INCREASING THE NITROGEN USE EFFICIENCY OF COOL-SEASON TURFGRASSES BY REGULATING NITRATE METABOLISM

Richard J. Hull
University of Rhode Island

Executive Summary

The purpose of this research is to understand the factors contributing to nitrogen use efficiency in turfgrasses and manipulate them in such a way that the need of turf for nitrogen fertilizers will be reduced. We have concentrated on the physiology of biomass partitioning between roots and shoots. A turfgrass that can allocate more of its photosynthetic product toward root growth will have a larger root system that will be better able to absorb nitrate and water from a larger soil volume. Such turfgrasses will be better able to tolerate drought conditions and derive a larger portion of their nitrogen requirements from that provided by the soil.

Nitrogen is available to turfgrass roots primarily in the form of nitrate (NO_3^-). Nitrate is produced in the soil when organic matter is metabolized by microbes, releasing its nitrogen as ammonium (NH_4^+) that in turn is oxidized by other microbes to NO_3^-. This NO_3^- is highly mobile and can leach with rainwater out of the soil and potentially contaminate ground water. The best protection of ground water quality is a dense grass root system that will absorb NO_3^- to supply the grass' need for nitrogen and sustain a root mass capable of continued NO_3^- uptake.

In this research project, we are examining the capacity of nine cultivars each of perennial ryegrass and creeping bentgrass to absorb NO_3^- and metabolize it within the grass plant in such a way that root growth is maintained and the turf remains vigorous and of high quality. We are testing the hypothesis that quality turf is most likely maintained when turfgrasses metabolize NO_3^- primarily in their roots with relatively little NO_3^- transported to and metabolized in the shoots. If NO_3^- is metabolized into amino acids (the building blocks of proteins) in the roots, there will be a stimulation of root growth with less nitrogen transported to the shoots to promote clipping growth. If NO_3^- is not metabolized in the roots but transported to the shoots, it will be metabolized into amino acids there and promote shoot growth at the expense of root production. This situation can be aggravated further under high temperature conditions that stimulate respiration more than photosynthesis making less carbon and energy available for transport to roots. It is now generally recognized that this reduced energy supply during hot weather is a major contributor to summer turf decline. This theory also explains why heavily fertilized turf is often more vulnerable to summer turf decline than less intensively managed turf.

Our research has shown that cultivars of perennial ryegrass and creeping bentgrass allocate more of their photosynthetic resources to shoot growth than to roots. These same grasses also metabolize most of the NO_3^- they absorb from the soil in their shoots which may explain this priority for shoot growth over root production. Generally, creeping bentgrass cultivars metabolize more NO_3^- in their roots than do perennial ryegrasses which may explain in part how bentgrasses can sustain themselves when maintained as a very closely mowed turf. In perennial ryegrass, we have also demonstrated a positive and significant relationship between NO_3^- metabolism in roots and the amount of roots produced. Our findings with these two turfgrasses has so far supported our proposed linkage between root centered NO_3^- metabolism and greater root growth with less shoot production. Research proposed for the remaining year of this project will concentrate on further testing our hypothesis and formulating turf management strategies that can use these findings to make present turfgrasses more efficient in their use of soil nitrogen. Preliminary studies designed to alter the genetics of turfgrasses so as to optimize root metabolism of NO_3^- will be initiated so that more nitrogen efficient turfgrasses will be available in the near future.

INCREASING THE NITROGEN USE EFFICIENCY OF COOL-SEASON TURFGRASSES BY REGULATING NITRATE METOBOLISM

A Report of Progress Prepared for the
United States Golf Association

November 1999

Principal Investigator: Richard J. Hull, Professor of Plant Physiology

Cooperating Investigators: Zhongchun Jiang, Postdoctoral Research Associate
Bridget Ruemmele, Assoc. Prof. Turfgrass Improvement

Graduate Student: John T. Bushoven, Graduate Research Assistant

Institution: University of Rhode Island
Plant Sciences Department
Kingston, RI 02881

Project Duration: 3 years (Feb. 1998 - Jan. 2001)

Project Objectives

1. To quantify each step in nitrate metabolism for selected cultivars of creeping bentgrass (*Agrostis palustris* Huds.) and perennial ryegrass (*Lolium perenne* L.).

