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^{15}N partitioning in tomato: vascular constraints versus tissue demand

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Abstract. Allocation of root-derived resources is influenced by tissue demand; however, vascular pathways mediate resource flow from roots to shoots. In vascularly constrained plants (i.e. sectored plants), effects of vascular connections likely limit homogenous resource delivery, especially when environmental resource distribution is patchy. Here, we quantify relative roles of vascular connections, demands by different leaves (i.e. by leaf age and size), and molecule size of transported N compounds (effective sectoriality: nitrate *v.* ammonium) on allocation of ^{15}N in the sectored tomato (*Solanum lycopersicum* L.). Vascular connections were the strongest predictor of both accumulation (amount per leaf; $P<0.0001$) and δ (estimate of concentration; $P<0.0001$) ^{15}N values in mature leaves, but young expanding leaves did not show such dramatically sectored uptake (accumulation: $P=0.0685$; δ: $P=0.0455$), suggesting that sectoriality is less strong in young expanding tissue, especially in the youngest leaf. In patchy environments sectoriality, then, should have large consequences for the ability of a plant to allocate resources in mature tissue; however, young leaves do not appear to experience such strong vascular constraints when building new tissue.

Keywords: ammonium, developmental state, effective sectoriality, ^{15}N isotope, nitrate, patchy resources, *Solanum*, vascular connections.

Introduction

The movement of mineral nutrients from roots to shoots is determined by the arrangement of vascular pathways that serve as conduits for resource delivery. Vascular conduits can be interconnected extensively in a net-like pattern (Orians *et al*. 2005), allowing free movement of resources among plant organs. However, they also can be much less thoroughly connected (Watson and Casper 1984; Hay and Sackville Hamilton 1996; Orians *et al*. 2002, 2004, 2005), contributing to a sectored plant body where not all plant organs are interconnected by vasculature. In plants with net-like vasculature, young, actively growing tissue or tissue with greater biomass is likely to make greater demand for, and thus receive more, resources via phloem (where supply is influenced by solute concentrations in phloem sap and unloading rates) and xylem (where transpiration rates and solute concentrations in xylem influence solute delivery). In sectored plants, resource transport is restricted to specific vascular pathways. As a consequence, at the plant level, available resources may not be distributed in proportion to demand in sectored plants, since not all leaves are connected to all roots. Unequal resource distribution associated with sectorial vascular connections should be particularly acute in patchy environments where only some sources (e.g. individual roots) have access to large quantities of resources (Orians and Jones 2001).

Resource allocation patterns in sectorial plants, then, are complex functions of at least two influences: vascular conduit arrangement and tissue demand. This complexity may also be increased in xylem by subtle interactions generated by the resources themselves, thereby altering the effective sectoriality. In a sectorial plant, xylem conduits connected end-to-end are clearly part of a single sector; yet, there can be some movement of xylem sap contents between adjacent xylem elements via intervessel pits (Zwieniecki *et al*. 2003). This flow through intervessel pits can allow xylem sap

Abbreviations used: LP, lateral pot; MP, main pot.

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to move from one otherwise clearly defined vascular sector to another. Recent evidence suggests hydraulic resistance to flux of xylem sap across these pits is modulated by variable hydraulic conductance across them (Zwieniecki *et al.* 2001, 2003) and pit membrane porosity (Sperry and Hacke 2004). Both conductivity and porosity can vary among species, while conductivity can also vary with the environment (e.g. ion concentration in the sap).

Although the physical arrangement of vasculature cannot change once development is complete, the degree of effective sectoriality in xylem for any one resource may vary depending on the molecule size of the resource moving through vascular pathways. Zwieniecki *et al.* (2001) have shown that an increase in xylem sap ionic concentration can lead to hydrogel contraction and increased conductance across pits (Zwieniecki *et al.* 2001), causing a 20% increase in axial flow and an even larger increase (50%) in flow between adjacent vessels. Thus, the xylem sap chemistry can influence effective sectoriality in resource allocation, independent of the static 'plumbing' of the plant. Furthermore, although membrane pore size varies among species, larger molecules are less likely to move between adjacent vessels (Choat *et al.* 2003; Sano 2004). Resource allocation patterns are thus potentially functions of tissue demand, static vascular architecture laid down during organ development, and effective sectoriality influenced by ionic concentrations in xylem sap and the size of the resource being transported. Definitive evidence for this latter driver of resource allocation — resource-dependent 'effective sectoriality' — has not been explicitly documented in the literature.

