J. Amer. Soc. Hort. Sci. 131(1):5–10. 2006.

Uptake and Assimilation of Nitrate and Iron in Two *Vaccinium* Species as Affected by External Nitrate Concentration

Rebecca L. Darnell[1] and Steven A. Hiss
Horticultural Sciences Department, University of Florida, Gainesville, FL 32611

Additional index words. ferric chelate reductase, nitrate reductase, southern highbush blueberry, sparkleberry, *Vaccinium arboreum*, *Vaccinium corymbosum*

Abstract. Most *Vaccinium* species have narrow soil adaptation and are limited to soils that have low pH, high available iron (Fe), and nitrogen (N) primarily in the ammonium (NH_4^+) form. *Vaccinium arboreum* Marsh. is a wild species that can tolerate a wider range of soil conditions, including higher pH and nitrate (NO_3^-) as the predominant N form. This wider soil adaptation may be related to the ability of *V. arboreum* to acquire Fe and NO_3^- more efficiently than cultivated *Vaccinium* species, such as *V. corymbosum* L. interspecific hybrid (southern highbush). Nitrate and Fe uptake, and nitrate reductase (NR) and ferric chelate reductase (FCR) activities were compared in these two species grown hydroponically in either 1.0 or 5.0 mM NO_3^-. Nitrate uptake rate (on a whole-plant and FW basis) and root NR activity were significantly greater in *V. arboreum* compared with *V. corymbosum*. Iron uptake on a FW basis was also greater in *V. arboreum*, and was correlated with higher root FCR activity than was found in *V. corymbosum*. Increased Fe and NO_3^- uptake/assimilation in *V. arboreum* were reflected in increased organ and whole-plant dry weights compared with *V. corymbosum*. *Vaccinium arboreum* appears to be more efficient in acquiring and assimilating NO_3^- and Fe than is the cultivated species, *V. corymbosum*. This may partially explain the wider soil adaptation of *V. arboreum*.

Production of cultivated blueberry (*Vaccinium* spp.) is limited to acidic (pH 4.0–5.5) soils with high organic matter, readily available Fe, and N primarily in the NH_4^+ form (Erb et al., 1993; Williamson and Lyrene, 1998). However, increased demand for fresh blueberries has led to the expansion of production acreage to areas with sub-optimal soil conditions (Korcak, 1989), such as high pH, low Fe availability, and N primarily in the NO_3^- form. Under these soil conditions, blueberry growth and productivity are restricted, possibly due to limitation in Fe and/or NO_3^- uptake and assimilation (Gough, 1996; Korcak, 1989).

Many dicotyledonous plants respond to Fe deficiency by increasing the activity of the plasmamembrane-bound ferric chelate reductase (FCR) (Curie and Briat, 2003; Hell and Stephan, 2003), which cleaves and reduces Fe^{3+}-chelates to free Fe^{2+}. The free cation is then transported across the membrane by a channel transporter specific for Fe^{2+} (Zaharieva and Romheld, 2000). Once inside the root, Fe^{2+} can be used in root metabolic processes or it can be re-oxidized and chelated to form Fe^{3+}-citrate, which is then transported in the xylem from root to shoot (Brown and Ambler, 1974; Marschner, 1991). The transport of Fe across the leaf plasmamembrane is a crucial step in utilization by the leaf and is regulated by Fe^{3+} reduction via the leaf FCR (Kosegarten et al., 1999). In herbaceous crops, root FCR activity is considered to be the rate limiting step in Fe acquisition (Grusak et al., 1993).

In most plants, NO_3^- is the major form of N taken up and assimilated; however, *Vaccinium* species supplied with NO_3^- as the sole N source often show decreased growth and yield compared with plants supplied with NH_4^+ as the N source (Finn et al., 1991; Korcak, 1989). These symptoms appear to be related to decreased uptake and assimilation of NO_3^- compared with NH_4^+ (Merhaut and Darnell, 1995; Poonnachit and Darnell, 2004). Nitrate assimilation is regulated by nitrate reductase (NR), a cytosolic-localized enzyme that reduces NO_3^- to nitrite (NO_2^-) as the first step in NO_3^- assimilation. Activity of NR is considered to be the rate-limiting step in NO_3^- uptake and assimilation and is often correlated with potential yield (Touraine et al., 2001).

Reduction of NO_3^- may occur in the root, leaves, or both, depending on external NO_3^- concentration and the plant species (Black et al., 2002; Gojon et al., 1991). Root NR activity in *Vaccinium* is similar to activities reported in roots of many other woody species (Black et al., 2002; Poonnachit and Darnell, 2004). However, most woody species exhibit significant leaf NR activity (Smirnoff et al., 1984), which is often markedly greater than corresponding root NR activities (Claussen and Lenz, 1999; Darnell and Stutte, 2001; Hucklesby and Blanke, 1987). In cultivated *Vaccinium* species, however, leaf NR activity is low or nondetectable (Claussen and Lenz, 1999; Smirnoff et al., 1984). Thus, the overall ability of *Vaccinium* to assimilate NO_3^- is reduced compared with many other woody plants. This may result in insufficient NO_3^- uptake and assimilation, and may be a limiting factor in growth of *Vaccinium* under NO_3^- conditions.

