Chapter 1

Determination of shade tolerance for three cool-season turfgrass species (Kentucky bluegrass, tufted hairgrass, and tall fescue) using $^{13}CO_2$ pulse chase procedures.

ABSTRACT

Reduced light conditions are said to comprise an estimated 20-25% of all managed turfgrass. To further understand the relative shade tolerance a simple study was initiated using three cool-season turfgrass species. In this experiment, Kentucky bluegrass (*Poa pratensis* L.), tufted hairgrass (*Descampsia caespotosa*), and tall fescue (*Festuca arundianacea* Schreb.) were labeled with $^{13}CO_2$ during photosynthesis to assess C allocation under full sun and reduced light conditions. The objective of this study was to compare photosynthetic carbon partitioning between cool-season turfgrass species grown under full sun and reduced light conditions. Results for this experiment determined that reduced light levels provided by the indoor research facility are not limiting enough for tall fescue and tufted hairgrass, thus suggesting their effective shade tolerance. Because tall fescue and tufted hairgrass are slow growing turfgrasses, the level of reduced light did not limit their photosynthetic requirements. Conversely, the $^{13}CO_2$ pulsing suggested that the shade tolerance of Kentucky bluegrass, a more aggressive growing turfgrass, to be insufficient under the reduced light level.

INTRODUCTION

The use of stable carbon (C) isotopes in agricultural and ecological research has become more frequent in recent years (Tieszen and Boutton, 1989). The two stable isotopes of carbon are ^{12}C and ^{13}C, which comprise 98.89 and 1.11%, respectively, of all C in nature. Early ^{12}C and ^{13}C research utilized natural variation in the relative abundances of these two stable isotopes. For example, cool-season (C_3) plant species discriminate against $^{13}CO_2$ during photosynthesis to a greater extent than do warm-season (C_4) plant species (O'Leary, 1981). This difference in $^{13}C/^{12}C$ ratio between C_3 and C_4 plants has been used to estimate the proportion of C_3 and C_4 species in diets of rangeland insects and large herbivores (Boutton *et al.*, 1980; Jones *et al.*, 1979). The difference in $^{13}C/^{12}C$ ratio has also been studied in root cores containing the two aforementioned functional groups (Svejcar and Boutton, 1985). The C isotope ratios of C_3 plants have also been shown to be correlated with water-use efficiency (Farquhar and Richards, 1984), which makes C isotope analysis a useful tool in ecophysiology and plant breeding research.

In addition to C isotopic studies, which capitalize on natural $^{13}C/^{12}C$ variation, plants can also be labeled with $^{13}CO_2$ during photosynthesis to assess C allocation. Boutton, *et al.* (1987) labeled rice (*Oryza sativa* L.) to obtain grain enriched in ^{13}C for use in human nutrition studies. Mordacq *et al.* (1986) studied C flow to roots and respiratory losses in a chestnut coppice using $^{13}CO_2$. Kouchi and Yoneyama (1984a,b) used a steady state $^{13}CO_2$ assimilation system and

long-term labeling to study accumulation, translocation, and metabolism of photosynthetically assimilated C in nodulated soybean (*Glycine max* L.) plants. The aforementioned ^{13}C tracer experiments implemented in agronomic or ecological context are relatively simple procedures for labeling plants with $^{13}CO_2$ (Svejcar, *et al.*, 1990). While much C labeling using $^{13}CO_2$ has been done, nothing has been done on turfgrass exposed to reduced light conditions (shade).

In this experiment, three cool-season turfgrass species were labeled with $^{13}CO_2$ during photosynthesis to assess C allocation under full sun and reduced light conditions. Reduced light conditions is said to comprise an estimated 20-25% of all managed turfgrass (Beard, 1973). With cool-season turfgrass species, differences in shade adaptation have also been found. Supina bluegrass (*Poa supina* Schrad.) is a cool season turfgrass that has been identified as having exceptional shade adaptation (Berner, 1984; Pietsch, 1989; Stier and Rogers, 2001) while tall fescue (*Festuca arundinacea*), creeping red fescue (*Festuca rubra* L. ssp. r*ubra*), creeping bentgrass (*Agrostis stolonifera* L.), and colonial bentgrass (*Agrostis capillaris* L.) have good shade tolerance. Kentucky bluegrass (*Poa pratensis* L.) has been identified as having poor shade tolerance (Dudeck and Peacock, 1992).