2. To determine which of these metabolic steps correlates best with nitrogen use efficiency under field conditions.

3. To assess the potential for increasing nitrogen use efficiency by optimizing the activity and location of those metabolic steps which are limiting.

Narrative of Progress

Status of the Problem:

Based on preliminary studies on Kentucky bluegrass conducted by Zhongchun Jiang, the metabolic steps in nitrate acquisition and metabolism which appear to be limiting for efficient nitrogen utilization by turfgrasses are: nitrate absorption by roots, nitrate reduction in roots and carbohydrate translocation from leaves to roots. Of these, nitrate reduction in roots and the energy supply (carbohydrates) provided to the roots from shoot photosynthesis are the most limiting. While significant variation in nitrate absorption kinetics has been observed among genotypes of several cool-season turfgrasses, most cultivars are capable of rapid nitrate uptake and this initial step in nitrate utilization rarely

appears to be limiting. This has also been observed by other investigators notably Dan Bowman and Jack Paul in studies conducted at Davis, California. The supply of nitrate via root absorption is more likely limited by root mass and surface area during the growing season than by the physiological capability of roots to absorb nitrate. Our work on Kentucky bluegrass has also indicated that nitrite reduction and ammonium assimilation appear to be more than adequate to match the rate of nitrate delivery within root cells or in leaves. Consequently activity of the enzymes nitrite reductase and glutamine synthetase do not appear to be limiting steps in nitrate utilization. For these reasons, our investigation has concentrated on the partitioning of nitrate reductase activity (NRA) between roots and shoots and the control exerted by carbohydrate supply on nitrate reduction in roots. This latter factor has long been recognized to be a crucial limitation to nitrate reduction in roots of many plants so it should not be surprising that carbohydrate supply may also limit NRA in roots of turfgrasses.

The general strategy of this investigation has been to quantify the partitioning of NRA between roots and shoots of several creeping bentgrass and perennial ryegrass cultivars differing in their field performance in NTEP trials. We also compared cultivars of diverse genetic backgrounds. Photosynthate partitioning to roots is being compared among cultivars to establish how well this factor correlates with root NRA. By altering turf management practices (mowing height, nitrogen fertility, etc.) so as to increase photosynthate partitioning to roots and determining what effect such practices have on root NRA, we hope to establish which is the controlling factor over NRA in roots: the properties of the nitrate reductase enzyme or the energy supply available to it. This must be understood before a systematic effort can be made to improve the nitrogen use efficiency of turfgrasses.

Methodology:

The technique employed to assay NRA is an *in vivo* method originally developed by Hageman in the late 1970s. The method measures the product of nitrate reductase (nitrite) in living tissues by imposing conditions which block its subsequent reduction to ammonium. This method has been optimized for leaf and root tissues of Kentucky bluegrass by Zhungchun Jiang and adapted to creeping bentgrass and perennial ryegrass by John Bushoven. In general, fresh tissues are incubated in darkness under anaerobic conditions at 30°C in a medium containing 0.1 M phosphate buffer (pH 7.5), 50 mM KNO_3 and 3% 2-propanol. The NRA is calculated based on the amount of nitrite released by the tissue into the incubation medium and expressed as μmoles of NO_2^- produced per gram of fresh tissue per hour. This method measures the capacity of the tissue to reduce nitrate (50 mM being saturating) under the prevailing activity of the enzyme and the availability of reducing equivalents (NADH). Because nitrite is reduced to ammonium in plastids, this is inhibited in leaf tissues by dark incubation and in roots by low oxygen supply which blocks mitochondrial oxidative respiration. More recent research has shown that reducing equivalents required for nitrite reduction to ammonium in non-chlorophyllous tissues is provided by the oxidative pentose phosphate pathway. Since this pathway does not require oxygen, it would not be inhibited by low oxygen

tensions and nitrate could be reduced to ammonium and assimilated. For this reason, 2.5 mM methionine sulfoximine, an inhibitor of glutamine synthetase, was included in the incubation medium used for root assays and NRA was based on both nitrite and ammonium accumulation. Cytosolic glycolysis apparently provides sufficient reducing equivalents to reduce nitrate to nitrite since the nitrate reductase enzyme is confined to the cytosol and only one NADH is required for each nitrate reduced. The 2-propanol increases plasma membrane permeability of tissue cells to both nitrate (influx) and nitrite (efflux) without otherwise altering cellular metabolism or integrity.