Vaccinium arboreum is a wild species native to the southeastern United States (Brooks and Lyrene, 1995) that tolerates a wider range of soil types than cultivated *Vaccinium* species. It typically grows on soils with pH 6.0–6.5, low organic matter (Lyrene, 1997), low Fe availability, and N primarily in the NO_3^- form due to rapid nitrification and/or ammonia volatilization (Mengel, 1994); i.e., soils that cultivated *Vaccinium* tolerate poorly (Brooks and Lyrene, 1995; Lyrene, 1997). The ability to tolerate these types of soils may be related to increased efficiency of Fe and/or NO_3^- acquisition/assimilation in *V. arboreum* compared with cultivated *Vaccinium*. The hypothesis being tested is that *V. arboreum* is better able to assimilate Fe and/or NO_3^- than is the cultivated species, *V. corymbosum* interspecific hybrid, under NO_3^--N conditions.

The objectives were to determine NO_3^- concentration effects on NR and FCR activities, NO_3^- and Fe uptake, and growth in the cultivated species, *V. corymbosum,* and the wild species, *V. arboreum*.

Received for publication 6 June 2005. Accepted for publication 11 July 2005. Florida Agricultural Experiment Station journal series R-10945.
[1]To whom reprint requests should be addressed. E-mail address: rld@ufl.edu

Materials and Methods

Seeds of the wild species, *V. arboreum*, were collected from a natural habitat of distinct plants (most likely representing a single genotype) at Manatee Springs, Fla., in Dec. 2002 and germinated on the surface of Canadian peat under intermittent mist in a greenhouse, with average daily temperatures of 25 ºC and photosynthetic photon flux (*PPF*) of 545 μmol·m^{-2}·s^{-1}. Propagation by seed was required since previous work indicated that stem cutting propagation was unsuccessful. In Feb. 2003, shoot cuttings of the southern highbush blueberry 'Misty' were rooted in 1 peat : 1 perlite (by volume) medium under intermittent mist in a greenhouse with average temperatures of 25 ºC and *PPF* of 545 μmol·m^{-2}·s^{-1}. As germination and rooting proceeded, plants of both species were transplanted to 1-L pots containing pine bark and maintained in the same greenhouse. On 6 May 2004, 10 plants of each species were selected and plant fresh weights (FW) determined. Plants were blocked by size and transferred into 2-L plastic bottles filled with a complete nutrient solution. Plastic bottles were wrapped with aluminum foil to eliminate light infiltration. The nutrient solution contained (mM): 0.5 K_2HPO_4, 1.0 $MgSO_4$, 0.5 $CaCl_2$, 0.09 Fe-diethylenetriaminopentaacetic acid (Fe-DTPA), 0.045 H_3BO_3, 0.01 $MnSO_4$, 0.01 $ZnSO_4$, and 0.2 μM Na_2MoO_4. The nitrogen source was either 1.0 or 5.0 mM KNO_3. For the 1.0 mM KNO_3 treatment, 4.0 mM KCl was added to balance K^+ in both nutrient solution treatments. The nutrient solutions were buffered at pH 5.5 with 10 mM 2-(4-morpholino)-ethane sulfonic acid (MES). This pH was chosen since it is in the optimum range for both species and pH was not a variable in this experiment. Solution pH was monitored daily with a portable pH meter (Accumet 1001; Fisher Scientific, Hampton, N.H.) and maintained at 5.5, using 0.1 N KOH or HCl. Aeration (1 L·min^{-1}) was provided to each bottle by an aquarium pump (Elite 801; Rolf C. Hagen, Mansfield, Mass.), connected to tygon tubing. Nutrient solutions were changed weekly. The amount of solution left in each bottle was recorded and used to determine the water use in each bottle on a weekly basis. Plant water use was corrected for evaporative losses using aerated bottles containing nutrient solution without plants.

Nitrate and iron uptake. Nitrate and Fe uptake were determined weekly by measuring depletion from the nutrient solutions. For nitrate uptake, 10 μL of the nutrient solution was taken from each sample bottle before the solutions were changed each week and diluted with 1.5 mL distilled water. Concentrated HCl (15 μL of 12.1 N) was added and samples were vortexed before reading spectrophotometrically (UV-160; Shimadzu, Kyoto, Japan) in quartz cuvettes at 210 nm.

Iron concentration left in the solution was determined using atomic absorption spectrophotometry (3030B; Perkin Elmer, Norwalk, Conn.) with a hallow cathode lamp as a light source and air-acetylene flame.

