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To cite this version:
Sanoussi Atta, Stephane Maltese, Pascal Marget, Roger Cousin. 15NO3 assimilation by the field Pea Pisumsativum L.. Agronomie, EDP Sciences, 2004, 24 (2), pp.85-92. 10.1051/agro:2004003 . hal-00886245

HAL Id: hal-00886245
https://hal.archives-ouvertes.fr/hal-00886245
Submitted on 1 Jan 2004

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15NO₃ assimilation by the field Pea *Pisum sativum* L.

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(Received 10 January 2003; accepted 8 January 2004)

Abstract – The objectives of this study were to determine the effect of low mineral supply on plant growth and the uptake and redistribution of mineral N by different plant organs according to the period of uptake. A glasshouse study was conducted on two pea genotypes, L833 and cv. Frisson, fed without or with 4 mM NO₃. Plants fed with 4 mM N were labelled for 5 days with ¹⁵N at three stages: 7 leaf stage, beginning of flowering, and beginning of seed filling. Plants were harvested at day 6 and at later stages. The results indicated for the two genotypes that supplying 4 mM N to the plants significantly increased their total dry weight up to the beginning of seed filling, whereas nodule dry weight was reduced. Genotype differences in N uptake and redistribution among plant organs were minor. When plants were labelled with ¹⁵N at early stages of growth, about 60% of total plant ¹⁵N was located in leaves. At maturity the proportion of ¹⁵N recovered in seeds was about 60% for both genotypes. When plants were labelled at the beginning of seed filling, ¹⁵N was mainly located in young organs such as upper leaves, pods and seeds. During seed fill the remobilisation of ¹⁵N to seeds occurred from all organs of the plant. At physiological maturity about 70% of ¹⁵N was located in seeds.

Pea *Pisum sativum* / ¹⁵N assimilation / remobilisation / redistribution / flowering / seed filling / physiological maturity

1. INTRODUCTION

One of the pea (*Pisum sativum* L.) breeder’s objectives is to develop varieties with improved harvest and nitrogen indices. These indices are determined both by genetic characteristics and by environmental conditions. The assimilation and distribution of nitrogen in the vegetative and the reproductive parts of grain legumes are key processes involved in the elaboration of seed N. Nitrogen accumulation by seeds during seed filling depends upon the external N supply (N retrieval from the soil and symbiotic fixation of atmospheric N₂) which generally cannot sustain the high demand of developing seeds [32]. Hence, seed growth throughout the plant involves N remobilisation [32, 36]. All organs undergo N remobilisation but the efficiency with which it can be transferred to growing seeds and the rate of remobilisation may vary according to the organ and the regime of N nutrition [35]. As an example, the remobilisation was estimated to be about 60% of fixed nitrogen for Vigna [34] and 40% for Soybean [18]: symbiotically fixed nitrogen was found to be more mobile than nitrogen originating from soil or fertiliser [26] and was transported more quickly to pods and seeds than nitrate nitrogen [14].

In forage legumes, it is generally accepted that the mobilisation of N from vegetative tissues to regrowing foliage after cutting is necessary for defoliation tolerance [6, 39]. In alfalfa, about 34% of N was mobilised from source organs to regrowing tissues within 30 days [6] and regrowth following defoliation is linked to the availability of N reserves in roots rather than that of C reserves [28]. In *Trifolium repens* L., about 55% and 70% of the nitrogen content of roots and stolons, respectively, were mobilised to support the regrowth of leaves [11]. Hence, regrowth after defoliation requires N reserves to initiate new shoot growth because of the strong decrease in nitrogenase activity and/or soil N uptake induced by shoot removal.

Isotopic techniques based on ¹⁵N labelling have proved to be the most effective means of measuring N fluxes between different tissues or organs, and for evaluating the respective contributions of N remobilised from storage tissues or recently acquired by uptake and fixation from the environment. As such, the mobilisation of N reserves during plant growth has been estimated using pulse-chase ¹⁵N labelling [5, 6, 9, 11, 38]. The aims of this work were: (i) to determine the effect of low mineral N (4 mM) supply on plant growth, and (ii) to quantify, by ¹⁵N labelling at different stages of plant growth, the contribution of N reserves to seed growth in field pea (*Pisum sativum* L.).

