GENETIC VARIATION IN NITRATE METABOLISM BY COOL-SEASON TURFGRASSES

BY

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ABSTRACT

Reports that NO_3^- contamination of groundwater and surface water bodies may result from fertilizer leaching and runoff have increased concerns about the N use efficiency (NUE) by turfgrasses since NO_3^- is the predominant form of inorganic nitrogen in the turf-soil environment. The rate of NO_3^- absorption by turf roots and the partitioning of NO_3^- assimilation and biomass between roots and shoots have been demonstrated to influence turfgrass NUE.

The first objective of this study was to assess if genetic variation exists in these processes among cultivars of perennial ryegrass (*Lolium perenne* L.) and creeping bentgrass (*Agrostis palustris* Huds.).

The second objective was to establish the relationship between the partitioning of NO_3^- assimilation to roots and total root biomass and to establish if these factors contribute to NUE in these same species. Cultivars were selected for this study on the basis of their performance scores in the National Turfgrass Evaluation Program. Seeds were germinated on washed silica sand and seedlings transplanted into aerated nutrient solution containing 1.0 mM NO_3^- and maintained in an environmental control chamber. Nitrate uptake rate (NUR) was determined by a solution depletion method. Nitrate reductase activity (NRA) was assayed by an *in vivo* assay modified for turfgrasses and partitioning of NO_3^- assimilation was calculated by multiplying tissue specific NRA by the fresh weight of that tissue. Tissue N content was analyzed using a micro-Kjeldahl procedure and NUE was calculated based on biomass produced per unit N.

Significant differences in NUR, NUE, NRA, and the partitioning of NRA and biomass between roots and shoots were found in both species. Among perennial ryegrass cultivars, only Secretariat exhibited significantly higher uptake rates when compared with Linn and among cultivars of creeping bentgrass, only Penncross exhibited significantly higher uptake rates when compared with 18th Green. Significant differences in shoot specific NRA were observed among cultivars of both perennial ryegrass and creeping bentgrass, whereas root specific NRA differed significantly only between SR-1020 and 18th Green creeping bentgrass.

Partitioning of biomass to roots differed significantly in both species and creeping bentgrass partitioned more to its roots than did perennial ryegrass. Partitioning of NRA to roots was also greater in creeping bentgrass than in perennial ryegrass, however, significant differences were observed only in perennial ryegrass. Shoot NUE differed significantly in both species and root NUE differed significantly only in creeping bentgrass. A significant and positive relationship between the partitioning of NRA to roots and an increase in root biomass in perennial ryegrass was demonstrated. However, this relationship was positive but not significant in creeping bentgrass. A negative relationship between the NRA in shoots and shoot NUE in both perennial ryegrass and creeping bentgrass was observed, although significant only in the former. A relationship between the NRA in roots and root NUE was not established in perennial ryegrass, but in creeping bentgrass, a significant negative correlation was demonstrated.

A negative relationship was almost always observed between NRA of the tissues most active in assimilating N and the NUE of those tissues.

This genetic variation in NUR, NRA and the partitioning of NRA indicates that increasing turfgrass NUE may be possible by optimizing the activity and location of these processes through breeding and selection programs. These results are also consistent with the basic hypothesis that NO_3^- assimilation concentrated in turfgrass roots correlates with greater biomass allocation to these roots. An increase in root biomass enables turfgrasses to acquire nutrients and water more effectively and less N will be lost during partial defoliation sustained during each mowing. These results indicate that NUE of perennial ryegrass and creeping bentgrass may be enhanced by selection and breeding of cultivars that assimilate NO_3^- predominantly within their roots.

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PREFACE

This thesis is written in the MANUSCRIPT FORMAT for submission to Crop Science, as specified in the STATEMENT ON THESIS AND DISSERTATION PREPARATION (LONG FORM), the Graduate School, University of Rhode Island.

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VARIATION IN NITRATE UPTAKE, NITRATE REDUCTION AND PARTITIONING OF NITRATE REDUCTION IN ROOTS AND SHOOTS OF PERENNIAL RYEGRASS AND CREEPING BENTGRASS

Abbreviations: NUR, nitrate uptake rate; NRA, nitrate reductase activity; NUE, nitrogen use efficiency.

ABSTRACT

The rate of NO_3^- absorption by turfgrass roots and the rate and site of NO_3^- assimilation have been demonstrated to influence turfgrass nitrogen use efficiency (NUE). The objective of this study was to assess if there is a genetic basis for variation in these processes among cultivars of perennial ryegrass (*Lolium perenne* L.) and creeping bentgrass (*Agrostis palustris* Huds.). Cultivars were selected on the basis of their performance scores in the National Turfgrass Evaluation Program. Seeds were germinated on washed silica sand and seedlings transplanted into aerated nutrient solution containing 1.0 mM NO_3^- and maintained in an environmental control chamber. Nitrate uptake rate (NUR) was determined by a solution depletion method. Nitrate reductase activity (NRA) was assayed by an *in vivo* assay modified for turfgrasses. Partitioning of NO_3^- assimilation was calculated by multiplying tissue specific NRA by the fresh weight of that tissue. Significant differences in NUR, NRA,

and the partitioning of NRA between roots and shoots were found in both species. Among perennial ryegrass cultivars, only Secretariat exhibited significantly higher uptake rates when compared with Linn and among cultivars of creeping bentgrass, only Penncross exhibited significantly higher uptake rates when compared with 18th Green. Significant differences in shoot specific NRA were observed among cultivars of both perennial ryegrass and creeping bentgrass, whereas root specific NRA differed significantly only between SR-1020 and 18th Green creeping bentgrass. Creeping bentgrass partitioned more NRA to the roots than perennial ryegrass, however, significant differences among cultivars were observed only among perennial ryegrasses. This genetic variation in NUR, NRA and the partitioning of NRA indicates that increasing turfgrass NUE may be possible by optimizing the activity and location of these processes through breeding and selection programs.

Key words: nitrate uptake, nitrate reductase activity, nitrogen use efficiency, *Lolium perenne*, *Agrostis palustris*.

INTRODUCTION

Nitrate is the predominant form of inorganic-N available to turfgrass roots (Petrovic, 1990), and its assimilation into amino acids is an energy-consuming process requiring approximately 20% of photosynthetically derived energy (Kaiser and Huber, 1994). The pathway for the acquisition and assimilation of NO_3^- by a turfgrass begins with the uptake of NO_3^- by root cells followed by its reduction and assimilation into amino acids and its subsequent transport to sites of metabolic need (Simpson, 1986). If any of these steps are inefficient, especially at low external nitrogen concentrations, the efficiency of N utilization by the turfgrass may be reduced.

The rate of NO_3^- uptake, although not thought to be rate limiting, differs among cultivars of several turfgrass species (Liu *et al.*, 1993) and is affected by external NO_3^- concentration (Bowman, 1989; Jiang and Hull, 1999). Reduction and assimilation of NO_3^-, following its uptake by roots, can occur in either roots or shoots of most grass plants and this spatial partitioning varies widely among species (Pate, 1973). External NO_3^- concentration also affects this partitioning of NO_3^- assimilation between roots and shoots, as does age and the carbohydrate status of the plant. Analysis of *in vivo* NRA has indicated that, as external NO_3^- concentrations increase, a greater proportion of total plant NRA is partitioned to the shoots of perennial ryegrass (Andrews *et al.*, 1992). *In vivo* analysis of NRA in perennial ryegrass also indicates that root activity is significantly lower than that in shoots and is negatively affected by partial defoliation (Smith and James, 1982; Boucaud and Bigot, 1989). In intensively managed areas turf is subjected to frequent and close mowing, which may adversely

affect overall turf N, nutrition since a greater proportion of reduced N must be derived from reserves or root-centered NRA. Translocation of NO_3^- to the shoots also redirects the flow of carbon from sucrose biosynthesis toward amino acid biosynthesis by modulating the activities of sucrose phosphate synthase and phosphoenolpyruvate carboxylase (Champigny and Foyer, 1992). This leads to a greater proportion of NO_3^- being reduced in the shoots, since the energy and carbon requirements for root NRA are dependent upon sucrose translocation from the shoots (Radin *et al.*, 1978). Greater partitioning of NO_3^- reduction and assimilation to turfgrass roots would therefore reduce the loss of absorbed N as a result of shoot removal during mowing and subsequently increase the efficiency of N utilization.

This study quantifies and assesses the variability in uptake and reduction of NO_3^- among perennial ryegrass and creeping bentgrass cultivars utilized on managed turf areas. The variability in partitioning of NRA between roots and shoots of these same species is also examined. This information will be useful for determining if the rate of NO_3^- uptake or the partitioning of NRA may limit the efficient utilization of NO_3^- – N by perennial ryegrass and creeping bentgrass and for assessing the potential for increasing NUE by optimizing the activity and location of these processes.

MATERIALS AND METHODS

Plant Materials and Culture

Seeds of perennial ryegrass (*Lolium perenne L.)* and creeping bentgrass (*Agrostis palustris* Huds.) cultivars selected on the basis of their performance scores in the National Turfgrass Evaluation Program (Table 1), were germinated on washed silica sand. After twenty days, seedlings were removed, the roots washed free of all sand and transplanted into either 250-ml Erlenmeyer flasks for NO_3^- uptake determination, or 20-L culture troughs (63" x 5" x 3.5") for NRA assays. Both flasks and troughs contained one-quarter-strength, aerated, modified N-free Hoagland's solution (Hoagland and Arnon, 1950) supplemented with 1 mM $NaNO_3$. Cultures were maintained in an environmental control chamber (ppfd 800 $\mu M\ m^{-2}\ s^{-1}$, 16-h photoperiod, and day/night temperatures of $25/20^0C$). Solutions were replaced once each week and tap water was added daily to compensate for evapotranspiration loss.

Nitrate Uptake Rate

Nitrate uptake rate was determined by a solution depletion method (Liu, 1992). Culture solution was replaced with fresh one-quarter-strength modified N-free Hoagland's solution supplemented with 1 mM $NaNO_3$ 24 hours prior to uptake determination. Initial volume (Vi) and final volume (Vf) of nutrient solutions were measured, and initial NO_3^- concentration (Ci) and final concentration (Cf) determined using a copperized cadmium reduction method (Keeney and Nelson, 1982). Nitrate uptake rate was calculated as follows: $NUR = \{[Vi(Ci) - Vf(Cf)]/R\}/t$ where R is root

fresh weight and t is the time between initial and final solution sampling. Uptake rates were expressed as μmol NO_3^- absorbed from culture solution per hour per gram fresh root based upon a 24-hour uptake period.

