AN ABSTRACT OF THE THESIS OF:

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Lasting, effective solutions to nitrogen (N) loss from container nurseries must address layers of complexity ranging in scale from whole system nursery management to gene expression. Group-based On-site Active Learning (GOAL) was developed to aid nursery managers and related stakeholders (e.g. neighbors, policy makers, regulating agencies, researchers) in developing a better understanding of how nitrogen flows through container nurseries and the effects of N management decisions over space and time. After completing GOAL, 94% of participants indicated that they learned a new idea or concept about N cycling in their container nursery. New ideas and concepts from peers and colleagues were gained by 100% of participants, 60% from researchers, and 60% developed their own ideas and concepts.

Controlled release fertilizers (CRFs), applied to reduce N losses, introduce substantial amounts of N to production systems, and their release patterns often do not match plant requirements. Commercially comparable plants were grown under a precision liquid fertilization regime with 68.7% (*Euonymus alatus* 'Compactus', slow growing) and 48.6% (*Weigela florida* 'Red Prince', fast growing) less N than was

introduced to the production system with CRF treatments yielding the highest dry weights and total plant N.

Various rates (25, 50 100, 200, and 300 $mg \cdot L^{-1}$) of ^{15}N depleted NH_4NO_3 (min 99.95% atom ^{14}N) were applied to three container-grown woody ornamentals. Estimation of N recovery determined by total N in the plant was significantly higher than estimation of N recovery determined by labeled fertilizer N in the plant at low N rates. Increasing fertilizer rates up to 100 $mg \cdot L^{-1}$ resulted in increased uptake of nitrogen derived from other sources (NDFO), and NDFO at low N concentrations was a significant portion of the total N in the plant. As a result, the nonisotopic total N method overestimates fertilizer N uptake three to four times in container-grown plants at N concentrations of 25 $mg \cdot L^{-1}$.

A pair of degenerate primers were developed that consistently amplify a ~635 nucleotide section of the *NRT1* gene (nitrate transport) in *Rhododendron* 'Unique' and *Cornus sericea*. PCR products were cloned and sequenced and 79.52 % of nucleotides matched between *Rhododendron* 'Unique' and *Cornus sericea*.

Nitrogen Use in Container-grown Woody Ornamentals

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

David R. Sandrock, Author

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CONTRIBUTION OF AUTHORS

Dr. Nahla Bassil assisted in the molecular portion of the thesis (Chapter 6) including protocol development, primer design and sequencing. Dr. Ray William assisted in the systems portion of the thesis (Chapter 3).

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CHAPTER 1

INTRODUCTION

Container production of woody ornamental plants is increasing because it offers advantages over traditional field production. These advantages include more plants per unit land area, faster plant growth, higher quality plants, lack of dependence on arable land, easier shipping and handling, and year-round sales (Davidson et al., 1988; Whitcomb, 1986). However, container production is less nitrogen (N) efficient than field production and requires greater N applications to be successful. Higher N application and less efficient N use has led to concern about N management in container nurseries.

This thesis investigates achieving greater N efficiency and reducing N pollution at three levels or scales:

Large – Understanding the complexity of N management from a whole systems perspective

Medium –Investigating the physiological events (field studies) associated with N use in container-grown plants

Small – Investigating N uptake potential with molecular techniques

Nitrogen (N) management in container nurseries is a complex system. Working within this system, owners, managers and employees of container nurseries routinely make N management decisions that have consequences for the immediate nursery environment (e.g. plant growth, yield, disease susceptibility, water quality) as well as areas beyond nursery boundaries (e.g. surface and groundwater quality, public perception). Effective and lasting solutions to N pollution must consider the interests of all stakeholders. Yet, because of individual perspectives, understanding, discussion, planning, and implementation of effective policies can be strained. The need exists for a simple, integrative model that encourages open, two-way communication between stakeholder groups with different views. At the whole nursery level, greater N efficiency can be achieved when stakeholders understand how N flows through nurseries and seek out strategies to reduce N inputs while maintaining commercial viability.

Due to their size and the diversity of crops produced, the most common strategy of wholesale container nurseries is to apply a blanket controlled-release fertilizer at planting to insure that N is available whenever the plants need it. This method of fertilization requires relatively little labor and is promoted as providing greater N efficiency. Yet, controlled-release fertilizers continue to introduce large amounts of N to the biosphere, and efficiency of uptake is often quite low due to over-application (to species with low N needs) and a lack of synchronization between N release and plant requirements. At the single plant or crop specific level, field studies

that reveal the actual N requirements of plant species and cultivars and determine possible methods of delivery can lead to greater N efficiency. Additionally, a greater understanding of N efficiency at various rates and how plants use fertilizer and other N sources over time will lead to greater N efficiency.

Woody ornamental plant nurseries are unique among horticultural enterprises in that they often grow hundreds or thousands of species and cultivars, all of which differ in their ability to take up N. Each species and cultivar has its own genetically coded N uptake potential (ability to produce and activate proteins necessary for N uptake) evolved from ages of adaptation to a particular growing environment. Molecular tools now offer accurate evaluation of an individual species or cultivar's unique uptake potential. Results from gene expression studies will lead to greater N use efficiency by enabling managers to provide N to match the specific N uptake potential of a particular taxon.

As concern about N pollution from agricultural sources increases, nursery owners and managers will face increasingly rigid requirements from regulating agencies. Pragmatic, long-term, sustainable solutions must emerge from multiscale research that considers multiple components of N use in container nurseries.

CHAPTER 2

LITERATURE REVIEW

INCREASING N LEVELS

The Haber-Bosch process, facilitating the fixation of nitrogen (N) gas to ammonia, was developed in 1914. As a result, use of N fertilizers has increased steadily since the 1940's. Approximately 80 million metric tons of N are fixed each year in the form of N fertilizers (Figure 2.1; Cheng, 2001). Globally, anthropogenic N inputs equal 210 million metric tons, and natural sources equal 140 million metric tons (Vitousek et al., 1997). Nitrogen pollution of surface and groundwater from agricultural sources is a global and local concern. Fertilizer N, mostly in the nitrate form, can enter surface and groundwater through runoff and leaching.

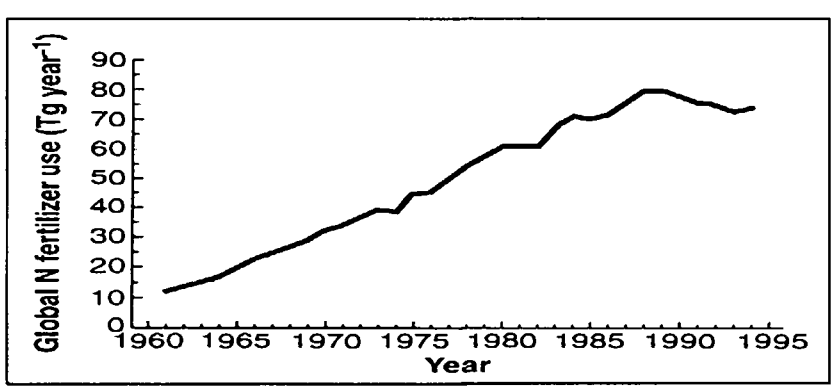

Figure 2.1: Global N fertilizer use (Cheng, 2001).

Nitrate in drinking water is a human health concern. When nitrate is ingested, microorganisms in the stomach may convert the nitrate to nitrite. Nitrite in the blood stream will convert hemoglobin (responsible for oxygen transport in the bloodstream) to methemoglobin (an ineffective oxygen transporter; Lee, 1970). This condition is known as methemoglobinemia or 'blue-baby syndrome' and can be fatal to infants. Earlier this century 'blue-baby' was a serious threat, but today it is treatable and rare. Other health concerns include birth defects and cancer caused by high nitrate levels. Research is being conducted in these areas, but to date, results are inconclusive (Mufford, 1991). The Public Health Service has defined 10 $mg \cdot l^{-1}$ in drinking water as potentially harmful to human health (Vitousek et al., 1997).

Most N inputs serve human needs such as agricultural production, but their environmental consequences are serious and long term. Vitousek et al. (1997) recognize the following environmental impacts of human alteration of the N cycle:

- loss of soil nutrients such as calcium and potassium
- acidification of soil, lakes, and streams in several regions
- increase in the transfer of N through rivers to estuaries and oceans
- loss of biological diversity (especially to plants that are efficient N users and the animals and microorganisms that depend on them)
- long-term decline in coastal marine fisheries

THE NURSERY INDUSTRY

Local and national production

The nursery and greenhouse industry is a substantial component of U.S. farm crop sales. According to the USDA National Agricultural Statistics Service (2002), the nursery and greenhouse industry accounted for 11.2% of total U.S. farm crop sales in 1998, making it the fourth largest crop commodity in the U.S.

Nursery and greenhouse crops are the number one agricultural commodity in Oregon, and the state is ranked third in the U.S. in nursery and greenhouse sales behind California and Florida. Oregon's 1999 sales were $584 million, up 10% from the 1998 value of $532 million. Nursery sales have grown 85% since 1990, and in 1999 Oregon nurseries occupied 41,100 acres. However, Oregon's nursery industry is not distributed throughout the state. In fact, five counties, Clackamas, Marion, Washington, Yamhill, and Multnomah, accounted for 86% of Oregon's nursery sales in 1999. Three of these counties, Multnomah, Washington, and Clackamas are the urban 'tri-county' area of metropolitan Portland, and their sales accounted for almost half the 1999 sales.

In Oregon, container production has shown more growth than any other sector of nursery and greenhouse sales. Container sales in 1999 increased $35 million from 1998 (19%), and accounted for two-thirds of the $52 million industry surge. Container sales rose from $52 million in 1997 to $223 million in 1999; a more than 30% increase representing over half of the entire sales growth of $92 million during the same period. Container sales now account for over 38% of total sales (OAN 'Digger', 2000).

Laws and regulations governing N use

Nitrogen pollution from agriculture is a concern in many parts of the world. In the United States the Federal Clean Water act of 1972 (amended 1977, 1981, and 1987) provides the Environmental Protection Agency (EPA) the authority to set pollution related standards and to delegate administrative and enforcement aspects of the law to state governments.

The Oregon Department of Environmental Quality (DEQ) and the Oregon Department of Agriculture (ODA) monitor water quality in Oregon, as well as other federal, state and local agencies. The DEQ, in compliance with the Federal Clean Water Act, identifies lakes, rivers, and streams that do not meet water quality standards such as water temperature, level of dissolved oxygen, nutrients, sediment load, pH, bacteria, and other factors. These standards are established to maintain beneficial uses of water such as irrigation, fisheries, aquatic life, recreation, aesthetics, livestock watering, and drinking water supplies. Watersheds that do not meet the DEQ's standards are subject to involvement by the ODA. The Agriculture Water Quality Management Program was developed as a result of legislation such as the Clean Water Act and is administered by the Natural Resources Division of the ODA. In 1993, the Oregon Legislature passed Senate Bill 1010 (ORS 568.900-568.933), the Agriculture Water Quality Management Act, which defines the ODA as the leading state agency working with agriculture to address issues such as nitrogen pollution in the state's waterways. In 1995, the Oregon Legislature passed Senate Bill 502 (ORS 561.191) reiterating that the ODA is the only agency responsible for regulating

agricultural activities that affect water quality. The Container Nursery Irrigation Water Management Program is a division of the ODA's Agricultural Water Quality Management Program. Under agreement between the ODA and the DEQ, the Container Nursery Irrigation Water Management Program provides nurseries with guidelines to insure minimal surface water pollution from production of container nursery stock (Oregon Department of Environmental Quality, 2002; Oregon Department of Agriculture, 2002). As concerns about N pollution increase, regulations on agricultural practices are likely to become more rigid.

N FERTILIZATION OF CONTAINER-GROWN WOODY PLANTS

The following categories address the inefficiencies experienced in container production of woody ornamental plants and the relevant research performed in each area. This thesis focuses specifically on N efficiency, but as water and N efficiency are inseparable, both are addressed as necessary.

Container- vs. field-grown methods

Container production of woody ornamental plants requires more N inputs and is less nitrogen-use efficient than field-grown production. Field-grown lining-out stock and established plants require N at a rate of 25 to 50 and 84 to 111 kg· hectare^{-1} (22 to 44 and 75 to 100 lbs/acre) per year, respectively (Green, unpublished). Container nursery stock is often fertilized with soluble fertilizers through overhead

irrigation at an N rate in excess of 505 to 594 kg· hectare^{-1} (450 to 530 lbs/acre) per year (Rathier and Frink, 1989).

Container nurseries require more water than field-grown operations. One acre-inch of water is 254,427 L· hectare^{-1} (27,154 gal/acre). In Oregon's Willamette Valley, 10 acre-inches of water (2,544,276 L· hectare^{-1}) are applied to field-grown nursery stock per growing season. In contrast, container nurseries apply 53 to 170 acre-inches of water (13.5 to 43.3 million L· hectare^{-1}) per growing season (Bluhm et al., 1980). In this case, if fertilizer N is constantly supplied through irrigation, the likelihood of excess N application increases dramatically.

Substrates

Container growing mixes consist primarily of pine, douglas-fir, or other types of bark. These products are used because they are an inexpensive by-product of the forestry industry, provide good drainage, and are lightweight. However, bark media do not retain capillary water because of large pore sizes and have almost no capacity to retain anions such as nitrate (NO_3^-: Furuta, 1976). Foster et al. (1983) determined that 100% of NO_3^- anions were leached from a milled pine bark substrate while only 6% of applied ammonium (NH_4^+) ions were leached.

Fertilizer form

Nitrogen fertilizers are usually applied in container nurseries as either liquid (overhead or drip irrigation), soluble granules (topdressed to the container or broadcast

over an area), or controlled release (incorporated at planting or topdressed during the season), and many studies have been performed comparing fertilizer forms. Various methods and calculations can be used to compare N leaching losses between liquid and controlled-release fertilizers, and readers must pay close attention to the methods used to insure an accurate interpretation of the results. Hershey and Paul (1982) demonstrated that depending on which method was used, leaching losses from liquid fertilization could appear to be more than 4 times, 2 times, or similar to leaching losses from controlled release fertilizers within the same study.

Several studies have concluded that controlled-release fertilizers result in just as much N loss from leaching as liquid fertilization at similar rates. Comparable or slightly larger pot chrysanthemums (based on fresh weight of tops) were grown with liquid fertilization at an N rate of 1.21 g/pot compared to a controlled release fertilizer (Osmocote 14-14-14) at N rates of 1.68, 2.52, and 3.36 g/pot. Moreover, N leaching losses from the liquid fertilization treatment of 1.21 g/pot [0.14 g/pot (12% of applied N)] were lower than the controlled-release treatments of 2.52 and 3.36 g/pot [0.77 g/pot (23% of applied N)] (results shown for 3.36 g/pot treatment; Hershey and Paul, 1982). A similar study showed that growing 'First Lady' marigolds (*Tagetes erecta*) in 0.5 L containers with the same amounts of total N delivered as a water soluble fertilizer, Osmocote, or Nutricote, demonstrated that a single large application of a controlled-release fertilizer at planting resulted in as much or more N loss by leaching as regular irrigation with solutions of water soluble fertilizer (Cox, 1993).

Likewise, several studies have shown that controlled release fertilizers result in less N loss from leaching than liquid fertilization regimes. Broschat (1995) tested the effect of a single fertilizer formulation (21N-3P-12K) and amount delivered to a pine bark based medium as liquid, soluble granules, lightly coated controlled-release, or heavily coated controlled-release. Results showed that *Spathiphyllum* Schott. 'Mauna Loa Supreme' and areca palm (*Chrysalidocarpus lutescens* H. Wendl.) grew as well and better, respectively, with liquid fertilization as with other delivery methods. Over the course of the six-month study, the liquid fertilization method resulted in lower nitrate leaching than the soluble granules but higher nitrate leaching than the controlled release fertilizers. Another study concluded that fertilization of potted chrysanthemums with controlled-release fertilizers resulted in reduced nutrient leaching losses compared with liquid fertilization (Catanzaro, 1998). Unfortunately, the results of this study are inconsequential because the *total amount* of N supplied in liquid treatments was far above (37% more) the *total amount* of N supplied in the slow release treatments, making it impossible to compare results. Rathier and Frink (1989) showed that andorra juniper and dwarf alberta spruce supplied with soluble fertilizer resulted in higher N losses due to leaching than plants supplied with equal amounts of N as controlled-release fertilizers when subjected to trickle and overhead irrigation.

Controlled-release fertilizers

Controlled-release fertilizers are the most common type of fertilizer used in container nurseries. In theory, controlled-release fertilizers offer advantages over

other fertilizer forms such as reduced build-up of soluble salts (EC), constant supply of nutrients, and reduced leaching losses. However, N release patterns can be sporadic due to fluctuations in temperature and moisture and often do not match the needs of the plant (Wright and Niemiera, 1987). Controlled release fertilizers with similar longevity ratings may have release patterns that differ in intensity and timing (Cabrera, 1997). Controlled-release fertilizers often release large quantities of N early in the season, before newly planted cuttings or starts have a sufficient root mass to exploit the abundant nutrient supply. This results in excessive N leaching early in the season (Hershey and Paul, 1983; Huett, 1997b; Rathier and Frink, 1989). Disproportionately large N release early in the season results in an N shortage later in the season. Huett (1997a) demonstrated the inability of single, large applications of controlled-release fertilizers at planting to adequately supply the nutritional needs of 4- and 10-week groundcover varieties. Other studies have shown that incorporating controlled-release fertilizers increases the amount of N recovered in the leachate as compared to surface applications (Cabrera, 1997; Cox, 1993).

Fertilizer N rate

Many studies have determined the effect of N rate on plant growth. Rooted cuttings of crape myrtle (*Lagerstroemia indica* L. × *Lagerstroemia fauriei* Koehne 'Tonto') were grown under liquid fertilization at N rates ranging from 15 – 300 $mg \cdot L^{-1}$ and then transplanted into the landscape for 16 weeks (Cabrera and Devereaux, 1999). At planting, plants grown at low N rates (up to 60 $mg \cdot L^{-1}$) during the nursery phase

demonstrated increased shoot biomass, shoot to root ratios, and leaf area with increasing N. Beyond 60 $mg \cdot L^{-1}$ these parameters decreased. As N rate increased, root biomass and plant height decreased linearly. Sixteen weeks after transplanting, plant biomass was significantly higher in plants grown at higher N rates (smallest plants at transplanting with the highest N concentrations), but plant shoot to root ratio and tissue N concentrations were not significantly different among treatments (Cabrera and Devereaux, 1999). 'Royalty' roses grown in a soilless substrate in microlysimeters displayed no significant differences in cumulative dry weight or number of flowers harvested despite N rates of 77 (half the recommended N rate for rose production), 154, and 231 $mg \cdot L^{-1}$ (Cabrera et al., 1993). Griffin et al. (1999) determined that 100 $mg \cdot L^{-1}$ delivered three times a week resulted in *Thuja* × ' Green Giant' plants with shoot and root dry weights as large as or larger than plants grown at a 320 $mg \cdot L^{-1}$ treatment. Using a linear plateau model, it was determined that the critical container solution N level was between 15 and 20 $mg \cdot L^{-1}$ for citrus plants grown in containers (Maust and Williamson, 1991). However, treatments in these experiments were applied daily and at a high volume (1 L/day) so that even at low N rates the total amount of N delivered was relatively high. Container-grown *Ilex crenata* Thunb. 'Helleri' accumulated more N, exhibited a higher shoot-to-root ratio, and initiated extension growth sooner when treated with 100 $mg \cdot L^{-1}$ N than when treated at lower N levels (Niemiera and Wright, 1982). Nitrogen recovery efficiency of 'Celebrate 2' poinsettias (*Euphorbia pulcherrima* Willd.) was higher (58% recovery) under a sliding scale fertilization regime than under a constant 200 $mg \cdot L^{-1}$

treatment (38% recovery). Both treatments resulted in similar, commercial grade plants, but the sliding scale fertilization regime used 41% less total N (Rose et al., 1994).

Application method

Four methods of fertilizer application are generally used in container production systems: topdressing, incorporation, liquid feed, and broadcast (Yeager et al., 1986). Topdressing is the application of granular fertilizer to the surface of the container. This practice is labor-intensive, and loss may occur when containers tip over. Incorporation involves mixing fertilizer with the growing media prior to potting. There is no fertilizer loss during application with this method, but incorporation does not adjust the amount of fertilizer available to crop demand. Heavy leaching can occur early in the season when fertilizer availability is high but plant growth and uptake is low (van der Boon and Niers, 1983). Liquid feed is the application of fertilizers through an irrigation system. This often involves reapplication of collected runoff. Liquid feed application is inefficient because of losses in non-target areas such as spaces between pots, aisles, and roadways. When overhead liquid feed was used to fertilize one acre of one-gallon containers (10,839 plants), 81% of the fertilizer was wasted (Yeager et al., 1986). Broadcasting granular fertilizers is perhaps the most inefficient method of application. When fertilizer was broadcast on one acre of one-gallon containers, 90% of the broadcast-applied fertilizer was wasted (Yeager et al.,

1986). As with liquid feed, most of the fertilizer ends up between pots, in the aisles, or in the roads.

Irrigation

Much of the irrigation water applied to container systems falls between pots, in aisles, and in roads. Several authors have demonstrated methods for improving irrigation efficiency. Triangular spacing, sprinkler selection and design, grouping plants according to water needs, trickle irrigation, and capillary irrigation have been demonstrated to reduce water usage by 10 to 80% (Whitcomb, 1984; Smucker, 1985; Burger et al., 1987; Ticknor and Green, 1987; Ross, 1988)

Plant spacing

Plants in container nurseries are spaced to allow adequate room for plant growth. If containers of any size are set edge-to-edge, 21% of the growing space is open. If spaced one half the pot diameter apart (1.5×), 65% of the bed area is open. If there is one diameter between containers (2× spacing), 80% of the growing space is open. Much of the fertilizer, if applied through liquid feed or broadcast, never reaches the containers but falls into open spaces (Green, unpublished).

Few improvements in spacing have been made. Whitcomb (1984) states that triangular spacing of containers can reduce non-target irrigation by 10% from 90% to 80%.

Nursery organization

Nurseries are most often organized by similar taxa regardless of their growth strategies and substrate demands. Plants at El Modeno Gardens, a 95-acre container nursery in California, are divided into 64 groups according to water needs. Plants are trickle irrigated to replenish specific crop evapotranspiration. This system reduced water use by 30%, fertilizer use by 50%, and nitrogen runoff by 91% (Whiteside, 1989).

^{15}N fertilizer tracers

In general, fertilizer N uptake is determined by the difference method (N in fertilized plants minus N in unfertilized plants) or by a ^{15}N fertilizer tracer. When various N rates are used, N recovery can be determined by the linear regression of total N in the crop on rates of applied N or by the linear regression of labeled fertilizer N in the plant on rates of labeled fertilizer N (Westerman and Kurtz, 1974).

The standard nonisotopic method of determining N recovery in field-grown agronomic crops is the difference method. This method is not suitable for long-term container-grown nursery crop studies because the potting substrate supplies insufficient N to sustain woody crops for the duration of a pragmatic experiment. Considering the low N content of most growing media used in container production (bark, sand, peat, etc.), fertilizer N recovery is often equated with total N in the plant. Therefore, it is common practice to determine N recovery efficiency in nursery crops by dividing the total N in the plant by the amount of applied N or by the linear

regression of total N in the crop on rates of applied N (Niemiera and Wright, 1982; Maust and Williamson, 1991; Cabrera and Devereaux, 1999; Griffin et al., 1999).

For many agronomic crops grown in the field, ^{15}N fertilizer tracers are regarded as a superior method because determinations of N fertilizers can be made more accurately (Hauck and Bremner, 1976) and treatment effects are detected with greater sensitivity (Russelle et al., 1981). In addition,^{15}N tracers serve to distinguish fertilizer N recovered by the plant from soil N recovered by the plant, and allows the calculation of fertilizer N efficiency without regard to residual soil N (Torbert et al., 1992).

Many studies have compared isotopic and nonisotopic methods of determining fertilizer N recovery (Westerman and Kurtz, 1974; Olson, 1980; Oslon and Swallow, 1984; Rao et al., 1991; Bronson et al., 2000). Nonisotopic determination of fertilizer N recovery is consistently higher than isotopic determination because of a phenomenon called added N interaction (ANI) in which the addition of fertilizer N increases the mineralization and availability of native soil N (Jenkinson et al., 1985; Hart et al., 1986; Wood et al., 1987; Azam et al., 1989; Chalk et al., 1990). Added N interaction causes isotopic methods of determining fertilizer N recovery to underestimate fertilizer N uptake due to mineralization-immobilization turnover (MIT). During MIT, labeled fertilizer N exchanges with native soil N (Jansson and Persson, 1982; Walters and Malzer, 1990). The non-labeled soil N is then taken up by the plant resulting in an underestimation of fertilizer N uptake. Not only does substitution occur, but also during MIT the isotopic composition of labeled fertilizer N

is markedly changed (Hauck, 1978). For example, remineralized ^{15}N depleted fertilizer will have a much higher ^{15}N concentration (diluted) than the labeled fertilizer before it was immobilized. Similarly, ANI causes nonisotopic methods of determining fertilizer N recovery to overestimate fertilizer N uptake because the additional N taken up after ANI cannot be distinguished from fertilizer N. Added N interactions are defined as "real" if N fertilizer actually increases N mineralization or if root exploration is greater in fertilized plants due to larger root systems than in unfertilized plants (Bronson et al., 2000). Added N interactions are defined as "apparent" if simple pool substitution occurs in which fertilizer N replaces native soil N (Jenkinson et al., 1985).

