately 24 cm^3) secured over the turf surface. Gas exchange measurements, foliar characteristics, and chlorophyll concentrations were determined 23-25 Nov. 1996 approximately seven weeks after the turf was moved into the CSSF. Carbon exchange rates (CERs) were collected on 23 Nov. between 1200-1600 h after CO_2 levels in ambient air had stabilized following large fluctuations earlier in the day. Data were analyzed as a 2 x 2 factorial in a RCB design with two species (Supina bluegrass and Kentucky bluegrass) and TE treatments (0.00 and 0.19 kg ha^{-1} trinexapac-ethyl) as main plots with five replications.

Table 62. Photosynthetically active radiation (PAR) of plots in the Covered Stadium Simulator Facility (CSSF), Hancock Turfgrass Research Center, East Lansing, MI.

Location	1996	
	October	November
	----------mol PAR day^{-1} ----------	
Outside†		
average	15.5	9.8
standard deviation	9.4	4.3
CSSF, ambient light ‡		
average	2.4	1.5
standard deviation	1.5	0.7
CSSF, supplemental light §		
average	5.5	5.1
standard deviation	1.1	0.7

† PAR was integrated daily using a pyranometer (Li-Cor, model PY 14226, Lincoln NE). Radiometric units (Ly day^{-1}) were converted to quantum units (mol PAR m^{-2} day^{-1}) based on the conversion units published by Thimijan and Heins (1983).

‡ PAR inside the CSSF was determined by measuring the percent of PAR transmitted into the CSSF at turf level with a portable spectroradiometer (Li-Cor, model LI-1800, Lincoln NE).

§ Supplemental light (approximately 100 µmol m^{-2} s^{-1}; 12 h photoperiod) was supplied with 400 W high pressure sodium lamps.

Experiment II: Nitrogen x PGR study

Portable plots were established outside in full sun conditions. Wooden boxes (1.2 x 1.2 x 0.15 m depth) were filled with sand (Table 79, Appendix). Sixteen holes (0.6 cm diam) were drilled on approximately 23 cm spacings in the bottom of each box to provide drainage. Starter fertilizer (13-25-12) was raked into the upper 2 cm of the sand surface to provide 76 kg N ha^{-1}, 64 kg P ha^{-1}, and 58 kg K ha^{-1}. Sixteen plots were sodded 29 August 1996 with Supina bluegrass 'Supranova' washed sod. Plots were irrigated as necessary to prevent moisture stress. Plots were mowed at 5 cm height at seven day intervals for the first 14 days, after which mowing height was gradually reduced to 3 cm height during the following 21 days. Thereafter, plots were mowed two to three times weekly to 3 cm height. Beginning 18 Sept. 1996 plots were fertilized with either a low N rate, 24 kg N ha^{-1} $month^{-1}$, or a high N rate, 96 kg N ha^{-1} $month^{-1}$ applied in split applications biweekly at 48 kg N ha^{-1}. Potassium was applied biweekly at 48 kg K ha^{-1} to all plots.

On 18 Sept. 1996 trinexapac-ethyl (0.19 kg ha^{-1}) was applied to four plots each fertilized with low or high N rates. Plots were moved into the CSSF on 4 October 1996 and arranged in a randomized complete block (RCB) design with four replications. High pressure sodium lamps (400 W) were suspended 2.7 m above the turf surface and provided a steady but reduced light condition of approximately 100 ± 9 µmol m^{-2} s^{-1}. The lamps were on a 12 h photoperiod (ppd) which totalled approximately 5 mol PAR m^{-2} day^{-1}, including ambient light (Table 62). Iprodione (3-(3,5-dichlorophenyl)-N-(1-methethyl)-2,4-dioxo-1-imidazolidinecarboximide), 5.93 kg ha^{-1}, was applied with a

CO_2-powered backpack sprayer on 2 November 1996 to control Microdochium patch (*Microdochium nivale*). Carbon exchange rates were determined 15 and 16 Nov. 1996 (one and two days after mowing, respectively) using an open gas exchange system with a polycarbonate chamber (surface area = 27 cm^2; volume approximately 200 cm^3). The same location on each plot was assayed on both dates. Leaf areas from CER sampling areas were determined 16 Nov. 1996. Samples for chlorophyll analysis were collected 18 Nov. and analyzed 20 Nov. 1996. Carbon exchange rates were determined again on 26 Nov. (1 day after mowing) using a smaller polycarbonate chamber (surface area = 4.9 cm^2; volume approximately 24 cm^3) to determine the effects of a greater flow rate:surface area on the CER. Leaf areas were determined from the sample areas the same day. Samples for chlorophyll analysis were collected 25 Nov. and analyzed 27 Nov. Photosynthetic measurements were collected between 0900-1200 h on all dates. Gas exchange and foliar data were analyzed as a 2 x 2 factorial with N rate (24 and 96 kg ha^{-1} $month^{-1}$) and TE (0.00 and 0.19 kg ha^{-1}) as main plots with four replications.