To explore variation in effective sectoriality as a function of the size of resource being transported, we note first that N taken up as ammonium is typically assimilated into larger amino acids within the roots to avoid ammonium toxicity (Bloom 1988; but see Schjoerring *et al.* 2002). In contrast, much of the nitrate acquired by roots is transported to and assimilated in leaves (Smirnoff and Stewart 1985; Lopes *et al.* 2004). We hypothesised, therefore, that effective sectoriality, and associated spatial constraints on N distribution from roots to shoots, would be more evident when ammonium is the source of nitrogen to sectorial plants than when nitrate is the N-source.

We used tomato to explore the relative influence of (1) tissue demand, (2) static vascular architecture laid down during development (plumbing), and (3) effective sectoriality (influenced by the size of the resource being transported) on the allocation of nitrogen taken up by roots. We used the stable isotope ^{15}N as it allows us to track transport and fate of supplied nitrogen (Dawson *et al.* 2002). A split-pot design was employed and isotopically labelled nitrate ($^{15}NO_3^-$) or ammonium ($^{15}NH_4^+$) applied to either main or lateral root pots to investigate differences in resource allocation based on vascular connections, leaf developmental state (influencing both the size and activity components of tissue demand), and the source of ^{15}N, nitrate or ammonium. From the high degree of sectoriality previously found in tomatoes, we predicted that vascular connections would be the main determinant of ^{15}N accumulation. Within a given sector, however, we expected leaf developmental state would play a strong role in resource distribution. Additionally, we tested whether ^{15}N applied as ammonium showed a more sectored distribution than ^{15}N applied as nitrate.

Materials and methods

Background information

In tomatoes, transport in both xylem (Orians *et al.* 2002; Zwieniecki *et al.* 2003) and phloem (Orians *et al.* 2000; Bledsoe and Orians 2006) is sectored. Orians *et al.* (2002) showed that when fertiliser is applied to isolated lateral roots, leaves with direct vascular connections to the fertilised roots were larger and had lower concentrations of phenolics. Although xylem is likely to be the dominant pathway for nutrient transport in this study, tomatoes do have both upward and downward phloem transport (Khan and Sagar 1966; Watson and Casper 1984), but phloem pathways are similarly restricted (Bledsoe and Orians 2006). Thus, even if xylem-to-phloem resource exchange occurs (*sensu* van der Schoot and van Bel 1989), patterns of sectoriality should be similar. Additionally, the vascular interconnections between leaves have been determined (Dimond 1966; Zwieniecki *et al.* 2003), and this allows us to test whether the source of nitrogen alters the effective sectoriality.

Tomato grows well with either ammonium or nitrate as the N source (Evans *et al.* 1996), unlike other species with specific N-source preferences (Wanek *et al.* 2002). In tomatoes, ammonium is typically assimilated in the roots (Bloom 1988) and 90% of nitrate is assimilated in the shoots (Andrews 1986). Additionally, Gao *et al.* (1996) found that nitrate reductase activity was 5–7 times higher in leaves than in roots across a range of supplied nitrate concentrations (up to 25 mM).

Plants

On 24 February and 11 March 2002, tomato (*Solanum lycopersicum* L.; Johnny's Hybrid Red Tall Vine Big Beef) seeds were sown in Peters Professional potting soil (Scotts Co., Marysville, OH) in the greenhouse at Tufts University, Medford, MA, USA. Natural light was supplemented with 400-W sodium halide light (16 : 8 light : dark cycle). At the outset, three or four seeds were planted per cone at a depth of 1.3 cm, and thinned to one seedling per cone. Seedlings were watered daily and fertilised with 20–20–20 (NPK) Peters fertiliser (Scotts Co.) ~1 month after sowing, and then twice more over the following two weeks. Plants initially received 5 mL of fertiliser at a concentration of 1 g L^{-1}, and this was increased to 5 mL at a concentration of 2 g L^{-1} as the plants grew.

Leaf phyllotaxy

Tomatoes have alternate leaves that are produced in a spiral manner, such that in two rotations around the stem, five leaves are produced at unique angles (Fig. 1*A*). This pattern is repeated with all new leaves such that the sixth leaf (leaf 6) and eleventh (leaf 11) are orthostichous with the first leaf (leaf 1) and these leaves share all vascular traces (Dimond 1966; Zwieniecki *et al.* 2003). Leaves on the opposite side (180°) of the stem (leaves 2, 5, 7, 10) share no vascular traces with leaves 1, 6, and 11. Adjacent leaves (less than 180°; leaves 3, 4, 8, 9) share partial vascular traces with leaves 1, 6, and 11.

