Enhanced Nutrient Absorption Kinetics Following Nitrate, Phosphate, and Potassium Deprivation

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ABSTRACT. Plant internal nutrient status is known to influence the kinetics of nutrient absorption, but little on this relationship has been reported for roses (Rosa spp. L.). The objectives of this experiment were to determine the influence of NO₃, PO₄, and K deprivation on plant tissue concentrations and relative growth rates and to quantify the influence of nutrient deprivation on absorption kinetic parameters. Rose plants growing in solution culture were deprived of N, P, or K for 0 to 20 days to establish differing tissue concentrations. Absorption kinetics were then determined based on the rate of NO₃, PO₄, or K depletion from solution over a range of concentrations. The data were fit to a modified Michaelis-Menten equation to account for the influence of internal nutrient status on absorption kinetics. Plants deprived of the nutrients for up to 20 d did not show significantly reduced root or plant fresh weight as compared with control plants. Plant tissue concentrations differed significantly by deprivation treatment and varied from 1.4% to 2.3% for N, 0.22% to 0.35% for P, and from 1.0% to 2.0% for K. Plants deprived of NO₃, PO₄, or K subsequently showed increased absorption rates. This was primarily expressed as an increased maximum absorption rate for NO₃ and PO₄. In contrast, K-deprived plants primarily exhibited an increased affinity (decreased Kₘ) for K. The results demonstrate the plasticity of rose plants to grow and absorb nutrients under varying internal nutrient concentrations. This work quantifies the influence of rose plant nutritional status on the kinetics of NO₃, PO₄, and K absorption. The knowledge would be useful to improve models for providing decision support for fertilization based on plant growth rates and internal nutrient status.

An increased efficiency of fertilization in horticultural systems requires a greater understanding of the factors that influence the nutrient absorption characteristics of plant roots. Nutrient absorption rates by roots are reported to vary according to plant species (Bassiri-Rad et al., 1999; Jungk, 1977), cultivar (Barber and Mackay, 1986; Kelly et al., 2000; Nielsen and Barber, 1978; Sharifi and Zebarth, 2006; Teo et al., 1992), growing conditions (Steingrobe and Schenk, 1994; Wheeler et al., 1998), plant age (Jungk and Barber, 1975; Malagoli et al., 2004), and the nutritional history of the plant (Jungk, 1977; Jungk et al., 1990; Lindgren et al., 1977; Subasinghe, 2006; Walker et al., 2001; Wheeler et al., 1998).

The relationship between nutrient concentration at the root surface [C (µM)] and net flux of the nutrient by the root [Iₙ (typically in pmol·cm⁻²·s⁻¹)] is often described mathematically by enzyme kinetics, as in the Michaelis-Menten function (Eq. [1]; modified from Claassen and Barber, 1974)

\[ I_n = \frac{I_{max} (C - C_{min})}{K_m + C - C_{min}} \]  

where \( I_{max} \) (pmol·cm⁻²·s⁻¹) is the maximum uptake rate, \( C_{min} \) (µM) is the solution concentration at which no flux occurs, and \( K_m \) (µM) is the Michaelis-Menten constant, which represents the concentration at which net flux is one-half of \( I_{max} \).

The influence of plant nutritional history on nutrient absorption in terms of Michaelis-Menten kinetics has been reported for many plant species (Glass, 2002; Reid, 1999). For example, sugarcane (Saccharum officinarum L.) plants grown with sub-optimal N and K in the nutrient solution for 3½ months showed increased absorption rates when subsequently exposed to these nutrients; this was expressed in terms of Michaelis-Menten kinetics as an increased \( I_{max} \) and decreased \( K_m \) (Subasinghe, 2006). A strong correlation between K concentration of barley (Hordeum vulgare L.) roots and \( I_{max} \) and \( K_m \) was reported by Siddiqi and Glass (1986). The Michaelis-Menten equation can be adapted to account for influence of plant (or root) ion concentration \( [C_p] \) on \( I_{max} \) and \( K_m \) as in Eqs. [2 and 3] (modified from Siddiqi and Glass, 1986).

\[ I_{max} = MaxI_{max} \cdot e^{\alpha C_p} \]  

\[ K_m = MinK_m \cdot e^{\beta C_p} \]

where \( MaxI_{max} \) and \( MinK_m \) are the theoretical maximum and minimum values for \( I_{max} \) and \( K_m \), respectively. Coefficient \( \alpha \) represents the relative effect that tissue concentrations have on \( I_{max} \). Within Eq. [2], negative \( \alpha \) signifies that \( I_{max} \) is negatively affected by increased tissue concentrations. Similarly, a positive value of \( \beta \) signifies that affinity decreases (\( K_m \) increases) with increasing tissue concentrations. For both \( \alpha \) and \( \beta \), values approaching 0 signify that tissue concentration has little effect on \( I_{max} \) or \( K_m \), respectively.
Cut flower roses are typically grown in greenhouses in containers holding soilless mixes, and plants are simultaneously irrigated and fertilized (fertilization) several times per day. The crop cycle typically takes 4 to 8 weeks depending on cultivar and environmental conditions. Plants are often managed to produce a flush of harvestable stems in time for a particular holiday, and this crop timing is initiated by removing existing flower stems or bending weak stems. During these crop cycles, rose plants exhibit cyclical patterns of NO$_3$, PO$_4$, K, Ca, and Mg absorption (Cabrera et al., 1995a). Typical of intensive plant production, commercial rose production uses high concentrations of N, P, and K fertilizer. It has been proposed that nutrient losses in an open irrigation system (i.e., where irrigation water leachate is not captured and reused) can be minimized by irrigating with clear water for a period of time and intermittently irrigating with fertilizer (Cabrera et al., 1996). To maintain plant yield, this fertilization strategy requires that plants deprived of a nutrient would have increased absorption rates when subsequently provided with the nutrient and that excess nutrient could be stored for use during the deprivation periods. This fertilization strategy appeared to be successful for roses; plants previously deprived of N for 4 to 16 d showed a two- to threefold increase in NO$_3$ uptake rates (Cabrera et al., 1996).