We now know more about the transcriptional and post-translational controls over nitrate reductase than was the case when this *in vivo* method was first described. Thus, the proper gene induction by nitrate and covalent regulation of enzyme activity by phosphorylation must also be taken into account in using this method. Preculturing plants in moderate nitrate levels (>50 μM) for at least 12 hours prior to assaying for NRA should provide for full gene induction. The dark anaerobic incubation conditions should also lower the availability of cytosolic ATP to preclude enzyme phosphorylation and inactivation. Some factors which may affect this method remain to be resolved fully. These include the impact of lowered carbohydrate supply in roots due to excision from the shoots and the normal oxygen tensions present in the root zone under field conditions. These factors are currently being explored and if necessary our method will be adjusted accordingly.

Seeds of perennial ryegrass and creeping bentgrass were germinated on washed silica sand. After twenty days, seedlings were removed from the sand, the roots washed free of adhering sand and the seedlings in groups of 30 transplanted into culture troughs (63' x 5" x 3.5") containing 20 L of one-quarter-strength aerated, modified, N-free Hoagland's solution supplemented with 1.0 mM $NaNO_3$. Solutions were replaced at weekly intervals. The culture troughs were maintained in a walk-in growth chamber under controlled temperature and a photosynthetic photon flux density of 800 $\mu mol/m^2/sec$ provided by two sodium halide lamps that were set for a 16-hour photoperiod. Day and night temperatures are accurately maintained at 75° and 65° F, respectively and relative humidity, while not controlled, remained at about 50%.

Turfgrass cultivars were selected in consultation with Bridget Ruemmele to include genotypes of diverse background and differing performance in the national NTEP trials (Tables 1 & 2). Seed of perennial ryegrass and creeping bentgrass cultivars was generously provided by various seed companies (Tables 1 & 2).

Statistical analyses were performed using the GLM, Duncan's Multiple Range Test and regression analysis procedures within the Statistical Analysis System (SAS Institute, Cary, NC).

Photosynthate partitioning within turf cultures will be determined by measuring mass distribution (root:shoot ratios) and ^{14}C-photosynthate partitioning

following exposure of leaves to $^{14}CO_2$. This latter work has not yet begun and the procedure will be fully described when we have results to report.

Results:

At the time NRA assays were conducted, the total biomass of grass cultures was roughly comparable between the two grass species (Table 3). However, perennial ryegrass consistently partitioned less biomass to roots than did creeping bentgrass. The total root mass recovered from the most highly root-endowed perennial ryegrass (Palmer III) was about equal to that of the least root endowed creeping bentgrass (PennLinks).

Cultivars of both perennial ryegrass and creeping bentgrass differed in their growth rates and mass partitioning between roots and shoots (Table 3). Among the nine perennial ryegrass cultivars assayed, Morning Star and Palmer III partitioned a greater proportion of their total biomass to roots than did Calypso II and Nighthawk. Among creeping bentgrass cultivars, 18th Green exhibited the highest root:shoot mass ratio although this did not differ significantly from six other cultivars. Penn G-2 and Seaside partitioned less of their total biomass to roots although again they differed significantly only from 18th Green.

Comparative assays of leaf and root NRA demonstrated significant differences among leaves of both perennial ryegrass and creeping bentgrass cultivars (Fig. 1 & 2). By comparison, only creeping bentgrass exhibited significant cultivar differences in root NRA (Fig. 2). The leaf specific NRA was much greater in perennial ryegrass than in creeping bentgrass while root specific activities among the two species were comparable. Greater variation in leaf NRA among cultivars was noted in perennial ryegrass than in creeping bentgrass. Leaves of Secretariat averaged a NRA that was more than 3x that of Linn, the cultivars exhibiting the highest and lowest leaf specific NRA, respectively. By comparison, the most active creeping bentgrass cultivar, SR-1020, averaged a leaf NRA that was not even twice that of PennLinks, the cultivar exhibiting the lowest NRA.

This species difference in NRA partitioning between leaves and roots was reflected in root:shoot NRA ratios (Figs. 3 & 4). In creeping bentgrass, the total plant NRA attributed to roots was about 2x that of perennial ryegrass. Surprisingly, cultivar differences in root:shoot NRA ratios among perennial ryegrass cultivars were significant while those among creeping bentgrass cultivars were not. The perennial ryegrass, Palmer III, expressed 3x more of its total plant NRA in its roots than did Manhattan III.