Treatments

Approximately 6 weeks after sowing, when plants were at the four-leaf stage, they were split-root potted into two 10.16-cm square pots taped together. One to three lateral roots below the second leaf were placed

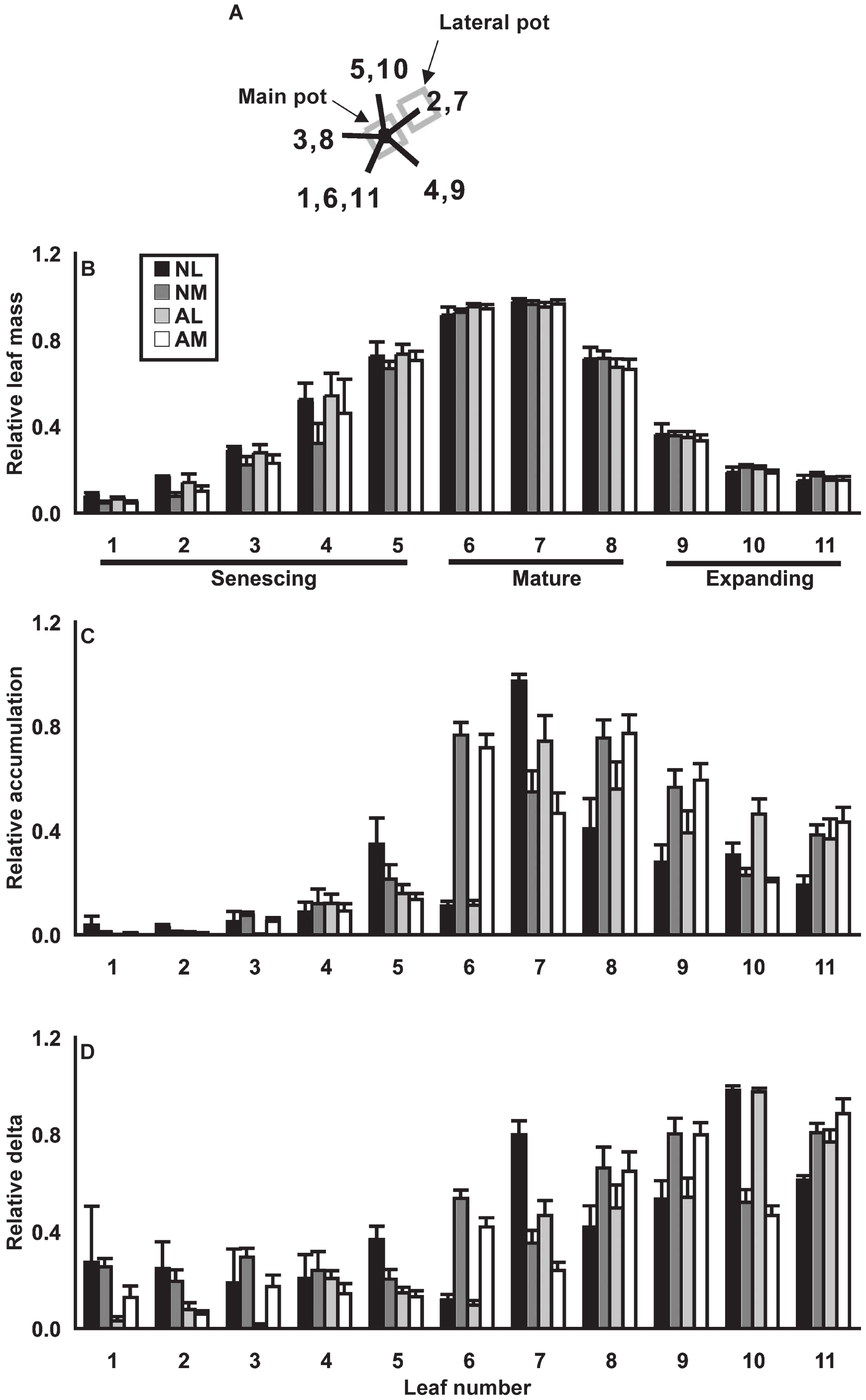

Fig. 1. (*A*) Tomato (*Solanum lycopersicum*) plants in experimental set up, including placement of main and lateral pots and phyllotactic leaf arrangement, and relationships between leaf number. (*B*) Relative leaf mass (mean ± 1 s.e.). (*C*) Relative ^{15}N accumulation (mean ± 1 s.e.). (*D*) Relative δ^{15}N values (mean ± 1 s.e.). Tomato plants are in one of four treatments: NL, nitrate-labelled lateral pot; NM, nitrate-labelled main pot; AL, ammonium-labelled lateral pot; AM, ammonium-labelled main pot. Leaves 2, 5, 7 and 10 are considered 'lateral' as they are directly connected to the root in the lateral pot, leaves 1, 6, and 11 are considered 'main' as they are not directly connected to the root in the lateral pot, and leaves 3, 4, 8, and 9 are considered 'partial' as they are directly connected to both the lateral and main pot roots. Leaves 1–5 are classified as senescing, leaves 6–8 are classified as mature and leaves 9–11 are classified as expanding.

