Nitrate uptake by red maple varies with root-zone temperature

by

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CHAPTER ONE. GENERAL INTRODUCTION

Goal

The goal of my research was to examine the variability of nitrate uptake by red maple (*Acer rubrum* L.) when the roots of the plants are exposed to different temperatures. My objectives were:

- to determine the kinetics of nitrate uptake at different root-zone temperatures by using 'Autumn Flame' and 'Franksred' ramets as representative of red maple, and

- to compare a method for determining the kinetics of nitrate uptake by using plants grown in soil with the standard solution-depletion method of plants growing in nutrient solution.

I used four experiments to achieve these objectives. Three of these were solution-depletion experiments to determine uptake kinetics. We used standard solution-depletion methods to determine the kinetics of nitrate uptake by red maple grown in nutrient solution and exposed to root-zone temperature treatments of 14, 24, and 34 °C. These experiments met my main objective. For the second objective, I modified a method originally designed to examine potassium uptake by agronomic crops growing in field conditions. I modified the method to determine nitrate-uptake kinetics using greenhouse-grown red maple with root zones at 14, 24, and 34 °C.

Thesis organization

This thesis is organized into five chapters. Chapter One is the general introduction. Chapter Two is a literature review that discusses the background of the research: use of nutrient uptake models, root-zone temperature, red maples, and temperature effects on plant physiology and nutrient uptake. Chapter Three is a research paper prepared for *Plant and Soil* entailing the solution-depletion studies I did. Chapter Four discusses the merits of the soil-based method for determining nitrate uptake kinetics and the results of an experiment I performed. Chapter Five contains the conclusion and significance of the research. The thesis follows the rules and style set by *Plant and Soil* whenever possible to maintain a consistent appearance. Literature citations, tables, and figures appear at the end of each chapter. Tables, figures, and equations are numbered separately within each chapter.

CHAPTER TWO. LITERATURE REVIEW

Temperature

General effects

Plants have to adjust to the root-zone temperature to maintain adequate nitrate uptake to meet internal demand for nitrogen. Root-zone temperature affects the environment plants are growing in, as well as the plant material itself. Increasing the temperature affects chemical reaction rates by increasing the number of molecular collisions and the proportion of molecules with enough activation energy to react during those collisions, as explained by the Arrhenius equation (Johnson and Thornley, 1985). The Arrhenius equation uses the Boltzman distribution, which estimates the distribution of molecules with enough energy to react (Nobel, 1983), to relate temperature and reaction rate (Equation 1). In this equation, A

$$k = Ae^{-E/RT} \tag{1}$$

is a constant, E is the activation energy, R is the Boltzman constant multiplied by Avogadro's number (the gas constant), and T is the temperature in degrees Kelvin. Johnson and Thornley (1985) describe forms of the Arrhenius equations for enzyme-mediated reactions.

Temperature also affects viscosity and diffusion (Johnson and Thornley, 1985), but neither is a linear relationship. For equations 2 and 3, E is the activation energy, R is the gas

$$\eta = \frac{c_\eta}{\sqrt{T}} e^{E/RT} \tag{2}$$

$$D = c_d \sqrt{T} e^{-E/RT} \tag{3}$$

constant, T is the temperature in Kelvin, and each has a constant c. The viscosity of a fluid is inversely proportional to temperature (Equation 2). Since viscosity measures water's ability to resist movement, an increase in temperature will increase the fluidity of water. Xylem water potentials in red maple (*Acer rubrum* L.) decrease at high root-zone temperature (Graves et al., 1989), and this could be due to a decrease in viscosity. Because nitrate delivery to the root is primarily by mass flow (Barber, 1995; Miller and Donahue, 1995), viscosity change could affect the delivery of nitrate to the root. For every 10 °C increase, viscosity should drop to 35% of its original value, according to the equation given by Johnson and Thornley (1985). Diffusion velocity increases with increasing temperature (Equation 3). This should increase the speed of delivery of nitrate to the root, although this delivery method is a relatively small proportion of overall nitrate delivery to the root. It could have implications where ammonium competes with nitrate as a nitrogen source because diffusive delivery of ammonium is a more important delivery mechanism than for nitrate.

Physiological effects

Membranes are integral to life, and membrane components are altered in response to temperature changes. Membranes function as barriers to diffusive reactions, catalyze transport, store energy in transmembrane electrochemical gradients, provide an organizational matrix for protein networks, anchoring proteins in general, and regulate energy utilization by controlling electrochemical channels and permeability. Phospholipids, the main constituents of the membrane, have different phase states that are determined by chemical composition and temperature (Hazel, 1997). The theory of homeoviscous

adaptation (Hazel, 1997) states that the organism will adapt the appropriate membrane structure to meet the fluidity requirements necessary for life. At high temperatures, plants must maintain proper arrangement in cell membranes to make membrane fusion possible, while still maintaining double-layer membrane stability. At lower temperatures, the plant must be able to avoid the gel state of the lamellar phase state to maintain fluidity and protein function (Hazel, 1997).

The extent of this adaptation will affect nitrate transport across membranes when roots are exposed to different temperatures. Nitrate transport is a carrier-mediated process, and evidence suggests that a correlation exists between membrane fluidity and protein denaturation (Hazel, 1997). The phospholipidic head-group and steroid insertion also help stabilize membrane fluidity and protein complexes (Hazel, 1997). Plants can counteract low temperatures by increasing the number of transporters or reducing the dissipation of concentration gradients. Membrane physiology is only one reason to suspect that root-zone temperature will affect nitrate uptake. Bhat (1982) suggests that changes in activation energy for the nitrate uptake process between 5 and 10 °C in apple (*Malus domestica* Borkh.) are associated with membrane phase transitions. Bravo-F and Uribe (1981) used potassium and phosphorus to show that ion uptake is even more responsive to temperature, especially at low temperatures, than respiration.

Root functions are temperature dependent (Miller, 1986). Temperature affects the anatomy of the root (Nielson, 1974). Cooler root-zone temperatures usually lead to whiter, thicker, and less branched roots with delayed cell maturation (Miller, 1986; Marschner, 1995). High temperatures can lead to filamentous roots (Barr and Pellett, 1972; Marschner, 1995).

Sustained temperatures over 50 °C can kill roots, and no root regeneration may occur. Plants exposed to daily maxima of 40-45 °C can have the root tips killed. These temperatures may seem high, but a black nursery container exposed to direct sun can reach over 45 °C, and it is common to see temperatures as high as 50 °C in such containers (Wong et al., 1971). Barr and Pellett (1972) observed black (necrotic) roots at 37 °C for several woody species. Time of exposure and rate of temperature change influence the degree of stress root-zone temperature puts on plants (Ingram, 1985). Wong et al. (1971) showed significant decline in root growth by black locust (*Robinia pseudoacacia* L.) exposed to 6 h of 35 °C, and note that summer temperatures in an exposed container could reach 35 °C for 8 h. Urban soils near paved surfaces have higher mean and maximum root-zone temperatures (Graves and Dana, 1987) than those away from the pavement in urban situations, or in a natural forest ecosystem. This was due to both direct sun and pavement heat retention.

Nitrate uptake

Plants use more nitrogen than any other mineral nutrient, and nitrate is a common source of nitrogen for plants. Lack of nitrogen is often a limiting factor for plant growth (Forde and Clarkson, 1999). Nitrate transport limits growth of photosynthetic cyanobacteria (*Synechococcus*) at low temperatures (Sakamoto and Bryant, 1999), showing that delivery to cells can limit growth, even if nitrate exists in the growth medium. Nitrate also acts as a signal for transcription of many genes (Wang et al., 2000), and is involved intricately in the carbon-nitrogen and hormonal balance of the plant (Marschner, 1995). Nitrate assimilation requires carbon skeletons and energy, so manipulation of assimilation has energy-balance

implications, including the possibility of alleviating stress in excessive high-light conditions (Marschner, 1995). Most plants prefer to have both ammonium and nitrate as nitrogen sources. This allows plants to regulate internal pH, and reduces the effect on soil pH that uptake of ammonium (decrease) and nitrate (increase) individually would have. Nitrate has an advantage over ammonium in plants because it can be transported in the xylem, and does not have to be assimilated in the roots (Marschner, 1995). When soil conditions are favorable for nitrification, nitrate is usually abundant in soil. Nitrate is highly mobile, because it reacts little with mineral-soil surfaces (it is usually repelled and relegated to solution phase) and is very soluble in water. For these reasons, nitrate is the most important form of the most limiting nutrient for plant growth in many environments.

Transporters

Nitrate is an anion. The cell constantly creates a negative potential across the plasma membrane, so nitrate transport is against an electrical gradient. Thus, nitrate transport is an energy-requiring process, as is shown by the Nernst equation (Taiz and Zeiger, 1998). Scientists who have measured the potential across the plasma membrane have developed a H^+/NO_3^- cotransport theory (Crawford and Glass, 1998) that describes nitrate transport across the plasma membrane. This theory supports the findings that a H^+-ATPase is involved in nitrate uptake (McClure et al., 1990; Crawford, 1995). The H^+-ATPase uses energy from ATP to pump H^+ outside of the root cell to create a proton-motive force. The transporters use this proton-motive force to move nitrate into the cell. The current model proposes that two protons per nitrate ion are co-transported (Crawford and Glass, 1998). Specifically, one proton binds to the transporter, and then nitrate, which is followed by another proton. Then

the transporter moves the nitrate and one of the protons across the plasma membrane and into the cytoplasm (Forde and Clarkson, 1999). Other mechanisms for transport include passive transport. Pouliquin et al. (2000) reported passive nitrate transport driven by electrochemical gradients. This could have implications for transport at high nitrate concentrations.

Several nitrate-transporting proteins make transport against this gradient possible. Researchers have identified two gene families, NRT1 and NRT2, for nitrate transport. These families have no sequence similarity and play different roles in nitrate transport. The encoded transporters can be induced by nitrate (Crawford and Glass, 1998). Research suggests that NRT1 genes code for low-affinity, nitrate-transport system (LATS) proteins, and NRT2 genes code for induced high-affinity, nitrate-transport system (IHATS) proteins (Liu et al., 1999). However, Liu et al. (1999) and Wang et al. (1998) gave evidence that it is possible for one transporter to comprise both IHATS and LATS, and this provides a direct link between the two "mechanisms."