To test one of our principal theories that greater root NRA should translate into a larger root biomass, we conducted linear regression analyses between total plant root:shoot NRA ratios and root biomass for the two species (Figs. 5 & 6). The relationship between NRA distribution between roots and shoots and total root mass proved to be positive and significant for perennial ryegrass but not significant

for creeping bentgrass. The significant relationship in perennial ryegrass likely reflects the greater range in root:shoot NRA ratios exhibited by its cultivars than the much smaller range observed among cultivars of creeping bentgrass.

Discussion:

Creeping bentgrass partitioned more of its photosynthetic resources toward root production than did perennial ryegrass (Table 3). Because a larger root system better equips turfgrasses for water and nutrient absorption, it should also confer greater resistance to stress conditions. This idea appears to be supported by the cultivar L-93 that exhibited the largest root mass of the nine cultivars compared, received the highest quality scores and is among the more heat resistant bentgrasses.

A high root:shoot mass ratio does not appear in itself always to contribute toward superior turf performance. Greater depth and extent of rooting is critical and that depends upon an adequate leaf surface to supply the energy needed to support root growth. This is true for Palmer III perennial ryegrass that was the highest rated cultivar in our comparison (Table 1) and also exhibited one of the largest root:shoot mass ratios along with the greatest root mass (Table 3). Morning Star had an equally high root:shoot mass ratio but was apparently photosynthetically less efficient being among the lowest producers of total biomass. But what about Secretariat that produced the lowest root and total biomass, had an intermediate root:shoot mass ratio but produced a turf that was among the highest ranked in the NTEP quality evaluations? Obviously photosynthetic efficiency and mass partitioning to roots are not the only physiological factors that contribute to turf quality.

Leaves of perennial ryegrass exhibited greater NRA per unit of tissue mass than did creeping bentgrass while the roots of both species recorded similar specific activities. However, because creeping bentgrasses generally had a larger root:shoot mass ratio (Table 3), it exhibited a greater root:shoot ratio in NRA distribution (Figs. 3 & 4). For these reasons Creeping bentgrass likely reduced and metabolized more nitrate in its roots and that translated into a slightly lower shoot N content (data not shown). These data do not permit us to establish a cause and effect relationship between mass partitioning to roots and nitrate metabolism in roots. However, because of the more significant differences in root:shoot NRA distribution recorded for perennial ryegrass (Fig. 3), a significant positive relationship between root mass and root:shoot NRA distribution was established (Fig. 5). Since creeping bentgrass cultivars failed to exhibit significant differences in root:shoot ratios for NRA, no significant relationship between root mass and root:shoot NRA distribution was observed (Fig. 6).

Our findings are consistent with the basic hypothesis that nitrate metabolism concentrated in the roots correlates with greater mass allocation to roots and a more stress resistant turfgrass. The limited number of genotypes included in this study may weaken the strength of our conclusions but so far, the hypothesis appears to be valid. There are of course factors that contribute to turf quality, e.g. large leaf angle and prostrate growth , that were not considered in this study and may account for

some cases where turf quality is high but not related to partitioning of either mass or NRA. Experiments scheduled for the year 2000 will involve more field observations and short term carbon partitioning studies that will provide additional evidence relevant to the objectives of this project.

Research Scheduled for 2000

Nitrate absorption rates of perennial ryegrass and creeping bentgrass cultivars will be compared to determine if the partitioning of NRA between roots and shoots has any impact on nitrate acquisition. The experimental part of this goal has been completed but sample and data analyses have not been completed.

Samples of roots and shoots from perennial ryegrass and creeping bentgrass are being collected from field plots (NTEP) of the same cultivars compared in the experiments described above. This will help establish if measurements made under controlled conditions have any relationship to what is observed in the field.

Leaves of all cultivars of both species compared in this study will be exposed to Carbon-14 (^{14}C) labeled CO_2 to determine current photosynthate partitioning between roots and shoots. Such measurements will enable us to determine if the partitioning of NRA between roots and shoots is influenced by the plant's ability to allocate photosynthetic resources to its roots. This will address one of the major questions in this research namely: is root:shoot partitioning of NRA controlled by genetic partitioning of the NR enzyme or by the distribution of photosynthetic product within the plant.

