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

Richard J. Hull
University of Rhode Island

Executive Summary - 2000

Among the objectives of the USGA's research grants program is the development of turfgrasses and a strategy for managing them that will significantly reduce the amount of fertilizer required to maintain high quality turf under golf course conditions. The achievement of this objective will lower the cost of turf management and further reduce the already minimal contributions golf courses make to ground and surface water contamination. This project has concentrated on increasing our understanding of the metabolic basis for efficient nitrogen use by the perennial cool-season turfgrasses creeping bentgrass and perennial ryegrass. We have attempted to understand the factors contributing to nitrogen use efficiency in turfgrasses and manipulate these factors 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 resources toward root growth will have a larger root system that will be better able to absorb nutrients 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 organic matter cycling within 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^-. Thus, regardless of the nitrogen source applied, that nitrogen will be available to the turf as nitrate. 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 root system that will absorb NO_3^- to supply the needs of the grass for nitrogen and sustain those roots throughout the year for continued NO_3^- uptake.

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 order to test our hypothesis that quality turf is most likely to occur when turfgrasses metabolize NO_3^- primarily in their roots with relatively little NO_3^- transported to and metabolized in the shoots. Our research has shown that cultivars of perennial ryegrass and creeping bentgrass allocate most of their photosynthetic resources to shoot growth and little to roots. These same grasses also metabolize most of the NO_3^- they absorb from the soil in their shoots which may explain their priority of shoot growth over root production. Generally, creeping bentgrass metabolizes more NO_3^- in its roots and also partitions a greater portion of its total biomass to root growth than does perennial ryegrasses. We have also observed that perennial ryegrass absorbs nitrate more rapidly from the soil than does creeping bentgrass but much of this nitrate is transported to and metabolized in leaves. This alone could explain why perennial ryegrass produces more shoot growth and less root growth. In perennial ryegrass, we have also observed a positive and significant relationship between NO_3^- metabolism in roots and the amount of roots produced. This may explain how bentgrasses, that absorb nitrate more slowly and metabolize more of it in the roots thereby promoting greater root growth, can sustain themselves when maintained as a very closely mowed turf. These findings have supported our theory linking root-centered NO_3^- metabolism and greater root growth with less shoot production. Research proposed for the future 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.

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

A Report of Progress Prepared for the
United States Golf Association

November 2000

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:

Research already reported from this project has established that genetic differences in nitrate reductase activity (NRA) can be demonstrated in both roots and leaves of turf-type creeping bentgrass and perennial ryegrass cultivars. It was also noted that in both grass species, NRA was greater in leaf tissues than it was in roots. Finally, leaves of perennial ryegrass exhibited greater NRA than did leaves of creeping bentgrass. Roots of both species expressed roughly equivalent levels of NRA although specific enzyme activity was generally greater in roots of creeping bentgrass than it was in perennial ryegrass. Although genetic differences in the capacity to reduce and assimilate nitrate-nitrogen were evident within both species,

the differences were modest and did not offer great hope for finding a genotype that was markedly superior in utilizing soil nitrate. While only nine cultivars of each species were evaluated, they did represent a broad range of performance levels in the standardized National Turfgrass Evaluation Trials (Table 1). Thus, it appears that commercially available cultivars of creeping bentgrass and perennial ryegrass constitute genotypes possessing adequate capabilities for nitrate uptake from soil solutions, nitrate reduction especially in shoot tissues and ammonium assimilation leading to the synthesis of amino acids and ultimately proteins. Consequently, selecting or breeding turfgrasses for greater capacities to absorb and metabolize nitrate would appear to offer limited promise for markedly improving the nitrogen use efficiency of these grasses.

However, nitrogen utilization is a whole plant process and in perennial grasses it is especially integrated with growth rates and the partitioning of both mass and nitrogen between roots and shoots. This transcends the capacities of specific tissues and forces us to confront the highly coordinated character of nitrogen acquisition and utilization in perennial grasses. Our research has begun to focus on these aspects of nitrate utilization by turfgrasses and our initial investigations into these areas will be the subject of this report.