Enzyme assays. Root and leaf NR activities were quantified four times during the course of the 15-week experiment, starting 5 weeks after the beginning of treatments. Root tips (1 cm long) or leaf disks (4 mm diameter) were cut, placed in a beaker filled with ice water, and transferred to the lab. Samples were weighed and ≈100 mg tissue FW were placed in each test tube (two tubes per treatment per replication). Two milliliters of assay solution, composed of 2% 1-propanol, 100 mM KH_2PO_4 (pH 7.5), and 30 mM KNO_3 were added to each test tube. One tube per treatment per replication was immediately filtered through Whatman No. 2 paper and used as the time 0 control. Samples were vacuum infiltrated for 5 min and incubated in a shaking water bath at 31 ºC and 100 rpm for 1 h in the dark. After incubation, the assay solution containing tissue samples was filtered and a 1-mL aliquot from each sample was removed to a new tube. One milliliter sulfanilamide (1% w/v in 1.5 N HCl) and 1 mL *N*-(1-naphthyl)-ethylenediaminedihydrochloride (0.02% w/v in 0.2 N HCl) were added. The samples were incubated at room temperature for 30 min. The absorbance of NO_2^- at 540 nm was determined spectrophotometrically (modified from Jaworski, 1971).

Root and leaf FCR activities were quantified four times during the experiment, beginning 7 weeks after the beginning of treatments. Root tips (1 cm long) or leaf disks (4 mm diameter) were cut, placed in a beaker filled with ice water, and transferred to the lab. Samples were weighed and ≈100 mg tissue FW were placed in a test tube. Tissues were rinsed in 0.2 mM $CaSO_4$ for 10 min before transferring to 2 mL assay solution, containing 10 mM $CaSO_4$, 5 mM MES (pH 5.5), 0.1 mM Fe-EDTA, and 0.3 mM sodiumbathophenanthroline disulfonic acid (Na_2-BPDS). Leaf tissues were vacuum infiltrated with assay solution for 10 min. Test tubes containing 2 mL assay solution without tissue were used as controls. Samples and control tubes were incubated for 1 h in a shaking water bath at 100 rpm and 23 °C in the dark. After incubation, a 1-mL aliquot from each tube was transferred into a cuvette and read spectrophotometrically at 535 nm (Shimadzu UV-160). The concentration of Fe(II)-BPDS produced was calculated using the molar extinction coefficient of 22.14 mM·cm^{-1} (Chaney et al., 1972).

Tissue N and Fe determination. Plants were harvested on 20 Aug. 2004, and total plant FW was determined. Plants were then separated into shoots (stems + leaves) and roots, and dry weight (DW) was determined after oven drying at 70 ºC to constant weight. Removal of root tips and leaf disks for FCR and NR activity measurements did not appear to have any impact on root or plant growth, as this represented a small fraction (<0.05%) of the total root or leaf FW. Dried samples were ground through a 20-mesh screen in a Wiley mill (Thomas Scientific, Swedesboro, N.J.) and were analyzed for total Kjeldahl nitrogen (TKN) and Fe. A second sample of dried tissue was ground through a 40-mesh screen and analyzed for NO_3^-.

For TKN, 100 mg tissue was placed into 50-mL digesting tubes. Popes Kjeldahl mixture (2.0 g of 18 K_2SO_4 : 1 $CuSO_4$, w/w) was added to each tube, followed by 2.5 mL sulfuric acid. Glass funnels were inserted in each tube before placing the tubes on a digestion block at 380 °C for 8–10 h. After cooling, the tubes were rinsed with 10–15 mL of distilled (DI) water, vortexed, and brought to 50 mL with DI water. Samples were then filtered (Whatman No. 8 filter paper) and analyzed using the Kjeldahl method (Horneck and Miller, 1998).

For Fe concentration, a dry ash digestion of the tissue was performed. Dried tissue (500 mg) was placed in a muffle furnace at 500 °C for 10–12 h. After cooling, 50 mL of 1 N HCl was added to each sample and the solutions were filtered (Whatman No. 8 filter paper). Iron concentration was determined using inductively coupled argon plasma spectrophotometry (SPECTRO Analytical Instruments, Kleve, Germany).

For NO_3^- concentration, dried tissue (500 mg) was placed in a 50-mL erlenmeyer flask, 50 mL of DI water was added, and samples were incubated on a lateral shaker for 30 min. The samples were filtered with Whatman No. 8 filter paper. The extract was analyzed for NO_3^- concentration by automated cadmium reduction method using a rapid flow analyzer (model 300; Alpkem Corp., Saskatoon, Sask.) at 540 nm (Anderson and Case, 1999).

Experimental design. Treatments were arranged in a 2 × 2 factorial (species × NO_3^- concentration) in a randomized complete-block design with five replications, using a single plant per replicate. Data were analyzed using SAS (SAS Institute, Cary, N.C.) Proc GLM. Mean separation was performed using least square means or *t* test. Daily NO_3^- and Fe uptake rates/plant and final plant FW and DW were analyzed using initial plant FW as a covariate to normalize for differences in initial plant size.

