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.

TABLE OF CONTENTS

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

LIST OF TABLES

MANUSCRIPT I

MANUSCRIPT II

LIST OF FIGURES

MANUSCRIPT II

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.

REFERENCES

Anderson, E.L., E.J. Kamprath, and R.H. Moll. 1984. Nitrogen fertility effects on accumulation, remobilization, and partitioning of N and dry matter in corn genotypes differing in prolificacy. Agronomy J. 76:397-404.

Andrews, M., J.D. Morton, M. Lieffering, and L. Bisset. 1992. The partitioning of nitrate assimilation between root and shoot of a range of temperate cereals and pasture grasses. Ann. Bot. 70:271-276.

Aslam, M., and A. Oaks. 1975. Effect of glucose on the induction of nitrate reductase in corn roots. Plant Physiol. 56:634-639.

Barta, A.L. 1976. Transport and distribution of $^{14}CO_2$ assimilate in Lolium perenne in response to varying nitrogen supply to halves of a divided root system. Physiol. Plant. 38:48-52.

Beard, J.B. 1973. Turfgrass science and culture. Prentice-Hall, Englewood Cliffs, NJ.

Bertauski, A.F., J.M. Swiader, and D.J. Wehner. 1997. Dry weight production and nitrogen efficiency traits in Kentucky bluegrass cultivars in nutrient solution and soil. Crop Sci. 37:1548-1553.

Botrel, A., and W.M. Kaiser. 1997. Nitrate reductase activation state in barley roots in relation to the energy and carbohydrate status. Planta. 204:496-501.

Cassman, K.G., M.J. Kropff, and J. Gaunt. 1993. Nitrogen use efficiency of rice reconsidered: what are the key constraints? Plant Soil. 155:359-362.

Champigny, M-L., and C. Foyer. 1992. Nitrate activation of cytosolic protein kinases diverts photosynthetic carbon from sucrose to amino acid biosynthesis. Basis for a new concept. Plant Physiol. 100:7-12.

Chapin III, S.F. 1980. The mineral nutrition of wild plants. Annu. Rev. of Ecology and Systematics. 11:233-260.

Cheeseman. J.M., R. Barreiro, M. Lexa. 1996. Plant growth modeling and the integration of shoot and root activities without communicating messenger-opinion. Plant and Soil. 185:51-64

Cisar, J.L., R.J. Hull, and D.T. Duff. 1989. Ion uptake kinetics of cool season turfgrasses. P. 233-235. In H. Ide and H. Takatoh (ed.) Proc. Intl. Turfgrasses Res. Conf., 6th, Tokyo. July 31-Aug.4, 1989.

Eastin, E.F. 1978. Total nitrogen determination for plant material containing nitrate. Anal. Bichem. 85:591-594.

Ericsson, T. 1988. Growth and shoot:root ratio of seedlings in relation to nutrient availability. Plant and Soil. 168/169:205-214.

Fiez, T.E., W.L. Pan, and B.C. Miller. 1995. Nitrogen use efficiency of winter wheat among landscape positions. Soil Sci. Soc. Am. J. 59:1666-1671.

Gascho, G.J., D.L. Anderson, and H.Y. Ozaki. 1986. Cultivar dependent sugarcane response to nitrogen. Agron J. 78:1064-1069.

Gonzalez Ponce, R., M.L. Salas, and S.C. Mason. 1993. Nitrogen use efficiency by winter barley under different climatic conditions. J. Plant Nutr. 16:1249-1261.

Hageman, R.H., and A.J. Reed. 1980. Nitrate reductase from higher plants. Methods Enzymol. 69:270-280.

Hageman, R.H., and R.J. Lambert. 1988. The use of physiological traits for corn improvement. *In* G. F. Sprague, J. W. Dudley (eds.), Corn and Corn Improvement. Agronomy Monogr. No. 18. P. 431-461 ASA-CSSA-SSSA. Madison, WI.

Harris, P., and W.J. Whittington. 1983. Effects of temperature, levels of nitrate supply and duration of light and growth on nitrate reductase activity in *Agrostis tenuis* and *Agrostis stolonifera*. New Phytol. 93:193-201.

Hoagland, D.R., and D.I. Arnon. 1950. The water-culture method for growing plants without soil. Calif. Agr. Exp. Stn. Circular 347:32.

Hocking, P.J., C.P. Meyer. 1991. Effects of enrichment and nitrogen stress on growth, and partitioning of dry matter and nitrogen in wheat and maize. Aust. J. Plant Physiol. 18:339-356.

Hull, R.J., and H. Liu. 1995. Nitrogen accumulation in a turf-soil ecosystem as a function of N source and grass species. Agronomy Abstracts. 87:127.

Jeschke, W.D., and J.S. Pate. 1991. Modeling of the partitioning, assimilation and storage of nitrate within root and shoot organs of castor bean (*Rhicinus communis* L.). J. Exp. Bot. 42:1091-1103.

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

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

Levin S.A, H.A. Mooney, and C. Field. 1989. The dependence of plant root:shoot ratios on internal nitrogen concentrations. Ann. Bot. 64:71-75.

Lexa, M., and J.M. Cheeseman. 1997. Growth and nitrogen relations in reciprocal grafts of wild-type and nitrate reductase-deficient mutants of pea (*Pisum sativum* L. var. Juneau). J. Exp. Bot. 48:1241-1250.

Li, X-Z., and A. Oaks. 1993. Induction and turnover of nitrate reductase in *Zea mays*: Influence of NO_3^-. Plant Physiol. 102: 1251-1257.

Lillo, C. 1994. Light regulation of nitrate reductase in green leaves of higher plants. Physiol. Plant. 90:616-620.

Liu , H., R.J. Hull, and D.T. Duff. 1993. Comparing cultivars of three cool season turfgrasses for nitrate uptake kinetics and nitrogen recovery in the field. P. 546-552. *In* R.N. Carrow et al (ed.). Inter. Turfgrass Soc. Res. J. vol 7. Intertec Publishing Corp., Overland Park KS.

Microsoft Excel 97. 1996. Microsoft Corp.

Nelson, S.H., and F.W. Sosulski. 1984. Amino acid and protein content of *Poa pratensis* as related to nitrogen application and color. Can. J. Plant Sci. 64:691-697.