The general strategy of these investigations has been to quantify the partitioning of biomass and NRA between roots and shoots of creeping bentgrass and perennial ryegrass cultivars differing in their field performance in NTEP trials. Photosynthate partitioning to roots was compared among cultivars to establish how well this factor correlates with root NRA. Because nitrate reduction and assimilation are energy demanding processes, the site of nitrate metabolism in a plant could well be a function of where its energy is concentrated. The immediate products of nitrate metabolism (nitrite and ammonium) are toxic and plants have evolved metabolic control mechanisms that inhibit initiation of this metabolic sequence (NRA) unless sufficient energy and carbon metabolites are available to carry the process to completion. Based on recent discoveries of biochemical control sites for nitrogen assimilation, we have formulated a hypothesis that turfgrasses will be more efficient in their use of nitrate and photosynthetic products if NRA is concentrated within roots rather than in the shoots. Cool-season turfgrasses, currently in use, partition most of their nitrate metabolism within their shoots. 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 and thereby reduce fertilizer requirements and minimize negative environmental impacts.

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 Zhongchun Jiang and further 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 NO_3^- 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 a 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 (MSX), 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. Recent evidence has indicated that MSX can also partially inhibit nitrate reductase so duration of exposure to the inhibitor was minimized. 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 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 and 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 will have to be standardized before critical physiological experiments can be conducted.

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 0.5-1.0 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 were accurately maintained at 75° and 65° F, respectively and relative humidity, while not controlled, remained at about 50%.

The same cultivars of creeping bentgrass and perennial ryegrass that had been selected in consultation with Bridget Ruemmele to include genotypes of diverse background and differing performance in the national NTEP trials (Table 1) were utilized in these studies.

Photosynthate partitioning within turf cultures was determined by measuring the mass of roots and shoots and biomass distribution within plants was expressed as root:shoot mass ratios. When total tissue mass of roots or shoots is multiplied by their tissue specific NRA, the total potential nitrate reduction rate for each tissue can be calculated. From such calculations, NRA partitioning between roots and shoots was determined.

Nitrate uptake rates by roots were determined using a solution depletion technique that we had developed for use with turfgrasses. Plant solution cultures (30 plants) obtained from 60-120 day-old sand cultures were acclimated to aerated nutrient solution containing 1.0 mM nitrate as described above. Prior to measuring nitrate uptake, solution cultures were maintained with no nitrate for 24 h after which time, they were transferred to a complete nutrient solution containing 1.0 mM nitrate. Nitrate depletion of the solution was determined after 12 h and nitrate uptake rate calculated based on the amount of nitrate absorbed divided by the root mass of the grass culture.

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).

Results and Discussion:

At the time NRA assays were conducted, the total biomass of grass cultures was roughly comparable between the two grass species (Table 1). However, perennial ryegrass consistently partitioned less biomass to its roots than did creeping bentgrass. The total root biomass recovered from Palmer III perennial ryegrass was about equal to that of Pennlinks creeping bentgrass: the greatest and poorest root producer from each species, respectively.

Comparative assays of leaf NRA demonstrated significant differences among cultivars of both perennial ryegrass and creeping bentgrass (Table 2). By comparison, only creeping bentgrass exhibited significant cultivar differences in root NRA (Table

2). Leaf specific NRA in perennial ryegrass was more than twice that of creeping bentgrass while root specific NRA among the two species was comparable. This similarity in enzyme activity both between and within species suggests that root NRA was at or near saturation under the experimental conditions used.