2. MATERIALS AND METHODS

2.1. Glasshouse experiment

Two genotypes of pea, cv. Frisson and line L833, were sown in pots and grown under glasshouse conditions during
summer 1993. These two genotypes, created at INRA Versailles, are both early flowering genotypes. Line L833 has large seeds (seed dry weight of 250 mg), while Frisson has small seeds (seed dry weight of 150 mg). Previous results [4] indicated that Frisson has the ability to maintain nitrogen fixation after flowering while nitrogen fixation ceases before flowering in the L833 genotype.

Seeds of the two genotypes were germinated for 2 weeks with de-ionised water. Plants were then grown in nutrient solutions, as described previously [20], throughout two experiments: in experiment 1, plants were supplied with 4 mM KNO₃ while in experiment 2 were grown without nitrogen (0 mM KNO₃) in the nutrient solution. The pH of the solution was adjusted to 5.6 with NaOH. For both experiments, seeds were inoculated at sowing by adding to the nutrient solution an appropriate strain of *Rhizobium leguminosarum* bv. *viciae* (MSDJ469 from N. Amarger, INRA Dijon). Four plants were grown on sand in each pot (three litres each) and watered daily with 300 ml of solution. The experimental design was a split plot with 2 factors and 3 repetitions. The first factor, which was in the main plot, was 3 batches of pots for ¹⁵N labelling at 3 growth stages. The second factor was the 2 genotypes. The temperature of the glasshouse was 20 °C day and 15 °C night with a photoperiod of 16 hours day/8 hours night. Natural light was supplemented for 16 h d⁻¹ with fluorescent tubes (Phyto, Claude GTE, Puteaux, France) supplying approximately 150 µmol photons m⁻² s⁻¹ at the canopy height.

### 2.2. Labelling experimental setup

Three batches of plants in experiment 1 were labelled with ¹⁵N. The first batch was labelled when plants were at the 7 leaf stage, about 34 days after sowing. Each pot of four plants received, over five days, a nutrient solution similar to that previously provided but with 4 mM N (KNO₃) containing 66.46% excess ¹⁵N. On day 6, unabsorbed ¹⁵NO₃ was then thoroughly washed through with de-ionised water and the plants were harvested. Thereafter the remaining labelled plants were grown in unlabelled culture solution, and three successive harvests were carried out at the flowering of the first fruiting node (48 days after sowing), at the beginning of seed filling (57 days after sowing) and at physiological maturity of the plant (74 days after sowing). The second and the third batches of plants were labelled and harvested for analysis similarly, but labelling was performed at the flowering of the first node and at the beginning of seed filling, respectively.

### 2.3. Harvesting of plants

Harvested plants were separated into different organs: roots, nodules, stem, upper leaves and lower leaves from above and below the first fruiting node, pods and seeds. Plant parts were dried for 48 hours at 85 °C, weighed and milled for further ¹⁵N and total N analysis.

### 2.4. ¹⁵N and total N determination and calculation

Total N and ¹⁵N concentration in the samples were determined at the Laboratoire de Biochimie et de Physiologie Moléculaire des Plantes (INRA Montpellier, France) with a Roboprep C/N analyser (PDZ Europa, Crewe, UK) coupled to a Tracermass spectrometer. For each organ the ¹⁵N atom excess, *Eo*, was calculated as follows:

\[
Eo = (A%t - A%c)/(A%s - A%c')
\]

where \( A%t = ¹⁵N \) atom % in samples treated with ¹⁵NO₃, \( A%c = ¹⁵N \) atom % in control plants fed with unlabelled solution, \( A%s = ¹⁵N \) atom % in the labelled solution, \( A%c' = ¹²N \) atom % in the control solution. The amount of labelled nitrogen in each organ, Q (organ), was then calculated using the dry weight (DW), the %N content and *Eo* according to the following equation:

\[
Q \text{ (organ)} = DW \times (%N / 100) \times Eo.
\]

The ratio R of nitrogen for each organ of the plant was calculated as follows:

\[
R = Q \text{ (organ)} / Q \text{ (plant)}
\]

where Q (plant) is the total amount of labelled nitrogen in the plant.