Osborne, B.A., and W.J. Whittington. 1981. Eco-physiological aspects of inter-specific and seasonal variation in nitrate utilization in the genus *Agrostis*. New Phytol. 87:595-614.

Pueke, A.D., W. Hartung, and W.D. Jeschke. 1994. The uptake and flow of C, N and ion between roots and shoots in *Ricinus communis* L. II. Grown with low or high nitrate supply. J. Exp. Bot. 45:733-740.

Radin, J.W. 1978. A physiological basis for the division of nitrate assimilation between roots and leaves. Plant Sci. Lett. 13:21-25.

Rao, S.C., and T.H. Dao. 1996. Nitrogen placement and tillage effects on dry matter and nitrogen accumulation and redistribution in winter wheat. Agronomy J. 88:365-371.

Samuelson, M.E., L. Eliasson, and C.M. Larson. 1992. Nitrate regulated and cytokinin responses of seminal roots of barley. Plant Physiol. 98:309-315.

SAS Institute Inc. 1990. SAS/STAT User's guide. Version 6. 4th ed. SAS Institute, Cary, NC.

Scheible W-R, A. Gonzales-Fontes, R. Morcuende, M. Lauerer, M. Geiger, J. Glabb, A. Gojon, E-D. Schultz, and M. Stitt. 1997. Tobacco mutants with a decreased number of functional *nia* genes compensate by modifying the diurnal regulation of transcription, pot-translational modification and turnover of nitrate reductase. Planta. 203:304-319.

Simpson, R.J., H. Lambers, and M.J. Dalling. 1982. Translocation of nitrogen in a vegetative wheat plant (*Triticum aestivum*). Physiol. Plant. 56:11-17.

Smith, S.M., and D.B. James. 1982. Control and variation of in vivo nitrate reductase activity in *Lolium perenne* L> cv. S24. II. Variation of activity associated with different light regimes. Plant and Soil. 68:231-239.

Snell, F.D., and C.T. Snell. 1949. P. 84. Colorimetric Methods of Analysis. Van Nostrand. Princeton, NJ

Sullivan, W.M., Z. Jiang, and R.J. Hull. 1999. Root morphology and its relationship with nitrate uptake in Kentucky bluegrass. Crop Sci. (in press).

Van Quy, L., and M.L. Champigny. 1992. NO_3^- enhances the kinase activity for phosphorylation of phosphoenolpyruvate carboxylase and sucrose phosphate synthase proteins in wheat leaves. Plant Physiol. 99:344-347.

Van Quy, L., T. Lamaze, and M.L. Champigny. 1991. Short-term effects of nitrate on sucrose synthesis in wheat leaves. Planta. 185:53-57.

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 $P \leq 0.05$.

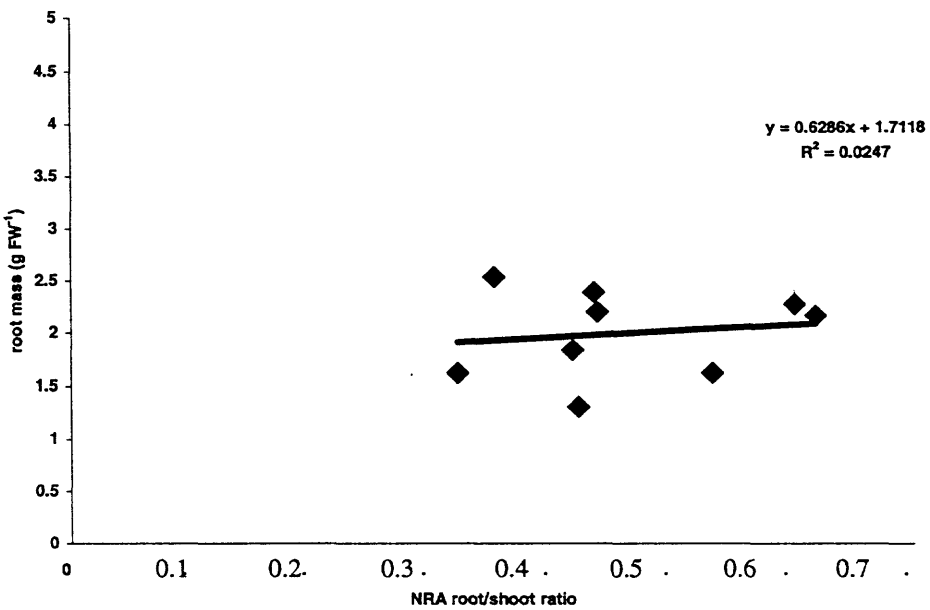

Figure 1. Relationship between whole plant NRA root:shoot ratio and root biomass in nine creeping bentgrass cultivars.

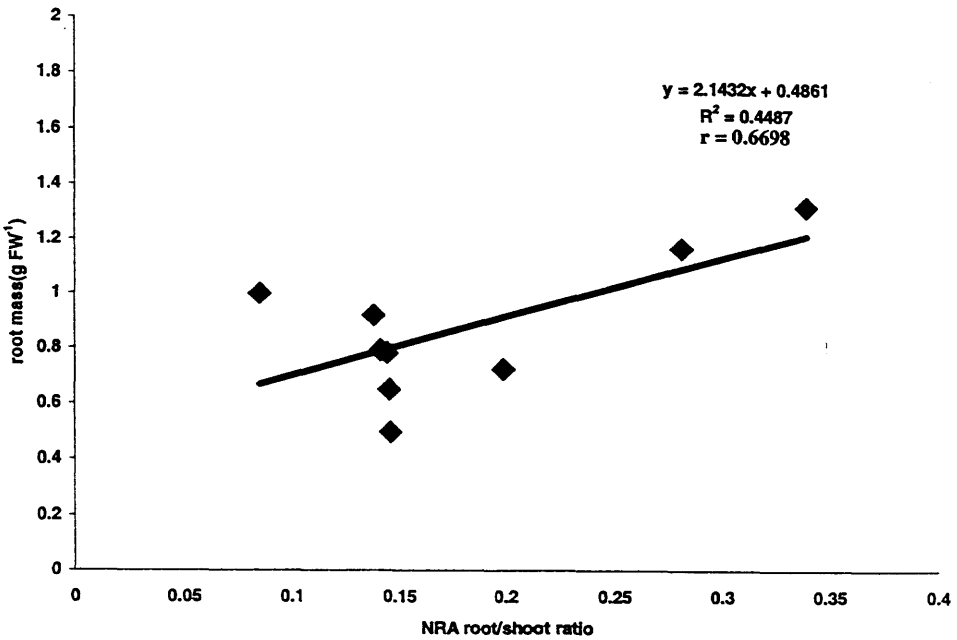

Figure 2. Relationship between whole plant NRA root:shoot ratio and root biomass in nine perennial ryegrass cultivars.