Nitrate Reductase Activity

Nitrate reductase activity was assayed using an *in vivo* method described by Hageman and Reed (1980) and optimized for cool season turfgrasses by Jiang and Hull (1998). After 60-90 days, shoots that included leaf and stem tissues were separated from roots and cut into 2 mm-long segments. Shoot segments and intact roots (0.2 g) were placed in 25 ml flasks containing 5 ml of incubation medium which consisted of 0.1 M potassium phosphate buffer (pH 7.5), 50 mM KNO_3, and 0.39 M 2-propanol. Incubation flasks were placed in a vacuum desiccator and evacuated to 6 mm Hg for 2 minutes. After releasing the vacuum, flasks were stirred to ensure tissues were submerged in incubation medium and again evacuated, followed by purging with N_2 gas. This evacuation/purging procedure was repeated once and flasks were immediately stoppered and transferred to a 30^0C water bath shaker and covered to exclude light. After 15 minutes, 0.5-ml aliquots were withdrawn from each flask to 10-ml test tubes to determine the initial NO_2^- concentration. Shoot and root NRA was based upon NO_2^- increase in the medium between 15 and 45 minutes and 15 and 120 minutes respectively. Nitrite concentration was determined by adding to the test tubes, 1 ml 29 mM sulfanilamide solution in 2.4 M HCl followed by 1 ml 11.6 mM N-(1-napthyl)-ethylenediamine dihydochloride in 0.12 M HCl (Snell and Snell, 1949). Absorbance was measured at 540 nm in a spectrophotometer (Bausch and Lomb

Spectronic 21). The NRA was expressed as μmole NO_2^- produced per gram fresh tissue per hour.

Statistical Design and Analysis

Individual assays were conducted as a randomized complete block design with four replications each. Experiments were conducted twice and statistical computations were performed using procedures within the Statistical Analysis System (SAS Institute, 1990). A general linear model procedure was used to establish significant differences among cultivars. Duncan's multiple range tests were used to separate significant cultivar means.

RESULTS

Nitrate Uptake Rate

On a root weight basis, perennial ryegrass absorbed NO_3^- at approximately twice the rate of creeping bentgrass (Table 2). Analysis of NO_3^- uptake among cultivars of both species demonstrated relatively few significant differences. Among perennial ryegrass cultivars, only Secretariat exhibited significantly higher uptake rates when compared with Linn, the remaining seven perennial ryegrass cultivars exhibited similar uptake rates (Table 2). Among cultivars of creeping bentgrass, only Penncross exhibited significantly higher uptake rates when compared with 18th Green (Table 2). As was also evident in the analysis of uptake rates among cultivars of perennial ryegrass, variation among the remaining seven creeping bentgrass cultivars was not significant.

Nitrate Reduction

On average, shoot specific NRA of perennial ryegrass was 230% greater that creeping bentgrass. Significant differences in shoot specific NRA were observed among cultivars of both perennial ryegrass and creeping bentgrass (Table 3). Greater variation in NRA was observed in shoots of perennial ryegrass than creeping bentgrass. Shoot specific NRA of Secretariat was 270% higher than that of Linn (the perennial ryegrass cultivars with the highest and lowest shoot specific NRAs, respectively (Table 3). By comparison, SR-1020, was 195% higher than Pennlinks (the creeping bentgrass cultivars with the highest and lowest shoot specific NRAs,

respectively) (Table 3). Variability in root specific NRA was even more limited among cultivars of both species. No significant differences were observed in root specific NRA among the nine perennial ryegrass cultivars. Significant differences were observed only between two creeping bentgrass cultivars, SR-1020 and Penncross. Average root specific NRAs were similar for perennial ryegrass and creeping bentgrass.

Analysis of total plant root:shoot NRA ratios demonstrated significant differences among cultivars of perennial ryegrass in their partitioning of NRA between roots and shoots (Table 4). Partitioning of NRA to roots in perennial ryegrass ranged from 8% to 22% of total plant NRA for Manhattan III and Palmer III, respectively (Table 4.). No significant differences were observed in whole plant partitioning of NRA between roots and shoots among cultivars of creeping bentgrass (Table 4.), where partitioning of NRA to roots ranged from 17% to 30% of total plant NRA for Penncross and SR 1020, respectively (Table 4). Creeping bentgrass generally reduced more of its NO_3^- in the roots than did perennial ryegrass.

DISCUSSION

Previous analyses of NO_3^- uptake have demonstrated significant genetic variations in perennial ryegrass and Kentucky bluegrass and these variations were shown to be affected by NO_3^- availability (Liu *et al.*, 1993; Jiang and Hull, 1998). Kinetic studies of NO_3^- uptake by perennial ryegrass have demonstrated that changes in NO_3^- availability, temperature, plant age or solution pH affect maximum uptake rates (Vmax) but do not significantly affect the affinity of the transport protein for NO_3^- (Km) (Bowman *et* al., 1989; Barber 1984). Studies involving NO_3^- uptake by Kentucky bluegrass have demonstrated that the high affinity NO_3^- induced uptake system is saturated when external NO_3^- concentrations exceed 1 mM (Jiang and Hull, 1998). Other studies have demonstrated that the rate of NO_3^- uptake at high external concentrations may be mediated by a constitutive or induced low affinity, nonsaturable system operating through NO_3^- specific channels. These systems are subject to negative feedback inhibition after prolonged exposure to NO_3^- (Siddiqi *et al* 1990). This inhibition may have influenced the measured uptake rates of the species examined in this study. Earlier investigations indicated significant genetic differences in NUR could be observed in Kentucky bluegrass cultivars cultured in solution containing 1 mM NO_3^- (Jiang and Hull, 1998).

The first objective of this study was to determine if the rate of NO_3^- uptake by perennial ryegrass and creeping bentgrass also varied among cultivars. Preliminary assays conducted to establish optimum solution NO_3^- concentrations, indicated that

1 mM NO_3^- was adequate to maintain a desired quality of perennial ryegrass and creeping bentgrass cultures. Maintaining cultures in 1 mM NO_3^- eliminated differential depletion of NO_3^- that was observed at lower concentrations due to differential growth rates of the selected cultivars. This concentration (14 mg L^{-1}) is higher than actual soil water NO_3^--N levels of 0.5 to 10 mg L^{-1} (Hallberg, 1989). However, during NO_3^- depletion of the solution, plants were exposed to a typical range of NO_3^- concentrations. Our results demonstrated that genetic variation exists in NO_3^- uptake in perennial ryegrass and creeping bentgrass when supplied with up to 1 mM NO_3^-. This genetic variability may provide breeders with a useful genetic parameter for the development of improved turfgrass cultivars.

The second objective of this study was to determine if genetic variability existed in NRA among perennial ryegrass and creeping bentgrass cultivars, and to assess if NO_3^- was preferentially reduced within shoots or roots of these species. Genetic variability in NRA within roots and shoots, and its contribution to total plant NO_3^- reduction have been observed previously in Kentucky bluegrass (Jiang and Hull, 1998), but has not been investigated in perennial ryegrass or creeping bentgrass. The *in vivo* method for quantifying NRA has been demonstrated to provide accurate estimates of *in situ* activity (Brunetti and Hageman, 1976), however, other studies have demonstrated higher levels of shoot and root NRA when assayed *in vitro* (Andrews, 1986, Wallace 1986). The *in vivo* assay conditions utilized in this study were described by Hageman and Reed (1980) and have been optimized for studies of NRA in Kentucky bluegrass to detect NRA that occurs only when the active enzyme is present and sufficient reducing equivalents are available (Jiang and Hull 1998). Using

this method, genetic variation in shoot NRA was observed in perennial ryegrass and creeping bentgrass, with greater variation found in perennial ryegrass. Analyses of root NRA indicated significant difference only between two creeping bentgrass cultivars and no significant differences among perennial ryegrass cultivars.

Creeping bentgrass was observed to partition up to 30 % of total plant NRA to roots. By comparison, the maximum NRA partitioned to perennial ryegrass roots never exceeded 22% of total plant NRA. These data are consistent with earlier reports that NO_3^- is reduced primarily in the shoots of turf and pasture grasses when roots are supplied with NO_3^- concentrations at or exceeding 1 mM (Jiang and Hull, 1999; Andrews *et al* 1992). The comparatively higher NRA partitioned to roots of creeping bentgrass may be related to the greater partitioning of photosynthetic resources towards root production (Table 5). The average root biomass of creeping bentgrass cultivars comprised up to 28% of total plant biomass. By comparison, perennial ryegrass root biomass never exceeded 11% of total plant biomass. A relative increase in the capacity of roots to reduce NO_3^- has been observed in wheat seedlings to be a direct result of increased carbohydrate levels when plants were nitrogen starved (Talouise *et al*, 1984). In a similar study, an increase in root NRA has also been positively correlated with an increase in the partitioning of photosynthate to roots of Kentucky bluegrass (Jiang and Hull, 1999). Photosynthate translocated from the shoots provides the carbon skeletons and energy necessary for the reduction and assimilation of NO_3^- in the roots (Oaks and Hirel, 1985). In perennial ryegrass, NO_3^- uptake rates averaged 250% greater than that of creeping bentgrass. Root specific NRA was similar for both species, which promoted more NO_3^- translocation to shoots

in perennial ryegrass. This promoted a greater shoot specific NRA, which in turn promoted more shoot growth at the expense of root growth.

The genetic variations in NUR, NRA and the partitioning of NRA between roots and shoots of perennial ryegrass and creeping bentgrass observed in this study indicate that increasing turfgrass NUE may be possible by optimizing the activity and location of these metabolic steps through breeding and selection programs. The relationship between these metabolic processes and NUE will be addressed in the following report.

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Table 1. National Turfgrass Evaluation Program mean quality ratings of perennial ryegrass (USDA ARS NTEP Final Report 1998 No. 99-10) and creeping bentgrass (USDA ARS NTEP Final Report 1994-97 No. 98-11) cultivars for Kingston, RI.