Because some of the questions raised by this research will be answered most directly by creating mutants that are genetically altered in their capability to metabolize and partition nitrate, initial efforts to develop a protocol for transforming creeping bentgrass has been initiated. This capability to transform specifically turfgrass genotypes will provide the tools for a logical extension of the current research.

Publications Derived from this Research

Jiang, Z and R. J. Hull. 1998. Interrelationships of nitrate uptake, nitrate reductase and nitrogen use efficiency in selected Kentucky bluegrass cultivars. Crop Science 38:1623-1632.

Jiang, Z. and R.J. Hull. 1999. Partitioning of nitrate assimilation between shoots and roots of Kentucky bluegrass. Crop Science 39:746-754.

Jiang, Z.; J.T. Bushoven and R.J. Hull. 1999. Photosynthate partitioning in perennial grasses can influence the site of nitrate metabolism. Plant Biology '99,

Abstracts of Annual Meeting, Am. Soc. Plant Physiol., July 24-28, Baltimore, MD.

Sullivan, W.M.; Z. Jiang; R.J. Hull and C.D. Sawyer. 1999. Nitrate uptake and root morphology of Kentucky bluegrass. Am. Soc. Hort. Sci. Annual Meeting, July 28-31, Minneapolis, MN. HortSci. 34:492-493.

Jiang, Z.; W.M. Sullivan; C.D. Sawyer and R.J. Hull. 1999. Genetic and temporal variation in nitrate uptake by Kentucky bluegrass. Am. Soc. Hort. Sci. Annual Meeting, July 28-31, Minneapolis, MN. HortSci. 34:493.

Jiang, Z. and R.J. Hull. 1999. *In vivo* assay of nitrate reductase activities in Kentucky bluegrass. Agron. Abstracts 91:128.

Bushoven, J.T.; Z. Jiang and R.J. Hull. 1999. Variation in nitrate reductase activity among cultivars of creeping bentgrass and perennial ryegrass. Agron. Abstracts 91:139.

Table 1. Perennial ryegrass cultivars selected for nitrate metabolism comparisons, the seed source and Rhode Island quality score in the 1995-98 NTEP trials.

Cultivar	Seed source	NTEP score
Calypso II	Roberts Seed Co.	5.3*
Figaro	DLF-Trifolium Inc.	3.6
Linn	Pennington Seed Co.	1.4
Manhattan III	Turf Merchants	4.8
Morning Star	Pennington Seed Co.	4.9
Nighthawk	Allens Seed Store Inc.	4.4
Palmer III	Lofts Seeds Inc.	6.6
Saturn II	Zajac Performance Seeds	5.2
Secretariat	Grassland West	6.1
LSD (P<0.05)		0.1

* Turf quality scores: 9 = excellent, 1 = dead grass

Table 2. Creeping bentgrass cultivars selected for nitrate metabolism comparisons, the seed source and Rhode Island quality score in the 1994-97 NTEP trials.

Cultivar	Seed source	NTEP score
18th Green	Zajac Performance Seeds	3.9*
L-93	Lofts Great Western Seed Co.	6.6
PennCross	Allens Seed Store Inc.	4.0
Penn G-2	Lesco Inc.	6.1
PennLinks	Allens Seed Store Inc.	5.2
Providence	Seed Research of Oregon	5.7
Seaside	Allens Seed Store Inc.	3.4
Southshore	Lofts Great Western Seed Co.	5.6
SR-1020	Seed Research of Oregon	4.9
LSD (P<0.05)		0.2

* Turf quality scores: 9 = excellent, 1 = dead grass

Table 3. Mass distribution of perennial ryegrass and creeping bentgrass cultivars utilized for comparative NRA measurements.