Results

Nitrate uptake rates on a whole-plant basis were significantly greater in *V. arboreum* compared with *V. corymbosum* starting 6 weeks after treatment and continuing through the end of the experiment (Fig. 1A). Only the beginning and ending points for NO_3^- uptake could be normalized for differences in plant FW, since these were the only times plant FW were measured. The specific NO_3^- uptake rates at the end of the first week of treatment were not significantly different between species, with *V. arboreum* averaging 2.0 μmol·g^{-1} FW per day and *V. corymbosum* averaging 1.4 μmol·g^{-1} FW per day ($P \leq 0.20$). However, by the end of the experiment (week 15), the specific rate of NO_3^- uptake was significantly greater in *V. arboreum* (1.9 μmol·g^{-1} FW per day) than it was in *V. corymbosum* (0.9 μmol·g^{-1} FW per day) ($P \leq 0.02$).

Increasing external NO_3^- concentration from 1.0 to 5.0 mM did not consistently increase NO_3^- uptake rates on a whole-plant basis, which ranged from 0.08 to 0.47 mmol/plant per day (Fig. 1B). However, total NO_3^- taken up during the experiment was higher at the 5.0 mM compared with the 1.0 mM NO_3^- concentration (22.9 vs. 17.4 mmol NO_3^-, respectively; $P \leq 0.04$). There was no interaction between external NO_3^- concentration and species on NO_3^- uptake rates.

Root NR activity was similar between species at 5 weeks after treatment, averaging 39 nmol NO_2^- per gram FW per hour (Fig 2). By 9 weeks after treatment, NR activity in *V. arboreum* roots was almost 2-fold greater than root activities in *V. corymbosum* (184 vs. 97 nmol NO_2^- per gram FW per hour, respectively). This difference continued throughout the experiment, and by week 15, root NR activity in *V. arboreum* was 3-fold higher than that in *V. corymbosum*. Leaf NR activities were low or nondetectable in both species, ranging from 0 to 2.4 nmol NO_2^- per gram FW per hour (data not shown). There was no effect of external NO_3^- concentration on root or leaf NR activity, and there were no interactions between external NO_3^- concentration and species on NR activity.

There was no consistent effect of species or external NO_3^- concentration on whole-plant Fe uptake rate, which varied throughout the experiment, ranging from 0.33 to 3.62 μmol/plant per day (data not shown). However, specific Fe uptake rates for the beginning and end of the experiment were greater in *V. arboreum* compared with *V. corymbosum* (0.04 vs. 0.01 μmol·g^{-1} FW per day, respectively, at week 1, $P \leq 0.05$; and 0.08 vs. 0.05 μmol·g^{-1} FW per day, respectively, at week 15, $P \leq 0.10$).

Root FCR activity in *V. arboreum* averaged ≈80 nmol·g^{-1} FW per hour throughout the experiment, significantly greater than root activity in *V. corymbosum*, which averaged ≈50 nmol·g^{-1} FW per hour (Fig. 3). There was no effect of external NO_3^- concentration on root FCR activity in either species, which averaged ≈65 nmol·g^{-1} FW per hour (data not shown). There was no interaction between external NO_3^- concentration and species on root FCR activity, except during week 10, when root FCR activity in *V. arboreum*

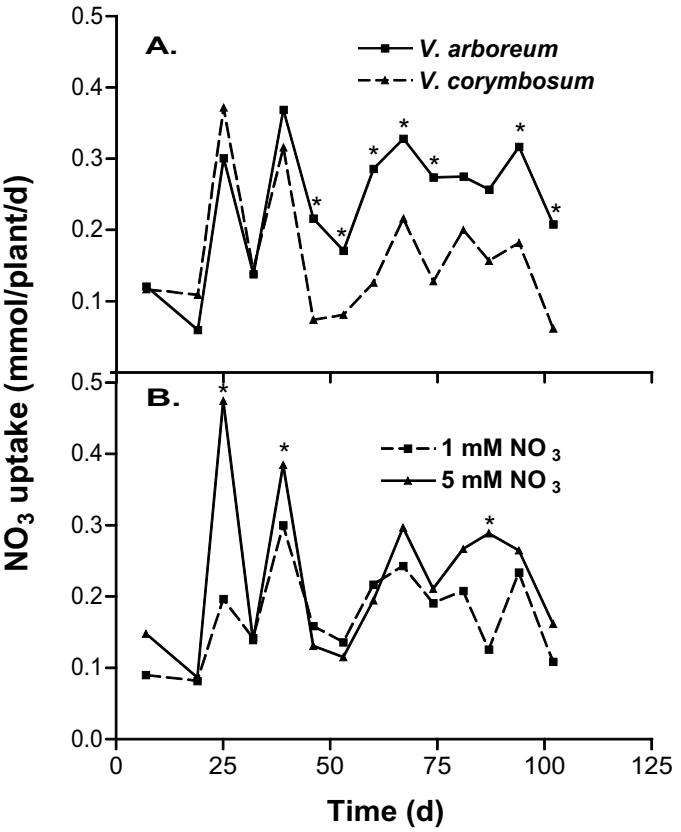

Fig. 1. Species (**A**) and nitrate concentration (**B**) effects on nitrate uptake in *Vaccinium corymbosum* and *V. arboreum*. Values are adjusted using initial whole-plant fresh weight as a covariate. * denotes significant differences within sampling date, $P \leq 0.05$, n = 5.