### 3. RESULTS

#### 3.1. Dry matter accumulation and partitioning in plant organs

Changes in dry matter accumulation in various plant organs throughout the plant life cycle for the two genotypes were recorded (Tab. I). Total plant dry weight increased greatly from sowing to physiological maturity for the two genotypes. Dry weight accumulation was greatest between the beginning of flowering (48 days after sowing) and physiological maturity (74 days after sowing). During this period, total plant dry weight tripled for the two genotypes. Leaves accumulated more dry matter than the other organs during plant growth (Tab. I). From the beginning of seed filling to physiological maturity, only seed dry matter increased significantly, from 0.46 to 5.77 g/plant for L833 and from 0.32 to 5.30 g/plant for Frisson. At physiological maturity, the total plant dry weight of L833 was higher than that of Frisson: 16.34 g and 12.17 g, respectively. The lower dry matter accumulation in Frisson was attributed to its smaller, semi-determinant growth habit. The contribution of seed to total dry weight was higher for Frisson (43.5%), compared with that for L833 (35.3%).

From sowing to the beginning of seed filling, the total plant dry weight was affected by the NO₃ concentration in the nutrient solution for the two genotypes (Tab. I). Plants fed with 4 mM N had a significantly higher total dry weight at the beginning of seed filling (10.08 g/plant and 7.91 g/plant, respectively, for L833 and Frisson) than the controls (8.64 g and 4.85 g/plant, respectively). The increase in total plant dry weight due to nitrate supply was greater for Frisson (about 60%) than for L833 (17%). For genotype L833, the main increases in total dry weight per plant at the beginning of seed filling were in roots (730 mg), stem (820 mg) and lower leaves (920 mg). For Frisson, the main increase in total dry weight concerned all plant organs except nodules (Tab. I). The supply of 4 mM N in the nutrient solution affected nodule dry weight. Nodule dry weight was greater with 4 mM N-supplied plants than with control plants for both genotypes and at all stages of growth. However, the increased nodules dry weight did not lead to a significant increase in total plant dry weight.
weight for the two genotypes was significantly lower \( (P = 0.001) \) during the whole plant cycle (Tab. I). From the beginning of seed filling to physiological maturity, the rate of dry matter accumulation in some organs such as leaves decreased or even reversed, particularly in plants fed with 4 mM N and especially for the L833 genotype. At the beginning of seed filling, the decrease in nodule dry weight due to nitrate supply was about 80% and 65% for L833 and Frisson, respectively. At physiological maturity, the effect of nitrate supply on plant dry matter was not significant for either of the two genotypes.

### 3.2. Total nitrogen accumulation and partitioning in plant organs

For both genotypes, vegetative organs accumulated the bulk of their nitrogen (N) from the 7 leaf stage to the beginning of seed filling (Fig. 1). During this period, leaves were the main site of N accumulation in the plant. At the beginning of seed filling, the total N per plant was about 360 mg and 300 mg for L833 and Frisson, respectively. The proportion of total plant N recovered in leaves at this time was about 80% and 65% for L833 and Frisson, respectively. At physiological maturity, the effect of nitrate supply on plant dry matter was not significant for either of the two genotypes.

