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

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Executive Summary

A long-standing paradox confronting turfgrass managers is the simple fact that high quality turf cannot be maintained without annual applications of nitrogen even when clippings are retained on the turf and no nitrogen is removed. This paradox is made all the more intriguing by recent research that shows very little nitrogen is lost from turf through nitrate leaching, ammonia volatilization or denitrification (usually less than 15% of that applied as fertilizer). Our research has shown that more than 2000 lbs. of nitrogen per acre can be recovered within the turf-soil ecosystem of long established turf. With that much nitrogen present in the turf environment, it would appear unlikely that additional applications are unnecessary. The answer to this paradox lies in the fact that in the spring when nitrogen is most needed by turfgrasses, soils are too cold to mineralize much of the organic nitrogen available. During the summer, when the soil is warm and nitrogen becomes available, turfgrass roots are starved for energy because of high respiratory demands by the shoots due to elevated air temperatures. Warm-season turfgrasses do not experience this problem because their leaf respiration does not increase as much during hot weather.

This research project is intended to find means for making cool-season turf more efficient in recovering soil nitrogen. One obvious approach would be to promote greater root development and less shoot growth. This would make the grass better able to absorb nitrate from a larger volume of soil while less energy is committed to rapid shoot growth. Plant growth regulators have been used to achieve this goal but their action is not long lasting and while they inhibit shoot growth they often fail to stimulate root development. We believe the location of nitrogen metabolism in turfgrasses may be the key to this problem.

Most soil nitrogen is available to turfgrass roots as nitrate and is readily absorbed in that form. However, before nitrate-nitrogen can be assimilated into amino acids and proteins it first must be reduced to ammonium. This reduction of nitrate occurs in two steps or reactions: nitrate reduction (NR) and nitrite reduction (NiR). In roots, the eight electrons (e^-) required for these two

$$NO_3^- + 2H^+ + 2e^- \xrightarrow{NR} NO_2^- + H_2O$$

$$NO_2^- + 8H^+ + 6e^- \xrightarrow{NiR} NH_4^- + 2H_2O$$

reactions come from the reduction of sugars produced during photosynthesis and translocated to the roots. In leaves, most of the eight electrons come directly from photosynthetic reactions. The ammonium (NH_4^+) is assimilated directly into the amide-nitrogen of glutamine which is a five-carbon amino acid.

The first reaction in this process is catalyzed by the enzyme nitrate reductase and this has been determined to be the rate limiting step in the chain of reactions leading to nitrogen assimilation. If nitrate is reduced in the roots, amino acids are produced there and root growth is promoted. If roots are unable to reduce nitrate as rapidly as it is absorbed from the soil, the nitrate can be transported to leaves where it will be reduced, assimilated into amino acids and stimulate shoot growth. When nitrate is reduced in leaves, photosynthetic products are diverted to shoot growth and away from roots. This lowered carbon flow to roots makes them even lass able to

reduce nitrate so more is transported to leaves and shoot growth is further stimulated and roots are not. This is what normally happens when turf receives nitrate from the soil or fertilizers.

The research conducted in this project will determine if the nitrate stimulation of shoot growth can be minimized by promoting nitrate reductase activity (NRA) within roots. We have found that in all Kentucky bluegrass cultivars studied, NRA is often ten times more active in the

Nitrate reductase activity (NRA) in leaves and roots of four Kentucky bluegrass cultivars.

	NRA		Root
Cultivar	Leaves	Roots	Shoot
	µmoles/gram/hour		
Liberty	7.44 a*	1.63 a	0.22
Freedom	6.91 a	0.86 b	0.12
Ram-1	6.54 ab	0.44 b	0.07
Glade	5.21 b	0.40 b	0.08

* Means followed by the same letter are not significantly different (P=0.05).

leaves than it is in roots. However, some cultivars (Liberty) did exhibit significantly greater NRA in their roots. We will determine if such cultivars produce greater root growth and if this contributes to more efficient nitrogen use. Currently we are extending this investigation to include diverse cultivars of perennial ryegrass and creeping bentgrass. Similar comparisons will be made and we will determine if greater root NRA correlates well with increased nitrogen use efficiency and field performance. We are also examining management practices that promote greater root growth (higher mowing heights, lower nitrogen fertilization, infrequent but through irrigation, etc.) to determine if they contribute to greater root NRA.

If this relationship between NRA in turfgrass roots and increased root growth is substantiated, efforts will be made to alter turfgrasses genetically to produce cultivars that have a more active nitrate reductase enzyme in their roots. This may produce turfgrasses that will utilize soil nitrate so efficiently that little if any fertilizer nitrogen will be required. This would all but eliminate nitrate leaching from turf and produce turf with a larger, stronger root system that would be more tolerant of drought, and root feeding insects. This research could greatly increase the over-all efficiency of turfgrass management.