MATERIALS AND METHODS

The accumulation and translocation of photosynthetically assimilated carbon using $^{13}CO_2$ was studied using established stands of Kentucky bluegrass (*Poa pratensis* L.), tufted hairgrass (*Descampsia caespotosa*), and tall fescue (*Festuca arundinacea* Schreb.) grown under full sun and reduced light conditions. The experimental design for this study was a completely randomized factorial design with three replications. On 18 May 2000, 18 (six of each species) 175 cm^2 pots were filled with sand and seeded outside at the Hancock Turfgrass Research Center at Michigan State University, East Lansing, MI. The seeding rates were 5, 10, and 35 grams of seed m^{-2}, for Kentucky bluegrass, tufted hairgrass, and tall fescue, respectively. Fertilizer was applied once every two weeks for the first six weeks using Lebanon Country Club (Lebanon, PA, USA) 13-25-12 Starter Fertilizer at 5 grams N m^{-2}. Beginning 30 June 2000 and continuing through 17 August 2001, 5 grams N m^{-2} were applied every three weeks using Lebanon Country Club 18-3-18 fertilizer. On 6 October 2000 all pots were moved into the indoor research facility at the Hancock Turfgrass Research Center to continue to grow under reduced light conditions. On 28 April 2001 three pots of each species were taken outside to acclimate to full sun conditions.

The indoor research facility at the Hancock Turfgrass Research Center is a 400 m^2 air-supported structure constructed of Ultralux® (Chemical Fabrics Corporation, Buffalo, NY, USA), a fiberglass fabric which transmits approximately

20% +/- 2% photosynthetically active radiation (Figure 1). Temperature, relative humidity, and light levels were recorded hourly during the treatment application until the last sampling date using a Spectrum Watchdog Data Logger Model 450 (Spectrum Technologies Inc., Plainfield, Illinois, USA).

On 14 August 2001 $^{13}CO_2$ labeling was done between 0900 h and 1200 h by placing individual turfgrass pots into sealed chambers with a Mylar® (Du Pont, Wilmington, DE, USA) cover for light transmittance. To generate $^{13}CO_2$ for ^{13}C labeling, one ml of $Ba^{13}CO_3$ (98 atom %, Isotec Inc., OH, USA) and one ml of 85% lactic acid (Baker, Phillipsburg, NJ, USA) were mixed using two five ml syringes. Once generated, the $^{13}CO_2$ was injected into the gastight chamber for labeling (Figure 2). The total CO_2 concentration following injection was greater than 900 ppm above ambient when the CO_2 levels were initially measured using a CIRAS-1 infrared gas analyzer (PP-Systems Inc., Haverhill, MA, USA). Because preliminary tests had shown daily photosynthesis to end earlier outside than inside, $^{13}CO_2$ labeling was done to all of the turfgrass species growing outside, then to the pots growing inside.

Plant samples were harvested at 1 and 24-h, and 7 days post $^{13}CO_2$ labeling. In addition, one set of unlabeled plants was harvested to obtain natural ^{13}C abundances. Plant samples were rinsed in double distilled water and separated into crown, roots, and shoots (Figure 3). The shoots consisted of all green plant material; where, the crown area was the compressed stem area and any junction from the first and last node; and finally, the roots were the remaining