### 3.3. Distribution of \( ^{15}N \) between organs at the end of the labelling period (Day 6)

During the labelling period, the amount of \( ^{15}N \) that was assimilated by the plants was similar for the two genotypes (Tab. III): about 11 mg at the 7 leaf stage and 15 mg at both the beginning of flowering and the beginning of seed filling stages. When plants were labelled with \( ^{15}N \) at the 7 leaf stage (Tab. III), there was no difference between genotypes in N distribution between plant organs. Leaves accumulated most of the \( ^{15}N \) (about 60%) while roots and stems only accumulated 25% and 15%, respectively. This N partitioning, however, varied between organs and between genotypes according to the labelling period.

When plants were labelled with \( ^{15}N \) at the beginning of flowering (Tab. III), the distribution of nitrogen accumulated in different parts of the plant varied between genotypes. Leaves were

---

**Table I.** Total plant dry weight of the two genotypes at different stages of sampling. BSF = beginning of seed filling.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>7 leaves</th>
<th>Beginning of flowering</th>
<th>BSF</th>
<th>Physiological maturity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 mM</td>
<td>4 mM</td>
<td>0 mM</td>
<td>4 mM</td>
</tr>
<tr>
<td>L833</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roots</td>
<td>0.41</td>
<td>0.53</td>
<td>0.32</td>
<td>0.54</td>
</tr>
<tr>
<td>Nodules</td>
<td>0.48</td>
<td>0.11</td>
<td>1.29</td>
<td>1.60</td>
</tr>
<tr>
<td>Stem</td>
<td>0.24</td>
<td>0.46</td>
<td>2.57</td>
<td>3.50</td>
</tr>
<tr>
<td>Lower leaves</td>
<td>0.76</td>
<td>1.20</td>
<td>2.04</td>
<td>2.12</td>
</tr>
<tr>
<td>Upper leaves</td>
<td></td>
<td></td>
<td>0.26</td>
<td>0.19</td>
</tr>
<tr>
<td>Pods</td>
<td></td>
<td></td>
<td>0.46</td>
<td>0.61</td>
</tr>
<tr>
<td>Seeds</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total plant</td>
<td>1.41</td>
<td>2.19</td>
<td>4.92</td>
<td>5.94</td>
</tr>
<tr>
<td>Frisson</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roots</td>
<td>0.33</td>
<td>0.48</td>
<td>0.29</td>
<td>0.41</td>
</tr>
<tr>
<td>Nodules</td>
<td>0.27</td>
<td>0.15</td>
<td>0.80</td>
<td>1.40</td>
</tr>
<tr>
<td>Stem</td>
<td>0.15</td>
<td>0.34</td>
<td>1.70</td>
<td>2.72</td>
</tr>
<tr>
<td>Lower leaves</td>
<td>0.54</td>
<td>1.00</td>
<td>1.16</td>
<td>1.75</td>
</tr>
<tr>
<td>Upper leaves</td>
<td></td>
<td></td>
<td>0.38</td>
<td>0.33</td>
</tr>
<tr>
<td>Pods</td>
<td></td>
<td></td>
<td>0.32</td>
<td>0.91</td>
</tr>
<tr>
<td>Seeds</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total plant</td>
<td>1.02</td>
<td>1.82</td>
<td>3.44</td>
<td>5.01</td>
</tr>
</tbody>
</table>

1 Roots + nodules.

*Significant, respectively, at the 0.05 and 0.01 probability level.

ns = non-significant.
still the main sink for newly absorbed $^{15}$N; about 75% of the whole plant for L833, but only 56% for Frisson. For Frisson, about 25% of $^{15}$N absorbed by the plant was recovered in the stem while this proportion was only 10% for L833. The proportion of $^{15}$N in the nodules and roots was similar for both genotypes: about 10% of the whole plant.

When plants were labelled with $^{15}$N at the beginning of seed filling, N partitioning between organs and between genotypes was found to vary greatly as compared with the labelling experiment conducted at an earlier stage (Tab. III). Nitrogen was mainly accumulated in young organs. Hence, for Frisson plants, seeds accumulated about 30% of the total $^{15}$N of the plant while about 25% was assimilated both by the stem and the young (upper) leaves. Pods accumulated 15% of the total $^{15}$N of the plant. For L833 plants, seeds, pods, upper leaves and stem accumulated an identical proportion of $^{15}$N (about 20%). For a given genotype, the proportion of $^{15}$N in nodules + roots and lower leaves was the same: about 5% for Frisson and 10% for L833.