	Cultivar	Mean quality rating †
Perennial ryegrass	Palmer III	6.5
	Secretariat	6.3
	Calypso II	6.3
	Saturn II	5.8
	Manhattan III	5.5
	Morning Star	4.9
	Nighthawk	4.5
	Figaro	4.3
	Linn	2.9
	LSD (0.05)	0.9
Creeping bentgrass	L-93	6.6
	Penn G-2	6.3
	Providence	5.7
	Southshore	5.6
	Pennlinks	5.2
	SR 1020	4.9
	Penncross	4.0
	18th Green	3.9
	Seaside	3.4
	LSD (0.05)	0.7

† 1-9; 9 = Ideal turf.

Table 2. Differences in NO_3^- uptake rate of nine perennial ryegrass and nine creeping bentgrass cultivars.

	Cultivar		NO_3^- uptake rate
			μmol g FW^{-1} h^{-1} †
Perennial ryegrass	Palmer III		7.04ab‡
	Secretariat		10.7a
	Calypso II		8.63ab
	Saturn II		7.95ab
	Manhattan III		7.96ab
	Morning Star		7.22ab
	Nighthawk		9.68ab
	Figaro		9.29ab
	Linn		5.79b
		Mean	8.25
Creeping bentgrass	L-93		2.67ab
	Penn G-2		3.78ab
	Providence		3.09ab
	Southshore		2.73ab
	Pennlinks		3.15ab
	SR 1020		3.74ab
	Penncross		4.00a
	18^{th} Green		2.56b
	Seaside		3.45ab
		Mean	3.24

† FW is fresh weight.

‡ Means followed by the same letter are not significantly different at $P \leq 0.05$.

Table 3. Differences in nitrate reductase activity of nine perennial ryegrass and nine creeping bentgrass cultivars.

	Cultivar		Nitrate reductase activity	
			Root	Shoot
			μmol NO_2^- g FW^{-1} h^{-1} †	
Perennial ryegrass	Palmer III		0.56a‡	1.95de
	Secretariat		0.50a	4.01a
	Calypso II		0.39a	3.28abc
	Saturn II		0.46a	3.49ab
	Manhattan III		0.28a	2.48cd
	Morning Star		0.38a	3.29abc
	Nighthawk		0.53a	2.70bcd
	Figaro		0.39a	2.79bcd
	Linn		0.38a	1.47e
		Mean	0.43	2.83
Creeping bentgrass	L-93		0.48ab	1.45ab
	Penn G-2		0.49ab	1.27ab
	Providence		0.49ab	1.17ab
	Southshore		0.59ab	1.49ab
	Pennlinks		0.34ab	0.82b
	SR 1020		0.69a	1.61a
	Penncross		0.27b	1.32ab
	18^{th} Green		0.35ab	0.89ab
	Seaside		0.38ab	0.95ab
		Mean	0.45	1.22

† FW is fresh weight.

‡ Means followed by the same letter are not significantly different at $P \leq 0.05$.

Table 4. Differences in potential root:shoot ratio of total plant nitrate reductase activity rate of nine perennial ryegrass and nine creeping bentgrass cultivars.

	Cultivar	Potential total plant NRA root:shoot ratio
Perennial ryegrass	Palmer III	0.34a†
	Secretariat	0.13bc
	Calypso II	0.14bc
	Saturn II	0.13bc
	Manhattan III	0.08bc
	Morning Star	0.148c
	Nighthawk	0.19bc
	Figaro	0.14bc
	Linn	0.28ab
Creeping bentgrass	L-93	0.35a
	Penn G-2	0.41a
	Providence	0.61a
	Southshore	0.43a
	Pennlinks	0.42a
	SR 1020	0.60a
	Penncross	0.32a
	18th Green	0.53a
	Seaside	0.43a

† Means followed by the same letter are not significantly different at $P \leq 0.05$.

Table 5. Differences in root and shoot biomass of nine perennial ryegrass and nine creeping bentgrass cultivars.

Cultivar	Root	Shoot	Root:shoot ratio
	g FW^{-1} †		
Perennial ryegrass			
Palmer III	1.32a‡	6.60ab	0.22a
Secretariat	0.49d	3.74c	0.16ab
Calypso II	0.65cd	6.67ab	0.10b
Saturn II	0.91bc	6.36ab	0.16ab
Manhattan III	0.78bcd	4.33bc	0.13ab
Morning Star	0.99abc	7.78a	0.23a
Nighthawk	0.72cd	6.88ab	0.11b
Figaro	0.79bcd	4.67bc	0.19ab
Linn	1.16ab	7.61a	0.17ab
Mean	0.87	6.07	0.14
Creeping bentgrass			
L-93	2.53a	6.23a	0.42ab
Penn G-2	1.84ab	6.62a	0.28b
Providence	2.17ab	6.27a	0.36ab
Southshore	2.39a	6.40a	0.38ab
Pennlinks	1.31b	3.59b	0.38ab
SR 1020	2.28ab	6.12a	0.37ab
Penncross	1.62ab	4.64ab	0.36ab
18^{th} Green	1.62ab	3.33b	0.50a
Seaside	2.20ab	5.98a	0.33b
Mean	2.00	5.46	0.37

† FW is fresh weight.

‡ Means followed by the same letter are not significantly different at $P \leq 0.05$.

MANUSCRIPT II

NITROGEN USE EFFICIENCY AND THE PARTITIONING OF NITRATE REDUCTASE AND BIOMASS BETWEEN ROOTS AND SHOOTS OF PERENNIAL RYEGRASS AND CREEPING BENTGRASS

Abbreviations: NUR, nitrate uptake rate; NR, nitrate reduction; NRA, nitrate reductase activity; NUE, nitrogen use efficiency.

ABSTRACT

Reports that NO_3^- contamination of groundwater and surface water bodies may result from fertilizer leaching and runoff have increased concerns about the N use efficiency (NUE) by turfgrasses. The partitioning of NO_3^- assimilation and biomass between roots and shoots have been demonstrated to influence NUE. The objectives of this study were to establish the relationship between the partitioning of NO_3^- assimilation to roots and total root biomass among cultivars of perennial ryegrass (*Lolium perenne* L.) and creeping bentgrass (*Agrostis palustris* Huds.) and to establish if these factors contribute to NUE in these same species. Cultivars were selected on the basis of their performance scores in the National Turfgrass Evaluation Program. Seeds were germinated on washed silica sand and seedlings transplanted into aerated nutrient solution containing 1.0 mM NO_3^- and maintained in an environmental control chamber. Nitrate reductase activity (NRA) was assayed by an *in vivo* assay modified for turfgrasses. Partitioning of NO_3^- assimilation was calculated by multiplying tissue

specific NRA by the fresh weight of that tissue. Tissue N content was analyzed using a micro-Kjeldahl procedure and NUE was calculated based on root biomass produced per unit N. Partitioning of biomass to roots differed significantly in both species. Creeping bentgrass partitioned more biomass to the roots than perennial ryegrass. Partitioning of NRA to roots was also greater in creeping bentgrass than in perennial ryegrass, however, significant differences were observed among cultivars only in perennial ryegrass. Shoot NUE differed significantly in both species, however, root NUE differed significantly in only creeping bentgrass. A significant and positive relationship between the partitioning of NRA to roots and an increase in root biomass in perennial ryegrass was demonstrated. However, this relationship was positive but not significant in creeping bentgrass. A negative relationship between the shoot NRA and shoot NUE in both perennial ryegrass and creeping bentgrass was observed, although significant only in the former. A relationship between the root NRA and root NUE was not established in perennial ryegrass, but in creeping bentgrass, a significant negative correlation was demonstrated. A negative relationship was almost always observed between the NRA of tissues most active in assimilating N and the NUE of those tissues. These results are consistent with the basic hypothesis that NO_3^- assimilation concentrated in turfgrass roots correlates with greater biomass allocation to their roots. These results indicate that NUE of perennial ryegrass and creeping bentgrass may be enhanced by selection and breeding of cultivars which assimilate NO_3^- predominantly within the roots.

Key words: nitrate uptake, nitrate reductase activity, nitrogen use efficiency, *Lolium perenne*, *Agrostis palustris*.

INTRODUCTION

Cool-season turfgrasses require the application of approximately 15-25 g N m^{-2} yr^{-1} in order to maintain acceptable quality (Beard, 1973). When this fertilizer N is combined with the approximately 10-15 g soil organic N per m^2 released through mineralization, 25-40 g N m^{-2} yr^{-1} are available to turf. Since no nitrogen is removed from turf when clippings are retained, these relatively high levels of available nitrogen suggest that turfgrasses are not very efficient in their utilization of available nitrogen (Hull and Liu, 1995).

Reports that NO_3^- contamination of groundwater and surface water bodies may result from fertilizer leaching and runoff have increased concerns about the N use efficiency (NUE) by turfgrasses. Currently, turfgrass performance is evaluated under a range of N fertility conditions through the National Turfgrass Evaluation Program (NTEP). However, because the appearance and vigor of turfgrasses are considered to be two major factors contributing to their overall performance, selection is often conducted under high N fertility and NUE is not a criterion. Under high N fertility, potential genetic variations in NUE among turf cultivars that may exist under low N fertility can be concealed.

Nitrogen use efficiency in other agronomically important crops is defined as the amount of biomass produced per unit of N present in the plant (Bertauski *et al.*, 1997). Genetic variation in NUE has been demonstrated in wheat [*Triticum aestivum*] (Fiez *et al.*, 1995; Rao and Dao, 1996), rice [*Oryza sativa* L.] (Cassman *et al.*, 1993), maize [*Zea mays* L.] (Anderson *et al.*, 1984), and barley [*Hordeum vulgare* L.]

(Gonzalez Ponce, *et al.*, 1993). In 1993, Liu *et al* observed that variation in the NUE of Kentucky bluegrass (*Poa pratensis* L.) and perennial ryegrass (*Lolium perenne* L.) did not differ significantly among cultivars. However, NO_3^- uptake efficiency by roots of cultivars within the same species did vary significantly. Genetic variations in NUE of Kentucky bluegrass were later reported to differ significantly and these differences were found to be related to shoot NUE and not to root absorption efficiency (Bertauski *et al.*, 1997).