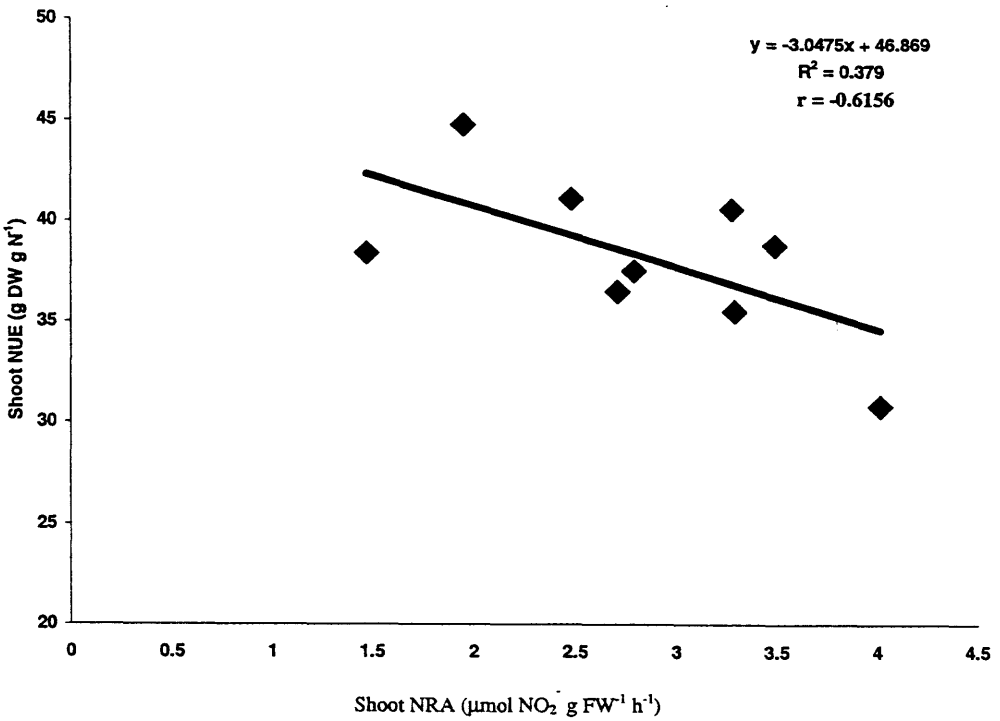

Figure 3. Relationship between shoot NUE and NRA in nine perennial ryegrass cultivars.

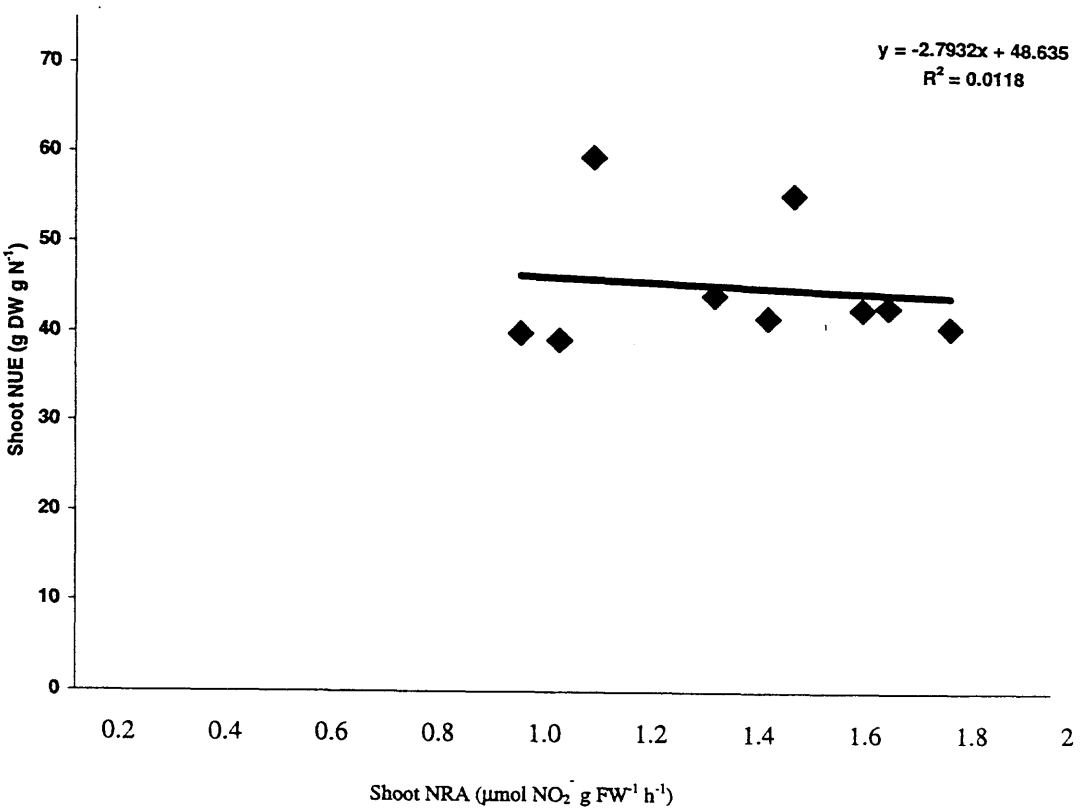

Figure 4. Relationship between shoot NUE and NRA in nine creeping bentgrass cultivars.