Gas exchange measurements

Carbon dioxide assimilation and related parameters were measured using an open system. The system was comprised of an ADC LCA2 infrared gas analyzer (IRGA), an air supply unit (ASU) capable of delivering up to 600 ml min^{-1} flow, a Parkinson leaf chamber (PLC) (Analytical Development Co. Ltd., Hoddesdon, England), and a polycarbonate assimilation chamber (PLC). Semi-flexible polyethylene tubing was used to connect the system components. Ambient air inside the CSSF was used as the air source and was drawn from a distance of at least 4 m away from the experimental site to

minimize CO_2 fluctuations due to the investigator. Air was drawn from approximately 0.3 m above the asphalt floor from a corner of the facility subject to little air movement which minimized CO_2 fluctuation. Air drawn from a height of 4 m inside the facility, or from outside the facility, had serious CO_2 fluctuations due apparently to furnace-emitted (heated) air. The CO_2 fluctuations prevented accurate measurements even when the air was passed through containers up to 250 L in attempts to dampen the CO_2 fluctuations. Ambient air (approximately $CO_2 = 349 \pm 5$ μL L^{-1} except on 26 Nov. when $CO_2 = 427 \pm 11$ μL L^{-1}) was passed into the ASU which pumped at a flow rate of 500 ml min^{-1}. The air was passed into a dome-shaped polycarbonate chamber (either 4.91 cm^2 opening, volume approximately 24 cm^3, or 27.3 cm^2 opening, volume approximately 200 cm^3) through in inlet port midway at or slightly below the turf surface. The chamber was secured over the turf surface using wire which was hooked over bolts at the chamber base and inserted into the turf. An exit port near the top of the chamber passed air into the IRGA for analysis of CO_2 concentration. Steady readings of CO_2 differential between the chamber and ambient air were achieved within one to two minutes. Immediately following gas exchange determination, a PLC was connected between the outlet port of the assimilation chamber and the IRGA for temperature and relative humidity measurements. The temperature and relative humidity of the ambient air were then measured. The photosynthetic photon flux density (PPFD) was determined during assimilation using a Li-Cor 190S quantum sensor (LiCor, Lincoln, NE). Photosynthetic parameters were calculated on both a turf surface area and leaf area basis using a BASIC

computer program (Moon and Flore, 1986). No attempt was made to inhibit the effects of soil respiration on CER.

Leaf area analysis

Leaf area of the turf was determined using a Li-Cor 300 leaf area meter (Li-Cor, Lincoln, NE). Leaf blades were excised from shoots and placed flat on sheet of clear contact paper. The contact paper was taped inside a folded piece of transparency paper; the sheets were then passed through the leaf area meter. The average of three readings were collected for each sample and the "blank" area of the contact paper plus tape was subtracted.

Chlorophyll analysis

Sections (1 cm length) were collected from the middle of the youngest, fully expanded leaf blades of 10 plants per plot. Leaf widths were measured for leaf area determination. The mass of each 10 segment sample was determined to evaluate fresh leaf weight. Chlorophyll was extracted according to the methods of Moran and Porath (1980) using the extinction coefficients and formulae determined by Inskeep and Bloom (1985). Chlorophyll was extracted from each 10 leaf segment sample in 3 ml *N,N*-Dimethylformamide during incubation in the dark for 48 h at 4 °C. Absorbance values were measured using a UV-Vis spectrophotometer to determine chlorophyll *a*, chlorophyll *b*, and total chlorophyll concentrations.

RESULTS

Experiment I: Species x PGR study

Species significantly affected CER, E, and g_s on a turf surface basis while trinexapac-ethyl did not affect gas exchange parameters (Table 63). No interactions occurred between species and TE on any gas exchange parameters. Supina bluegrass CER on a turf surface area basis was over 50% greater than CER of Kentucky bluegrass and significantly different at p=0.05 (Table 64). Higher transpiration rate and stomatal conductance of Supina bluegrass corresponded with the greater CER compared to lower values observed from Kentucky bluegrass. Trinexapac-ethyl did not significantly enhance CER although CER was 36% greater in treated versus control plots. On a leaf area basis neither species or TE affected CER (Table 65). Values of gas exchange parameters were quite similar between the two species on a leaf area basis (Table 66).

Species and TE both significantly affected LAI, fresh leaf weight, and chlorophyll levels (Table 67). There were no significant interactions between species and TE. Supina bluegrass turf had a greater LAI and lower fresh leaf weight but less chlorophyll compared to Kentucky bluegrass (Table 68). Trinexapac-ethyl resulted in greater LAI and increased chlorophyll levels in both species. Chlorophyll a:b was not affected by any treatment.

Experiment II: Nitrogen x PGR study

Nitrogen and trinexapac-ethyl did not have a significant effect on CER or other gas exchange parameters of Supina bluegrass when evaluated on a turf area basis (Table 69). A higher than normal (approximately 350 $\mu L\ L^{-1}\ CO_2$) ambient CO_2 level and decreased

Table 63. Mean squares and treatment effects of species and trinexapac-ethyl on photosynthetic parameters† of turfgrass maintained in reduced light conditions (approximately 5 mol PAR day^{-1}), turf surface area basis, 23 November 1996.