Increased external NO_3^- concentrations have been reported to decrease the NUE of corn and sugarcane (Anderson *et al.*, 1984; Gascho *et al.*, 1986). In a study, with Kentucky bluegrass, Jiang and Hull (1998) reported that shoot NUE was also negatively correlated with NO_3^- uptake rate (NUR) and NO_3^- reduction (NR). They concluded that high external NO_3^- concentrations stimulated NUR and increased transport of NO_3^- to the shoots where it was subsequently reduced and assimilated. This resulted in a low C/N ratio and a subsequent decrease in root:shoot ratios. Such relationships between the presence of NO_3^- in the shoots and a decrease in root:shoot ratios is consistent with earlier studies in which NO_3^- was observed to function as a signal metabolite (Champigny and Foyer, 1992; Van Quy *et al.*, 1991). In these studies, the modulation by NO_3^- of two key enzymes involved in photosynthate partitioning redirected the flow of carbon away from sucrose synthesis and root growth toward amino acid synthesis and shoot growth. If NO_3^- is first reduced and assimilated in the roots and subsequently transported to the shoots as amino acids, turfgrass NUE may increase since a greater root:shoot ratio will occur, thereby

enabling turfgrasses to acquire nutrients and water more effectively. Also, less N will be lost during partial defoliation sustained during each mowing.

The objective of this study was to establish the relationship between the partitioning of NR to roots and total root biomass among cultivars of perennial ryegrass and creeping bentgrass and to establish if these factors contribute to NUE in these same species.

MATERIALS AND METHODS

Plant Materials and Culture

Seeds of perennial ryegrass (*Lolium perenne L.)* and creeping bentgrass (*Agrostis palustris* Huds.) cultivars selected on the basis of performance scores from the National Turfgrass Evaluation Program (Table 1), were germinated on washed silica sand. After twenty days, seedlings were removed, the roots washed free of all sand and transplanted into 20-L culture troughs (63" x 5" x 3.5"). The troughs contained one-quarter-strength aerated, modified N-free Hoagland's solution (Hoagland and Arnon, 1950) supplemented with 1 mM $NaNO_3$. Cultures were maintained in an environmental control chamber (ppfd 800 $\mu mol\ m^{-2}\ s^{-1}$, 16-h photoperiod, and day/night temperatures of $25/20^0C$). Solutions were replaced once each week and tap water was added daily to compensate for evapotranspiration loss.

Plant Biomass

Biomass of roots and shoots was measured on a gram fresh weight basis immediately prior to analysis for NRA. Upon removal from solution culture, roots were blotted dry with tissue paper to remove free water, and total plant weight was determined. Shoots that included leaf and stem tissues were separated from roots and the weights of both roots and shoots determined individually.

Nitrate Reductase Activity

Nitrate reductase activity was assayed using an *in vivo* method described by Hageman and Reed (1980) and optimized for cool-season turfgrasses by Jiang and

Hull (1998). After 60-90 days, shoots that included leaf and stem tissues were separated from roots and cut into 2 mm-long segments. Shoot segments and intact roots (0.2 g) were placed in 25-ml flasks containing 5 ml of incubation medium which consisted of 0.1 M potassium phosphate buffer (pH 7.5), 50 mM KNO_3, and 0.39 M 2-propanol. Incubation flasks were placed in a vacuum desiccator and evacuated to 6 mm Hg for 2 minutes. After releasing the vacuum, flasks were stirred to ensure tissues were submerged in incubation medium and again evacuated, followed by purging with N_2 gas. This evacuation/purging procedure was repeated once and the flasks were immediately stoppered and transferred to a 30^0C water bath shaker and covered to exclude light. After 15 minutes, 0.5-ml aliquots were withdrawn from each flask to 10-ml test tubes to determine the initial NO_2^- concentration. Shoot and root NRA was based upon NO_2^- increase in the incubation medium between 15 and 45 minutes and 15 and 120 minutes, respectively. Nitrite concentration was determined by adding to the test tubes, 1 ml 29 mM sulfanilamide solution in 2.4 M HCl followed by 1 ml 11.6 mM N-(1-napthyl)-ethylenediamine dihydochloride in 0.12 M HCl (Snell and Snell, 1949). Absorbance was measured at 540 nm in a spectrophotometer (Bausch and Lomb Spectronic 21). The NRA was expressed as μmole NO_2^- produced per gram fresh tissue per hour.

Total Nitrogen Content

Frozen tissues were oven dried at 70^0C until no further decrease in weight was observed. Tissues were cut into 1.0-1.5 mm segments and 0.14 g samples were analyzed for total N content using a micro-Kjeldahl procedure (Eastin, 1978).

Statistical Design and Analysis

Individual assays were conducted as a randomized complete block design with four replications each. Experiments were conducted twice and statistical computations were performed using procedures within the Statistical Analysis System (SAS Institute, 1990). A general linear model procedure was used to evaluate differences among cultivars. Duncan's multiple range tests were used to identify significant differences among cultivar means. Regression analyses to establish relationships between NRA, plant tissue biomass and NUE were performed using the data analysis package within Microsoft Excel 97.

RESULTS

Biomass partitioning

Total biomass of solution grown perennial ryegrass and creeping bentgrass cultivars were roughly comparable at the time they were harvested for NRA analysis (Table 3). However, in perennial ryegrass, partitioning of biomass to roots never exceeded 17% of total plant biomass whereas creeping bentgrass partitioned up to 33% of total plant biomass to roots (Table 3). Total root biomass of Palmer III perennial ryegrass was approximately equal to that of Pennlinks creeping bentgrass, the cultivars of each species with the highest and lowest root biomass respectively. Significant genetic variation in rate of growth and the partitioning of biomass between roots and shoots of both species was observed (Table 3). Among the nine perennial ryegrass cultivars examined, Palmer III and Morning Star partitioned significantly more biomass to roots, 17% and 15% respectively, than Calypso II and Nighthawk which partitioned only 9% of total plant biomass to its roots. Among the nine creeping bentgrass cultivars, significant differences in biomass partitioning were observed between 18th Green and Penn G-2. Partitioning of only 22% of total biomass to roots occurred in Penn G-2, whereas 18th Green and L-93 partitioned 33% and 29% of total plant biomass to roots, respectively. Significantly more total biomass was partitioned to the roots of L-93 and Southshore than Pennlinks, the cultivars with the greatest and least partitioning of biomass to roots respectively.

Potential partitioning of NRA

Partitioning of NO_3^- reduction between roots and shoots under these conditions was calculated by multiplying tissue specific NRA by the fresh weight of that tissue and dividing total root NRA by total shoot NRA. Analysis of perennial ryegrass root:shoot NRA ratios demonstrated significant differences among cultivars in the partitioning of NRA between roots and shoots (Table 2). The NRA in roots of perennial ryegrass ranged from 8% to 22% of total plant NRA for Manhattan III and Palmer III, respectively. No significant differences in whole plant partitioning of NRA between roots and shoots were observed among cultivars of creeping bentgrass, where partitioning of NRA to roots ranged from 17% to 30% of total plant NRA (Table 2). Creeping bentgrass generally partitioned more of its NO_3^- reduction to roots than did perennial ryegrass.

Nitrogen use efficiency

Nitrogen use efficiency was calculated based upon the amount of root or shoot biomass (Table 3) produced per unit N (Table 5) and expressed as g DW mg N^{-1} (Table 5). Significant differences in total shoot N were observed among cultivars of both species but total root N differed only in creeping bentgrass (Table 4). These differences were reflected in the calculated NUE of both species where the root NUE was approximately twice that of shoots (Table 5). Among perennial ryegrass cultivars, Morning Star exhibited significantly lower shoot NUE than Palmer III, while Secretariat was significantly lower than that of Palmer III, Calypso II and Morning Star. The NUE in perennial ryegrass roots did not differ significantly among the nine

cultivars examined (Table 5). In creeping bentgrass, significant differences were observed both in root and shoot NUE. Seaside exhibited significantly higher shoot NUE than the remaining cultivars with the exception of Penncross which itself was significantly higher than 18th Green and Pennlinks (Table 5). Pennlinks exhibited the highest root NUE but was only significantly higher than Providence, the cultivars with the highest and lowest root NUE, respectively.

DISCUSSION

The importance of nitrogen use efficiency as a factor in increased yield of forage grasses (Hageman and Lambert, 1988), and appearance of turfgrasses (Nelson and Sosulski, 1984), has long been apparent. However, the physiological basis for NUE in turfgrasses has received little attention. Genetic variation in NUE has been demonstrated in relation to N supply in corn and Kentucky bluegrass. (Anderson *et al.*, 1984; Bertauski *et al.*, 1997), and has been shown to be related to root capacity for NO_3^- absorption and reduction (Jiang and Hull, 1998).

The rate at which NO_3^- is absorbed by turfgrass roots has been demonstrated to vary significantly among cultivars of Kentucky bluegrass and perennial ryegrass (Liu *et al.*, 1993; Cisar *et al.*, 1989). Significant variations in the rate at which NO_3^- is subsequently reduced have also been reported in turf (Osborne and Whittington, 1981; Smith and James, 1982; Jiang and Hull, 1998). The activity of nitrate reductase (NRA) is influenced by temperature (Harris and Whittington, 1983), light (Lillo, 1994), NO_3^- supply (Li and Oaks, 1993), and carbohydrate supply (Jiang and Hull, 1999). In several turf species, partial defoliation has also been shown to affect the capacity of roots or shoots to reduce NO_3^- either by altering the partitioning of photosynthate (Alsam and Oaks, 1975; Radin *et al.*, 1978) or by enzyme inactivation (Botrel and Kaiser, 1996). It has also been reported that approximately 70% of total plant NO_3^- reduction occurs in the roots of perennial ryegrass maintained at 0.5 mM NO_3^- concentrations (Andrews *et al.*, 1992). Significant and positive relationships between root biomass and NO_3^- uptake and between root morphology and NO_3^- uptake

have been demonstrated in Kentucky bluegrass (Sullivan *et al.*, 1999). Cheeseman *et al.*, (1996) suggested that models describing N and C allocation in plants, may be limited in their ability to describe realistic biomass partitioning as they do not account for NO_3^- transport to shoots or its reduction there. The objective of this study was to determine if the partitioning of NRA to the roots of perennial ryegrass and creeping bentgrass cultivars was related to biomass partitioning to roots and to determine if these factors contribute to NUE in these same species.

Our results demonstrated a significant and positive relationship between the partitioning of NRA to roots and an increase in root biomass in perennial ryegrass (Figure 2, $r = 0.67$). However, this relationship, was positive but not significant in creeping bentgrass (Figure 1, $r = 0.16$). These results are consistent with earlier reports which demonstrated that NO_3^- translocated to the shoots, prior to its reduction and assimilation, is correlated with a decrease in root:shoot biomass ratios in both annual and perennial species (Levin *et al.*, 1989; Hocking and Meyer, 1991; Scheible *et al.*, 1997). The presence of NO_3^- in the shoots alters the flow of carbon from the synthesis of sucrose, a requirement for root growth, to amino acid production, which promotes shoot growth (Van Quy and Champigny, 1992). However, the regulation of NRA and the flow of NO_3^- and organic N between roots and shoots have also been demonstrated to minimize differences in growth (Lexa and Cheeseman, 1997).