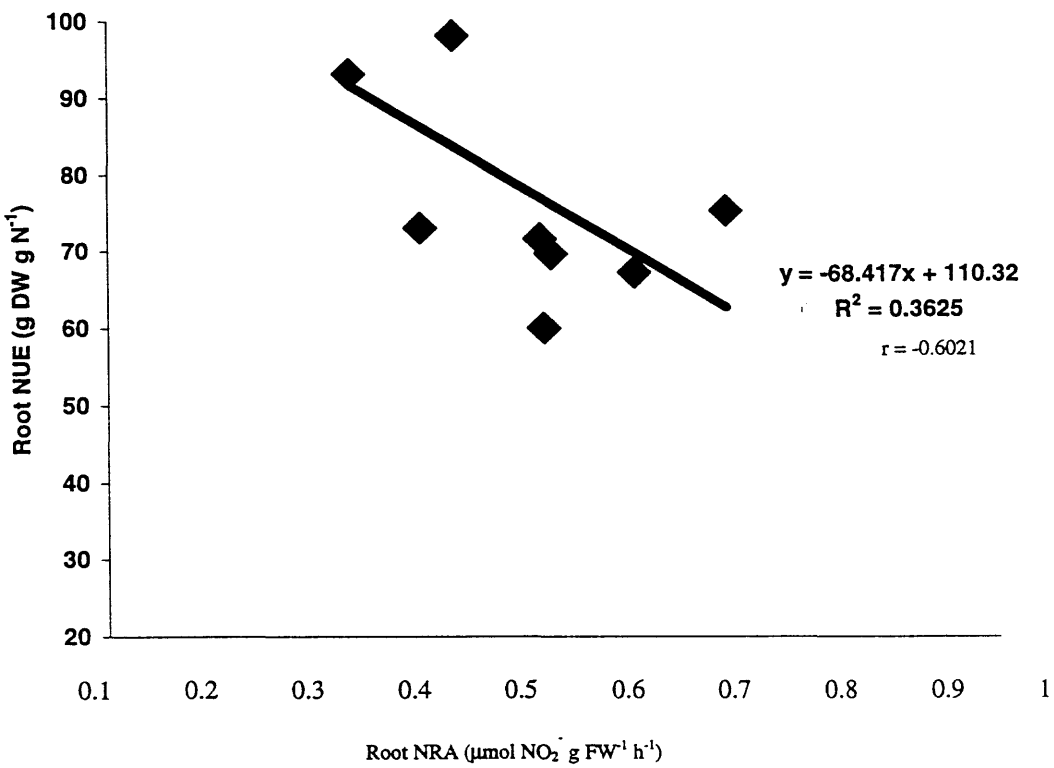

Figure 5. Relationship between root NUE and NRA in nine creeping bentgrass cultivars

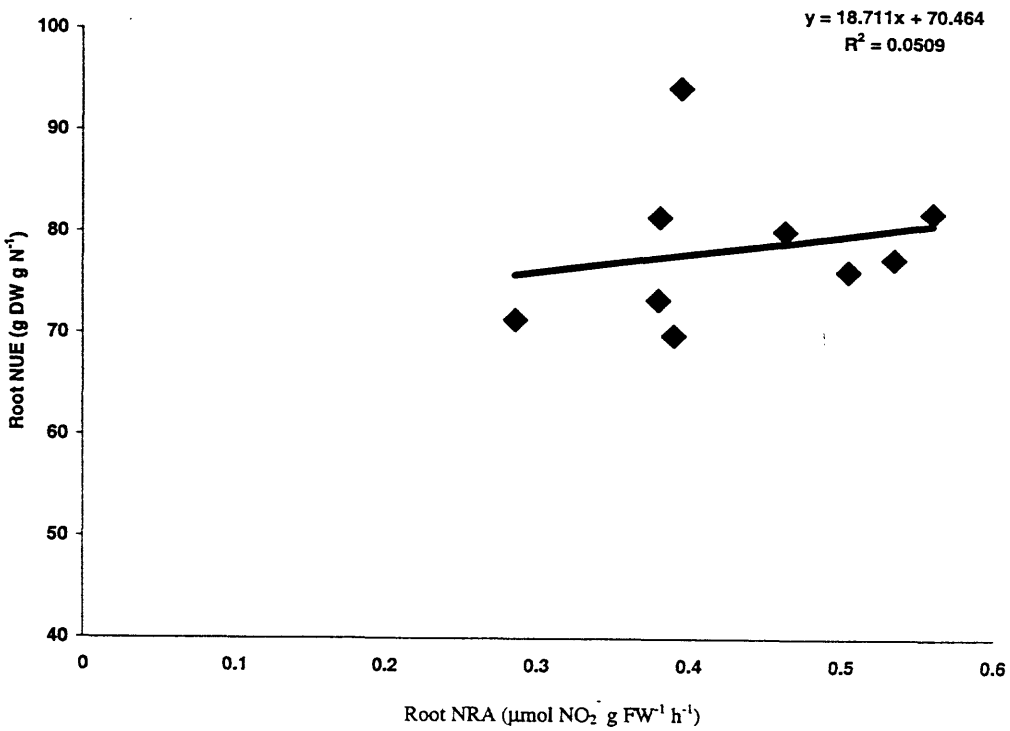

Figure 6. Relationship between root NUE and NRA in nine perennial ryegrass cultivars