MOLECULAR ASPECTS OF NITRATE UPTAKE

Introduction

Nitrogen is the mineral macronutrient required in the highest amount by plants, and its deficiency is often the growth-limiting factor for plants in natural and agricultural systems. N is present in the biosphere as di-nitrogen gas (N_2), ammonia (NH_3), ammonium (NH_4), nitrate (NO_3), and organic N (amino acids and other peptides). Plants display preferences for different N forms depending upon their habitats. For example, white spruce has a strong preference for NH_4 (Kronzucker et al., 1997), and some artic sedges prefer amino acids (Chapin et al., 1993). However, NO_3 is the predominant form assimilated by plants in most situations. In agricultural

situations, NH_4 and NO_3 are the most important forms for plant growth and productivity. Regardless of habitat and preferred form, plants must compete with soil microbes and environmental processes for the acquisition of N and have therefore evolved strategies to optimize N uptake and assimilation. N uptake and assimilation require transport across cell membranes at many points along the assimilation pathway. This review will focus on the initial uptake of nitrate across the plasma membrane of root cells.

Energetics

Nitrate reaches the roots surface by bulk flow through the soil solution. Nitrate uptake into epidermal and cortical root cells requires energy, even when the external NO_3 concentration is relatively high (mM range). For example, NO_3 uptake from 10 mM external nitrate, with 5 mM cytoplasmic NO_3 and an electrical potential difference across the plasma membrane of –150 mV requires approximately 13 $kJ \cdot mol^{-1}$ at 20 C. This figure increases by ~5 $kJ \cdot mol^{-1}$ for each tenfold decline in external NO_3 (Crawford and Glass, 1998). The energy required for NO_3 uptake is provided by a proton motive force (pmf; Glass and Siddiqi, 1995). In addition, NO_3 uptake results in the depolarization of the plasma membrane (an increase in the positive charge inside the cell). Considering these factors, it has been proposed that NO_3 uptake is achieved by a $2H^+/1NO_3$ symport mechanism (Meharg and Blatt, 1995).

Once inside the cell, there are four fates of NO_3:

1) it is reduced and incorporated into amino acids

2) it is transported to the xylem for long distance transport

3) it is stored in the vacuole

4) it is effluxed out of the cell

Transportation to the xylem and storage in the vacuole also require transport across a membrane. Despite the energy requirement for NO_3 uptake, there appears to be a significant passive efflux of NO_3 from the cell that is saturable and NO_3-selective (Grouzis et al., 1997). The rate of efflux increases with increasing external NO_3 concentrations, and NO_3 efflux has been shown to be NO_3 inducible (Aslam et al., 1996a).

Kinetics

Plots of NO_3 uptake velocity vs. external NO_3 concentrations yield curves that exhibit two or more phases of uptake. Current theories suggest that roots exhibit three kinetically distinct NO_3 uptake systems:

CHATS- constitutive high affinity transport system

IHATS- inducible high affinity transport system

LATS- low affinity transport system (constitutive; Aslam et al.,1992; Glass and Siddiqi, 1995)

Possessing a variety of NO_3 uptake systems allows plants to take up and assimilate adequate amounts of N over a wide range of NO_3 concentrations.

HATS (CHATS and IHATS)

The HATS are involved in the uptake of NO_3 at low concentrations. Genetic and physiological evidence suggests that the HATS have at least two distinct components with differing affinities for NO_3 and different regulation pathways. The CHATS is constitutive, and is expressed in the absence of NO_3. The IHATS is inducible, and induction occurs within hours to days of exposure to NO_3. The K_m values for the HATS are in the range of 7-110 μM, and the V_{max} values are in the range of 2-9 $\mu mol \cdot g^{-1}$ fresh weight h^{-1}(Peuke and Kaiser, 1996). Specifically, the CHATS is characterized by low K_m and V_{max} values, 6-20 μM and 0.3-0.82 $\mu mol \cdot g^{-1}$ fresh weight h^{-1}, respectively. The IHATS is characterized by higher K_m and V_{max} values, 20-100 μM and 3-8 $\mu mol \cdot g^{-1}$ fresh weight h^{-1}, respectively (Crawford and Glass, 1998). Of the two systems, the CHATS has the higher affinity for NO_3, but has a very low capacity for NO_3 uptake (Forde and Clarkson, 1999). Isolation of an *Arabidopsis* mutant defective in the CHATS (Wang and Crawford, 1996) indicates that the CHATS and the IHATS are encoded by two different genes. It is thought that the CHATS is necessary to allow the initial uptake of NO_3 which in turn can induce the IHATS. An alternative view is that passive NO_3 uptake through anion channels would be enough to induce the IHATS (Miller and Smith, 1996). Is the CHATS truly constitutive or is the NO_3 contained in seeds used for studies enough to induce a basal level of uptake? The finding that the IHATS is not induced under low NO_3 levels (amount contained in seeds; Aslam et al., 1992) suggests that the CHATS is either

much more sensitive to induction at low NO_3 concentrations, or it is indeed constitutive.

LATS

The LATS is involved in the uptake of NO_3 at high concentrations. The K_m values for the LATS are in the range of 170-25,000 μM, and the V_{max} values are in the range of 8-700 $\mu mol \cdot g^{-1}$ fresh weight h^{-1}(Peuke and Kaiser, 1996). The LATS can significantly contribute to NO_3 uptake at concentrations above 250 μM, and does not saturate at NO_3 concentrations as high as 50 mM (Crawford and Glass, 1998). The uptake rate increases linearly between 5 and 100 mM. LATS was previously thought to be passive (Glass et al., 1990; Siddiqi et al., 1990), but subsequent studies of the electrical potential across the plasma membrane of barley roots indicate that the NO_3 concentration in the cytochrome (3-5 mM) is greater than that which could be achieved by passive transport (Zhen et al., 1991).

Nitrate uptake genes

Genes encoding NO_3 transporters have been identified in several fungi, bacteria, algae and higher plants. By screening for poor growth on NO_3 and/or chlorate resistance (ClO_3; the chlorine analog of NO_3), mutants emerged which were defective in NO_3 uptake. Characterization of the mutants and isolation of the genes responsible have led to the identification of two distinct gene families related to NO_3

uptake: *NRT1* and *NRT2*. *NRT1* and *NRT2* play distinct roles in nitrate uptake, and have no sequence similarity.

NRT1

The *NRT1* family encodes transport proteins with dual or low affinity for NO_3. The first member of the *NRT1* family was identified from a chlorate resistant mutant of *Arabidopsis* called *chl1* (Braaksma and Feenstra, 1973; Tsay et al., 1993). Analysis of mRNA expression patterns indicates that CHL1 expression is NO_3 inducible and found primarily in roots (Tsay et al., 1993). This mutant is unique in that it displays wild-type levels of NO_3 reductase activity but reduced levels of NO_3 uptake. The gene was cloned and shown to encode a protein with 12 putative membrane-spanning regions, a membrane topology found in most cotransporters (Tsay et al., 1993). The CHL1 protein was expressed in *Xenopus* oocytes to test for NO_3 transport and shown to be a dual affinity transporter with 2 K_m values of 35 μM and 8 mM. This indicates that CHL1 is a component of both high- and low- affinity uptake systems. High NO_3 (>1mM) and acidification of the medium depolarized the oocyte membrane, similar to the response observed in plant root cells. Uptake of NO_3 into oocytes increased with expression of CHL1.

Two *NRT1* homologues have been identified in tomato: *LeNRT;1* and *LeNRT;2* (Lauter et al., 1996). Both are expressed mainly in roots, but *LeNRT;1* is expressed constitutively while *LeNRT;2* is induced by NO_3.

Two *NRT1* homologues have been identified and cloned from *Brassica napus* (Muldin and Ingemarsson, 1995). One of these, *BnNRT1;2*, when expressed in *Xenopus* oocyte, proves to be an effective transporter of nitrate and capable of transporting amino acids, particularly histidine (Zhou et al., 1998).

NRT2

The *NRT2* family encodes transport proteins that contribute to the IHATS. The first member of this family to be identified was the *CRNA* gene from a chlorate resistant mutant of *Aspergillus nidulans*. The first higher plant members of the *NRT2* family (*HvNRT2A* and *HvNRT2B*) were identified from barley (Trueman et al., 1996). Other members of the *NRT2* family include genes from *Nicotinia plumbaginifolia* (Quesada et al., 1997), soybean (Amarasinghe et al., 1998), *Arabidopsis* and *L. japonicus*. The mRNA transcripts of these genes are found primarily in roots and are downregulated by reduced forms of nitrogen.

Regulation of nitrate uptake

The pathway of NO_3 uptake is highly regulated and has been studied in higher plants (Crawford and Arst, 1993; Hoff et al., 1994; Daniel-Vedele and Caboche, 1996; Huber et al., 1996). Regulation is necessary to coordinate root uptake of N with shoot demand during the growth cycle of the plant. The regulation of NO_3 uptake is quite different from the regulation of other ions because NO_3 uptake is induced by the substrate.

The roles of NO_3 reductase and nitrite reductase have been closely studied because they are readily assayed and because specific cDNA probes are available. Nitrate transport proteins are more difficult to assay. Only recently have the relevant probes been available to study their expression at the mRNA and protein levels.

Nitrate reductase activity is highly regulated in plants (Daniel-Vedele and Caboche, 1996; Huber et al., 1996). This raises the question of whether or not nitrate reductase is the key regulatory step in the N assimilation pathway and whether the changes seen in NO_3 uptake at different NO_3 concentrations are a result of changes in nitrate reductase activity. Studies on algae indicate that this is not the case; NO_3 uptake is just as highly regulated as nitrate reductase activity (Quesada and Fernandez, 1994; Pistorious et al., 1978; Florencio and Vega, 1982). Studies in higher plants concur. A nitrate reductase deficient barley mutant was used to demonstrate that NO_3 uptake was induced independent of any nitrate reductase activity (Warner and Huffaker, 1989). Higher plant NO_3 transporter genes have been shown to be inducible (Tsay et al., 1993; Trueman et al.,1996; Quesada et al., 1997; Amarasinghe et al., 1998) and feedback-repressible (Quesada et al., 1997; Amarasinghe et al., 1998) at the mRNA level. Separate regulation of nitrate reductase activity and NO_3 uptake in higher plants is not surprising when one considers that reduction of NO_3 is only one of the four possible fates of NO_3 in the cell (Forde and Clarkson, 1999).

Evidence from protein synthesis and RNA inhibition studies suggests that induction involves the synthesis of a new transporter protein (Hole et al., 1990; Aslam et al., 1993; Siebrecht et al., 1995). Evidence from molecular studies confirms that the

abundance of LATS (*NRT1*) and HATS (*NRT2*) NO_3 transporter genes increases rapidly when N starved roots are exposed to NO_3 (Tsay et al., 1993; Trueman et al.,1996; Quesada et al., 1997; Amarasinghe et al., 1998; Krapp et al., 1998).

The CHATS and IHATS are both upregulated in response to NO_3. In barley and white spruce the CHATS activity increased threefold upon exposure to NO_3 (Aslam et al., 1992; Kronzucker et al., 1995). The IHATS is induced by NO_3 and nitrite (Aslam et al., 1992; Siddiqi et al., 1990; Aslam et al., 1993; Kronzucker et al., 1995). In klondike barley, the induced IHATS flux was 30 times higher than the CHATS flux (Siddiqi et al., 1990), and in CM72 barley the IHATS flux was 10 times higher than the CHATS flux (Aslam et al., 1992). The IHATS activity often overshoots plant demand and is downregulated rapidly after initial exposure to NO_3 (Glass and Siddiqi, 1995; Forde and Clarkson, in press). Studies of *Nicotinia* and *Arabidopsis* indicate that several forms of N, including NO_3, NH_4, and amino acids, may contribute to this downregulation, and that downregulation can occur at the mRNA level (Quesada et al., 1997; Krapp et al., 1998).

Is the NO_3 signal perceived on the outside of the plasma membrane or on the inside? In other words, which is more important for regulation, the concentration of NO_3 in the cytoplasm or the concentration of nitrate outside the root cell?

In *Chlamydomonas*, the evidence suggests that concentration of NO_3 in the cytoplasm is important for induction. *Chlamydomonas* cells engineered to overexpress nitrate reductase no longer expressed the NO_3 transporter genes when exposed to NO_3 (Navarro et al., 1996). The authors hypothesized that the high nitrate

reductase activity reduced the cytoplasmic NO_3 concentration to a level below that required to induce transcription of the transporter genes. In higher plants it is theorized that the loading of N into the xylem for transport to the shoot is regulated by the concentration of recycling amino acids. This loading, in turn, is likely to have an effect on the concentration of various cytoplasmic pools of N compounds. The affected cytoplasmic pools of N compounds are probably responsible for regulation of NO_3 uptake through effects on transcriptional activity, and possibly through direct effects on transport activity (Imsande, 1994; Marschner et al., 1997).

In contrast, evidence from a study on barley indicates that it may have a receptor mechanism outside the plasma membrane. A low concentration (10 μM) was sufficient to achieve full induction of the IHATS in barley roots (Aslam et al., 1993). Even short pulses of NO_3 (70 μM for 5 minutes) were able to induce the IHATS in barley seedlings. In another study using barley, it was observed that the abundance of nitrate reductase mRNA fell rapidly within 30 minutes of withdrawing external NO_3, even though tissue NO_3 concentrations did not decrease over the same time period (Sueyoshi et al., 1995). These studies suggest that it is the external NO_3 concentration that is important for NO_3 transport regulation.

Ammonium has been shown to have an inhibitory effect on the accumulation of NO_3 in plants (Rufty et al., 1982; Lee and Drew, 1989). In contrast, low concentrations of NH_4 in the nutrient solution have been shown to stimulate NO_3 uptake in plants (Bloom and Sukrapanna, 1990). The stimulation of NO_3 uptake by low concentrations of NH_4 has been proposed to be due to the acidification of the

medium and the subsequent increase in the proton gradient across the plasma membrane which, in turn, promotes the NO_3 uptake (Smart and Bloom, 1998). The inhibitory effect may be observed within minutes (short term) or it may require hours or days (long term). The actual mechanism is controversial. Is the NO_3 influx pathway reduced or is the NO_3 efflux pathway increased (Glass et al., 1985; Ullrich, 1992; Aslam and Huffaker, 1994)? A common theory of the short term inhibitory effect of NH_4 on NO_3 uptake is that exposure to NH_4 depolarizes the plasma membrane therefore reducing the proton motive force necessary for NO_3 uptake by the $2H/NO_3$ symport mechanism (Ullrich, 1992). The long term inhibitory effect of NH_4 on NO_3 uptake is probably due to a feedback mechanism involving amino acids and other products of ammonium assimilation rather than NH_4 itself. Treating roots with methionine sulphoximine, an inhibitor of the first step of NH_4 assimilation, relieved NH_4 inhibition of net NO_3 uptake (Breteler and Siegerist, 1984; Lee et al., 1992). Reports suggest internal and external pools of NH_4 may have a direct effect on NO_3 uptake (Aslam et al., 1996b), but this study determined NO_3 uptake by NO_3 depletion of the external solution. The effect of the NH_4 may have stimulated NO_3 efflux rather than inhibited NO_3 influx.

IHATS for NO_3 is regulated through negative feedback from a product(s) that monitors the N status of the tissue. Exposing roots to exogenous amino acids reduces NO_3 uptake, and decreasing internal amino acid concentrations can stimulate NO_3 uptake (Atilio and Causin, 1996). In soybean plants, NO_3 uptake was inhibited by a number of externally applied amino acids (Muller and Touraine, 1992). This effect

was later shown to be an effect on NO_3 influx and not NO_3 efflux (Muller et al., 1995). In maize cell suspensions, the total pool size of exogenously applied amino acids was correlated with NO_3 uptake but no correlation existed between specific amino acids and uptake inhibition (Padgett and Leonard, 1996). The question remains as to whether a single amino acid plays a regulatory role in NO_3 uptake through feedback inhibition, and if so, which one?

The internal NO_3 pool has been hypothesized to have a direct effect on NO_3 influx. Using a nitrate reductase deficient mutant of barley, researchers demonstrated a marked decrease in NO_3 influx within 5 days of exposure to a low concentration of NO_3, despite the reduced amount of reduced N products (King et al., 1993).

THE PLANTS

Cornus sericea L. (formerly *Cornus stolonifera* Michx. F.)

C. sericea, red osier dogwood, is a member of Cornaceae and is native from Newfoundland to central Alaska and south to Virginia, Kentucky, Nebraska California, Arizona, New Mexico and northern Mexico (Dirr, 1998; Little, 1953). It is adapted to a wide range of soil and climactic conditions and is often found in wet, swampy sites in the wild. *C. sericea* is best suited for zones 2-7 and will not tolerate extreme heat or humidity (Dirr, 1998).

C. sericea is a vigorous, multi-stemmed, deciduous shrub that spreads by stolons. It has opposite, simple, 5 to 13 cm (2 to 5 inches) long leaves which are

medium green in summer becoming purple to red in fall. Its upright stems vary in color from yellow-green to bright red and are covered with lenticels. Its dull-white flowers are born in spring in 3.8 to 6.3 cm (1.5 to 2.5 inches), flat-top cymes. The fruit that follows is a 0.76 cm (0.3 inch) drupe that starts green and turns white. *C. sericea* has a fibrous root system and is easily transplanted from containers, bare root, or balled-and-burlapped.

C. sericea is easy to propagate from softwood and hardwood cuttings. Seed propagation requires fall planting or a 2 to 3 month cold stratification; 5° C (41°F) for 60 to 90 days is recommended (Dirr, 1987, 1998). Softwood cuttings have been rooted with 90% success anytime there were leaves on the plant when subjected to a 1000 ppm IBA quick-dip. Hardwood cuttings placed in the field in late winter give 90% to 100% success with no treatment (Dirr, 1998).

C. sericea 'Cardinal' is a large 2.4 to 3 m (8 to 10 ft) form with bright red stems. It was introduced in 1987 by Dr. Harold Pellet of the Minnesota Landscape Arboretum.

C. sericea 'Isanti' is also an introduction of the Minnesota Landscape Arboretum. It is more compact 1.5 to 1.8 m (5 to 6 ft) than *C. sericea* 'Cardinal' with shorter internodes.

C. sericea 'Kelseyi' is a low-growing, compact form 61 to 76 cm (24 to 30 inches) with a mounded habit. The stem and fall leaf color of 'Kelseyi' is less impressive than the species (Dirr, 1998).

***Euonymus alatus* (Thunb.) Sieb.**

E. alatus, the winged euonymus, is a member of Celastraceae and native from northeastern Asia and Japan to central China (Bean, 1981; Dirr, 1998). It is a deciduous shrub that tolerates various soil, pH and light conditions; however, it is not tolerant of heavy wet soils and performs best in full sun.

E. alatus is a slow growing, mounded or horizontal shrub which is usually broader than high. Contrary to how it is often listed, *E. alatus* can reach a size of 4.6 to 6.1 m (15 to 20 feet; Dirr, 1998). Leaves are simple, opposite to sub-opposite, serrated and 2.5 to 7.6 cm (1 to 3 inches) long. Leaves are dark green in summer and turn a brilliant red in fall. Stems of *E. alatus* are green or brown with two to four 6.3 to 13 mm (0.25 to 0.5 inch) corky wings. The small 6.3 mm (0.25 inch) yellow-green flowers occur in three-flowered cymes in spring and merit little ornamental attention. The subsequent fruit is a 6.3 to 8.5 mm (0.25 to 0.33 inch) capsule which exposes an orange-red seed when it dehisces; though brilliant, the seeds have little ornamental impact as they are borne under the foliage (Bean, 1981; Dirr, 1998).

E. alatus 'Compactus' is a slightly smaller form [3 m (10 ft)] but is by no means a small shrub. The branches are smaller and more densely borne. The corky extensions on the stem of 'Compactus' are less pronounced than the species (Dirr, 1998).

***Weigela florida* (Bunge.) A. DC.**

W. florida, old fashioned weigela, is a member of Caprifoleaceae and is native to Japan. It is extremely adaptable but prefers well-drained soil and full sun.

W. florida is found in various sizes and habits depending on the cultivar. Often, the gray-brown branches are arching to the ground. The 5.1 to 11 cm (2 to 4.5 inches) leaves are opposite, simple and medium green in summer. Fall color is yellow to orange at best but often dull brown. Seeds are 2.54 cm (1inch) long, two-valved capsules of no ornamental significance (Dirr, 1998).

W. florida is one of the easiest plants to propagate. Seeds can be sown directly, and softwood cuttings root readily from June to August (Dirr, 1998).

W. florida 'Minuet' is a compact, dwarf form reaching 76 cm (30 inches) in height. Foliage is dark green and tinged with purple. Flowers are 3.8 cm (1.5 inches) long, slightly fragrant and ruby-red on the outside of the corolla. The lobes are purple-lilac and the throat is yellow. *W. florida* 'Minuet' was released by Agricultural Canada and is a cross between 'Folis Purpureis' and 'Dropmore Pink' (Dirr, 1998).

W. florida 'Red Prince' has a red flower and grows to 1.5 to 1.8 m (5 to 6 ft) tall. It was introduced by Iowa State University (Dirr, 1998).

W. florida 'White Knight' has white flowers and grows to 1.5 to 1.8 m (5 to 6 ft) tall. It is also an introduction of Iowa State University (Dirr, 1998).

W. florida Wine and Roses® 'Alexandra' is a compact form [1.2 to 1.5 m (4 to 5 ft)] with dark burgundy-purple leaves and bright pink flowers (Dirr, 1998).

SYSTEMS

A background of systems thinking

The word "system" has been defined in many different ways. For this manuscript, I am not concerned with the everyday contentless use of "system" (Flood and Jackson, 1991). Rather, I am focusing on a stronger connotation that defines a situation of complexity. The following are a few excerpts that define "system" as it will be used throughout this text.

1. A system is a complex and highly interlinked network of parts exhibiting synergistic properties; the whole is greater than the sum of its parts (Flood and Jackson, 1991).
2. A system, formally, is a set of components that interact with each other (Clayton and Radcliffe, 1996).
3. A system consists of a number of elements and the relationships between the elements (Flood and Jackson, 1991).
4. A system is a set of two or more interrelated elements of any kind. It is not an ultimate indivisible element but a whole that can be divided into parts (Ackoff, 1974).
5. Viewed structurally, a system is a divisible whole; but viewed functionally it is an indivisible whole in the sense that some of its essential properties are lost when it is taken apart (Ackoff, 1974).

The science of systems thinking is traditionally accepted as emerging in the 1940s in response to the failure of mechanistic thinking to explain biological

phenomena (Flood and Jackson, 1991). To fully understand this emergence, a brief history is appropriate.

Aristotle stated 2300 years ago that, "the whole is more than the sum of its parts." This dictum remained a central part of scientific and philosophical thought for the centuries that followed. However, Aristotle's descriptive-metaphysical conception of the universe was negated and bypassed by the Scientific Revolution of the 16th and 17th centuries (von Bertalanffy, 1975). Aristotle's view of the world was replaced by a causal, mathematical view associated with 16th and 17th century contemporaries such as Galileo and Descartes. The second maxim of Descartes' *Discours de la Méthode* " to break down every problem into as many separate, simple elements as might be possible," was a polar contrast to Aristotle's view. Galileo concurred with Descartes and put forth a similar idea of the "resolutive" method (von Bertalanffy, 1975). Thus was born the mechanistic age and the familiar paradigm of modern science that a whole can be understood by reducing it to and understanding its parts - reductionism. The reductionist method worked great for causal chains and was at the root of the subsequent successes experienced in physics. However, the treatment of complex systems, particularly biological and social phenomena, still remained and mechanistic explanations and reductionism offered little insight. Around the turn of the 20th century, a reemergence of Aristotelian theory (under new names and descriptions) occurred, championed by philosophers such as Hans Adolf Eduard Driesch (1867-1941) and Henri Bergson (1859-1941) and scientists such as Paul Weiss, Walter B. Cannon and Ludwig von Bertalanffy (Flood, 1999). In response, a debate began that

indicated increasing doubts in the ability of the traditional paradigm of classical science (reductionism) to explain complex phenomena (von Bertalanffy, 1975). This was the beginning of the transition to the "Systems Age" (Ackoff, 1974).

Even though the notion of systems had been around for quite some time, it remained a philosophical discipline rather than a scientific one because mathematical techniques were lacking and the successes of traditional reductionist science resisted any change in the scientific paradigm (von Bertalanffy, 1975). Ludwig von Bertalanffy first formulated the notion of general systems theory in the 1930s and 1940s, providing some mathematical definitions for system components. Others followed, and the science of systems thinking was born.

By their nature, the production, distribution, consumption, and study of horticultural crops occur as part of a complex web that is constantly under the influence of nature, economics, politics, and many other social and natural effects. Many of the obstacles faced by growers, policy makers, and scientist are not separable cause and effect, linear problems; instead, they are situations that consist of complex systems of strongly interacting problems (messes; Ackoff, 1999). Traditional methods of inquiry (reductionism and closed system theory) often fail to account the complexity of relationships between components in a horticultural system and the forces that act upon them. Concepts of physics are helpless in appreciating the dynamics of such situations (Flood, 1999). Therefore, it may be hypothesized that a different inquiry method (systems approach) will be more beneficial to understanding and solving problems of complexity in horticulture.

Science, as a discipline, adheres to a strict set of rules, the scientific method. This method, as stated previously, has worked quite well for physics but may not be the best criteria for dealing with complex adaptive systems encountered in biology, ecology, agriculture and other fields. It is important that we never allow our thinking to become unduly constrained by disciplinary boundaries to the point where we might fail to observe deep underlying continuities in terms of organizational processes and structures (Clayton and Radcliffe, 1996).

Panarchy and the renewal cycle

Recognizing patterns, behaviors, and cycles plays an important role in understanding the functions and evaluating the consequences of complex systems as they perform across temporal and spatial scales. To investigate, this thesis employs the renewal cycle introduced by C.S. Holling (1986) and further developed in a book called "Panarchy" (Gunderson and Holling, 2002).

The renewal cycle is developed from a theory of ecosystem succession (Clements, 1916) based on the presence of and sequential domination by opportunistic species (*r*-species) in unpredictable environments and equilibrium species (*K*-species) in predictable environments (MacArthur, 1960). *r*-species have a high reproductive potential, short life, high dispersal properties, small size and resistance to extremes and represent initial, exploitive species in a community. In contrast, *K*-species have lower reproductive potential, longer life, lower dispersal rates, larger size and effective competitive abilities and represent climax species of a community (Pianka, 1970).