Source	CER ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	E ($mmol\ m^{-2}\ s^{-1}$)	g_s ($mmol\ m^{-2}\ s^{-1}$)	Ci ($\mu mol\ CO_2\ mol\ CO_2^{-1}$)	g_m ($mmol\ m^{-2}\ s^{-1}$)	WUE, 1 x 10^{-3} ($mol\ CO_2\ mol\ H_2O^{-1}$)
Replication	0.313	0.124	226.552	1062.181	5.603	1.841
Species (S)	1.331*	0.522*	883.253*	1504.765	10.382	0.538
Trinexapac-ethyl (TE)	0.677	0.033	39.340	131.687	6.555	1.342
S x TE	0.246	0.043	74.846	571.594	1.255	0.050
Error	0.274	0.102	174.425	1026.379	4.879	1.098
CV, %	43.08	47.46	51.88	10.98	51.46	50.60

* Significant at the 0.05 probability level.

† CER, carbon exchange rate; E, transpiration; g_s, stomatal conductance; Ci, internal leaf CO_2 , g_m , mesophyll conductance; WUE, water use efficiency.

Table 64. Photosynthetic differences between Supina bluegrass and Kentucky bluegrass in reduced light conditions (approximately 5 mol PAR day^{-1}), turf surface area basis, 23 November 1996.

Treatment	CER † ($\mu mol\ m^{-2}\ s^{-1}$)	E ($mmol\ m^{-2}\ s^{-1}$)	C_i ($\mu mol\ mol^{-1}$)	g_s ($mmol\ mol^{-1}$)	g_m ($mmol\ mol^{-1}$)	WUE, 1 x 10^{-3} ($mol\ CO_2\ mol\ H_2O^{-1}$)
Species						
Supina bluegrass	1.47	0.84	300.33	32.10	5.01	1.91
Kentucky bluegrass	0.96*	0.51*	282.98	18.81*	3.57	2.24
Trinexapac-ethyl ($kg\ ha^{-1}$)						
0.00	1.03	0.72	294.22	26.86	3.72	1.82
0.19 ‡	1.40	0.63	289.09	24.06	4.86	2.33

* Significant at the 0.05 probability level.

† CER, carbon exchange rate; E, transpiration; g_s, stomatal conductance; Ci, internal leaf CO_2 , g_m , mesophyll conductance; WUE, water use efficiency.

‡ Applied 18 Sept. 1996.

Table 65. Mean squares and treatment effects of species and trinexapac-ethyl on photosynthetic parameters† of turfgrass maintained in reduced light conditions (approximately 5 mol PAR day^{-1}), leaf area basis, 23 November 1996.

Source	CER ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	E ($mmol\ m^{-2}\ s^{-1}$)	g_s ($mmol\ m^{-2}\ s^{-1}$)	Ci ($\mu mol\ CO_2\ mol\ CO_2^{-1}$)	g_m ($mmol\ m^{-2}\ s^{-1}$)	WUE, 1×10^{-3} ($mol\ CO_2\ mol\ H_2O^{-1}$)
Replication	0.055*	0.012	19.793	1062.181	1.000	1.870
Species (S)	0.003	0.005	11.674	1504.765	0.000	0.522
Trinexapac-ethyl (TE)	0.001	0.030	41.876	131.687	0.001	1.316
S x TE	0.000	0.000	0.242	571.594	0.017	0.055
Error	0.014	0.015	23.974	1026.379	0.266	1.103
CV, %	35.11	63.08	67.69	10.98	43.47	50.65

* Significant at the 0.05 probability level.

† CER, carbon exchange rate; E, transpiration; g_s, stomatal conductance; Ci, internal leaf CO_2 , g_m , mesophyll conductance; WUE, water use efficiency.

Table 66. Photosynthetic differences between Supina bluegrass and Kentucky bluegrass in reduced light conditions (approximately 5mol PAR day^{-1}), leaf area basis, 23 November 1996.

Treatment	CER † ($\mu mol\ m^{-2}\ s^{-1}$)	E ($mmol\ m^{-2}\ s^{-1}$)	C_i ($\mu mol\ mol^{-1}$)	g_s ($mmol\ mol^{-1}$)	g_m ($mmol\ mol^{-1}$)	WUE, 1 x 10^{-3} ($mol\ CO_2\ mol\ H_2O^{-1}$)
Species						
Supina bluegrass	0.35	0.21	300.33	8.00	1.18	1.91
Kentucky bluegrass	0.32	0.18	282.98	6.50	1.19	2.24
Trinexapac-ethyl ($kg\ ha^{-1}$)						
0.00	0.33	0.23	294.22	8.68	1.18	1.82
0.19‡	0.34	0.16	289.09	5.79	1.20	2.33

† CER, carbon exchange rate; E, transpiration; g_s, stomatal conductance; Ci, internal leaf CO_2 , g_m , mesophyll conductance; WUE, water use efficiency.

‡ Applied 18 Sept. 1996.

Table 67. Mean squares and significance of treatment effects on leaf area index (LAI), fresh leaf weight, and chlorophyll concentration of turfgrasses in reduced light conditions (approximately 5 mol PAR day^{-1}), 24 Nov. 1996.

			chlorophyll (μg cm^{-2} leaf tissue)			
Source	LAI	Fresh leaf wt. (μg cm^{-2})	Chl *a*	Chl *b*	Chl total	Chl a:b
Replication	1.984	2.513**	23.547*	2.924*	41.765*	0.040
Species (S)	7.308**	2.537*	404.011**	42.166**	706.860**	0.009
Trinexapac-ethyl (TE)	5.629*	9.098**	196.753**	23.285**	354.987**	0.014
S x TE	0.981	0.035	6.555	3.329†	19.247	0.136
Error	0.696	0.347	5.120	0.710	7.985	0.06
CV, %	22.21	6.64	9.12	10.39	8.58	7.97

*, ** Significant at the 0.05 and 0.01 probability levels, respectively.