The similarity in tissue specific NRA between the two turfgrass species resulted in greater total NRA partitioned to roots in creeping bentgrass because its root mass was more than double that of perennial ryegrass. When the total potential nitrate reduction in roots and leaves was computed, the ratio of root to shoot NRA in creeping bentgrass was almost six times that of perennial ryegrass (Table 2). This resulted in an average root contribution to total plant nitrate assimilation of 13.5% for creeping bentgrass but only 3.9% for perennial ryegrass. Significant differences in the partitioning of NRA between roots and shoots were noted among perennial ryegrass cultivars but not among those of creeping bentgrass. This was probably due to the greater range in root biomass among ryegrass cultivars (~3X) while the root mass of bentgrasses cultivars was greater but differed by less than 2X. With root specific NRA being almost equal between the two species, the greater mass differences among ryegrass cultivars resulted in significant differences in root contributions to total plant NRA. Thus, while bentgrass roots were the site of more nitrate assimilation than were roots of ryegrass, cultivars of perennial ryegrass exhibited greater variation in their root contribution to nitrate assimilation than did cultivars of creeping bentgrass. This variation might be exploited by turfgrass breeders to select for greater nutrient use efficiency by perennial ryegrasses.

Nitrate absorption by perennial ryegrass roots was about 2.5 times greater than that of creeping bentgrass (Table 3). Since root specific NRA of both species was similar and appeared to be saturated at the nitrate levels provided (1.0 mM), the greater nitrate absorbed by perennial ryegrass roots was probably translocated to the shoots where it was stored or reduced and assimilated. The somewhat greater nitrogen content of perennial ryegrass shoots reflects the increased delivery of nitrate from the roots (Table 3). The greater supply of nitrate to shoots also promoted slightly greater shoot growth and diverted photosynthate away from root growth (Table 1). Increased amounts of nitrate delivered to leaves of perennial ryegrass induced greater leaf specific NRA in ryegrass shoots (Table 2). Conversely, creeping bentgrass absorbed nitrate at a lesser rate permitting more of the nitrate to be reduced and assimilated within the roots. While the root specific NRA was similar for both species the greater root biomass of creeping bentgrass resulted in substantially more nitrate being metabolized within its root system: almost 10% more than was reduced in roots of perennial ryegrass (Table 2).

These results support our basic theory that nitrate metabolism concentrated within the roots will promote greater root growth with less shoot growth. To test the idea that greater root NRA should translate into greater root biomass, we conducted linear regression analyses between total plant root:shoot NRA ratios and

root biomass for the two species (Fig. 1). This relationship between the distribution of total NRA 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 biomass and root:shoot NRA ratios exhibited by its cultivars than the much smaller range in values observed among cultivars of creeping bentgrass.

Creeping bentgrass partitioned more of its photosynthetic resources toward root production than did perennial ryegrass (Table 1). 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 also among the more heat resistant bentgrasses (Table 1).

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 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. 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 shoot biomass, had an intermediate root:shoot mass ratio, but produced a turf that was among the highest ranked in the NTEP quality evaluations (Table 1)? Secretariat also exhibited the greatest nitrate uptake rate (Table 2) and had the highest nitrogen content in its leaves (Table 3) which translated into a low nitrogen use efficiency of 30.8 g dry weight per mg tissue nitrogen. Obviously photosynthetic efficiency and mass partitioning to roots are not the only physiological factors that contribute to turf quality.

Our findings do not reject the 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. What has not been established is the cause and effect of this relationship between root centered nitrogen metabolism and greater root:shoot ratio. We do not know if greater root NRA is a result of increased enzyme levels (greater gene expression or less enzyme inactivation) in the roots or greater energy transport to roots from the shoots. Either one of these conditions could cause increased NRA in roots but which is cause and which effect? Our future research will resolve this question

Research Scheduled for 2001

Selected cultivars of creeping bentgrass and perennial ryegrass will be subjected to conditions that will enhance photosynthate partitioning to the roots. Such experimentally imposed conditions as elevated atmospheric CO_2 or reduced O_2 levels will increase photosynthetic efficiency and make more energy available for export to the roots. Greater mowing heights and increased light levels should have similar effects. If such conditions increase root NRA, the idea that nitrate metabolism in roots is energy limited will be supported. On the other hand, concentrating NRA in the roots by supplying low but steady levels of solution nitrate so as not to exceed the capacity of roots to reduce and assimilate nitrate may promote a high root:shoot mass ratio. This would support the idea that nitrogen metabolism in roots is enzyme limited.

