Nitrate Concentration Effects on NO₃-N Uptake and Reduction, Growth, and Fruit Yield in Strawberry

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ABSTRACT. Strawberries (Fragaria ×ananassa Duch. ‘Osogrande’) were grown hydroponically with three NO₃-N concentrations (3.75, 7.5, or 15.0 ms) to determine effects of varying concentration on NO₃-N uptake and reduction rates, and to relate these processes to growth and fruit yield. Plants were grown for 32 weeks, and NO₃-N uptake and nitrate reductase (NR) activities in roots and shoots were measured during vegetative and reproductive growth. In general, NO₃-N uptake rates increased as NO₃-N concentration in the hydroponics system increased. Tissue NO₃ concentration also increased as external NO₃-N concentration increased, reflecting the differences in uptake rates. There was no effect of external NO₃-N concentration on NR activities in leaves or roots during either stage of development. Leaf NR activity averaged $\approx 360 \text{ nmol NO}_3^{-}\text{formed/g fresh weight (FW)/h}$ over both developmental stages, while NR activity in roots was much lower, averaging $\approx 115 \text{ nmol NO}_3^{-}\text{formed/g FW/h}$. Vegetative organ FW, dry weight (DW), and total fruit yield were unaffected by NO₃-N concentration. These data suggest that the inability of strawberry to increase growth and fruit yield in response to increasing NO₃-N concentrations is not due to limitations in NO₃-N uptake rates, but rather to limitations in NO₃ reduction and/or assimilation in both roots and leaves.

Several studies have examined effects of N levels on strawberry (Fragaria ×ananassa) growth and fruit yield (Albregts and Howard, 1986; Lamarre and Learue, 1995; Locascio and Saxena, 1967), but most of these field studies have given conflicting results. For example, some studies report no increase in yield as N rates increased from 50 to 200 kg·ha⁻¹ (Lamarre and Learue, 1995; Locascio and Saxena, 1967), while others report a significant increase in that range (Albregts and Howard, 1986). Although field studies are the best and easiest means of determining optimum N rate for particular cultivars under particular environmental conditions, they are not designed to examine the effects of N levels on the rate and/or extent of N uptake and assimilation and relate these physiological processes to fruit yield.

The nitrogen form (i.e., NO₃⁻ vs. NH₄⁺) taken up preferentially by plants depends partly on species. In many crops, including strawberry, N uptake rates and/or growth and yield responses are greater when N is supplied as NO₃-N compared with NH₄-N (Gammore-Neumann and Kafkafi, 1985; Kafkafi, 1990). Nitrate reductase (NR) activity is considered to be the rate-limiting step in NO₃ assimilation, and its activity was reported to be highly correlated with plant growth and yield (Shen et al., 1993). Induction of NR is regulated, in part, by cytoplasmic NO₃⁻ concentrations; as NO₃⁻ levels increase, NR mRNA and protein increase (Campbell, 1996). Since NO₃⁻ influx rate and cytoplasmic NO₃⁻ concentrations increase as the external NO₃⁻ concentration increases (Kronzucker et al., 1995), it might be expected that NR activity, plant growth, and yield would also increase. However, most NO₃⁻ assimilation studies are short term, and long-term effects of external NO₃⁻ concentrations on these parameters are unknown in many crops, including strawberry. Additionally, most research on NO₃⁻ assimilation has focused primarily on annual herbaceous plants. Although strawberry is often referred to as a herbaceous perennial, it is in fact, a woody perennial, as evidenced by lignification and production of woody tissue with age (Larsen, 1994). The objectives of the present study were to 1) determine effects of different external NO₃-N concentrations on NO₃⁻ uptake and NR activity and 2) correlate any changes observed in NO₃⁻ uptake/assimilation with changes in growth and fruit yield in strawberry.

Materials and Methods

In February 1996, runners of the short day cultivar Osogrande (still attached to the mother plants) were planted in a greenhouse in 60×45×10 cm trays containing 1 perlite: 1 vermiculite (by volume) until rooted. Day/night greenhouse temperatures averaged 24/18 °C with a 16 h photoperiod. Natural daylength ranged from 11 to 13 h during this time, and daylength extension was provided by high pressure sodium lamps [photosynthetic photon flux (PPF) =700 µmol·m⁻²·s⁻¹].