Table 68. Effects of species and trinexapac-ethyl on foliage and chlorophyll of turfgrasses in reduced light conditions, approximately 5 mol PAR day^{-1}, 24 Nov. 1996.

Treatment	LAI	Fresh leaf wt. ($\mu g\ cm^2$)	chlorophyll ($\mu g\ cm^{-2}$ leaf tissue) Chl *a*	Chl *b*	Chl total	Chl *a*:*b*
Species						
Supina bluegrass	4.36	8.52	20.32	6.66	29.98	3.10
Kentucky bluegrass	3.15**	9.23*	29.31**	9.56**	38.87**	3.06
Trinexapac-ethyl (kg ha^{-1})						
0.00	3.22	8.20	21.68	7.03	28.71	3.11
0.19 †	4.29*	9.55**	27.95**	9.19**	37.13**	3.06

*, ** Significant at the 0.05 and 0.01 probability levels, respectively.
† Applied 18 Sept. 1996.

Table 69. Mean squares and treatment effects of photosynthetic characteristics† of Supina bluegrass affected by trinexapac-ethyl and nitrogen rate in reduced light conditions (approximately 5 mol PAR day^{-1}), turf surface area basis.

Source	CER ($\mu mol\ m^{-2}\ s^{-1}$)	E ($mmol\ m^{-2}\ s^{-1}$)	C_i ($\mu mol\ mol^{-1}$)	g_s ($mmol\ mol^{-1}$)	g_m ($mmol\ mol^{-1}$)	WUE, 1 x 10^{-3} ($mol\ CO_2\ mol^{-1}\ H_2O$)
27.3 cm^2 assimilation chamber						
15 Nov. 1996, 24 h after mowing						
Replication	0.662*	0.004	9171.106*	0.687	117.530	6.188
Nitrogen (N)	0.010	0.000	62.450	0.526	24.182	0.366
Trinexapac-ethyl (TE)	0.028	0.009	395.513	10.049	18.512	0.286
N x TE	0.089	0.032*	4293.853	37.454*	21.414	5.244
Error	0.162	0.004	1573.685	6.951	45.771	1.786
CV, %	43.23	32.55	19.45	37.31	109.51	28.95
16 Nov. 1996, 48 h after mowing						
Replication	0.122	0.006	3298.292	7.419	27.152	4.588
Nitrogen (N)	0.476	0.004	13825.645	4.233	109.412	18.041
Trinexapac-ethyl (TE)	0.010	0.001	9.938	1.594	8.851	0.069
N x TE	0.245	0.058	7578.136	84.502	5.546	7.385
Error	0.153	0.012	4470.106	16.822	24.036	5.111
CV, %	35.29	45.45	36.62	49.01	63.84	42.67
4.9 cm^2 assimilation chamber						
26 Nov. 1996, 24 h after mowing						
Replication	0.727	0.169	232.507	1074.779	8.948	4.679
Nitrogen (N)	0.086	0.014	34.486	156.688	1.600	0.222
Trinexapac-ethyl (TE)	0.092	0.012	42.935	573.004	2.117	2.415
N x TE	0.086	0.042	81.406	14.119	0.766	0.065

Table 69 (cont'd.)

Error	0.500	0.084	218.729	574.758	3.077	1.228
CV, %	30.68	40.98	44.61	6.98	25.99	30.84

*, ** Significant at the 0.05 and 0.01 probability levels, respectively.

† CER, carbon exchange rate; E, transpiration; g_s, stomatal conductance; Ci, internal leaf CO_2; g_m, mesophyll conductance; WUE, water use efficiency.

assimilation area:flow rate ratio (smaller versus larger chamber) did not result in different treatment effects although the CER, E, and g_s rates were higher than on previous dates at a "typical" ambient CO_2 level (Table 70). Small and large chamber sizes (flow rate approximately 500 ml min^{-1}) resulted in similar values when compared between the species x PGR and nitrogen x PGR studies.

Carbon exchange rates on a turf area basis were lower at the high N rate compared to the low N rate although treatment effects were not significant at p=0.05 (Table 70). Carbon exchange rates 48 h after mowing were slightly greater compared to 24 h after mowing but there were still no significant differences among treatments. An interaction occurred on 15 Nov. 1996 between species and TE on E and g_s when gas exchange parameters were determined on a turf area basis. Twenty-four hours after mowing, E and g_s of turf maintained at high N and treated with TE were significantly greater than untreated, high N turf or low N turf regardless of treatment (Table 71). This interaction was not significant on a leaf area basis and was not observed when the experiment was repeated on 26 Nov. 1996.

On a leaf area basis nitrogen was the only treatment effect to produce any significant effects (Table 72). TE had no effect and there were no interactions. The high nitrogen rate increased CER, E, g_s, and g_m although the results were only significant for CER (p=0.10), E, and g_s on one date 24 h after mowing and were not significant 48 h after mowing (Table 73).

Both nitrogen rate and TE significantly affected LAI and chlorophyll content of Supina bluegrass (Table 74). There were no significant interactions on foliage or

Table 70. Effects of nitrogen and trinexapac-ethyl on photosynthetic parameters‡ of Supina bluegrass in reduced light conditions (approximately 5 mol PAR day^{-1}), turf surface area basis.