Grass - Cultivar	Roots	Shoots	Root/shoot
	grams		
Perennial ryegrass			
Calypso II	0.65 cd*	6.68 ab	0.102 b
Figaro	0.79 bcd	4.67 bc	0.186 ab
Linn	1.16 ab	7.61 a	0.167 ab
Manhattan III	1.00 abc	7.78 a	0.130 ab
Morning Star	0.78 bcd	4.33 bc	0.225 a
Nighthawk	0.72 cd	6.88 ab	0.108 b
Palmer III	1.32 a	6.60 ab	0.219 a
Saturn II	0.92 bc	6.37 ab	0.155 ab
Secretariat	0.50 d	3.74 c	0.161 ab
Creeping bentgrass			
18th Green	1.63 ab	3.33 b	0.494 a
L-93	2.54 a	6.23 a	0.419 ab
PennCross	1.63 ab	4.65 ab	0.355 ab
Penn G-2	1.84 ab	6.62 a	0.282 b
PennLinks	1.31 b	3.60 b	0.383 ab
Providence	2.18 ab	6.27 a	0.361 ab
Seaside	2.20 ab	5.99 a	0.332 b
Southshore	2.40 a	6.41 a	0.382 ab
SR-1020	2.28 ab	6.12 a	0.371 ab

* Means in a column for each species followed by the same letter are not significantly different ($P<0.05$); Duncan's Multiple Range Test.

Figure 1. Comparison of leaf and root NRA in perennial ryegrass. (Means with same letter are not significantly different $P<=0.05$)

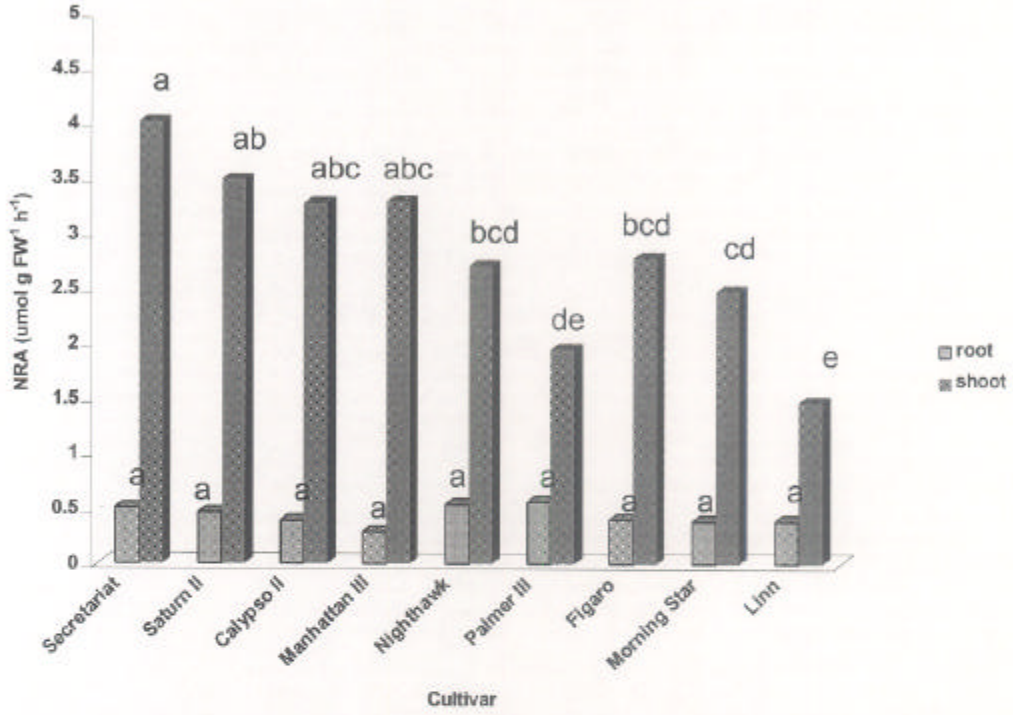

Figure 2. Comparison of leaf and root NRA in creeping bentgrass. (Means with same letter are not significantly different $P<=0.05$)