We have previously studied NH$_4$, PO$_4$, and K kinetic parameters and N, P, and K storage and allocation within a plant and used this information to model NO$_3$ and K absorption over rose crop cycles (Mattson et al., 2006; Silberbush and Lieth, 2004). Although Eq. [2] was applied by Silberbush and Lieth (2004) to account for changes in NO$_3$ and K absorption by roses during a crop cycle, no experimental data were available on the effect of internal nutrient status on NO$_3$, PO$_4$, and K absorption kinetics in rose plants.

The objectives of this study were 1) to determine the influence of NO$_3$, PO$_4$, or K deprivation for 0 to 20 d on the subsequent tissue concentration and growth rates of rose plants; and 2) to quantify the influence of internal nutrient status (deprivation treatment) on kinetic parameters for these nutrients.

**Materials and Methods**

**Plant culture.** One hundred 5-month-old ‘Kardinal’ rose plants on ‘Natal Briar’ rootstock were established in half strength Hoagland’s solution (Hoagland and Arnon, 1950) culture in a greenhouse with six plants per 16-L container. The solution was kept aerated by continuously bubbling air into the solution. The nutrient solution was replaced every 5 d. At solution replacement, FW was recorded (after roots had been blotted with tissue paper to remove excess water) and root volume was measured through water displacement in a volumetric cylinder. During the establishment period and the first 18 d of the deprivation treatments, the greenhouse average daily temperature was 23.3 °C and the average daily light integral was 13.9 mol·m$^{-2}$·d$^{-1}$. On day 18, the plants were moved to a controlled environment chamber with a 16-h photoperiod at 590 μmol·m$^{-2}$·s$^{-1}$ photosynthetic photon flux (average daily light integral 34.0 mol·m$^{-2}$·d$^{-1}$) and 85% relative humidity. Average daily temperature was 22.7 °C (day temperature 24 °C, night temperature 20 °C). The relative growth rate [RGR (g·g$^{-1}$·d$^{-1}$)] of plants and roots (estimated as root volume) during the 20-d deprivation period was calculated as in Eq. [4]:

$$RGR = \frac{\ln FW_{t_2} - \ln FW_{t_1}}{t_2 - t_1}$$  \[4\]

where $t_1$ and $t_2$ are successive measurement dates. FW was chosen rather than dry weight (DW) so that measurements were nondestructive.

The NO$_3$ deprivation treatments were initiated on 12 Sept. (day 0). All plants received the following macronutrients: 0.5 mm KH$_2$PO$_4$, 3.5 mm KCl, 1.0 mm MgSO$_4$, and 2.0 mm CaCl$_2$·2H$_2$O; and micronutrients: 18 μm Fe-EDTA, 2 μm CuSO$_4$·5H$_2$O, 4 μm ZnSO$_4$·7H$_2$O, 0.2 μm H$_2$MoO$_4$·H$_2$O, 28 μm MnCl$_2$·4H$_2$O, and 4 μm H$_2$BO$_3$. Plants receiving N were also provided with 8 mm KNO$_3$, whereas the plants not receiving N were given 8 mm KCl. The solution was chosen to provide an approximately full-strength Hoagland’s concentration for the nutrient of interest (to ensure ample supply at all times), approximately half-strength concentration for the other micronutrients, and full-strength micronutrient concentration (Hoagland and Arnon, 1950).

The K deprivation treatments were initiated on 15 Sept. (day 0) on a different set of 24 plants. Similar to the NO$_3$ deprivation experiment, plants were deprived of K for 20, 10, 5, or 0 d. All plants received the following macronutrients: 0.25 mm Ca(H$_2$PO$_4$)·H$_2$O, 4.0 mm Ca(NO$_3$)$_2$, and 1.0 mm MgSO$_4$; and
micronutrients as in the NO$_3$ experiment. The plants receiving K were provided with 4.0 mM KCl, whereas the plants not receiving K were provided with 4.0 mM NaCl. Nutrient solution replacement and plant measurements were conducted as described previously.

The PO$_4$ deprivation treatments were initiated on 19 Sept. (day 0) following the same protocol as for NO$_3$ and K. All plants received the following macronutrients: 4.0 mM KNO$_3$, 1.0 mM MgSO$_4$, and 2.0 mM Ca(NO$_3$)$_2$; and micronutrients as in the NO$_3$ experiment. The plants receiving PO$_4$ were provided with 0.5 mM KH$_2$PO$_4$, whereas the plants not receiving PO$_4$ were provided with 0.5 mM KCl. Nutrient solution replacement and plant measurements were conducted as described previously.

**Determination of depletion curves.** On the morning of day 20 after the start of each deprivation treatment, a nutrient depletion experiment was conducted to measure nutrient absorption rates as a plant draws down the nutrient of interest. The five largest plants within a treatment (based on FW) were selected for the depletion trials. This occurred on 2, 5, and 9 Oct. for the NO$_3$, K, and PO$_4$ experiments, respectively.

For the NO$_3$ experiment, each plant was moved to a 250-mL glass flask with 210 mL of nutrient solution containing the standard macronutrients and micronutrients for the N deprivation treatment without NO$_3$ plus the addition of 500 $\mu$M KNO$_3$. Air was continuously bubbled into the solution to maintain oxygen saturation. Every hour for 12 h, a 3-mL nutrient solution sample was taken to determine NO$_3$ concentration; and the plant and container were weighed to determine water loss to transpiration. At the end of the 12-h period, each plant was partitioned into roots, stems, and leaves; FW was measured; and plants were immediately placed in an oven and dried at 60 °C for 5 d. A 12-h depletion period was chosen so that the results would be more representative of absorption rate changes that could be sustained over a time scale of several hours rather than instantaneous changes.

