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Seasonal Patterns of ^{13}C Partitioning Between Shoots and Nodulated Roots of N_2- or Nitrate-fed *Pisum sativum* L.

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The effect of nitrogen source (N_2 or nitrate) on carbon assimilation by photosynthesis and on carbon partitioning between shoots and roots was investigated in pea (*Pisum sativum* L. 'Baccara') plants at different growth stages using ^{13}C labelling. Plants were grown in the greenhouse on different occasions in 1999 and 2000. Atmospheric [CO_2] and growth conditions were varied to alter the rate of photosynthesis. Carbon allocation to nodulated roots was unaffected by N source. At the beginning of the vegetative period, nodulated roots had priority for assimilates over shoots; this priority decreased during later stages and became identical to that of the shoot during seed filling. Carbon allocation to nodulated roots was always limited by competition with shoots, and could be predicted for each phenological stage: during vegetative and flowering stages a single, negative exponential relationship was established between sink intensity (percentage of C allocated to the nodulated root per unit biomass) and net photosynthesis. At seed filling, the amount of carbon allocated to the nodulated root was directly related to net photosynthesis. Respiration of nodulated roots accounted for more than 60 % of carbon allocated to them during growth. Only at flowering was respiration affected by N supply: it was significantly higher for strictly N_2-fixing plants (83 %) than for plants fed with nitrate (71 %). At the vegetative stage, the increase in carbon in nodulated root biomass was probably limited by respiration losses.

Key words: Roots, nodules, legumes, C partitioning, symbiotic nitrogen fixation, *Pisum sativum* L., ^{13}C labelling.

INTRODUCTION

It has long been suggested that symbiotic N_2 fixation requires more carbon than absorption of nitrate by roots and so would impair carbon accumulation by whole plants (Minchin and Pate, 1973; Silsbury, 1977; Ryle *et al*., 1979*b*). However, relationships between C and N nutrition remain unclear. Some authors have reported symptoms of N deficiency (stress) in the early stages of growth for strictly N_2-fixing pea plants (Oghoghorie and Pate, 1971); others have observed optimal growth and N nutrition for field-grown pea plants regardless of the N source (Sagan *et al*., 1993; Crozat *et al*., 1994; Voisin *et al*., 2002*a*). Nitrate supply to pea in the field was rarely found to increase yields, except when provided late in the growth cycle (Jensen, 1986). The same was observed for soybean (Isfan, 1991; Yinbo, 1997) even though it is more sensitive to N supply and N source than other legumes (Crozat *et al*., 1994). Biomass accumulation and partitioning within the plant also differ with N source, particularly in the glasshouse where light may be more limiting than it is in the field, with strictly N_2-fixing plants having less root biomass but more nodule biomass than plants supplied with nitrate (Oghoghorie and Pate, 1971; Pate *et al*., 1979; Ryle *et al*., 1979*b*; Butler and Ladd, 1985; Jensen, 1986; Schulze *et al*., 1999). Understanding the relationship between biomass accumulation and the mode of N nutrition of legumes (i.e. symbiotic N_2 fixation *vs*. absorption of mineral N from the soil) requires a better understanding of carbon partitioning and use within the plant. However, photosynthetic carbon input is difficult to compare among plants relying on different N sources, since nitrate often increases both leaf area and photosynthesis and thus stimulates shoot and root biomass (Mahon and Child, 1979; Lodeiro *et al*., 2000).

Differences in shoot and nodulated root biomass could be explained by differences in carbon use efficiency (i.e. the ratio of C increment in biomass over C increment in biomass plus respiration). Carbon use efficiency may be unaffected by N source, as in cowpea (Atkins *et al*., 1980), or it may be greater for nitrate-assimilating than for N_2-fixing plants, as in lupin (Pate *et al*., 1979), pea and faba bean (Schulze *et al*., 1999). Carbon allocated to below-ground organs has various possible fates which can be modified by nitrate supply: carbon may be incorporated into root and nodule biomass; respired for synthesis and maintenance processes; or used to provide carbon skeletons for the amino compounds that are translocated to the shoots. It should be noted that in temperate legumes, such as pea, nitrate is largely reduced in roots, whereas it is mainly reduced in shoots of most tropical legumes (Wallace, 1986). The percentage of photosynthetic C allocated to nodulated roots and the amount of C respired may be higher for nodulated than non-nodulated roots (Pate *et al*., 1979; Ryle *et al*., 1979*a*, *b*; Atkins *et al*., 1980; Schulze *et al*., 1999). These results are likely to vary during the growth cycle, with the degree of variation depending on the species (Herridge and Pate, 1977; Atkins *et al*., 1978).