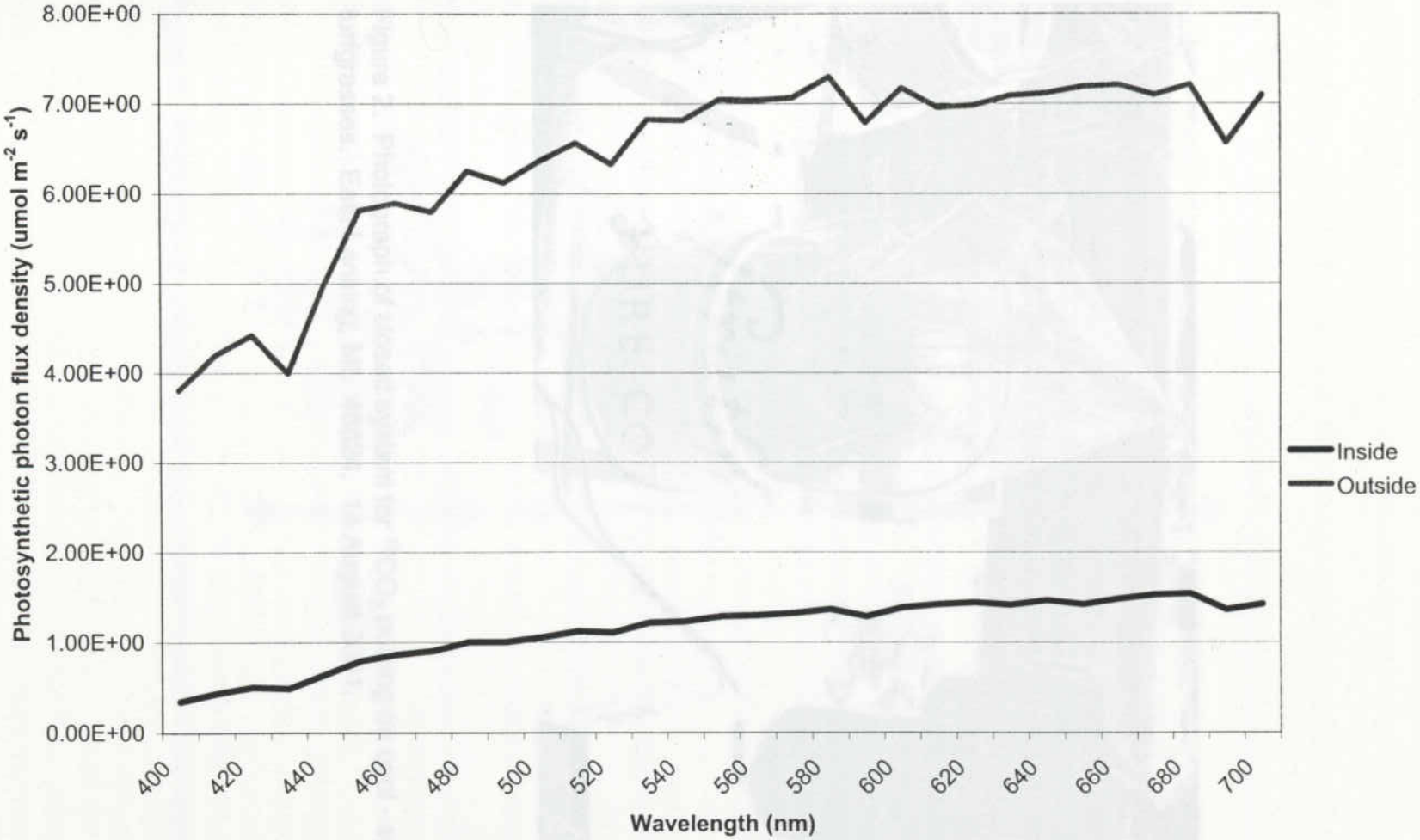

Figure 1. Photosynthetic photon flux density of sunlight and ambient light inside the indoor research facility, East Lansing, MI. 48824. 1430 h, 19 June 2000.