The K depletion experiment was similar with the following modifications. A different set of plants was moved to flasks containing the K deprivation experiment solution with the addition of 625 $\mu$M KCl. Every hour for 12 h, a 3-mL nutrient solution sample was taken. When hourly nutrient samples and plant/container weights were taken, deionized water was added to the container to make up for water loss to sampling and transpiration during the previous hour (i.e., solution volume was maintained at 210 mL). At the end of the 12-h period, plants were measured and harvested.

For the PO$_4$ experiment, the depletion experiment was similar to the K depletion experiment. The nutrient solution contained the standard macronutrients and micronutrients for the PO$_4$ deprivation experiment without PO$_4$ plus the addition of 225 $\mu$M KH$_2$PO$_4$. Nutrient solution samples were taken every hour for 12 h. At the end of 12-h period, plants were measured and harvested.

**Solution and tissue analysis.** The nutrient solution samples were analyzed for the nutrient of interest. Solution concentrations were determined as follows: NO$_3$ by diffusion conductivity with an ammonia/nitrate analyzer (model TL200; Timberline Instruments, Boulder, CO); K by flame emission with an atomic absorption spectrometer (model SpectrAA Model 55; Varian, Palo Alto, CA); and H$_2$PO$_4$ by the stannous chloride colorimetric method with a colorimeter (model PL800; Brinkmann Instruments, Westbury, NY).

The roots, stems, and leaves of each plant were ground to pass through a 40-mesh screen and sent to a laboratory for analysis (University of California Agriculture and Natural Resources Analytical Laboratory, Davis). For the NO$_3$ experiment, total N was determined using an induction furnace and thermal conductivity detector (Windham, 1997) and NO$_3$-N by 2% acetic acid extraction followed by flow injection analysis (Wendt, 1999). For the K experiment, K was determined through 2% acetic acid extraction (Johnson and Ulrich, 1959) followed by atomic absorption spectrometry. For the PO$_4$ experiment, total P was analyzed using nitric acid/hydrogen peroxide microwave digestion (Sah and Miller, 1992) followed by atomic absorption spectrometry and PO$_4$-P by 2% acetic acid extraction followed by flow injection analysis (Prokopy, 1995). Whole plant tissue concentrations were calculated using root, stem, and leaf tissue concentrations and their corresponding DW.

**Nutrient uptake calculations and statistical analysis.** Using the depletion data, hourly plant $I_n$ was calculated as the difference in the nutrient content at the beginning of the interval $(t_i)$ to the end of the interval $(t_{i+1})$ minus that removed from sampling at time $t_i$, as in Eq. [5]:

$$ V_i C_n - V_{i+1} C_{n+1} - V_S C_H = [5] $$

where $V$ is the solution volume, $V_S$ is the sample volume (3 mL), and $C$ is the solution concentration at time, $i$. Absorption of each nutrient was scaled in terms of plant DW to determine uptake rates in units of $\mu$mol g$^{-1}$ h$^{-1}$.

All statistical analyses were conducted with SAS (version 9.1; SAS Institute, Cary, NC). Data were subjected to analysis of variance using the General Linear Model (SAS Proc GLM) to identify differences in means based on nutrient deprivation treatment. When significant differences were found, Tukey’s honestly significant difference method was used to compare treatment means. The kinetic parameters were estimated by fitting Eq. [1] to the relationship between $I_n$ ($\mu$mol g$^{-1}$ h$^{-1}$) and solution concentration ($C$) using nonlinear regression (SAS PROC NLIN). To quantify the effect of plant tissue nutrient concentration [$C_p$ (in percent or grams of nutrient per gram DW x 100)] on absorption kinetics, the data on $C_p$, $C$, and $I_n$ ($\mu$mol g$^{-1}$ h$^{-1}$) were fit to Eq. [1] with Eqs. [2] and [3] substituted for $I_{max}$ and $K_m$, respectively, using nonlinear regression.

**Results**

**Plant growth parameters.** In the NO$_3$ experiment, the root FW at day 0 (deprivation initiation) was greatest for the control treatment and least for the –20 (Table 1). There were no other differences in root or plant FW during the rest of the 20-d period. Plant FW increased from 17 g at day 0 to 32 to 39 g at day 20. There was weak evidence of decreased plant FW for the –10 and –20 treatments ($P = 0.079$). By day 20, the plant RGR of the control treatment was greater than that of the –10 and –20 treatments.

Potassium deprivation had no effect on root FW, plant FW, and root RGR during the 20-d treatment period (data not presented). Plant RGR did not differ between treatments, except at day 20 when plant RGR was greatest for the K control treatment (0.027 ± 0.007 g g$^{-1}$) and least for the –10 treatment (–0.003 ± 0.001, $P = 0.008$).
Phosphorus deprivation had no effect on root FW, plant FW, and plant RGR during the 20-d treatment period. Minor differences in root RGR were present at day 5 and 10 but not on subsequent dates (data not presented).

Potassium and P deprivation treatments did not significantly affect root, stem, leaf, or whole plant DW by the end of the 20-d period (Table 2). Nitrates deprivation decreased leaf DW of the –20 plants as compared with the –5 and control treatments (Table 2). There was weak evidence that NO₃ deprivation decreased whole plant DW (P = 0.064) (Table 2).

**Plant tissue concentrations.**

Nitrates deprivation significantly decreased total N and NO₃-N concentrations in rose plant tissues (Fig. 1). The total N concentration of plants was 2.4% for control plants and 1.4% for –20 plants (Fig. 1). Whole plant NO₃-N did not differ among the –5, –10, and –20 treatments and averaged 0.003%, whereas control plants had a 10-fold greater NO₃-N concentration (0.03%) (Fig. 1).