Leaves of grasses compared in this study will be exposed to Carbon-14 (^{14}C) labeled CO_2 during imposition of experimental variables to determine current photosynthate partitioning between roots and shoots. Such measurements will enable us to determine more directly 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 be a valuable tool for monitoring the immediate impact of experimentally imposed conditions on the photosynthate partitioning within turfgrasses.

Because some of the questions raised by this research can 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 produce specifically transform turfgrass genotypes will provide the tools for a logical future extension of the current research.

Publications Related to 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.

Jiang, Z. and R.J. Hull. 2000. Diurnal patterns of nitrate assimilation in Kentucky bluegrass. J. Plant Nutrition 23:443-456.

Sullivan, W.M.; Z. Jiang and R.J. Hull. 2000. Root morphology and its relationship with nitrate uptake in Kentucky bluegrass. Crop Science 40:765-772.

Sullivan, W.M.; Z. Jiang and R.J. Hull. 2000. Nitrate assimilation and carbon-14 partitioning between shoots and roots of tall fescue. Abstract No. 137, 97th Inter. Conf. ASHS, Lake Buena Vista, Florida. HortScience 35:413.

Jiang, Z.; J.T. Bushoven; R.J. Hull and W.M. Sullivan. 2000. Use of methionine sulfoximine for *in vivo* nitrate and nitrite reductase activity assays. Abstract No. 603, Plant Biology 2000 Program, Am. Soc. Plant Physiol. page 128.

Bushoven, J.T.; Z. Jiang and R.J. Hull. 2001. Variation in the uptake and metabolism of nitrate in perennial ryegrass and creeping bentgrass. Crop Sci. (submitted).

Bushoven, J.T. and R.J. Hull. 2001. Nitrogen use efficiency is linked to nitrate reductase activity and biomass partitioning between roots and shoots of perennial ryegrass and creeping bentgrass. Int. Turfgrass Soc. Jour. (submitted).

Table 1. Turf quality scores and mass distribution of perennial ryegrass and creeping bentgrass cultivars utilized for comparative nitrogen use measurements.

Grass - Cultivar	NTEP Score		Roots	Shoots	Root/shoot
			grams		
Perennial ryegrass					
Calypso II	6.3*		0.65 cd**	6.68 ab	0.102 b
Figaro	4.3		0.79 bcd	4.67 bc	0.186 ab
Linn	2.9		1.16 ab	7.61 a	0.167 ab
Manhattan III	5.5		1.00 abc	7.78 a	0.130 ab
Morning Star	4.9		0.78 bcd	4.33 bc	0.225 a
Nighthawk	4.5		0.72 cd	6.88 ab	0.108 b
Palmer III	6.5		1.32 a	6.60 ab	0.219 a
Saturn II	5.8		0.92 bc	6.37 ab	0.155 ab
Secretariat	6.3		0.50 d	3.74 c	0.161 ab
		Mean	0.87	6.07	0.14
Creeping bentgrass					
18th Green	3.9		1.63 ab	3.33 b	0.494 a
L-93	6.6		2.54 a	6.23 a	0.419 ab
PennCross	4.0		1.63 ab	4.65 ab	0.355 ab
Penn G-2	6.3		1.84 ab	6.62 a	0.282 b
PennLinks	5.2		1.31 b	3.60 b	0.383 ab
Providence	5.7		2.18 ab	6.27 a	0.361 ab
Seaside	3.4		2.20 ab	5.99 a	0.332 b
Southshore	5.6		2.40 a	6.41 a	0.382 ab
SR-1020	4.9		2.28 ab	6.12 a	0.371 ab
		Mean	2.00	5.46	0.37

* USDA, ARS, NTEP Final Report 1998 No. 99-10 & Final Report 1994-97 No. 98-11, for Kingston, RI. (1-9; 9 = Ideal turf).