in one pot (lateral pot, LP) and the remaining roots were placed in the second pot (main pot, MP). These plants were grown in MetroMix 510 (Scotts Co.) and Turface (Profile Products LLC, Buffalo Grove, IL) in a 2 : 1 ratio. Fertiliser (30 mL of 2 g L^{-1}) was added to both the main and lateral pots after split-root potting. Twice over the next 2 weeks, 50 mL of fertiliser at 2.0 g L^{-1} was added to the main pot and 50 mL at 0.4 g L^{-1} was added to the lateral pot. Plants were grown to the 11-leaf stage.

^{15}N fertilisation

The uptake of ^{15}N was compared when it was added to fertiliser as ammonium *v.* added as nitrate and when either source of ^{15}N was added to the lateral pot (LP) *v.* to the main pot (MP), making a total of four treatments. A 2.5 g L^{-1} solution of Peters fertiliser was prepared as described above and all plants received 40 mL in both LP and MP. For the ammonium-treated plants, each plant received 105.2 mg L^{-1} ^{15}N-ammonium nitrate (98 atom% ^{15}N), applied either to the MP (MP labelled: $n = 18$) or to the LP (LP labelled; $n = 15$). Similar treatments occurred for nitrate-treated plants (LP labelled: $n = 16$ plants; MP labelled: $n = 17$ plants), except that 101.6 mg ammonium ^{15}N-nitrate (98 atom% ^{15}N) was added instead of labelled ammonium. A third set of plants ($n = 10$), in which only fertiliser solution (no labelled nitrogen) was added to the main and lateral pots, acted as controls. It should be noted that all plants received both nitrate and ammonium in the fertiliser solution but the isotope label was only added to one of the nitrogen sources. All labelled solutions were added ~2 months after sowing.

Tomatoes were then watered twice a day for the next 5 d, at which time all plants were harvested. For each of the four experimental treatments as well as the control, three focal plants were chosen, for which all leaves were analysed for isotope content. In all other plants, leaves 6–11 were analysed. All leaves were dried at 60°C to a constant mass. Leaves were then ground with a KLECO ball mill (model 4200, Garcia Machine, Visalia, CA) and sent to University of California Stable Isotope Facility (Davis, CA) to be analysed for ^{15}N with a Europe Scientific Integra continuous-flow mass spectrometer. Both $\delta^{15}N$ values and mass-based N content were determined. Our sample sizes were reduced in each treatment population (nitrate: LP = 8, MP = 16; ammonium: LP = 13, MP = 15; control = 3) because of pests or the growth of roots from other sectors into the lateral pot.

Gas-exchange measurements

To determine which leaves would draw the most resources, we examined gas-exchange rates on a second set of tomato plants of similar developmental stage to the fertilised plants. The two populations were quite similar in leaf mass for a given leaf number (Fig. 2), with the gas-exchange plants having slightly larger leaves in general. Additionally, while we did not measure leaf area on the ^{15}N treatment plants, leaf area was linearly related to leaf mass in the gas-exchange plants (r=0.998, P<0.001), suggesting that leaf mass can be a good representative of leaf area in the ^{15}N plants. Because $\delta^{15}N$ was greatest in young leaves (see Results), we examined whether the amount of ^{15}N would be related to leaf demand. To do this, we measured gas exchange with a Li-Cor 6400 gas-exchange system (Li-Cor, Lincoln, NE) on three split-root treated tomato plants (Johnny's Celebrity F1) at the 9- to 10-leaf stage on 2 and 3 May 2003. Light was provided by a blue / red LED light source in a temperature-controlled leaf cuvette with a CO_2 mixer attached. To avoid diurnal variation in measures, all measurements were taken between 1000 and 1430 hours. Leaves were allowed to equilibrate before measurements were taken. Transpiration was measured at constant external conditions with average (±1 s.d.) as follows: light = 499.9 (±0.4) µmol photons m^{-2} s^{-1}, temperature = 25.3 (±0.4)°C, external CO_2 concentration = 389.6 (±0.6) µmol mol^{-1}, and vapor pressure deficit = 19.4 (±0.8) mmol mol^{-1}.