Potassium deprivation significantly decreased K concentration in plant tissues (Fig. 2). Tissue concentrations were always least for the –10 and –20 treatments and did not differ significantly between these two treatments. The greatest K concentration differences were found in the roots, varying from 0.5% for the –20 plants to 2.2% for control plants.

Phosphorus deprivation significantly reduced root, stem, leaf, and whole plant total P and PO₄-P tissue concentrations (Fig. 3). Plants deprived of P for 10 or 20 d did not differ significantly in total P or PO₄-P tissue concentrations. Whole plant P was greatest for the control treatment (0.35%), least for the –10 and –20 treatments and averaged 0.003%, whereas control plants had a 10-fold greater NO₃-N concentration (0.03%) (Fig. 1).

**Kinetic parameters.**

Representative depletion curves are shown in Figure 4. For the depletion data, it was noted that control plants did not initially show positive Iᵣ (i.e., nutrient content in solution increased); this may represent loss from the apoplast of roots. Plants that had been deprived of NO₃ or K showed an initial high rate of net flux during the first hour. Plants that had been deprived of NO₃ exhibited a low

### Table 1. Fresh weight and relative growth rate of ‘Kardinal’ roses during the 20-d deprivation experiment.*

<table>
<thead>
<tr>
<th>Day</th>
<th>Treatment</th>
<th>Fresh wt (g)</th>
<th>Relative growth rate (g·g⁻¹·d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Root</td>
<td>Plant</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>–20</td>
<td>5.0 ± 0.0 b</td>
<td>17.2 ± 0.5 a</td>
</tr>
<tr>
<td></td>
<td>–10</td>
<td>5.2 ± 0.3 ab</td>
<td>16.7 ± 0.3 a</td>
</tr>
<tr>
<td></td>
<td>–5</td>
<td>5.2 ± 0.3 ab</td>
<td>16.9 ± 0.6 a</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>6.0 ± 0.0 a</td>
<td>17.5 ± 0.4 a</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.017–*</td>
<td>0.637–NS</td>
</tr>
<tr>
<td>5</td>
<td>–20</td>
<td>6.8 ± 0.3 a</td>
<td>20.8 ± 1.0 a</td>
</tr>
<tr>
<td></td>
<td>–10</td>
<td>7.0 ± 0.5 a</td>
<td>21.1 ± 0.9 a</td>
</tr>
<tr>
<td></td>
<td>–5</td>
<td>6.3 ± 0.3 a</td>
<td>20.2 ± 0.6 a</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>7.3 ± 0.4 a</td>
<td>21.3 ± 1.6 a</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.385–NS</td>
<td>0.873–NS</td>
</tr>
<tr>
<td>10</td>
<td>–20</td>
<td>6.8 ± 0.3 a</td>
<td>25.5 ± 1.4 a</td>
</tr>
<tr>
<td></td>
<td>–10</td>
<td>7.2 ± 0.5 a</td>
<td>26.0 ± 1.5 a</td>
</tr>
<tr>
<td></td>
<td>–5</td>
<td>6.5 ± 0.4 a</td>
<td>26.0 ± 0.8 a</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>7.8 ± 0.3 a</td>
<td>25.8 ± 0.8 a</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.125–NS</td>
<td>0.989–NS</td>
</tr>
<tr>
<td>15</td>
<td>–20</td>
<td>6.8 ± 0.3 a</td>
<td>31.0 ± 1.7 a</td>
</tr>
<tr>
<td></td>
<td>–10</td>
<td>7.5 ± 0.2 a</td>
<td>32.0 ± 2.1 a</td>
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<td>33.5 ± 1.3 a</td>
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<td></td>
<td>P</td>
<td>0.455–NS</td>
<td>0.631–NS</td>
</tr>
<tr>
<td>20</td>
<td>–20</td>
<td>6.5 ± 0.4 a</td>
<td>31.9 ± 2.7 a</td>
</tr>
<tr>
<td></td>
<td>–10</td>
<td>6.9 ± 0.7 a</td>
<td>32.9 ± 2.7 a</td>
</tr>
<tr>
<td></td>
<td>–5</td>
<td>5.9 ± 0.5 a</td>
<td>38.1 ± 1.6 a</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>5.9 ± 0.2 a</td>
<td>38.9 ± 1.1 a</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.400–NS</td>
<td>0.079–NS</td>
</tr>
</tbody>
</table>

*Plants were deprived of NO₃ for 20 d (--20), 10 d (--10), 5 d (--5), or 0 d (control) before experiment termination. Data are means ± SE of six plants.