Kinetics

Nitrate uptake has been broken down mechanistically into the constitutive high-affinity nitrate-transport system (CHATS), IHATS, and LATS (Crawford and Glass, 1998). Recent results show LATS has constitutive and inducible components (Huang et al., 1996), and CHATS increases in the presence of nitrate (Forde and Clarkson, 1999). Values for the K_m and I_{max} of CHATS range from 6-20 μM and 0.3-0.82 μmol g^{-1} h^{-1} (grams of root fresh weight), respectively. IHATS values of K_m and I_{max} range from 20-100 μM and 3-8 μmol g^{-1} h^{-1}, respectively (Crawford and Glass, 1998). Plants exposed to nitrate (or nitrite) can take several hours to several days for self-induction of IHATS. LATS responds linearly to the

concentration of nitrate in the growing medium, and can significantly contribute to uptake above 250 μM (Crawford and Glass, 1998).

Kelly et al. (2000) reported I_{max} nitrate values ranging from 157.0 to 590.8 nmol m^{-2} s^{-1} and K_m values ranging 204 from to 524 μM for red maple. Nitrate uptake in aspen (*Populus tremuloides* Michx.) exhibits HATS that is inducible, and the I_{max} is ten-fold higher than when not induced with nitrate. The induced plants had I_{max} of 3.00 μmol g^{-1} h^{-1} (grams of fresh weight) and a K_m of 11.69 μM (Min et al., 2000). However, some forest species have much lower IHATS I_{max} values: 0.354 and 0.29 μmol g^{-1} h^{-1} for spruce (*Picea glauca* Voss.) (Kronzucker et al., 1995) and pine (*Pinus contorta* Douglas ex Arias) (Min et al., 2000), respectively. The K_m values were 13.74 and 153.4 μM, respectively. The spruce values are averages from Table II in Kronzucker et al. (1995). Bhat (1982) reported a constant uptake (concentration independent) of nitrate outside of 1-20 μM in the soil solution for apple. Bhat (1982) then concluded that concentration dependence of uptake rate below 20 °C is of "limited relevance" to apple roots in soil, as such nitrate concentrations are not prevalent. Treatments over 20 °C resulted in more concentration dependence on uptake.

Effectors

Nitrate influx is partially offset by a nitrate efflux system. Aslam et al. (1996) provide evidence that nitrate efflux is induced by nitrate, and that RNA and protein synthesis are required for nitrate-induced nitrate efflux. However, the literature is conflicting on how ammonium reduces nitrate uptake. In the short term, ammonium might contribute to a depolarization of the plasma membrane, which reduces the proton-motive force for nitrate

uptake (Crawford and Glass, 1998). Ammonium assimilation also releases protons into the cell that reduce the proton-motive force, until pumped out outside by the H^+-ATPase. Ammonium stimulates efflux in barley (*Hordeum vulgare* L.) plants that are pre-loaded with nitrate, but nitrate efflux was not affected by ammonium in plants that were low in internal nitrate (Aslam et al., 1994). Vidmar et al. (2000) point out that ammonium can affect nitrate uptake on many levels, and that it is possible that ammonium might have direct effects on transport systems, but also transcriptional effects on nitrate uptake by the products of ammonium assimilation. This means that ammonium is an additional supply of nitrogen and contributes to the internal pool of nitrogen, which leads to down-regulation of nitrate uptake. Ammonium treatments of several hours decrease nitrate influx (Vidmar et al., 2000). Some argument arises in the literature about preference of nitrogen source for plants. It is becoming increasing clear that many higher plants prefer a ratio with more ammonium than nitrate (BassiriRad et al., 1999; Min et al., 2000), but this can be affected by soil factors, like pH and temperature (Marschner, 1995). Nitrite (NO_2^-), another mineral form of nitrogen, is a competitive inhibitor of nitrate uptake. Nitrite can be a source of nitrogen to plants if it is available in the soil in high enough concentrations (Aslam et al., 1992).

General nutrition and health, which must be determined by species, will affect nitrogen demand. Plants adjust nitrate uptake so that internal pools of nitrate remain constant (Imsande and Touraine, 1994). The theoretical mechanism of this regulation is organic acid cycling, possibly with potassium, from the leaves to the roots, and back to the leaves. One of these proposals is that potassium is taken up with nitrate and accompanies nitrate in the xylem to the leaves, where nitrate is exchanged for malate. Potassium accompanies malate to the roots (Imsande and Touraine, 1994). Here, the malate is metabolized, and ^-OH is

released into the medium, which accounts for pH increases in growing media where nitrate is the nitrogen source. Proposed feedback includes amino acid cycling in the phloem that reduces nitrate uptake (Imsande and Touraine, 1994).

Measured nitrate uptake will be dependent on use of nitrate for nitrogen assimilation, vacuolar storage, or efflux. Nitrate is ultimately reduced by nitrate and nitrite reductase to be assimilated into amino acids. However, regulation of the reductases and nitrate uptake at the mRNA level is separate (Forde and Clarkson, 1999). The chain of nitrogen assimilation can also play a role in nitrate uptake by determining the rate-limiting step. The vacuole occupies most of the space inside a plant cell and contains most of the nitrate within a cell (Belton et al., 1985). The transport of nitrate from the cytoplasm to the vacuole could be a limiting step. Köhler and Raschke (2000) reported anion channels for loading xylem can have different affinities for nitrate versus chloride, and these authors note that it is common for anion channels in plants to have high nitrate affinities. Nitrate is assimilated in both the roots and shoots of plants, but temperature and the accompanying cation alter the root to shoot assimilation ratio (Marschner, 1995).

Temperature

Previous studies show that nutrient acquisition increases with temperature to an optimum and then declines (Barber, 1995). Quantifying the root-zone temperature effect on uptake requires a separate investigation for each nutrient and species combination (Barber, 1995). The relative preference of the plant between nitrogen sources affects the magnitude of nitrate uptake response to root-zone temperature (Marschner, 1995).

Nitrate uptake was less when root zones were 12 °C versus 25 °C for winter wheat (*Triticum aestivum* L.), and nitrate uptake decreased the most when the accompaning ion was K^+, as opposed to either NH_4^+ or Ca^{2+} (Kharitonashvili and Alekhina, 1986). Week-long uptake measurements of hydroponically grown snapdragon (*Antirrhinum majus* L.) showed maximum nitrate uptake occurs when the root zone is at 22.5 °C (Hood and Mills, 1994). For nitrate concentrations of 60 to 200 μM, Bhat (1982) observed an increase in uptake per unit surface area between 5 and 25 °C for apple.

Red Maple

Red maples are a highly diverse group of trees that are a favorite for domestic planting. It is one of the most planted species by municipalities in the northeastern United States (Townsend, 1977). The native range of red maple is from southern Canada to the southern United States and from the Midwest to the East Coast. Because of this large variety of habitat, red maple offers opportunity to examine intra-species variation and environmental adaptability. Townsend (1977) showed that red maples vary in growth rate, cold-hardiness, winter preparation, and fall coloration. Greater height, diameter, and winter injury were associated with southern trees. The northern trees had better fall coloration, earlier defoliation, budset, and flushing. These same traits (except color) were also inversely correlated to amount of rainfall in the native environment.

Shoot and root growth of red maples is dependent on the root-zone temperature (Graves et al., 1989). Shoot water potential decreases as the root-zone temperature increases. Plants grown with root zones at 24 °C had the lowest resistance to water loss through the

leaves (lowest diffusive resistance) and the highest leaf osmotic potential compared to plants at 18 and 36 °C. It seems clear from the data presented by Graves et al. (1989) and Wilkins et al. (1995) that temperature in the root zone has an influence on carbon partitioning between roots and shoots. In maples grown at 34 °C, the stem elongation was longer and the third-order root shorter (and apparently thicker and fewer in number) than those grown at 28 °C (Wilkins et al., 1995). BassiriRad et al. (1999) showed that red maple has a higher uptake rate for ammonium than for nitrate, which has implications for research protocols that call for both ions in the growing medium.

I chose 'Autumn Flame' and 'Franksred' (Red Sunset®) to represent red maple in my research. Both of these cultivars were selected in Oregon, but the native origin of both is unknown. 'Autumn Flame' appears to be slower growing than 'Franksred', and 'Autumn Flame' has a more branched appearance than 'Franksred' in our greenhouse. Plant dry mass and stem length for 'Autumn Flame' were similar for plants with root-zones at 28 and 34 °C, but the longest third-order root was 50% shorter at 34 than 28 °C (Wilkins et al., 1995). In the same study, 'Franksred' had less plant dry mass, shorter stems, and shorter third-order roots at 34 than 28 °C. This is evidence that 'Autumn Flame' is more resistant to high root-zone temperatures than 'Franksred'. 'Autumn Flame' has: larger diameter roots, shorter roots, lower root length per gram of root, and lower root-surface area, and higher nitrate uptake rate per unit root surface area than 'Franksred'. Both 'Franksred' and 'Autumn Flame' have similar total uptake over time (Kelly et al., 2000).

Uptake Models

Uptake models are mathematical representations of biological and natural processes. Models can allow researchers to test hypotheses relating to complex processes by using variables that are relatively easy to measure or calculate (Kelly et al., 1992), and models provide a common platform for discussion between researchers. This research focuses on the root surface. Several models of what happens at the root surface exist. The Barber model (Barber, 1995), which is based on earlier models, uses Michaelis-Menten enzyme kinetics concepts to explain the influx process, while others use a variable called root-absorption power (Yanai, 1994).

Equation 4 is the Michaelis-Menten equation. V is the dependent variable and is the

$$V = \frac{V_{\max} C_l}{Km + C_l} \tag{4}$$

rate of enzymatic reaction. V_{max} is the theoretical maximum velocity of the enzymatic reaction, when substrate saturates the enzyme. C_l is the substrate concentration. K_m is the concentration where the reaction rate is half of V_{max}. Equation 5 is the modified Michaelis-

$$In = \frac{\mathrm{Imax}(C_l - \mathrm{Cmin})}{Km + C_l - C\,\mathrm{min}} \tag{5}$$

Menten equation used in nutrient influx modeling. The basic idea is the same, but V, velocity, is re-termed influx, and includes the C_{min} variable. C_{min} describes the concentration when net uptake ceases, and influx and efflux reach equilibrium. Alternatively, C_{min} can be omitted and a single efflux parameter can be subtracted from the Michaelis-Menten equation (Barber, 1995). Researchers have used sensitivity analysis to determine that I_{max} is the most responsive kinetic parameter in model calculations of influx (Williams and Yanai, 1996;

Barber, 1995; Kelly et al., 1992). The responsiveness of influx to I_{max} is greater as concentration increases, and when mass flow delivers nutrients to the root faster than I_{max} (Barber, 1995).