### 3.4. Redistribution of $^{15}$N between organs during plant growth

In order to study N remobilisation within plants and between organs, plants were grown under $^{14}$N after the $^{15}$N labelling period. (Fig. 2). During plant growth, N remobilisation occurred between the different organs of the plants. The proportion of N remobilised in the plant varied widely according to the genotype and the labelling period. When plants were labelled with $^{15}$N at the 7 leaf stage, the proportion of N in the stem remained constant for both genotypes until the beginning of seed filling: about 13% (Fig. 2A). During seed filling this proportion remained similar for L833 while it decreased for

### Table II. Total N accumulation by plant parts at the beginning of seed filling (P1) and during seed filling (P2). Plants were labelled with 4 mM N.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Component</th>
<th>Period 1 (P1)</th>
<th>Period 2 (P2)*</th>
<th>P2/P1 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frisson</td>
<td>Nodules + roots</td>
<td>23.5</td>
<td>6.0</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Stem</td>
<td>35.0</td>
<td>–13.7</td>
<td>–39</td>
</tr>
<tr>
<td></td>
<td>Lower leaves</td>
<td>39.4</td>
<td>–8.6</td>
<td>–22</td>
</tr>
<tr>
<td></td>
<td>Upper leaves</td>
<td>94.4</td>
<td>–37.2</td>
<td>–39</td>
</tr>
<tr>
<td></td>
<td>Pods</td>
<td>44.0</td>
<td>–24.5</td>
<td>–56</td>
</tr>
<tr>
<td></td>
<td>Seeds</td>
<td>59.2</td>
<td>221.8</td>
<td>374</td>
</tr>
<tr>
<td></td>
<td>Whole plant</td>
<td>295.5</td>
<td>143.7</td>
<td>49</td>
</tr>
<tr>
<td>L833</td>
<td>Nodules + roots</td>
<td>32.9</td>
<td>–1.9</td>
<td>–6</td>
</tr>
<tr>
<td></td>
<td>Stem</td>
<td>42.4</td>
<td>–11.1</td>
<td>–26</td>
</tr>
<tr>
<td></td>
<td>Lower leaves</td>
<td>94.6</td>
<td>–41.0</td>
<td>–43</td>
</tr>
<tr>
<td></td>
<td>Upper leaves</td>
<td>97.4</td>
<td>–48.6</td>
<td>–50</td>
</tr>
<tr>
<td></td>
<td>Pods</td>
<td>55.2</td>
<td>–27.0</td>
<td>–49</td>
</tr>
<tr>
<td></td>
<td>Seeds</td>
<td>36.9</td>
<td>255.5</td>
<td>693</td>
</tr>
<tr>
<td></td>
<td>Whole plant</td>
<td>359.3</td>
<td>125.9</td>
<td>35</td>
</tr>
</tbody>
</table>

* (P2) = total N accumulated from the beginning of seed filling to physiological maturity.
NO₃ assimilation by the field Pea *Pisum sativum* L.

For both genotypes, the proportion of ¹⁵N in the nodules and roots decreased from the 7 leaf stage to the beginning of flowering. It remained constant until the beginning of seed filling, and then decreased at physiological maturity. The proportion of ¹⁵N in the leaves, which was relatively constant during the vegetative stage of plant growth, decreased dramatically throughout seed filling. From the beginning of seed filling to physiological maturity, the proportions of ¹⁵N in pods and lower and upper leaves were halved for L833. Similarly, the proportion of ¹⁵N in lower leaves was also halved for Frisson during seed filling. However, for this genotype, the variation in ¹⁵N during this period was greater for upper leaves and pods. The proportion of ¹⁵N in upper leaves decreased from 30% at the beginning of seed filling to 7% at physiological maturity. That of pods decreased from 10% to 2%. The remobilisation of N occurred from older organs toward young organs of the plant. Thus, from flowering to the beginning of seed filling, N lost by old leaves was gained mainly by new and also young plant organs: upper leaves, pod and seeds. At maturity, ¹⁵N was mainly located in seeds: about 45% for L833 and 60% for Frisson.

