GENETIC VARIATION IN NITRATE METABOLISM BY COOL-SEASON TURFGRASSES

BY

JOHN T. BUSHOVEN

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE

REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

PLANT SCIENCES

UNIVERSITY OF RHODE ISLAND

1999

MASTER OF SCIENCE THESIS

OF

JOHN T. BUSHOVEN

APPROVED:

Thesis Committee

Major Professor

DEAN OF THE GRADUATE SCHOOL

UNIVERSITY OF RHODE ISLAND

1999

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.

ACKNOWLEDGMENTS

I would like to express my sincerest gratitude to Dr. Richard J. Hull not only for his guidance and insight for this project but also for his thoughtful discussions on the role of plant physiology in the "molecular age". I would also like to thank Dr. W. Michael Sullivan for sharing his knowledge of statistical applications in the plant sciences. Dr. Bridgett Ruemmele for her extensive knowledge of turfgrass science and Dr. Jose Amador for his ecological perspective of the plant/soil system. My thanks to all fore mentioned for serving as members of my graduate committee and their review of this manuscript. My thanks also to Dr. Joel Chandlee for serving as committee chair and providing his expertise in molecular genetics. I thank Dr. Zhongchun Jiang for his expertise in the development of and modifications to, the procedures utilized for this project and also for the many long insightful discussions. I would also like to thank the United States Golf Association for their financial support of this project.

My thanks to the faculty and staff of the University who shared their expertise and facilities. My thanks to Raina Huebner, Neil Valdepeñas, Heather Ford, Heather Taylor, MariCruz Merino and Kathleen Mc Pherson for their work in the laboratory.

Most importantly, I would like to express my deepest thanks to my beautiful wife Karin for her endless love and support during these last two years and for the many long days and nights spent together as she shared with me her brilliant intellect.

And finally, I dedicate this manuscript to the memory of my late father, who taught me all the most important of life's lessons.

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 $P \leq 0.05$.

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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