Treatment	CER ($\mu mol\ m^{-2}\ s^{-1}$)	E ($mmol\ m^{-2}\ s^{-1}$)	C_i ($\mu mol\ mol^{-1}$)	g_s ($mmol\ mol^{-1}$)	g_m ($mmol\ mol^{-1}$)	WUE, 1 x 10^{-3} ($mol\ CO_2\ mol^{-1}\ H_2O$)
27.3 cm^2 assimilation chamber §						
Nitrogen ($kg\ ha^{-1}\ month^{-1}$)¶	15 Nov. 1996, 24 h after mowing, 346 ± 3 $\mu L\ L^{-1}$ ambient CO_2, 148 ± 18 $\mu mol\ m^{-2}\ s^{-1}$ PAR					
24	0.96	0.20	201.94	6.88	4.95	4.77
96	0.91	0.20	205.90	7.25	7.41	4.47
Trinexapac-ethyl ($kg\ ha^{-1}$)						
0.00	0.89	0.18	198.95	6.27	7.25	4.75
0.19 #	0.97	0.22	208.89	7.86	5.10	4.48
Nitrogen ($kg\ ha^{-1}\ month^{-1}$)	16 Nov. 1996, 48 h after mowing, 347 ± 2 $\mu L\ L^{-1}$ ambient CO_2, 162 ± 19 $\mu mol\ m^{-2}s^{-1}$ PAR					
24	1.28	0.23	153.16	7.86	10.29	6.36
96	0.94	0.26	211.95	8.88	5.06	4.24†
Trinexapac-ethyl ($kg\ ha^{-1}$)						
0.00	1.08	0.23	183.34	8.05	6.94	5.23
0.19	1.13	0.25	181.76	8.68	8.42	5.36
4.9 cm^2 assimilation chamber						
Nitrogen ($kg\ ha^{-1}\ month^{-1}$)	26 Nov. 1996, 24 h after mowing, 427 ± 11 $\mu L\ L^{-1}$ ambient CO_2, 166 ± 13 $\mu mol\ m^{-2}\ s^{-1}$ PAR					
24	2.38	0.74	340.09	34.62	7.06	3.71
96	2.23	0.68	346.35	31.68	6.43	3.48
Trinexapac-ethyl ($kg\ ha^{-1}$)						
0.00	2.23	0.73	349.21	34.79	6.38	3.20
0.19	2.38	0.68	337.24	31.51	7.11	3.98

Table 70 (cont'd).

† Significant at the 0.10 probability level.
‡ CER, carbon exchange rate; E, transpiration; g_s, stomatal conductance; Ci, internal leaf CO_2; g_m, mesophyll conductance; WUE, water use efficiency.
§ Flow rate was 0.5 L min^{-1} through both chambers on all dates.
¶ Nitrogen was supplied as urea. The low rate was applied at four week intervals, the high rate was split into two biweekly applications each month.
# Applied 18 Sept. 1996.

Table 71. Interaction of N rate and trinexapac-ethyl on transpiration (E) and stomatal conductance (g_s) of Supina bluegrass maintained in reduced light conditions of approximately 5 mol PAR day^{-1}.

	ambient CO_2 = 346 ± 3 µL L^{-1}			
	E		g_s	
	trinexapac-ethyl (kg ha^{-1}) †			
N rate (kg ha^{-1})	0.00	0.19	0.00	0.19
24	0.22	0.18	7.62	6.15
96	0.13	0.27	4.92	9.57
LSD (0.05)	0.10		4.2	

† Applied 18 Sept. 1996.

Table 72. Mean squares and treament effects of photosynthetic characteristics† of Supina bluegrass affected by trinexapac-ethyl and nitrogen rate in reduced light conditions (approximately 5 mol PAR day^{-1}), leaf area basis.

Source	CER ($\mu mol\ m^{-2}\ s^{-1}$)	E ($mmol\ m^{-2}\ s^{-1}$)	C_i ($\mu mol\ mol^{-1}$)	g_s ($mmol\ mol^{-1}$)	g_m ($mmol\ mol^{-1}$)	WUE, 1 x 10^{-3} ($mol\ CO_2\ mol^{-1}\ H_2O$)
			27.3 cm^2 assimilation chamber			
	15 Nov. 1996, 24 h after mowing, 346 ± 3 $\mu L\ L^{-1}$ ambient CO_2, 148 ± 18 $\mu mol\ m^{-2}\ s^{-1}$ PAR					
Replication	0.072*	0.000	9171.106*	0.546	15.871	6.188
Nitrogen (N)	0.053†	0.005**	62.450	6.337*	10.808	0.366
Trinexapac-ethyl (TE)	0.004	0.000	395.513	0.025	5.748	0.286
N x TE	0.00	0.001	4293.853	1.160	5.653	5.244
Error	0.015	0.000	1573.685	0.663	6.228	1.786
CV, %	37.16	27.21	19.45	30.92	110.58	28.95
	16 Nov. 1996, 48 h after mowing, 347 ± 2 $\mu L\ L^{-1}$ ambient CO_2, 162 ± 19 $\mu mol\ m^{-2}\ s^{-1}$ PAR					
Replication	0.012	0.000	3298.292	1.766	1.116	4.588
Nitrogen (N)	0.010	0.012	13825.645	13.268	1.300	18.041
Trinexapac-ethyl (TE)	0.016	0.001	9.938	0.870	0.133	0.069
N x TE	0.002	0.002	7578.136	2.814	0.828	7.385
Error	0.007	0.004	4470.106	5.661	1.299	5.111
CV, %	21.68	68.17	36.62	72.20	46.07	42.67
			4.9 cm^2 assimilation chamber			
	26 Nov. 1996, 24 h after mowing, 427 ± 11 $\mu L\ L^{-1}$ ambient CO_2, 166 ± 13 $\mu mol\ m^{-2}\ s^{-1}$ PAR					
Replication	0.079	0.007	1074.779	10.435	0.872*	4.681
Nitrogen (N)	0.034	0.003	156.688	7.426	0.187	0.221
Trinexapac-ethyl (TE)	0.022	0.008	573.004	20.521	0.101	2.418
N x TE	0.017	0.004	14.119	8.702	0.114	0.065
Error	0.022	0.003	574.758	9.071	0.138	1.227