The tissue specific NRA in the shoots of perennial ryegrass was greater than that of creeping bentgrass, whereas specific NRA in roots was similar in both species (Table 2). However, because creeping bentgrass partitioned a greater amount of biomass to roots than did perennial ryegrass (Table 3), it exhibited a greater

partitioning of NRA to roots than did perennial ryegrass (Table 2). The slightly lower total N content observed in creeping bentgrass shoots than in perennial ryegrass (Table 5) may have been due to the greater partitioning of NRA to the roots of creeping bentgrass. However, it has been demonstrated that when NRA occurs in the roots, a considerable proportion of the reduced N which is transported to shoots, may be retranslocated to roots, a portion of which then may be recycled back to shoots (Jeschke and Pate, 1991). In plants that reduce N predominantly in their shoots, Simpson *et al* (1982) demonstrated that up to 79% of N transported from roots to shoots was retranslocated to roots, of which 50% was recycled back to the shoot, and the remainder incorporated into root tissue.

A negative relationship between the shoot NRA and shoot NUE in both perennial ryegrass and creeping bentgrass was observed (Figure 3 and 4), although significant at $P < 10\%$, only in the former ($r = 0.62$). This negative relationship has been demonstrated in earlier studies of NUE in Kentucky bluegrass, in which shoot NRA had the strongest negative effect on NUE (Jiang and Hull, 1998). A significant relationship between the NRA in roots and root NUE was not established in perennial ryegrass (Figure 6), but in creeping bentgrass, a significant negative correlation was demonstrated between root NUE and NRA in roots (Figure 5, $r = 0.60$, $P < 10\%$). A negative relationship was almost always observed between the NRA of tissues most active in assimilating N and the NUE of those tissues. These relationships suggest that the site of greatest N assimilation is also the site of N utilization, indicating that translocation of N to other organs is limited in these species. This appears to be confirmed when NUR and root NUE are correlated for creeping bentgrass (Figure 8),

but not for perennial ryegrass (Figure 9). The positive but not significant correlation observed between NUR and NUE in creeping bentgrass (Figure 7 and 8) indicates that less N is translocated to the shoots of this species or that NO_3^- uptake by roots is in better balance with root NRA so that C:N ratios remains constantly higher.

This increase in NUE as a function of increased NUR is consistent with earlier reports which associated the retention of N in roots with increased root growth and increased N interception (Chapin, 1980). A positive correlation between NUR and NUE has also been demonstrated in Kentucky bluegrass and was suggested to result from NUR stimulation of NRA (Jiang and Hull, 1998). Earlier investigations have demonstrated that transport of photosynthate from shoots to roots of perennial ryegrass is influenced by the form and level of N supply (Barta, 1976), and that a greater proportion of biomass is partitioned to roots at low N availability (Ericsson, 1988). Peuke *et al* (1994), suggested that this increase in root:shoot ratio is not only caused by export of sucrose from shoots to the roots, but also by export of N, which can exceed the import of N from roots to shoots. In a similar study, roots have been demonstrated to grow at different rates, in proportion to the amount of N which they were absorbing (Samuelson *et al.*, 1992).

The results demonstrated in this study are consistent with the basic hypothesis that NO_3^- assimilation concentrated in turfgrass roots correlates with greater biomass allocation to these roots. Since an increase in root biomass enables turfgrasses to acquire nutrients and water more effectively, these results indicate that nitrogen use efficiency of turfgrasses may be enhanced by selection and breeding of cultivars which assimilate NO_3^- predominantly within their roots.

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Table 1. National Turfgrass Evaluation Program mean quality ratings of perennial ryegrass (USDA ARS NTEP Final Report 1998 No. 99-10) and creeping bentgrass (USDA ARS NTEP Final Report 1994-97 No. 98-11) cultivars for Kingston, RI.

	Cultivar	Mean quality rating †
Perennial ryegrass	Palmer III	6.5
	Secretariat	6.3
	Calypso II	6.3
	Saturn II	5.8
	Manhattan III	5.5
	Morning Star	4.9
	Nighthawk	4.5
	Figaro	4.3
	Linn	2.9
	LSD (0.05)	0.9
Creeping bentgrass	L-93	6.6
	Penn G-2	6.3
	Providence	5.7
	Southshore	5.6
	Pennlinks	5.2
	SR 1020	4.9
	Penncross	4.0
	18th Green	3.9
	Seaside	3.4
	LSD (0.05)	0.7

†1-9; 9 = Ideal turf

Table 2. Differences in nitrate reductase activity rate of nine perennial ryegrass and nine creeping bentgrass cultivars.

Cultivar	Nitrate reductase activity		Potential total plant NRA root:shoot ratio
	Root	Shoot	
	μmol NO_2^- g FW^{-1} h^{-1}†		
Perennial ryegrass			
Palmer III	0.56a‡	1.95de	0.34a
Secretariat	0.50a	4.01a	0.13bc
Calypso II	0.39a	3.28abc	0.14bc
Saturn II	0.46a	3.49ab	0.13bc
Manhattan III	0.28a	2.48cd	0.08bc
Morning Star	0.38a	3.29abc	0.14c
Nighthawk	0.53a	2.70bcd	0.19bc
Figaro	0.39a	2.79bcd	0.14bc
Linn	0.38a	1.47e	0.28ab
Mean	0.43	2.83	
Creeping bentgrass			
L-93	0.48ab	1.45ab	0.35a
Penn G-2	0.49ab	1.27ab	0.41a
Providence	0.49ab	1.17ab	0.61a
Southshore	0.59ab	1.49ab	0.43a
Pennlinks	0.34ab	0.82b	0.42a
SR 1020	0.69a	1.61a	0.60a
Penncross	0.27b	1.32ab	0.32a
18th Green	0.35ab	0.89ab	0.53a
Seaside	0.38ab	0.95ab	0.43a
Mean	0.45	1.22	

† FW is fresh weight.

‡ Means followed by the same letter are not significantly different at $P \leq 0.05$.

Table 3. Differences in root and shoot biomass of nine perennial ryegrass and nine creeping bentgrass cultivars.

Cultivar	Root	Shoot	Total	Root: shoot ratio
		g FW^{-1} †		
Perennial ryegrass				
Palmer III	1.32a‡	6.60ab	7.92	0.22a
Secretariat	0.49d	3.74c	4.23	0.16ab
Calypso II	0.65cd	6.67ab	7.32	0.10b
Saturn II	0.91bc	6.36ab	7.27	0.16ab
Manhattan III	0.78bcd	4.33bc	5.11	0.13ab
Morning Star	0.99abc	7.78a	8.77	0.23a
Nighthawk	0.72cd	6.88ab	7.60	0.11b
Figaro	0.79bcd	4.67bc	5.46	0.19ab
Linn	1.16ab	7.61a	8.71	0.17ab
Mean	0.87	6.07	6.93	0.14
Creeping bentgrass				
L-93	2.53a	6.23a	8.76	0.42ab
Penn G-2	1.84ab	6.62a	8.46	0.28b
Providence	2.17ab	6.27a	8.44	0.36ab
Southshore	2.39a	6.40a	8.79	0.38ab
Pennlinks	1.31b	3.59b	4.90	0.38ab
SR 1020	2.28ab	6.12a	8.40	0.37ab
Penncross	1.62ab	4.64ab	6.26	0.36ab
18^{th} Green	1.62ab	3.33b	4.95	0.50a
Seaside	2.20ab	5.98a	8.18	0.33b
Mean	2.00	5.46	7.46	0.37

† FW is fresh weight.

‡ Means followed by the same letter are not significantly different at $P \leq 0.05$.

Table 4. Differences in N content of nine perennial ryegrass and nine creeping bentgrass cultivars.

	Cultivar		N content	
			mg N g DW^{-1} †	
			Root	Shoot
Perennial ryegrass	Palmer III		12.2a‡	22.5c
	Secretariat		13.6a	32.4a
	Calypso II		14.6a	24.8bc
	Saturn II		12.4a	25.8bc
	Manhattan III		13.7a	24.8bc
	Morning Star		15.1a	28.3ab
	Nighthawk		15.5a	27.7abc
	Figaro		9.53a	26.9bc
	Linn		13.0a	26.0bc
		Mean	13.3	26.6
Creeping bentgrass	L-93		13.9abc	23.0abc
	Penn G-2		14.6abc	23.9abc
	Providence		16.9a	23.3abc
	Southshore		15.4ab	24.0abc
	Pennlinks		11.4bc	26.2a
	SR 1020		13.8abc	25.0ab
	Penncross		11.1bc	18.4bc
	18^{th} Green		10.4bc	26.7a
	Seaside		9.34c	17.3c
		Mean	12.9	23.1

† DW is dry weight.

‡ Means followed by the same letter are not significantly different at $P \leq 0.05$.

Table 5. Differences in N use efficiency of nine perennial ryegrass and nine creeping bentgrass cultivars.

	Cultivar		N use efficiency	
			g DW mg N^{-1} †	
			Root	Shoot
Perennial ryegrass	Palmer III		82.1a‡	44.7a
	Secretariat		76.3a	30.8c
	Calypso II		69.9a	40.6ab
	Saturn II		80.3a	38.8abc
	Manhattan III		73.4a	41.1ab
	Morning Star		71.4a	35.5bc
	Nighthawk		77.5a	36.5abc
	Figaro		94.3a	37.5abc
	Linn		81.6a	38.3abc
		Mean	78.5	38.2
Creeping bentgrass	L-93		71.6ab	42.7bc
	Penn G-2		69.7ab	41.8bc
	Providence		60.0b	44.2bc
	Southshore		67.3ab	43.0bc
	Pennlinks		102a	40.0c
	SR 1020		75.3ab	40.7bc
	Penncross		93.1ab	55.4ab
	18^{th} Green		73.0ab	39.2c
	Seaside		98.1ab	59.6a
		Mean	78.9	45.2

† DW is dry weight.

‡ Means followed by the same letter are not significantly different at $P \leq 0.05$.