On 21 Apr., rooted daughter plants were detached from the mother plants, roots were cleaned, and plants were transferred to a growth chamber (E15; Conviron, Controlled Environments, Inc., Asheville, N.C.). Chamber conditions were initially set at 25/15 °C day/night, with a 14-h photoperiod of 600 µmol·m⁻²·s⁻¹ PPF to encourage vegetative growth. On 18 June, chamber conditions were changed to 20/16 °C day/night and a 12-h photoperiod to promote reproductive growth. PPF during this stage remained at 600 µmol·m⁻²·s⁻¹. The light source was a combination of 16 VHO (very high output) fluorescent lamps (160 W) plus 12 extended life tungsten incandescent lamps (60 W). The experimental setup consisted of three separate hydroponic systems using a recirculating (i.e., solutions were not changed during the experiment) nutrient film technique (NFT) (Graves, 1983). The solutions were modified half-strength Hoagland’s (Arnon and Hoagland, 1940), in which the NO₃⁻ concentrations were 3.75, 7.5, or 15.0 ms. These concentrations were chosen based on previous work examining NO₃-N effects on strawberry production in an NFT hydroponic system (Stutte, 1996). Nitrate was added as KNO₃ and Ca(NO₃)₂. In order to counter balance K and Ca concentration in the 3.75 and 7.50 ms treatments,
initial starting solutions for these contained 1.75 and 0.5 mM KCl, respectively, and 2.75 and 1.5 mM CaSO₄, respectively. Solution pH was manually maintained between 5.5 and 6.5 by addition of H₃PO₄.

Daily N use was determined by measuring solution NO₃⁻ deple- tion with a nitrate probe (Cardy, Spectrum Tech., Inc., Plainfield, Ill.) and/or spectrophotometrically (Goldsmith et al., 1973). Ten microliters nutrient solution was diluted with 1.5 mL H₂O, followed by addition of 15 µL 12 n HCl. Absorbance was read immediately at 210 nm (UV-160; Shimadzu Scientific Instruments, Inc., Columbia, Md.). Daily N depletion in each treatment was calculated, and the original N concentrations were restored on a daily basis by addition of concentrated nutrient replenishment solutions.

Each NFT system contained four replicate growth trays (11.4 × 85.7 × 6.4 cm), with each tray holding five plants. Solution volumes were 40 L and the flow rate through each tray averaged 1 L min⁻¹. Daily records of water use, acid additions for pH control, and nutrient replenishment solution additions were maintained.

At bloom, flowers were tagged and hand pollinated. Ripe fruit were harvested and number and fresh weight (FW) were determined. Fruit were dried at 70°C for 96 h to determine dry weight (DW).

Net carbon exchange rates (NCERs) and NR activities were measured during the vegetative and reproductive stages. Single leaf NCER measurements were taken on one recently matured leaf on each plant using a portable, closed gas exchange system (LI-6200; LI-COR, Lincoln, Nebr.). Measurements were taken in the growth chambers on 11 June, when all plants were still in the vegetative stage, and on 9 Oct., during the middle of the reproductive stage. Three measurements were taken per leaf and averaged.

Root and shoot NR activity was measured in mid-June and mid-October using a modified in vivo procedure (Jaworski, 1971). About 250 mg young, white root tissue or ≈50 mg mature leaf tissue were incubated in 30 mM KNO₃ (buffered with 100 mM KH₂PO₄, pH 7.5) at 31°C for 1 h in the dark. After incubation, 1-mL aliquots were reacted with 1 mL 58.0 mM sulfanilamide and 1 mL 0.8 mM (1-naphthyl)ethylenediamine and absorbance was measured at 540 nm.

The experiment was terminated after 32 weeks and plants were harvested and separated into remaining flowers, developing fruit, leaves, crowns, and roots. Tissue FW was recorded and tissues were dried for DW measurements.

Total N in leaves, roots, crowns, and fruit, and crown was determined using a modification of the automated combustion method (Sweeney, 1989). Dried tissue was ground to pass a 20-mesh (1.27-mm-mesh) screen. A 150 mg sample of dried tissue was encased in a tin container and ignited in a resistance furnace (model TN-300; Leco, St. Joseph, Mich.) at 1050°C. An aliquot of the combustion gas was passed through a copper catalyst to remove O₂ and convert nitrous oxides to N₂, then passed over absorber columns to remove H₂O and CO₂ from the sample. The N concentration was determined by thermal conductivity.