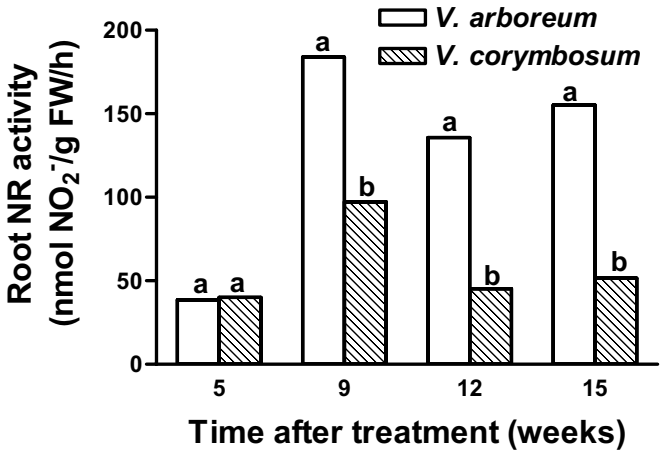

Fig. 2. Species effect on root nitrate reductase (NR) activity in *Vaccinium corymbosum* and *V. arboreum*. Mean separation within sampling date by *t* test, $P \leq 0.05$.

grown at 1 mM NO_3^- was significantly greater (≈110 nmol·g^{-1} FW per hour) than root activity at 5 mM NO_3^- (≈67 nmol·g^{-1} FW per hour) and greater than activity measured in *V. corymbosum* roots at either external NO_3^- concentration (≈47 nmol·g^{-1} FW per hour). Leaf FCR activity was not affected by either external NO_3^- concentration or species, and averaged 25 nmol·g^{-1} FW per hour throughout the experiment.

Total and organ FW and DW were significantly greater in

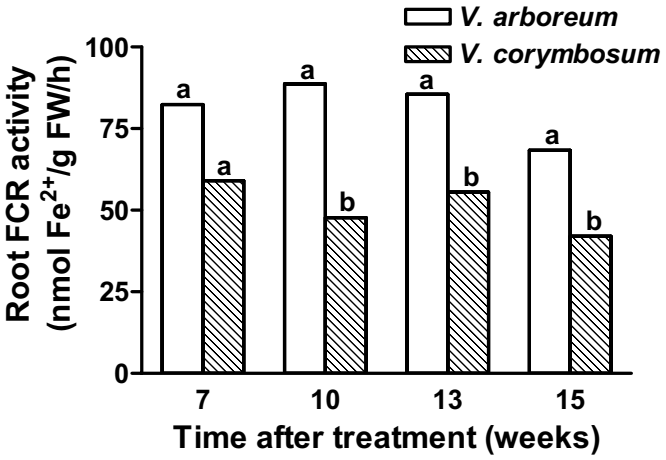

Fig. 3. Species effect on root ferric chelate reductase (FCR) activity in *Vaccinium corymbosum* and *V. arboreum*. Mean separation within sampling date by *t* test, $P \leq 0.05$.

Table 1. The main effects of species and external NO_3^- concentration on final whole-plant FW and DW of roots, shoots, and whole plants of *Vaccinium corymbosum* and *V. arboreum*. Values are adjusted means using initial whole-plant FW as a covariate (n = 5).

	Final	DW (g)		
	plant FW (g)	Root	Shoot	Plant
Species				
V. arboreum	130.4 a[z]	17.0 a	24.4 a	41.3 a
V. corymbosum	82.9 b	9.3 b	8.7 b	18.1 b
NO_3^- (mM)				
1.0	115.8 a	13.0 a	17.2 a	30.2 a
5.0	97.5 a	13.3 a	15.9 a	29.2 a

[z]Mean separation within columns and within species or NO_3^- concentration by *t* test, $P < 0.05$.

V. arboreum compared with *V. corymbosum* by the end of the experiment (Table 1). There was no effect of external NO_3^- concentration on final plant FW, DW, or individual organ DWs. There was no interaction between external NO_3^- concentration and species on FW or DW.

Vaccinium corymbosum shoots (leaves + stems) had significantly greater TKN concentrations than did *V. arboreum* shoots, which resulted in increased whole-plant TKN concentrations in *V. corymbosum* (Table 2). There was no difference in root TKN concentrations between species. Whole-plant NO_3^- concentration in *V. corymbosum* was significantly greater than in *V. arboreum*, due to higher root concentrations, which averaged ≈91 μmol·g^{-1} DW in *V. corymbosum* compared with ≈54 μmol·g^{-1} DW in *V. arboreum*. *Vaccinium corymbosum* roots also had higher Fe concentrations (≈1.7 mg·g^{-1} DW) compared with roots of *V. arboreum* (≈1.2 mg·g^{-1}DW), and consequently, higher whole-plant Fe concentrations. No differences in shoot NO_3^- or Fe concentrations were observed between species.