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These observations have led to the hypothesis, mentioned above, that higher C costs are incurred by symbiotic N_2 fixation than nitrate assimilation (Pate *et al*., 1979; Ryle *et al*., 1979*a*, *b*; Atkins *et al*., 1980; Schulze *et al*., 1999). However, it remains difficult to quantify and compare carbon costs associated with both symbiotic N_2-fixation activity and nodule biosynthesis and maintenance (Phillips, 1980). The strategy of adaptation to the higher C costs of symbiotic N_2 fixation varies among species, with some having greater photosynthetic capacities when relying on symbiotic N_2 fixation (e.g. faba bean), whilst others (e.g. pea) suffer the additional C costs at the expense of root tissue (Schulze *et al*., 1994, 1999).

The objectives of this study were to determine how nitrogen sources modify carbon fluxes during growth of pea plants, and to understand the rules that drive C distribution and use. Carbon partitioning between shoots and nodulated roots, and C loss by respiration of nodulated roots were quantified in relation to the mode of N nutrition using ^{13}C. Atmospheric [CO_2] and sowing date were altered to provide treatments to alter net photosynthesis and biomass partitioning within the plant.

MATERIALS AND METHODS

Plant material and growing conditions

Pea plants (*Pisum sativum* L. 'Baccara') were sown in 5 l plastic pots (eight seeds per pot) on three occasions (15 Sep. 1999, 2 Mar. 2000, 26 May 2000) and were inoculated with *Rhizobium leguminosarum* bv *vicieae*. The growing medium was a 1 : 1 (v/v) mixture of sterilized attapulgite and clay balls (diameter 2–6 mm). This medium has no buffering effect, allows gases to diffuse easily and is inert to gas, thus avoiding interference with CO_2 and O_2 measurements. At emergence, plants were thinned to four per pot. Since plants were grown in a naturally lit glasshouse (Dijon, France), delaying the date of sowing was a means of varying growing conditions, owing to natural variations in temperature, radiation and photoperiod. The temperature in the glasshouse was maintained at over 5 °C during winter, and the roof opened automatically when the temperature exceeded 20 °C. The genotype used (Baccara) requires long days for flower initiation and has a facultative vernalization requirement. Supplementary artificial light was provided in autumn 1999 (total day length 16 h d^{-1}) to induce flowering. Plants were watered regularly and maintained at water-retention capacity using nutrient solution (P, K, S, Mg, Ca, Na, Fe, oligoelements: Co, B, Mn, Zn, Mo, Cu) according to plant transpiration needs. Timing of irrigation was controlled by two balances carrying one pot each, which automatically triggered watering of all pots of a treatment when the cumulated transpiration since the last watering exceeded one-tenth of the soil water reserve.

Experimental treatments

For each sowing date, two different N treatments were applied to obtain various rates of symbiotic N_2 fixation. As it is difficult to preclude nodulation on pea roots throughout the growth cycle, a level of nitrate supply that reduces nodule biomass (and symbiotic N_2 fixation) by 50 % compared with that of control plants (only fixing N_2) was chosen during a preliminary experiment: half of the plants (90 pots) were provided with N-free nutrient solution (N_0 treatment, plants fixing N_2 only) and the other half with 5 mol m^{-3} N as KNO_3 in the nutrient solution (N_5 treatment, plants assimilating both NO_3 and N_2).

For each group of plants corresponding to different sowing dates, three pots of each treatment were selected for ^{13}C labelling at three characteristic stages: 'vegetative' (ten nodes on the main stem); 'flowering' (flowers on the sixth node of the main stem, before the beginning of seed filling); and 'seed filling' (between the final stage of seed abortion and the start of physiological maturity). The early flowering stage (two reproductive nodes) was also studied in 1999.

In 2000, in order to obtain a range of photosynthetic rates for a given phenological stage, three different concentrations of CO_2 in air (150, 360 and 750 µl l^{-1}) were applied to the different plant sets (three sowing dates) during ^{13}C labelling.

^{13}C labelling

The equipment used for ^{13}C labelling was similar to that used by Warembourg *et al*. (1982) and described by Voisin *et al*. (2000). Roots and shoots were maintained in separate compartments: pots of plants were inserted into individual air-tight plastic containers to separate root and shoot atmospheres. Air leaks between aerial and root atmospheres were prevented by using physiological moulding material (Qubitac; Qubit Systems Inc., Kingston, Canada) and silicone rubber (RTV; Zundel Kohler, Illzach, France) around the stems of plants. The pots with separate root atmospheres were placed in a transparent, air-tight labelling chamber (0·95 × 0·95 × 1·5 m) made of Plexiglas.