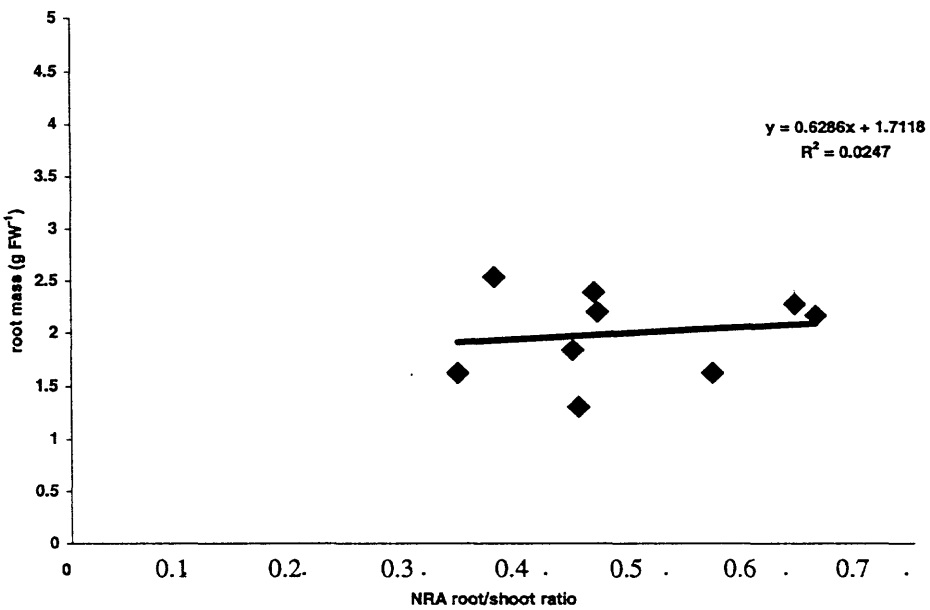

Figure 1. Relationship between whole plant NRA root:shoot ratio and root biomass in nine creeping bentgrass cultivars.

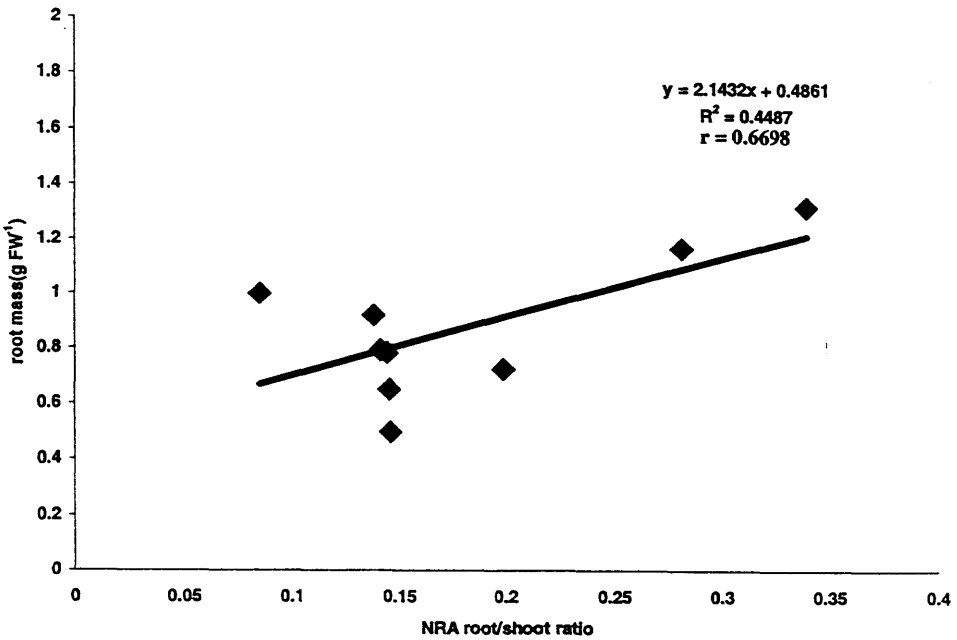

Figure 2. Relationship between whole plant NRA root:shoot ratio and root biomass in nine perennial ryegrass cultivars.

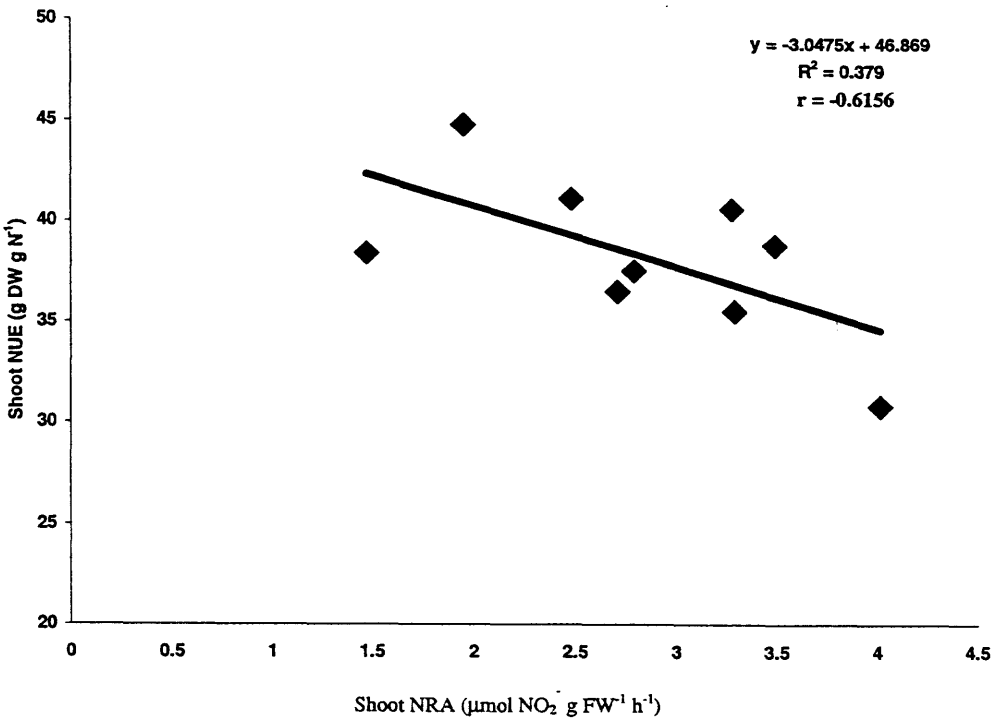

Figure 3. Relationship between shoot NUE and NRA in nine perennial ryegrass cultivars.

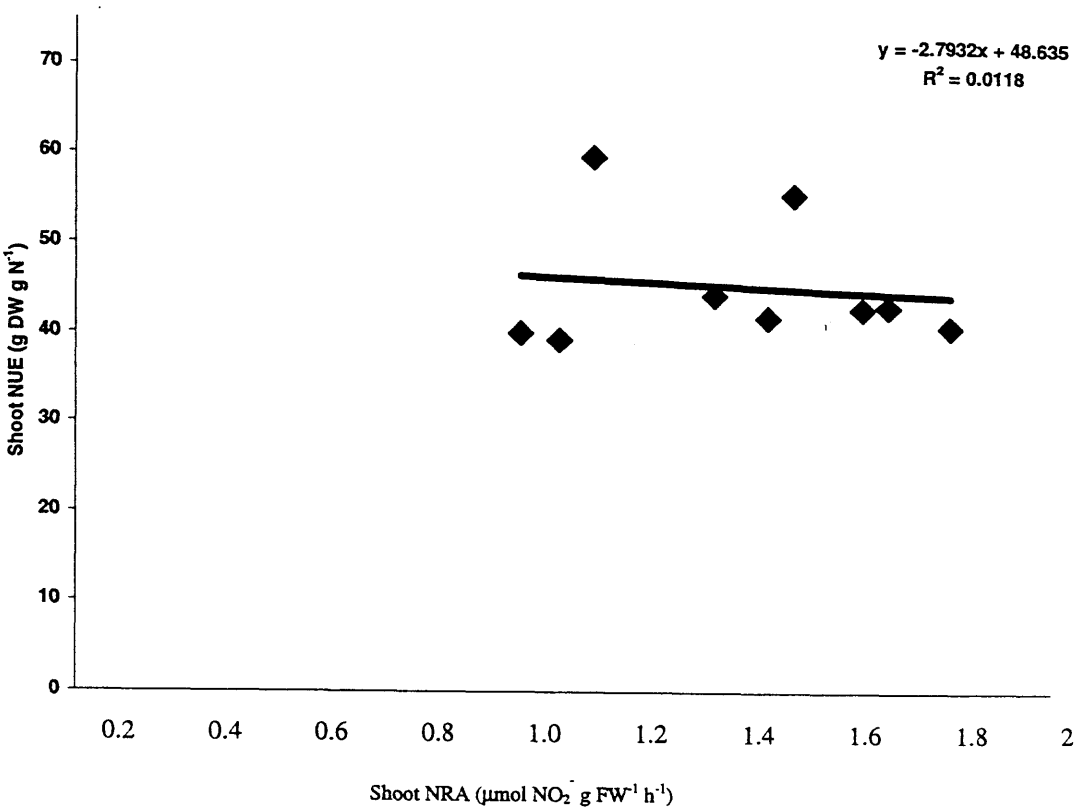

Figure 4. Relationship between shoot NUE and NRA in nine creeping bentgrass cultivars.