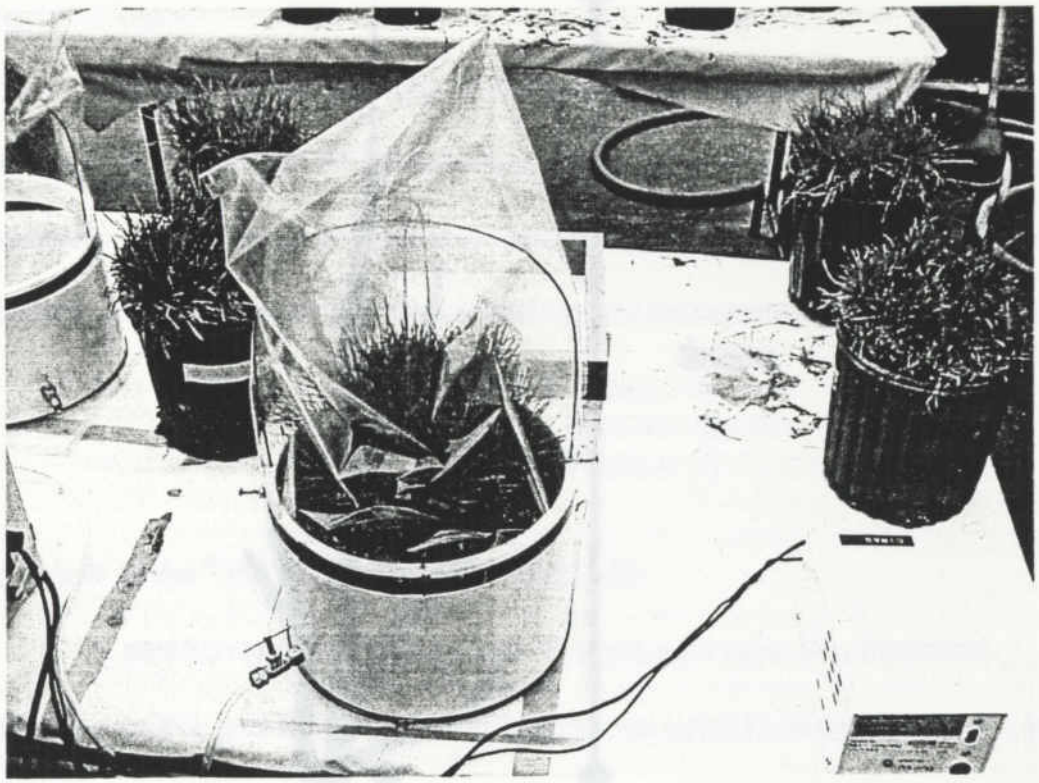

Figure 2. Photograph of closed system for $^{13}CO_2$ pulsing on cool - season turfgrasses. East Lansing, MI. 48824. 14 August 2001.

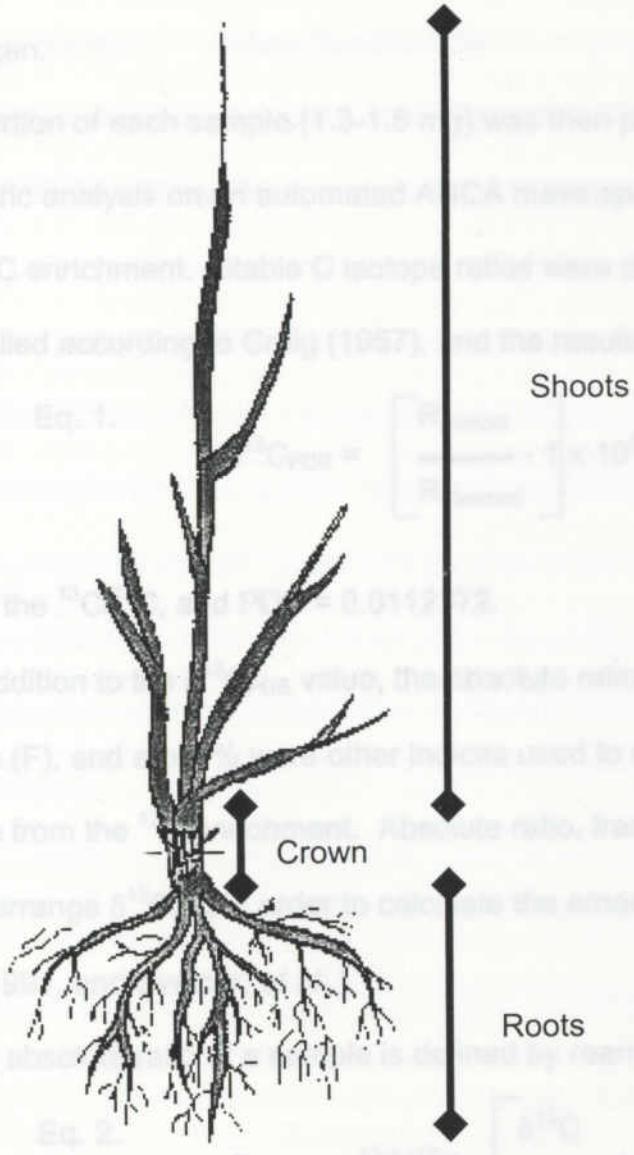

Figure 3. Illustration of turfgrass plant tissue shoot - crown - root separation.

plant tissue growing below ground. Separated plant parts were placed in aluminum foil, frozen in liquid nitrogen, and then transferred to a freezer at -80 °C. Next, the plant samples were put into a freeze drier for 24 hours. After 24 hours, the plant samples were ground into a fine powder using a mortar and liquid nitrogen.