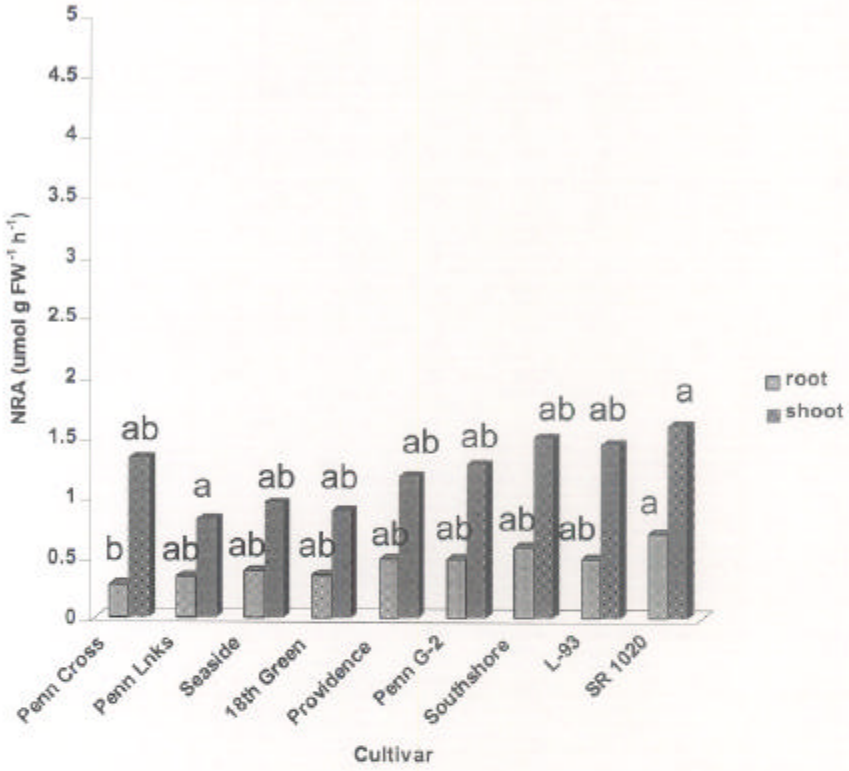

Figure 3. Root:shoot NRA ratios in perennial ryegrass. (Means with same letter are not significantly different $P<=0.05$)

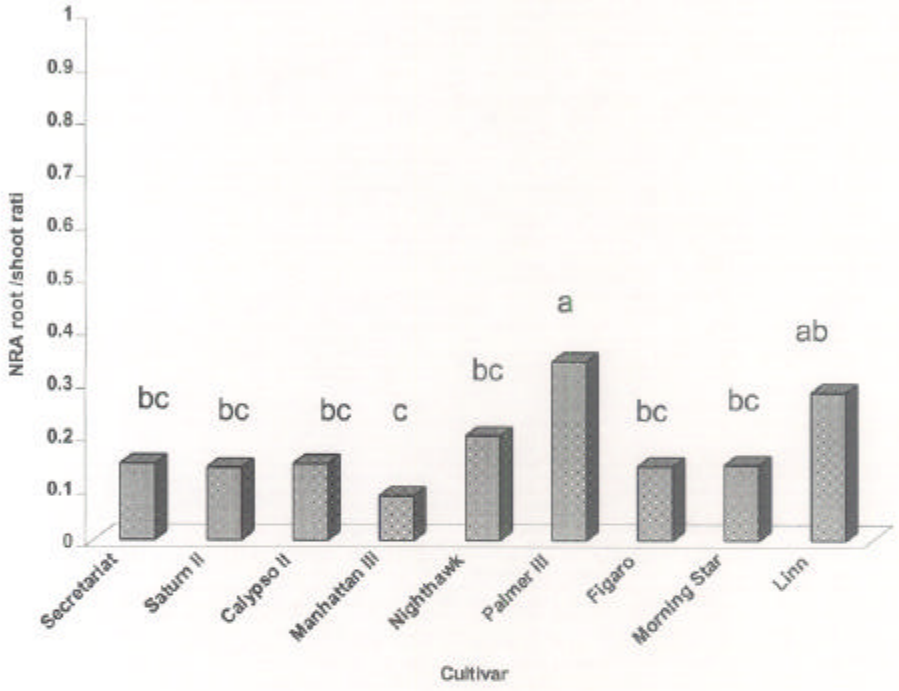

Figure 4. Root:shoot NRA ratios in creeping bentgrass. (No significant differences $P<=0.05$)