Total extractable NO₃⁻N in leaves, roots, crown, and fruit was determined spectrophotometrically using a modification of the chromotropic acid method (Dahneke, 1990; Sims and Jackson, 1971). Briefly, 0.5 g of dried and ground tissue was extracted in a saturated Ca(OH)₂ solution with a small amount of decolorizing carbon added, then filtered through Whatman 2 paper and adjusted to 50 mL. Two milliliters 0.04 mM antimony dissolved in concentrated H₂SO₄ was added to the extract, and placed in a 0°C ice water bath. Once the sample cooled, 1 mL 0.1% chromotropic acid and 4.5 mL concentrated H₂SO₄ was added. The samples reached maximum color development after 20 min at 20°C, and absorbance at 410 nm was read spectrophotometrically. Total reduced N in tissue was calculated by subtracting NO₃⁻N from total N.

The entire experiment was repeated in 1998. Results from the two experiments were consistent, so data were combined for analysis. Data were analyzed as a randomized complete block design with eight replications and five plants per treatment in each replication. Data were subjected to analysis of variance and mean separation, using least square means or paired t-test, or regression analysis where appropriate (SAS Inst. Inc., Cary, N.C.).

**Results and Discussion**

Cumulative N uptake per plant increased as external NO₃⁻N concentration increased (Fig. 1). The rate of NO₃⁻N uptake during vegetative growth was determined by performing a linear regression of cumulative N uptake per plant for this period (weeks 1 to 11). A similar analysis was done for the reproductive period (weeks 12 to 32). These analyses were applied since a high linear correlation coefficient (r² = 0.99) was found for each of these growth stages at all external NO₃⁻N concentrations. Nitrogen uptake rates during vegetative growth (weeks 1 to 11) increased as external NO₃⁻N concentration increased. During this period, uptake from the 3.75 mM NO₃⁻ solution averaged 2.6 mmol N/plant/week, while uptake from the 15 mM solution averaged 3.4 mmol N/plant/week. During the reproductive stage (weeks 12 to 32) weekly N uptake rate in the 15 mM treatment was greater (≈2.2 mmol/plant/week) than in the 3.75 or 7.5 mM treatments, which were similar (≈1.7 mmol/plant/week). When normalized on a FW or DW basis, these rates are similar to NO₃⁻ uptake rates observed in hydroponically grown strawberry (Ganmore-Neumann and Kafkafi, 1985), as well as hydropionically grown blueberry (Vaccinium corymbosum L. interspecific hybrid) (Merhaut and Durnell, 1995), citrus (Citrus sinensis L.) (Serna et al., 1992), and spruce (Picea glauca [Moench] Voss.) (Kronzucker et al., 1995). Ganmore-Neumann and Kafkafi (1985) also reported that NO₃⁻ uptake rates were higher in fruiting compared with nonfruiting strawberry plants. Temporal differences prevented a statistical comparison of NO₃⁻ uptake rates in fruiting vs. nonfruiting strawberries in our study; however, rates during reproductive development were lower than those measured during the vegetative stage. Although strawberry fruit accumulate significant amounts of N (≈20% of total plant N in our study), much of the N may be remobilized from other organs. Albregts and Howard (1981) found that fruit N levels in field-grown strawberry increased concomitantly with decreasing N levels in all other organs, and concluded that significant remobilization of N to fruit occurred. The similar or greater NO₃⁻ uptake rates observed in the present study during vegetative compared to reproductive stages suggest that N accumulation in the fruit is, at least partially, dependent on remobilized N.

The increased NO₃⁻ uptake with increasing external NO₃⁻N concentration was reflected in tissue NO₃⁻ concentrations (Table 1). In general, NO₃⁻ concentration increased in all organs except the fruit as the NO₃⁻N concentration increased from 3.75 to 15 mM NO₃⁻. This suggests that the maximum NO₃⁻ storage potential of the fruit was reached at the lowest external NO₃⁻N concentration (3.75 mM), so that further increases in external NO₃⁻N concentration did not increase fruit tissue NO₃⁻.

External NO₃⁻N concentration had negligible effect on reduced or total N concentrations in roots, crowns, or fruit (Table 1). Leaf concentration of reduced and total N increased as external NO₃⁻ concentration increased from 3.75 to 7.5 mM, with no additional increase as the external NO₃⁻N concentration increased to 15.0 mM.
Table 1. Effects of external NO₃-N concentration on tissue NO₃-N, reduced N, and total N concentrations in hydroponically grown 'Osogrande' strawberries (n = 12).