Clements' (1916) theory of ecosystem succession implies that communities reach and maintain a stable state in the *K* phase of succession.

Holling's (1986) renewal cycle adds a "backloop" to the succession model and introduces two more phases besides *r* and *K*. The first additional phase occurs after the *K* phase and is defined as "creative destruction" (Holling, 1986). This phase represents an event that serves to release bound resources (accumulated during the progression from *r* to *K*) back to the system and is assigned the symbol Ω. The other phase, introduced by the addition of the backloop, is termed "reorganization" or "renewal". It occurs after the phase and is given the symbol α. The α phase is characterized by increasingly available resources (released during) and the reorganization of resources and consumers (Holling, 1986; Gunderson and Holling, 2002). After the α phase, the system of interest enters an *r* phase, and the cycle continues.

The renewal cycle has historically been applied to ecological or natural systems. However, researchers from various disciplines have attempted to apply the model to human, economic, political, cultural and natural systems as well as systems requiring the interaction of humans and nature (Gunderson and Holling, 2002). Three parameters (potential, connectedness and resilience) have been identified as significant and meaningful measurables in the analysis of renewal cycle applications. Each of these parameters may increase and decrease as a system evolves through the four phases of the renewal cycle. Furthermore, individual renewal cycles do not exist as separate entities, cut off from the rest of the world. Instead they exist as nested cycles

with smaller, faster cycles below and larger slower cycles above (Gunderson and Holling, 2002). Cycles at different scales are connected by events whose consequences may effect cycles both above and below them.

The word "panarchy" was developed by the authors of the book to capture the adaptive and evolutionary nature of renewal cycles, and is a combination of "Pan", from the Greek god Pan (universal god of nature) and "hierarchy". "Pan" represents the dynamic and somewhat unpredictable nature of systems and "archy" (from hierarchy) emphasizes the nested nature of individual renewal cycles (Gunderson and Holling, 2002).

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CHAPTER 3

GROUP-BASED ON-SITE ACTIVE LEARNING (GOAL): TECHNIQUE FOR INVESTIGATING NITROGEN MANAGEMENT IN CONTAINER NURSERIES

ABSTRACT

Nitrogen (N) management in container nurseries is a complex system. Working within this system, nursery owners, managers and employees routinely make N management decisions that have consequences for the immediate nursery environment (e.g. plant growth, yield, disease susceptibility, water quality) as well as areas beyond nursery boundaries (e.g. surface and groundwater quality, public perception). Research approaches often address parts of the system associated with the immediate nursery environment and purpose. As a result, best management practices that contribute to greater N use efficiency have been developed. Research approaches that consider the whole system reveal novel relationships and patterns that identify areas for future research and may direct future management decisions. To investigate N management from a whole system perspective, a group of nursery managers from Oregon and scientists from Oregon State University met three times between 2001 and 2003. Growers drew their N management systems and identified components, relationships and feedback loops using an ActionGram technique. From

this information, researchers developed Group-based On-site Active Learning (GOAL). GOAL combines ActionGrams and the Adaptive Cycle at container nursery sites. In this case N flow and management in container production systems served as the topic of active learning. Managers and employees from four wholesale container nurseries evaluated the GOAL exercise. After completing GOAL, 94% of participants indicated that they learned a new idea or concept about N cycling in their container nursery. 100% of participants gained new ideas and concepts from peers and colleagues present at the meeting. 60% of participants gained new ideas and concepts from researchers and 60% developed some of their own ideas and concepts. GOAL is a learning tool that provides a simple, convenient, interactive format for investigating complex systems.

INTRODUCTION

Container production of woody ornamental plants is gaining popularity because it offers advantages over field production. These advantages include more plants per unit land area, faster plant growth, higher quality plants, lack of dependence on arable land, easier shipping and handling, and year-round sales (Davidson et al., 1988; Whitcomb, 1986). Yet, container production requires the use of a porous substrate, usually a mix of bark, sand, or peat moss with little water holding capacity (Furuta, 1976; Rathier and Frink, 1989) and little ability to retain nitrate (NO_3^-) ions (Foster et al. 1983). Consequently, the substrate must be watered often to maintain

adequate moisture, and nitrogen (N) levels must be replenished frequently. Combined, these two conditions lead to substantial N leaching losses from container nurseries and subsequent concern over NO_3–N contamination of surface and ground water.

Management of N in container nurseries is a complex system of decisions and actions with consequences that effect both the immediate time and space embodied in the nursery as well as scales far beyond the nursery boundaries. For instance, N management decisions will affect NO_3^- levels at the nursery as well as potentially effecting NO_3^- levels in the local watershed. Therefore, multiple stakeholders exist (eg. neighbors, environmental groups, regulating agencies, academics, consumers, political factions, etc.) beyond the obvious economic interests of the nursery owner/manager. Effective and lasting solutions to N pollution must consider the interests of all stakeholders. Yet, because of individual perspectives, understanding, discussion, planning, and implementation of effective policies can be strained. The need exists for a simple, integrative model that encourages open, two-way communication between stakeholder groups with different views.

Reductionist research investigates parts of the system resulting in improved water usage, water recycling, controlled release fertilizers, catch strips and other best management practices that contribute to greater N use efficiency. However, the system as a whole may behave differently than the individual parts. Research approaches that consider the whole system reveal novel relationships and patterns that identify areas for future research and may direct future management decisions.

The objectives of this project were (i) to gather information from nursery managers about their respective N management systems, (ii) to develop an interactive exercise for learning about and communicating N management in container nurseries, and (iii) to have the exercise evaluated by nursery managers.

MATERIALS AND METHODS

A group of nursery managers and researchers from Oregon State University identified the components and relationships important to N management systems used in container nurseries. The group met three times: 23 Feb. 2001, 30 Oct. 2001, and 4 Mar. 2002. Four managers and three researchers attended the first meeting. Two managers and three researchers attended the second meeting. Six managers and three researchers attended the third meeting. At each meeting, participants were provided large sheets of newsprint and asked to draw their respective N management systems according to the following directions:

1) List or name the components of your N management system.

2) Use arrows to indicate relationships between components.

3) Identify feedback loops. Are they positive or negative? Do they balance?

Participants could work alone or in groups (two or three) and were asked to complete their ActionGram (William, 2002) in approximately 15 minutes. After diagrams were complete, each person or group shared their diagram, and participants commented or asked questions. After presentations, each person or group was allowed five minutes

to revise their diagram by adding or changing items discussed. After revisions, the group reassembled for a final debriefing of the N management diagramming exercise.

After the meetings, researchers summarized components and relationships of the ActionGrams. Analysis of the ActionGrams led to the pursuit of an integrative theory that accounted for the major drivers of N management systems while also considering the relationships of N management to related systems (e.g. pest management, water management, environment, public, neighbors, politics, etc.). During this development, we became aware of the adaptive cycle and Panarchy (Gunderson and Holling, 2002). This theory fit our criteria and represented a plant production cycle and the practices involved in the operation of a container nursery.

The Adaptive Cycle is developed from a theory of ecosystem succession (Clements, 1916) based on the presence of and sequential domination by opportunistic (exploitation) species (*r*-species) in unpredictable environments and equilibrium (conservation) species (*K*-species) in predictable environments (MacArthur, 1960). *r*-species have a high reproductive potential, short life, high dispersal properties, small size and resistance to extremes and represent initial, exploitive species in a community. In contrast, *K*-species have lower reproductive potential, longer life, lower dispersal rates, larger size and effective competitive abilities and represent climax species of a community (Pianka, 1970). Clements' (1916) theory of ecosystem succession implies that communities reach and maintain a stable state in the *K* phase of succession (Fig. 3.1-A).

Holling's (1986) Adaptive Cycle adds a "backloop" to the succession model and introduces two more phases, Ω (release) and α (reorganization), that complete the cycle (Fig. 3.1-B). Ω occurs after the *K* phase and is defined as "creative destruction" (Holling, 1986). This phase represents a release of bound resources (accumulated during the progression from *r* to *K*) back to the system followed by immediate "reorganization" of resources and processes, assigned the symbol α (Holling, 1986; Gunderson and Holling, 2002). After the α phase, the system resumes an *r* phase, and the cycle continues.

Three parameters, potential, connectedness and resilience, are significant measures of renewal and are incorporated by imposing a set of axes on the Adaptive Cycle (Fig. 3.1-C). Potential may be defined in biological (e.g. biomass, nutrient accumulation, physical structure), social (e.g. network of relationships, trust, friendships), or economic (e.g. value of product, value of acquired skills, inventions) contexts. Connectedness is defined as the internal control that a system can exert over external variability and reflects the strength of connections between system components. Resilience is defined as the capacity of a system to experience disturbance and still maintain its fundamental functions and controls (Gunderson and Holling, 2002). Each of these parameters will increase and decrease as a system evolves through the four phases of the Adaptive Cycle.

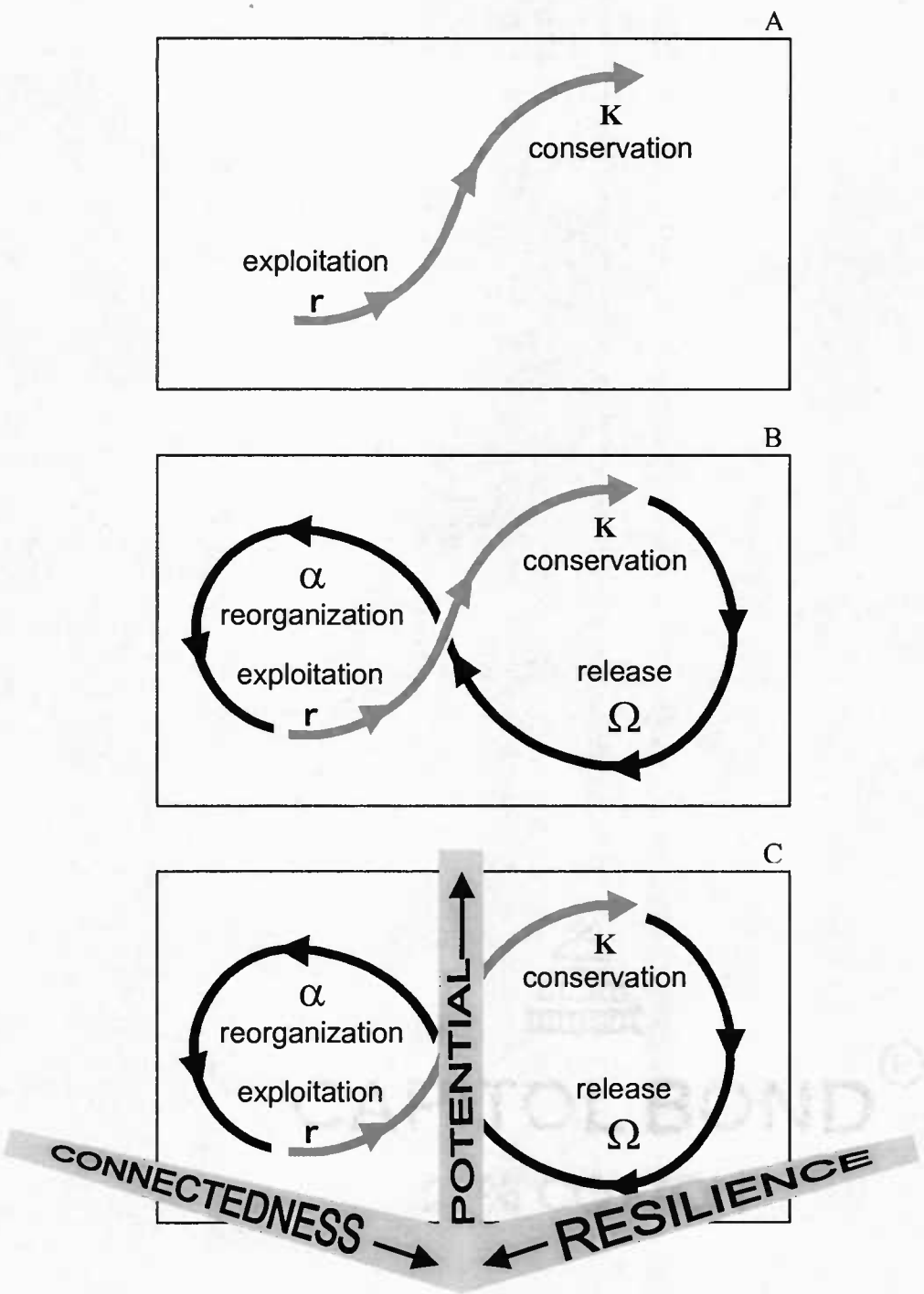


Fig. 3.1. Development of the Adaptive Cycle. (A) A succession model or growth curve illustrating the progression from r (exploitation) to K (conservation). (B) Addition of a backloop that introduces Ω (release) and α (reorganization) phases to the cycle. (C) Application of axes representing potential, connectedness, and resilience. Each parameter increases in the direction of the arrow.

Initial analysis revealed that nursery production cycles follow a pattern similar to the Adaptive Cycle. The r (exploitation), K (conservation), Ω (release), and α (reorganization) phases of the Adaptive Cycle are analogous to the new crop, mature crop, shipping, and reorganization phases of a container nursery production cycle, respectively.

Group-based On-site Active Learning (GOAL) combines the ActionGram exercise with a diagramming and discussion session centered on the Adaptive Cycle. The ActionGram exercise is intended to focus participants on the subject, and the Adaptive Cycle session is intended to promote interaction among participants as well as with the model. Materials needed for the ActionGram exercise include large pieces of paper and markers. A laminated poster was developed for the Adaptive Cycle session (Fig. 3.2). The format had to (1) be simple, (2) facilitate the investigation of time and spatial scales, (3) be interactive, and (4) be mobile, reusable, and easily prepared. The poster was designed with PowerPoint (Microsoft, Redmond, Wash.) and is 122 × 92 cm. Because the poster is laminated it can be written or drawn on with Expo (Sanford, Bellwood, Ill.) dry erase markers and easily cleaned with a dry cloth. The Adaptive Cycle addresses the time scale of a container production cycle and is surrounded by borders representing the nursery, watershed, state, continent, biosphere, and atmosphere. These boundaries allow participants to address spatial considerations related to N management in container nurseries. To measure potential, connectedness, and resilience of the system at different phases, a set of axes is imposed on the diagram (Fig. 3.3).

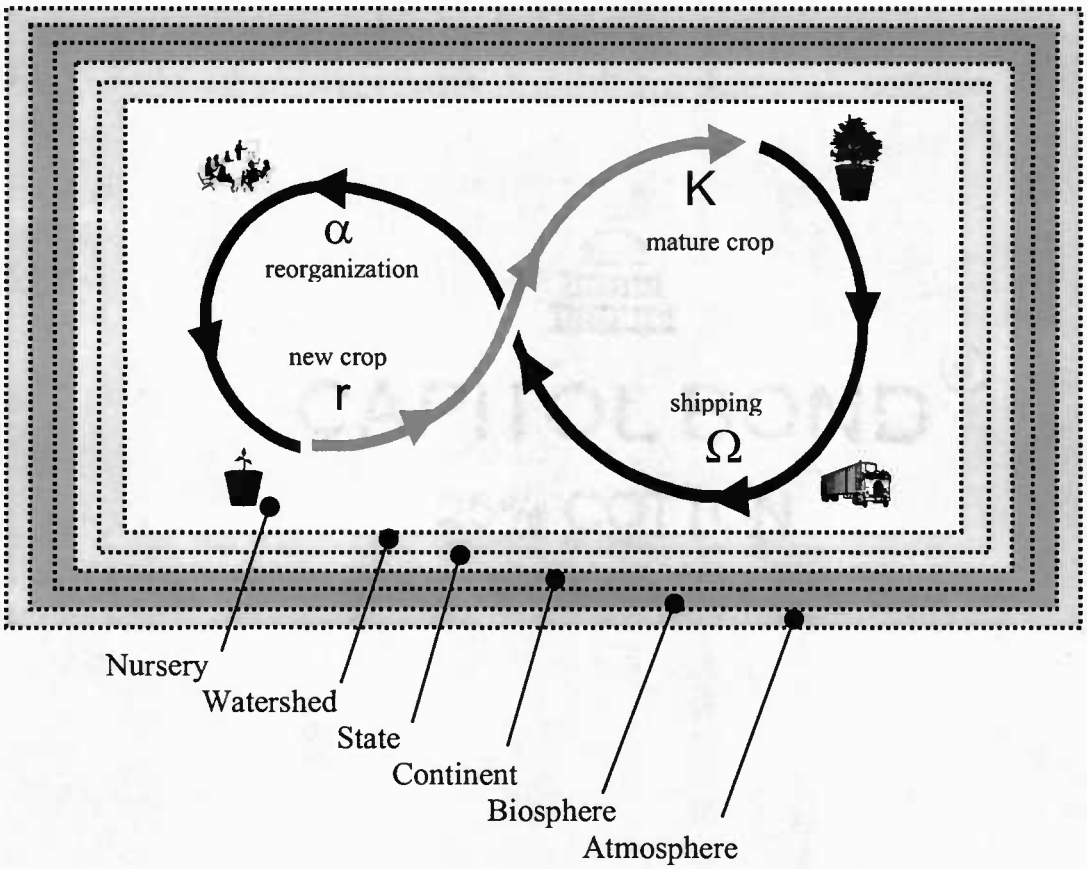


Fig. 3.2. A laminated poster containing a nursery production cycle imposed on the adaptive cycle. Different colored boundaries represent special scales from the nursery to the atmosphere. r = exploitation phase, K = conservation phase, Ω = release phase, and α = reorganization phase. The poster is the central element of the Group-based On-site Active Learning (GOAL) exercise.

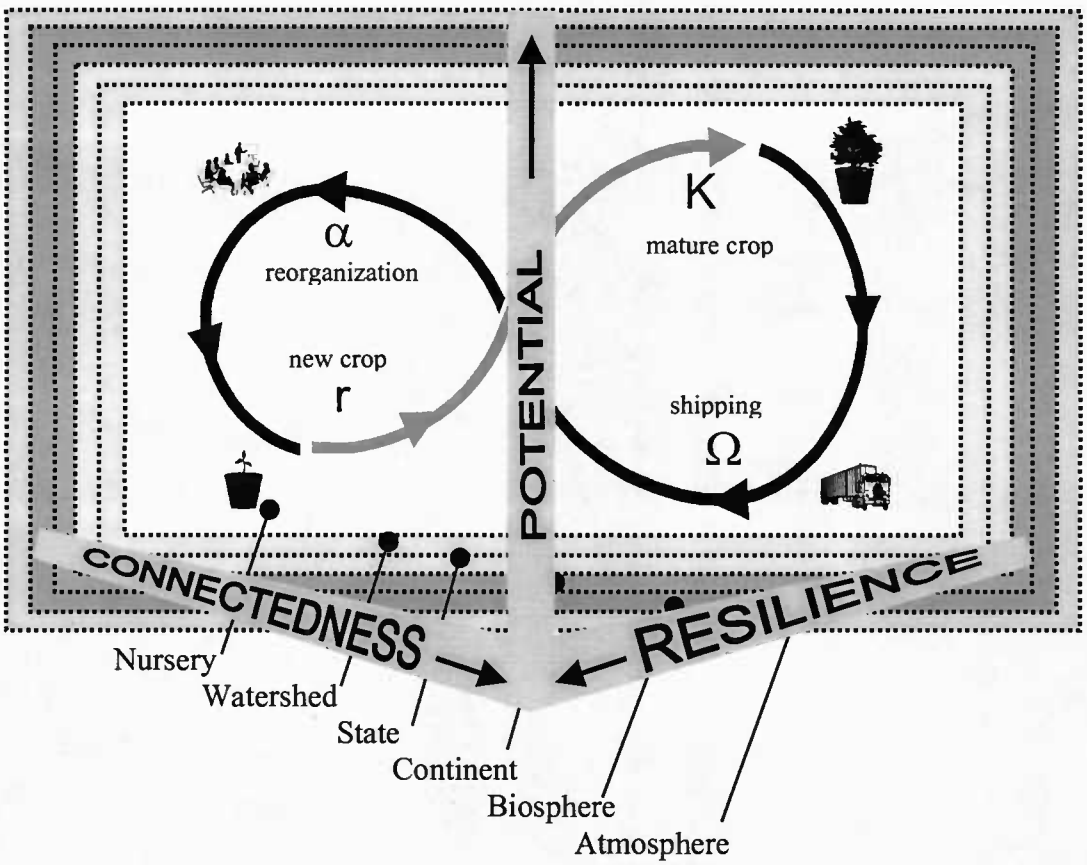


Fig. 3.3. The adaptive nursery cycle with three axes (potential, connectedness, and resilience) imposed on it. Each parameter increases in the direction of the associated arrow.

To evaluate GOAL as a learning tool, the exercise was conducted on-site at four container nurseries. At each nursery, participants were asked to draw N flow within their respective nursery (ActionGram). This step focused participants on N flow and management. After the diagrams were completed, each participant explained his/her diagram to the group, and participants commented or asked questions. Next, the Adaptive Cycle poster was introduced to the group. The introduction included a brief history of Adaptive Cycle theory and an explanation of the various phases and boundaries. In addition, the participatory nature of the poster (drawing on it, erasing, and easy cleaning) was demonstrated. Participants began working with the model by drawing/writing on the poster in response to two questions:

1) Where does N enter the system?
2) Where does N leave the system?

As discussion proceeded, other questions were presented including:

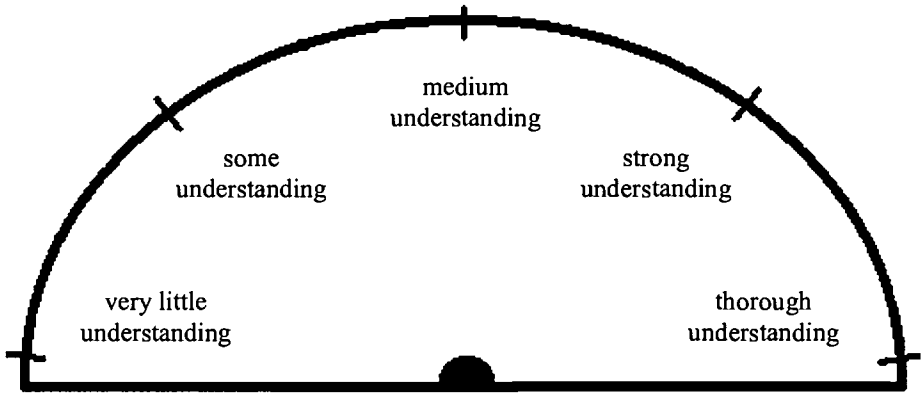
1) What are the sources of N?
2) Ultimately, where does N used in container production systems originate?
3) Ultimately, where does N go that is used in container production systems?

After 30 to 45 minutes of interaction centered on the Adaptive Cycle poster, participants were asked to complete an evaluation form (Fig. 3.4). The evaluation employs a post/pre measure of learning (Davis, 2003; Pratt, 2001) followed by four questions specific to the participant's experience with the exercise. A total of 19 responses were collected across four nurseries. Two sample t-tests were performed using SAS v. 8.02 (SAS Institute, Cary, N.C.).

Nitrogen (N) Flow in Container Nurseries
David Sandrock, Oregon State University

Evaluation and assessment

5) Imagine that the meter below is a measure of your understanding of N flow in your nursery. Draw an arrow representing where on the meter your understanding of N flow is right now, *after* today's meeting. Next, draw an arrow representing where on the meter your understanding of N flow was *before* today's meeting. Indicate which arrow is which.



1) Did the position of the arrows change?

2) What things did you learn today that caused the position of the arrows on your meter to change?

3) If the position of your arrows did not change, please explain why.

4) The ideas and concepts that I learned today came from:
   a. peers and colleagues
   b. researchers
   c. development of my own ideas

Fig. 3.4. Evaluation form presented to nursery managers after their participation in GOAL.

RESULTS AND DISCUSSION

Results from the ActionGrams indicated that nursery managers viewed N management as a dynamic system (i.e. changing over space and time) integrated with other practices (e.g. water management, pest management, labor) essential to successful management of a container nursery. While the core components and relationships were similar, results from the ActionGrams revealed that individual circumstances within a nursery created specific combinations unique to that nursery and led to the pursuit of an integrated model.

Figure 3.5 A-D shows four diagrams developed by managers participating in the GOAL exercise at their respective nursery. Common themes in all diagrams include recycling of irrigation runoff during the production season, loss of N during the production season, and spatial recognition of the source and fate of N fertilizers used for container plant production. Participants from each meeting assigned the majority of N inputs within the r to K phase. Initial N inputs such as liquid feeding or incorporation of controlled-release fertilizers, a common practice in container nurseries, occur near the r phase when connectedness and potential of the nursery cycle are relatively low but resilience is relatively high. Fertilizer N is delivered at r to increase the potential of the system; however, excess N at r will leak from the system (or be scavenged by weeds, microbes, etc). Even with fast growing plants, recommended N rates exceed plant demand resulting in N losses accumulating faster than N uptake by the plant from r to K (Sandrock, 2004). This is why it is essential that controlled-release fertilizer patterns match the needs of the crop (Sandrock, 2004).