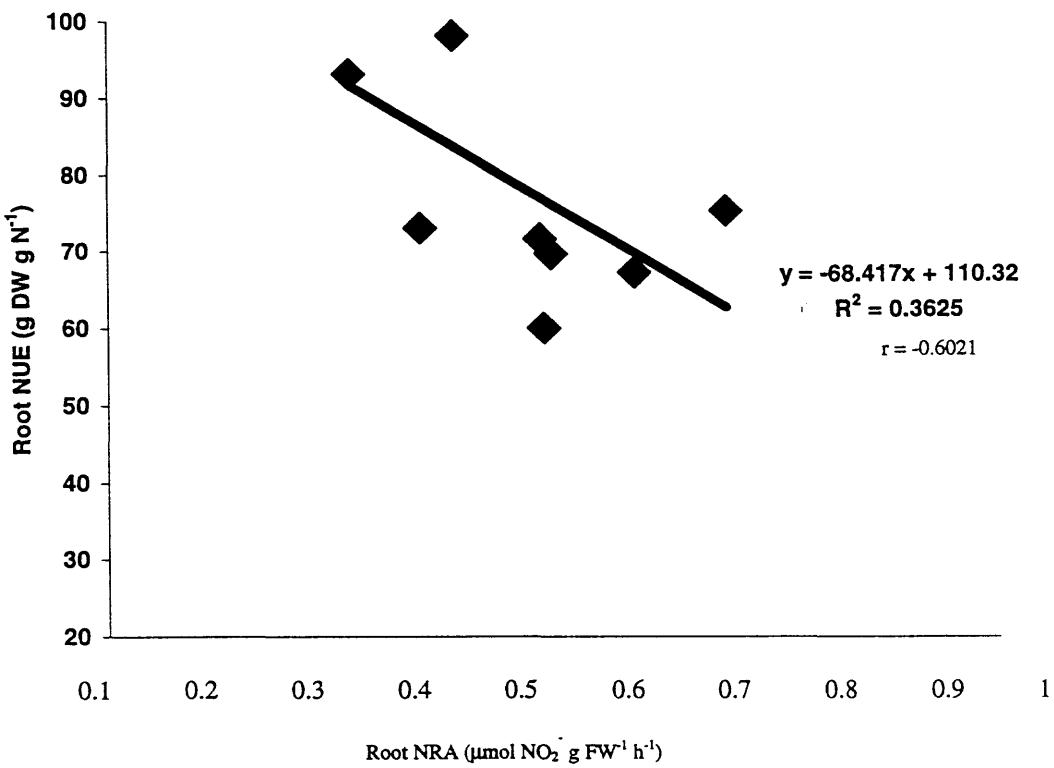

Figure 5. Relationship between root NUE and NRA in nine creeping bentgrass cultivars

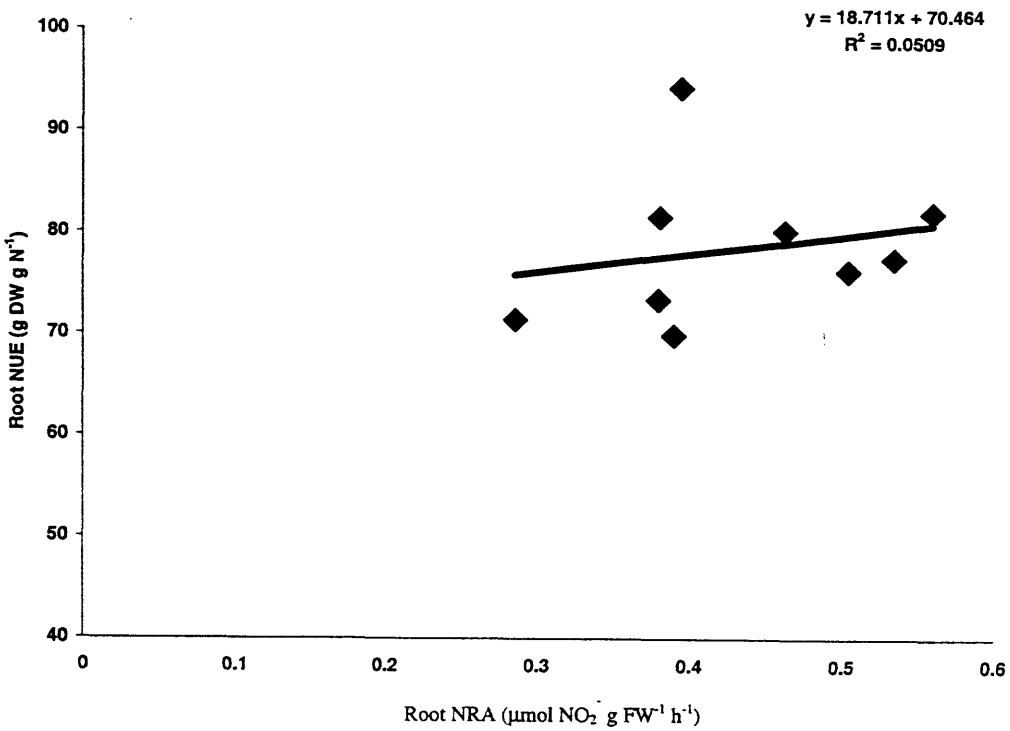

Figure 6. Relationship between root NUE and NRA in nine perennial ryegrass cultivars

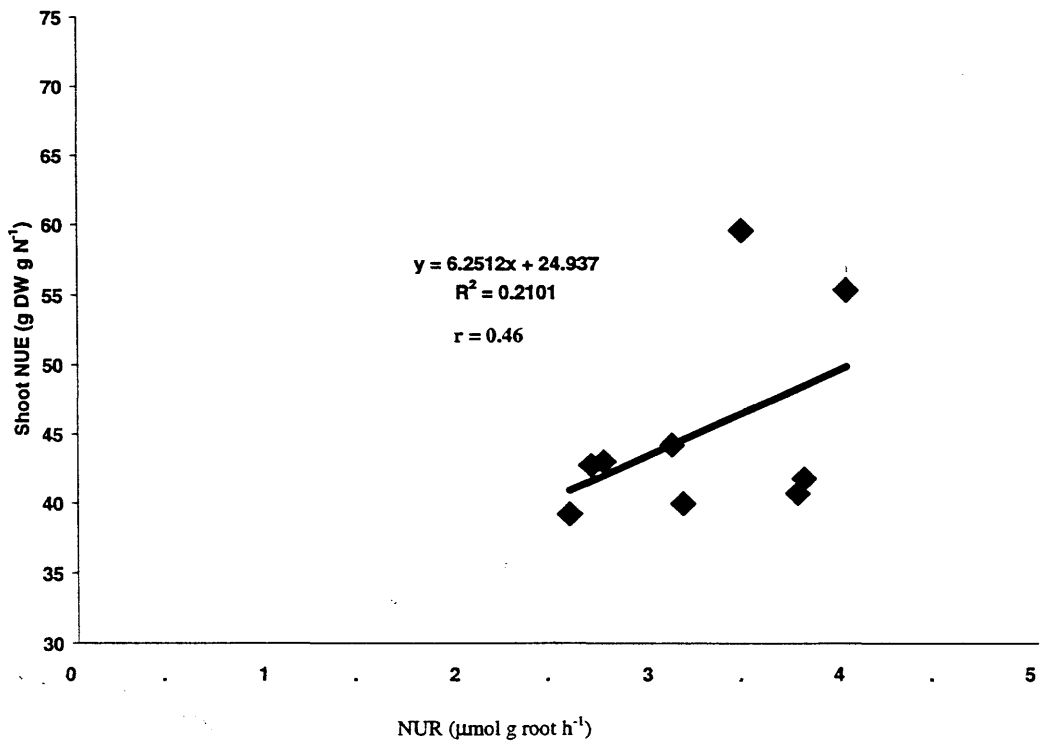

Figure 7. Relationship between NUR and shoot NUE in nine creeping bentgrass cultivars

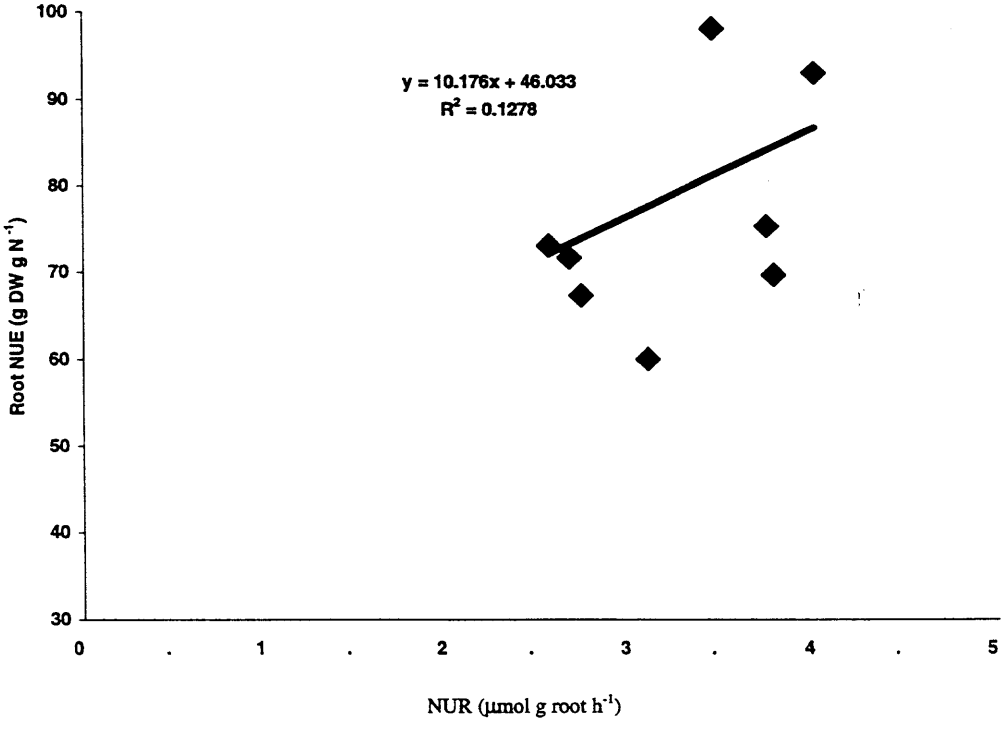

Figure 8. Relationship between NUR and root NUE in nine creeping bentgrass cultivars