In general, external NO_3^- concentration had little effect on root, shoot, or whole-plant TKN, NO_3^-, or Fe concentrations, with the exception of higher root, and therefore whole-plant, NO_3^- concentrations in *V. corymbosum* compared with *V. arboreum*. There were no interactions between species and external NO_3^- concentration on tissue levels of TKN, NO_3^-, or Fe.

Discussion

Vaccinium arboreum clearly has increased capacity for NO_3^- and Fe assimilation compared with *V. corymbosum*, as indicated by higher root NR and FCR activities. The average root NR activity in *V. arboreum* was 2- to 3-fold higher than root activity in *V. corymbosum*. Root NR activities in both species were lower than activities reported for some woody species, such as apple (*Malus ×domestica* Borkh.) (Lee and Titus, 1992) and calamondin (*Citrus madurensis* Lour.) (Hucklesby and Blanke, 1987), but higher than root activities reported in strawberry (*Fragaria ×ananassa* Duch.) (Darnell and Stutte, 2001).

Many woody plants exhibit significant leaf NR activity (Smirnoff et al., 1984), often greater than the corresponding root NR activities (Claussen and Lenz, 1999; Darnell and Stutte, 2001). However, leaf NR activities in *V. arboreum* and *V. corymbosum* were undetectable in the present study, a finding supported by work with other cultivated and wild *Vaccinium* species (Claussen and Lenz, 1999; Smirnoff et al., 1984;). Thus, total NR activity in *Vaccinium* is markedly lower compared with other woody species, resulting in reduced overall ability to assimilate NO_3^-.

Although total NR activity in *Vaccinium* is generally lower than in other plants, *V. arboreum* exhibited increased capacity for NO_3^- uptake and reduction compared with *V. corymbosum*. The higher NR activity in *V. arboreum* was reflected in increased NO_3^- uptake rates on a FW basis at the end of the experiment, as well as increased NO_3^- uptake rates on a whole-plant basis throughout the experiment. Higher NR activity also corresponded well with lower root and plant NO_3^- concentrations and increased root, shoot, and whole-plant DW of *V. arboreum* compared with *V. corymbosum*. However, the lower plant TKN concentrations in *V. arboreum* did not reflect the greater capacity for NO_3^- reduction. This was likely due to the greater shoot and root DW in *V. arboreum* resulting in dilution of the reduced N concentration. Thus, total TKN content was greater in *V. arboreum* compared with *V. corymbosum* (478 vs. 254 mg, respectively; $P \leq 0.001$), which reflected the increased capacity for NO_3^- reduction in *V. arboreum*. The increased capacity for NO_3^- uptake and assimilation exhibited by *V. arboreum* would enable this species to grow on soils containing NO_3^- as the predominant N form; conditions that *V. corymbosum* would not tolerate as well.

External NO_3^- concentration had no consistent effect on NO_3^- uptake rate or root NR activity in either species. Nitrate reductase is an NO_3^--inducible enzyme, and increasing NO_3^- concentration increases NR activity in many plants (Bussi et al., 1997; Chen et al., 2004). However,

Table 2. The main effects of species and external NO_3^- concentration on root, shoot, and whole-plant total Kjeldahl nitrogen (TKN), NO_3^-, and Fe concentrations in *Vaccinium corymbosum* and *V. arboreum*

	TKN (mg·g^{-1} DW)			NO_3^- (μg·g^{-1} DW)			Fe (mg·g^{-1} DW)		
	Root	Shoot	Plant	Root	Shoot	Plant	Root	Shoot	Plant
Species									
V. arboreum	12.8 a[z]	11.0 b	11.6 b	53.9 b	21.0 a	31.6 b	1.22 b	0.05 a	0.37 b
V. corymbosum	14.6 a	13.7 a	14.1 a	90.7 a	22.1 a	56.4 a	1.68 a	0.06 a	0.91 a
NO_3^- (mM)									
1.0	13.5 a	12.2 a	12.6 a	31.1 b	21.1 a	26.8 b	1.37 a	0.06 a	0.63 a
5.0	14.0 a	12.5 a	13.0 a	113.5 a	22.0 a	61.2 a	1.53 a	0.04 a	0.66 a

[z]Mean separation within columns and within species or NO_3^- concentration by *t* test, $P < 0.05$.

increasing external NO_3^- concentrations from 3.75 to 15 mM in strawberry did not increase root or leaf NR activity, although tissue NO_3^- concentrations did increase (Darnell and Stutte, 2001). Similarly, Lee and Titus (1992) reported no significant increase in leaf, root, or stem NR activity in apple as external NO_3^- concentrations increased from 1 to 15 mM. In the present study, increasing external NO_3^- from 1.0 to 5.0 mM increased cumulative NO_3^- uptake (without increasing NR activity), and therefore increased root and whole-plant NO_3^- concentrations. The additional NO_3^- in root tissues may represent vacuolar storage, but may also reflect NO_3^- accumulation in the root apoplast and therefore may not truly represent tissue NO_3^- concentration. The lack of effect of external NO_3^- concentration on plant DW or TKN concentration is consistent with the lack of effect on NR activity and suggests that NO_3^- concentrations greater than 1.0 mM resulted in luxury consumption.