When plants were labelled at the beginning of flowering, N remobilisation from vegetative parts of the plant increased as compared with the preceding growth stage (Fig. 2B). From flowering to the beginning of seed filling, the ¹⁵N remobilised by lower leaves of both genotypes was mainly gained by upper leaves and pods. The proportion of ¹⁵N in nodules, roots and stems remained constant during this period for L833. This constancy was also observed for nodules and roots for Frisson while the proportion of stem ¹⁵N was halved. During seed filling, the remobilisation of ¹⁵N to seeds occurred from all plant organs of both genotypes except lower leaves of Frisson where the proportion reached a plateau. For this genotype, remobilisation from lower leaves occurred between the beginning of flowering and the beginning of seed filling. At physiological maturity, the proportion of ¹⁵N in seeds was about 60% for both genotypes.

When plants were labelled at the beginning of seed filling (Fig. 2C), about 20% and 30% of total plant ¹⁵N was located in L833 and Frisson seeds, respectively. Then, during seed

### Table III. Amount of ¹⁵N assimilated by different plant organs during the 5 days of feeding. Plants were sampled at Day 6.

<table>
<thead>
<tr>
<th>Period of ¹⁵N feeding</th>
<th>L833</th>
<th>Frisson</th>
<th>L833</th>
<th>Frisson</th>
<th>L833</th>
<th>Frisson</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/plant</td>
<td>mg/plant</td>
<td>mg/plant</td>
<td>mg/plant</td>
<td>mg/plant</td>
<td>mg/plant</td>
</tr>
<tr>
<td>Nodules + roots</td>
<td>2.43 (23)</td>
<td>2.72 (24)</td>
<td>1.21 (9)</td>
<td>1.37 (9)</td>
<td>1.56 (10)</td>
<td>0.75 (5)</td>
</tr>
<tr>
<td>Stem</td>
<td>1.37 (13)</td>
<td>1.47 (13)</td>
<td>1.33 (10)</td>
<td>3.81 (25)</td>
<td>3.28 (21)</td>
<td>3.61 (24)</td>
</tr>
<tr>
<td>Lower leaves</td>
<td>6.77 (64)</td>
<td>7.13 (63)</td>
<td>10.37 (73)</td>
<td>8.54 (56)</td>
<td>1.41 (9)</td>
<td>0.9 (6)</td>
</tr>
<tr>
<td>Upper leaves</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.44 (22)</td>
<td>3.46 (23)</td>
</tr>
<tr>
<td>Pods</td>
<td>1.09 (8)</td>
<td>1.53 (10)</td>
<td>3.12 (20)</td>
<td>2.1 (14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seeds</td>
<td>2.81 (18)</td>
<td>4.21 (28)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total plant</td>
<td>10.57 (100)</td>
<td>11.32 (100)</td>
<td>14.00 (100)</td>
<td>15.25 (100)</td>
<td>15.62 (100)</td>
<td>15.03 (100)</td>
</tr>
</tbody>
</table>

¹( ) : % of total ¹⁵N of the plant.
filing, most younger plant organs such as pods, upper leaves and stem (including upper stem) lost an appreciable part of their N. At the same time, during this period of seed filling, the proportion of $^{15}$N in seed increased to reach 70% at physiological maturity for both genotypes.

4. DISCUSSION

In order to improve the yield of a seed crop such as a pulse legume, it is highly desirable for the plant breeder to gain a thorough understanding of the processes involved in the delivery of materials to the developing seeds, and especially of the relative importance of already stored reserves as opposed to current uptake in meeting the requirements of the seeds. This paper provides such information concerning N in *Pisum sativum*.