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

Project Objectives

1. To quantify each step in nitrate metabolism for selected cultivars of Kentucky bluegrass (*Poa pratensis* L.), 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 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. 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 will concentrate 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 will be 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 will try to compare cultivars of diverse genetic backgrounds. Photosynthate partitioning to roots will also be 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. 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 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.

Initial NRA assays were conducted on greenhouse grown plants cultured in half-strength nitrogen-free Hoagland's solution supplemented with 1.0 mM $NaNO_3$. Turfgrass sod cultures were grown hydroponically in this solution or in sand irrigated with the nutrient solution. During exposure to experimental conditions, turf cultures were transferred to a growth room under controlled temperature and a photosynthetic photon flux density of 800 $\mu mol/m^2/sec$ provided by a sodium halide lamp. Because the greenhouse conditions were unavoidably variable and such variation can influence the general metabolic status of plants, all turf cultures are currently grown in a walk-in environmental control chamber which was generously restored to operating condition by the Rhode Island Turfgrass Foundation. Day and night temperatures are accurately maintained at 75° and 65° F, respectively and irradiance is 800 $\mu mol/m^2/sec$ provided by high intensity fluorescence tubes and two sodium halide lamps.

Turfgrass cultivars were selected in consultation with Bridget Ruemmele to include genotypes of diverse background and differing performance in the national NTEP trials (Tables 1-3). Seed of Kentucky bluegrass cultivars was provided by Dr. Ruemmele from NTEP stocks. Seed of perennial ryegrass and creeping bentgrass cultivars was generously provided by various seed companies (Tables 1 & 2).

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 work has not yet begun and the procedure will be fully described when we have results to report.

Results:

Much of the preliminary work on this project has been conducted on Kentucky bluegrass by Dr. Jiang and has been documented in his doctoral dissertation and in reports currently in press (see publications). Some of his results

on comparative NRA in leaves and roots are summarized in Table 3. Significant differences were observed in leaf NRA among the 14 cultivars compared. The data shown in Table 3 are derived from leaf samples collected during late summer from NTEP field plots. Root NRA was determined from solution cultured mini-turfs grown at 1.0 and 0.1 mM nitrate. It is evident that leaf NRA was substantially greater than that in the roots. These represent some of our better measurements of root NRA in which precautions were taken to minimize enzyme inactivation following harvest and detachment from the shoots. A more recent study is summarized in Table 4 where plants were grown in 5mM NO_3^- prior to assaying for NRA. Here both shoot and root activities of the enzyme were generally greater than in earlier experiments. Higher leaf activity was often matched by higher root activity. However, total plant nitrate reduction occurs mostly in the shoots but as much as 25% has been observed to be contributed by the roots. Further refinements in making NRA measurements of root samples may increase the size of their contribution to total nitrate metabolism but currently we have found most nitrate to be reduced in the leaves of cool-season turfgrasses.

Our data are too preliminary and incomplete to assess any relationship between NRA in leaves or roots with field performance of turfgrasses (Table 3 & 4). John Bushoven has mastered the maintenance of perennial ryegrass and creeping bentgrass cultivars under controlled solution culture. Nitrate and nitrite reduction are being measured in leaves and roots and the partitioning of nitrogen reduction within turfgrass plants is being calculated. In addition, total tissue nitrate and nitrite as well as total nitrogen are being measured. Initial results of these studies will be available for our summer report.

Research Scheduled for 1999

Some methodology questions concerning the *in vivo* assay for NRA and NiRA are currently being resolved and will be completed by the end of the current year. Root and shoot assays of NRA and NiRA will be completed for the twelve perennial ryegrass and creeping bentgrass cultivars selected (Tables 1 & 2) by mid-year. Metabolic and storage pools of nitrate and nitrite in roots and leaves will be determined as will the total nitrogen present within these organs.

During the 1999 growing season, these same cultivars will be evaluated under field conditions in the Rhode Island NTEP trials. NRA of leaf tissues, clipping yields and root:shoot ratios will be determined, as well as turf quality scores. These data will enable us to calculate whole plant growth parameters and determine their relationship with nitrate metabolic functions. Nitrogen use efficiency estimates will be made and correlated with nitrate metabolic parameters derived from field grown and controlled environment cultures.