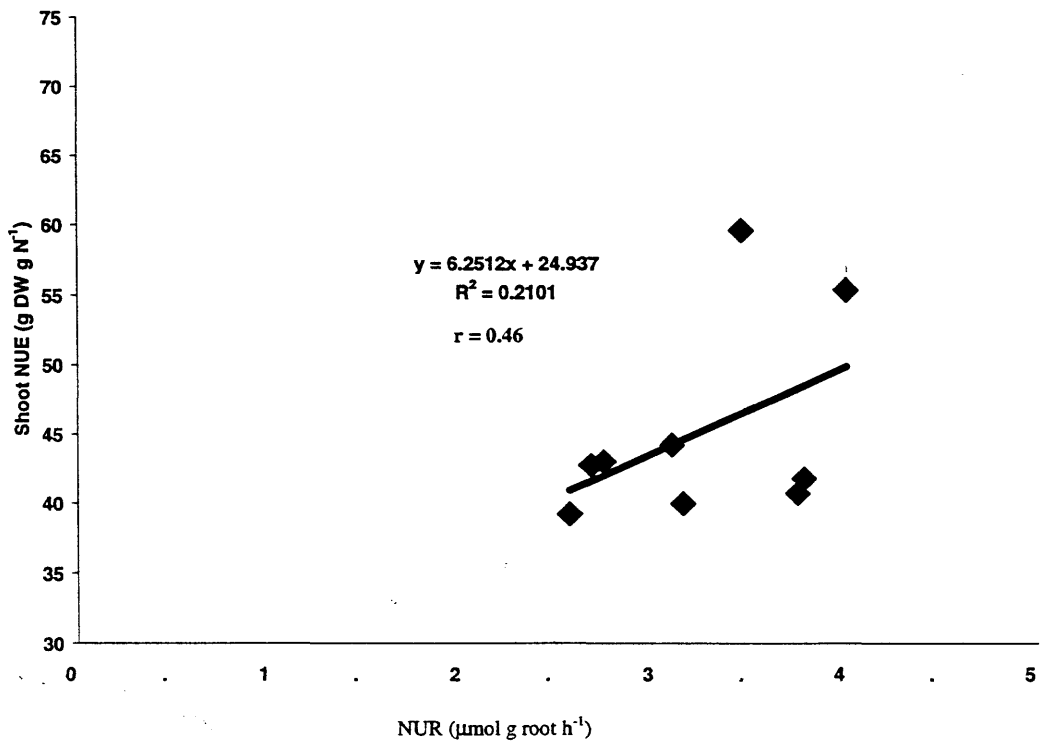

Figure 7. Relationship between NUR and shoot NUE in nine creeping bentgrass cultivars

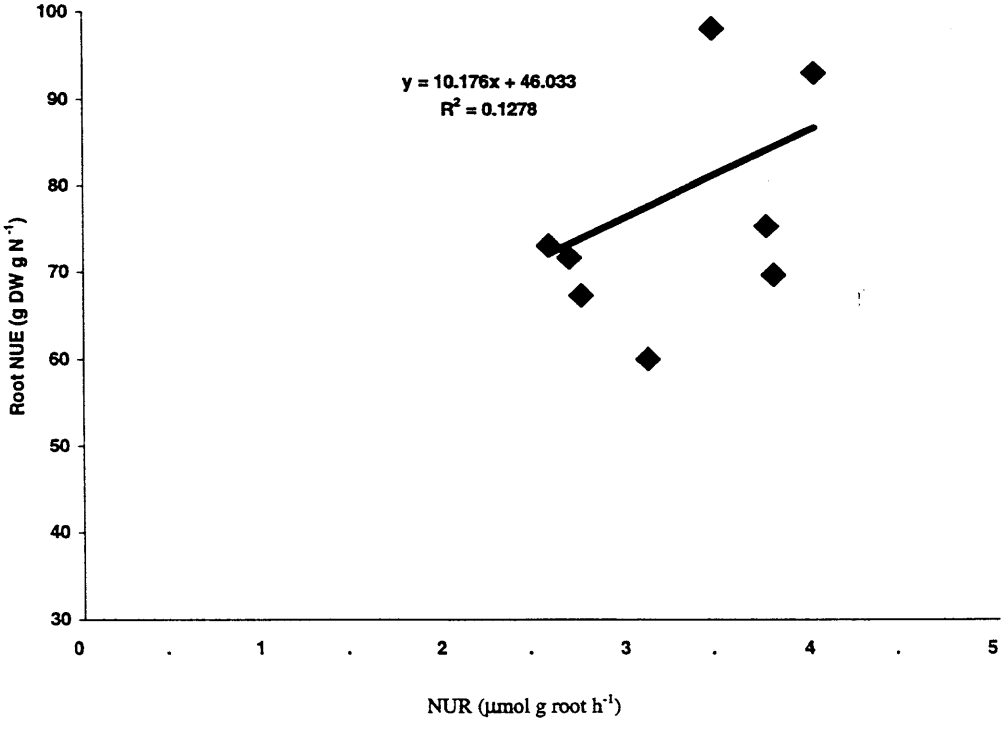

Figure 8. Relationship between NUR and root NUE in nine creeping bentgrass cultivars