At each chosen CO_2 concentration (150, 360 and 750 µl l^{-1}), shoots were exposed to a ^{13}C-enriched atmosphere for 10 h: the CO_2 concentration was measured continuously using an infrared gas analyser (IRGA; PP system; Ciras, Montigny le Bretonneux, France) and maintained by automatic CO_2 injection. To obtain a constant and uniformly labelled atmosphere, all CO_2 in the atmosphere within the chamber was trapped for 20 min at the beginning of the experiment; the required CO_2 concentration (150, 360 or 750 µl l^{-1}) was then achieved and maintained by injecting a mixture of CO_2 with a constant ^{13}C : ^{12}C ratio (10 atom %^{13}C). ^{13}C enrichment of the atmosphere around the shoot during the labelling period was assessed by mass spectrometry of samples taken regularly.

To study the fate of photosynthates, labelling was followed by a non-labelling period (chase) lasting 4 d in 1999 and 2 d in 2000 (Montange *et al*., 1981; Kouchi *et al*., 1986), with plants being held at 360 µl l^{-1} CO_2. Within the chamber, temperature and humidity of the air around the shoots were maintained using an air-conditioning unit. Light was supplied by four 400 W sodium lamps placed on each side of the enclosure and two 1000 W mercury lamps situated above it. The resultant photosynthetic active radiation varied from 600–900 µmol m^{-2} from the bottom

TABLE 1. *$\delta^{13}C$ isotopic content (δ‰^{13}C) of carbon in strictly N_2-fixing pea plants (N_0) and in plants supplied with nitrate*

	Vegetative stage		Flowering		Seed filling	
Isotopic content of C (δ‰ ^{13}C)	Control	Labelled	Control	Labelled	Control	Labelled
N_0 treatment						
Leaves and stems	–28·7	597	–29·5	528	–22·0	157
Pod walls	–	–	–28·3	1022	–24·0	71
Seeds	–	–	–27·3	1289	–27·9	452
Roots	–27·5	127	–28·4	122	–28·6	94
Nodules	–28·3	396	–28·5	96	–29·0	206
N_5 treatment						
Leaves and stems	–28·9	614	–29·3	558	–27·0	250
Pod walls	–	–	–28·4	1040	–26·9	156
Seeds	–	–	–26·2	1232	–27·0	682
Roots	–27·9	214	–28·2	267	–28·6	126
Nodules	–27·9	197	–28·0	229	–28·5	109

Pea plants were sown on 26 May 2002. Values are means for three pots before (control plants) and after (labelled) exposure to $^{13}CO_2$ labelling.

to the top of the canopy. Soil water was adjusted gravimetrically each day. Throughout labelling and during the chase, the soil atmosphere entering the pots was CO_2-free air. Respiration of nodulated roots was measured by trapping CO_2 from the pots by bubbling the gas stream through NaOH solution (0·5 mol.l^{-1}). The whole system was monitored by computer using Dasylab software (Newport Omega, Trappes, France).

Harvesting and measurements

Plants were harvested at the beginning of the labelling experiment (control plants) and at the end of the chase period (labelled plants). All plants in each pot were harvested together and separated into shoots, roots and nodules. Dry matter was determined after oven drying at 80 °C for 48 h. Concentrations of C and N were determined by the Dumas procedure on ground samples. Their ^{13}C enrichment (atom%^{13}C) was measured using a dual-inlet mass spectrometer (Fisons Isochron; Micromass, Villeurbanne, France).

Respiration of nodulated roots was measured by titration of the NaOH solution used to trap the CO_2 evolved. ^{13}C enrichment of the respired CO_2 was measured using a mass spectrometer on a gaseous sample produced by acidification of the trap solutions. The total amount of respiration was obtained by summation of the daily respiration values from the start of labelling until harvest.

Calculations

Isotopic determinations were carried out according to the isotope dilution principle. The percentage of carbon that was derived from the labelled source (%^{13}C; I) can be calculated by:

$$I = 100\ (S - C)/(A - C)$$

where C is atom%^{13}C untreated plants (control) and A is atom%^{13}C labelled atmosphere, where S is atom%^{13}C sample in [plant material (shoot, roots, nodules) or respired C] (Deléens *et al*., 1994). For each component (plant sample, CO_2), the quantity of carbon derived from photosynthesis during the labelling period (Q_C) was calculated using I, dry matter (M) and carbon (C) determinations:

$$Q_C = M(C/100)(I/100)$$

Statistics

For each treatment, values were the average of three measurements (three pots). Analysis of variance was performed using the GLM procedure of SAS, and means were compared using the least significant difference test (LSD) at the 0·05 probability level (SAS Institute, 1987).