**Table 2. Dry weight of compartments of ‘Kardinal’ roses after harvest at day 20.**

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Deprivation treatment</th>
<th>Root dry wt (g)</th>
<th>Stem dry wt (g)</th>
<th>Leaf dry wt (g)</th>
<th>Plant dry wt (g)</th>
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</thead>
<tbody>
<tr>
<td>NO₃</td>
<td>–20</td>
<td>1.0 ± 0.1 a</td>
<td>3.6 ± 0.3 a</td>
<td>3.3 ± 0.4 b</td>
<td>7.9 ± 0.8 a</td>
</tr>
<tr>
<td></td>
<td>–10</td>
<td>0.9 ± 0.2 a</td>
<td>3.6 ± 0.3 a</td>
<td>3.7 ± 0.3 ab</td>
<td>8.1 ± 0.7 a</td>
</tr>
<tr>
<td></td>
<td>–5</td>
<td>1.0 ± 0.1 a</td>
<td>4.4 ± 0.2 a</td>
<td>4.7 ± 0.3 a</td>
<td>10.1 ± 0.6 a</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.9 ± 0.1 a</td>
<td>4.0 ± 0.1 a</td>
<td>4.7 ± 0.2 a</td>
<td>9.6 ± 0.3 a</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.325–NS</td>
<td>0.148–NS</td>
<td>0.015–*</td>
<td>0.064–NS</td>
</tr>
<tr>
<td>K</td>
<td>–20</td>
<td>1.2 ± 0.1 a</td>
<td>5.1 ± 0.6 a</td>
<td>6.1 ± 1.0 a</td>
<td>12.3 ± 1.5 a</td>
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<tr>
<td></td>
<td>–10</td>
<td>1.2 ± 0.1 a</td>
<td>4.5 ± 0.4 a</td>
<td>5.5 ± 0.3 a</td>
<td>11.2 ± 0.7 a</td>
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<td></td>
<td>–5</td>
<td>1.2 ± 0.1 a</td>
<td>5.1 ± 0.5 a</td>
<td>6.1 ± 0.6 a</td>
<td>12.4 ± 1 a</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>1.2 ± 0.2 a</td>
<td>5.5 ± 0.2 a</td>
<td>6.7 ± 0.4 a</td>
<td>13.5 ± 0.7 a</td>
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<td></td>
<td>P</td>
<td>0.985–NS</td>
<td>0.429–NS</td>
<td>0.596–NS</td>
<td>0.510–NS</td>
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<tr>
<td>PO₄</td>
<td>–20</td>
<td>1.4 ± 0.1 a</td>
<td>6.3 ± 0.4 a</td>
<td>6.2 ± 0.6 a</td>
<td>13.8 ± 0.9 a</td>
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<td></td>
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<td>1.4 ± 0.1 a</td>
<td>6.3 ± 0.6 a</td>
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<td>14.6 ± 1.5 a</td>
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<td>15.8 ± 0.8 a</td>
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<td></td>
<td>Control</td>
<td>1.3 ± 0.1 a</td>
<td>5.8 ± 0.3 a</td>
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<td>13.3 ± 0.8 a</td>
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<td></td>
<td>P</td>
<td>0.446–NS</td>
<td>0.632–NS</td>
<td>0.330–NS</td>
<td>0.371–NS</td>
</tr>
</tbody>
</table>

*Plants were growing in nutrient solution that was deprived of NO₃, K, and PO₄ for 20 d (--20), 10 d (--10), 5 d (--5), or 0 d (control). Data are means ± SE of five plants.

**Means for fresh weight or relative growth rate followed by the same letter are not significantly different from other treatments at the same day based on Tukey’s honestly significant difference test (P = 0.05).

**Probability values from analysis of variance comparing treatment fresh weight and relative growth rate at each day; NS (nonsignificant) P ≥ 0.05, * P < 0.05, ** P < 0.01, *** P < 0.001.
rate of NO$_3$ absorption for $\approx$3 h before increased rates were observed, likely representing the time required for induction of the NO$_3$-inducible high affinity transport system (IHATS). When any of the aforementioned cases were observed, the initial $I_n$ values were excluded so that the results represent steady-state $I_n$.

In the NO$_3$ experiment, kinetic parameters ($I_{\text{max}}, K_m$, and $C_{\text{min}}$) did not differ among the –5, –10, and –20 deprivation treatments (Table 3). For the NO$_3$ control plants, solution depletion occurred at a slower rate and solution NO$_3$ concentration did not approach a final equilibrium. Consequently, we were not able to fit the control data to a Michaelis-Menten relationship (Eq. [1]) and obtain information about solution concentration affect on influx rate represented as parameters $K_m$ and $C_{\text{min}}$. However, the treatment results could still be used to determine the relatively stable influx rate that occurred at high solution concentration. As a means of comparing the four treatments, Table 4 presents average $I_n$ of the treatments at a NO$_3$ solution concentration of 500 μM (near the initial concentration for the depletion experiment). Deprivation treatment significantly influenced $I_n$ at 500 μM NO$_3$ with the greatest $I_n$ found for plants deprived of NO$_3$ for 20 d (1.66 μmol·g$^{-1}$·h$^{-1}$) and the least $I_n$ for the control treatment (0.71 μmol·g$^{-1}$·h$^{-1}$).

In the K experiment, the primary influence of K deprivation on net flux was on increasing the affinity for K absorption at low solute concentrations (Table 3). The apparent $I_{\text{max}}$ was not significantly influenced by deprivation treatment and varied from 2.1 to 2.5 μmol·g$^{-1}$·h$^{-1}$. K$_m$ did not differ significantly among deprivation treatments. The concentration at which no net flux of PO$_4$ occurred ($C_{\text{min}}$) was significantly greater for the –20 treatment (42 μM) than in the –5 and –10 treatments (9 to 10 μM).

A significant fit to Eqs. [1 to 3] was found for the relationship between $C$, $C_p$, and $I_n$ (Table 5). The $I_{\text{max}}$ parameter was greatest for NO$_3$ (3.09 μmol·g$^{-1}$·h$^{-1}$) followed by K (2.59 μmol·g$^{-1}$·h$^{-1}$).
Experiment for plants deprived of NO$_3$ and K for 10 to 20 d. Cabrera et al. (1996) found no yield reduction in rose stems grown for one crop cycle with a 4- or 8-d N deprivation followed by a 4-d supply period. This was attributed to two- to threefold increased absorption rates after deprivation and may also be the result of the ability of rose plants to store and mobilize N (Cabrera et al., 1995b). Plants grown with 16 d deprivation did show decreased yield and plant DW. We have previously found that storage in base plant parts could provide one-fourth of the N, P, and K required by new rose flower shoots (Mattson, 2007). Longer-term experiments with N, P, or K deprivation are necessary to determine the influence of nutrient supply on plant growth parameters. For example, when roses were fertilized continuously at N concentrations ranging from 30 to 220 mg L$^{-1}$ for 13 months, low N supply did not influence yield until after the first crop cycle; and leaf N concentration was significantly related to plant yield only during the last half of the experimental period (Cabrera, 2000). Researchers conducting experiments with long-term nutrient stress have often reported increased allocation to roots (Glass, 2002; Subasinghe, 2006). In our experiments, root FW, DW