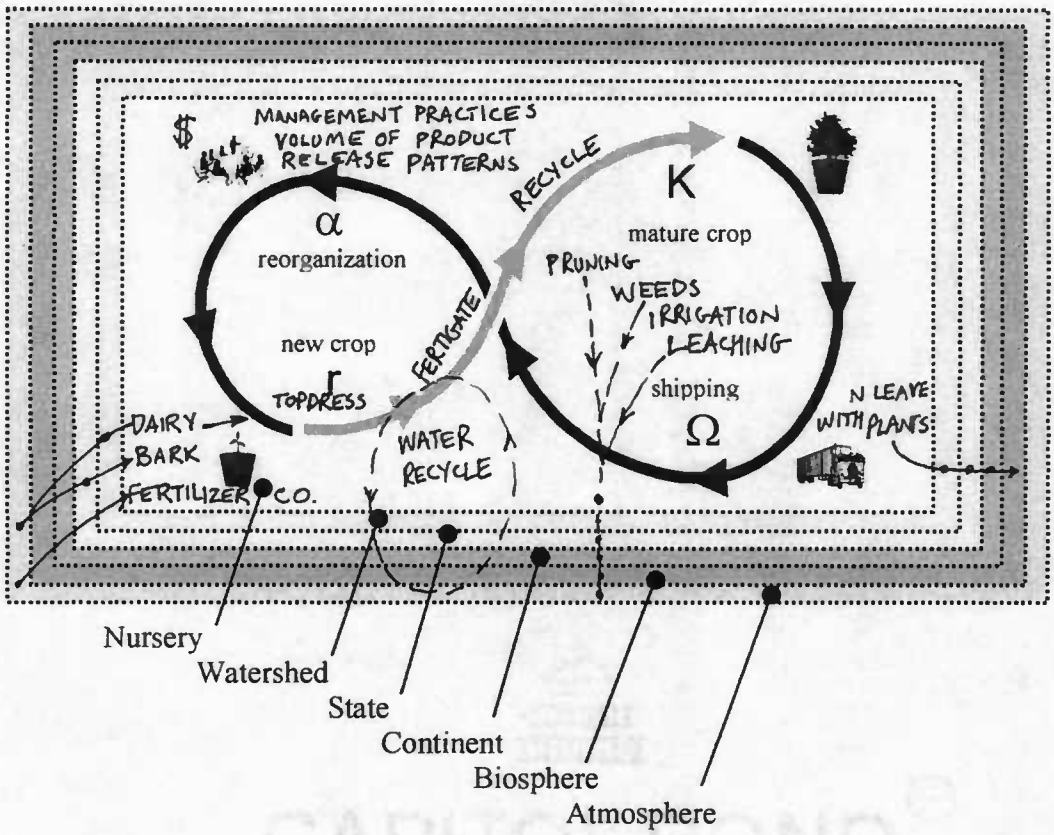


Fig. 3.5. Diagrams (A-D) developed by groups of managers and employees from four wholesale container nurseries. Participants were introduced to the model as seen in Fig. 3.2 and then drew or wrote in their perspectives on N management and flow in their respective nursery. Diagrams were re-drawn by the author to facilitate formatting to the text.

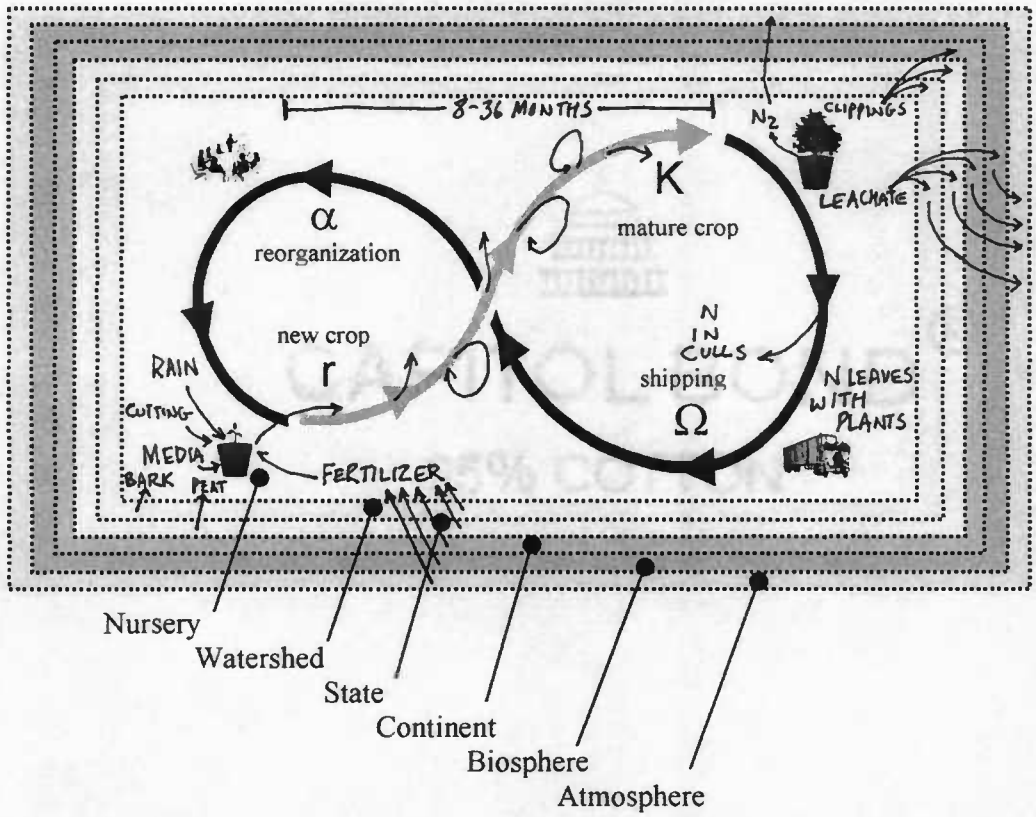


Fig. 3.5 B (continued)

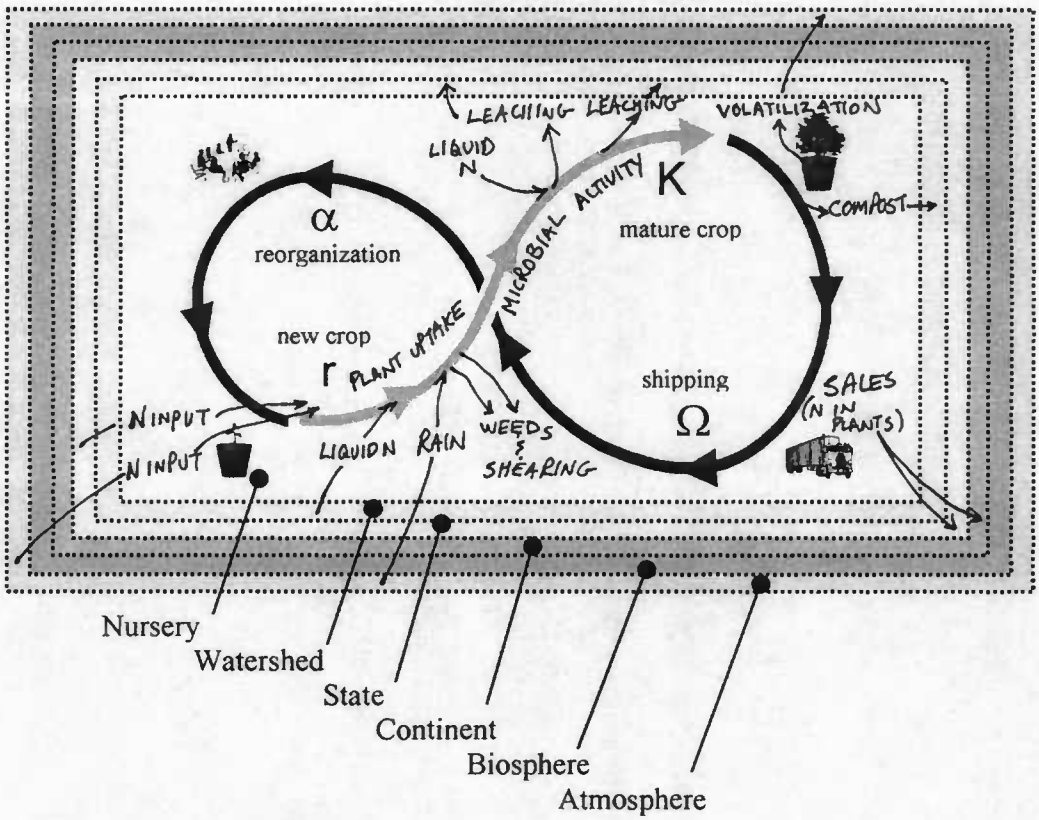


Fig. 3.5 C (continued)

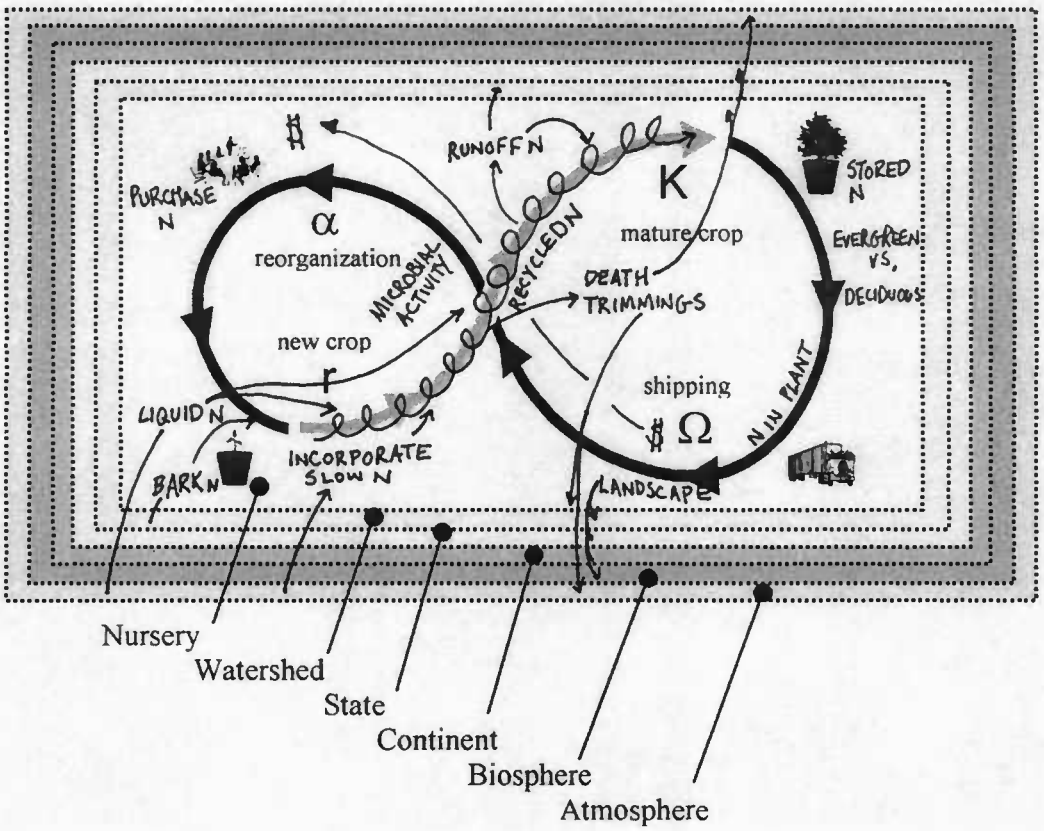


Fig. 3.5 D (continued)

Resilience at r is high because the crop is young and invested resources low. If a surprise or disaster (e.g. freeze, hurricane) were to occur and damage or eliminate the crop at this phase, it is likely that management and labor would have time and resources available to "bounce back" and start a new crop.

Potential and connectedness of the system increase and resilience decreases as the new crop matures. Participants identified periodic topdressing, liquid feeding, and recycling of runoff water as additional N inputs that occur as the system moves from r to K. Addition of N to the system promotes growth of the crop and potential (monetary value, biomass, etc.) increases. Likewise, the addition of N increases the connectedness of the system as it moves from r to K, and components of the system become more dependent on each other for survival. For instance, plants near the K phase are more dependent on a regular irrigation schedule than plants near r. As potential and connectedness are increasing, resilience decreases. If the same surprise or disaster mentioned above were to occur near K and damage or eliminate the crop, losses would be much greater because of the invested capital, and it is doubtful that managers and workers would be able to replace the crop or its value. Obviously, managers hope to convert the high potential at K to dollars by selling the crop, but the crop becomes vulnerable at K, and there are opportunities for pest or diseases to take advantage of the high potential for their own gains.

Participants identified multiple N "leaks" as the system moved from r to K including N leaching, N volatilization, removal of N in weeds, and loss of N from pruning. Fate of these N losses were discussed and the groups concluded that

although N lost from containers remains for some time within the nursery (compost piles and collection ponds) or in the local watershed (leaching and runoff), it ultimately returns to the atmosphere via biological processes. Many of the participants recognized that the process of introducing fertilizer N to the biosphere was occurring faster than the biological processes responsible for returning N to the endogenous atmospheric pool, thus causing concern about N contamination in the environment.

Each nursery group identified the substantial amount of N that leaves the nursery grounds when plants are sold. Participants affirmed that the goal of nursery managers was to have as much of the applied N in the plant or accompanying container when it leaves the property. Sale of nursery plants serves as a type of "creative destruction" ushering in the Ω and α phases of the cycle. The Ω phase is characterized by low potential because the crop is gone and payment has not yet been received.

The adaptive nursery cycle moves relatively quickly from Ω to α as connectedness decreases (e.g. fewer relationships between plants and cultural practices, labor and management, labor and plants) and potential and resilience increase. The system regains potential as accounts are paid (economic potential). Resilience increases as the production cycle approaches α because the system becomes more liquid (economically) and adjustments within the nursery (e.g. to labor, production strategies, expansion) are relatively easy at this stage compared to other phases of the cycle. Resilience remains high during the α and r phases because the system is able to readily adapt or change to outside influences. For example, if labor

availability decreases, managers can scale back production plans, or if market trends change, managers can adjust production strategies and goals to avoid losses and potentially benefit from those changes. The α phase is a period of reorganization when money that was received for plants sold can be routed to buy supplies (including N fertilizer) for the next cycle. This is also the period when decisions about production strategies, use of space, expansion, and labor are made in preparation for beginning the cycle again at the r phase.

When asked about GOAL, 18 of the 19 participants (94%) stated in response to question two of the evaluation form (Fig. 3.4) that the position of their arrow changed on the post/pre meter. By assigning the distance between each tick mark on the meter as two units (8 total units on the meter) the average distance that a participant's arrow moved was 1.9 units. The mean post evaluation (5.7 units, SD = 0.7) was significantly higher ($\alpha = 0.05$) than the mean pre evaluation (3.8 units, SD = 1.4). Seven participants (37%) indicated that their arrows moved from "medium understanding" to "strong understanding".

Responses to question three included:

- better understanding of the complexity of nitrogen cycles. I became more aware of the inputs and outputs.
- about nutrition of the plants and where the nutrition comes from
- where nitrogen is derived, % in the air, nitrogen goes somewhere other than on plants

- bigger picture, N from atmosphere, larger perspective than my department... nurserywide
- where N comes from, where N goes, global impact
- think more about the big picture of N use
- group talk with input from different areas
- variety of sources of N
- inputs from others which I had not considered-views of others from a different vantage point
- most enlightening was thinking about outside receptions and how we might prepare/analyze our data and practices
- had not thought about the idea of N flow in terms of our nursery
- we tend to only think about what happens 'on site', but as we discussed the cycle of our product you gain an understanding of the impact you may have 'off site'
- that nitrogen is not only used in the pots and for the plants, but in fact many things affect the loss of nitrogen into the general public, also that the perception is that the nursery industry causes a large amount of runoff into the water supply
- N levels in recycled water
- cycle of N, where it comes from and where it goes
- original sources of N, discussion of multiple fates of N

- I felt I gained an understanding of the complexity of the topic. I do not know as much as I thought.
- The picture of N flow is now much more broad. I was thinking more of a single plant picture previously vs. where N is obtained, produced, and ultimately ends up.
- sources and destinations of N in the cycle. How the different boundaries are affected.

Only one participant responded to question four, indicating that the position of their arrow did not change. Their response was:

- I may be biased, but I think I have a pretty good handle on our systems.

In response to question five, 100% of participants indicated that they gained new ideas and concepts from peers and colleagues present at the meeting. 60% of participants gained new ideas and concepts from researchers, and 60% developed their own ideas and concepts.

Nursery managers regard N management as a dynamic system closely integrated with other nursery practices (e.g. water management, pest management, timing of production and sales, labor) and having consequences within the nursery (e.g. plant growth, economic return) as well as beyond the boundaries of the nursery (e.g. pollution, regulations, public perception). Managers concurred that the adaptive cycle is an accurate representation of container nursery production, matching the processes, events, and choices encountered during a production cycle. GOAL

provides a simple but robust context for investigation of N flow and management validated by measured learning among participants. GOAL will be valuable in facilitating discussion and understanding of complex issues associated with nursery production (e.g. N, water, and pest management) amongst nursery personnel and with stakeholders from other organizations (e.g. regulating agencies, environmentalist, the public). GOAL promotes participatory investigation of complex issues and serves as a teaching or extension tool to measure subsequent learning.

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CHAPTER 4

ISOTOPIC AND NONISOTOPIC ESTIMATION OF NITROGEN FERTILIZER UPTAKE IN CONTAINER-GROWN WOODY ORNAMENTALS

ABSTRACT

Accurate methods for determining the fate and recovery efficiency of nitrogen (N) fertilizer applied to container-grown nursery crops are essential to industry compliance with regulations as well as development of innovative fertilizer programs. The objectives of this study were (i) to use ^{15}N techniques to determine the fate of fertilizer N, (ii) to compare nonisotopic and isotopic methods of determining N recovery, and (iii) to determine the relative importance of fertilizer and non-fertilizer N at rates of 25, 50 100, 200, and 300 $mg \cdot L^{-1}$ in container-grown *Euonymus alatus* (Thunb.) Sieb., *E. alatus* 'Compacta', *Cornus sericea* L. 'Cardinal', *C. sericea* 'Isanti', *Weigela florida* (Bunge) A. DC. 'Red Prince', and *W. florida* 'Alexandra' (Wine and Roses). In all species, root and shoot N increased with N rate, and at each rate more N was stored in the roots than in the shoots. Estimation of N recovery determined by the linear regression of total N in the plant on rates of applied N was significantly higher for all species and at each N rate than estimation of N recovery determined by linear regression of labeled fertilizer N in the plant on rates of labeled

fertilizer N applied. Increasing fertilizer rates up to 100 $mg \cdot L^{-1}$ resulted in increased uptake of N derived from other sources (NDFO). NDFO at low N concentrations was a significant portion of the total N in the plant. As a result, the difference in estimation of percent N recovery between each method was larger at lower N concentrations for all species. Assuming that the ^{15}N tracer is the more accurate method of estimating fertilizer N uptake, the nonisotopic total N method overestimates fertilizer N uptake substantially, as much as three to four times in container-grown plants at N concentrations of 25 $mg \cdot L^{-1}$.

INTRODUCTION

Public concern regarding nitrate (NO_3^-) leaching from container nurseries and the potential contribution to surface and ground water contamination requires the development of reliable methods for determining the fate of nitrogen (N) fertilizers applied to nursery crops. In general, fertilizer N uptake is determined by the difference method (N in fertilized plants minus N in unfertilized plants) or by a ^{15}N fertilizer tracer. When various N rates are used, N recovery can be determined by the linear regression of total N in the crop on rates of applied N or by the linear regression of labeled fertilizer N in the plant on rates of labeled fertilizer N (Westerman and Kurtz, 1974).

The standard nonisotopic method of determining N recovery in field-grown agronomic crops is the difference method. This method is not suitable for long-term

container-grown nursery crop studies because the potting substrate supplies insufficient N to sustain woody crops for the duration of a pragmatic experiment. Considering the low N content of growing substrate used in container production (e.g. bark, sand, peat), fertilizer N recovery is often equated with total N in the plant. Therefore, it is common to determine N recovery efficiency in nursery crops by dividing the total N in the plant by the amount of applied N or by the linear regression of total N in the crop on rates of applied N (Niemiera and Wright, 1982; Maust and Williamson, 1991; Cabrera and Devereaux, 1999; Griffin et al., 1999).

For many agronomic crops grown in the field, ^{15}N fertilizer tracers are regarded as a superior method because determinations of N fertilizers can be made more accurately (Hauck and Bremner, 1976) and treatment effects are detected with greater sensitivity (Russelle et al., 1981). In addition,^{15}N tracers serve to distinguish fertilizer N from soil N, and allow the calculation of fertilizer N efficiency without regard to residual soil N (Torbert et al., 1992).

Many studies have compared isotopic and nonisotopic methods of determining fertilizer N recovery (Westerman and Kurtz, 1974; Olson, 1980; Oslon and Swallow, 1984; Rao et al., 1991; Bronson et al., 2000). Nonisotopic determination of fertilizer N recovery is consistently higher than isotopic determination because of a phenomenon called added N interaction (ANI) in which the addition of fertilizer N increases the mineralization and availability of native soil N (Jenkinson et al., 1985; Hart et al., 1986; Wood et al., 1987; Azam et al., 1989; Chalk et al., 1990). Added N interaction causes isotopic methods of determining fertilizer N recovery to

underestimate fertilizer N uptake due to mineralization-immobilization turnover (MIT). During MIT, labeled fertilizer N exchanges with native soil N (Jansson and Persson, 1982; Walters and Malzer, 1990). The non-labeled soil N is then taken up by the plant resulting in an underestimation of fertilizer N uptake. Not only does substitution occur, but also during MIT the isotopic composition of labeled fertilizer N is markedly changed (Hauck, 1978). For example, remineralized ^{15}N depleted fertilizer will have a much higher ^{15}N concentration (diluted) than the labeled fertilizer before it was immobilized. Similarly, ANI causes nonisotopic methods of determining fertilizer N recovery to overestimate fertilizer N uptake because the additional N taken up after ANI cannot be distinguished from fertilizer N. Added N interactions are defined as "real" if N fertilizer actually increases N mineralization or if root exploration is greater in fertilized plants due to larger root systems than in unfertilized plants (Bronson et al., 2000). Added N interactions are defined as "apparent" if simple pool substitution occurs in which fertilizer N replaces native soil N (Jenkinson et al., 1985).

The objectives of this study were (i) to use ^{15}N techniques to determine the fate of fertilizer N in three woody ornamental species commonly grown in container nurseries, (ii) to compare nonisotopic and isotopic methods of determining N recovery in container-grown woody plants, and (iii) to determine the relative importance of fertilizer and non-fertilizer N at various rates.

MATERIALS AND METHODS

On 1 Apr., uniform rooted cuttings of *E. alatus, E. alatus* 'Compacta', *C. sericea* 'Cardinal', *C. sericea* 'Isanti', *W. florida* 'Red Prince', and *W. florida* 'Alexandra' (Wine and Roses) were potted into 3.8 L containers. At planting, 10 plants of each taxon were partitioned into shoots and roots, dried, weighed, ground to pass a 0.85 mm sieve, and analyzed for total N by the Kjeldahl procedure (Horneck et al., 1989).

E. alatus represented a slow growing species while *W. florida* and *C. sericea* represented fast growing species. Within each species there was one taxon with dwarf characteristics and one taxon with non-dwarf or standard growth characteristics. Within *E. alatus*, the species represented the non-dwarf type while the cultivar 'Compacta' represented the dwarf type. Within *W. florida*, 'Red Prince' represented the non-dwarf type while 'Alexandra' represented the dwarf type. Within *C. sericea*, 'Cardinal' represented the non-dwarf type while 'Isanti' represented the dwarf type.

The growing substrate consisted of 7 fresh Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco.] bark (initial pH of 3.6) : 2 sphagnum peat moss : 1 silica sand (0.65 mm) by volume. The substrate was amended with 0.883 kg Micromax (The Scotts Co., Marysville, Ohio), 1.77 kg ag lime ($CaCO_3$), 1.77 kg dolomite ($CaCO_3$ + $MgCO_3$), 1.05 kg 8-9 month slow release phosphorous (P; The Scotts Co.), and 1.18 kg 8-9 month slow release potassium (K; The Scotts Co.) all per m^3. Substrate pH at transplanting (after all amendments were added) was 5.6.

Experiments were conducted outdoors in full sun on a gravel pad at Oregon State University's Lewis Brown Horticulture Farm (Corvallis, Ore.) in a completely

randomized design. Water from the horticulture farm had a NO_3^- concentration of 4.54 $mg \cdot L^{-1}$.

Treatments consisted of five concentrations of N: 25, 50 100, 200, and 300 $mg \cdot L^{-1}$ delivered as liquid, double-labeled ^{15}N depleted NH_4NO_3 (min 99.95% atom ^{14}N; Isotec, Miamisburg, Ohio). Treatments began on 9 Apr., and were applied approximately every other day until 9 Sept. At each application, each container received the same volume (enough to maintain a 25% leaching fraction for the driest containers). During the first two months of the experiment, treatments were applied with a Wheaton Unispense Peristaltic Pump (Wheaton Science Products, Millville N.J.), while volumes were low, and were later applied with 354.8 ml plastic bottles modified to fill and drain to the required amount. From 9 Apr. to 18 May plants received 100, 150, 200, or 250 mL of fertilizer solution at each treatment as needed to maintain an approximate 25% leaching fraction. From 22 May to 10 Sept. plants received 300 mL of fertilizer solution at each treatment. After 16 June, supplemental irrigation was delivered on days when the plants did not receive treatments. Overhead irrigation rates began at 300 $mL \cdot d^{-1}$ and reached 500 $mL \cdot d^{-1}$ by the end of the study.

Before spring growth (11 Mar.), three single-plant replications from each taxon and N concentration were harvested. Plants were partitioned into roots and shoots. Potting substrate was removed from the root system with a high-pressure hose nozzle. All tissues were dried, weighed, ground to pass a 0.425 mm sieve, and subjected to ^{15}N analysis (Isotope Services, Los Alamos N.M.). Percent total N and percent ^{15}N were determined.

Regression analysis and means separations were performed using SAS v. 8.02 (SAS Institute, Cary, N.C.).

RESULTS AND DISCUSSION

Growth and N data from cultivars within a species were not significantly different and were therefore pooled. Labeled N in roots and shoots of *C. sericea*, *W. florida*, and, *E. alatus* 11 months after planting increased with N rate (Fig. 4.1A). At each rate, more N was stored in the roots than in the shoots. At lower N rates, unlabeled N was a substantial portion of the total N in the plant. Dry weights of roots and shoots increased for all species as N rates increased (Fig. 4.1B). Root to shoot ratios were higher at low N rates and decreased with increasing N rates.

