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Translocation of ^{14}C-photosynthate, Carbohydrate Content, and Nitrogen Fixation in *Phaseolus vulgaris* L. during Reproductive Development[1]

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Abstract. **Leaves at nodes 4 or 8 of greenhouse grown beans, *Phaseolus vulgaris* L., cv. Puelba 152, were briefly exposed to $^{14}CO_2$ at 35, 48, 63, or 70 days after planting. Prior to flowering, over 85% of the recovered ^{14}C-activity translocated in 24 hours from node 4 was in roots, nodules, and lower stem. At flowering, radioactivity translocated to the lower stem decreased but correspondingly increased in nodules. Roots sequestered 45% of translocated-^{14}C throughout the life of the node-4 leaf. About 80% of the ^{14}C-activity exported from node 8 at flowering was in middle and upper stem sections, but during pod-fill over 85% moved into the pods and less than 1% to the nodulated root system. Starch concentration in the lower stem increased continuously from flowering, but in other plant parts declined after early pod-fill. At mid pod-fill, the concentration of soluble sugars in nodules and roots declined and reached a common value in stem sections. Nitrogen (C_2H_2) fixation decreased rapidly after peaking at early pod-fill. This decline, which was accompanied by loss of lower leaves, occurred in the presence of a high concentration of starch in the stem.**

Leaves on lower nodes are the major contributors of photosynthate to roots and lower stems in bean (6, 13, 17), but data are not available on their contribution to nodules. Since canopy closure reduces light penetration to the lower leaves and decreases their photosynthetic activity, the dependence of nodules on photosynthate from lower leaves could be a major factor limiting N_2 fixation. High plant densities greatly reduce N_2 fixation of individual bean plants (9). The availability of photosynthate to below-ground parts also depends on competition from sinks higher in the plant (12, 13). Information is lacking, however, on the effect of reproductive development on translocation patterns in nodulated beans. This study compares the contribution of leaves at nodes 4 and 8 to various plant parts in relation to plant development, carbohydrate content, and N_2 fixation.

Materials and Methods

The black seeded 'Puebla 152' bean (CIAT designation P498) is a Type III growth habit with determinant mainstem and indeterminant branches and has demonstrated capacity for abundant nodulation and N_2 fixation (9).

Uniform seeds were inoculated with a peat culture of *Rhizobium phaseoli* (CIAT strain 57) and planted 3 per 15 cm plastic pot containing a sterilized soil:peat (4:1) medium supplemented with 3 g hydrated lime (pH 6.5), 500 mg triple superphosphate, and 250 mg K_2SO_4 per pot. No nitrogen fertilizer was added. Seeds were covered with approximately 1 cm of sterilized sand. Plants were thinned to 1 per pot and staked after primary leaves developed. Branches were removed as they appeared to restrict photosynthate movement to the main shoot. Plants were grown in a greenhouse from March to June at 2 populations, by spacing pots at 45 × 15 cm and 45 × 45 cm, in a randomized-block design with 6 replications. Since plant density had little or no significant effect on the results, the data were combined for each stage of development. Each datum presented, therefore, is the mean of 12 values.

At 35, 48, 63, and 70 days after planting (preflowering, flowering, early pod-fill, and mid pod-fill, respectively) plants were selected for uniformity and removed for exposure of the node-4 or 8 leaf (numbering from the cotyledonary node) to $^{14}CO_2$. The node-8 leaf had not appeared by day 35 and the node-4 leaf (second trifoliate) abscised by day 70. At about 11:00 AM, a leaf was sealed in a polyethylene bag (27 × 28 cm) along with a gelatin capsule containing 50 μCi Na_2-$^{14}CO_2$ (specific activity 20 μCi/μmole) and perchloric acid injected to dissolve the capsule and generate $^{14}CO_2$. Plants were placed in indirect light, about 600 $\mu E/m^2$ sec^{-1} of photosynthetically active radiation, to prevent leaf damaging heat buildup in the bags. The bag was removed after 1 hr and plants were returned to their original positions and harvested 23 hr later.

Harvested plants were divided into nodules, roots, and 4-node shoot sections corresponding to nodes 1–4, 5–8, and above 8, which were designated lower, middle, and upper, respectively. Each shoot section was further separated into stem, leaves, and pods. The leaf exposed to $^{14}CO_2$ was discarded. Plant parts were dried at 55°C for 48 hr, weighed, and ground to 40 mesh. A 20 mg aliquot of each part was suspended in 15 ml of a scintillation cocktail (38 g Cab-O-Sil, 250 mg dimethyl POPOP, and 4 g PPO per liter of toluene) and ^{14}C-activity determined with a Packard Tri-Carb Scintillation Counter. Quench was monitored using an external standard and corrected for by a quench curve derived from internal standards. The ^{14}C-activity in each plant part (counts adjusted to total dry weight) is expressed as percent of the total recovered activity per plant. No attempt was made to measure or correct for respirational losses of ^{14}C during the 24 hr translocation period.

At each harvest separate plants not exposed to $^{14}CO_2$ were used to estimate N_2 fixation (acetylene reduction), dry weight distribution and carobhydrate (ethanol soluble and starch) content. Nitrogen (C_2H_2) reduction was determined on individual root systems using the sequence described by Graham and Rosas (8) with ethylene production assayed on a gas chromatograph using a 3.6m (12-ft) Poropak R column operated at 50°C with helium carrier gas. The rate of C_2H_2 reduction (μmole/plant per hr) was calculated using peak areas.