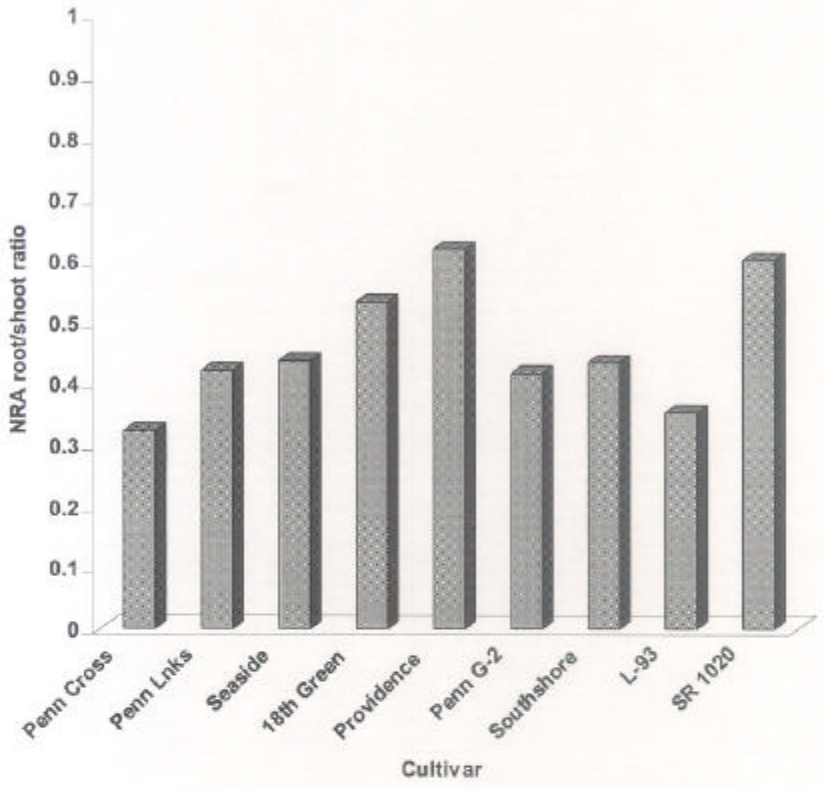

Figure 5. Relationship between total plant root:shoot NRA ratios and root biomass in perennial ryegrass. (Significant at $P = 0.05$)

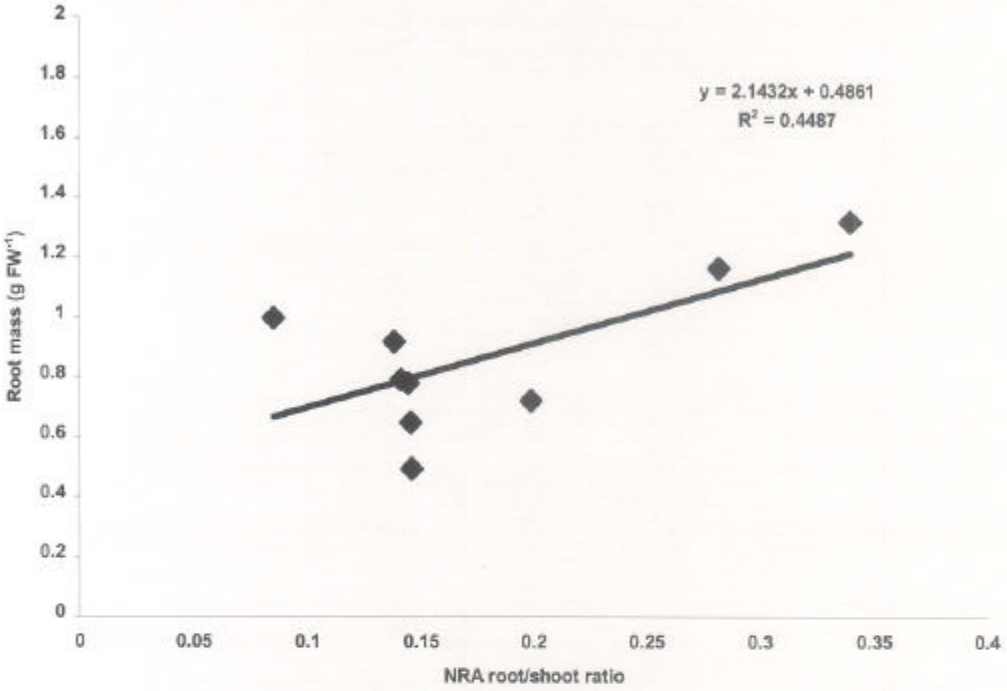

Figure 6. Relationship between total plant root:shoot NRA ratios and root biomass in creeping bentgrass. (Not significant at $P <= 0.05$)