N recovery determined by the linear regression of total N in the plant on rates of applied N and linear regression of labeled fertilizer N in the plant (nitrogen derived from fertilizer; NDFF) on rates of labeled fertilizer N applied both increased as N concentration increased from 25 to 300 $mg \cdot L^{-1}$ (Table 4.1). Estimation of N recovery determined by the linear regression of total N in the plant on rates of applied N was significantly higher for all species and at each N rate than estimation of N recovery determined by linear regression of labeled fertilizer N in the plant on rates of labeled fertilizer N applied (Table 4.1). These results agree with several studies performed with agronomic crops grown in soil (Westerman and Kurtz, 1974; Olson, 1980; Oslon

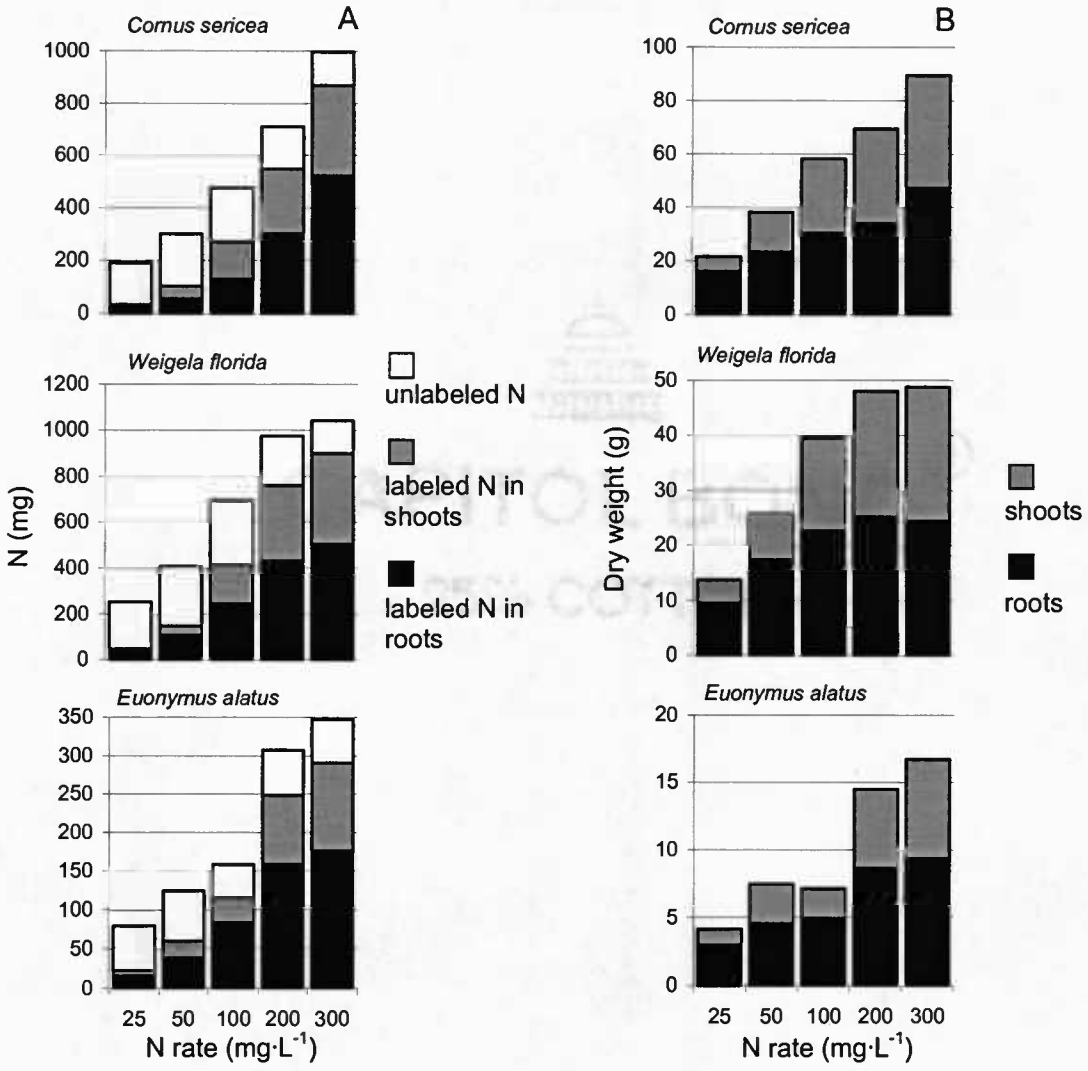

Fig. 4.1. (A) Average (n=6) unlabeled plant N and labeled N (delivered as double-labeled ^{15}N depleted NH_4NO_3 at 25, 50, 100, 200, and 300 $mg \cdot L^{-1}$) in the roots and shoots (no leaves) and (B) average dry weights of roots and shoots of container-grown *Cornus sericea*, *Weigela florida*, and *Euonymus alatus* 11 months after planting. Plants were grown in 3.8 L containers and sampled on 11 Mar. before spring growth began.

Table 4.1. Average total N, average N derived from fertilizer (NDFF), % N, and % NDFF of 11-month old[z] container-grown *Cornus sericea*, *Weigela florida*, and *Euonymus alatus* grown at N concentrations of 25, 50, 100, 200, and 300 $mg \cdot L^{-1}$.

	Species											
	Cornus sericea				*Weigela florida*				*Euonymus alatus*			
N rate ($mg \cdot L^{-1}$)	Total N (mg)	NDFF (mg)	% N	% NDFF	Total N (mg)	NDFF (mg)	% N	% NDFF	Total N (mg)	NDFF (mg)	% N	% NDFF
25	191.15[y,x]	33.83	0.91	0.17	252.43	47.56	1.84	0.36	80.05	22.41	2.02	0.61
50	299.37	104.19	0.80	0.28	406.70	168.73	1.59	0.60	124.94	60.62	1.72	0.85
100	475.41	269.72	0.83	0.47	693.38	414.47	1.76	1.06	158.39	116.03	2.51	1.89
200	710.72	546.97	1.08	0.85	974.18	759.60	2.07	1.62	307.06	248.90	2.09	1.69
300	996.56	868.08	1.15	1.01	1042.36	898.07	2.14	1.85	347.24	290.61	2.14	1.78
Linear	***	***	**	***	***	***	***	***	***	***	NS	***
r^2	0.955	0.981	0.316	0.780	0.852	0.924	0.398	0.844	0.609	0.687	0.022	0.403
Quadratic	***	***	**	***	***	***	**	***	***	***	NS	***
r^2	0.957	0.981	0.323	0.800	0.951	0.968	0.399	0.896	0.623	0.708	0.078	0.606

[z] Data collected 11 Mar. 2002 before spring growth.
[y] All values are means of six single-plant replicates.
[x] All total N and NDFF values across rows within a species are significantly different ($P \leq 0.05$) by the paired t-test (SAS).
NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001 respectively.

and Swallow, 1984; Azam, 1991; Bronson et al., 2000). Linear models fit each series of data significantly, and graphically the lines appear to be parallel. However, slight improvements in r^2 values are recognized in each series when fitted to a quadratic model, indicating that the lines are not parallel. The distance between each line represents N in the plant derived from some source other than fertilizer N (NDFO; e.g. mineralization of the substrate, irrigation water). In *W. florida* and *C. sericea*, average NDFO increased across N rates of 25 to 100 $mg \cdot L^{-1}$, reached a plateau between 100 and 150 $mg \cdot L^{-1}$, and then declined (Fig. 4.2). The increase in NDFO indicates that increasing fertilizer rates up to 100 $mg \cdot L^{-1}$ results in increased uptake of NDFO. This trend is most likely a "real" ANI resulting from increased root mass at higher N concentrations that is able to take up more mineralized N from the container substrate. However, it may be an'apparent' ANI caused by the immobilization and subsequent mineralization (MIT) of labeled fertilizer N which would produce a diluted ^{15}N signal. Regardless, the ANI does not continue to increase as it might in a soil system under nontoxic N levels. The plateau and subsequent decline in NDFO may be partially due to dilution of the NDFO in the presence of increasing amounts of NDFF.

When water is limiting, addition of N can result in earlier N depletion and less utilization of soil N (Campbell and Paul, 1978). Containers of *C. sericea* and *W. florida* fertilized at high N rates (fast growth with large root masses) remained dryer than containers at low N rates (fast growth but with less root mass to exploit soil moisture; data not shown). Moisture status may contribute to the decline in NDFO at higher N concentrations (> 100 $mg \cdot L^{-1}$).

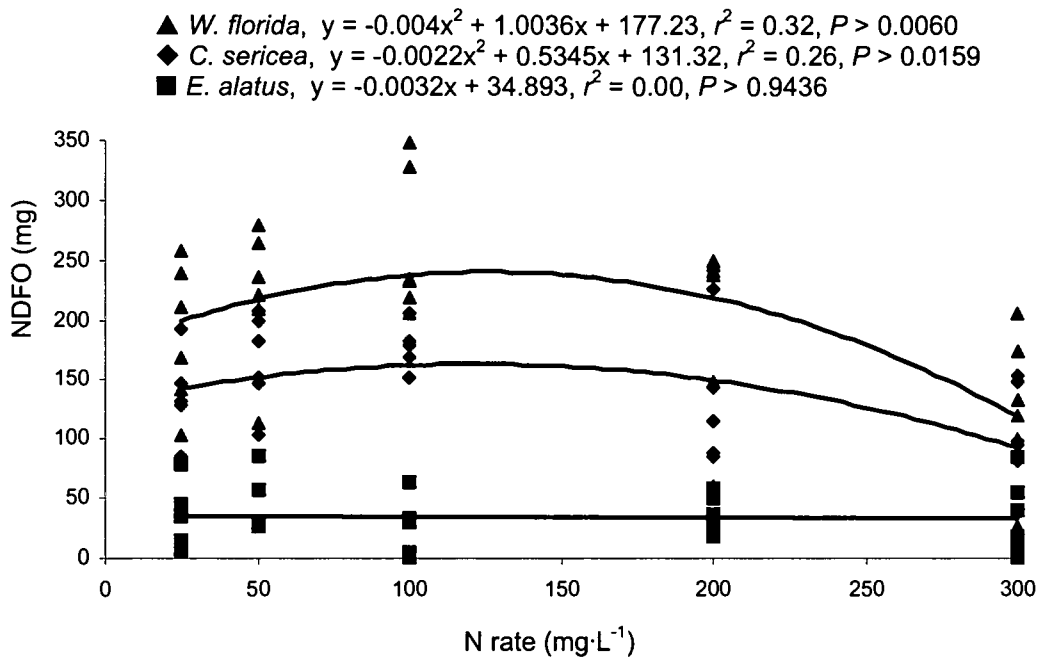

Fig. 4.2. Nitrogen (N) derived from sources other than fertilizer N (NDFO) for *Cornus sericea, Weigela florida*, and *Euonymus alatus* grown in 3.8 L containers at N concentrations of 25, 50, 100, 200, and 300 $mg \cdot L^{-1}$.

For *E. alatus*, the lack of significant change in NDFO across N rates (Fig. 4.2) is most likely due to its slow growth rate. Increase in root weight of *E. alatus* across N rates was relatively small (Fig. 4.1B) facilitating little or no increase in NDFO from 25 to 100 $mg \cdot L^{-1}$. Similarly, there was no decrease in NDFO above 150 $mg \cdot L^{-1}$ because *E. alatus* root systems were never restricted by container size as the faster growing *W. florida* and *C. sericea* were.

A 3.8 L container of 7 Douglas fir bark : 2 sphagnum peat moss : 1 silica sand (by volume) contains approximately 0.7 kg of bark and 0.1 kg of peat moss. Douglas fir bark and sphagnum peat moss are approximately 0.12 and 0.83 % N respectively (Bollen, 1969). Therefore, 0.7 kg of Douglas fir bark at 0.12 % N contains 840 mg of potentially mineralized organic N. Likewise, 0.1 kg of sphagnum peat moss at 0.83 % N contains 830 mg of potentially mineralized organic N. Assuming an N recovery of ~40 %, these combined (1670 mg) media sources of N could account for the total NDFO. Yet, Douglas fir bark and sphagnum peat moss at C:N ratios of 400:1 and 58:1 respectively (Bollen, 1969) would require substantial N input, particularly in the presence of significant microbial activity, before a net N mineralization could be expected. This, coupled with increased root mass, may be the mechanism for increased average NDFO for *W. florida* and *C. sericea*, across N rates of 25 to 100 $mg \cdot L^{-}$.

For each species, percent NDFO decreased with increasing N concentrations (Fig. 4.3). As N concentrations increased, plants took up more fertilizer N (Table 4.1) rendering NDFO a smaller portion of total N. NDFO at low N concentrations is a

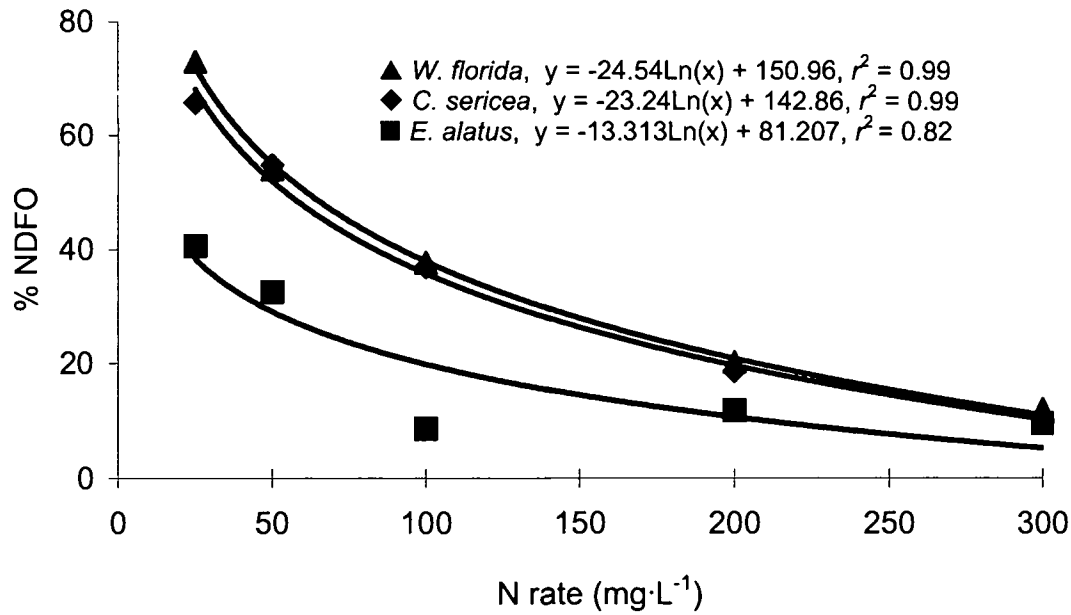

Fig. 4.3. Percent nitrogen (N) derived from sources other than fertilizer N (NDFO) for *Cornus sericea, Weigela florida*, and *Euonymus alatus* grown in 3.8L containers at N concentrations of 25, 50, 100, 200, and 300 $mg \cdot L^{-1}$. Each point represents the mean of six values.

significant portion of the total N in the plant. Therefore, the difference in estimation of percent N recovery between each method was larger at lower N concentrations for all species (Fig. 4.4). The difference decreased as N concentration increased, and NDFO became a smaller portion of total N. Assuming that the ^{15}N tracer is the more accurate method of estimating fertilizer N uptake (Hauck, 1978), the total N method overestimates fertilizer N uptake substantially (Fig. 4.5). Overestimation is more prominent at lower N concentrations where it was three to four times the ^{15}N estimations.

For each species, NDFF recovery efficiency (labeled fertilizer N in the plant / labeled fertilizer N applied × 100) was positively correlated (quadratic) with root weight (Fig. 4.6A), and negatively (*W. florida* and *C. sericea*) or not (*E. alatus*) correlated with root to shoot ratios (data not shown). These relationships indicate that fertilizer N recovery efficiency in container-grown woody ornamental plants is more influenced by total root weight than root to shoot ratio. However, dry weight of roots is not the only determining factor of fertilizer N recovery efficiency. Plotting NDFF recovery efficiency versus total N (Fig. 4.6B) indicates that each species has an inherent N recovery capacity. *W. florida* has a higher NDFF recovery efficiency across the range of measured plant N and a higher percent N across all N rates (Table 4.1) than *C. sericea*, another fast growing species. Naturally, *W. florida* has a higher NDFF recovery efficiency than *E. alatus*, a slow growing species, across the range of measured plant N. Fertilizer N recovery efficiency is a result of both factors, and neither is independent of the other.

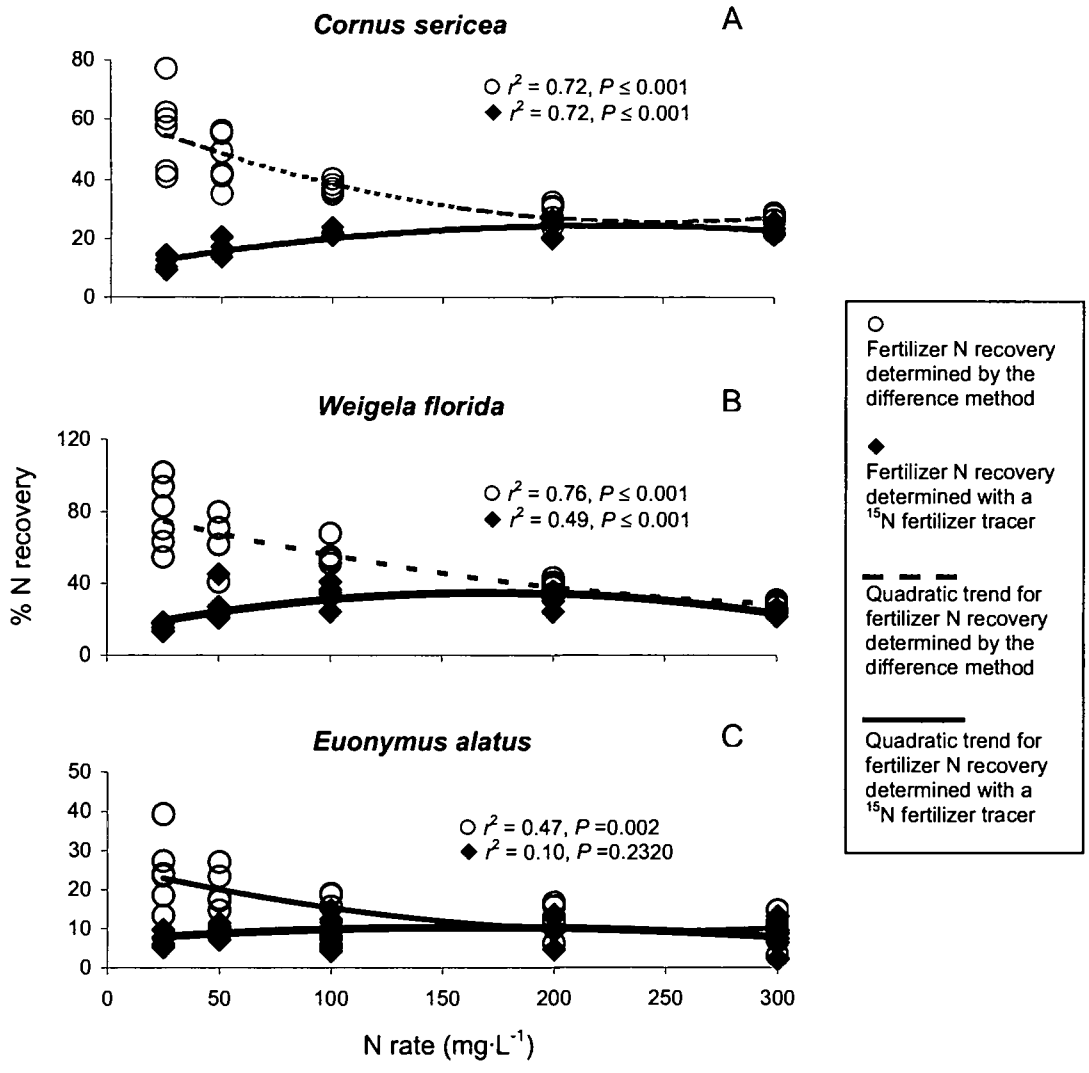

Fig. 4.4. Fertilizer nitrogen (N) recovery of (A)*Cornus sericea*, (B)*Weigela florida*, and (C)*Euonymus alatus* determined by the total N method (total N in the plant / total N applied + total N from the cutting) and with a ^{15}N fertilizer tracer (total ^{15}N in the plant / ^{15}N applied). Plants were grown for 11 months in 3.8 L containers at N concentrations of 25, 50, 100, 200, and 300 mg·L^{-1}.

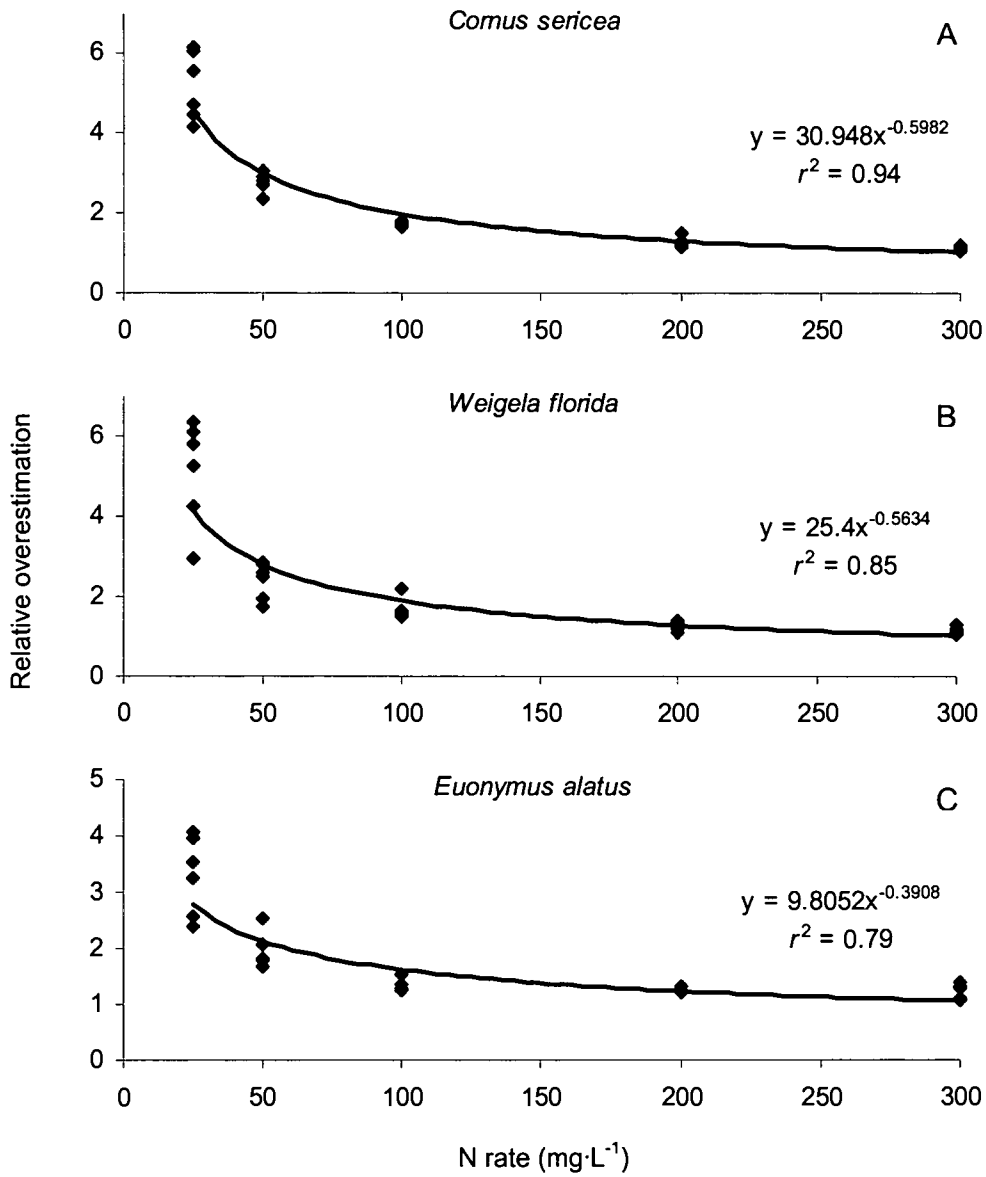

Fig. 4.5. Relative overestimation of N recovery for (A) *Cornus sericea*, (B) *Weigela florida*, and (C) *Euonymus alatus* by the linear regression of total N in the plant on rates of applied N (nonisotopic) over linear regression of labeled fertilizer N in the plant on rates of labeled fertilizer N applied (isotopic). Plants were grown for 11 months in 3.8 L containers at N concentrations of 25, 50, 100, 200, and 300 $mg \cdot L^{-1}$.