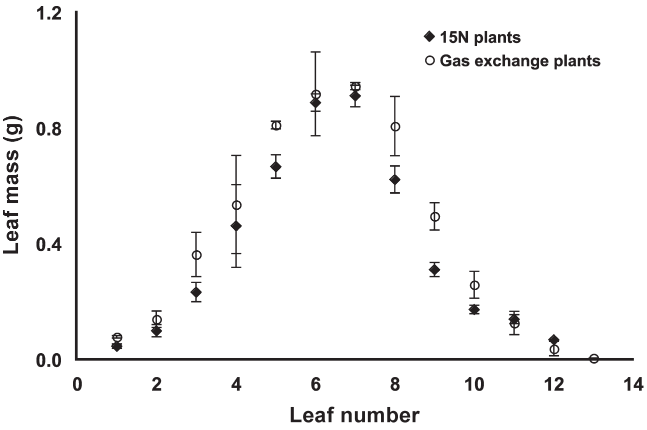

Fig. 2. Relationships between leaf number and mean values (±1 s.e.) of leaf mass for ^{15}N and gas exchange plants.

Isotope analyses

Abundance of ^{15}N in the leaves was calculated as (Dawson *et al.* 2002):

$$\delta^{15}N = (R_{sample} / R_{standard} - 1) \times 1000,$$

with R_{sample} and $R_{standard}$ representing the $^{15}N/^{14}N$ ratios of the sample and standard, and $R_{standard} = 0.0036765$. Total ^{15}N per leaf was calculated as:

$$\text{Total } \mu g\ ^{15}N = \text{Total N (sample)} \times \text{biomass (leaf)} \times (\text{mass\%} \times 0.01).$$

We determined ^{15}N accumulation in micrograms by subtracting background levels of ^{15}N from means of control plants. Since different plants take up different amounts of isotope, accumulation and $\delta^{15}N$ values are presented as relative values, normalised by the maximum leaf value for each plant. We also present leaf mass values as relative leaf mass by normalising all leaf mass values to the largest leaf mass in each plant. Relative accumulation, $\delta^{15}N$ values and relative leaf mass values were $\log_{10}$ transformed for statistical analyses.

The distribution of leaf mass by leaf number showed clear distinctions with leaves 1–5 senescing; leaves 6–8 expanded (mature); and leaves 9–11 expanding (expanding; Fig. 1*B*). Because plants were split-root potted at the 4-leaf stage with leaf 5 already expanding, only the mature and expanding leaf categories (6–11) were included in statistical analyses. We also categorised leaves by their connections to the root in the lateral pot (Orians *et al.* 2002). Leaves 7 and 10 have direct vascular connections to and are on the same side of the plant as the lateral root and are termed 'lateral'; leaves 6 and 11 lack direct vascular connections to, and are on the opposite side of the plant from, the lateral root and are termed 'main'. Leaves 8 and 9 share partial vascular connections to the lateral root and are termed 'partial.'

Statistical analyses

Relative ^{15}N accumulation and relative δ values were analysed by Linear mixed effects models, treating plant as a random effect, with (1) pot receiving the isotope label (= pot; main and lateral), (2) leaf developmental state (= leaf number), and (3) effective sectoriality (= ^{15}N source; nitrate and ammonium) treated as fixed factors, as well as (4) relative leaf mass (= relative mass) treated as a covariate. Interactions between leaf number and either pot or ^{15}N source, as well as the three-way interaction between leaf number, pot, and ^{15}N source were also examined as described below. All analyses were run in R, version 2.1.1 (R Development Core Team 2005). To compare differences among vascular connections, we examined mature leaves

(6 *v.* 7 *v.* 8) and expanding leaves (9 *v.* 10 *v.* 11) separately. For each of these models, the leaf number × pot interaction term tests whether differences in transport based on vascular connections occurred among leaves, and leaf number × pot × ^{15}N source interaction term tests whether different ^{15}N sources showed more sectored transport. We first ran the model with the three-way interaction term, and if not significant, we ran the model again with just the two-way interaction term. To examine differences between mature and expanding leaves, we compared main leaves (6 *v.* 11), as these were the only leaves that had all of their connected roots in only one pot (main). Both lateral and partial leaves had only 1–3 connected roots in the lateral pot and the rest were in the main pot. For these models, the leaf number × pot interaction term tests whether differences in transport based on vascular connections occurred between these mature and expanding leaves, and the leaf number × ^{15}N source interaction term tests whether there are differences in ^{15}N source between these mature and expanding leaves.