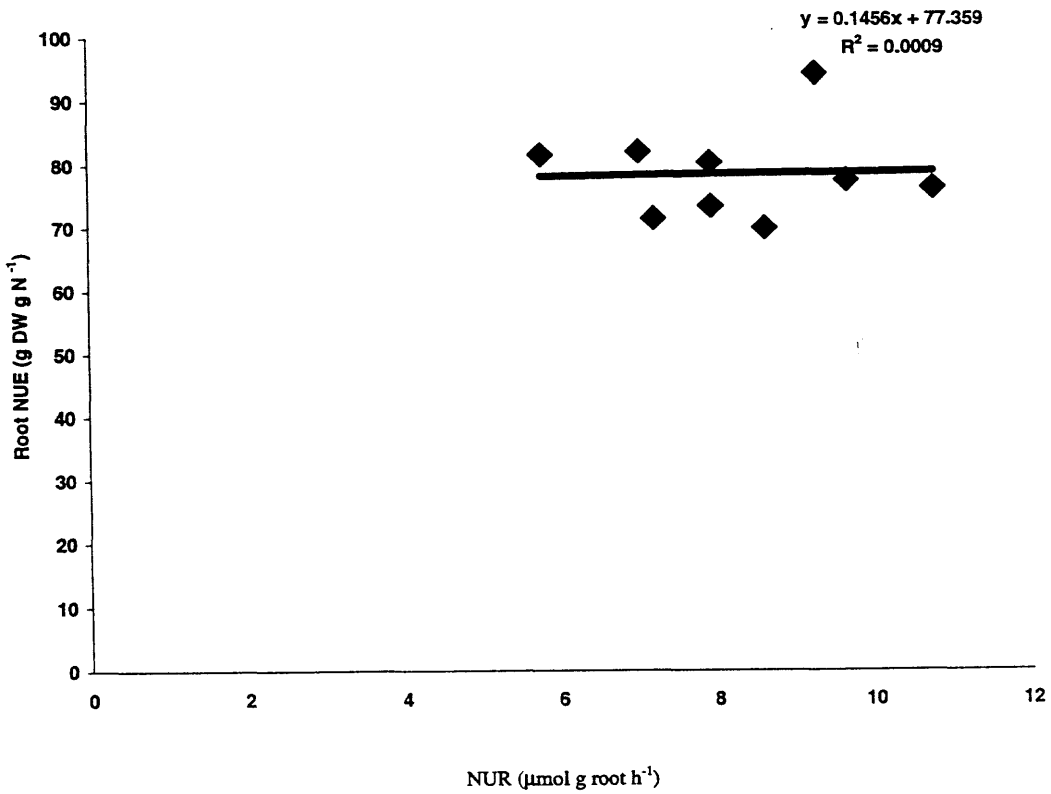

Figure 9. Relationship between NUR and root NUE in nine perennial ryegrass cultivars

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

BIBLIOGRAPHY

Anderson, E.L., E.J. Kamprath, and R.H. Moll. 1984. Nitrogen fertility effects on accumulation, remobilization, and partitioning of N and dry matter in corn genotypes differing in prolificacy. *Agronomy Journal.* 76:397-404.

Andrews, M., J.D. Morton, M. Lieffering, and L. Bisset. 1992. The partitioning of nitrate assimilation between root and shoot of a range of temperate cereals and pasture grasses. *Annals of Botany.* 70:271-276.

Andrews, M. 1986. The partitioning of nitrate assimilation between root and shoot of higher plants. *Plant, Cell and Environment.* 9:511-519.

Aslam, M., and A. Oaks. 1975. Effect of glucose on the induction of nitrate reductase in corn roots. *Plant Physiology.* 56:634-639.

Aslam, M., R.L. Travis, and R.C. Huffacker. 1992. Comparative kinetics and reciprocal inhibition of nitrate and nitrate uptake in roots of uninduced and induced barley seedlings. *Plant Physiology.* 99:1124-1133.

Barber, S.A.1984 *Soil nutrient bioavailibility.* John Wiley and Sons, NY. 398 pp.

Barta, A.L. 1976. Transport and distribution of $^{14}CO_2$ assimilate in Lolium perenne in response to varying nitrogen supply to halves of a divided root system. *Physiologiae. Plantarum.* 38:48-52.

Beard, J.B. 1973. *Turfgrass science and culture.* Prentice-Hall, Englewood Cliffs, NJ.

Bertauski, A.F., J.M. Swiader, and D.J. Wehner. 1997. Dry weight production and nitrogen efficiency traits in Kentucky bluegrass cultivars in nutrient solution and soil. *Crop Science.* 37:1548-1553.

Boucaud, J., and J. Bigot. 1989. Changes in the activities of nitrogen assimilation enzymes of *Lolium perenne* L. during regrowth after cutting. *Plant and Soil.* 114:1221-125.

Botrel, A., and W.M. Kaiser. 1997. Nitrate reductase activation state in barley roots in relation to the energy and carbohydrate status. *Planta.* 204:496-501.

Bowman, D.C., and J.L. Paul. 1988. Uptake and assimilation of NO_3^- and NH_4^+ by nitrogen-deficient perennial ryegrass turf. *Plant Physiology.* 88:1303-1309.

Bowman, D.C., J.L. Paul, and W.B. Davis. 1989. Nitrate and ammonium uptake by nitrogen-deficient perennial ryegrass and Kentucky bluegrass turf. *Journal of the American Society for Horticultural Science.* 114:421-426.

Brunetti, N., and R.H. Hageman. 1976. Comparison of in vivo and in vitro assays of nitrate reductase in wheat (Tritium aestivum) seedlings. *Plant Physiology*. 58:583-587.

Campbell, W.H. 1999. Nitrate reductase structure, function and regulation: bridging the gap between biochemistry and physiology. *Annual Review of Plant Physiology and. Plant Molecular Biology*. 50:277-303.

Canvin, D.T., and K.C. Woo. 1979. The regulation of nitrate reduction in spinach leaves. *Canadian Journal of Botany*. 57:1155-1160.

Cassman, K.G., M.J. Kropff, and J. Gaunt. 1993. Nitrogen use efficiency of rice reconsidered: what are the key constraints? *Plant and Soil*. 155:359-362.

Champigny, M-L., and C. Foyer. 1992. Nitrate activation of cytosolic protein kinases diverts photosynthetic carbon from sucrose to amino acid biosynthesis. Basis for a new concept. *Plant Physiology*. 100:7-12.

Chapin III, S.F. 1980. The mineral nutrition of wild plants. *Annual Review of Ecology and Systematics*. 11:233-260.

Cheeseman. J.M., R. Barreiro, M. Lexa. 1996. Plant growth modeling and the integration of shoot and root activities without communicating messenger-opinion. *Plant and Soil*. 185:51-64

Cheng, C.L., G.N. Acedo, J. Dewdney, H.M. Goodman, and M.A. Conkling. 1991. Differential expression of two Arabidopsis nitrate reductase genes. *Plant Physiology.* 96:275-279.

Cisar, J.L., R.J. Hull, and D.T. Duff. 1989. Ion uptake kinetics of cool season turfgrasses. p. 233-235. In H. Ide and H. Takatoh (ed.) *Proceedings of the. International. Turfgrasses Research Conference 6th*, Tokyo. July 31-Aug.4, 1989.

Crawford, N.M., W.H. Campbell, and R.W. Davis. 1986. *Proceedings of the National Academy of Sciences.* U.S.A. 83:8073-8076.

Deng, M.D., T. Moureaux, I. Cherel, J.P. Boutin, and M. Caboche. 1991. Effects of nitrogen metabolites on the regulation and circadian expression of tobacco nitrate reductase. *Plant Physiology and Biochemistry.* 29:239-247.

Eastin, E.F. 1978. Total nitrogen determination for plant material containing nitrate. *Annals of Biochemistry.* 85:591-594.

Ericsson, T. 1988. Growth and shoot:root ratio of seedlings in relation to nutrient availability. *Plant and Soil.* 168/169:205-214.