<table>
<thead>
<tr>
<th>Organ</th>
<th>External NO₃-N concn (mM)</th>
<th>Tissue N concn (mg g⁻¹ dry wt)</th>
<th>NO₃-N Reduced N Total N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>3.75</td>
<td>0.81 b</td>
<td>14.4 b</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>1.68 a</td>
<td>19.2 a</td>
</tr>
<tr>
<td></td>
<td>15.0</td>
<td>2.03 a</td>
<td>17.2 ab</td>
</tr>
<tr>
<td>Roots</td>
<td>3.75</td>
<td>0.53 b</td>
<td>30.7 a</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>1.36 a</td>
<td>29.0 b</td>
</tr>
<tr>
<td></td>
<td>15.0</td>
<td>1.73 a</td>
<td>29.5 ab</td>
</tr>
<tr>
<td>Crown</td>
<td>3.75</td>
<td>0.52 c</td>
<td>12.0 a</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>1.15 b</td>
<td>11.7 a</td>
</tr>
<tr>
<td></td>
<td>15.0</td>
<td>1.98 a</td>
<td>12.0 a</td>
</tr>
<tr>
<td>Fruit</td>
<td>3.75</td>
<td>1.16 a</td>
<td>12.7 c</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>1.04 a</td>
<td>13.6 b</td>
</tr>
<tr>
<td></td>
<td>15.0</td>
<td>1.19 a</td>
<td>14.9 a</td>
</tr>
</tbody>
</table>

³Mean separation within columns and within organs by least square means, P ≤ 0.05.

Table 2. Nitrate reductase activity in leaves and roots of 'Osogrande' strawberry at two developmental stages (n = 8).

<table>
<thead>
<tr>
<th>Organ</th>
<th>Vegetative stage</th>
<th>Reproductive stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>360.4 a</td>
<td>236.4 a</td>
</tr>
<tr>
<td>Roots</td>
<td>116.3 b</td>
<td>99.2 b</td>
</tr>
</tbody>
</table>

³Mean separation within columns by paired t-test, P ≤ 0.05.

Leaf N concentrations at 7.5 and 15.0 mM external NO₃-N concentrations averaged =20 mg g⁻¹ DW, lower than the 30 mg g⁻¹ DW considered to be sufficient (Albregts and Howard, 1981). However, plants did not exhibit leaf chlorosis or any other leaf problems. Total N concentrations in each of the other organs were similar to those reported previously (Albregts and Howard, 1978; Ganmore-Neumann and Kaufmann, 1985).

Leaf NR activity was significantly greater than root NR activity at all external NO₃-N concentrations (interaction was nonsignificant) and both developmental stages, averaging 360 and 115 nmol·g⁻¹ FW/h, respectively, during vegetative growth, and 235 and 100 nmol·g⁻¹ FW/h, respectively, during reproductive growth (Table 2). Similar activities in strawberry roots and crowns were reported by Claussen and Lenz (1999). In most woody plants, NR predominates in the roots, with little activity found in the shoots (Lee and Titus, 1992; Seith et al., 1994), except under high NO₃-N conditions. Under these conditions, the root system NR is thought to be saturated, and the excess NO₃⁻ is translocated to the leaves for reduction by leaf NR (Lee and Titus, 1992). Although strawberry is a true woody perennial, localization of NR activity is more similar to that of herbaceous plants (Chu et al., 1989; Hucklesby and Blanke, 1987). Regardless of NR localization, NR activities in strawberry tissues were much lower than activities found in many other woody (Bussi et al., 1997; Hucklesby and Blanke, 1987; Lee and Titus, 1992) and herbaceous crops (Hucklesby and Blanke, 1987; Sivasankar and Oaks, 1995).