Table 72 (cont'd.)

CV, %	33.37	43.28	6.98	46.98	28.23	30.83

*, ** Significant at the 0.10, 0.05 and 0.01 probability levels, respectively.

† CER, carbon exchange rate; E, transpiration rate; g_s, stomatal conductance; Ci, internal leaf CO_2; g_m, mesophyll conductance; WUE, water use efficiency.

Table 73. Effects of nitrogen and trinexapac-ethyl on photosynthetic parameters‡ of Supina bluegrass in reduced light conditions (approximately 5 mol PAR day^{-1}), leaf area basis.

Treatment	CER ($\mu mol\ m^{-2}\ s^{-1}$)	E ($mmol\ m^{-2}\ s^{-1}$)	C_i ($\mu mol\ mol^{-1}$)	g_s ($mmol\ mol^{-1}$)	g_m ($mmol\ mol^{-1}$)	WUE, 1 x 10^{-3} ($mol\ CO_2\ mol^{-1}\ H_2O$)
			27.3 cm^2 assimilation chamber †			
Nitrogen ($kg\ ha^{-1}\ month^{-1}$)	15 Nov. 1996, 24 h after mowing, 346 ± 3 $\mu L\ L^{-1}$ ambient CO_2, 148 ± 18 $\mu mol\ m^{-2}\ s^{-1}$ PAR					
24	0.28	0.06	201.945	2.00	1.44	4.77
96	0.39†	0.09**	205.896	3.26*	3.08	4.47
Trinexapac-ethyl ($kg\ ha^{-1}$)						
0.00	0.35	0.07	198.949	2.59	2.86	4.75
0.19 §	0.32	0.08	208.893	2.67	1.66	4.84
Nitrogen ($kg\ ha^{-1}\ month^{-1}$)	16 Nov. 1996, 48 h after mowing, 347 ± 2 $\mu L\ L^{-1}$ ambient CO_2, 162 ± 19 $\mu mol\ m^{-2}s^{-1}$ PAR					
24	0.37	0.07	153.156	2.38	2.76	6.36
96	0.42	0.12	211.948	4.21	2.19	4.24†
Trinexapac-ethyl ($kg\ ha^{-1}$)						
0.00	0.42	0.10	183.340	3.53	2.56	5.23
0.19	0.36	0.09	181.764	3.06	2.38	5.36
			4.9 cm^2 assimilation chamber			
Nitrogen ($kg\ ha^{-1}\ month^{-1}$)	26 Nov. 1996, 24 h after mowing, 427 ± 11 $\mu L\ L^{-1}$ ambient CO_2, 166 ± 13 $\mu mol\ m^{-2}\ s^{-1}$ PAR					
24	0.40	0.12	340.09	5.73	1.21	3.71
96	0.50	0.15	346.35	7.09	1.43	3.48
Trinexapac-ethyl ($kg\ ha^{-1}$)						
0.00	0.49	0.16	349.21	7.54	1.40	3.20
0.19	0.41	0.11	337.24	5.28	1.24	3.98

Table 73 (cont'd.)

†, *, ** Significant at the 0.10, 0.05, and 0.01 probability levels, respectively.
‡ CER, carbon exchange rate; E, transpiration rate; g_s, stomatal conductance; Ci, internal leaf CO_2; g_m, mesophyll conductance; WUE, water use efficiency.

Table 74. Mean squares and significance of treatment effects on leaf area index (LAI), fresh leaf weight, and chlorophyll concentration of Supina bluegrass in reduced light conditions (approximately 5 mol PAR day^{-1}), 17 Nov. 1996.

		Fresh leaf wt.	Chlorophyll (μg cm^{-2})			
Source	LAI	(μg cm^{-2})	Chl a	Chl b	Chl Total	Chl a:b
Replication	1.943	0.819	16.305*	1.843*	29.105*	0.004
Nitrogen (N)	5.736**	0.148	93.364**	6.799**	150.492**	0.017
Trinexapac-ethyl (TE)	2.190†	6.945*	47.163**	3.851**	77.925**	0.002
N x TE	0.040	0.805	2.273	0.473	4.785	0.013
Error	0.504	0.938	3.261	0.337	5.611	0.005
CV, %	26.33	9.94	6.61	7.08	6.67	2.06

*, ** Significant at the 0.10, 0.05, and 0.01 probability levels, respectively.

chlorophyll content. The high nitrogen rate significantly reduced LAI although chlorophyll content was increased (Table 75). Trinexapac-ethyl significantly increased LAI, fresh leaf weight, and chlorophyll concentration. The ratio of chlorophyll *a:b* was not affected by any treatment.