Evans, H.J., and A. Nason. 1953. Pyridine nucleotide nitrate reductase from extracts of higher plants. *Plant Physiology.* 28:233-254.

Fiez, T.E., W.L. Pan, and B.C. Miller. 1995. Nitrogen use efficiency of winter wheat among landscape positions. *Soil Science Society of America Journal.* 59:1666-1671.

Galangau, F., F. Daniel-Vedele, T. Moreaux, M.F. Dorbe, M.T. Leydecker, and M. Caboche. 1988. Expression of leaf nitrate reductase genes from tomato and tobacco in relation to light dark regimes and nitrate supply. *Plant Physiology.* 88:383-388.

Gascho, G.J., D.L. Anderson, and H.Y. Ozaki. 1986. Cultivar dependent sugarcane response to nitrogen. *Agronomy Journal.* 78:1064-1069.

Glaab, J. and W.M. Kaiser. 1993. Rapid modulation of nitrate reductase in pea roots. *Planta.* 191:173-179.

Glass, A.D., J.E. Shaff, and L.V. Kochian. 1992. Studies of the uptake of nitrate in barley, IV Electrophysiology. *Plant Physiology.* 99:456-463.

Gonzalez Ponce, R., M.L. Salas, and S.C. Mason. 1993. Nitrogen use efficiency by winter barley under different climatic conditions. *Journal of Plant Nutrition.* 16:1249-1261.

Hageman, R.H., and A.J. Reed. 1980. Nitrate reductase from higher plants. *Methods in Enzymology.* 69:270-280.

Hageman, R.H., and R.J. Lambert. 1988. The use of physiological traits for corn improvement. *In* G. F. Sprague, J. W. Dudley (eds.), Corn and Corn Improvement. *Agronomy Monograph. no. 18.* p. 431-461 ASA-CSSA-SSSA. Madison, WI.

Halberg, G.R. 1989. Nitrate in ground water in the United States. *In* R. F. Follet (ed.), *Nitrogen Management and Ground Water Protection.* pp 35-74. Amsterdam, Elsevier.

Hansen, G.K. 1980. Diurnal variation in root respiration rates and nitrate uptake as influenced by nitrogen supply. *Physiologiae Plantarum.* 48:421-427.

Harris, P., and W.J. Whittington. 1983. Effects of temperature, levels of nitrate supply and duration of light and growth on nitrate reductase activity in *Agrostis tenuis* and *Agrostis stolonifera. New Phytologist.* 93:193-201.

Heldt, H.W., and U.I. Flugge. 1992. Metabolite transport in plant cells. pp21-47. *In* A.K. Tobin. (ed.). *Plant Organelles.* Cambridge University Press, Cambridge, UK.

Hoagland, D.R., and D.I. Arnon. 1950. The water-culture method for growing plants without soil. *California Agricultural Experiment Station Circular* 347:32.

Hocking, P.J., C.P. Meyer. 1991. Effects of enrichment and nitrogen stress on growth, and partitioning of dry matter and nitrogen in wheat and maize. *Australian Journal of Plant Physiology.* 18:339-356.

Huber, J.L., S.C. Huber, W.H. Campbell, and M.G. Redinaugh. 1992. Reversible light/dark modulation of spinach leaf nitrate reductase activity involves protein phosphorylation. *Archives of Biochemistry and Biophysics*. 296:58-65.

Hull, R.J., and H. Liu. 1995. Nitrogen accumulation in a turf-soil ecosystem as a function of N source and grass species. *Agronomy Abstracts*. 87:127.

Jeschke, W.D., and J.S. Pate. 1991. Modeling of the partitioning, assimilation and storage of nitrate within root and shoot organs of castor bean (*Rhicinus communis* L.). *Journal of Experimental Botany*. 42:1091-1103.

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

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

Kaiser, W.M., and E. Brendle-Behnisch. 1991. Rapid modulation of spinach leaf nitrate reductase activity by photosynthesis. *Plant Physiology*. 96:363-367.

Kaiser, W.M., and D. Spill. 1991. Rapid modulation of spinach leaf nitrate reductase activity by photosynthesis. II in vitro modulation by ATP and AMP. *Plant Physiology.* 96:368-375.

Kaiser, W.M., and S.C. Huber. 1994. Postranslational regulation of nitrate reductase in higher plants. *Plant Physiology.* 106:817-821.

Keeney, D.R., and D.W. Nelson. 1982. Nitrogen-Inorganic forms. P. 643-698. *In* A.L. Page et al (ed.) *Methods of soil analysis.* Part 2 2nd ed. Agronomy Monograph. 9 ASA and SSSA, Madison, WI.

Levin S.A, H.A. Mooney, and C. Field. 1989. The dependence of plant root:shoot ratios on internal nitrogen concentrations. *Annals of Botany.* 64:71-75.

Lexa, M., and J.M. Cheeseman. 1997. Growth and nitrogen relations in reciprocal grafts of wild-type and nitrate reductase-deficient mutants of pea (*Pisum sativum* L. var. Juneau). *Journal of Experimental Botany.* 48:1241-1250.

Li, X-Z., and A. Oaks. 1993. Induction and turnover of nitrate reductase in *Zea mays*: Influence of NO_3^-. *Plant Physiology.* 102: 1251-1257

Lilo, C., and A. Henrikson. 1984. Comparative studies of diurnal variations of nitrate reductase activity in wheat, oat and barley. *Physiologiae Plantarum.* 62:89-94.

Lillo, C. 1994. Light regulation of nitrate reductase in green leaves of higher plants. *Physiologiae Plantarum*. 90:616-620.

Liu, H. 1992. Evaluating three turfgrasses for nitrogen, phosphorus, and potassium nutrient uptake efficiency. *Ph.D. Dissertation*, Univ. of RI. pp 208.

Liu , H., R.J. Hull, and D.T. Duff. 1993. Comparing cultivars of three cool season turfgrasses for nitrate uptake kinetics and nitrogen recovery in the field. P. 546-552. *In* R.N. Carrow et al (ed.). *International Turfgrass Society Research Journal. vol. 7.* Intertec Publishing Corp., Overland Park KS.