Researchers can modify the steady-state Barber model by adding parameters or inserting mathematical functions in place of constants. As an example, Yanai (1994) showed how to modify uptake models for growing roots. However, inconsistencies in the use of the model can be problematic. Influx can be reported on the basis of root fresh weight, length, or surface area (Claassen and Barber, 1974). This leads to problems in comparison between experiments, because often the information needed to convert between these root parameters is not provided in the research article.

Equation 6 is the inner boundary of the Barber model, and a basic assumption of this

$$D_e b \frac{\partial C_l}{\partial r} + v_o C_l = \frac{\mathrm{Imax}(\mathrm{C_l} - \mathrm{Cmin})}{Km + C_l - C\,\mathrm{min}} \qquad (6)$$

research. It relates a steady state flow that includes mass flow and diffusion to the transfer of nutrient through the root surface into the plant root. Effective diffusion coefficient (D_e), soil buffer power (b), and the $\partial C_l/\partial r$ component describe nutrient movement to the root surface by diffusion. For the $\partial C_l/\partial r$ component, r is the radial distance from the root axis, where r_0 would be the root radius, and C_l is the concentration of the nutrient in the liquid phase. Movement of nutrient to the root surface by mass flow is described by v_0 (water flux into the root) and C_l.

Soil parameters are used to determine the amount of nutrient that is delivered to the root surface. Equation 7 is the effective diffusion coefficient equation (Van Rees et al.,

$$D_e = \frac{D_l \theta_v f}{b} \qquad (7)$$

1990). θ_v represents the volumetric water content. The modified Millington-Quark tortuosity relation (Jury et al., 1991), equation 8, estimates the tortuosity (f) of water movement through

$$\xi(\theta_v) = \frac{\theta_v^{10/3}}{\phi^2} = f \tag{8}$$

the soil. Soil porosity is represented by ϕ. Equation 9 defines the soil buffer power (Van

$$b = \theta_v + \rho_b K_d = \frac{\partial C_t}{\partial C_l} \tag{9}$$

Rees et al., 1990). Soil bulk density is represented by ρ_b. K_d is the kinetic coefficient for the relation $C_s = K_d C_l$ (C_s is the solid-phase nutrient concentration).

Uptake Measurements

The solution-depletion method (Claassen and Barber, 1974) is widely used for determining concentration versus time for uptake calculations. Uptake (net influx) can be calculated by determining the slope between each sampling point, or by the first derivative of the regression of concentration versus time. The depletion method allows observations over a wide concentration range without having nutrient-concentration treatments for kinetic analysis. Bhat (1982) used established apple trees growing in soil, but then the investigators exposed sections of intact roots, immersed the roots in a small vessel containing nutrient solution, and measured the depletion of the nutrient in solution. BassiriRad et al. (1999) developed a similar method that also uses the depletion method for field-grown plants. The method involves carefully excavating fine roots from established plants growing in soil, and measuring the nutrient depletion of a very small amount of solution (2 mL) for an hour or less. They used concentration treatments to develop kinetic parameters. Seeling and

Claassen (1990) developed a method for evaluating uptake kinetics in soil, which is not a depletion method, and also requires concentration treatments. Others have used flowing-solution systems that keep constant-concentration treatments. This approach has been criticized because it may not adequately represent nutrient availability in the rhizosphere (Van Rees, 1994).

Van Rees (1994) discusses several methods for determining K_m, I_{max}, and C_{min} from depletion data by using methods including those of Bhat (1981) and Claassen and Barber (1974). Van Rees (1994) discusses the necessity of full depletion, and warns that depletion data should first be fit with a regression technique so that each successive sampling point is less, before calculating the kinetic variables. This avoids scatter in plots of Michaelis-Menten transforms, and allows for more accurate calculations of C_{min}.

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CHAPTER THREE. NITRATE UPTAKE BY RED MAPLE VARIES WITH ROOT-ZONE TEMPERATURE

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Abstract

Red maple (*Acer rubrum* L.) occurs in a range of habitats over much of the eastern United States and southeastern Canada. This adaptability is one reason why red maple is an important forest tree and landscaping species. Nutrient uptake is a complex process influenced by plant factors, the growing medium, and interactions between the two. Mathematical models allow us to separate the multiple factors involved in nutrient uptake processes, which allows us to examine and isolate factors that might impact uptake. Root-zone temperature response must be quantified for each plant species and nutrient combination, and research has not yet been done on the effect root-zone temperature has on nutrient uptake by red maple. Our objective was to determine the influence of root-zone temperature on nitrate uptake kinetics by using 'Autumn Flame' and 'Franksred' (Red Sunset®) as representatives of red maple. We conducted three experiments. Red maples were grown with root zones at 14, 24, and 34 °C for three, four, and six weeks for experiment one, two, and three, respectively. At the end of the treatment period, we used standard solution-depletion techniques to assess nitrate uptake over a 14-hour period. 'Franksred' responded to root zones at 14 °C with less decrease in root-surface area from the

maxima at 24 °C than 'Autumn Flame', while the reverse trend was true at 34 °C. Cultivar uptake means differed only in experiment one. A linear, concentration-independent estimate of I_{max} dominated uptake below 540 μM. Averaged over both cultivars in all experiments, I_{max} estimates were 120, 150, and 170 nmol m^{-2} s^{-1} for the root-zone treatments 14, 24, 34 °C, respectively. K_m increased with root-zone temperature and had means of 88, 140, and 190 μM, while C_{min} decreased and had means of 66, 38, and 18 μM for the 14, 24, and 34 °C treatments, respectively. We conclude that it is necessary to account for root-zone temperature when estimating nitrate uptake in red maple, and our results suggest that only a single concentration–independent constant for nitrate uptake is necessary for uptake calculations below 540 μM.

Introduction

Root-zone temperature can vary spatially, and can reach extremes in urban landscapes and production of plants in containers (Graves and Dana, 1987; Wong et al., 1971). Root structure and function are temperature dependent (Miller, 1986), and cool root-zone temperatures can lead to shorter, thicker, and whiter roots (Marschner, 1995; Nielson, 1974) that appear to have delayed maturation. Root temperature has a profound effect on respiration, translocation, and transpiration, which makes predicting the effects of root-zone temperature on nutrient uptake difficult. Previous studies show that nutrient acquisition increases with temperature to an optimum, and then declines, but quantifying the root-zone temperature effect on uptake requires a separate investigation for each nutrient and species combination (Barber, 1995).

Red maple is a highly diverse species that is adapted to a wide range of landscape applications. Red maple occurs naturally from southern Canada to the southern United States and varies in growth rate, cold hardiness, and winter preparation (Townsend, 1977). Red maple offers an opportunity to examine intra-species variation and environmental adaptability. 'Autumn Flame' and 'Franksred' differ in their capacity to function at different root-zone temperatures (Wilkins et al., 1995) and degrees of water stress (Zwack and Graves, 1999). 'Autumn Flame' produces shorter and thicker roots than 'Franksred,' while the latter produces longer roots and more root surface area (Kelly et al., 2000). Kelly et al. (2000) also suggested that these cultivars might differ in nutrient uptake due to differences in root-surface area. We used cultivars in this research because it allows us to reduce experimental error, more easily compare our experiments, and compare our research with previous research done with root-zone temperature and nutrient uptake. The evidence presented shows that 'Autumn Flame' and 'Franksred' differ in the key areas of production of root-surface area with root-zone temperature treatments, and nitrate uptake. Therefore, we selected these two cultivars to represent red maple. It is unknown how root-zone temperature affects nitrate acquisition for red maple.

The Barber-Cushman nutrient-uptake model (Barber and Cushman, 1981) is a simplified mathematical model that approximates the complex process of nutrient uptake. Kelly et al. (1992, 1994) discussed the merits of using a computerized version of this model to evaluate uptake responses by tree species. The model uses Michaelis-Menten kinetics to explain uptake at the root surface. While uptake can be Michaelis-Menten-like, nitrate uptake is multi-faceted (Imsande and Touraine, 1994), has at least three uptake mechanisms (Crawford and Glass, 1998), and has shown an inconclusive inter-cultivar relationship in red

maples (Kelly et al., 2000). Researchers hope that by quantifying the nutrient uptake kinetic parameters for the model, they can use the parameter values to estimate the uptake kinetics of plants in any situation. This is possible because the model separates the multiple processes of nutrient uptake, and researchers can use the appropriate environmental and kinetic values for each situation. Without modifications, the model ignores the effect of root-zone temperature on uptake. The objective of this research was to determine the kinetics of net nitrate uptake at three root-zone temperatures (14, 24, and 34 °C) that represent early spring soil conditions, mid-season conditions, and extreme conditions, respectively. We chose 34 °C as our high temperature because 36 °C in the root zone results in much smaller plants (Graves et al., 1989), which would have compromised our ability to make comparisons.

Materials and Methods

Experiment One

Plant preparation. We took green-stem single-node cuttings of 'Franksred' and 'Autumn Flame' from juvenile greenhouse-grown stock plants on May 18, 1999. We rooted these cuttings by using subirrigation (Zhang and Graves, 1995). After four weeks, we moved the cuttings to 1.8-L stainless steel pots that contained Hoagland's #1 nutrient solution (Hoagland and Arnon, 1950) modified by using 0.1 mM EDDHA chelated $Fe(NO_3)_3$ as the iron source. Each pot contained four plants. All nutrient solutions used were adjusted to an initial pH of 5.8. We changed the nutrient solutions once a week throughout the experiment, unless noted. We adjusted nutrient solution strength as noted to maintain vigorous growth. We added deionized water daily to replace water lost to evapotranspiration.

We did not apply temperature treatments immediately to allow the plants to adjust to the hydroponic solution. The first three weeks, we used 25% strength nutrient solution. In the fourth week, we switched the nutrient solution to 50% strength, and the following week, we moved the plants to similar pots that had controlled-temperature nutrient solution.

Experimental design and conditions. The treatment pots consisted of a stainless steel liner and a PVC outer jacket. The gap between liner and jacket was filled with water that circulated to a water bath (RTE 111 and EX 220, Neslab Instruments, Inc., Newington, NH) capable of keeping the whole-system water temperature within 0.1 °C. The pots were aerated with compressed air through tubes submerged in the nutrient solution.

The experimental design consisted of two blocks with two replications completely randomized within each block. Each temperature treatment was applied to both cultivars; this resulted in eight pots at each temperature. We used data logging equipment (CR23X Micrologger, Campbell Scientific, Logan, UT) to track air temperature, relative humidity (CS500 Temperature and Relative Humidity Probe, Cambell Scientific), water temperature (CS Model 107 Temperature Probe, Campbell Scientific), and photosynthetic photon flux (LI190SB Quantum Sensor, LI-COR, Lincoln, NE). The quantum sensors were located 10 cm above the base of the pots.