$$\mu\text{mol·g}^{-1} \cdot \text{h}^{-1}$$ and PO$_4$ (0.938 $\mu\text{mol·g}^{-1} \cdot \text{h}^{-1}$). For all three ions, $I_{\text{max}}$ was negatively affected by increased tissue concentrations as coefficient $\alpha < 0$ (Table 5). The greatest absolute $\alpha$ was found for PO$_4$ ($\alpha = -4.17$), demonstrating that $I_{\text{max}}$ is more responsive to tissue concentration for PO$_4$ than for NO$_3$ or K. Potassium $I_{\text{max}}$ was influenced very little by tissue concentration ($\alpha = -0.149$). A positive $\beta$ was found for K ($\beta = 1.06$) meaning that K affinity decreases ($K_m$ increases) as tissue K increases (Table 5). This parameter was not fit for NO$_3$ and PO$_4$ because $K_m$ was not significantly influenced by deprivation treatment for these ions (Table 3). Figure 5 shows the relationship among $C$, $C_p$, and $I_0$ as fit to Eqs. [1 to 3] for the solution and tissue concentration ranges of our experiments. The graphs visually demonstrate the kinetic parameter features presented in Table 5; for example, PO$_4$ absorption at a $C$ of 300 $\mu$M varied from 0.16 to 0.34 as $\mu\text{mol·g}^{-1} \cdot \text{h}^{-1}$ as tissue P decreases from 0.4% to 0.2% (Fig. 5).

**Discussion**

**PLANT GROWTH AND TISSUE CONCENTRATIONS.** The plants that were deprived of NO$_3$, PO$_4$, or K in the nutrient solution for up to 20 d did not have significantly lower root or plant FW than control plants (Tables 2–4). This result may be partially the result of plant-to-plant variation in FW within a treatment, because a reduced plant RGR was found by the end of the

Fig. 4. Depletion of NO$_3$, K, and PO$_4$ from 210 mL of solution by ‘Kardinal’ roses over time. Plants were deprived of the respective nutrients for 20 d (–20), 10 d (–10), 5 d (–5), or 0 d (control) before the nutrient depletion measurements. Data for each treatment are from single representative plants.
of existing nutrient within the tissue once the nutrient was withheld from solution. Rose plants under continuous N supply or periods of 4, 8, and 16 d deprivation followed by a 4-d supply had leaf N concentrations of 3.4%, 3.1%, 2.7%, and 2.8%, respectively (Cabrera et al., 1996). Leaf N, P, and K concentrations of the control plants in our experiment are similar to those of Carlson and Bergman (1966) and Gabriels and Meneve (1973).

### Table 3. Michaelis-Menten parameters for the relationship between net nutrient flux ($I_n$) and uptake rate for ‘Kardinal’ roses.

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Deprivation treatment</th>
<th>$I_{\text{max}}$ (µmol g$^{-1}$ h$^{-1}$)</th>
<th>$K_m$ (µM)</th>
<th>$C_{\text{min}}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO$_3$</td>
<td>–20</td>
<td>2.00 ± 0.16 a$^*$</td>
<td>86.4 ± 29.1 a</td>
<td>4.7 ± 2.6 a</td>
</tr>
<tr>
<td></td>
<td>–10</td>
<td>1.43 ± 0.36 a</td>
<td>79.2 ± 31.7 a</td>
<td>3.6 ± 2.5 a</td>
</tr>
<tr>
<td></td>
<td>–5</td>
<td>1.21 ± 0.17 a</td>
<td>31.6 ± 9.9 a</td>
<td>3.7 ± 3.1 a</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>— x</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>$P^*$</td>
<td></td>
<td>0.18–NS</td>
<td>0.330–NS</td>
<td>0.935–NS</td>
</tr>
<tr>
<td>K</td>
<td>–20</td>
<td>2.39 ± 0.18 a</td>
<td>33.4 ± 3.8 a</td>
<td>0.4 ± 0.2 a</td>
</tr>
<tr>
<td></td>
<td>–10</td>
<td>2.20 ± 0.21 a</td>
<td>28.8 ± 3.2 b</td>
<td>1.3 ± 0.8 a</td>
</tr>
<tr>
<td></td>
<td>–5</td>
<td>2.08 ± 0.33 a</td>
<td>44.1 ± 7.9 b</td>
<td>0.9 ± 0.5 a</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>2.52 ± 0.30 a</td>
<td>223.0 ± 70.4 a</td>
<td>— x</td>
</tr>
<tr>
<td>$P$</td>
<td></td>
<td>0.691–NS</td>
<td>0.005–**</td>
<td>0.656–**</td>
</tr>
<tr>
<td>PO$_4$</td>
<td>–20</td>
<td>0.38 ± 0.02 a</td>
<td>43.0 ± 8.8 a</td>
<td>41.9 ± 11.2 a</td>
</tr>
<tr>
<td></td>
<td>–10</td>
<td>0.38 ± 0.03 a</td>
<td>47.1 ± 7.3 a</td>
<td>10.3 ± 3.6 b</td>
</tr>
<tr>
<td></td>
<td>–5</td>
<td>0.42 ± 0.06 a</td>
<td>56.1 ± 13.8 a</td>
<td>8.5 ± 4.2 b</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.18 ± 0.04 b</td>
<td>28.5 ± 11.6 a</td>
<td>— y</td>
</tr>
<tr>
<td>$P$</td>
<td></td>
<td>0.034–*</td>
<td>0.520–NS</td>
<td>0.008–**</td>
</tr>
</tbody>
</table>

$^*$Plants were deprived of NO$_3$, K, or PO$_4$ for 20 d (–20), 10 d (–10), 5 d (–5), or 0 d (control) before nutrient depletion measurements to determine the parameters. Michaelis-Menten parameters were determined using nonlinear regression to fit data on $I_n$ versus solution concentration for individual plants. Data are means ± se of five plants.