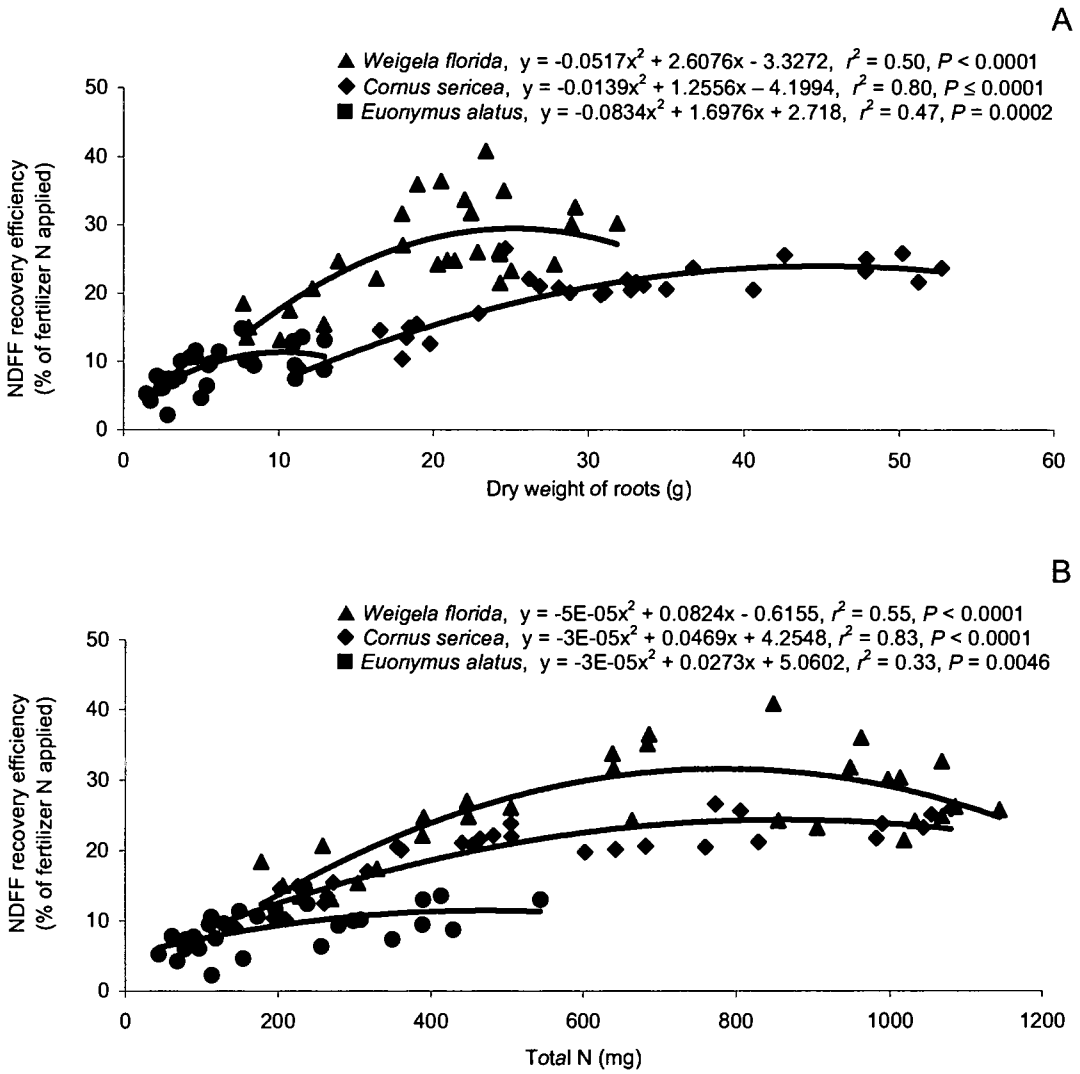

Fig. 4.6. Effect of (A) dry weight of roots and (B) total plant N on recovery efficiency of N derived from fertilizer (NDFF; labeled fertilizer N in the plant / labeled fertilizer N applied × 100) for *Cornus sericea, Weigela florida*, and *Euonymus alatus* grown in 3.8 L containers at N concentrations of 25, 50, 100, 200, and 300 $mg \cdot L^{-1}$. (n = 30 for each species).

Percent fertilizer N recovery, determined by the ^{15}N method, increases slightly from N rates of 25 to 300 $mg \cdot L^{-1}$ (Fig. 4.4), but at the same time actual fertilizer N lost (FNL) increases dramatically (Table 4.2). This is because fertilizer N applied (FNA) increases substantially from 25 to 300 $mg \cdot L^{-1}$ (i.e. 307 mg N at 25 $mg \cdot L^{-1}$ versus 3690 mg N at 300 $mg \cdot L^{-1}$) while percent fertilizer N lost (FNL; the inverse of percent fertilizer N recovery) only decreases slightly (Table 4.2). For example, in *C. sericea*, 89 % of 307 mg (FNA at an N rate of 25 $mg \cdot L^{-1}$) results in 273.2 mg actual FNL, while 76.5 % of 3690 mg (FNA at an N rate of 300 $mg \cdot L^{-1}$) results in much greater FNL, 2821.9 mg, even though N recovery is increasing.

Results from this study indicate that NDFO can be a substantial portion of total N in containerized plants grown at low (< 100 $mg \cdot L^{-1}$) N rates. Increasing amounts of NDFO in containerized plants grown at N rates of 25 to 100 $mg \cdot L^{-1}$ suggests that an ANI is occurring even in a bark-based substrate with relatively little N. This is most likely a 'real' ANI resulting from increased root mass at higher N rates leading to increased uptake of N mineralized from the media. The NDFO causes a difference in the estimation of N recovery between isotopic and nonisotopic methods, and the difference is larger at lower N concentrations. These results emphasize the necessity for developing rigorous techniques for determining N recovery in container-grown woody ornamental crops and the importance of ANI in the interpretation of results.

Table 4.2. Average fertilizer N loss and percent fertilizer N loss from 11-month old[z] containerized *Cornus sericea*, *Weigela florida*, and *Euonymus alatus* grown in 3.8 L containers at N concentrations of 25, 50, 100, 200, and 300 $mg \cdot L^{-1}$.

		Species					
		Cornus sericea		*Weigela florida*		*Euonymus alatus*	
N rate ($mg \cdot L^{-1}$)	FNA[y] (mg)	FNL (mg)	% FNL	FNL (mg)	% FNL	FNL (mg)	% FNL
25	307	273.2[x]	89.0	259.4	84.5	284.6	92.7
50	615	510.8	83.1	446.3	72.6	554.4	90.1
100	1230	960.3	78.1	815.5	66.3	1114.0	90.6
200	2460	1913.0	77.8	1700.4	69.1	2211.1	89.9
300	3690	2821.9	76.5	2791.9	75.7	3399.4	92.1
Linear		***	***	***	NS	***	NS
r^2		0.998	0.541	0.990	0.041	0.996	0.000
Quadratic		***	***	***	***	***	NS
r^2		0.998	0.722	0.996	0.492	0.997	0.103

[z] Data collected 11 Mar. 2002 before spring growth.
[y] FNA = fertilizer nitrogen applied, FNL = fertilizer nitrogen lost (FNA- fertilizer N in the plant).
[x] All values are means of six single-plant replicates.
NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001 respectively.

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CHAPTER 5

LIQUID FERTILIZATION REDUCES NITROGEN INPUTS IN CONTAINER PRODUCTION OF TWO WOODY ORNAMENTALS: COMPARISON WITH A CONTROLLED-RELEASE FERTILIZER

ABSTRACT

To determine the relative efficiency of a controlled-release fertilizer (CRF) to a liquid feed program based on specific nitrogen (N) requirements, N fertilization budgets for *Euonymus alatus* (Thunb.) Sieb. 'Compactus' (a slow growing taxon) and *Weigela florida* (Bunge.) A. DC. 'Red Prince' (a fast growing taxon) were established. Based on these budgets, daily (D) and bi-weekly (BW) liquid N delivery regimes were developed and tested against an industry standard CRF [Osmocote 18-6-12 (The Scotts Co., Marysville, Ohio; 18N-2.6P-9.9K)]. Plants were grown in 3.8 L containers in 7 douglas-fir bark : 2 sphagnum peat moss : 1 0.65 mm silica sand (by volume) outdoors in full sun on a gravel pad for 140 days. For *E. alatus* 'Compactus', total dry weight and total N content of the D and BW treatments (1015 mg N applied) were not significantly different (α=0.05) from the topdress high rate of 18g Osmocote per container (3240 mg N applied), which had the highest values. However, the D and BW liquid feed treatments introduced 68.7% less N to the production system. Total dry weight and total N content of *W. florida* 'Red Prince' were highest when

Osmocote was incorporated at the high rate of 7.12 kg m^{-3} (2994 mg N applied) but did not differ significantly (α=0.05) from the D and BW treatments (1837 mg N applied), which introduced 48.6% less total N to the production system. Sliding scale liquid fertilization (D and BW) resulted in significantly higher (α=0.05) percent N recovery (total N in the plant / total N applied) than in the controlled-release treatments in both *E. alatus* 'Compacta' and *W. florida* 'Red Prince'. Results indicate that with liquid fertilization comparable woody nursery stock can be produced with significantly less N inputs than are recommended with CRFs.

INTRODUCTION

Container production of woody ornamental plants is increasing because it offers advantages over traditional field production. These advantages include more plants per unit land area, faster plant growth, higher quality plants, lack of dependence on arable land, easier shipping and handling, and year-round sales (Davidson et al., 1988; Whitcomb, 1986).

Achieving efficient nitrogen (N) fertilization in container nurseries is difficult because most nurseries grow hundreds of species and cultivars with unique N requirements, at one site. Plants at the same site require N in different amounts and at different times during the growing season for optimum growth, so no generalization about N fertilization can be made (Whitcomb, 1986). In addition, container production requires the use of a porous substrate, usually a mix of bark, sand, or peat

moss, with little water holding capacity (Furuta, 1976; Rathier and Frink, 1989) and little ability to retain nitrate (NO_3^-) ions (Foster et al., 1983). Consequently, the substrate must be watered often to maintain adequate moisture, and N levels must be replenished frequently. Combined, these two conditions lead to substantial N leaching from container nurseries, and subsequent concern over NO_3–N contamination of surface and ground water.

Use of controlled-release fertilizers (CRFs) to reduce NO_3-N leaching has become widely adopted among container nurseries, but nurseries also use CRFs because they require less labor to apply than other fertilization methods, and manufacturers claim that they reduce build-up of soluble salts while providing a constant supply of nutrients (Bunt, 1988; Maynard and Lorenz, 1980). Several studies have concluded that CRFs reduce NO_3–N leaching from container production systems (Broschat, 1995; Catanzaro, 1998; Rathier and Frink, 1989). Other studies have demonstrated that CRFs can result in NO_3–N leaching levels as high or higher than water-soluble fertilizers in container grown plants (Cox, 1993; Hershey and Paul, 1982)

In theory, CRFs reduce NO_3–N leaching losses by holding N in the container for an extended period and releasing it slowly, as the plant needs it. However, N release patterns from CRFs often do not match the needs of the plant and can be unpredictable due to fluctuations in environmental conditions (Cabrera, 1997; Huett, 1997a; Wright and Niemiera, 1987). In addition to mismatched timing, blanket fertilization with a CRF may provide too much N to some taxa, resulting in excessive

N loss, and not enough N to other taxa, resulting in a reduction of plant size and quality or requiring the additional input of liquid fertilizer N.

In contrast to the use of CRFs, we propose that the most effective way to reduce NO_3–N leaching from container nurseries is to supply N based on the individual requirements of the plant, thus reducing the total amount of N introduced to the production system. To that end, we designed a two-year experiment to test the hypothesis that commercially comparable woody ornamental plants can be grown under a sliding scale fertilization regime based on plant requirements (timing and amount) with substantially less N input than with an industry standard CRF. The first objective was to determine the growth and N uptake patterns for *Euonymus alatus* 'Compacta', a slow growing taxon and *Weigela florida* 'Red Prince', a fast growing taxon. The second objective was to design a sliding scale liquid fertilization program to deliver N according to plant requirements. The third objective was to compare plant growth, N status, and N recovery under the sliding scale program to plants fertilized with an industry standard CRF.

MATERIALS AND METHODS

Establishment of growth curves and N requirements (2001)

Uniform rooted cuttings of *E. alatus* 'Compacta', a slow growing taxon and *W. florida* 'Red Prince', a fast growing taxon were potted into 3.8 L containers on 1 Apr.

2001. At planting, 10 plants of each taxon were partitioned into shoots and roots, dried, and ground to pass a 0.85 mm sieve.

The growing substrate consisted of 7 fresh douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco.] bark (initial pH of 3.6) : 2 sphagnum peat moss : 1 silica sand (0.65 mm) by volume. The substrate was amended with 0.883 kg Micromax (The Scotts Co., Marysville, Ohio), 1.77 kg ag lime ($CaCO_3$), 1.77 kg dolomite ($CaCO_3$ + $MgCO_3$), 1.05 kg 8-9 month slow release phosphorous (P; The Scotts Co.), and 1.18 kg 8-9 month slow release potassium (K; The Scotts Co.) all per m^3. Substrate pH at transplanting (after all amendments were added) was 5.6.

The experiment was conducted under full sun on a gravel pad at the Lewis Brown Horticulture Farm in Corvallis, Ore. Each taxon was represented by 60 single-plant replications in a completely randomized design. Water from the horticulture farm had a NO_3 concentration of 4.54 mg· L^{-1}.

Treatments began on 9 Apr. and ended on 4 Sept. and were applied approximately every other day. All plants received 200 mg· L^{-1} N as ammonium nitrate (NH_4NO_3; Western Farm Services, Tangent, OR). Treatment volumes were adjusted throughout the experiment to maintain an approximate 25% leaching fraction. From 9 Apr. to 18 May all plants received between 150 and 250 mL solution per application. After 18 May, all plants received 300 mL solution per application. After 16 June, supplemental irrigation was delivered on days when plants did not receive solution applications. During the first two months of the experiment, treatments were applied with a Wheaton Unispense Peristaltic Pump (Wheaton Science Products,

Millville N.J.), while volumes were low, and were later applied with 354.8 mL plastic bottles modified to fill and drain to the required amount.

Plants were sampled four times at one-month intervals beginning on 14 May and ending 6 Aug. At each sampling, four plants of each taxon were partitioned into leaves, roots, and shoots. Potting substrate was removed from the root system with a high-pressure hose nozzle. All tissues were dried, weighed, ground to pass a 0.85 mm sieve, and analyzed for total N by the Kjeldahl procedure (Horneck et al., 1989).

Sliding scale fertilization vs. CRF (2002)

Uniform rooted cuttings of *E. alatus* 'Compactus' and *W. florida* 'Red Prince' were transplanted into 3.8 L containers on 1 Apr. 2002. At planting, 10 plants of each taxa were partitioned into shoots and roots, dried, weighed, and ground to pass a 0.85 mm sieve.

Substrate and amendments for plants receiving the sliding scale liquid N treatments were the same as for the previous year (2001). The substrate for plants receiving CRF treatments was amended with 0.883 kg Micromax (The Scotts Co.), 1.77 kg ag lime ($CaCO_3$), 1.77 kg dolomite ($CaCO_3 + MgCO_3$) all per m^3. The pH of both substrates at transplanting (after all amendments were added) was 5.8-6.0.

The experiment was conducted under full sun on a gravel pad at Oregon State University's Lewis Brown Horticulture Farm (Corvallis, Ore.). Containers were watered manually until treatments began on 6 Apr. The NO_3^- concentration of the irrigation water and water used to premix solutions was 4.54 mg$\cdot$ L^{-1}.

The experiment was conducted in a completely randomized factorial design with two treatments; plant and N application. The two plant types, *E. alatus* 'Compacta' (E) and *W. florida* 'Red Prince' (W), received eight different N treatments. Four N treatments were designed to represent industry standards (following the manufacturer's recommendations). They included topdressing 8-9 month controlled release Osmocote 18-6-12 (The Scotts Co.; 18N-2.6P-9.9K) at medium (TM; 14 g/container) and high (TH; 18 g/container) rates and incorporating at medium (IM; 5.34 $kg \cdot m^{-3}$) and high (IH; 7.12 $kg \cdot m^{-3}$) rates. Osmocote 18N-2.6P-9.9K, although it is an 8-9 month release CRF, was used in this 140 day experiment because other studies established that the effective N release under nursery conditions is approximately 120 to 150 days (Huett and Gojel, 2000; Meadows and Fuller, 1983). Industry standard treatments were applied at planting. Three N application levels were developed based on growth curves and N uptake patterns established in 2001 and included the delayed (30 d) topdress of Osmocote 18N-2.6P-9.9K at a high [TH (L) 18 g/container] rate and daily (D) and bi-weekly delivery of required N as NH_4NO_3. The eighth N treatment was 200 $mg \cdot L^{-1}$ (COMP) delivered three times per week and was included as a comparison to the previous year's data from which N budgets were developed. Each treatment combination contained five single-plant replications.

Liquid fertilizer treatments (D, BW, and COMP), were applied using 354.8 mL plastic bottles modified to fill and drain to the required volume. Each day, plants either received fertilizer N solution or water according to their N treatment schedules.

Table 5.1. Water (H_2O) volumes and nitrogen (N) concentrations for daily and bi-weekly sliding scale liquid fertilization treatments.

		Period							
		6 May – 19 May		20 May – 16 June		17 June – 14 July		15 July – 19 Aug.	
Species	N trt.	H_2O (mL)	[N] mg· L^{-1}	H_2O (mL)	[N] mg· L^{-1}	H_2O (mL)	[N] mg· L^{-1}	H_2O (mL)	[N] mg· L^{-1}
E[z]	D[y]	150	0	150	50	200	50	200	75
E	BW	150	0	150	175	200	175	200	262.5
W	D	150	50	200	75	250	75	400	56.25
W	BW	150	175	200	262.5	250	262.5	400	196.8

[z] E=*Euonymus alatus* 'Compacta', W= *Weigela florida* 'Red Prince'
[y] D= daily applications, BW= bi-weekly applications

D and BW treatments within a species received the same amount of water or solution on any given day but differed in the N concentration (Table 5.1). Plants subjected to the CRF treatments (TM, TH, IM, IH, and DTH) received water at 200 $mL \cdot d^{-1}$ from 6 May – 26 May, 300 $mL \cdot d^{-1}$ from 27 May – 14 July, and 400 $mL \cdot d^{-1}$ from 15 July – 19 Aug. Likewise, COMP received the same amounts of water but received 200 $mg \cdot L^{-1}$ N on Monday, Wednesday, and Friday. No additional irrigation was supplied.

Leachate was collected on 3 May and 2 July using the pour-through extraction method (Wright, 1984). pH and electrical conductivity (EC) were determined with the Accumet AR20 pH/Conductivity meter (Fisher Scientific, USA).

Plants were destructively harvested on 20 Aug. and partitioned into leaves, roots, and shoots. Potting substrate was removed from the root system with a high-pressure hose nozzle. All tissues were dried, weighed, ground to pass a 0.85 mm sieve, and analyzed for total N by the Kjeldahl procedure (Horneck et al., 1989).

Percent N recovery was calculated as total Kjeldahl N in the plant / total fertilizer N applied. Analyses of variance (ANOVA) and mean separations were performed using SAS v. 8.02 (SAS Institute, Cary, N.C.).

RESULTS AND DISCUSSION

Year 1 (2001)

Previous studies indicate that N concentrations of 100-200 $mg \cdot L^{-1}$ in the substrate solution result in optimum growth of various ornamentals (Hershey and Paul,

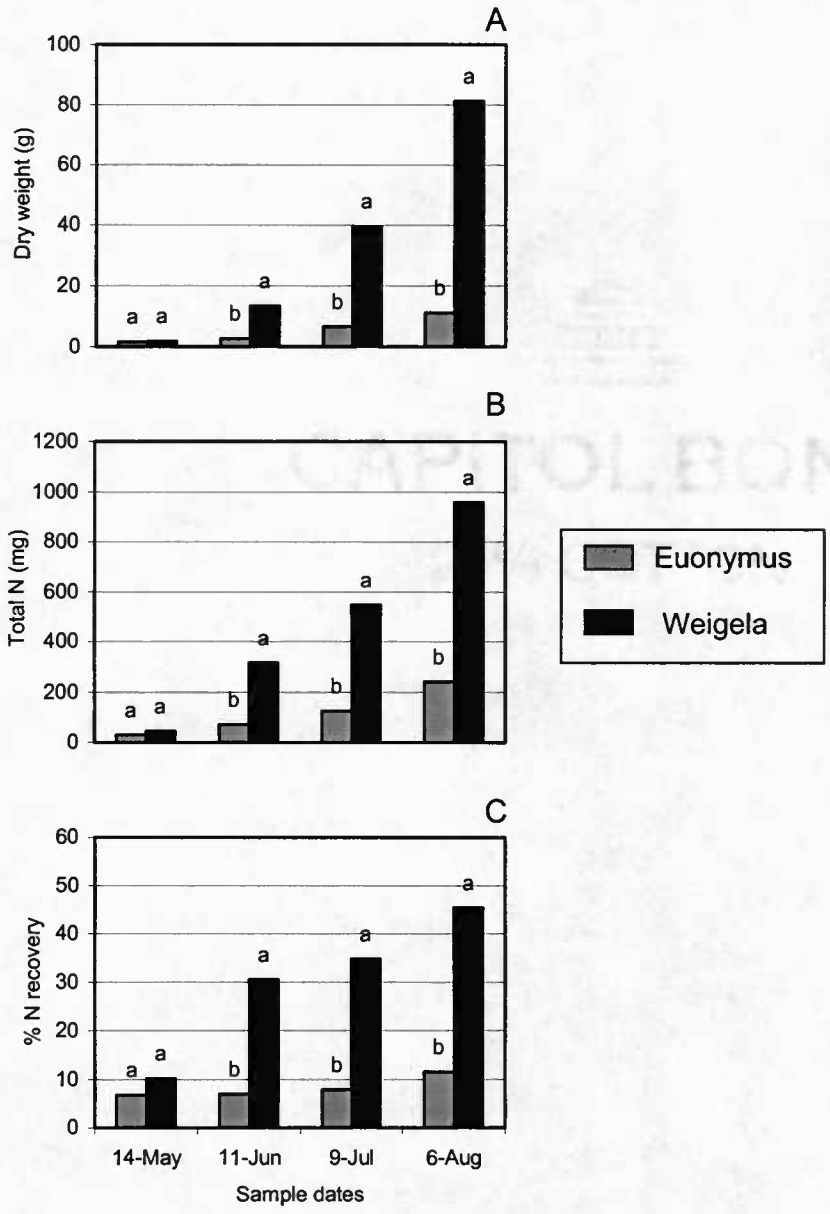

Fig 5.1. Dry weight (A), total N content (B), and percent N recovery (C) of *Euonymus alatus* 'Compacta' and *Weigela florida* 'Red Prince'. All plants were grown from rooted cuttings outdoors in full sun in 3.8 L containers and supplied N at a concentration of 200 $mg \cdot L^{-1}$ approximately every other day. Treatment volumes were adjusted to maintain a 25% leaching fraction. Means within a sample date with the same letter are not significantly different (α=0.05) by a two-sample t-test.

1982; Wright and Niemiera, 1987). At 200 mg· L^{-1}, *W. florida* 'Red Prince' gained more total dry weight, more total N, and exhibited higher N recovery rates than *E. alatus* 'Compacta' (Fig. 5.1A-C). Prior to 14 Mar., total dry weight and total N were similar for the two taxa, but thereafter *W. florida* 'Red Prince' had statistically higher values than *E. alatus* 'Compacta' for all dependent variables. These results indicate the biological limit of *E. alatus* 'Compacta' and *W. florida* 'Red Prince' N uptake when the substrate solution is maintained near 200 mg· L^{-1} N for five months. Clearly, a substantial difference in N requirements of two commonly grown woody ornamentals exists, thereby validating the need for plant specific N delivery to achieve efficiency of N use. Sliding scale treatments applied in year 2 (2002) were designed from total dry weights, total N, periodic N recovery, and seasonal N recovery measured or calculated in year 1 (2001).

Year 2 (2002)

Total dry weights of the D and BW treatments and total N content of the BW treatment for *E. alatus* 'Compacta' were similar to the TH treatment that produced the highest total dry weight and highest total N content (Table 5.2). Total N content of the D treatment for *E. alatus* 'Compacta' was less than the TH treatment but similar to all other CRF treatments. Based on dry weight, and total N content in the case of the BW treatment, the D and BW sliding scale liquid treatments and the TH treatment resulted in commercially comparable plants, but the D and BW treatments introduced 68.7% less N to the production system (Table 5.2).

Table 5.2. Dry weight, total N content, and N recovery of container-grown *Euonymus alatus* 'Compacta' and *Weigela florida* 'Red Prince' under controlled-release and sliding scale liquid fertilizer treatments.

		Plant taxa					
		E. alatus 'Compacta'			*W. florida* 'Red Prince'		
Treat.	N applied (mg)	Dry weight (g)	Total N (mg)	N recovery (%)	Dry weight (g)	Total N (mg)	N recovery (%)
IH[z]	2994	5.6 b[y]	137.1 b	4.6 d	58.7 a	914.4 a	30.5 b
TH	3240	11.1 a	239.5 a	7.4 cd	51.5 a	554.2 cd	17.1 d
TH (L)	3240	8.2 ab	166.1 ab	5.1 cd	55.0 a	503.5 d	15.5 d
IM	2230	9.4 ab	217.7 ab	9.8 c	54.2 a	672.8 bc	30.2 b
TM	2520	7.0 ab	156.1 ab	6.2 cd	52.3 a	597.4 cd	23.7 c
D(E)	1015	8.1 ab	150.9 b	14.9 b	---	---	---
BW(E)	1015	8.8 ab	202.4 ab	19.9 a	---	---	---
D(W)	1837	---	---	---	51.9 a	784.4 ab	42.7 a
BW(W)	1837	---	---	---	53.4 a	803.1 ab	43.7 a
COMP	2900	5.4 b	143.3 b	4.9 cd	51.3 a	913.7 a	31.5 b

[z] IH=incorporate at the high rate (7.12 $kg \cdot m^{-3}$), TH=top-dress at the high rate (18 g/container), TH (L)=top-dress at the high rate 30 days after planting, IM=incorporate at the medium rate (5.34 $kg \cdot m^{-3}$), TM=topdress at the medium rate (14 g/container), D (E)= daily liquid treatments to *E. alatus* 'Compacta', BW (E)= Bi-weekly liquid treatments to *E. alatus* 'Compacta', D (W)= daily liquid treatments to *W. florida* 'Red Prince', BW (W)= Bi-weekly liquid treatments to *W. florida* 'Red Prince', COMP= 200 $mg \cdot L^{-1}$ N delivered Mon., Wed. and Fri.