The day before we recorded uptake measurements for block one, we measured transpiration and photosynthesis (LI6400, LI-COR, Lincoln, NE) on the newest fully expanded leaf. We set photosynthetic photon flux (PPF) at 400 μmol s^{-1} m^{-2} and CO_2 flow at 400 μmol s^{-1} inside the leaf chamber during measurements.

Daily maximum and minimum temperatures averaged 31 and 23 °C in the greenhouse during the treatment period. PPF averaged 250 μmol s^{-1} m^{-2} over the entire

experiment during the daily 16 hr photoperiod. High-pressure sodium lamps provided supplemental irradiance from 0600-2200 HR.

Uptake measurements. A week after we moved the plants to the root-zone temperature treatments, we changed the nutrient solution concentration to 20%. We changed the solutions by block on succeeding days so that each block was exposed to low-nitrate solutions for the same period. We changed the solution to a 10% modified Hoagland's with low nitrogen, 48 h before uptake measurements. The solution contained: 0.25 mM K_2SO_4, 0.5 mM $CaSO_4$, 0.05 mM KH_2PO_4, 0.10 mM $MgSO_4$, 0.01 mM Fe-EDDHA, and 0.1 mL/L Hoagland's micronutient stock (Hoagland and Arnon, 1950). The solution contained 0.03 mM NO_3^- because the iron source contained $Fe(NO_3)_3$. We performed the solution-depletion measurements three weeks after the root-zone temperature treatments began.

The solution used for depletion measurement contained: 170 μM KNO_3, 170 μM $Ca(NO_3)_2$, 170 μM K_2SO_4, 330 μM $CaSO_4$, 100 μM KH_2PO_4, 200 μM $MgSO_4$, 0.1 mL/L Hoagland's micronutrient stock, and 10 μM Fe-EDDHA that contained 30 μM NO_3^-. To avoid thermal shock, we adjusted the nutrient solution to the treatment temperature before we added it to the pots. Uptake measurements for block one began at 0830 HR and ended at 2200 HR, while block two began at 0815 HR and ended at 2200 HR the following day. We used a peristaltic pump (Manostat, Barrington, IL) to take 2-mL samples over intervals of 15-60 min. Peristaltic pumps replenished the pots with deionized water to replace withdrawn solution and evapotranspirated water. The nitrate concentration of the withdrawn solution was analyzed by using an infrared analytical technique (Crumpton et al., 1992).

Harvest. We harvested the plants 12 hr after uptake measurements were completed. We excised the roots, patted them with paper towels to remove excess water, and recorded

the fresh weight. We froze the roots until we determined root length by the line-intersect method (Tennant, 1975). We assumed that root growth was negligible during the uptake-measurement period. We determined mean root surface area by using root length and fresh weight (Mackay and Barber, 1985). We removed the leaf blades from the stem, leaving the petioles on the stem. Leaves were refrigerated until we performed leaf area measurements on a 3100 Area Meter (LI-COR, Lincoln, NE). We dried leaves, stems, and roots at 60 °C, and recorded dry weights.

Data analysis. Significance was set at the $P = 0.05$ level for all tests. We used analysis of variance (ANOVA) to assess treatment effects and to calculate the mean-square error necessary for the least significant difference (LSD) statistic (SAS Institute Inc., 1985). We considered all interactions, but we retained main-effect interactions and only the interactions that were significant. This approach kept non-significant effects as part of experimental error. For comparing means, we used the LSD as described by Steel et al. (1997).

We analyzed uptake kinetics by comparing the regression coefficients of the depletion curves, using pots as experimental units (PROCs ANOVA and REG, SAS Institute Inc., 1985). We used the quadratic model as a preliminary estimation of the presence of Michaelis-Menten kinetics. A t-test with the null hypothesis that the second power coefficient is zero was used to compare the linear model with the linear coefficient of a quadratic model. We transformed the data for use in the Hanes plot (Hanes, 1932) to estimate I_{max} and K_m.

Experiment Two

We performed experiment two like experiment one, with the exceptions listed here. We began propagation of 'Autumn Flame' cuttings on March 30, 2000. The plants were moved to aerated 20% Hoagland's solution in 1.8-L pots on April 29. The nutrient solution contained 90 mg/L of 2-(4-Morpholino)-Ethane Sulfonic Acid (MES) as a pH buffer. Each pot had three plants. This nutrient solution was replaced on Mondays and Thursdays each week until uptake preparations began. We started the temperature treatments on May 25, 2000.

High-pressure sodium lamps provided supplemental irradiance from 0700-2300 HR. PPF averaged 209 μmol s^{-1} m^{-2} over the daily 16-hr photoperiod. Daily mean minima and maxima were 23 and 27 °C in the greenhouse.

Uptake preparations began with staggered solution changes. We changed blocks one, two, and three on June 24, 25, and 26, respectively. The nutrient solution was changed to 10% Hoagland's with low nitrate. We measured nitrate uptake for block one, two, and three on June 26, 27, and 28, respectively. We harvested the plants on June 27, 28, and 29 for block one, two, and three, respectively.

Root-surface area was estimated from the relationship of fresh weight and root-surface area from experiment one. We did not measure dry weights or leaf surface area.

We measured photosynthesis and transpiration on June 5, 2000. We measured dissolved oxygen (YSI 55 Dissolved Oxygen meter, Yellow Springs Instrument Co., Yellow Springs, OH) in the nutrient solution of each pot on June 5. The oxygen sensor was immersed in each pot and allowed to equilibrate for about 30-60 s until a stable reading was recorded. The sensor self-calibrated for each temperature.

Experiment Three

We performed experiment three like experiment two, with the exceptions listed here. 'Autumn Flame' and 'Franksred' were propagated in tissue culture and transplanted to 200-mL clay pots (RP-025 Ceramo Company, Jackson, MO) in a greenhouse potting mix (by volume: 40% peat, 40% perlite, 20% top soil) on May 11, 2000. We transferred them to a nutrient solution on June 30. The plants were subjected to root-zone temperature treatments immediately. Pots were smaller, 1.1 L, but otherwise similar to those used in experiments one and two. Each pot contained two plants. We determined root radius and surface area by using the methods in experiment one.

Uptake measurements were performed with solution that contained 290 μM nitrate. Staggered solution changes, uptake measurements, and plant harvests began, by block, on August 12, 14, and 15, respectively. We measured nitrate uptake on July 31 to evaluate the rate of uptake by the red maples. All blocks were exposed to the low-nitrate nutrient solution for 48 hr. After July 31, all the blocks were placed back into the maintenance solution until uptake preparations began on August 12.

We measured photosynthesis and transpiration on August 14. PPF averaged 265 $\mu mol\ s^{-1}\ m^{-2}$ during the entire experiment over the daily 16-hr photoperiod. Daily mean minima and maxima were 23 and 34 °C.

Analysis of uptake differed from experiments one and two, after attempts at using the Hanes (1932) plot failed. We linked a numerical integrator to a non-linear estimator (PROC NLIN, SAS Institute, Inc., 1985). In this method, the NLIN procedure estimates the Michaelis-Menten parameters K_m, I_{max}, and C_{min} and model parameter C_o (initial

concentration), while it integrates the least-squares curve of concentration over time. We bound all parameters to be larger than zero, but bound K_m to be larger than C_{min} and smaller than the concentration at time zero. This gave us the least-squares fit to our depletion data for each pot. Standard errors of the parameter estimates differed considerably between pots, so we used a weighted ANOVA to compare means. We used $(1/\sqrt{se})$ as the weight in our ANOVA test (PROC GLM, SAS Institute, Inc., 1985). To separate the means, we used the probabilities generated by the GLM procedure to test the hypothesis that each pair of treatment means were equal. We performed linear regression on the first 3 hr of depletion data for comparison to the NLIN procedure.

Results

Experiment One

Plant measurements. 'Franksred' produced longer roots with more root surface area, but 'Autumn Flame' roots were thicker (Table 1). Roots at 14 °C had a larger radius, compared to roots grown at 24 and 34 °C (Table 1). Root length was not affected by temperature (Table 1). Pot means of root fresh weight differed only between 14 °C and 34 °C treatments (Table 1). Total plant dry weights were not different between treatments, and ranged from 22.7 g to 46.4 g per pot. No temperature-by-cultivar interactions occurred. Leaf area was unaffected by treatments and ranged from 0.254 to 0.451 m^2. Photosynthesis did not differ by cultivar, but increased as root-zone temperature increased; 14, 24, and 34 °C,

and had means of 4.0, 5.8, and 7.1 μmol m^{-2} s^{-1}, respectively. Transpiration was not different for any temperature treatment, or between cultivars.

Uptake. Total net nitrate uptake was greatest at 24 °C, while uptake at 34 °C was higher than at 14 °C (Table 2). Considering uptake on a root-surface-area basis, the 24 and 34 °C treatments were not different, but were higher than at 14 °C (Table 2). 'Franksred' exceeded 'Autumn Flame' for total net nitrate uptake, but the two cultivars were equivalent for uptake per unit root surface area (Table 2). No temperature-by-cultivar interactions were observed. The maples removed nitrate from the solution to analytical limits, <0.007 mM, within 24 h.

A linear model provided the best fit for the nitrate-depletion data. When we divided the total net uptake by the unit root surface area to perform a *t*-test, the p value of the test between a quadratic and linear model was 0.063. While the R^2 values for the quadratic model were slightly higher, ten of the 24 pots had a negative second-order coefficient. If plants depleted pots to analytical limits or C_{min} before the uptake period was over, we dropped the data after that point to avoid an artificial quadratic relationship.

Experiment Two

Dissolved oxygen decreased as root-zone temperature increased, and had means of 9.27, 6.86, and 5.51 mg/L ($LSD_{(0.05)} = 0.371$) for the 14, 24, and 34 °C treatments, respectively. Oxygen levels maintained a steady state for each pot during the measurement period.

Root-surface area and fresh weight were not different (Table 1). Photosynthesis did not differ by treatment and averaged 5.9 μmol m^{-2} s^{-1}. Transpiration averaged 2.1, 3.4, and

3.4 mmol m^{-2} s^{-1} for the 14, 24, and 34 °C treatments, respectively, and only the plants at 14 °C differed ($LSD_{(0.05)}$ = 1.1 mmol m^{-2} s^{-1}).

R-square values of the depletion data regression averaged 0.89 and 0.85 for quadratic and linear, respectively, and the t-test showed significance between the two models, but the data were more variable over time than in experiment one. Five of 24 pots had negative second-order coefficients that made uptake highest at the lowest concentrations. Hanes transform resulted in unacceptable estimates of K_m and I_{max}. We chose the linear model of uptake, as in experiment one. Total uptake was highest at 24 and 34 °C (Table 2). Uptake on a root-surface area basis was higher at 34 than 14 °C (Table 2).