$^*$Means for the kinetic parameters followed by the same letter are not significantly different from other treatments within the same experiment based on Tukey’s honestly significant difference test ($P = 0.05$).

$^*$A significant Michaelis-Menten relationship was not found for the plants in this treatment. $I_n$ averaged 0.71 ± 0.07 µmol g$^{-1}$ h$^{-1}$ between the concentrations of 400 and 600 µM NO$_3$.

$^*$Probability values from analysis of variance comparing treatment Michaelis-Menten parameters within each experiment; NS (nonsignificant) $P \geq 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

$^*$During the deprivation period, the solution concentration for this treatment did not approach 0, so $C_{\text{min}}$ was unable to be estimated.

### Deprivation and kinetic parameters.

Plants deprived of NO$_3$, PO$_4$, and K showed increased absorption rates when subsequently exposed to these nutrients as compared with control plants; this was primarily expressed as an increased $I_{\text{max}}$ for NO$_3$ and PO$_4$-deprived plants and an increased affinity (reduced $K_m$) for K-deprived plants (Table 3). The reviews by Reid (1999) and Glass (2002) note that increased $I_{\text{max}}$ is consistently reported for many plant species when they have been deprived of NH$_4$, NO$_3$, PO$_4$, and K but that $K_m$ changes are consistently reported only for K and NH$_4$. Our NO$_3$ results are similar to those of Cabrera et al. (1996), who found that NO$_3$ uptake rates increased by two- to threefold when rose plants were deprived of NO$_3$ for 4 to 16 d. The increased NO$_3$ and PO$_4$ uptake rates we found after decreased nutrient availability are in agreement with other studies for NO$_3$ (Cárdenas-Navarro et al., 1999; Hole et al., 1990; Raman et al., 1995; Siddiqui et al., 1990; Subasinghe, 2006) and PO$_4$ (Clark et al., 2000; Jungk 1977; Jungk et al., 1990). The NO$_3$ $K_m$ of one sugarcane cultivar decreased with increasing tissue N stress, whereas it was unaffected for the other cultivar (Subasinghe, 2006). The PO$_4$ $I_{\text{max}}$ of arabidopsis [Arabidopsis thaliana (L.) Heynh.] plants decreased from 118 to 43 nmol/plant per hour when they were grown in solution with 10 versus 250 µM P for 15 d before depletion measurements (Clark et al., 2000).

Changes in kinetic parameters have been reported within 4 h of manipulation of nutrient supply in rice (Oryza sativa L.) (Raman et al., 1995). Our experiment did not find an increased $I_{\text{max}}$ with K deprivation. This may be a product of fitting to Michaelis-Menten kinetics. The high $K_m$ of control plants also means that net flux of K by control plants was lower than that of the deprivation treatments at the K concentration range used in this experiment (calculated from Table 3).

The changes in kinetic parameters that we observed with nutrient deprivation presumably represent plant regulation that would act to optimize nutrient absorption under conditions of low nutrient availability. From our experiment, we cannot say whether changes in absorption kinetics with varying internal nutrient status are the result of feedback regulation by the ion itself or whether internal nutrient status serves only as an indicator of internal regulation taking place by some other mechanism. Molecular studies have revealed the multifaceted nature of nutrient transporter expression and regulation. In the case of nitrate uptake in arabidopsis, gene expression of one of the nitrate IHATS transporters (NRT2.1) is promoted by light and sugars, induced by NO$_3$, and repressed by reduced N metabolites such as NH$_4$ and amino acids (Miller et al., 2007).

Furthermore, the AtNRT2.1 protein is subject to posttranslational regulation and requires an additional protein to facilitate targeting to the plasma membrane (Miller et al., 2007). Whether there is molecular relevance in fitting kinetic parameters to $C_p$.
it appears to be a useful empirical method to predict plant demand/uptake. In terms of ion transport systems, an increase in $I_{\text{max}}$ is attributed to an increase in the number of ion transporters (Glass, 2002). Several high-affinity PO$_4$ transporters are known to be induced by plant phosphate deficiency (Raghothama and Karthikeyan, 2005). Although changes in $K_m$ are not well understood, they may represent allosteric regulation of transport sites or the expression of other transporters that differ in kinetic characteristics (Reid, 1999).

**PLANT TISSUE CONCENTRATION AND KINETIC PARAMETERS.** By imposing various nutrient deprivation treatments on rose plants, we were able to obtain different plant tissue concentrations. We found a significant increase in NO$_3$, PO$_4$, and K absorption kinetics under decreased plant tissue concentrations (Table 5). Our results are in agreement with

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Parameters</th>
<th>Coefficients</th>
<th>Curve fit</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO$_3$</td>
<td>Max$I_{\text{max}}$ (μmol·g$^{-1}$·h$^{-1}$) Min$K_m$ (μM) $C_{\text{min}}$ (μM)</td>
<td>α β</td>
<td>$R^2$ Significance of fit ($P$)</td>
</tr>
<tr>
<td>NO$_3$</td>
<td>3.09 26.7 3.43</td>
<td>-0.595 0$^a$</td>
<td>0.87 &lt; 0.001</td>
</tr>
<tr>
<td>K</td>
<td>2.58 11.7 1.06</td>
<td>-0.149 1.06</td>
<td>0.93 &lt; 0.001</td>
</tr>
<tr>
<td>PO$_4$</td>
<td>0.938 49.7 3.58</td>
<td>-4.17 0$^a$</td>
<td>0.85 &lt; 0.001</td>
</tr>
</tbody>
</table>

$^aK_m$ was not significantly influenced by deprivation treatment (Table 3); therefore, this coefficient was not considered in the fitting procedure.