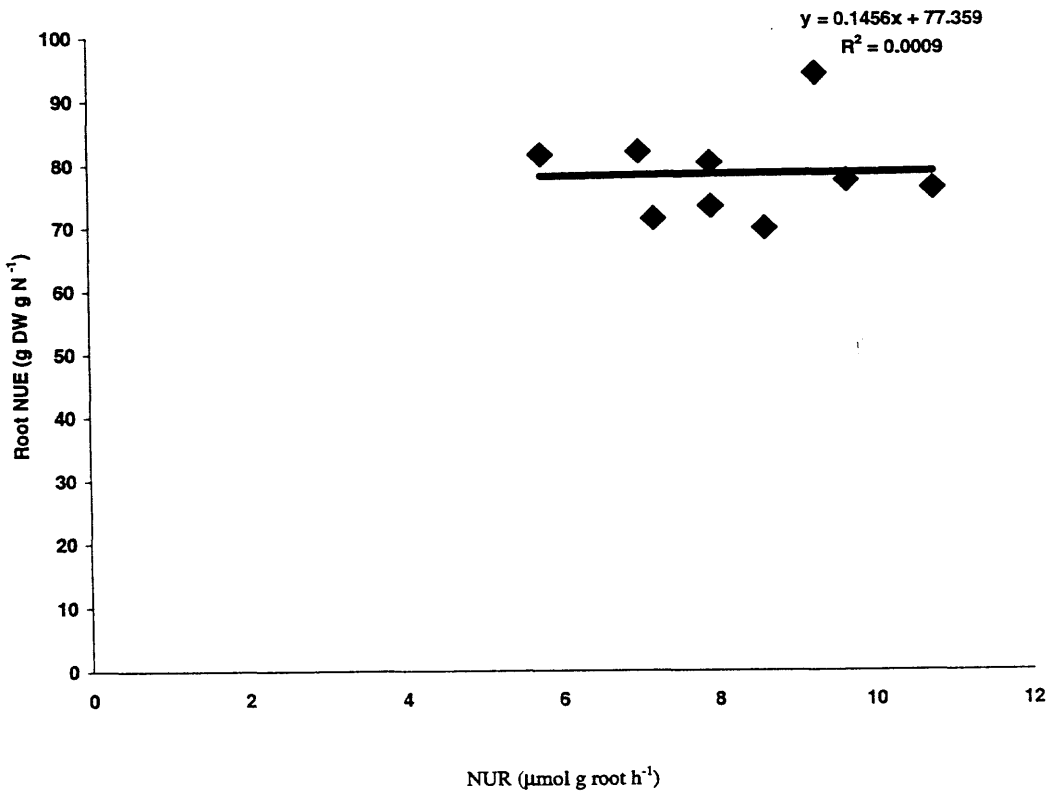

Figure 9. Relationship between NUR and root NUE in nine perennial ryegrass cultivars

APPENDIX A

LITERATURE REVIEW

Nitrate (NO_3^-) uptake by roots is an active process, which involves specific ion transport proteins (Warner and Huffaker, 1989). These proteins function either as an $OH^-(HCO_3^-)/ NO_3^-$ antiporter (Touraine *et al*, 1992) or as a $2H+/ NO_3^-$ symporter (Glass *et al*, 1992). Nitrate uptake is carried out by four different systems (Siddiqi *et al.*, 1990; Alsam *et al.*, 1992; Liu *et al.*, 1999). At high external NO_3^- concentrations, linear constitutive and inducible low affinity systems operate, facilitated by the same protein. At low external NO_3^- concentrations, two high affinity saturable systems operate: One (Km for NO_3^- 7 μm) is constitutive and the second (Km for NO_3^- 15-34 μm) is induced by NO_3^- (Alsam *et al.*, 1992). The level of expression of the inducible high affinity system is subject to negative feedback regulation dependent on the nitrogen status of the plant (Siddiqi *et al.*, 1990).

Nitrate uptake in turfgrasses at low external NO_3^- concentrations has been shown to follow Michaelis-Menten saturation kinetics (Cisar, 1989; Liu *et al.*, 1993). The transport affinity for NO_3^- (Km) and its subsequent maximum uptake rate (Vmax) values have been shown to vary among species and among cultivars within the same species (Liu *et al.*, 1993). Diurnal patterns of NO_3^- uptake have been observed to result from variations in availability of metabolic energy (Pearson *et al.*, 1981; Hansen, 1980), NO_3^- accumulation (Mattsson *et al.*, 1991; Siddiqi *et al.*, 1990), and amino acid transport between roots and shoots (Muller *et al.*, 1995).

The reduction of NO_3^- to ammonium (NH_4^+) in either roots or shoots involves a two step process: Nitrate (oxidation state +5) reduction to nitrite (NO_2^-) (oxidation state +3) in the cytosol, followed by NO_2^- reduction to NH_4^+ (oxidation state –3) in the plastids. This highly reduced form of nitrogen is assimilated into amino acids, which are then transported to sites of metabolic activity (Simpson 1986). The reduction of NO_3^- to NH_4^+ is therefore an energy-consuming process, requiring 8 mole equivalents of electrons (or 4 moles NAD(P)H) per mol of NO_3^- reduced. In order to maintain a C/N ratio of 10 (normal for herbaceous plants) about 20% of photosynthetically produced reducing equivalents are required for NO_3^- reduction to occur (Kaiser and Huber, 1994). In addition to these energy requirements, the primary product of NO_3^- reduction, NO_2^-, is cytotoxic and regarded to be mutagenic as it diazotizes amino groups. HNO_2 is also a weak acid that, in its undissociated form, can easily penetrate biomembranes, leading to acidification of cells or of subcellular compartments. The reduction of NO_3^- by the enzyme nitrate reductase (NR) is, therefore, considered to be a control point in the assimilatory pathway of NO_3^--N (Kaiser and Huber, 1994) and will be the central focus of this study.

First identified in 1953, (Evans and Nason), the role of NR has been the subject of intensive research. The reactions catalyzed by NR have been understood best by viewing the enzyme as a redox system with an internal electron transport chain (Campbell, 1999). The enzyme is a homodimer composed of two identical 100-115 kDa subunits, each containing one equivalent of flavin adenine dinucleotide (FAD), heme-Fe, and Mo-molybdopterin (Mo-MPT). The reducing power for NR (EC 1.6.6.1) is supplied by NADH or by NAD(P)H in the case of the bispecific NR (EC 1.6.6.2.).

Three isoenzymes of NR have been isolated: constitutive NADH and NAD(P)H forms and an inducible NADH-specific form (Streit *et al.*, 1987). The major source of cytosolic NADH for NR is a malate/oxaloacetate (OAA) shuttle that operates in green leaves between the chloroplast and cytosol (Heldt and Flugge, 1992). In this shuttle, NADPH produced by photosystem I (PSI) in the chloroplast mediates the reduction of OAA to malate (OAA + NADPH-→ malate + NADP) that is transferred via a malate/OAA anti-transporter to the cytosol. In the cytosol, malate oxidation to OAA is coupled to the reduction of of NAD (Malate + NAD → OAA + NADH). The OAA is cycled back to the chloroplast in exchange for another malate, and the NADH produced can be used in the reduction of NO_3^- (Oaks, 1994 Canvin and Woo, 1979). The generation of mitochondrial NADH in anaerobic conditions may also be transported to the cytosol via a malate/oxaloacetate shuttle (Naik and Nicholas, 1986).

NR is regulated by several mechanisms including protein turnover, transcriptional or post-translational modification. The NR protein has been shown to be degraded rapidly with a half-life of a few hours (Li and Oaks, 1993). Nitrate supply has beeen shown to promote an increase in the steady state level of mRNA encoding NR in a variety of plant species (Cheng *et al.*, 1991; Crawford *et al.*, 1986). In light-grown plants, the level of NR decreases after two days in darkness and increases rapidly after return to light (Deng *et al.*,1991; Cheng *et al.*, 1991). Diurnal vartiation in NR activity (NRA) has been recognized for some time (Lillo, 1984), with a major peak in NRA occuring at the end of the dark period (Galangau *et al.*, 1988). A circadian rythym in the level of NR mRNA is maintained in continuous light but disappears slowly in the dark and it has been observed that the addition of fructose,

glucose or sucrose induces the expression of NR, whereas addition of glutamate or glutamine results in its down-regulation (Deng *et al.*, 1991). NRA has been observed to decline when the rate of photosynthetic CO_2 assmilation was low and oxygen levels were high (Kaiser and Brendle-Behnisch, 1991) and increase in response to oxygen deficiency (Glaab and Kaiser, 1993). The addition of ATP and Mg^{2+} leads to inactivation of NR *in vitro* and can be reversed upon removal of ATP (Kaiser and Spill, 1991). It has now been demonstrated that NR is subject to covalent modification by phosphorylation resulting in inactivation by ATP (Huber *et al.*, 1992). Thus, protein phosphorylation represents an important mechanism for the control of carbon and nitrogen partitioning in higher plants (Kaiser and Huber, 1994).

Analysis of xylem sap indicated that the shoots of perennial ryegrass were the major site of NO_3^- reduction and assimilation (Bowman and Paul ,1988). In several grass species, the partial removal of shoots has been shown to reduce the root capacity for NO_3^- reduction and assimilation by reducing the partitioning of photosynthate to the roots (Oaks and Hirel, 1985; Radin, *et* al., 1978). This poses a problem for intensively managed turfgrasses, since partial shoot removal occurs regularly thereby increasing reliance upon root-centered NO_3^- reduction and assimilation for its N supply.

APPENDIX B

Suppliers of selected perennial ryegrass and creeping bentgrass cultivars.

Perennial ryegrass		**Creeping Bentgrass**	
Cultivar	**Supplier**	**Cultivar**	**Supplier**
Palmer III	Lofts New England 20 Beck Rd. Arlington, MA 02174	L-93	Lofts Great Western Seed Co. 810 Jackson St. Albany, OR 97321
Secretariat	Grassland West 908 Port Dr. Clarkson, WA 99403	Penn G-2	Lesco 2005 Lake Rd. Rocky River, OH 44116
Calypso II	Roberts Seed Co. 33095 Hwy 99E Tangent, OR 97389	Providence	Seed Research of OR 27630 Llewellyn Rd. Corvallis, OR 97333
Saturn II	Zajac Performance Seeds 175 Scaravel Hill Rd. Albany, OR 97321	Southshore	Lofts Great Western Seed Co. 810 Jackson St. Albany, OR 97321
Manhattan III	Turf Merchants 33390 Tangent Loop Tangent, OR 97389	Pennlinks	Allens Seed Store Inc. 693 South County Trail Exeter, RI 02882
Morning Star	Pennington Seed Inc. of OR 270 Hansard Ave. Lebanon, OR 97355	SR 1020	Seed Research of OR 27630 Llewellyn Rd. Corvallis, OR 97333
Nighthawk	Allens Seed Store Inc. 693 South County Trail Exeter, RI 02882	Penncross	Allens Seed Store Inc. 693 South County Trail Exeter, RI 02882
Figaro	DLF-Trifolium Inc. 5757 NE Hwy 20 Corvallis, OR 97330	18th Green	Zajac Performance Seeds 175 Scaravel Hill Rd. Albany, OR 97321
Linn	Pennington Seed Inc. of OR 270 Hansard Ave. Lebanon, OR 97355	Seaside	Allens Seed Store Inc. 693 South County Trail Exeter, RI 02882

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