RESULTS

Plant biomass partitioning related to N source and growing conditions

An example of the isotopic content of pea plants is given in Table 1. The different N treatments and growing conditions altered both plant biomass accumulation and partitioning (Table 2). Total biomass was significantly larger for plants grown with nitrate (N_5 treatment) than for those fixing N_2 only (N_0 treatment; Table 2). For each sowing date, plants from the N_5 treatment had greater (30–60 %) shoot biomass than those from the N_0 treatment. Roots of plants in both N treatments were nodulated, but plants supplied with nitrate produced 35–110 % more root biomass and 60–70 % less nodule biomass compared with those only fixing N_2 (Table 2). Considering the different growing periods, biomass of all parts was significantly smaller for plants grown in autumn 1999 compared with that of plants grown in spring and summer 2000 (Table 2). Overall, total growth decreased as the sowing date was delayed from March to September.

Net photosynthesis related to $^{13}CO_2$ concentration

The amount of labelled C in plant parts at harvest, and in CO_2 from root respiration after each ^{13}C experiment, was

TABLE 2. *Biomass and total N concentration at the seed filling stage for strictly N_2-fixing plants (N_0 treatment) and for plants supplied with nitrate (N_5 treatment) for each experiment*

Plant set (sowing date)	Total biomass (g per plant)	Shoot biomass (g per plant)	Root biomass (g per plant)	Nodule biomass (g per plant)
15 September 1999				
N_5	8·9 ± 2·1	8·4 ± 2·1	0·39 ± 0·12	0·04 ± 0·02
N_0	6·6 ± 0·3	6·2 ± 0·2	0·29 ± 0·06	0·13 ± 0·09
3 March 2000				
N_5	22·9 ± 1·8	21·6 ± 1·8	1·21 ± 0·40	0·07 ± 0·05
N_0	17·3 ± 0·7	16·4 ± 0·7	0·73 ± 0·03	0·17 ± 0·01
16 March 2000				
N_5	20·5 ± 1·7	19·4 ± 1·7	1·02 ± 0·11	0·05 ± 0·02
N_0	14·4 ± 1·9	13·5 ± 1·8	0·71 ± 0·09	0·16 ± 0·01
26 May 2000				
N_5	13·0 ± 1·6	12·1 ± 1·5	0·83 ± 0·07	0·04 ± 0·01
N_0	8·2 ± 1·4	7·7 ± 1·3	0·40 ± 0·07	0·10 ± 0·03

Plant were grown in a glasshouse on different occasions in 1999 and 2000.

used to estimate net C assimilation by photosynthesis during each period of exposure. Combining the different sowing dates and CO_2 concentrations, net C assimilation by photosynthesis ranged from 51 to 124 mg per plant d^{-1} at the vegetative stage, from 110 to 346 mg per plant d^{-1} at flowering and from 112 to 378 mg per plant d^{-1} at seed filling (Fig. 1).

C allocation to nodulated roots

Carbon partitioning between shoots and nodulated roots was related to daily total net C assimilated at each phenological stage (Fig. 1). Carbon allocated to nodulated roots was calculated as the sum of C increment in nodulated root biomass plus nodulated root respiration that originated from the labelling period.

The percentage of photosynthetic C allocated to nodulated roots decreased during growth for both N treatments, from around 45 % at the vegetative stage (Fig. 1A) to less than 10 % at seed filling (Fig. 1C). During the vegetative (Fig. 1A) and flowering (Fig. 1B) stages, C allocation to nodulated roots decreased linearly when net C-photosynthesis increased, regardless of N treatment. Slopes of the regressions between C allocation to nodulated roots and net C-photosynthesis decreased with phenology. At the seed filling stage (Fig. 1C), allocation remained stable (around 7 % of net C-photosynthesis) regardless of the rate of net C-photosynthesis. At flowering (Fig. 1B), values were systematically lower in 1999 than in 2000, as was nodulated root biomass (Table 2), suggesting that relationships between C allocation to nodulated roots and net C-photosynthesis depend upon initial biomass of nodulated roots.

At each phenological stage, the amount of C allocated to nodulated roots increased with net photosynthesis (Fig. 1D–F), despite the fact that the percentage of C allocated to the nodulated roots decreased (or remained stable) with increasing net C-photosynthesis (Fig. 1A–C). In all cases, allocation followed the same relationships irrespective of the different nitrogen sources (Fig. 1).

As mentioned above, carbon allocation to nodulated roots depended upon nodulated root biomass (Fig. 1B and E): for vegetative and flowering points, there was a single relationship between allocation per unit biomass (% C allocated to the nodulated roots per unit of nodulated root biomass) and net photosynthesis, regardless of N treatment or growth stage (Fig. 2).

C-respiration by nodulated roots

Nodulated root respiration was normalized by expressing it as a percentage of net C-photosynthesis, thus enabling comparisons to be made among plants of different sizes at different stages (Fig. 3). Differences among N treatments were not significant, regardless of the growth stage (Fig. 3). Averaged over both N treatments, the percentage of net photosynthesis respired by nodulated roots decreased during growth from 31 % at the vegetative stage to 6 % at seed filling (Fig. 3).