Supplying 4 mM NO$_3$ in the culture solution significantly increased total plant dry weight up to the beginning of seed filling (Tab. I) for both genotypes, indicating the role of NO$_3$ as starter N during early plant growth. This result confirms previous results [17] which demonstrated that soybean dependent on both N$_2$ fixation and sub-optimal levels of mineral N has higher biomass production than soybean grown with an excess of mineral N. Mineral N provides a N source to plants at the early growth stage before symbiotic N$_2$ fixation begins. Further, in seedlings of many nodulated legumes, such as common bean (*Phaseolus vulgaris* L.) and soybean (*Glycine max* L.), there is typically a period of “N stress” between 15 and 20 days after emergence, due to a lack of synchronisation between the depletion of N in the cotyledons and the beginning of N$_2$ fixation and transport [16]. The “N hunger” period would only be found in epigeal nodulated legumes such as cowpea and common bean [37]. For these legumes, symbiotically fixed N$_2$ is at first used for nodule development rather than nutrition of shoots and in addition there is a higher N request by the first foliage leaves of these plants. However, within an epigeal pattern, the patterns of N partitioning to the nodules and the symptoms of N hunger were different between a small- and a large-seeded cowpea cultivar [2].

But, at physiological maturity, the effect of nitrate supply on plant dry matter was not significant and this effect on redistribution among plant organs, except nodules, was minor, for both of the genotypes. Thus, the method using low mineral N supply on plant growth and $^{15}$N labelling was adapted to follow the remobilisation. In order to determine the effect of 4 mM NO$_3$ on the organs, plants were separated into different parts (Tab. I). The results indicated a dramatic decrease in nodule dry weight in NO$_3$-labelled plants, which was only about 20% of the control from the 7 leaf stage to the beginning of seed filling. Hence, N$_2$ fixation in these plants was presumably also reduced [4].

At maturity the efficiency of dry matter transfer to the seeds was very low for the two genotypes, about 40%. This efficiency was higher for total N, about 60% (Fig. 1), indicating N remobilisation from vegetative organs to supply seeds’ requirements. In order to determine the proportion of mineral N assimilated and redistributed by each organ, plants were labelled with $^{15}$N at early and later stages of growth. The results demonstrated that the period of application of the $^{15}$N labelling was more important than the variety used (Tab. III). Moreover, the partitioning of $^{15}$N at the end of the labelling period indicated that N from NO$_3$ was preferentially distributed to developing organs such as upper leaves, pods, seeds and the (upper) stem. Remobilisation of $^{15}$N between organs occurred throughout the period of plant growth (Fig. 2). N previously accumulated in vegetative parts was transferred to seeds. Therefore N availability in a plant, at a given time, can be considered as one common pool accessible to all seeds and equitably divided among them [21, 22]. Leaves were found to be the most important N source for seeds because they accumulated more N than any other tissue [3] and their N was more easily retrieved by seeds [26]. In the same way, mobilisation of N reserves seems to be a prerequisite for shoot regrowth in many species such as *Lolium perenne* [27], *Medicago sativa* [19, 39] and *Trifolium subterraneum* [12]. In cereals such as wheat and maize, leaves and stem have been found to be the main source of seed N [8].

The dynamic nature of the balance between the accumulation and mobilisation of reserve N compounds within the plant can be gauged from the changing pattern of source and sink interactions during its life cycle. The synthesis and accumulation of N reserves occur when a storage tissue is a net N importer (sink) [30]. This occurs when N acquisition by the plant exceeds that required to support synthesis of new tissue. In contrast, when N uptake and/or N$_2$ fixation are insufficient to meet the N growth demands, previously stored N is then mobilised, and storage organs behave as net exporters (source). For example, in cereals, a large proportion of the N previously accumulated in leaves is mobilised to sustain grain filling when N uptake is limited by soil N availability and/or N uptake is down-regulated [30]. Additionally, N storage has been demonstrated to occur in tubers or roots of *Cyperus intybus* [1], *Helianthus tuberosus* [25], *Solanum tuberosum* [31], *Ipomea batatas* [23], * Dioscorea rotundata* [15] and *Vitis vinifera* [10]. In these species, N reserves are mobilised primarily to support growth during the spring. For forage grass species, defoliation induces large changes in N source/sink relationships, with roots and remaining sheath (i.e. stubble) tissues acting as source organs, and regrowing shoot tissues becoming strong sinks [39].