Vaccinium arboreum exhibited greater rates of Fe uptake on a FW basis at the beginning and end of the experiment compared with *V. corymbosum*. This increased specific uptake rate correlated with the increased root FCR activity measured in *V. arboreum*, supporting the hypothesis that *V. arboreum* is better able to assimilate Fe than is the cultivated species, *V. corymbosum*. The higher root FCR activity in *V. arboreum* compared with *V. corymbosum* differs from previous work, in which no differences in root FCR activity were found between these two species when grown at 0.09 mM Fe and 5.0 mM NO_3^- (Poonnachit and Darnell, 2004). Although no consistent interactions between external NO_3^- concentration and species on root FCR activity were found in the present study (the only significant interaction occurring at week 10), *V. arboreum* generally exhibited greater FCR at the lower compared with the higher external NO_3^- concentration (87.2 vs. 74.5 nmol·g^{-1} FW per hour, respectively) although differences were not significant ($\leq$0.27). Studies with herbaceous plants suggest that high external NO_3^- concentrations in the root or leaf apoplast may decrease FCR activity (Kosegarten et al., 2001). This is apparently due to NO_3^-/H^+ symport across the membrane, which would increase apoplastic pH above the optimum for FCR activity (Moog and Bruggemann, 1994). It may be that high NO_3^- concentration has a similar effect on FCR activity in *V. arboreum*, although this has not been tested.

Although specific rates of Fe uptake at the beginning and end of the experiment were greater in *V. arboreum* compared with *V. corymbosum*, root (and therefore whole-plant) Fe concentrations were lower. This may be due to a dilution effect, since root and plant DWs were significantly greater in *V. arboreum* compared with *V. corymbosum*. Alternatively, it may represent precipitation of iron as hydroxide or phosphate salts in the apoplast of roots, and may not be a true representation of tissue Fe concentration (Hell and Stephan, 2003).

The results from the present study indicate that the wild species, *V. arboreum*, is more efficient in acquiring and assimilating NO_3^- and Fe than is the cultivated species, *V. corymbosum*, under the experimental conditions used. This is supported by higher NR and FCR activities, increased specific uptake rates of NO_3^- and Fe, and increased DW in *V. arboreum* compared with *V. corymbosum*. Our previous work supported our current finding that *V. arboreum* is more efficient at NO_3^- accumulation and assimilation than is *V. corymbosum* (Poonnachit and Darnell, 2004). However, increased Fe uptake and assimilation by *V. arboreum* was not observed in that study, although the methods used were identical in both studies. Differences may be due to the NO_3^- concentration used in that study (5.0 mM), and may indicate that the increased efficiency of Fe uptake and assimilation in *V. arboreum* compared with *V. corymbosum* is manifested better under low NO_3^- concentrations.

The increased efficiency in NO_3^- and Fe uptake and assimilation exhibited by *V. arboreum* compared with *V. corymbosum* occurred at a pH that was within the optimum range for both species. Additional work examining pH effects on NO_3^- and Fe uptake and assimilation in these two species is required in order to determine if this increased efficiency is a primary factor in the ability of *V. arboreum* to grow in higher pH (6.0–6.5) soils than does *V. corymbosum*.

Vaccinium arboreum does not have economical value; however, it may be a useful source of genes for soil adaptation in breeding programs, and/or a potential rootstock for cultivated *Vaccinium* species. Additionally, root NR activity may be useful as a tool to screen *Vaccinium* genotypes for broader soil adaptation.

Literature Cited

Anderson, K.A. and T.E. Case. 1999. Evaluation of plant extraction techniques and effect on commonly used analytical methods of detection. Commun. Soil Sci. Plant Anal. 30:1479–1495

Black, B.L., L.H. Fuchigami, and G.D. Coleman. 2002. Partitioning of nitrate assimilation among leaves, stems and roots of poplar. Tree Physiol. 22:717–724.

Brooks, S.J. and P.M. Lyrene. 1995. Characteristics of sparkleberry × blueberry hybrids. Proc. Fla. State Hort. Soc. 108:337–339.

Brown, J.C. and J.E. Ambler. 1974. Iron-stress response in tomato (*Lycopersicon esculentum*) 1. Sites of Fe reduction, absorption and transport. Physiol. Plant. 31:221–224.

Bussi, C., A. Gojon, and L. Passama. 1997. In *situ* nitrate reductase activity in leaves of adult peach trees. J. Hort. Sci. 72:347–353

Chaney, R.L., J.C. Brown, and L.O. Tiffin. 1972. Obligatory reduction of ferric chelates in iron uptake by soybeans. Plant Physiol. 50:208–213.

Chen, B.M., Z.H. Wang, S.X. Li, G.X. Wang, H.X. Song, and X.N. Wang. 2004. Effects of nitrate supply on plant growth, nitrate accumulation, metabolic nitrate concentration and nitrate reductase activity in three leafy vegetables. Plant Sci. 167:635–643.