[y] Means within columns followed by the same letter are not significantly different (α=0.05) by Duncan's multiple range test.

In the case of *W. florida* 'Red Prince', total dry weights and total N content of the D and BW treatments were similar to the IH treatment that produced the highest total dry weight and highest total N content (Table 5.2). The D and BW sliding scale liquid and IH treatments resulted in commercially comparable plants, but the D and BW treatments introduced 38.7% less N to the production system.

These results agree with other studies that indicate plant N requirements change with growth stage (Argo and Biernbaum, 1991; King and Stimart, 1990). Rose et al. (1994) were able to produce commercially comparable poinsettias (*Euphorbia pulcherrima* Willd.) with a sliding-scale fertilization regime based on plant requirements while introducing 41% less N to the production system than the standard constant rate fertilization regime.

Furthermore, several studies have found that N release patterns from CRFs often do not match the needs of the plant and can be unpredictable due to fluctuations in temperature and moisture (Cabrera, 1997; Huett, 1997a; Wright and Niemiera, 1987; Yeager and Cashion, 1993). Release of large quantities of N early in the season, before newly planted cuttings have a sufficient root mass to exploit the abundant nutrient supply, results in excessive NO_3–N leaching (Hershey and Paul, 1982; Huett, 1997b; Rathier and Frink, 1989). Consequently, large N release early in the season results in a N shortage later in the season. Liquid fertilization based on specific plant N requirements eliminates N losses due to the mismatch of CRF N release and plant requirements while introducing less total N to the production system.

Sliding scale liquid fertilization (D and BW) resulted in higher (α=0.05) N recovery than in the CRF treatments in both *E. alatus* 'Compacta' and *W. florida* 'Red Prince' (Table 5.2). D and BW treatments in *E. alatus* 'Compacta' resulted in 14.9% and 19.9% N recovery, respectively, while the TH treatment resulted in only 7.4% recovery. Likewise, D and BW treatments in *W. florida* 'Red Prince' resulted in 42.7% and 43.7% recovery, respectively, while the TH treatment resulted in 30.9% recovery. These data concur with Rose et al. (1994) who found that in poinsettia, N recovery was ~ 50% higher with a sliding scale treatment based on plant requirements than in a constant rate treatment. N recovery, though dependent on many factors, is most strongly a function of the amount of N applied. Increased N recovery in the D and BW treatments resulted from lower amounts of applied N since total N content was similar across treatments.

Across CRF treatments (TH, IH, TM, and IM), *E. alatus* 'Compacta' consistently had less total N uptake and N recovery than *W. florida* 'Red Prince' (Table 5.2). Recommended rates of CRF application are highly generalized and give nursery managers little guidance for application to the wide diversity of crops produced at one site (Hicklenton and Cairns, 1992). Therefore, CRF fertilization programs based on a single application and N rate implemented across a variety of taxa will inevitably not match the N requirements of all plants and will likely provide an excess of N to many. At a given CRF N rate, slower growing plants, like *E. alatus* 'Compacta' with lower N uptake potentials will accumulate less N over the growing season and therefore exhibit lower N recoveries.

After 20 weeks, the pH of the substrate for all treatments ranged from 6.1 to 6.6, and the electrical conductivity (EC) ranged from 0.19 to 0.84 dS/m. In *E. alatus* 'Compacta', liquid treatments yielded lower pHs and higher ECs in the substrate than the CRF treatments (data not shown). However, all values were within the recommended range for container-grown woody nursery crops (Wright, 1984).

Results from this study indicate that liquid fertilization based on plant N requirements introduces less total N to the production cycle and results in higher N recovery rates than fertilization with an industry standard CRF. With precision liquid fertilization, gains in N recovery can be achieved in two ways. First, matching timing and amount of N applications to plant requirements will result in greater N recovery and reduce NO_3-N leaching. Second, recognizing that different taxa can have substantially different N requirements and supplying N based on those respective requirements will avoid the recovery inefficiency experienced with single rate applications of CRFs.

To implement a liquid fertilization program on a commercial scale, nurseries would have to determine N requirements of each taxon produced, organize taxa according to N needs, and develop a precision delivery method for water and fertilizer solution. Such a fertilization regime may be best suited for high value crops grown in large containers. As an example, El Modeno Gardens, a 95-acre container nursery in California, divided their plants into 64 groups according to water needs and used trickle irrigation to replenish specific crop evapotranspiration (Whiteside, 1989). Implementation may prove costly initially, but faced with increasing health and

environmental regulations the nursery industry will have to engage in proactive thinking and implement innovative management strategies to remain profitable (Yeager, 1992).

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CHAPTER 6

PCR PRIMERS FOR AMPLIFICATION OF AN *NRT1* SEQUENCE IN *CORNUS* AND *RHODODENDRON*

ABSTRACT

Genes encoding nitrate uptake proteins in higher plants belong to either the *NRT1* or *NRT2* gene family. The *NRT1* gene family encodes transport proteins with dual or low affinity for nitrate. Members of the *NRT1* gene family have been cloned and sequenced in *Arabidopsis thaliana* (L.) Heynh, tomato (*Lycopersicon esculentum* Mill), rape (*Brassica napus* L.), and tobacco (*Nicotiana plumbaginifolia* Viv.) The objectives of this experiment were to use previously established sequences (i) to design primers that consistently distinguish pieces of the *NRT1* gene suitable downstream applications and (ii) to clone, determine the sequence of, and compare the resulting sequence(s) from *Cornus sericea* L., *C. sericea* 'Kelseyi' and *Rhododendron* 'Unique'. A primer pair, MD2-1 (5'-ATGTTACCAAYWTGGGCMAC-3') and MD2-2 (5'-GCCAMWARCCARTAGAAAT-3'), was designed that consistently amplified a 635 bp PCR fragment in the fifth exon of a putative arabidopsis *NRT1* gene. Each sequence was determined to be 635 nucleotides (with primers) in length. Up to 79.52 % of nucleotides were identical between the *C. sericea* 'Kelseyi' and *R.*

'Unique' sequences. These putative *NTR1* sequences will be used to investigate the expression of the *NRT1* gene in *C. sericea* and *R.* 'Unique' across a growing season. These results also serve as the initial step in sequencing the complete *NRT1* gene in *C. sericea* and *R.* 'Unique'.

INTRODUCTION

Nitrogen (N) uptake has been studied extensively in woody ornamental plants. These studies have relied on traditional experimental methods from plant physiology and chemistry. Recently developed molecular techniques enable researchers to now explore the molecular mechanism (gene and protein expression, gene regulation, etc.) of N uptake.

The *NRT1* gene family encodes transport proteins with dual or low affinity for nitrate. The first member of the *NRT1* family was identified from a chlorate resistant mutant of *Arabidopsis thaliana* (L.) Heynh called *chl1* (Braaksma and Feenstra, 1973; Tsay et al., 1993). Analysis of mRNA expression patterns indicates that CHL1 expression is nitrate inducible and found primarily in the roots (Tsay et al., 1993). This mutant is unique in that it displays wild-type levels of nitrate reductase activity but reduced levels of nitrate uptake. The gene was cloned and shown to encode a protein with 12 putative membrane-spanning regions, a membrane topology found in most cotransporters (Tsay et al., 1993). The CHL1 protein was expressed in *Xenopus* oocytes to test for nitrate transport and shown to be a dual affinity transporter with 2

K_m values of 35 μM and 8 mM. This indicates that CHL1 is a component of both the high- and low- affinity uptake systems. High nitrate (>1mM) and acidification of the medium depolarized the oocyte membrane, similar to the response observed in plant root cells. Uptake of nitrate into oocytes increased with expression of CHL1.

Genes belonging to the NRT1 family have been identified and characterized in other plants. Two *NRT1* homologues have been identified in tomato (*Lycopersicon esculentum* Mill.): *LeNRT.1* and *LeNRT.2* (Lauter et al., 1996). Both are expressed mainly in the roots, but *LeNRT.1* is expressed constitutively while *LeNRT.2* is induced by nitrate. Two *NRT1* homologues have been identified and cloned from rape (*Brassica napus* L.; Muldin and Ingemarsson, 1995). One of these, *BnNRT1.2*, when expressed in *Xenopus* oocytes, proves to be not only an effective transporter of nitrate, but also capable of transporting amino acids, particularly histidine (Zhou et al., 1998). Two homologues have been identified and characterized in tobacco (*Nicotiana plumbaginifolia* Viv.): *NpNRT1.1* and *NpNRT1.2* (Fraisier et al., 2001). Northern blot analysis showed that *NpNRT1.2* expression was restricted to roots, whereas *NpNRT1.1* was expressed at a basal level in plant organs.

The objectives of this experiment were (i) to design primers that consistently distinguish pieces of the *NRT1* gene and (ii) to clone, sequence, and compare the resulting sequence(s).

MATERIALS AND METHODS

On 10 Feb., leaves were harvested from *Cornus sericea*, *Cornus sericea* 'Kelseyi' (forced in the greenhouse for 30 d), and *Rhododendron* 'Unique'. Genomic DNA was extracted and prepared using the CTAB method (Doyle and Doyle, 1990).

NRT1 homologues were identified by entering the original *CHL1* sequence into a nucleotide-nucleotide BLAST (Altschul et al., 1990) search.

A putative map of the introns and exons of the CHL1 gene in arabidopsis was developed by aligning the genomic, mRNA, and coding sequences using the CLUSTAL W multiple sequence alignment program (Thompson et al., 1994)

Two degenerate primer pairs were designed by identifying conserved sequences in the alignment of *NRT1* nucleotide sequences of arabidopsis (accession number L10357), tomato (accession number X92852), tobacco (accession numbers AB102805 and AJ277084), and rape (accession number AJ278966). Alternatively, Blockmaker and CODEHOP (Consensus-degenerate hybrid oligonucleotide primers; Rose et al., 1998) were used to design primer pairs for the same accessions.

Polymerase chain reaction (PCR) was performed with each combination of primer pairs using the Eppendorf Master Cycler thermocycler (Eppendorf AG, Hamburg, Germany). The PCR reaction (15 μL total volume / reaction) was prepared in a 96-well PCR plate by mixing the following components: 9.4 μL PCR grade H_2O, 1.5 μL 10 x PCR buffer, 0.9 μL $MgCl_2$ (25 mM), 0.9 μL dNTP (2.5 mM), 0.5 μL forward and reverse primers (10 μM), 0.3 μL Amplitaq Gold polymerase (Applied Biosystems, Foster City, CA), and 1 μL DNA (10 $ng \cdot \mu L^{-1}$). The PCR program

consisted of 12 cycles of 45 sec at 94°C, 45 sec at 62°C, and 45 sec at 72°C followed by 30 cycles of 45 sec at 94°C, 45 sec at 50°C, and 45 sec at 72°C. Following PCR, 3.5 μL of 6 X loading dye (Promega, Madison, WI) were added to each PCR products, and results were separated on a 1% agarose gel at 95 V. Gel images were analyzed with Quantity One software (BioRad).

PCR products were ligated into the pCR2.1 TOPO vector and transformed into competent *Escherichia coli* One Shot® cells (Invitrogen, Carlsbad, CA). Positive colonies were selected, the expected size of the insert was confirmed by PCR and restriction endonuclease digestion (EcoRI), and plasmid DNA was isolated using the Perfectprep® Plasmid Mini kit (Eppendorf AG, Hamburg, Germany). Sequencing was done at the Central Services Lab (Oregon State University) with the ABI 3100 capillary sequencer. Nucleotide sequences of *C. sericea* 'Kelseyi' and *Rhododendron* 'Unique' were entered into a nucleotide-nucleotide BLAST (Altschul et al., 1990) search to identify similarity to other genes in the *NRT1* family. Additionally, sequences were realigned to the arabidopsis sequence and aligned to each other with the CLUSTAL W multiple sequence alignment program (Thompson et al., 1994)

RESULTS AND DISCUSSION

A nucleotide-nucleotide BLAST search (Altschul et al., 1990) identified several *NRTI* homologues or partial sequences. A putative map of the *NRT1* gene in

arabidopsis was designed for reference (Fig. 6.1). The map revealed five exons within a ~4200 bp sequence.

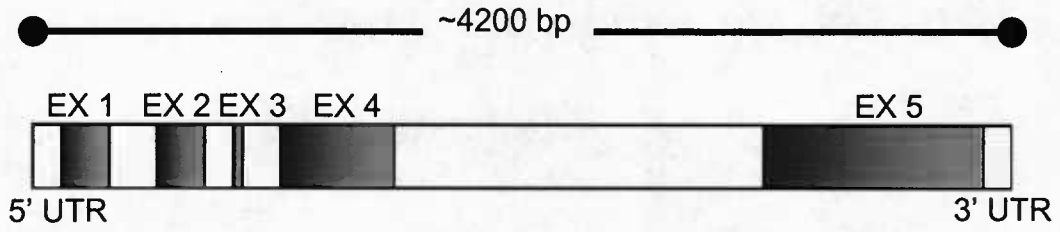

Fig. 6.1. Putative map of the *NRT1* gene in *Arabidopsis thaliana*. EX = exon, UTR = untranslated region

DNA extraction from leaf tissue of *C. sericea* and *R.* 'Unique' using the CTAB method yielded high-molecular weight (12 Kb) genomic DNA as observed in Fig. 6.2. DNA extraction was genotype-dependent as indicated by the larger amount of DNA obtained from *C. sericea* 'Kelseyi' and *C. sericea* as compared to the DNA isolated from *R.* 'Unique'.

One of the five degenerate primer pairs yielded positive results when employed in a PCR reaction with genomic DNA from leaf tissue of each of the taxa. The primer pair was MD2-1 (5'-ATGTTACCAAYWTGGGCMAC-3') and MD2-2 (5'-GCCAMWARCCARTAGAAAT-3') and is located in the fifth exon of the putative arabidopsis *NRT1* map. PCR product size was determined to be ~620 bp by counting the number of nucleotides between the respective primers. PCR reactions consistently yielded bands at ~620 nucleotides in each taxon (Fig. 6.3 A-B).

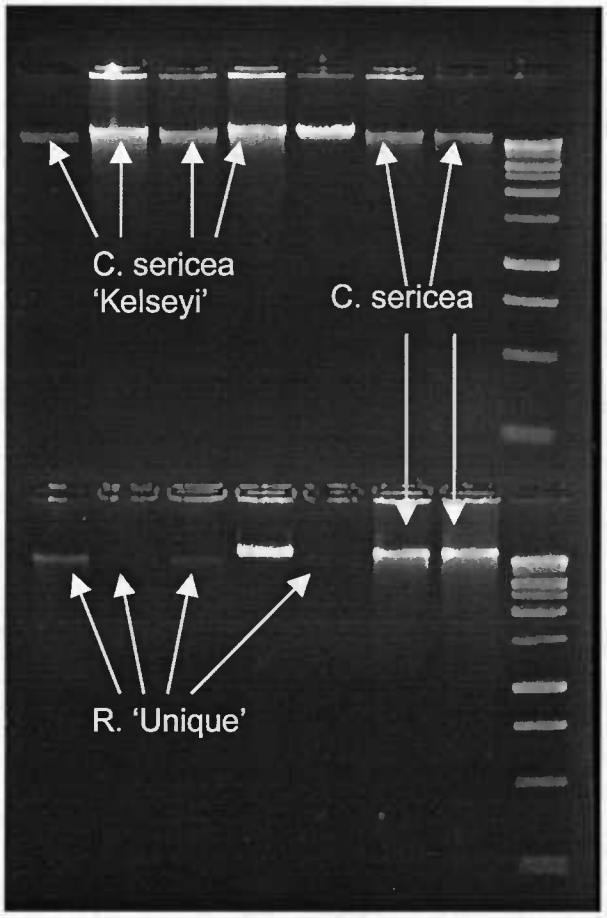

Fig. 6.2. One percent agarose gel showing bands representing 12 Kb high-molecular weight DNA extracted by the CTAB method.

Fig. 6.4 A-B shows the sequences for *C. sericea* 'Kelseyi' and *R.* 'Unique' as primed by the T7 forward promoter and M13 forward primer, respectively (MD2-1 and MD2-2 primers shown in gray), and Fig. 6.5 shows the alignment of these sequences. 79.52 % of nucleotides were a perfect match between the *C. sericea* 'Kelseyi' and *R.* 'Unique' sequences (CLUSTALW alignment score of 3489, * = direct match). Each sequence was determined to be 635 nucleotides in length.

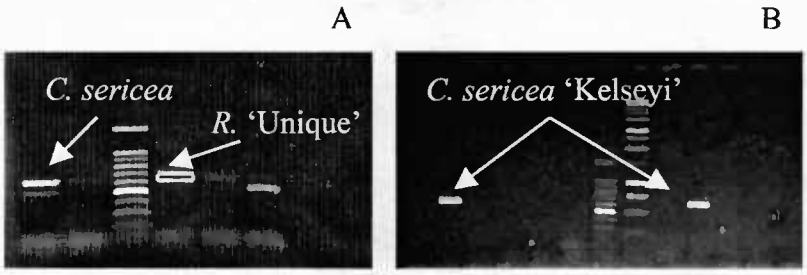

Fig. 6.3. PCR products of (A) *Rhododendron* 'Unique' and *Cornus sericea* and (B) *Cornus sericea* 'Kelseyi'. The brightest band in the middle of the 100 bp (base pair) ladder represents 500 bp. Visual reference establishes the PCR products between 600 and 700 bp. Band size quantitation with Quantity One quantitation software (BioRad) determined the bands from *C. sericea* 'Kelseyi' in lanes two and ten of gel B to be 618.12 and 612.37 bp, respectively.

(A) *C. sericea* 'Kelseyi':

ATGTTACCAATTTGGGCCACCACTATAATATTCTGGACTGTTT
ATGCCCAAATGACCACATTTTCAGTGTCACAAGCTACTACCA
TGGACCGCCGCATCGGCAAATCATTCCAAATTCCGGCAGCGT
CACTGACAGTTTTCTTCGTCGGTAGCATTCTCTTGACCGTCCC
GGTCTATGACCGAGTTATCGTTCCGTTCGCACGGAGAGTGCT
TAAGAACCCTCTAGGGCTTACCCCATTGCAACGTATAGGTGT
TGGTCTAGTCATGTCAATTTTTGCAATGGTAGCAGCTGCACT
TACTGAACTCAAACGACTACACGTCGCACAATCACATGGTAT
GACGGACAATGTAGGAGATGTGATCCCACTTAGTGTATTTTG
GTTGGTCCCACAATTCTTCTTGGTGGGGTCCGGCGAGGCCTT
CACATATATTGGGCAGCTTGATTTTTTCTTAAGGGAGTGCCC
TAAAGGGATGAAGACCATGAGCACAGGGCTGTTTTTAAGCA
CACTTTCACTAGGGTTTTTCTTCAGCTCTCTATTGGTTTCTAT
AGTGCACAAGGTGACCGGGGACAAAAGGCCATGGCTAGCTG
ATAATCTCAACCAAGGGAAGCTTTATGATTTCTANTGGCTAG
TGGC

(B) *R.* "Unique':

ATGTTACCAATTTGGGCCACCACAATCATGTTCTGGACAATA
TATGCCCAGATGACTACATTTTCAGTCTCCCAAGCCACTACA
ATGAACCGCCACCTTGGGAAATCGTTTAAAATTCCGGCTGCT
TCTCTCACCGCTTTCTTCGTCGGCAGCATTCTATTAACTGTGC
CAGTCTACGACCGGATTGTTGTGCCGATAGCAAGAAAATTGC
TTAGAAACCCCCAAGGTCTCACCCCATTGCAACGCATTGGCG
TTGGTCTAGTCTTCTCAATATTCGCCATGGTGGCAGCCGCTCT
CACCGAAATCAAGAGGTTGCACGTGGCACGATCGCACGGCT
TGACAAACGATCCGACAACTGTGGTTCCGCTGACGGTGTTTT
GGTTGATTCCACAATTCTTCTTCGTGGGGTCCGGCGAGGCGT
TTATTTATATTGGCCAGCTAGATTTTTTCCTGAGGGAGTGTCC
CAAGGGCATGAAGACCATGAGCACAGGGCTATTTTTGAGCA
CCCTTGCATTAGGGTTTTTCCTTAGCTCTATTTTGGTTACCAT
TGTGCACACAGTAACTGGGGATAGAAGGCCATGGCTAGCTG
ATAATCTCAACCAAGGGAGGCTCTACAATTTCTACTGGCTTG
TGGC