To investigate C utilization within the root systems, nodulated root respiration was then expressed as a percentage of C allocated to nodulated roots. Nodulated root respiration always accounted for more than 60 % of the total C allocated to nodulated roots (Fig. 4). At the vegetative stage this percentage decreased with increasing net photosynthesis (Fig. 4A), whereas it remained constant during flowering (Fig. 4B) and seed filling (Fig. 4C). At flowering, and only at that stage, the percentage of C allocated to nodulated roots that was respired differed significantly between the two N treatments, being approx. 83 % for the strictly N_2-fixing plants and 71 % for those supplied with nitrate (Fig. 4B).

DISCUSSION

Our calculations of net photosynthesis included carbon incorporated into both shoot and nodulated root biomass, plus nodulated root respiration, but did not account for shoot respiration. However, in pea plants, shoot respiration is usually a constant proportion of net photosynthesis (15 %) and is not influenced by N nutrition (Pate *et al*., 1979; Ryle

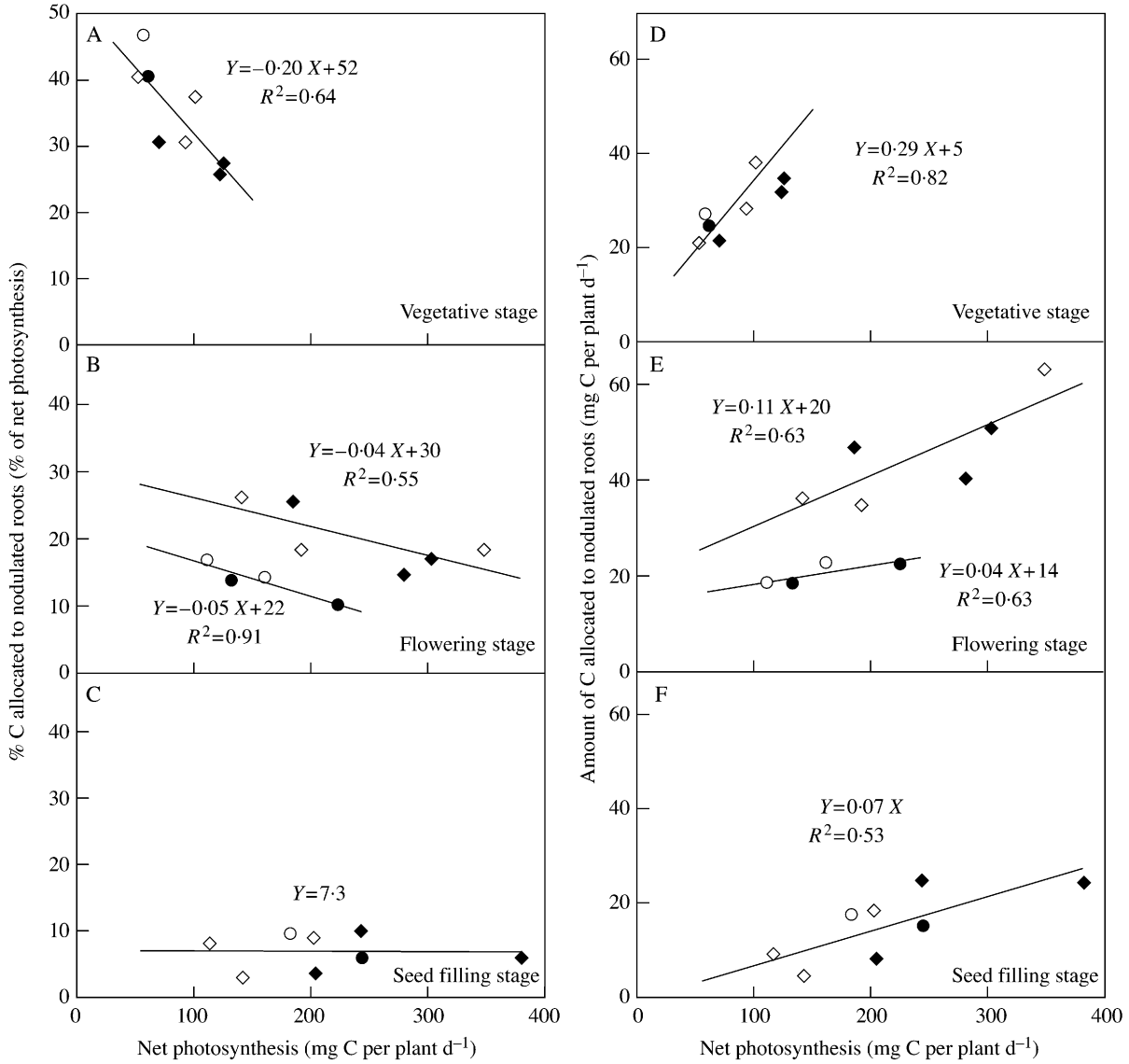

FIG. 1. Carbon allocation to nodulated roots (calculated as C increase in biomass plus nodulated root respiration) expressed as a percentage of net photosynthesis (A–C), or as mg C (D–F) during growth (A and D, vegetative; B and E, flowering; C and F, seed filling stages) of *Pisum sativum*, plotted as a function of net C-assimilation for two N treatments: N_2-fixing plants (N_0 treatment) (open symbols) or plants supplied with nitrate (N_5 treatment) (closed symbols), for experiments conducted in 1999 (circles) and 2000 (diamonds).