Results

Whole-leaf transpiration rates were highest in mature leaves (Fig. 3) suggesting these leaves will have the greatest total accumulation of ^{15}N isotope. Plants in all four treatments (pot receiving the isotope label: main *v.* lateral and ^{15}N source: ammonium *v.* nitrate) had greater accumulation of ^{15}N than controls (data not shown). All leaves accumulated some ^{15}N, suggesting that sectoriality is not absolute; however, accumulation and δ values varied by the vascular connections, developmental state, and to some degree effective sectoriality (source of ^{15}N; Fig. 1*C*, *D*). We describe the role of each below.

Relationships among mature and expanding leaves

The three-way interaction term was not significant in any model (mature leaves: δ, $P=0.8081$; accumulation, $P=0.6203$; expanding leaves: δ, $P=0.3160$; accumulation, $P=0.3341$), suggesting that nitrate and ammonium do not show differences in transport based on vascular connections. After removing the three-way interaction term, the resulting models showed that both accumulation and δ^{15}N were strongly restricted by vascular connections in mature leaves but much less so in expanding leaves, especially the youngest, leaf 11 (Table 1, Fig. 1*C*, *D*). The most significant contributions to both accumulation and δ^{15}N values in mature leaves were the main effect of pot and the interaction of pot with leaf number (Table 1). The main leaf 6 (supplied by roots found only in the main pot) shows this relationship most clearly; it had much higher relative accumulation and δ^{15}N values when ^{15}N was applied to the main pot (Fig. 1*C*, *D*). The lateral leaf 7 showed the opposite pattern with higher values when ^{15}N was applied to the lateral pot; however the differences were less striking than in leaf 6 because leaf 7 is connected to some roots in the main pot. The partial leaf 8 showed no strong differences in values, confirming that it is able to accumulate nitrogen from both the main and lateral pots.

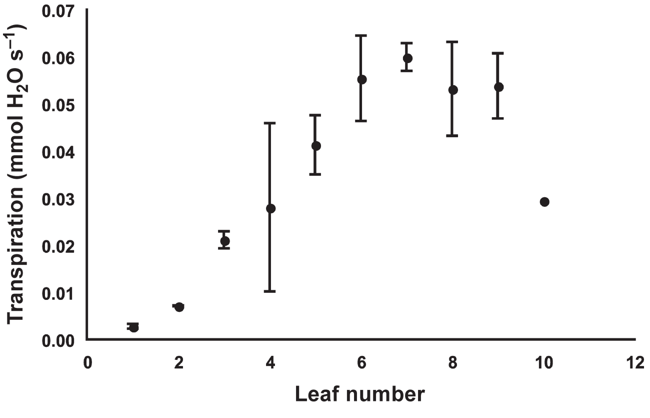

Fig. 3. Relationship between leaf number and mean (±1 s.e.) transpiration rate (values presented as rates per leaf).

Relationships across developmental states in main leaves

Leaves 6 and 11 were used to examine effects of developmental state on nitrogen accumulation and isotopic signature; only these leaves were chosen for the analysis because they alone are a comparable pair of mature and expanding leaves that are fed by roots in only one pot, the main pot (Fig. 1*A*). Developmental stage was a significant predictor of accumulation and δ^{15}N values, especially in combination with pot type (Table 1, Fig. 1*C*, *D*). Leaf 6 had much higher accumulation and δ values when ^{15}N was applied to the main pot; however, leaf 11 showed little difference in its values between main and lateral pot plants (Fig. 1*C*, *D*). Additionally, the interaction between leaf number and ^{15}N source was significant in both the δ and accumulation models (Table 1), suggesting differences in nitrate and ammonium transport with developmental state. In this analysis, relative leaf mass also predicted ^{15}N accumulation. This result confirmed those found in the gas-exchange measurements, where the mature leaves had higher leaf-level gas-exchange rates, suggesting that they should receive more resources per leaf. Finally, expanding leaves had higher δ values, suggesting that more of the N in expanding leaves is comprised by ^{15}N.