Experiment Three

'Franksred' produced longer roots than 'Autumn Flame' (Table 1). A general trend was that 'Franksred' produced larger plants than 'Autumn Flame,' although root fresh weights (Table 1), root-surface area (Table 1), and dry weights trended this way, only the root-surface area interaction is statistically different between cultivars. Plants at 24 °C had the highest root surface area, length, and fresh weight, but 14 and 34 °C were not different (Table 1). Plants at 24 °C produced the most dry weight with a mean of 10.5 g, while 14 and 34 °C were not different with means of 5.0 and 5.9 ($LSD_{(0.05)}$ = 1.9 g), respectively. The same dry weight effect occured when the plant was divided into roots, leaves and stem. Root radius was not different between cultivars or root-zone temperatures (Table 1). 'Franksred' had a higher leaf surface area mean of 0.109 m^2, while 'Autumn Flame' averaged 0.085 m^2 ($LSD_{(0.05)}$ = 0.0205 m^2). Leaf surface area had means of 0.0736, 0.147, and 0.0696 m^2 for the

treatments 14, 24, and 34 °C, respectively, but 14 and 34 °C treatments were not different ($LSD_{(0.05)} = 0.0251$ m^2).

Cultivar-by-temperature interactions showed that 'Franksred' deviated less from optimum growth at lower temperatures, but deviated more from maximum growth at higher temperatures than 'Autumn Flame.' For 'Franksred,' root-surface area declined 37% from 24 to 14 °C, but declined 53% from 24 to 34 °C, while 'Autumn Flame' declined 48% and 24%, respectively. Leaf-surface area and root length showed similar trends.

Photosynthetic rate was not different between cultivars. Photosynthetic rate for 14, 24, and 34 °C averaged 7.0, 5.1, and 9.5 $\mu mol\ m^{-2}\ s^{-1}$ ($LSD_{(0.05)} = 1.7\ \mu mol\ m^{-2}\ s^{-1}$) 'Franksred' transpired 5.7 $mmol\ m^{-2}\ s^{-1}$, while 'Autumn Flame' averaged 4.2 $mmol\ m^{-2}\ s^{-1}$ ($LSD_{(0.05)} = 1.0$ $mmol\ m^{-2}\ s^{-1}$). Root-zone temperature did not affect transpiration.

Uptake. Cultivar means did not differ for maximum total net nitrate uptake (Table 2), or for any of the kinetic parameter estimates (Table 3), but there were interactions for the K_m estimates. K_m estimates for plants at 34 °C increased from 24 °C for 'Autumn Flame', but decreased for 'Franksred.' Maximum total uptake was lowest at 14 °C, while maximum uptake at 24 and 34 °C did not differ (Table 2). Plants at 34 °C produced the highest I_{max} estimates, while plants at 14 and 24 °C were not different (Table 3). For the K_m parameter, only plants at 14 and 34 °C means were different (Table 3). The increase in K_m at 34 °C was due to the increase in 'Autumn Flame.' A strong decreasing trend existed for C_{min} as root-zone temperature increases (Table 3), but 24 and 34 °C were not different ($P = 0.09$). While the trend was the same for both cultivars, 'Autumn Flame' appeared to show a more dramatic drop in C_{min} at 34 °C. When we inserted C_o, and our estimates of C_{min}, K_m, and I_{max} into the

modified Michaelis-Menten equation, the uptake estimates ranged from 44 to 95% of the estimated I_{max}, but averaged 66%. Linear estimates over the first three hours of uptake measurement averaged 70% of I_{max}.

Discussion

This research is consistent with evidence that these cultivars differ in their response to stress (Wilkins et al., 1995; Zwack and Graves, 1999). Experiment three showed that 'Franksred' had a lower optimum root-zone temperature for root growth than 'Autumn Flame'. This supports the diversity of the red maple species and our use of these cultivars as represent of red maple.

In a study conducted at ambient temperatures, Kelly et al. (2000) speculated that 'Autumn Flame' has a higher uptake rate per unit area of root surface (I_{max}). They found that total uptake was equivalent for 'Autumn Flame' and 'Franksred', although 'Autumn Flame' produced less root-surface area. We found similar differences in root surface area between the two cultivars (Table 1) as observed by Kelly et al. (2000). Both root-radius and root-length responses were similar to those reported by Kelly et al. (2000). However, the nitrate uptake rates per unit root surface area we found did not differ (Table 1). Only results from experiment one contrast Kelly et al. (2000) in that 'Franksred' removed more total nitrate from solution than 'Autumn Flame.'

The real interest in this experiment was the effect of temperature on all the kinetic parameters, including K_m and C_{min}. Unfortunately, only experiment three yielded these, but they yield a general trend that the uptake system becomes stronger with increasing

temperature (Fig.1). The increase in K_m might be an artifact of our method because the increase in I_{max} is larger in magnitude between 24 °C and 34 °C than the accompanying decrease in C_{min} (Table 3). At 14 °C, low uptake might have been a simple metabolic rate effect, and was not because of a lack of root surface area. Treatment means confirm the pattern that total nutrient uptake increases to an optimum root-zone temperature, then declines (Barber, 1995) (Fig. 1). The regression quadratic of the maximum total uptake values for all experiments peaked at 26 °C (Fig.1). I_{max} continued to increase in an almost linear fashion (Fig. 1). Previous work shows that 36 °C is a critical root-zone temperature for red maple (Graves et al., 1989), so we might expect a large drop in I_{max} near 36 °C. Differences in the root morphology between root-zone temperatures appeared typical of other species (Marschner, 1995; Nielson, 1974), where the 14 °C treatment had whiter roots and the 34 °C were noticeably darker and spindlier, but only in experiment one were roots at 14 °C thicker (Table 1). The 34 °C treatment had reduced root surface that might be attributable to accelerated maturation, which occurs when roots are grown at high root-zone temperature (Marschner, 1995).

In experiments one and two, where the maples had more time to grow in and adjust to the hydroponic nutrient solution, above ground plant material appeared healthy and could not be distinguished by root-zone temperature. However, in experiment three where plants were exposed to temperature treatments immediately, chlorosis was prevalent in the 14 and 34 °C treatments, which is shown by the large discrepancy in dry weights from plants grown either at 14 or 34 °C treatments, and the larger plants grown at 24 °C. This provided an emphasis for the importance of root-zone temperature on younger, less-established plants.

We chose the linear model of nitrate depletion for experiments one and two because the fit of a second-order polynomial did not fit all the pots with the assumptions of Michaelis-Menten kinetics. The ten pots in experiment one and five in experiment two that had the highest rate at the lowest concentrations (negative second-order coefficients) represented all treatments, and this pattern of uptake violated the assumptions of Michaelis-Menten kinetics. For experiment one, we originally tried to fit the Hanes transform (Hanes, 1932), but this resulted in I_{max} and K_m values with opposite signs, which is another violation of Michaelis-Menten kinetics and helps confirm our decision to drop the quadratic model as the best fit. The Hanes plot method of determination failed for all three experiments.

The concentrations where uptake is linear for some nutrients was reported to be over 1000 μM (Marschner, 1995), however, Crawford and Glass (1998) report the low affinity transport system (LATS) has a linear response, and is a heavy contributor to nitrate uptake at concentrations above 250 μM in some model systems. However, the LATS linear relationship refers to a linear relationship between concentration and uptake rate, while our results show a constant uptake rate independent of concentration (Table 2).

It is likely that the linear relationship observed in experiments one and two represented the I_{max} of the high affinity transport system (HATS) that dominated uptake during the measuring period. This is logical because we started the uptake measurements in the concentration range between the disputed LATS and HATS transition area, where the HATS would be essentially I_{max}. Crawford and Glass (1998) report that IHATS values of K_m and I_{max} range from 20-100 μM and 3-8 μmols g^{-1} h^{-1}, respectively (Crawford and Glass, 1998). Min et al. (2000) reported I_{max} values for nitrate IHATS for a deciduous species, trembling aspen (*Populus tremuloides* Michx.), of 3.00 μmol g^{-1} h^{-1}. If we divide our

maximum uptake at 24 °C by root fresh weight as Min et al. (2000), and convert from seconds to hours, we find 2.7, 1.4, and 4.5 μmol g^{-1} h^{-1}, for experiments one, two, and three, respectively. The I_{max} values for experiments one and two are all 1-3 μmols g^{-1} h^{-1}, which falls short of the estimates of I_{max} reported by Crawford and Glass (1998). However, experiment three averages were 3.7, 4.5, and 6.2 μmols g^{-1} h^{-1} for the root-zone treatments of 14, 24, and 34 °C, respecitively. This shows that our experiment three I_{max} estimates are in agreement with estimates of I_{max} from a variety of species reviewed by Crawford and Glass (1998). Plants in the other two experiments did not show as much demand for nitrate. The differences in uptake for each experiment probably result from red maple attempting to maintain a consistent internal pool of nitrate (Imsande and Touraine, 1994), despite root surface area differences, and adjusting I_{max} to meet the needs of the plant.

The remainder of the HATS kinetics in experiments one and two was not observed because plants in some of the pots did not deplete the measurement solution to a low enough concentration to show Michaelis-Menten-like kinetics, and the HATS K_m value has been reported as low as 20 μM (Crawford and Glass, 1998). However, our results from experiment three showed larger estimates for red maple (Table 3). Furthermore, fewer measurements taken later in the uptake period gave lower resolution and extra weight in regression calculations to earlier measurements taken at closer intervals. Regardless, the dominance of the linear pattern of uptake (Fig. 2) might justify the use of a single constant value to estimate uptake at a given root-zone temperature below 540 μM. Even experiment three, where there was clear evidence of Michaelis-Menten kinetics, the linear relationship over all the data has an average R^2 of 0.85. It is unlikely that concentrations low enough to induce a dramatic

decrease in uptake occur in fertilized environments, and research at two different forest sites revealed soil solution nitrate concentrations above those investigated here until their measurement in October (Kelly and Mays, 1999). Further work needs to address the LATS of nitrate uptake for red maple.

In experiment three, the variances for our kinetic parameter estimates were unequal for each pot, so we used a weighted ANOVA. The standard weight for analysis is ($1/se^2$) (Hedges et al., 1999). After looking at these standard weights for experiment three, most of the weight was in a few pots. We decided it was necessary to use a weight that still emphasized the more precise estimates, but represented estimates from all pots. We decided on ($1/\sqrt{se}$) as the best alternative.