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

Table 2. Nitrate reductase activity (NRA) and partitioning between roots and shoots in nine cultivars each of perennial ryegrass and creeping bentgrass.

Grass - Cultivar	NRA		Potential total NRA root:shoot ratio	Percent root contribution to potential total NRA
	Root	Shoot		
	μmol NO_2^-/g FW/h			%
Perennial ryegrass				
Calypso II	0.39 a*	3.28 abc	0.01 b	1.34 b
Figaro	0.39 a	2.79 bcd	0.05 ab	3.96 ab
Linn	0.38 a	1.47 e	0.07 a	5.92 a
Manhattan III	0.28 a	2.48 cd	0.02 b	1.52 b
Morning Star	0.38 a	3.29 abc	0.04 ab	3.32 ab
Nighthawk	0.53 a	2.70 bcd	0.02 ab	1.96 ab
Palmer III	0.56 a	1.95 e	0.06 ab	5.27 ab
Saturn II	0.46 a	3.49 ab	0.03 ab	2.36 ab
Secretariat	0.50 a	4.01 a	0.02 ab	2.05 ab
Mean	0.43	2.83	0.03	3.87
Creeping bentgrass				
18th Green	0.35 ab*	0.89 ab	0.23 a	17.4 a
L-93	0.48 ab	1.45 ab	0.14 a	12.1 a
PennCross	0.27 b	1.32 ab	0.12 a	9.7 a
Penn G-2	0.49 ab	1.27 ab	0.12 a	10.3 a
PennLinks	0.34 ab	0.82 b	0.16 a	13.6 a
Providence	0.49 ab	1.17 ab	0.21 a	15.8 a
Seaside	0.38 ab	0.95 ab	0.16 a	13.0 a
Southshore	0.59 ab	1.49 ab	0.18 a	14.6 a
SR-1020	0.69 a	1.61 a	0.21 a	15.2 a
Mean	0.45	1.22	0.17	13.5

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

Table 3. Nitrate uptake rate by roots and N concentration in roots and shoots of nine cultivars each of perennial ryegrass and creeping bentgrass

Grass - Cultivar	Nitrate uptake rate	Nitrogen concentration	
		Roots	Shoots
	µmol/g FW/h	mg N/g DW	
Perennial ryegrass			
Calypso II	8.63 ab*	14.6 a	24.8 bc
Figaro	9.29 ab	9.5 a	26.9 bc
Linn	5.79 b	13.0 a	26.0 bc
Manhattan III	7.96 ab	13.7 a	24.8 bc
Morning Star	7.23 ab	15.1 a	28.3 ab
Nighthawk	9.89 ab	15.5 a	27.7 abc
Palmer III	7.04 ab	12.2 a	22.5 c
Saturn II	8.63 ab	12.4 a	25.8 bc
Secretariat	10.8 a	13.6 a	32.4 a
Mean	8.29	13.3	26.6
Creeping bentgrass			
18th Green	2.57 b*	10.4 bc	26.7 a
L-93	2.67 ab	13.9 abc	23.0 abc
PennCross	4.00 a	11.1 bc	18.4 bc
Penn G-2	3.78 ab	14.6 abc	23.9 abc
PennLinks	3.15 ab	11.4 bc	26.2 a
Providence	3.10 ab	16.9 a	23.3 abc
Seaside	3.46 ab	9.3 c	17.3 c
Southshore	2.73 ab	15.4 ab	24.0 abc
SR-1020	3.74 ab	13.8 abc	25.0 ab
Mean	3.24	12.9	23.1

* 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. Relationship between potential total plant NRA root: shoot ratio and root biomass of nine cultivars each of perennial ryegrass (A) and nine creeping bentgrass (B).