Although it was not a planned component of the study, N rate was observed to affect Microdochium patch (*Microdochium nivale*) (Table 76). Microdochium patch severely damaged turf maintained at high N rates while turf at low N rates sustained significantly less damage (Table 77).

DISCUSSION

Carbon exchange rates (approximately 1 μmol CO_2 m^{-2} s^{-1}, turf area basis) were comparable to results obtained using an open system to determine CER of Kentucky bluegrass during sod establishment in similarly reduced light conditions of 150 μmol m^{-2} s^{-1} PAR (Karnok and Augustin, 1981). Karnok and Augustin (1981) reported increasing assimilation rates on a sward area basis with increasing days after mowing which corresponded to increased shoot height. Since fine turf is normally mowed frequently (e.g., one or two day intervals) the photosynthetic rate within one to two days following mowing was deemed important in the current study.

Morgan and Brown (1983) concluded the optimal LAI of bermudagrass for photosynthesis was approximately 4.7 at 1600-2000 μmol m^{-2} s^{-1} PAR while lesser LAIs resulted in significantly lower CER. The optimal LAI for cool-season turfgrasses in reduced light conditions is unknown but the higher LAI of Supina bluegrass was

Table 75. Effects of nitrogen rate and trinexapac-ethyl on leaf area index (LAI), fresh leaf weight, and chlorophyll concentration of Supina bluegrass in reduced light conditions (approximately 5 mol PAR day^{-1}), 17 Nov. 1996.

Treatment	LAI	Fresh leaf wt: ($\mu g\ cm^{-2}$)	Chlorophyll ($\mu g\ cm^{-2}$) Chl a	Chl b	Chl total	Chl a:b
Nitrogen (kg ha^{-1} $month^{-1}$)						
24	3.3	9.64	24.92	7.55	32.46	3.30
96	2.1**	9.84	29.75**	8.86**	38.59**	3.36
Trinexapac-ethyl (kg ha^{-1})						
0.00	2.3	9.1	25.61	7.71	33.32	3.32
0.19‡	3.1†	10.4*	29.05**	8.69**	37.73**	3.34

†, *, ** Significant at the 0.10, 0.05, and 0.01 probability levels, respectively.
‡ Applied 18 Sept. 1996.

Table 76. Mean squares and treatment effects on Microdochium patch (*Microdochium nivale*) effects on Supina bluegrass in reduced light conditions (approximately 5 mol PAR day^{-1}), 18 Nov. 1996.

	Turfgrass		
Source of variation	Color	Density	Quality
Replication	0.099	421.229	1.307
Nitrogen rate (N)	0.391	6123.063**	62.106**
Trinexapac-ethyl (TE)	8.266**	742.563	8.266
N x TE	0.391	60.063	2.641
Error	0.488	175.229	5.307
CV, %	9.35	18.690	45.23

** Significant at the 0.01 probability level.

Table 77. Effects of nitrogen rate and trinexapac-ethyl on Microdochium patch (*Microdochium nivale*) damage to Supina bluegrass in reduced light conditions (approximately 5 mol PAR day^{-1}), 18 Nov. 1996.

	Turfgrass		
Treatment	Color†	Density‡	Quality§
Nitrogen rate (kg ha^{-1} $month^{-1}$)¶			
24	7.3	90.4	7.1
48	7.6	51.2**	3.1**
Trinexapac-ethyl (kg ha^{-1})			
0.00	6.8	64.0	4.4
0.19 ††	8.2**	77.6	5.8

** Significant at the 0.01 probability level.

† Color was rated visually on a one to nine scale, one = chlorotic, yellow turf, nine= dark green turf color with 5 a minimum rating for acceptable color.

‡ Percent turf cover, visual estimate.

§ Quality was rated visually on a one to nine scale, one=100% necrotic turf, nine=dense, uniform, ideal turf with 5 a minimum rating for acceptable turf.

¶ Nitrogen was applied as urea

†† Applied 18 Sept. 1996.

apparently responsible for most or all of the difference in CER between the two species a sward area basis. There were no significant gas exchange differences between species on a leaf area basis. Supina bluegrass plants have a prostrate growth habit and stolons with short internodes and numerous tillers which apparently provided a greater leaf area for photon capture and gas exchange compared to Kentucky bluegrass which exhibits an increasingly more vertical growth habit as PPFD declines (Wilkinson and Beard, 1973).

The high N rate did not increase photosynthesis on a turf area basis because the amount of foliage was significantly decreased. High disease incidence associated with the high N rate may have caused a reduction in foliage although areas which appeared to be relatively unaffected by disease were chosen for CER measurements. The direct relationship between N rate and photosynthesis in non light-limiting situations appears to be largely dependent on the increased leaf biomass stimulated by higher N rates which affect carbon partitioning (Belanger et al., 1994; Gastal and Belanger, 1993; Nelson et al., 1993; Walker and Ward, 1973). In the current study, the high N rate may have stimulated excessive shoot growth early on after being placed in the reduced light conditions and depleted the carbohydrate pool necessary to sustain foliar growth and development. The high N rate may also have stimulated respiration which would have depleted the pool of nonstructural carbohydrates and resulted in reduced tillering.