Liu, K-H., C-Y., Huang, and Y-F. Tsay. 1999. CHL1 is a dual-affinity nitrate transporter of Arabidopsis involved in multiple phases of nitrate uptake. *Plant Cell.* 11:865-874.

Mattsson, M., and J.K. Schjoerring. 1996. Ammonia emission form young barley plants: influence of N source, light/dark cycles and inhibition of glutamine synthetase. *Journal of Experimental Botany*. 47:477-484.

Microsoft Excel 97. 1996. Microsoft Corp.

Muller, B., P. Tillard, and B. Touraine. 1995. Nitrate fluxes in soybean seedling roots and their response to amino acids: An approach using ^{15}N. *Plant Cell Environment.* 18:1267-1278.

Naik,. M.S., and D.J.D. Nicholas. 1986. Malate metabolism and its relation to nitrate assimilation in plants. *Phytochemistry.* 25:571-576.

Nelson, S.H., and F.W. Sosulski. 1984. Amino acid and protein content of *Poa pratensis* as related to nitrogen application and color. *Canadian Journal of Plant Science.* 64:691-697.

Oaks, A.. and B. Hirel. 1985. Nitrogen metabolism in roots. *Annual Review of Plant Physiology.* 36:345-360.

Oaks, A. 1994. Efficiency of nitrogen utilization in C_3 and C_4 cereals. *Plant Physiology.* 106:407-414.

Osborne, B.A., and W.J. Whittington. 1981. Eco-physiological aspects of inter-specific and seasonal variation in nitrate utilization in the genus *Agrostis. New Phytologist.* 87:595-614.

Pate, J.S. 1973. Uptake, assimilation and transport of nitrogen compounds by plants. *Soil Biology and Biochemistry.* 5:109-119.

Pearson, C.J., R.J. Volk, and W.A. Jackson. 1981 Daily changes in nitrate influx, efflux, and metabolism in maize and pearl millet. *Planta*. 152:319-324.

Petrovic, A.M. 1990. The fate of nitrogenous fertilizers applied to turfgrass. *Journal of Environmental Quality*. 19:124-130.

Pueke, A.D., W. Hartung, and W.D. Jeschke. 1994. The uptake and flow of C, N and ion between roots and shoots in *Ricinus communis* L. II. Grown with low or high nitrate supply. *Journal of Experimental Botany*. 45:733-740.

Radin, J.W. 1978. A physiological basis for the division of nitrate assimilation between roots and leaves. *Plant Science Letters*. 13:21-25.

Rao, S.C., and T.H. Dao. 1996. Nitrogen placement and tillage effects on dry matter and nitrogen accumulation and redistribution in winter wheat. *Agronomy Journal*. 88:365-371.

Samuelson, M.E., L. Eliasson, and C.M. Larson. 1992. Nitrate regulated and cytokinin responses of seminal roots of barley. *Plant Physiology*. 98:309-315.

SAS Institute Inc. 1990. *SAS/STAT User's guide*. Version 6. 4th ed. SAS Institute, Cary, NC.

Scheible W-R, A. Gonzales-Fontes, R. Morcuende, M. Lauerer, M. Geiger, J. Glabb, A. Gojon, E-D. Schultz, and M. Stitt. 1997. Tobacco mutants with a decreased number of functional *nia* genes compensate by modifying the diurnal regulation of transcription, post-translational modification and turnover of nitrate reductase. *Planta.* 203:304-319.

Siddiqi, M.Y., A.D.M. Glass, T.J. Ruth, and T.W. Rufty, Jr. 1990. Studies of the uptake of nitrate in barley. I. Kinetics of $^{13}NO_3^-$ influx. *Plant Physiology.* 93:1426-1432.

Simpson, R.J., H. Lambers, and M.J. Dalling. 1982. Translocation of nitrogen in a vegetative wheat plant (*Triticum aestivum*). *Physiologiae Plantarum.* 56:11-17.

Simpson, R. J. 1986. Translocation and metabolism of nitrogen: whole plant aspects. P. 71-96. *In* H. Lambers, J.J. Neeteson, and I. Stulen (eds.). *Fundamental, Ecological and Agricultural Aspects of Nitrogen Metabolism in Higher Plants.* Martinus Nijhoff Pub., Dordecht, Netherlands.

Smith, S.M., and D.B. James. 1982. Control and variation of in vivo nitrate reductase activity in *Lolium perenne* L. cv. s24. II. Variation of activity associated with different light regimes. *Plant and Soil.* 68:231-239.

Snell, F.D., and C.T. Snell. 1949. p. 84. *Colorimetric Methods of Analysis*. Van Nostrand. Princeton, NJ

Streit, L, B.A. Martin, and J.E. Harper. 1987. A method for the separation and partial purification of three forms of nitrate reductase present in wild-type soybean leaves. *Plant Physiology*. 84:654-657.

Sullivan, W.M., Z. Jiang, and R.J. Hull. 1999. Root morphology and its relationship with nitrate uptake in Kentucky bluegrass. *Crop Science*. (in press).

Talouise, A., G. Guiraud, A. Moyse, F. Marolle, and M.L. Champigny. 1984. Effect of previous nitrate deprivation on ^{15}N-nitrate absorption and assimilation by wheat seedlings. *Journal of Plant Physiology*. 116:113-112.

Touraine, B., B. Muller, and C. Grignon. 1992. Effect of phloem translocated malate on NO_3^- uptake by roots of intact soybean plants. *Plant Physiology*. 99:1118-1123.

Van Quy, L., and M.L. Champigny. 1992. NO_3^- enhances the kinase activity for phosphorylation of phosphoenolpyruvate carboxylase and sucrose phosphate synthase proteins in wheat leaves. *Plant Physiology*. 99:344-347.

Van Quy, L., T. Lamaze, and M.L. Champigny. 1991. Short-term effects of nitrate on sucrose synthesis in wheat leaves. *Planta*. 185:53-57.

Wallace, W. 1986. Distribution of nitrate assimilation between the root and shoot of legumes and a comparison with wheat. *Physiologiae Plantarum*. 66:630-636.

Warner, R.L., and R.C. Huffaker. 1989. Nitrate transport is independent of NADH and NAD(P)H nitrate reductases in barley seedlings. *Plant Physiology*. 91:947-953.