Fig. 6.4. Sequences of *Cornus sericea* 'Kelseyi' and *Rhododendron* 'Unique' as primed by the T7 forward promoter and M13 forward primer, respectively (MD2-1 and MD2-2 primers shown in gray).

```
ATGTTACCAATTTGGGCCACCACTATAATATTCTGGACTGTTTATGCCCAAATGACCACA
ATGTTACCAATTTGGGCCACCACAATCATGTTCTGGACAATATATGCCCAGATGACTACA
********************** ** ** ********  * ******** ***** ***

TTTTCAGTGTCACAAGCTACTACCATGGACCGCCGCATCGGCAAATCATTCCAAATTCCG
TTTTCAGTCTCCCAAGCCACTACAATGAACCGCCACCTTGGGAAATCGTTTAAAATTCCG
******** ** ***** ***** *** ****** * * ** ***** **  ********

GCAGCGTCACTGACAGTTTTCTTCGTCGGTAGCATTCTCTTGACCGTCCCGGTCTATGAC
GCTGCTTCTCTCACCGCTTTCTTCGTCGGCAGCATTCTATTAACTGTGCCAGTCTACGAC
** ** ** ** ** * ************ ******** ** ** ** ** ***** ***

CGAGTTATCGTTCCGTTCGCACGGAGAGTGCTTAAGAACCCTCTAGGGCTTACCCCATTG
CGGATTGTTGTGCCGATAGCAAGAAAATTGCTTAGAAACCCCCAAGGTCTCACCCCATTG
**  ** * ** *** * *** * * * ******  ***** * *** ** *********

CAACGTATAGGTGTTGGTCTAGTCATGTCAATTTTTGCAATGGTAGCAGCTGCACTTACT
CAACGCATTGGCGTTGGTCTAGTCTTCTCAATATTCGCCATGGTGGCAGCCGCTCTCACC
***** ** ** ************ * ***** ** ** ***** ***** ** ** **

GAACTCAAACGACTACACGTCGCACAATCACATGGTATGACGGACAATGTAGGAGATGTG
GAAATCAAGAGGTTGCACGTGGCACGATCGCACGGCTTGACAAACGATCCGACAACTGTG
*** ****  *  * ***** **** *** ** **  ****  ** **     *  ****

ATCCCACTTAGTGTATTTTGGTTGGTCCCACAATTCTTCTTGGTGGGGTCCGGCGAGGCC
GTTCCGCTGACGGTGTTTTGGTTGATTCCACAATTCTTCTTCGTGGGGTCCGGCGAGGCG
 * ** ** *  ** ********* * ************** *****************

TTCACATATATTGGGCAGCTTGATTTTTTCTTAAGGGAGTGCCCTAAAGGGATGAAGACC
TTTATTTATATTGGCCAGCTAGATTTTTTCCTGAGGGAGTGTCCCAAGGGCATGAAGACC
** *  ******** ***** ********* * ******** ** ** ** *********

ATGAGCACAGGGCTGTTTTTAAGCACACTTTCACTAGGGTTTTTCTTCAGCTCTCTATTG
ATGAGCACAGGGCTATTTTTGAGCACCCTTGCATTAGGGTTTTTCCTTAGCTCTATTTTG
************** ***** ***** *** ** *********** * ****** * ***

GTTTCTATAGTGCACAAGGTGACCGGGGACAAAAGGCCATGGCTAGCTGATAATCTCAAC
GTTACCATTGTGCACACAGTAACTGGGGATAGAAGGCCATGGCTAGCTGATAATCTCAAC
*** * ** *******  ** ** ***** * ****************************

CAAGGGAAGCTTTATGATTTCTANTGGCTAGTGGC
CAAGGGAGGCTCTACAATTTCTACTGGCTTGTGGC
******* *** **  ******* ***** *****
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Fig. 6.5. Alignment of *Cornus sericea* 'Kelseyi' and *Rhododendron* 'Unique' sequences amplified with the MD2-1 and MD2-2 primer pair. 79.52 % of nucleotides were a perfect match between sequences (CLUSTALW alignment score of 3489, * = direct match).

Reentering the *C. sericea* 'Kelseyi' and *R.* 'Unique' sequences into nucleotide-nucleotide BLAST searches (Altschul et al., 1990) produced similar results. The best match for each search was the recently cloned *PpNRT1* gene from peach [*Prunus persica* (L.) Batsch.; accession number AB089677; Y. Nakamura, K. Masuda, and Y. Umemiya, unpublished) followed by sequences from tobacco, corn (*Zea mays* L.), narcissus (*Narcissus pseudonarcissus* L.), rice (*Oryza sativa* L.), tomato, and arabidopsis. The *C. sericea* 'Kelseyi' and *R.* 'Unique' sequences were 81.26 % and 79.21 % similar to that of the *PpNRT1* sequence, respectively.

With this work, we have succeeded in isolating and identifying a 635 bp sequence from *C. sericea* and R. 'Unique' corresponding to exon five of the *CHL1* gene in arabidopsis. Results will facilitate detection of gene expression and complete sequencing of *CHL1* homologues in *Cornus, Rhododendron,* and other woody plants.

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CHAPTER 7

SUMMARY AND CONCLUSIONS

Nitrogen (N) management in container nurseries, and the associated concern over N pollution, involves a complex system of interrelated components ranging in temporal scale from milliseconds (e.g. gene expression, protein transport) to centuries (e.g. adaptive response of flora and fauna to increased environmental N levels) and spatial scale from nanometers (e.g. molecular aspects) to kilometers (e.g. watersheds, rivers, biosphere). To improve management decisions and develop effective, lasting solutions to N loss, data from traditional reductionist research at several scales must continue to be collected and eventually integrated through whole systems research approaches.

Investigation of complex systems can be overwhelming, especially if multiple stakeholders exist with individual perspectives. Group-based On-site Active Learning (GOAL) was developed to aid nursery managers and related stakeholders (e.g. neighbors, policy makers, regulating agencies, researchers) in developing a better understanding of how nitrogen flows through container nurseries and the effects of N management decisions over space and time. Managers concurred that the adaptive cycle is an accurate representation of container nursery production, matching the

processes, events, and choices encountered during a production cycle. GOAL is an effective method to investigate N management and may be adapted to facilitate the investigation of other complex systems.

During the GOAL exercise, growers expressed an interest in knowing the efficiency of N uptake at various rates and the relative importance of non-fertilizer N to fertilizer N. The ^{15}N tracer study from Chapter 4 of this thesis addresses these questions. Various rates (25, 50 100, 200, and 300 mg· L^{-1}) of ^{15}N depleted NH_4NO_3 (min 99.95% atom ^{14}N) were applied to container-grown *E. alatus, C. sericea*, and *W. florida.* At N rates of 25 to 300 mg· L^{-1}, fertilizer N recovery determined with a ^{15}N tracer increases slightly while fertilizer N loss increases dramatically. At low N concentrations, N derived from other sources (NDFO; e.g. water, bark, peat) was a significant portion of the total N in the plant. Increasing fertilizer rates up to 100 mg· L^{-1} resulted in increased uptake of NDFO and suggests that an added N interaction (ANI) is occurring even in a bark-based substrate with relatively little N. This is most likely a 'real' ANI resulting from increased root mass at higher N rates leading to increased uptake of N mineralized from the media. The NDFO causes a difference in the estimation of N recovery between isotopic and nonisotopic methods, and the difference is larger at lower N concentrations. Assuming that the ^{15}N tracer is the more accurate method of estimating fertilizer N uptake, the nonisotopic total N method overestimates fertilizer N uptake substantially, as much as three to four times in container-grown plants at N concentrations of 25 mg· L^{-1}. From these results we conclude:

1) Conditions that promote large losses of N from container systems only result in slight improvements in fertilizer N uptake.
2) ANI can occur in container-grown plants.
3) Fertilizer strategies for container-grown plants should be designed to take advantage of N in the substrate.
4) Results of determining N uptake in container-grown plants can differ drastically; interpretation of results must consider the methods.
5) Non-isotopic, total N estimation of N uptake overestimates fertilizer N uptake in container-grown plants.

Nursery managers recognize that different plants have different N requirements, and they question if controlled-release fertilizers (CRF) are the most N efficient option. Early in the development of the GOAL exercise, growers and managers expressed that by knowing the specific N requirements of plants they could potentially reorganize production areas and implement precision fertilization strategies. The N uptake study in Chapter 5 of this thesis addresses these questions and concerns.

To determine their relative efficiency, liquid N delivery regimes were developed for *E. alatus* 'Compactus' (a slow growing taxon) and *W. florida* 'Red Prince' (a fast growing taxon) and tested against an industry standard CRF. For *E. alatus* 'Compactus', total dry weight and total N content of the liquid N treatments were not significantly less than the CRF treatment with the highest values. Yet, the liquid feed treatments introduced 68.7% less N to the production system. Likewise,

total dry weight and total N content of *W. florida* 'Red Prince' were highest with a CRF but did not differ significantly from the liquid N treatments, which introduced 48.6% less total N to the production system. Liquid N fertilization resulted in higher N recovery than the CRF treatments in both *E. alatus* 'Compacta' and *W. florida* 'Red Prince'. From these results we conclude:

1) Comparable woody nursery stock can be produced with significantly less N inputs than are recommended with CRFs.
2) Liquid fertilization based on plant N requirements introduces less total N to the production cycle and results in higher N recovery rates than fertilization with an industry standard CRF.

Throughout the GOAL exercise, growers and managers identified that the production of multiple taxa (hundreds or thousands) with different capacities for N added complexity to their N management systems. While the preceding studies indicate that there are options to increase N efficiency through production practices, the question remains: Why does the capacity for N uptake differ across plant taxa? Recently developed molecular techniques enable researchers to address this question and explore the mechanisms (gene and protein expression, gene regulation, etc.) of N uptake. Chapter 6 of this thesis is a "first step" in using molecular techniques to determine the N uptake potential of various woody ornamentals.

A pair of degenerate primers was designed that consistently amplified a 635 nucleotide PCR fragment of the *NRT1* gene from *Cornus sericea* L., *C. sericea* 'Kelseyi' (putative high nitrate users) and *Rhododendron* 'Unique' (putative low

nitrate user). The fragment corresponds to exon five of the *CHL1* (nitrate transport) gene in arabidopsis. Up to 79.52 % of nucleotides were identical between the *C. sericea* 'Kelseyi' and *R.* 'Unique' fragments, and the sequences were 81.26 % and 79.21 % similar to the recently confirmed *PpNRT1* sequence in *Prunus persica* L. (Peach), respectively. From these results we conclude:

1) A highly conserved, 635 nucleotide fragment of the *NRT1* gene is present in *C. sericea,* high nitrate-use plants, and *R.* 'Unique', plants traditionally thought to prefer ammonium (NH_4^+).
2) The potential exists for each species to express the *NRT1* gene

The degenerate primer pair will be used to investigate the expression of the *NRT1* gene in *C. sericea* and *R.* 'Unique' across a growing season by subjecting seasonally collected root mRNA to RT PCR. The 635 nucleotide sequence will also serve as the initial step in sequencing the complete *NRT1* gene in *C. sericea* and *R.* 'Unique'.

In this thesis, data were collected at the whole systems, plant physiological, and molecular level. Integration of the data suggests that knowledge of specific N requirements (determined with physiological experiments) and of specific N uptake potential (determined with molecular studies) will enable growers to produce commercially competitive plants with substantially less fertilizer N. Implementation of changes to achieve this new production strategy (e.g. reorganization of production space, installation of drip irrigation, reassessment of crops grown) will require a clear understanding of N management systems (achieved with GOAL).

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APPENDIX

The following tables present statistical analysis of data presented in this thesis.

Table 1. Analysis of variance (ANOVA) tables for data presented in Fig. 4.2.

Quadratic model for NDFO (mg) in *Cornus*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	17731	8865.31880	4.85	0.0159
Error	27	49372	1828.57499		
Corrected Total	29	67102			

Root MSE	42.76184	R-Square	0.2642
Dependent Mean	140.42575	Adj R-Sq	0.2097
Coeff Var	30.45157		

Quadratic model for NDFO (mg) in *Weigela*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	50833	25416	6.22	0.0060
Error	27	110250	4083.31579		
Corrected Total	29	161082			

Root MSE	63.90083	R-Square	0.3156
Dependent Mean	198.75861	Adj R-Sq	0.2649
Coeff Var	32.14997		

Table 1. (continued)

Linear model for NDFO (mg) in *Euonymus*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	3.15216	3.15216	0.01	0.9436
Error	28	17309	618.18814		
Corrected Total	29	17312			

Root MSE	24.86339	R-Square	0.0002
Dependent Mean	34.46432	Adj R-Sq	-0.0355
Coeff Var	72.14240		

Table 2. Analysis of variance (ANOVA) tables for data presented in Fig. 4.4.

Quadratic model for N efficiency determined by the difference method in *Cornus*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr >F
Model	2	3714.82314	1857.41157	34.84	<.0001
Error	27	1439.35330	53.30938		
Corrected Total	29	5154.17643			

Root MSE	7.30133	R-Square	0.7207
Dependent Mean	39.25944	Adj R-Sq	0.7001
Coeff Var	18.59763		

Quadratic model for N efficiency determined by a ^{15}N method in *Cornus*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	560.79805	280.39902	35.10	<.0001
Error	27	215.67960	7.98813		
Corrected Total	29	776.47765			

Root MSE	2.82633	R-Square	0.7222
Dependent Mean	19.12980	Adj R-Sq	0.7017
Coeff Var	14.77448		

Table 2. (continued)

Quadratic model for N efficiency determined by the difference method in *Weigela*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	9196.54943	4598.27471	42.05	<.0001
Error	27	2952.78886	109.36255		
Corrected Total	29	12149			

Root MSE	10.45766	R-Square	0.7570
Dependent Mean	53.03289	Adj R-Sq	0.7390
Coeff Var	19.71919		

Quadratic model for N efficiency determined by a ^{15}N method in *Weigela*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	914.14079	457.07040	13.08	0.0001
Error	27	943.72262	34.95269		
Corrected Total	29	1857.86341			

Root MSE	5.91208	R-Square	0.4920
Dependent Mean	26.36830	Adj R-Sq	0.4544
Coeff Var	22.42117		

Table 2. (continued)

Quadratic model for N efficiency determined by the difference method in *Euonymus*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	813.99721	406.99861	12.01	0.0002
Error	27	914.96199	33.88748		
Corrected Total	29	1728.95920			

Root MSE	5.82130	R-Square	0.4708
Dependent Mean	15.68035	Adj R-Sq	0.4316
Coeff Var	37.12477		

Quadratic model for N efficiency determined by a ^{15}N method in *Euonymus*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	26.74558	13.37279	1.54	0.2320
Error	27	234.00422	8.66682		
Corrected Total	29	260.74980			

Root MSE	2.94395	R-Square	0.1026
Dependent Mean	8.91663	Adj R-Sq	0.0361
Coeff Var	33.01636		

Table 3. Analysis of variance (ANOVA) tables for data presented in Fig. 4.6.

Quadratic model for the effect of root weight (mg) on NDFF efficiency in *Cornus*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	621.44449	310.72225	54.11	<.0001
Error	27	155.03315	5.74197		
Corrected Total	29	776.47765			

Root MSE	2.39624	R-Square	0.8003
Dependent Mean	19.12980	Adj R-Sq	0.7855
Coeff Var	12.52622		

Quadratic model for the effect of root weight (mg) on NDFF efficiency in *Weigela*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	918.29396	459.14698	13.19	0.0001
Error	27	939.56945	34.79887		
Corrected Total	29	1857.86341			

Root MSE	5.89906	R-Square	0.4943
Dependent Mean	26.36830	Adj R-Sq	0.4568
Coeff Var	22.37178		

Table 3. (continued)

Quadratic model for the effect of root weight (mg) on NDFF efficiency in *Euonymus*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	122.61970	61.30985	11.98	0.0002
Error	27	138.13011	5.11593		
Corrected Total	29	260.74980			

Root MSE	2.26184	R-Square	0.4703
Dependent Mean	8.91663	Adj R-Sq	0.4310
Coeff Var	25.36656		

Quadratic model for the effect of total N (mg) on NDFF efficiency in *Cornus*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	647.20794	323.60397	67.59	<.0001
Error	27	129.26971	4.78777		
Corrected Total	29	776.47765			

Root MSE	2.18810	R-Square	0.8335
Dependent Mean	19.12980	Adj R-Sq	0.8212
Coeff Var	11.43816		

Table 3. (continued)

Quadratic model for the effect of total N (mg) on NDFF efficiency in *Weigela*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	1019.57563	509.78781	16.42	<.0001
Error	27	838.28778	31.04770		
Corrected Total	29	1857.86341			

Root MSE	5.57205	R-Square	0.5488
Dependent Mean	26.36830	Adj R-Sq	0.5154
Coeff Var	21.13161		

Quadratic model for the effect of total N (mg) on NDFF efficiency in *Euonymus*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	85.83895	42.91948	6.63	0.0046
Error	27	174.91085	6.47818		
Corrected Total	29	260.74980			

Root MSE	2.54523	R-Square	0.3292
Dependent Mean	8.91663	Adj R-Sq	0.2795
Coeff Var	28.54472		

Table 4. Analysis of variance (ANOVA) tables for data presented in Table 4.1.

Linear model for total N (mg) in *Cornus*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	2510919	2510919	594.70	<.0001
Error	28	118221	4222.17508		
Corrected Total	29	2629140			

Root MSE	64.97827	R-Square	0.9550
Dependent Mean	534.64181	Adj R-Sq	0.9534
Coeff Var	12.15361		

Linear model for NDFF (mg) in *Cornus*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	2835721	2835721	1445.38	<.0001
Error	28	54934	1961.92465		
Corrected Total	29	2890655			

Root MSE	44.29362	R-Square	0.9810
Dependent Mean	364.55761	Adj R-Sq	0.9803
Coeff Var	12.14996		

Table 4. (continued)

Linear model for percent N in *Cornus*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.46813	0.46813	12.94	0.0012
Error	28	1.01278	0.03617		
Corrected Total	29	1.48091			

Root MSE	0.19019	R-Square	0.3161
Dependent Mean	0.95290	Adj R-Sq	0.2917
Coeff Var	19.95864		

Linear model for percent NDFF in *Cornus*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	3.07105	3.07105	99.28	<.0001
Error	28	0.86612	0.03093		
Corrected Total	29	3.93717			

Root MSE	0.17588	R-Square	0.7800
Dependent Mean	0.55411	Adj R-Sq	0.7722
Coeff Var	31.74029		

Table 4. (continued)

Quadratic model for total N (mg) in *Cornus*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	2516298	1258149	301.04	<.0001
Error	27	112842	4179.33878		
Corrected Total	29	2629140			

Root MSE	64.64781	R-Square	0.9571
Dependent Mean	534.64181	Adj R-Sq	0.9539
Coeff Var	12.09180		

Quadratic model for NDFF (mg) in *Cornus*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	2835955	1417978	699.92	<.0001
Error	27	54700	2025.92621		
Corrected Total	29	2890655			

Root MSE	45.01029	R-Square	0.9811
Dependent Mean	364.55761	Adj R-Sq	0.9797
Coeff Var	12.34655		

Table 4. (continued)

Quadratic model for percent N in *Cornus*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.47870	0.23935	6.45	0.0051
Error	27	1.00221	0.03712		
Corrected Total	29	1.48091			

Root MSE	0.19266	R-Square	0.3232
Dependent Mean	0.95290	Adj R-Sq	0.2731
Coeff Var	20.21854		

Quadratic model for percent NDFF in *Cornus*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	3.15048	1.57524	54.06	<.0001
Error	27	0.78669	0.02914		
Corrected Total	29	3.93717			

Root MSE	0.17069	R-Square	0.8002
Dependent Mean	0.55411	Adj R-Sq	0.7854
Coeff Var	30.80506		

Table 4. (continued)

Linear model for total N (mg) in *Weigela*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	2551526	2551526	161.28	<.0001
Error	28	442985	15821		
Corrected Total	29	2994510			

Root MSE	125.78108	R-Square	0.8521
Dependent Mean	673.81043	Adj R-Sq	0.8468
Coeff Var	18.66713		

Linear model for total NDFF (mg) in *Weigela*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	3083524	3083524	341.03	<.0001
Error	28	253173	9041.90107		
Corrected Total	29	3336697			

Root MSE	95.08891	R-Square	0.9241
Dependent Mean	457.68589	Adj R-Sq	0.9214
Coeff Var	20.77602		

Table 4. (continued)

Linear model for percent N in *Weigela*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.89336	0.89336	18.49	0.0002
Error	28	1.35300	0.04832		
Corrected Total	29	2.24636			

Root MSE	0.21982	R-Square	0.3977
Dependent Mean	1.88080	Adj R-Sq	0.3762
Coeff Var	11.68764		

Linear model for percent NDFF in *Weigela*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	8.85927	8.85927	143.05	<.0001
Error	28	1.73407	0.06193		
Corrected Total	29	10.59334			

Root MSE	0.24886	R-Square	0.8363
Dependent Mean	1.10993	Adj R-Sq	0.8305
Coeff Var	22.42109		

Table 4. (continued)

Quadratic model for total N (mg) in *Weigela.*

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	2847431	1423716	261.36	<.0001
Error	27	147079	5447.37220		
Corrected Total	29	2994510			

Root MSE	73.80632	R-Square	0.9509
Dependent Mean	673.81043	Adj R-Sq	0.9472
Coeff Var	10.95357		

Quadratic model for total NDFF (mg) in *Weigela.*

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	3230805	1615402	411.89	<.0001
Error	27	105892	3921.93469		
Corrected Total	29	3336697			

Root MSE	62.62535	R-Square	0.9683
Dependent Mean	457.68589	Adj R-Sq	0.9659
Coeff Var	13.68304		

Table 4. (continued)

Quadratic model for percent N in *Weigela*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.89831	0.44915	9.00	0.0010
Error	27	1.34805	0.04993		
Corrected Total	29	2.24636			

Root MSE	0.22345	R-Square	0.3999
Dependent Mean	1.88080	Adj R-Sq	0.3554
Coeff Var	11.88032		

Quadratic model for percent NDFF in *Weigela*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	9.40902	4.70451	107.25	<.0001
Error	27	1.18433	0.04386		
Corrected Total	29	10.59334			

Root MSE	0.20944	R-Square	0.8882
Dependent Mean	1.10993	Adj R-Sq	0.8799
Coeff Var	18.86933		

Table 4. (continued)

Linear model for total N (mg) in *Euonymus*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	315432	315432	43.67	<.0001
Error	28	202224	7222.29038		
Corrected Total	29	517656			

Root MSE	84.98406	R-Square	0.6093
Dependent Mean	203.53802	Adj R-Sq	0.5954
Coeff Var	41.75341		

Linear model for total NDFF (mg) in *Euonymus*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	317429	317429	61.38	<.0001
Error	28	144810	5171.79899		
Corrected Total	29	462240			

Root MSE	71.91522	R-Square	0.6867
Dependent Mean	147.71381	Adj R-Sq	0.6755
Coeff Var	48.68551		

Table 4. (continued)

Linear model for percent N in *Euonymus*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.11995	0.11995	0.63	0.4358
Error	28	5.37196	0.19186		
Corrected Total	29	5.49191			

Root MSE	0.43801	R-Square	0.0218
Dependent Mean	2.09686	Adj R-Sq	-0.0131
Coeff Var	20.88905		

Linear model for percent NDFF in *Euonymus*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	4.50875	4.50875	18.97	0.0002
Error	28	6.65338	0.23762		
Corrected Total	29	11.16213			

Root MSE	0.48746	R-Square	0.4039
Dependent Mean	1.36144	Adj R-Sq	0.3826
Coeff Var	35.80506		

Table 4. (continued)

Quadratic model for total N (mg) in *Euonymus*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	322442	161221	22.30	<.0001
Error	27	195214	7230.15433		
Corrected Total	29	517656			

Root MSE	85.03031	R-Square	0.6229
Dependent Mean	203.53802	Adj R-Sq	0.5950
Coeff Var	41.77613		

Quadratic model for total NDFF (mg) in *Euonymus*

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	327388	163694	32.77	<.0001
Error	27	134852	4994.52601		
Corrected Total	29	462240			

Root MSE	70.67196	R-Square	0.7083
Dependent Mean	147.71381	Adj R-Sq	0.6867
Coeff Var	47.84384		

Table 4. (continued)

Quadratic model for percent N in *Euonymus*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.42773	0.21387	1.14	0.3347
Error	27	5.06418	0.18756		
Corrected Total	29	5.49191			

Root MSE	0.43308	R-Square	0.0779
Dependent Mean	2.09686	Adj R-Sq	0.0096
Coeff Var	20.65398		

Quadratic model for percent NDFF in *Euonymus*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	6.76270	3.38135	20.75	<.0001
Error	27	4.39943	0.16294		
Corrected Total	29	11.16213			

Root MSE	0.40366	R-Square	0.6059
Dependent Mean	1.36144	Adj R-Sq	0.5767
Coeff Var	29.64959		

Table 5. Analysis of variance (ANOVA) tables for data presented in Table 4.2.

Linear model for fertilizer N loss (mg) in *Cornus*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	26905352	26905352	13712.2	<.0001
Error	28	54940	1962.14703		
Corrected Total	29	26960292			

Root MSE	44.29613	R-Square	0.9980
Dependent Mean	1295.84239	Adj R-Sq	0.9979
Coeff Var	3.41833		

Linear model for percent fertilizer N loss in *Cornus*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	419.72237	419.72237	32.94	<.0001
Error	28	356.75528	12.74126		
Corrected Total	29	776.47765			

Root MSE	3.56949	R-Square	0.5405
Dependent Mean	80.87020	Adj R-Sq	0.5241
Coeff Var	4.41385		

Table 5. (continued)

Quadratic model for fertilizer N loss (mg) in *Cornus*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	26905604	13452802	6641.70	<.0001
Error	27	54689	2025.50500		
Corrected Total	29	26960292			

Root MSE	45.00561	R-Square	0.9980
Dependent Mean	1295.84239	Adj R-Sq	0.9978
Coeff Var	3.47308		

Quadratic model for percent fertilizer N loss in *Cornus*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	560.79805	280.39902	35.10	<.0001
Error	27	215.67960	7.98813		
Corrected Total	29	776.47765			

Root MSE	2.82633	R-Square	0.7222
Dependent Mean	80.87020	Adj R-Sq	0.7017
Coeff Var	3.49490		

Table 5. (continued)

Linear model for fertilizer N loss (mg) in *Weigela*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	26163228	26163228	2897.96	<.0001
Error	28	252788	9028.15037		
Corrected Total	29	26416017			

Root MSE	95.01658	R-Square	0.9904
Dependent Mean	1202.71411	Adj R-Sq	0.9901
Coeff Var	7.90018		

Linear model for percent fertilizer N loss in *Weigela*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	75.17119	75.17119	1.18	0.2865
Error	28	1782.69222	63.66758		
Corrected Total	29	1857.86341			

Root MSE	7.97920	R-Square	0.0405
Dependent Mean	73.63170	Adj R-Sq	0.0062
Coeff Var	10.83663		

Table 5. (continued)

Quadratic model for fertilizer N loss (mg) in *Weigela*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	26310076	13155038	3352.69	<.0001
Error	27	105941	3923.72368		
Corrected Total	29	26416017			

Root MSE	62.63963	R-Square	0.9960
Dependent Mean	1202.71411	Adj R-Sq	0.9957
Coeff Var	5.20819		

Quadratic model for percent fertilizer N loss in *Weigela*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	914.14079	457.07040	13.08	0.0001
Error	27	943.72262	34.95269		
Corrected Total	29	1857.86341			

Root MSE	5.91208	R-Square	0.4920
Dependent Mean	73.63170	Adj R-Sq	0.4544
Coeff Var	8.02926		

Table 5. (continued)

Linear model for fertilizer N loss (mg) in *Euonymus*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	39785668	39785668	7697.34	<.0001
Error	28	144725	5168.75442		
Corrected Total	29	39930394			

Root MSE	71.89405	R-Square	0.9964
Dependent Mean	1512.68619	Adj R-Sq	0.9962
Coeff Var	4.75274		

Linear model for % fertilizer N loss in *Euonymus*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.02198	0.02198	0.00	0.9616
Error	28	260.72782	9.31171		
Corrected Total	29	260.74980			

Root MSE	3.05151	R-Square	0.0001
Dependent Mean	91.08337	Adj R-Sq	-0.0356
Coeff Var	3.35024		

Table 5. (continued)

Quadratic model for fertilizer N loss (mg) in *Euonymus*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	39795514	19897757	3983.11	<.0001
Error	27	134879	4995.53307		
Corrected Total	29	39930394			

Root MSE	70.67909	R-Square	0.9966
Dependent Mean	1512.68619	Adj R-Sq	0.9964
Coeff Var	4.67242		

Quadratic model for percent fertilizer N loss in *Euonymus*.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	26.74558	13.37279	1.54	0.2320
Error	27	234.00422	8.66682		
Corrected Total	29	260.74980			

Root MSE	2.94395	R-Square	0.1026
Dependent Mean	91.08337	Adj R-Sq	0.0361
Coeff Var	3.23215		

Table 6. Analysis of variance (ANOVA) tables for data presented in Table 5.2.

For: *Euonymus alatus* 'Compactus'

Class	Levels	Values
treat	8	IH IM Orange TDH TDH(L) TDM White Yellow

Number of observations 40

Dependent Variable: Total dry weight of *Euonymus alatus* 'Compactus'

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	128.7698000	18.3956857	2.10	0.0724
Error	32	280.3541600	8.7610675		
Corrected Total	39	409.1239600			

R-Square	Coeff Var	Root MSE	Total dry weight Mean
0.314745	37.37732	2.959910	7.919000

Dependent Variable: Total N in *Euonymus alatus* 'Compactus'

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	50874.4499	7267.7786	2.06	0.0780
Error	32	113103.5300	3534.4853		
Corrected Total	39	163977.9799			

R-Square	Coeff Var	Root MSE	Total N Mean
0.310252	33.65651	59.45154	176.6420

Table 6. (continued)

Dependent Variable: N recovery (%) in *Euonymus alatus* 'Compactus'

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	2.37568722	0.33938389	2.90	0.0183
Error	32	3.74863203	0.11714475		
Corrected Total	39	6.12431925			

R-Square	Coeff Var	Root MSE	N recovery Mean
0.387910	14.79284	0.342264	2.313715

For: *Weigela florida* 'Red Prince'

Class	Levels	Values
treat	8	Blue Green IH IM TDH TDH(L) TDM White

Number of observations 40

Dependent Variable: Total dry weight of *Weigela florida* 'Red Prince'

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	211.372717	30.196102	0.84	0.5655
Error	32	1155.355280	36.104852		
Corrected Total	39	1366.727997			

R-Square	Coeff Var	Root MSE	Total dry weight Mean
0.154656	11.22231	6.008731	53.54275

Table 6. (continued)

Dependent Variable: Total N in *Weigela florida* 'Red Prince'

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	889538.296	127076.899	11.31	<.0001
Error	32	359527.757	11235.242		
Corrected Total	39	1249066.053			

R-Square	Coeff Var	Root MSE	Total N Mean
0.712163	14.76392	105.9964	717.9424

Dependent Variable: N recovery (%) in *Weigela florida* 'Red Prince'

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	2.95654423	0.42236346	23.74	<.0001
Error	32	0.56925388	0.01778918		
Corrected Total	39	3.52579811			

R-Square	Coeff Var	Root MSE	N recovery Mean
0.838546	9.928488	0.133376	1.343368