On a leaf area basis, the high N rate had a tendency to increase photosynthesis although this was significant only on one of the three dates. This result concurs with Walker and Ward (1973) who reported photosynthetic rates of centipedegrass [*Eremochloa ophiuroides* (Munro.) Hack.] were directly dependent on N rate. The higher

N rate may have resulted in greater enzyme, particularly Rubisco, production (Ogata et al., 1983; Stitt and Schulze, 1994) and/or greater mesophyll conductance (g_m) (Bolton and Brown, 1980).

The lack of significant effect of TE on photosynthesis in RLC is not surprising. GA-inhibitors (paclobutrazol, flurprimidol) which act to inhibit *ent*-kaurene oxidation to *ent*-kaurenoic acid have been associated with both increases and decreases in photosynthetic rates in strawberries (Archbold and Houtz, 1988). Trinexapac-ethyl, however, inhibits the latter stages of GA biosynthesis, primarily by inhibiting hydroxylation at the 3ß position of $GA_{20}$to produce a biologically active GA_1 (Rademacher, 1991). Several other differences exist between trinexapac-ethyl and other GA-inhibitors commonly used on turf which may influence their effects on plant physiology: 1) trinexapac-ethyl is foliar-absorbed (Vitolo et al., 1990), while paclobutrazol and flurprimidol are drenched into the ground for root uptake (Watschke et al., 1992), and 2) trinexapac-ethyl may be less phytotoxic than paclobutrazol and flurprimidol (Watschke and DiPaola, 1995).

It is important to understand the mechanism(s) by which trinexapac-ethyl affects turfgrass growth and physiology in order to successfully use trinexapac-ethyl to maintain high quality turf in RLC. Green et al. (1990) reported flurprimidol significantly reduced the ET rate of St. Augustinegrass for 5 weeks after application. Although the ET components were not split into the respective components of evaporation and transpiration, it was implied the reduced leaf extension rate was responsible for lowering the ET. Such data are important as decreased transpiration in RLC will further inhibit photosynthate production, an undesirable effect.

TE did significantly enhance leaf area and chlorophyll concentrations of both species and of Supina bluegrass across nitrogen rates but did not signficantly affect CER, even on a sward area basis. The effects of TE on photosynthesis may have been complicated by reduced senescence and increased LAI since increased leaf age and greater canopy development have been reported to reduce individual leaf photosynthetic rate (Morgan and Brown 1983).

The improved turf quality associated with TE on turf in RLC may be related only to darker green leaf color and increased leaf area and/or tillering. Reduced leaf senescence rate and additional tillering could have been stimulated by TE side effects on other hormones or by TE altering carbohydrate levels and partitioning in the plants. GA-inhibitors have been shown to affect levels of other hormones such as abscisic acid in wheat (Buta and Spaulding, 1990) but their effects on hormones in turf is not known. Research on PGR effects on carbohydrate partitioning in turf is scarce. The key publication in the area, produced prior to the release of TE, indicates even GA-inhibitors with similar modes of action (paclobutrazol and flurprimidol) vary in their effect on assimilate partitioning (Hanson and Branham 1987). It is interesting to note that both paclobutrazol and flurprimidol did significantly decrease photoassimilate partitioning to roots four weeks after treatment (Hanson and Branham, 1987) although this may have been a transient response and not resulted in long-term effects. In the long term, reduced photoassimilate partitioning to roots could decrease turf quality and growth due to reduced root production. Studies on root growth of turf treated with flurprimidol or TE indicated these compounds had either no effect or had a beneficial effect on root growth

(Dernoeden, 1984; Elam, 1993; McCarty et al., 1990). Studies on the effects of GA-inhibitors on photosynthate partitioning and hormone levels in turfgrass in RLC are warranted.

Chlorophyll concentration did not affect photosynthetic rates ($r^2 = 0.07$). Differences in chlorophyll concentration were often statistically significant at p=0.05 when analyzed between species, between N rates, and between TE and untreated plots, but were not great enough to result in different photosynthetic rates. The quantity of photosynthetically active radiation, not chlorophyll, limited the CER. Species and TE did have a significant role in turf color (Ch. 2), however, and for practical reasons species and TE must be considered when managing turf in reduced light conditions. Chlorophyll *a:b* ratios were typical of "sun" plants, approximately 3 (Nobel, 1991), and were not affected by any treatments.

CONCLUSION

The relative shade tolerance of Supina bluegrass compared to Kentucky bluegrass appeared to be related to a greater LAI and not to superior gas exchange properties (e.g. CER, transpiration, stomatal resistance). The high N rate did not sufficiently enhance Supina bluegrass photosynthetic rates to offset problems associated with the lower LAI compared to the low N rate or the problem of the increased Microdochium patch incidence (*Microdochium nivale*). Trinexapac-ethyl did not seem to affect gas exchange parameters of photosynthesis. It is likely TE improved turf quality in RLC by

mechanisms other than enhanced photosynthesis, possibly by altering photosynthate partitioning.