A portion of each sample (1.3-1.5 mg) was then prepared for mass spectrometric analysis on an automated ANCA mass spectrophotometer to evaluate ^{13}C enrichment. Stable C isotope ratios were determined and correction factors applied according to Craig (1957), and the results are expressed as

Eq. 1.
$$\delta^{13}C_{PDB} = \left[\frac{R_{sample}}{R_{standard}} - 1 \right] x\ 10^3$$

where R is the $^{13}C/^{12}C$, and PDB = 0.0112372.

In addition to the $\delta^{13}C_{PDB}$ value, the absolute ratio (R), fractional abundance (F), and atom % were other indices used to determine stable isotope abundance from the ^{13}C enrichment. Absolute ratio, fractional abundance and atom % rearrange $\delta^{13}C_{PDB}$ in order to calculate the amount of ^{13}C in plant tissues (Boutton, 1991, and Svejcar, *et al.*,).

The absolute ratio of a sample is defined by rearrangement of Eq. 1 as:

Eq. 2.
$$R_{sample} = {}^{13}C/{}^{12}C = \left[\frac{\delta^{13}C}{1000} + 1 \right] x\ R_{PDB}$$

The fractional abundance is related to R by the equation:

Eq. 3. $$F = \frac{^{13}C}{^{13}C + ^{12}C} = \frac{R}{R + 1}$$

Atom % is used to express isotopic enrichment in samples highly enriched in ^{13}C:

Eq. 4. $$\text{Atom \%} = F \times 100$$

Statistical analysis was done using Agriculture Research Manager, version 6.18 (Gylling Data Management, Inc. Brookings, SD, USA).

RESULTS AND DISCUSSION

Atom % ($^{13}C/^{12}C$) differences in plant tissue sampling were significantly higher in the shoots versus the crown and roots one hour after $^{13}CO_2$ pulsing for all three species tested. At 168 hours after $^{13}CO_2$ pulsing, only Kentucky bluegrass had significant differences for ^{13}C partitioning (atom %) when comparing the reduced light versus full sun treatments (Table 1). Trends for the regression of ^{13}C partitioning in the shoots of all three species tested (atom % relative to the control) suggests that only Kentucky bluegrass has altered photosynthesis when subjected to reduced light conditions (Figure 4). These differences suggest that the level of reduced light conditions during testing, significantly impacted the photosynthetic process of Kentucky bluegrass, and not the other two species tested (tall fescue and tufted hairgrass).

Evidence suggests that Kentucky bluegrass under reduced light levels is using photosynthetic CO_2 more efficiently for structural synthesis, particularly in the shoots. These findings suggest that Kentucky bluegrass is not tolerant to the reduced light conditions of the indoor research facility; therefore, photosynthate CO_2 is being used for structural growth in the etiolated shoots.

Table 1. Analysis of variance for atom % ($^{13}C/^{12}C$) significance using $^{13}CO_2$ pulse chase technique on three cool-season turfgrasses grown in full sun and reduced light conditions. E. Lansing, MI. 48824. 14 August 2001.

		MSE (1×10^{-4})								
		Kentucky bluegrass			Tall fescue			Tufted hairgrass		
SOURCE	DF	1 hr	48 hrs	168 hrs	1 hr	48 hrs	168 hrs	1 hr	48 hrs	168 hrs
Total	17									
R	2	5	1	6	2	3	3	14	19	8
Tissue† (A)	2	1300*	4	20*	210*	4	5	426*	6	1
Location‡ (B)	1	193*	5	27*	1	9	3	22	2	14
AB	2	142*	1	10	0	5	2	1	0	3
ERROR	10	9	10	3	4	3	2	9	7	4

* Indicates significance at the 0.05 probability level.

† Reduced light conditions were from the indoor research facility with about 20% +/- 3% full sun transmittance.