et al., 1979*b*; Schulze *et al*., 1999). Thus, if there is a difference in carbon use efficiency, it is more likely to appear in below-ground organs. The amount of carbon allocated to nodulated roots included both nodulated root respiration and C accumulated in biomass. It is a net value since some of the carbon that reaches nodulated roots via the phloem is transported back to the shoot through the xylem as amino-compound skeletons (Pate and Herridge, 1978).

Carbon partitioning between shoots and nodulated roots

Percentages (5–50 %) and patterns of net C allocated to nodulated roots of pea (Fig. 1A–C) are similar to those described for cowpea (Herridge and Pate, 1977). However, they contrast with the high (around 50 %) and stable values reported for lupin (Atkins *et al*., 1978; Pate and Herridge, 1978). Although several authors have suggested that C supply to nodulated roots was higher than to non-nodulated roots (Pate *et al*., 1979; Ryle *et al*., 1979*b*; Atkins *et al*., 1980), and was therefore dependent upon the N source, this was not the case in the present study (Fig. 1A–C). However, until flowering, the percentage of C allocated to nodulated roots varied with photosynthetic rate (Fig. 1A and B). Since plants grown with nitrate usually had more shoot and leaf biomass, differences previously observed (Pate *et al*., 1979; Ryle *et al*., 1979*b*; Atkins *et al*., 1980) among plants relying on different N sources can be explained by differences in photosynthetic rate.

The percentage of net photosynthetic C allocated to nodulated roots was considered to be an indicator of the sink

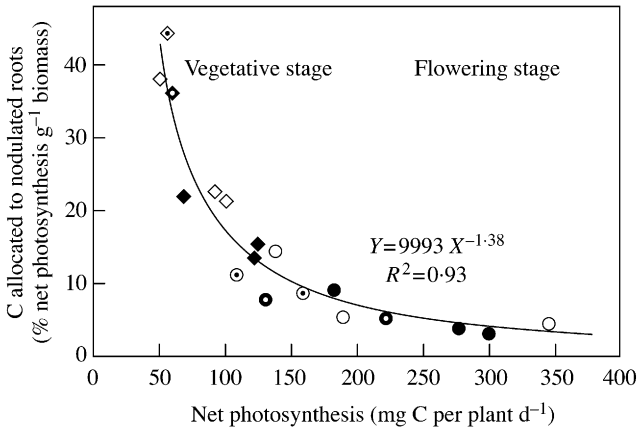

FIG. 2. Percentage of net photosynthesis allocated to nodulated roots of *Pisum sativum* expressed per unit of nodulated root biomass and plotted as a function of net C-assimilation for two N treatments: N_2-fixing plants (N_0 treatment) (open symbols), or plants supplied with nitrate (N_5 treatment) (closed symbols), at the vegetative (diamonds) and flowering (circles) stages. Symbols with a dot in the centre represent data from experiments conducted in 1999; all other data are from 2000.

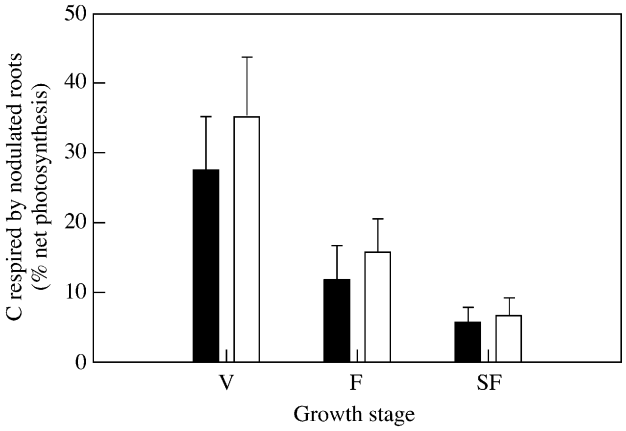

FIG. 3. Percentage of daily net photosynthesis respired by nodulated roots of *Pisum sativum* during growth in two N treatments: N_2-fixing plants (N_0 treatment) (open bars) or plants supplied with nitrate (N_5 treatment) (filled bars). Values are means for the different growing conditions. V, Vegetative stages; F, flowering; SF, seed filling stages. Bars represent s.d.