Discussion

In this study, we examined the roles of vascular connections, effective sectoriality, and developmental stage in determining nitrogen distribution in tomato. Vascular connection was the strongest factor influencing accumulation (leaf number × pot: $P<0.0001$, Table 1) and δ^{15}N (leaf number × pot: $P<0.0001$) values in mature leaves, consistent with a high degree of sectoriality in tomatoes (Orians *et al.* 2002). However, the effects of tissue demand were not limited to within a sectored pathway. Even though the highest values were within sectors directly connected to roots receiving the isotope label, larger leaves in all sectors had greater ^{15}N accumulation and younger

Table 1. Linear mixed effects model results

Relationships were examined between either relative δ or accumulation ^{15}N values and relative leaf mass, leaf number, pot type, ^{15}N source and interactions between leaf number and pot and leaf number and ^{15}N source. Differences were compared among mature leaves (leaves 6–8), among expanding leaves (leaves 9–11), and between mature and expanding main leaves (leaves 6, 11). In each model, plant was the random factor. Significant variables are in bold ($P \le 0.05$)

Leaf type	Variable	*B*	*P*
Mature	Relative δ		
	Intercept	**1.747**	**0.0095**
	Relative leaf mass	−0.531	0.0974
	Leaf number: 6 *v.* 7 *v.* 8	**−0.265**	**0.0057**
	Pot: main *v.* lateral	**−2.083**	**<0.0001**
	^{15}N source: nitrate *v.* ammonium	**−0.113**	**0.0279**
	Leaf number × pot	**0.274**	**<0.0001**
	Relative accumulation		
	Intercept	**1.976**	**0.0048**
	Relative leaf mass	0.452	0.1719
	Leaf number: 6 *v.* 7 *v.* 8	**−0.264**	**0.0079**
	Pot: main *v.* lateral	**−2.331**	**<0.0001**
	^{15}N source: nitrate *v.* ammonium	−0.032	0.5353
	Leaf number × pot	**0.293**	**<0.0001**
Expanding	Relative δ		
	Intercept	0.237	0.6906
	Relative leaf mass	0.201	0.0753
	Leaf number: 9 *v.* 10 *v.* 11	−0.033	0.5955
	Pot: main *v.* lateral	−0.790	0.0540
	^{15}N source: nitrate *v.* ammonium	0.012	0.6976
	Leaf number × pot	**0.081**	**0.0455**
	Relative accumulation		
	Intercept	−0.122	0.8579
	Relative leaf mass	**1.171**	**<0.0001**
	Leaf number: 9 *v.* 10 *v.* 11	0.035	0.6150
	Pot: main *v.* lateral	−0.918	0.0509
	^{15}N source: nitrate *v.* ammonium	**0.101**	**0.0075**
	Leaf number × pot	0.085	0.0685
Expanding *v.* mature leaves	Relative δ		
	Intercept	**1.015**	**0.0002**
	Relative leaf mass	0.273	0.0623
	Leaf number: 6 *v.* 11	**−0.078**	**0.0199**
	Pot: main *v.* lateral	**−1.447**	**<0.0001**
	^{15}N source: nitrate *v.* ammonium	**−0.321**	**0.0046**
	Leaf number × pot	**0.125**	**<0.0001**
	Leaf number × ^{15}N source	**0.033**	**0.0085**
	Relative accumulation		
	Intercept	**1.205**	**0.0001**
	Relative leaf mass	**1.119**	**<0.0001**
	Leaf number: 6 *v.* 11	−0.068	0.0753
	Pot: main *v.* lateral	**−1.656**	**<0.0001**
	^{15}N source: nitrate *v.* ammonium	−0.251	0.0530
	Leaf number × pot	**0.135**	**<0.0001**
	Leaf number × ^{15}N source	**0.035**	**0.0216**

leaves had greater δ^{15}N values (Fig. 1*C*, *D*). Furthermore, younger leaves showed a much smaller influence of vascular connections on accumulation (leaf number × pot: P=0.0685, Table 1) and δ^{15}N (leaf number × pot: P=0.0455) values than mature leaves, especially the youngest expanding leaf 11 (Fig. 1*C*, *D*). Taken together these results suggest a partial breakdown of sectoriality, particularly in younger tissue.

Vascular connections versus tissue demand

Strongly sectored patterns of resource movement as seen in this study can have large ecological consequences.

In patchy environments, restricted pathways in sectored plants can influence within-plant heterogeneity in nutrient distribution, chemical signalling, or disease transmission (Orians *et al.* 2000, 2004; Tyree and Zimmermann 2002; Schittko and Baldwin 2003). Such heterogeneity then can lead to disparity among sectors in plant function (e.g. growth rates) or form (e.g. leaf shape), and ultimately may affect species interactions (e.g. differential herbivory; Jones *et al.* 1993; Orians and Jones 2001; Orians *et al.* 2002).