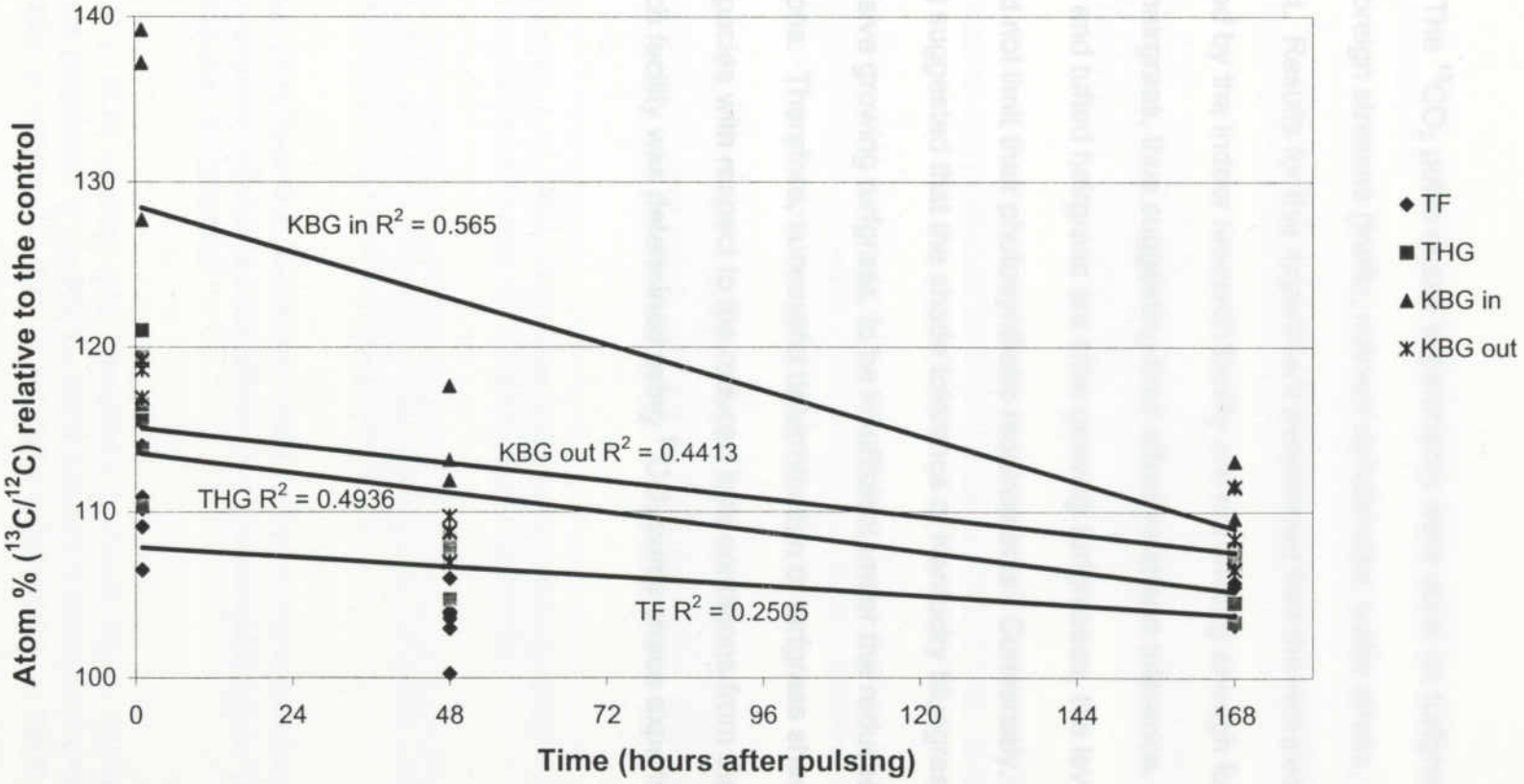

Figure 4. Regression of ^{13}C partitioning, for atom %; $^{13}C/^{12}C$ relative to the control, for the shoots of tall fescue (TF), tufted hairgrass (THG), and Kentucky bluegrass (KBG) after $^{13}CO_2$ pulsing. E. Lanisng, MI. 48824. 14 August 2001.

CONCLUSIONS

The $^{13}CO_2$ pulse chase experiments were done on turfgrasses where no other foreign stresses (traffic, nutrient deficiencies, water stress, etc.) where present. Results for this experiment determined that the reduced light levels provided by the indoor research facility are not limiting enough for tall fescue and tufted hairgrass, thus suggesting their effective shade tolerance. Because tall fescue and tufted hairgrass are slow growing turfgrasses, the level of reduced light did not limit their photosynthetic requirements. Conversely, the $^{13}CO_2$ pulsing suggested that the shade tolerance of Kentucky bluegrass, a more aggressive growing turfgrass, to be insufficient under the reduced light conditions. Therefore, successful determination of turfgrass shade tolerance for each species with respect to the reduced light conditions from the indoor research facility was determined using $^{13}CO_2$ pulse chase experiments.

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