The regression technique we used for experiment three resulted in I_{max} estimates that occur at greater concentrations than we investigated. This resulted in I_{max} values higher than we could estimate for experiments one and two. Even the estimates from experiment three linear regression (over the first three hours) fall short of the I_{max} estimates, but they are in close agreement with the influx estimated at time zero (when concentration is C_0).

We accepted that IHATS dominates uptake over the concentrations investigated for experiments one and two, because we did not find an alternative. Experiments one and two do not exhibit the characteristic Michaelis-Menten curve, and do not lend themselves to the method that we used to determine the kinetics for experiment three. However, attempts at confining linear regression estimates of I_{max} only to the first three hours of sampling provided estimates with very high treatment standard errors. We attempted the Hanes transform for experiment three, but like experiment one, we did not get acceptable estimates of the parameters. This was because of the unevenness the raw depletion data create (Fig. 2), and

was the reason we had to use a least squares estimate of concentration in the determination of the kinetic parameters. Van Rees (1994) warned about the problems of getting good kinetic estimates without curve fitting the depletion data. This unevenness is evident from all experiments, and could arise from several sources. This prevented us from using Michaelis-Menten transforms, sliding parabolas, or splines (Claassen and Barber, 1974; DuChateau, 1972). One possibility for error was the variability in the length of time from when we sampled, to when each sample was analyzed in the lab. All samples taken at the same time were sampled within a few minutes, but each sample time was not analyzed consecutively. Consecutive series might have been analyzed minutes or days apart, and the resulting small fluctuations could have caused some of the unevenness in our depletion data. Standard curve-fitting methods would be too sensitive to these time-dependent changes in samples, so we must be more aware of this problem for future uptake measurements.

We hypothesized, after experiment one, that transpiration optimized nitrate uptake by aiding mass flow delivery to the root, even in a stirred solution. It appeared that transpiration had an optimum root-zone temperature close to that of maximum net total uptake. This was not the case in the other two experiments. In experiment three, while the means were not different, it appears the transpiration increases with root-zone temperature. This hypothesis would be better tested using plants grown in a soil-like medium, where transpiration may play a bigger role in mass flow than in a stirred nutrient solution.

The fact that all three experiments differ is both a bane and a blessing. The first two experiments started with 4-week old rooted cuttings, but experiment two was started earlier in the growing season, in fact, early enough to run experiment three in the same year, where experiment three was run at essentially the same time of year as experiment one. In an

attempt to get the plants large enough to deplete the pots to C_{min} within one photoperiod (16 hr), we let the plants grow for 4 weeks without treatments in the first and second experiment, and we had the plants spend an extra week in the treatments in the second experiment. However, plant age and growth flushes have an affect on nutrient uptake (Marschner, 1995), and Kronzucker et al. (1995) show that older roots have reduced nitrate uptake rates. Even though we did not measure the age distribution of the roots in the pot, the better way to achieve depletion to C_{min} is to use a smaller volume of nutrient solution, which we did in experiment three. However, the advantage of having experiments differ is that it shows the range of kinetics for red maple. Kelly et al. (1992) point out the danger of quantifying kinetic parameters under one set of conditions and applying them in another. Since we did this work assuming use in a nutrient uptake model, having multiple conditions for quantifying the model parameters is better than a single set of conditions.

Nitrate uptake is a temperature-dependent process, and the extrapolated maximum total uptake is highest for root zones at 26 °C in red maple. Red maple increased maximum total uptake until the optimum, but lack of root-surface area reduced uptake thereafter, although I_{max} increased over the entire investigated range of 14-34 °C (Table 1). While both cultivars seemed to respond similarly to the treatments, cooler temperatures affected 'Autumn Flame' root growth more, while higher root-zone temperatures affected 'Franksred' root growth more. Much like Kelly et al. (2000), our observations in this study show that nitrate uptake by red maple is variable and puts into question whether or not the kinetic parameters are necessary for modeling nutrient uptake under field conditions. Research examining nitrate uptake at higher concentrations is necessary to clarify this. This would

allow researchers using models to use a single concentration-independent constant below 540 μM instead of Michaelis-Menten parameters.

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Table1. Root parameter means for red maple grown in Hoagland's nutrient solution with three root-zone temperature treatments. Means for root-zone treatments are pooled across cultivars, and cultivar means are pooled across treatments. Means for experiment one, two, and three are composites of four, three, and two plants, respectively. Root length and mean radius were not determined for experiment two.

Treatment	Length (m)		Mean Radius (μm)		Surface Area (m^2)			Fresh Weight (g)		
	Experiment									
	1	3	1	3	1	2	3	1	2	3
Root zone (°C)										
14	75.0	14.8	391	371	0.182	0.170	0.034	35.6	32.6	6.4
24	79.2	26.8	365	354	0.178	0.186	0.058	32.1	33.5	10.4
34	66.1	15.3	364	390	0.150	0.175	0.035	27.1	31.3	6.8
$LSD_{(.05)}$	16.1	5.6	23	100	0.035	0.047	0.011	8.2	8.6	2.3
Cultivar										
Autumn Flame	62.9	16.2	397	374	0.156	0.177	0.038	31.0	32.5	7.2
Franksred	84.0	21.7	350	369	0.184	-	0.047	32.3	-	8.5
$LSD_{(.05)}$	13.1	4.5	18.0	60	0.029	-	0.009	19.7	-	1.9

Table 2. Net nitrate uptake means for red maple at different root-zone temperatures. Experiments one and three used equal amounts of 'Autumn Flame' and 'Franksred', while experiment two used only 'Autumn Flame.' Experiments one, two, and three had four, three, and two plants per pot, respectively. Means are on a per-pot basis. Experiments one and two did not deplete the pots to a low-enough concentration to show Michaelis-Menten kinetics, and therefore, we used linear regression to estimate I_{max} for the nitrate high affinity transport mechanism in red maple. Weighted analysis for experiment three prohibits the use of a LSD. Individual t-tests ($P = 0.05$) were used to separate means in experiment three, and means followed by the same letter are not different. Means for root-zone treatments are pooled across cultivars, and cultivar means are pooled across treatments.

	Maximum total net nitrate uptake ($nmol\ s^{-1}$)			Maximum net nitrate uptake/root surface area (I_{max}) ($nmol\ m^{-2}\ s^{-1}$)		
	Experiment					
	1	2	3	1	2	3
Root zone (°C)						
14	17.5	10.4	6.5 [a]	97	61.2	195 [a]
24	24.4	13.0	13.1 [b]	139	72.5	232 [a]
34	19.0	13.1	11.8 [b]	128	77.2	318 [b]
$LSD_{(0.05)}$	1.5	2.2	-	16	15.0	-
Cultivar						
Autumn Flame	19.0	12.2	10.2 [a]	122	70.3	260 [a]
Franksred	21.6	-	10.7 [a]	121	-	237 [a]
$LSD_{(0.05)}$	1.2	-	-	13.2	-	-

Table 3. Nitrate uptake kinetic parameters for red maple, derived from experiment three. Only experiment three produced K_m and C_{min} values. Micropropagated red maple plants were grown in 20% Hoagland's solution and uptake measured by standard depletion techniques on pots that contained two plants. Six pots contained each cultivar at each root-zone temperature, for a total of 36 pots. Means are the result of a weighted ANOVA procedure after calculating each parameter on a per-pot basis using a non-linear estimation procedure. Means for root-zone treatments are pooled across cultivars, and cultivar means are pooled across treatments. Individual t-test with alpha equal to 0.05 were used to separate means. Values within the same column followed by the same letter are not different.

Treatment	I_{max} (nmol m^{-2} s^{-1})	K_m (µmol)	C_{min} (µmol)
Root zone (°C)			
14	195 [a]	90 [a]	66 [a]
24	232 [a]	139 [ab]	38 [b]
34	318 [b]	189 [b]	18 [bc]
Cultivar			
Autumn Flame	260 [a]	158 [a]	37 [a]
Franksred	237 [a]	120 [a]	44 [a]

Fig 1. Temperature dependence of nitrate uptake by red maple grown in Hoagland's nutrient solution with the root zone maintained at either 14, 24, or 34 °C. The quadratic regressions lines are derived from the results of three experiments. The first experiment used 4-week old single-node cuttings of both 'Autumn Flame' and 'Franksred', and the plants were allowed to grow 4 weeks in the nutrient solution before they spent three weeks in the root-zone treatments. The second experiment differed because we only used 'Autumn Flame', and the plants spent 4 weeks in the treatments. The third experiment used both cultivars, the plants were immediately exposed to the root-zone treatments and spent six weeks in them. Each pot contained 4, 3, and 2 plants for experiments 1, 2, and 3 respectively. Uptake measurements started with nutrient solutions of 540 μM in 1.8-L pots for experiments one and two, while experiment three started with 290 μM nitrate in 1.1-L pots. Not all uptake means were different (Table 2). Maximum uptake reveals the standard response curve to uptake when root-zone temperatures change. I_{max} reveals that the uptake system per unit of root surface gains capacity as root-zone temperature increases. The decrease in root surface at higher temperatures decreases maximum uptake.