Activity of NR in roots and leaves did not reflect differences in external NO₃-N concentrations, NO₃-N uptake, or organ NO₃⁻ concentrations among the three treatments. The combined NR activities found in roots and leaves averaged 238 nmol NO₂/g FW/h during the vegetative stage, and 167 nmol NO₂/g FW/h during the reproductive stage, regardless of external NO₃-N concentration (data not presented). Since NR is an NO₃-inducible enzyme (Campbell, 1996), this suggests that the NR systems in roots and leaves of strawberry were saturated at the 3.75 mM NO₃⁻ treatment. This is similar to conclusions reached by Lee and Titus (1992) who observed that apple [Malus sylvestris (L.) Mill. var. domestica (Borkh.) Mansf.] leaf and root NR activity did not increase as external NO₃⁻ concentration increased from 1 to 15 mM. Estimates of weekly NR activity per whole root and leaf during the reproductive stage (using NR activity measurements from mid-October and final root and leaf FW taken at the end of November) indicate that the measured NR activity accounts for only ≈45% of the weekly N uptake during that time (assuming NO₃⁻ reduction occurs only in the light). This is analogous to the situation in spruce (Kronzucker et al., 1995), and although only a rough calculation, it supports the premise that the NR systems were saturated at 3.75 mM NO₃⁻. The additional NO₃⁻ taken up probably represents both specific binding of NO₃⁻ in the apoplast and unidirectional flux into vacuoles for storage (Kronzucker et al., 1995).

Leaf NCER averaged 8.9 ± 1.2 µmol m⁻² s⁻¹ of CO₂ uptake during vegetative growth and 12.6 ± 0.6 µmol m⁻² s⁻¹ during reproductive growth, and was unaffected by NO₃-N concentration during either stage (data not presented). These rates are similar to those found by Choma et al. (1982) in strawberry and suggest that strawberry leaf N concentrations of ≈20 mg g⁻¹ DW, as found in our study, do not limit photosynthesis. Lack of response of leaf NCER to increasing NO₃-N concentration contrasts with work by Moon et al. (1990), which showed that CO₂ assimilation, DW, and organ number increased in two ecotypes of Fragaria chiloensis (L.) Duchn. as N increased from 7 to 14 mM. In that study, however, N was supplied as 40% NH₄-N and 60% NO₃-N. Previous work has shown that strawberry DW increases when plants are supplied with both N sources, compared to either source alone (Ganmore-Neumann and Kafkafi, 1985). Thus, use of NO₃⁻ as the sole N source may not result in optimum growth and fruit yield in strawberry. Although it was not possible to compare statistically due to the temporal difference in measurements, data suggest that leaf NCER may be higher during reproductive compared to vegetative growth. This would agree with previous work in strawberry (Choma et al., 1982; Forney and Breen, 1985) and other fruit crops (Gucci et al., 1991; Palmer, 1992).

Organ FW and DW, fruit number, individual fruit FW, and total fruit yield were unaffected by external NO₃-N concentration. Leaf, root, and crown FWs averaged 26.6, 29.9, and 18.8 g/plant, respectively, across all external NO₃-N concentrations, while leaf, root and crown DWs averaged 12.6, 5.5, and 7.5 g/plant, respectively. There was a mean of 9 fruit/plant, with an average individual weight of 9.3 g/fruit. Total fruit FW and DW averaged 82.7 and 10.4 g/plant, respectively. These data support the leaf CO₂ assimilation data and indicate that lack of NO₃-N concentration effects observed on the single CO₂ assimilation measurement days reflected the overall pattern of CO₂ assimilation and/or partitioning during the experiment. FW allocation to fruit averaged 50% of total plant FW for all treatments, while DW allocation to fruit averaged ≈30%. This is similar to the DW allocation to strawberry fruit found by Schafffer et al. (1986), which averaged ≈35% of total DW.

Our results indicate that under these experimental conditions, NO₃-N uptake rates and intracellular NO₃⁻ concentrations in strawberry increase in response to increasing external NO₃-N concentration. However, neither root nor leaf NR activities were affected by external changes in NO₃-N concentration. Additionally, strawberry
Fig. 1. Cumulative N uptake over 32 weeks in ‘Osogrande’ strawberry grown hydroponically at 3.75, 7.5, or 15.0 mM NO₃⁻N. Data are averaged for all plants within a treatment over 2 years (n = 2).

growth and fruit yield were not affected by these treatments. This suggests that growth and fruit yield of strawberry is limited not by its ability to take up NO₃⁻N, but by its ability to reduce and assimilate NO₃⁻ into the tissue. This limitation may be due to low levels of NR mRNA, protein and/or NR activity, lack of reductants (NADH, NADPH), and/or lack of available carbohydrate skeletons required for assimilation.

**Literature Cited**