During early summer, cultures of perennial ryegrass and creeping bentgrass will be will be established in a solid base medium (sand or particulate clay) that can easily be removed from the roots. These cultures will be maintained in column lysimeters from which leachate can be collected and rooting depth and mass

determined. Photosynthate partitioning within these cultures will be estimated by calculating root:shoot mass ratios and by exposing shoots to $^{14}CO_2$ and determining ^{14}C partitioning among various plant parts (leaves, crowns, roots). NRA of leaves and roots will also be monitored and its relationship to photosynthate delivery rate into roots will be assessed.

Publications Derived from this Research

Jiang, Z. 1998. Utilization of nitrate-nitrogen by Kentucky bluegrass. Ph.D. Dissertation, University of Rhode Island, Kingston, RI.

Jiang, Z and R. J. Hull. 1998. Interrelationships of nitrate uptake, nitrate reductase and nitrogen use efficiency in selected Kentucky bluegrass cultivars. Crop Science (In press).

Jiang, Z. and R.J. Hull. 1999. Partitioning of nitrate assimilation between shoots and roots of Kentucky bluegrass. Crop Science (In press).

Table 1. Perennial ryegrass cultivars selected for nitrate metabolism comparisons, the seed source and national quality score in the 1991-94 NTEP trials.

Cultivar	Seed source	NTEP score
BrightStar	Turf Seed Co.	6.2
Calypso II	Roberts Seed Co.	5.6
Figaro	DLF-Trifolium Inc.	*
Linn	Pennington Seed Co.	3.5
Manhattan III	Turf Merchants	5.8
Mine-O-Mine	Cascade International Seed Co.	*
Morning Star	Pennington Seed Co.	6.1
Nighthawk	Allens Seed Store Inc.	6.2
Palmer III	Lofts Seeds Inc.	6.1
PennFine	Roberts Seed Co.	5.1
Saturn II	Zajac Performance Seeds	5.8
Secretariat	Grassland West	*
LSD (P = 0.05)		0.1

* Cultivars not included in 1991-94 NTEP trials

Table 2. Creeping bentgrass cultivars selected for nitrate metabolism comparisons, the seed source and national quality score in the 1997 NTEP trials.

Cultivar	Seed source	NTEP score
Crenshaw	Lofts Seeds Inc.	5.8
18th Green	Zajac Performance Seeds	5.1
L-93	Lofts Great Western Seed Co.	6.6
Lopez	Fine Lawn Research Inc.	5.5
PennCross	Allens Seed Store Inc.	5.2
Penn G-2	Lesco Inc.	6.4
PennLinks	Allens Seed Store Inc.	5.8
ProCup	Forbes Seed & Grain Inc.	5.5
Providence	Seed Research of Oregon	6.2
Seaside	Allens Seed Store Inc.	4.3
Southshore	Lofts Great Western Seed Co.	6.0
SR-1020	Seed Research of Oregon	5.9
SR-7200*	Seed Research of Oregon	-
LSD		0.2

* A velvet bentgrass cultivar

Table 3. Kentucky bluegrass cultivars selected for nitrate metabolism comparisons, their shoot and root NRA and national quality score in the 1991-95 NTEP trials.

	NRA		NTEP score	
Cultivar	Leaves	Roots	High maint.	Low maint.
	µmol/g/h			
Baron	5.58 a*	-	5.5	4.8
Blacksburg	3.92 bc	-	6.0	-
Eclipse	3.85 bc	0.31	6.0	-
Freedom	4.14 bc	-	5.9	4.8
Glade	5.15 ab	0.60	5.9	-
Huntsville	4.11 bc	0.15	-	-
Kenblue	4.45 abc	-	4.6	4.2
Liberty	4.64 abc	-	5.6	4.7
Limousine	4.39 abc	-	5.9	-
Livingston	3.80 bc	0.20	5.7	4.8
Merit	5.75 a	1.08	5.4	4.8
Princeton 104	3.93 bc	-	5.9	-
Ram-1	4.11 bc	-	5.7	5.0
Suffolk	3.52 c	0.65	5.7	4.7
LSD	-	-	0.2	0.2

* Means followed by the same letter are not significantly different (P = 0.05). Leaves assayed for NRA were collected from high maintenance NTEP field plots.

Table 4. Nitrate reductase activity in leaves and roots of four Kentucky bluegrass cultivars.

	NTEP	NRA		Root
Cultivar	Score	Leaves	Roots	Shoot
		µmoles/gram/hour+		
Liberty	5.6	7.44 a*	1.63 a	0.22
Freedom	5.9	6.91 a	0.86 b	0.12
Ram-1	5.7	6.54 ab	0.44 b	0.07
Glade	5.9	5.21 b	0.40 b	0.08

* Means followed by the same letter are not significantly different (P=0.05).

+ Plants were cultured in 5 mM NO_3^- under growth chamber conditions prior to NRA assay.