Fig. 5. The relationship between solution concentration ($C_s$) and plant nutrient concentration ($C_p$) on net flux ($I_n$) fit to Eqs. [1 to 3] using nonlinear regression. The curves were calculated using the values obtained from NLIN of data from individual ‘Kardinal’ rose plants as presented in Table 5.
several studies that report a correlation between root or plant tissue concentration and absorption rates of NO$_3$ (Cárdenas-Navarro et al., 1999; Mattsson et al., 1991; Siddiqi et al., 1990), PO$_4$ (Clark et al., 2000; Jungk et al., 1990), and K (Siddiqi and Glass, 1986). Our findings are in contrast with those of Steingrobe and Schenk (1994), who reported that the NO$_3$ $I_{\text{max}}$ of lettuce (Lactuca sativa L.) plants is not correlated to tissue N concentration, but instead to plant RGR. Wheeler et al. (1998) achieved various RGR of lettuce plants by growing them under three light levels and three NO$_3$ concentrations; they reported that NO$_3$ absorption rates are related to plant growth rates and a plant’s “history of growth limitations.” We speculate that the difference between the lettuce work and our work may be the result of the relative capacity for nutrient storage in the two plant systems. Roses have a large capacity to store N, P, and K and mobilize them to new growing tissue (Cabrera et al., 1995b; Mattsson, 2007). We found relatively few RGR differences for plants deprived of nutrients for 5 to 10 d. If plants show a sustained RGR at the same time that they have reduced tissue nutrient levels, it makes sense that net flux per gram would be increased when plants are subsequently exposed to the nutrients. In the case of lettuce, reduced nutrient availability likely quickly led to decreased growth rates and hence subsequently reduced nutrient absorption. Mattsson et al. (1991) found that NO$_3$ supply was highly correlated to barley plant growth rates under low nutrient supply, but at higher supply rates, additional N was stored rather than used for structural growth. They also noted a decrease in $I_{\text{max}}$ with an increase in plant N storage. In the case of roses, RGR is somewhat uncoupled from nutrient status, whereas in lettuce, it may be more synchronous with nutrient status. We believe our choice of units for $I_o$ in Eqs. [1 and 2] (i.e., net flux per gram of plant DW) is useful in that it allows us to represent net flux according to plant demand. For example, when a plant is growing quickly, if much biomass is added while total plant nutrient content does not increase in a similar fashion, a dilution in nutrient concentration will occur. The decreased internal nutrient status will lead to increased absorption rates as in Table 5.

When using depletion experiments to determine kinetic parameters, one must be careful with the initial measurements. In our experiments, roots were not rinsed before experiment initiation; loss of nutrients from the root apoplast likely explains the initial efflux of nutrients, particularly from control plants. Clark et al. (2000) also noted a substantial efflux of P early in their nutrient depletion studies and that efflux increased with increased tissue P status. Plants deprived of K or PO$_4$ showed rapid uptake during the first measurement period, which is likely explained by rapid adsorption to the root apoplast. The K data of Teo et al. (1992) also show this rapid initial influx. In the case of NO$_3$-deprived plants, maximum absorption rates were reached $\approx$3 h from initiation of the depletion experiment. The inducible component of NO$_3$ transport system at low concentrations is well known (Hole et al., 1990; Siddiqi et al., 1990); and this phenomenon has also been observed for rose roots (Cabrera et al., 1996).

During the 12-h NO$_3$ depletion experiment, the solution CI concentration was 5.5 mm. Because CI is known to competitively inhibit NO$_3$ absorption (Bar-Yosef et al., 2006; Silberbush et al., 2005), it is likely that our calculated NO$_3$ kinetic parameters underestimate NO$_3$ uptake under conditions of minimal solution CI. For example, in the model by Silberbush et al. (2005), NO$_3$ $I_{\text{max}}$ declines by $\approx$2% for every 1 mm increase in solution CI.

**Potential application to fertilization strategy.** This work quantifies the influence of rose plant nutritional status on the kinetics of NO$_3$, PO$_4$, and K absorption. It would be useful to connect the model for nutrient absorption under varying $C_s$ and $C_p$ (Eq. [2]) with a model for plant growth given environmental conditions. This could enable decision support for fertilization based on plant growth rates and internal nutrient status. In the current experiment, the relationship among $C_p$, $C_s$, and $I_o$ for each nutrient was measured at one time point during a crop cycle. Because rose plant nutrient absorption is known to vary during the crop cycle (Cabrera et al., 1995a; Mattsson, 2007), it would be necessary to also relate Eq. [2] to crop developmental stage. The nutrient uptake measurements in this experiment took place 24 to 31 d after crop cycle initiation. Therefore, given our environmental conditions, depletion experiments took place when flower shoots had unfolded all their leaves and had well developed flower stems; this places them at the stage of most rapid nutrient absorption (Cabrera et al., 1995a; Mattsson, 2007). It would be interesting to repeat this experiment at the phenological stage that represents minimum nutrient absorption, which typically occurs for roses between the stage of budbreak and the appearance of visible flower buds. It should also be noted that the results of this project cannot be extrapolated to $C_s$ or $C_p$ outside the ranges used in the experiment. In the case of $C_s$, much higher solution concentrations are commonly used in commercial horticulture. Nutrient absorption at higher $C_s$ involves the low affinity transport system, which likely responds differently to internal nutrient status.

The finding that roses show increased absorption rates with decreased tissue levels and the finding that short-term nutrient deprivation does not affect plant growth rate suggest that plant yield could be maintained using a variety of fertilization strategies. In open irrigation systems, intermittent periods of phloem-mobile macronutrient supply, as suggested by Cabrera et al. (1996), could reduce runoff to the environment because fertilization would happen periodically rather than continuously; and when fertilization does occur, roots would have an increased capacity to absorb these nutrients. In closed irrigation systems, models predicting nutrient absorption could be used to estimate the fraction of nutrient remaining in recaptured water after irrigation events. This information could be used to maintain nutrient concentrations and nutrient balances at an optimal level. More research is needed to test these fertilization strategies before they could be applied in a commercial setting.

**Literature Cited**