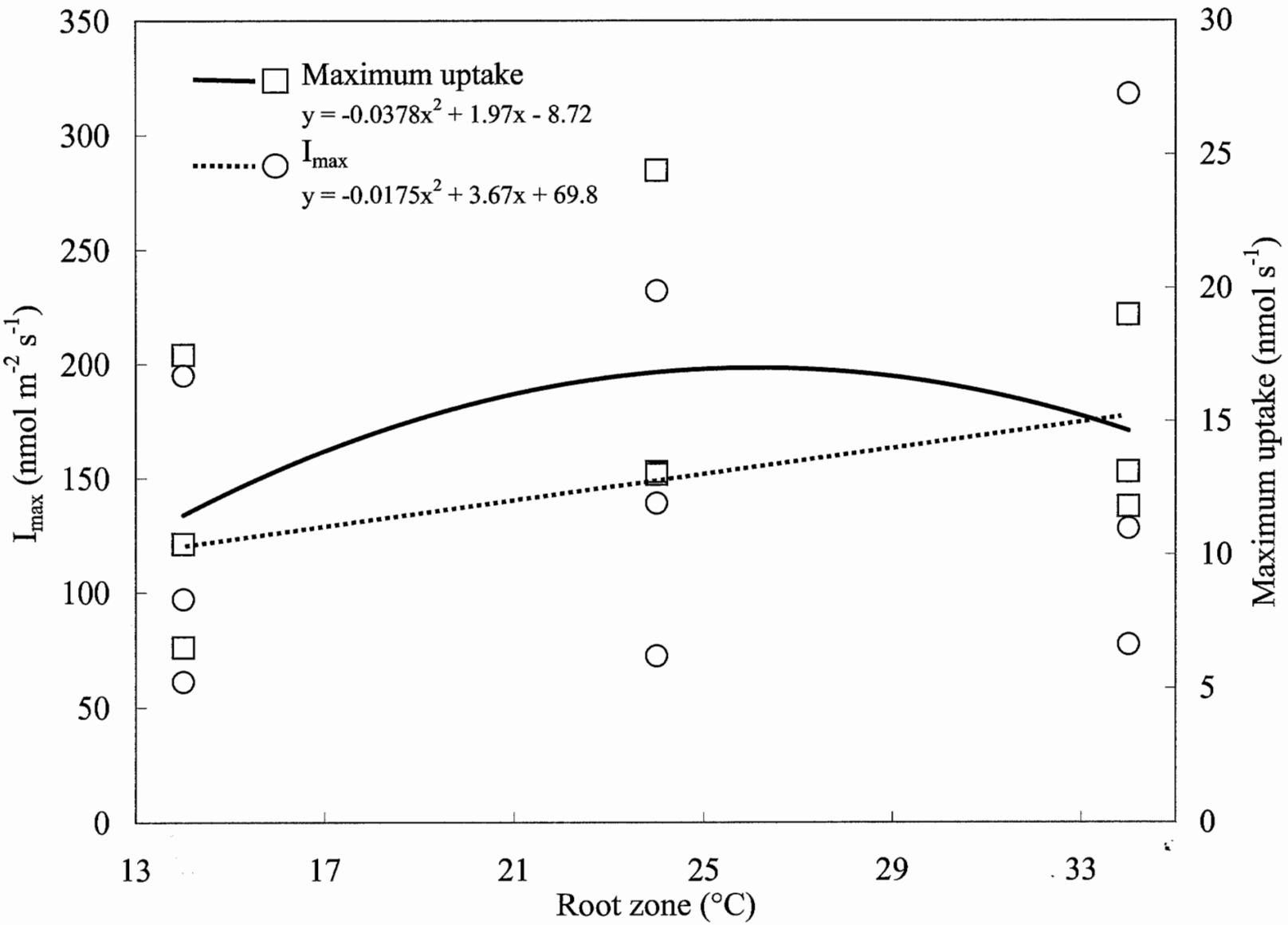
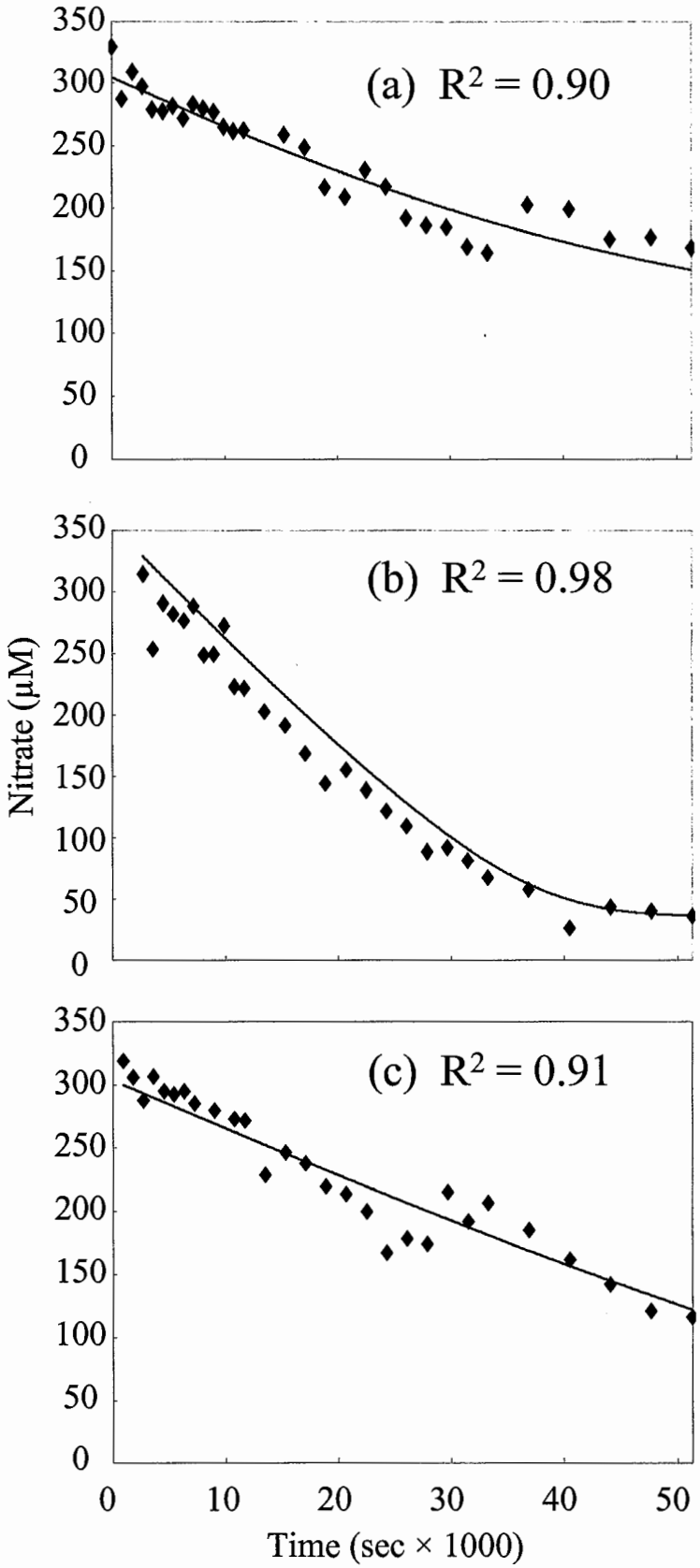

Maximum uptake
y = -0.0378x2 + 1.97x - 8.72
Imax
y = -0.0175x2 + 3.67x + 69.8
Imax (nmol m-2 s-1)
Maximum uptake (nmol s-1)
Root zone (°C)
350
300
250
200
150
100
50
0
30
25
20
15
10
5
0
13
17
21
25
29
33

Fig. 2. Nitrate depletion in 1.1-L pots by red maple grown in nutrient solution from experiment 3. Pots with 'Autumn Flame' and root-zones at 14 (a), 24 (b), and 34 (c) °C treatments are represented in the graphs. However, both cultivars and all temperatures had depletion patterns that looked similar to each of these examples. The fit is predicted by the Michaelis-Menten parameters we estimated by non-linear regression. Even (c), with the most curvature, has a linear component (I_{max}) that dominates the IHATS component of nitrate uptake over the concentration range investigated.


(a) $R^2 = 0.90$
(b) $R^2 = 0.98$
(c) $R^2 = 0.91$
Nitrate (μM)
Time (sec × 1000)
0
50
100
150
200
250
300
350
0
10
20
30
40
50

CHAPTER FOUR. DETERMINATION OF NITRATE UPTAKE KINETICS BY USING A SOIL METHOD

Introduction

The goal of this study was to determine if the method developed by Seeling and Claassen (1990) could be adapted for determining the kinetics of nitrate uptake by woody species in the greenhouse. I will compare nitrate uptake kinetics derived by soil method with the solution-depletion method by using red maple (*Acer rubrum* L.).

Seeling and Claassen's (1990) method differs from the standardized solution-depletion study in several ways. It allows researchers to examine nutrient uptake kinetics of plants growing in soil by using model equations to estimate the concentration of the nutrient at the root surface (Seeling and Claassen, 1990). In the solution-depletion method, we immerse the roots in a stirred nutrient solution, and we assume that the concentration at the root surface is equivalent to the concentration of the nutrient in the bulk solution. This necessitates that we take into account the zone of influence of the average root in the soil method (Yanai, 1994). The soil method allows researchers to determine uptake over several weeks or months, compared to the solution depletion method that determines uptake over several hours.

This method should expose greater differences in nitrate uptake based on root-zone temperature than plants grown in nutrient solution. Soil-like media is a more confining environment than solution culture, because it introduces pore spaces that restrict availability of nutrients and restricts fluid movement and exchange. The soil method determines the

uptake kinetics over longer time intervals, making it valuable to examiners looking at seasonal variation. Increasing temperature dramatically decreased solution-phase nitrate over 24 hr in a laboratory study using samples of the same soil (Kelly, 1993), but in the solution-depletion study, all the nitrate is solution-phase all the time, unless exchange with the root takes place.

Potassium is delivered to the root mostly by diffusion (Barber, 1995), but nitrate is delivered to the root mostly by mass flow. Knowing this, Seeling and Claassen (1990) used an equation that only has the diffusion component of delivery. Yanai (1994) described equations that account for both mass flow and diffusion, which will better describe nitrate delivery to the roots than the equation used by Seeling and Claassen (1990).

Nitrogen undergoes chemical reactions in the soil that inter-converts nitrogen between mineral (NH_4^+, NO_2^-, and NO_3^-), immobilized, and gaseous forms. These conversions are harder to control than in the solution-depletion method, and they are hard to monitor. We cannot neglect ammonium in a soil because soil organic matter degradation produces ammonium, and we know that red maple show a preference for ammonium versus nitrate (BassiriRad et al., 1999; M L Adam unpublished data). Because of the complications of using a real soil, we used a silica sand initially devoid of organic matter, and supplied nitrogen from a nitrate-only source.

Materials and Methods

Cuttings were taken as in the solution-depletion study (Chapter Three), except that they were taken on July 1, 1999, and we used only 'Autumn Flame' in this experiment. The

rooted cuttings were 11 weeks old when we placed them in the controlled-temperature vessels described in Chapter Two. Instead of nutrient solution, the growing medium consisted of silica sand (Granusil® 4030, Unimin Corp., Ottawa, MN) mixed with 1.89 g of 12-0-42 polymer-coated potassium nitrate (POLYON® Pursell Technologies, Inc., Sylacauga, AL). According to the manufacturer, this fertilizer has a constant rate of release over a wide range of soil water contents, and increasing temperature accelerates release of nitrate. We measured a bulk density of 1.7 kg L^{-1} for this mix, and used this measurement in all calculations. The pots were filled each day with deionized water to field capacity, and received 50 mL of 100% strength Hoagland's solution with no nitrogen (Hoagland and Arnon 1950) once a week. All pots were covered with lids made from 2.5-cm thick foam building insulation, to reduce heat transfer and water loss between the sand surface and the surrounding environment.