strength of nodulated roots compared with that of shoots. Variations of this indicator with net photosynthetic rate allowed a general relationship to be established for carbon partitioning between shoots and nodulated roots. The increased sink strength of nodulated roots when net photosynthesis decreased indicated the priority of C allocation to nodulated roots over that to shoots (Fig. 1A–C). This priority decreased as the plant aged; the slope of the linear regression between % C allocated to nodulated roots and net photosynthesis decreased to zero between the vegetative (Fig. 1A) and seed filling (Fig. 1C) stages. At seed filling, despite low sink strength (7 % of net photosynthesis), the priority of nodulated roots for C equalled that of shoots. Regression slopes at flowering (Fig. 1B), established separately for data collected in 1999 and 2000 (which differed in nodulated root biomass; Table 2), were not significantly different, suggesting that C partitioning between shoots and nodulated roots did not depend on the biomass of nodulated roots or on the form of N supplied (Table 2).

Is the sink strength of nodulated roots limited by photosynthesis?

At each phenological stage, the amount of photosynthetic C allocated to nodulated roots increased when net photosynthesis was enhanced (Fig. 1D–F). This suggests that nodulated roots were never saturated with assimilate, despite having a higher priority than the shoots, as discussed above. The slope of the linear regression between the amount of C allocated to nodulated roots and net photosynthesis decreased during growth (Fig. 1–F), indicating that the nodulated root was probably less well supplied with C as growth progressed, presumably in relation to variation in demand for C for synthesis and/or functioning of nodulated roots.

From the present data, it was impossible to determine the sink size of nodulated roots, as stabilization of C uptake by nodulated roots (C allocation to nodulated roots) with C supply by photosynthesis was not observed. At seed filling (Fig. 1F), carbon allocation to nodulated roots may have reached a plateau at approx. 20 mg per plant d^{-1} when net photosynthesis was over 250 mg C per plant d^{-1}.

CO_2 respiration by nodulated roots

The percentage of net photosynthesis that was respired by nodulated roots was not affected by N nutrition (Fig. 3), as previously shown for cowpea (Atkins *et al.*, 1978). However, the proportion, and its variation during growth, may differ among species. The percentage of net photosynthesis respired by nodulated roots increased from 30 to 50 % during growth for lupin (Pate and Herridge, 1978), and from 20 to 40 % for cowpea (Herridge and Pate, 1977), whereas it decreased from 31 to 6 % for pea in the present experiment (Fig. 3).

Respiration was the main component of C utilization within nodulated roots (Fig. 4), probably limiting C accumulation in nodulated root biomass. Within nodulated roots, the percentage of C lost by respiration decreased with net photosynthesis at the vegetative stage in both N treatments (Fig. 4A) and followed the same pattern as % C allocated to nodulated roots (Fig. 1A). From flowering, the percentage of C allocated to nodulated roots that was respired did not vary, despite changes in net photosynthesis, showing identical priority for respiration and increase in biomass. At flowering, however, the percentage of C allocated to nodulated roots that was respired was significantly higher for the strictly N_2-fixing plants than for those supplied with nitrate (Fig 4B), suggesting a difference in carbon use and/or carbon use efficiency according to the N source, i.e. a higher C cost for the process of symbiotic fixation. At the seed filling stage, the percentage of C