Nevertheless, tissue demand (determined by both leaf mass and age) also strongly influenced ^{15}N distribution. Such patterns were especially apparent from comparisons of developmental state in main leaves. Leaf 11, in particular, had comparable accumulation and δ^{15}N values between main and lateral pot isotope treatments, suggesting a breakdown of sectoriality in this very young leaf. Additionally the role of vascular connections was much stronger in comparisons among mature leaves (leaves 6–8) than among expanding leaves (leaves 9–11). These results, then, imply that the strength of the vascular constraints is higher in fully formed, mature tissues, and leakage between sectors is greater in young tissue where pathways are still forming and perhaps not yet fully lignified or close to the apical meristem (further from the root). The mechanism behind this breakdown in sectoriality is unclear and cannot be attributed to supply by a differential pathway (xylem *v.* phloem) to some tissues, since previous studies have found both xylem and phloem pathways to be highly sectored in tomatoes (Orians *et al.* 2000, 2002; Zwieniecki *et al.* 2003; Bledsoe and Orians 2006). However, the results are particularly intriguing and merit further investigation; they suggest that young leaves are not dependent on particular vascular pathways to supply their expanding tissue. One would predict that such plasticity in resource supply would be particularly advantageous in patchy environments.

Larger leaves proved to have the strongest total demand, leading to differential ^{15}N distribution. It seems likely that the large leaves had greater demands at the whole-leaf scale because of greater mass flow associated with greater transpiration. The larger amount of water being transpired would carry more of the ^{15}N to the larger leaves, resulting in greater accumulation. Conversely small young leaves were in the process of expanding and adding new tissue, while older mature leaves had finished expanding. Thus, a larger proportion of the N in young leaves came from the recently acquired ^{15}N (higher δ^{15}N values).

^{15}N source: nitrate versus ammonium

The ^{15}N source did not influence the distribution of ^{15}N based on vascular sectoriality. This result suggests either that the size of the molecule in which ^{15}N is transported does not matter, or that ^{15}N assimilation and transport is more complex than simply that nitrate is primarily transported to the leaves for assimilation while ammonium is assimilated first into larger molecules (amino acids) before transport to the leaves. This possibility merits further exploration to determine whether nitrate reduction and ammonium transport are more plastic in tomatoes than previously realised (Smirnoff and Stewart 1985; Bloom 1988; Gao *et al.* 1996; Schjoerring *et al.* 2002). Small but significant differences were seen in main leaves for accumulation and δ^{15}N values of nitrate *v.* ammonium between the expanding leaf 11 and the mature leaf 6. The robustness of this pattern deserves further attention, as the differences were small. If indeed real, perhaps nitrate reductase activity or nitrate storage is differentially distributed among leaves (Smirnoff and Stewart 1985). Overall, our results suggest that both forms of N (nitrate or amino acids derived from ammonium) are similarly constrained by the degree of sectoriality of the vasculature.

Recently Zwieniecki *et al.* (2003) found that high ionic concentration in xylem sap facilitates flow between vessels, apparently through changes in the hydrogel properties of intervessel pit membranes (Zwieniecki *et al.* 2001). The tomato plants in our experiments were well fertilised and this may have increased xylem sap concentrations to a level that facilitated intervessel flow. Yet the growth of many plants is nutrient limited and such limitation may lead to reduced xylem sap concentrations (Lorenz 1976). If so, nutrient-limited plants may be functionally more sectored. Future experiments should examine the effects of differential soil nutrient concentrations on xylem sap ionic composition, hydrogel properties, hydraulic resistance and patterns of N accumulation among leaves.

Implications and future directions

Vascular constraints in sectored plants can have large consequences for nutrient allocation patterns, especially when resource distribution in the environment is patchy. In fact, vascular constraints can override the demands of large leaves and lead to within-plant heterogeneity. Nevertheless, accumulation was not absolutely restricted to within sectors, indicating that some breakdown in sectoriality exists, especially in young tissue. Further investigations into the mechanisms by which sectoriality changes with developmental state or distance from roots are especially warranted as sectoriality can affect a species' tolerance of differential resource distribution in the environment (e.g. patchy *v.* homogenous) and ecological interactions (e.g. herbivory or competition). Finally, our results indicate that nitrate and ammonium have similar effective sectoriality in tomato, and it would be interesting to determine whether, indeed, different nitrogen-containing molecules are similarly constrained or whether greater nitrate reductase activity is occurring in the roots than has been previously reported. Additionally, differences in effective sectoriality may still occur for other nutrients or in other species, especially if assimilation of different nutrients occurs in different parts of the plant body.

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