Claussen, W. and F. Lenz. 1999. Effect of ammonium or nitrate nutrition on net photosymthesis, growth, and activity of the enzymes nitrate reductase and glutamine synthetase in blueberry, raspberry and strawberry. Plant Soil 208:95–102.

Curie, C. and J. Briat, 2003. Iron transport and signaling in plants. Ann. Rev. Plant Biol. 54:183–206.

Darnell, R. and G.W. Stutte. 2001. Nitrate concentration effects on NO_3-N uptake and reduction, growth, and fruit yield in strawberry. J. Amer. Soc. Hort. Sci. 125:560–563.

Erb, W.A., A.D. Draper, and H.J. Swartz. 1993. Relation between moisture stress and mineral soil tolerance in blueberries. J. Amer. Soc. Hort. Sci. 118:130–134.

Finn, C.E., J.J. Luby, C.J. Rosen, and P.D. Ascher. 1991. Evaluation in vitro of blueberry germplasm for higher pH tolerance. J. Amer. Soc. Hort. Sci. 116:312–316.

Gojon, A., C. Bussi, C. Grignon, and L. Salsac. 1991. Distribution of NO_3^- reduction between roots and shoots of peach tree seedlings as affected by NO_3^- uptake rate. Physiol. Plant. 82:505–512.

Gough, R.E. 1996. Blueberries—North and south. J. Small Fruit Viticult. 4:71–106.

Grusak, M.A., L.V. Kochian, and R.M. Welch. 1993. Spatial and temporal development of iron (III) reductase activity in root systems of *Pisum sativum* (Fabaceae) challenged with iron-deficiency stress. Amer. J. Bot. 80:300–308.

Hell, R. and U.W. Stephan. 2003. Iron uptake, trafficking, and homeostasis in plants. Planta 216:541–551.

Horneck, D.A. and R.O. Miller, 1998. Determination of total nitrogen

in plant tissue, p. 75–83. In: Y.P. Kalra (ed.). Handbook of reference methods for plant analysis. CRC, Boca Raton, Fla.
Hucklesby, D.P. and M.M. Blanke. 1987. Limitation of nitrogen assimilation in plants. I. Photosynthesis, nitrate content and distribution and pH-optima of nitrate reductase in apple, citrus, cucumber, spinach and tomato. Gartenbauwissenschaft. 52:176–180.
Jaworski, E.G. 1971. Nitrate reductase assay in intact plant tissues. Biochem. Biophys. Res. Commun. 43:1274–1279.
Korcak, R.F. 1989. Variation in nutrient requirements of blueberries and other calcifuges. HortScience 24:573–578.
Kosegarten, H., B. Hoffmann, and K. Mengel. 2001. The paramount influence of nitrate in increasing apoplastic pH of young sunflower leaves to induce Fe deficiency chlorosis, and the regreening effect brought about by acidic foliar sprays. J. Plant Nutr. Soil Sci. 164:155–163.
Kosegarten, H., B. Hoffmann, and K. Mengel. 1999. Apoplastic pH and Fe^{3+} reduction in intact sunflower leaves. Plant Physiol. 121:1069–1079.
Lee, H.J. and J.S. Titus. 1992. Nitrogen accumulation and nitrate reductase activity in MM. 106 apple trees as affected by nitrate supply. J. Hort. Sci. 67:273–281.
Lyrene, P.M. 1997. Value of various taxa in breeding tetraploid blueberries in Florida. Euphytica 94:15–22.
Marschner, H. 1991. Symposium summary and future research areas, p. 365–372. In: Y. Chen and Y. Hadar (eds.). Iron nutrition and interactions in plants. Kluwer, The Netherlands.
Mengel, K. 1994. Iron availability in plant tissues—Iron chlorosis on calcareous soils. Plant Soil 165:275–283.
Merhaut, D.J. and R.L. Darnell. 1995. Ammonium and nitrate accumulation in containerized southern highbush blueberry plants. HortScience 30:1378–1381.
Moog, P.R. and W. Bruggemann. 1994. Iron reductase systems on the plant plasma membrane—A review. Plant Soil 165:241–260.
Poonnachit, U. and R. Darnell. 2004. Effect of ammonium and nitrate on ferric chelate reductase and nitrate reductase in *Vaccinium* species. Ann. Bot. 93:399–405.
Smirnoff, N, P. Todd, and G.R. Stewart.1984.The occurrence of nitrate reduction in the leaves of woody plants. Ann. Bot. 54:363–374.
Touraine, B., F. Daniel-Vedele, and B.G. Forde. 2001. Nitrate uptake and its regulation, p. 1–36. In: P.J. Lea and J-F. Morot-Gaudry (eds.). Plant nitrogen. Springer-Verlag, Berlin.
Williamson, J.W. and P.M. Lyrene. 1998. Florida's commercial blueberry industry. Inst. of Food and Agricultural Sciences, Univ. Florida. Publ. HS 742.
Zaharieva, T. and V. Römheld. 2000. Specific Fe^{2+} uptake system in Strategy I plants inducible under Fe deficiency. J. Plant Nutr. 23:1733–1744.