On September 14, we randomly harvested ten plants as representatives of initial plant size and nutrient status. We had 18 pots, for each of the three root-zone temperatures 14, 24, or 34 °C. We planted one 'Autumn Flame' in each of 48 pots. Two additional pots at each temperature did not have plants in them, but were used to monitor evapotranspiration. We harvested 24 plants on October 5. Six more were harvested three weeks later. Additionally, we added 0.94 g of the controlled-release potassium nitrate to six pots, leached six other pots with one pour volume, and filled the rest of the pots to field capacity. We extended the experiment for an additional three weeks, and then harvested the remaining 18 plants. We dried plants at 67 °C for at least 72 hr and ground them through a 1-mm mesh screen in a Wiley mill. Total plant nitrogen was determined using a modified micro-Kjeldahl approach

(Nelson and Sommers, 1980; Jones, 1991) in conjunction with a nitroprusside-salicylate assay (Wall et al., 1975) by using flow injection analysis (Smith and Scott, 1990).

The sand medium was subsampled to determine solid-phase nitrate, solution-phase nitrate, phosphorous, and pH. We sampled solution-phase nitrate and ammonium by adding water to a sand column to field capactity, equilibrating for 24 hr, and eluting 15mL of solution, as described by Adams (1974). Solid-phase nitrate was determined by 2M KCl extraction (Mulvaney, 1996). The withdrawn solution was analyzed by using an infrared analytical technique (Crumpton et al., 1992).

Data analysis

We derived an equation (Equation 1) for mass flow delivery of nitrate to the root,

$$In = C_l v_0 \tag{1}$$

which neglects diffusion. The equation hypothesizes that influx (I_n) should equal solution-phase nitrate (or other nutrient) concentration (C_l) multiplied by the radial water flux toward the root at the root surface (v_o). We derived the equation from Yanai (1994) that describes nutrient delivery by mass flow and diffusion. This derived equation is essentially equation 5.7 from Barber (1995) without the diffusion component. Equation 1 requires the assumption that nitrate concentration is uniform in the pot, much like plants grown in nutrient solution. We also assume that the roots are equally distributed throughout the pots.

We used equation four from Seeling and Claasen (1990) to calculate influx, but modified the equation (Equation 2) by replacing the root length variable with root surface-

$$In = \frac{(U_f - U_i)(\ln RSA_f - \ln RSA_i)}{(RSA_f - RSA_i)(t_f - t_i)} \tag{2}$$

area (*RSA*). We measured nitrogen content of the plant (*U*), and time (*t*) at the beginning (*i*) and the end (*f*) of the three-week time intervals used. We transformed this influx estimate and average solution concentration of nitrate for use in the Hanes plot (Hanes, 1932) that produces I_{max} and K_m estimates after linear regression (PROCs ANOVA and REG, SAS Institute Inc., 1985).

Significance was set at the $P = 0.05$ level for all tests. We used analysis of variance (ANOVA) to assess treatment effects and to calculate the mean-square error necessary for the least significant difference (LSD) statistic (SAS Institute Inc., 1985). We considered all interactions, but we retained main-effect interactions and only the interactions that were significant. This approach keeps non-significant effects as part of experimental error. For comparing means, we used the LSD as described by Steel et al. (1997).

Results

Calculated I_{max} values for 14 and 24 °C treatments are negative, while for 34 °C it is positive (Table 1). K_m values were negative for 14 and 34 °C treatments, while 24 °C was positive (Table 1). Calculated water flux into the root was highest for 34 °C, while the 14 and 24 °C treatment were not different.

The sand-medium pH ranged from 6.79 to 9.29, and treatment means were 7.8, 7.8, and 8.2 for the root-zone temperatures 14, 24, 34 °C, respectively. Phosphate averaged 37 μM in each pot over all treatments. Ammonium averaged 13 and 9 μM for solid phase and solution phase, respectively.

Equation 1 predictions of influx were different from those calculated by Equation 2 that used the whole-plant nitrogen data.

Discussion

Our attempt to derive I_{max} and K_m values was complicated by a lack of fit to the Hanes transform, much like what happened in the solution-depletion determination (Chapter Three), and confirmed by the low R^2 values obtained from regression. Plots of the data for concentration versus influx show an erratic pattern. Because we lacked enough concentration treatments, we disregarded the concentration treatments and produced concentration versus uptake on a per pot basis over the temperature treatments. This was also unsuccessful. It is unfathomable to have K_m values that are negative, and the milli-molar concentrations for K_m (Table 1) are an order of magnitude higher than those we reported in Chapter Three and at least three times higher than the largest K_m values reported by Kelly et al. (2000) for red maple.

While I think that this method shows promise, several problems must be corrected before it can be used. A suitable buffer must be found that does not provide a large amount of carbon, or microorganism immobilization of nitrate might have to be accounted for. A phosphate buffer of 1 mM KH_2PO_4 and 0.05 mM K_2HPO_4 (pH 6.0) does not affect nitrate uptake research (BassiriRad et al., 1999). Presumably, the ratio of KH_2PO_4 to K_2HPO_4 can be adjusted to maintain pH and not affect nitrate uptake. However, our research was over a longer time frame than BassiriRad et al. (1999), so it is unclear if the longer period of exposure to higher phosphate would be detrimental to nitrate uptake research. Not much

nitrate was transformed into ammonium, as shown by the low ammonium concentrations. Several plants showed signs of nutrient deficiency, specifically purple coloration in the lower leaves. This might have been low phosphorous availability.

It was difficult to get accurate solid-phase nitrate concentrations (if a researcher wanted to consider diffusive nitrate delivery to the root) because of the fertilizer beads. The use of controlled-release potassium nitrate did not create constant delivery and uniformity. The beads did not stay uniformly distributed during sub-sampling, which created problems with analysis.

Future investigators should modify the sand medium we chose. We selected silica sand because it has a low exchange capacity and very low organic matter. The sand was much too fine to allow drainage, and too coarse for macro-pore formation, and an aerobic layer formed at field capacity in our containers. This caused bunched roots at the top of this layer, and large areas of root necrosis below this. Equation 1 shows that volumetric water content must be kept constant or measured for the concentration versus influx data to be meaningful. We attempted to do this by keeping the pots at field capacity. This might be improved by designing a system that would keep the volumetric water content constant, such as a Marriott jar.

More concentration treatments (fertilizer treatments) and replications would be necessary to accumulate enough data for meaningful regressions to find the K_m and I_{max} values. Each concentration treatment would be a single data point for regression, and this would necessitate a larger experiment. Even the method we used for experiment three in Chapter Three requires more concentration treatments.

However, it is not a more simplistic approach in terms of time, space, and cost. A method similar to that of BassiriRad et al. (1999) might be appropriate to examine as an alternative. It uses attached roots grown in soil to measure the the depletion of a nutrient in a very small sample tube. It has the disadvantages of not being able to analyze the whole root system at once, and averages uptake in time periods that are less than an hour. However, the method does allow researchers to grow plants without daily monitoring or special treatment in the greenhouse until uptake measurements. We need to further modify the method described by Seeling and Claassen (1990) before application of temperature treatments, but I think a soil method can be valuable in elucidating root-zone temperature differences in uptake kinetics because it better simulates real-world root conditions than plants grown in nutrient solution.

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Table 1. Kinetics of nitrate uptake by 'Autumn Flame' red maple. Plants were grown in silica sand with controlled-release potassium nitrate as the nitrogen source. Hoagland's no-nitrogen nutrient solution was applied once a week to supply the other nutrients. Kinetic parameters are averages of three weeks. Opposing signs on both the I_{max} and K_m estimates show that the derived kinetics violate the assumptions of Michaelis-Menten kinetics.

Root zone (°C)	Root surface area (m^2)	Average influx (nmol m^{-2} s^{-1})	V_o† (nm/s)	K_m (μM)	I_{max} (nmol m^{-2} s^{-1})	R^{2}††
14	0.091	27	8.4	-1.60	-21	0.038
24	0.12	16	8.2	2.88	-5.3	0.10
34	0.050	29	12	-1.41	48	0.011
LSD (0.05)	0.026	27	2.6			

† V_o is water flux through the root surface (m^3 of water through m^2 of root surface per second).

†† Regression R-square from Hanes plot; used to determine K_m and I_{max} estimates.

CHAPTER FIVE. GENERAL CONCLUSION

Nitrate uptake by red maple is a temperature dependent process, and we estimate maximum total uptake to be highest for root-zones at 26 °C. Red maple increased maximum total uptake until the optimum, but lack of root-surface area reduced uptake thereafter, although I_{max} increased over the entire investigated range of 14-34 °C in the solution-depletion experiments. While both cultivars seemed to respond similarly to the treatments, the 14 °C treatment affected 'Autumn Flame' root growth more, while the 34 °C treatment reduced 'Franksred' root growth more, relative to the other cultivar. Our observations in the solution-depletion study show that nitrate uptake by red maple does not show a consistent pattern and puts into question whether or not the kinetic parameters are necessary for dealing with modeling under field conditions. Research examining nitrate uptake at higher concentrations is necessary to clarify this. This would allow researchers using models to use an estimate of I_{max}, or a single concentration-independent constant, below 540 μM instead of Michaelis-Menten parameters. I conclude that high root-zone temperatures affect root surface area, which, in turn, affects total maximum uptake.

Secondly, modelers need to question if the resolution provided by Michaelis-Menten kinetics is necessary for modeling most horticultural and forest situations. I suggest that, in most instances, a simple linear concentration-independent nitrate uptake rate is necessary for these modeling situations. This is not an excuse for carelessness in researching the kinetics, because if the investigator is interested in the enzyme kinetics of uptake they would use

higher-resolution, more-controlled techniques than I did in this work. The difference is the precision of how I_{max} is measured.

The propagation process is crucial in determining the quality of work. Regardless of how the red maple were propagated it is necessary to have as uniform ramets as possible. It cannot be over-stated the importance of having enough uniform plant material to do uptake work. Uptake depends on so many growth-stage and plant-demand factors that non-uniform plants cause problems with uptake measurements, especially when it comes time to decide when the plants are ready for uptake measurements.

The soil method was an attempt to find an alternative to the solution-depletion method that also better-simulated natural conditions. It is not easier, and a better evaluation would require more equipment and a more elaborate set up. This field method is probably best left out in the field. We hoped to use the soil method in answering if changes in nitrate uptake could be attributed to changes in transpiration. Determining the transpiration response to root-zone temperature was elusive in the nutrient-solution work. The soil method produced such poor resolution that we cannot separate any treatment effects. Neither experiment allows us to draw any conclusions about the correlation between the concurrent changes in transpiration and uptake when the root zones are at 14, 24, or 34 °C.