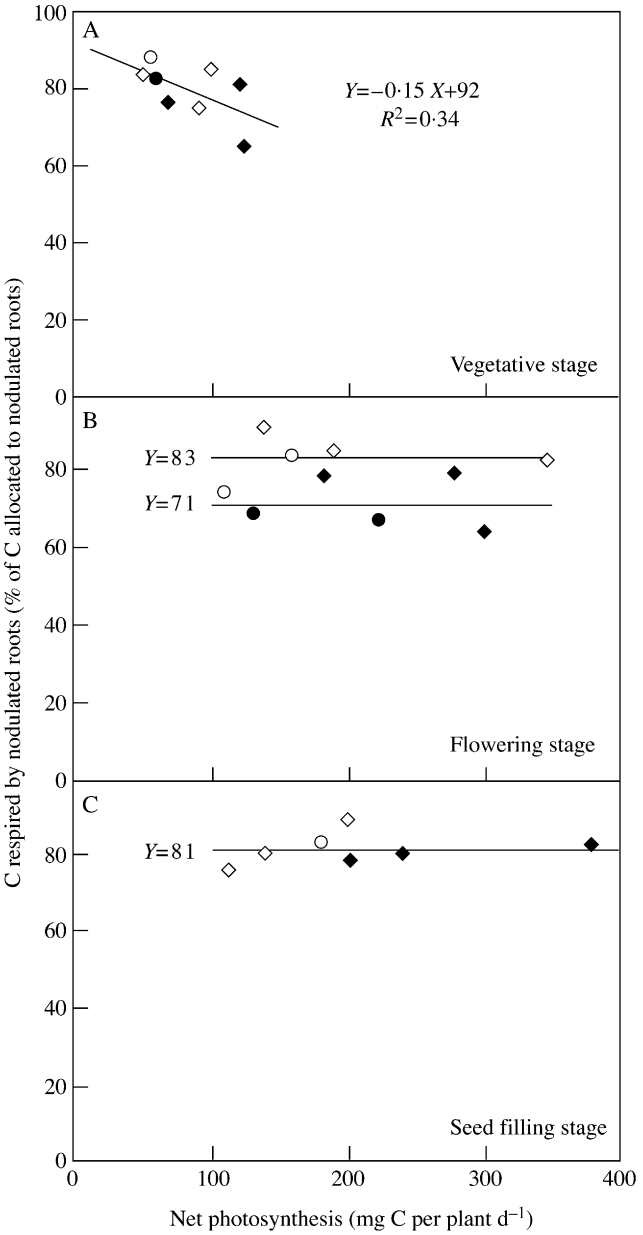

FIG. 4. Carbon respiration by nodulated roots, expressed as a percentage of carbon allocated to nodulated roots during growth (A, vegetative; B, flowering; C, seed filling stages) of *Pisum sativum*, plotted as a function of net C-assimilation for two N treatments: N_2-fixing plants (N_0 treatment) (open symbols), or plants supplied with nitrate (N_5 treatment) (closed symbols), for experiments conducted in 1999 (circles) and 2000 (diamonds).

allocated to the nodulated roots that was respired remained constant and was independent of N nutrition regime.

C partitioning at each growth stage

At the vegetative stage, establishment of nodulated roots seemed to take priority over shoot growth (Fig. 1A), even if shoot growth obviously limited the amount of C allocated to the nodulated root (Fig. 1D). At this early stage, when organs are forming, demand for assimilates may never totally be satisfied for shoots or nodulated roots. Nodulated root growth may be limited by the high C costs incurred by synthetic processes in the whole plant (Fig. 4A).

Sink strength of nodulated roots was lower at flowering than during vegetative growth (Fig. 1B) owing to the appearance of reproductive organs that constitute a competitive sink for assimilates (Jeuffroy and Warembourg, 1991). Growth of nodulated roots may also have been almost complete at this stage (Tricot, 1993). Carbon would then be used mainly for maintenance and symbiotic N_2-fixation activity within nodulated roots. Since these processes both depend upon biomass (Tricot-Pellerin *et al.*, 1994), C allocation to nodulated roots at flowering may depend on the biomass of nodulated roots, as shown by the single relationship established between the percentage of C allocated to the nodulated root per unit biomass (sink intensity) and net photosynthesis (Fig. 2) for both years, with plants differing in biomass of nodulated roots (Table 2). Data from the vegetative stage followed the same single relationship (Fig. 2). Thus, carbon allocation to nodulated roots at the vegetative stage can be calculated as a function of net photosynthesis using two different relationships (Figs 1D and Fig. 2, derived from Fig. 1). Of these, only the relationship shown in Fig. 1D depends upon nodulated root biomass. This suggests that there are two components that determine carbon allocation to nodulated roots, and that the component linked to biomass is dominant at the flowering stage only. At flowering, the greater proportion of the percentage C supplied to the nodulated root respired in strictly N_2-fixing plants compared with those supplied with nitrate (Fig. 4B) suggests higher C costs for the strictly N_2-fixing plants, induced by demands related to nodule maintenance and/or nodule activity.

At seed filling (Fig. 1F), the amount of C allocated to the nodulated root was directly related to photosynthesis. In contrast to previous stages when C allocation to the nodulated root depended upon nodulated root biomass (Fig. 2), this showed that nodulated roots behaved as a passive sink at the end of the growth cycle (Fig. 1F). Carbon may be mainly used for maintenance at this late stage, as nodulated roots become less active and senesce (Voisin *et al.*, 2002*b*) at a rate that depends on C supply to them.

The predictive relationships established in this study will be useful for a better understanding of legume C accumulation patterns since, as pointed out by Pate and Armstrong (1996), C integration and use in nodulated roots have often been neglected, despite their obvious requirement for assimilates and their importance in water and nutrient uptake. Moreover, the percentage of net photosynthesis allocated to nodulated roots can be substantial (up to 45 % at the vegetative stage) and respiratory losses can be more than 60 % of this allocation. It was shown that C use within nodulated roots may depend upon N nutrition at flowering. However, further investigation is needed to determine whether synthesis, maintenance or N accumulation, associated either with N_2 fixation or with nitrate assimilation, were affected.

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