In this study, it has been shown that the proportion of $^{15}$N recovered by seeds at maturity varied according to the period of $^{15}$N uptake. More labelled N was recovered in seeds when it was applied later together with the nutrient solution. When plants were labelled with $^{15}$N at the 7 leaf stage, the proportion of N in seeds at physiological maturity was about 45% for L833 and 60% for Frisson (Fig. 2A). This proportion was about 60% and 70%, respectively, if plants were labelled at the beginning of flowering (Fig. 2B) and the beginning of seed filling (Fig. 2C) for both genotypes. These results confirm previous results [33] which recorded in *Pisum arvense* an efficiency of N transfer to seeds of 51% and 74% when plants were labelled, respectively, at the seven leaf stage and flowering. They also confirm those obtained in *Vicia faba* [13] which reported an efficiency of 60% and 76%, respectively, at the beginning of flowering and the beginning of seed filling, using $^{15}$N feeding. In this study concerning *Pisum sativum*, the $^{15}$N analysis at different stages of plant development allowed a better understanding of the processes involved in N remobilisation. When plants were labelled at the 7 leaf stage, the remobilisation of N
occurred from older organs toward younger ones. Hence, each leaf along the stem was successively a N sink then a N source. Finally, at physiological maturity, most of the $^{15}$N was located in seeds. When plants were labelled at the beginning of seed filling, the proportion of $^{15}$N in seeds appeared higher than during the previous treatment.

The consequences of low storage of N compounds before shoot removal have been shown in *Medicago sativa* [28] and in *Lolium temulentum* L. [29]. A shortage of N reserves results in reduced shoot yield in both species, but the forage legume seems to be more affected. Such a difference between fixing and non-fixing forage species may be explained by the higher plasticity of mineral N uptake as nitrate or ammonium by roots of the latter, which is able to compensate by an increase in uptake rate, whereas a compensatory increase in N$_2$ fixation and/or inorganic N uptake rates may not be possible in the fixing forage species [6].

Much of the genetic improvement in crop productivity over the past 50 years has been conducted under high N input conditions [30]. Selection in this environment has reduced the N storage capacity of grassland species. Species associated with environments in which levels of available N are generally low rely more on N storage than species usually found in N-rich environments [38]. Likewise, shoot regrowth of *Lolium temulentum*, which is a ruderal species, appears to be limited by the capacity of N reserves [29]. In contrast, *Lolium perenne* can compensate for low N reserves status by rapidly increasing root N uptake [24]. It has also been argued from a wider ecological perspective [7] that the most efficient mechanisms for N recycling and storage are likely to be found in species adapted to low N fertility environments. This has significant consequences for identifying genetic material for breeding programmes aimed specifically at enhancing N use efficiency. *Pisum sativum*, which has a high N fixation capacity, is a species adapted to low N fertility. Remobilised N is used for seed filling rather than for the synthesis of new vegetative organs. In this study, Frisson exhibited harvest N indexes higher than L833. This resulted from greater N remobilisation in lower leaves, from slower vegetative development at the beginning of flowering and from slower growth of the stem and leaves during seed filling. These results allow better characterisation of the plant idiotype, which could be of use to plant breeders.

Acknowledgements: The authors would like to acknowledge the $^{15}$N analysis of Alain Gojon and Pascal Tillard, INRA Montpellier, France.

REFERENCES


[23] Li H., Oba K., Major soluble proteins of sweet potato roots and changes in proteins after cutting, infection, or storage, Agric. Biol. Chem. 49 (1985) 737–744.