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These plants were divided, dried, weighed, and ground in the same manner as those dosed with $^{14}CO_2$. Soluble carbohydrates were extracted by suspending a 100 mg sample in 20 ml of boiling 80% ethanol and refluxing for 2 hr. The plant residue was then boiled for 3 min in 50 ml of distilled water and incubated 3 hr at 50°C with 10 ml 0.25% amyloglucosidase and 1 ml of 1.0 M sodium actetate buffer (pH 4.2) to hydrolyze starch (15). Soluble carbohydrates and starch were determined with anthrone (19). Starch hydrolyzate values were multiplied by 0.9 to correct for hydrolytic water.

Results and Discussion

Dry matter and carbohydrates. Total plant dry weight reached a maximum at, and was maintained during, pod-fill (Table 1). During this period all stem sections lost dry weight but this was compensated for by pod gains. A majority of pod weight was borne above node 8. Root dry weight increased 90% after flowering and more than one-third was gained during pod-fill when other vegetative parts decreased or ceased to accumulate dry matter. This late root growth has not been observed in field studies (10), and may have resulted from the removal of potential pod-producing branches which reduced the shoot sink and increased the availability of photosynthate to the roots.

The concentration of soluble sugars in each of the 3 stem sections appeared to stabilize at about 74 mg/g dry weight by mid pod-fill, while the concentration in roots and nodules was lowest at this stage (Table 2). Possibly the similar sugar content reached in the stem sections was indicative of completion of structural development following cessation of elongation.

The stem sections accumulated extremely high concentrations of starch, reaching over 60% of the dry weight of the middle stem (Table 2). The highest starch values are several times greater, and occurred earlier in plant development than those in this and similar cultivars when field grown (8). From flowering to early pod-fill the starch concentration in the 3 stem sections remained high or increased, but decreased in the middle and upper sections as pod-fill continued. However, in the lower stem, which had few pods, the concentration of starch continued to rise. This accumulation of starch in stems during pod-filling has been previously observed and may indicate that beans are inefficient in their use of photosynthate or provide inadequate sink capacity for the source present (1, 6). Adams et al. (1) made visual comparisons of the starch levels in bean cultivars and found the content at the fifth internode of 'Puebla 152' (black) was moderately high at first flowering and mid pod-fill and decreased to only medium levels at pod maturity.

^{14}C-distribution. At preflowering, over 85% of the ^{14}C-photosynthate from the node-4 leaf was recovered in the nodules, root, and lower stem section (Fig. 1b). The results for lower stem and root parallel those obtained in other work (6, 17). The roots contained 45% of the recovered radioactivity and this level of distribution continued into pod-filling. Nodules accumulated only 3.5% of the radioactivity at the preflowering stage. Sucrose is the major sugar in host cells of active bean nodules and becomes highly labeled upon exposing plant tops to $^{14}CO_2$ (2).

At flowering, the proportion of radioactivity translocated from the node-4 leaf to the lower stem declined by about 40%, while the fraction moving into nodules increased over 5-fold. Nodules are strong sinks for photosynthate (14, 16) and, in this study, the increased radioactivity in nodules was at the expense of accumulation in the lower stem. Again, plant parts above node 4 contained less than 15% of the total radioactivity. A portion of this may have cycled through the roots and nodules during the 24 hr translocation period.

Over 90% of the recovered radioactivity translocated from the node-8 leaf at flowering was found in the shoot sections immediately above and below this node (Fig. 1a). At this time the upper stem section was still elongating while the middle stem was rapidly accumulating starch and increasing in dry matter (Table 1 and 2). Some leaves above node 8 were immature and were appreciable sinks for ^{14}C-photosynthate. Together, all reproductive parts contained less than 5% of the ^{14}C-photosynthate translocated from the node-8 leaf, and less than 1% each was recovered in the root, nodules, or lower stem at this or later developmental stages.

Partitioning of radioactivity from the node-4 leaf changed little between flowering and early pod-fill (Fig. 1b). There was,

Table 2. Concentration of ethanol-soluble sugars and starch in nodules, roots, and stems of bean plants at different stages.

Days after planting	Nodule	Root	Stem sections		
			Lower	Middle	Upper
	Soluble sugars (mg/g dry wt)				
35	83.45	79.56	70.03	103.25	---
48	44.01	86.39	40.15	52.18	48.04
63	51.76	69.93	73.02	77.26	159.89
70	32.90	41.68	73.49	74.23	75.47
LSD 5%	13.37	15.04	10.65	12.83	32.31
	Starch (mg/g dry wt)				
35	148.27	17.53	231.33	152.93	---
48	87.20	35.85	203.70	683.10	70.81
63	57.45	50.08	305.70	628.35	475.87
70	64.40	32.66	403.13	363.83	315.46
LSD 5%	23.79	9.00	98.09	82.40	159.11

Table 1. Dry weight of bean plants at different growth stages.

Days after planting	Root	Dry weight (g/plant)									
		Stem[z]			Leaves			Pods			Total[y]
		LR	MD	UP	LR	MD	UP	LR	MD	UP	
35	1.96	.86	.85	--	2.91	1.80	--	--	--	--	8.74
48	1.94	1.05	1.16	.48	2.70	3.29	.69	.00	.10	.13	11.97
63	2.78	1.50	2.35	1.79	1.65	3.44	2.82	.00	1.84	2.99	22.01
70	3.71	1.11	1.36	1.13	.58	2.81	2.85	.67	1.75	4.55	21.34
LSD 5%	.72	.14	.27	.32	.38	.75	.78	ns	1.06	1.73	1.94

[z]Shoot sections: LR = lower, nodes 1–4; MD = middle, nodes 5–8; UP = upper, nodes >8.

[y]Includes nodule dry weight (Fig. 2).

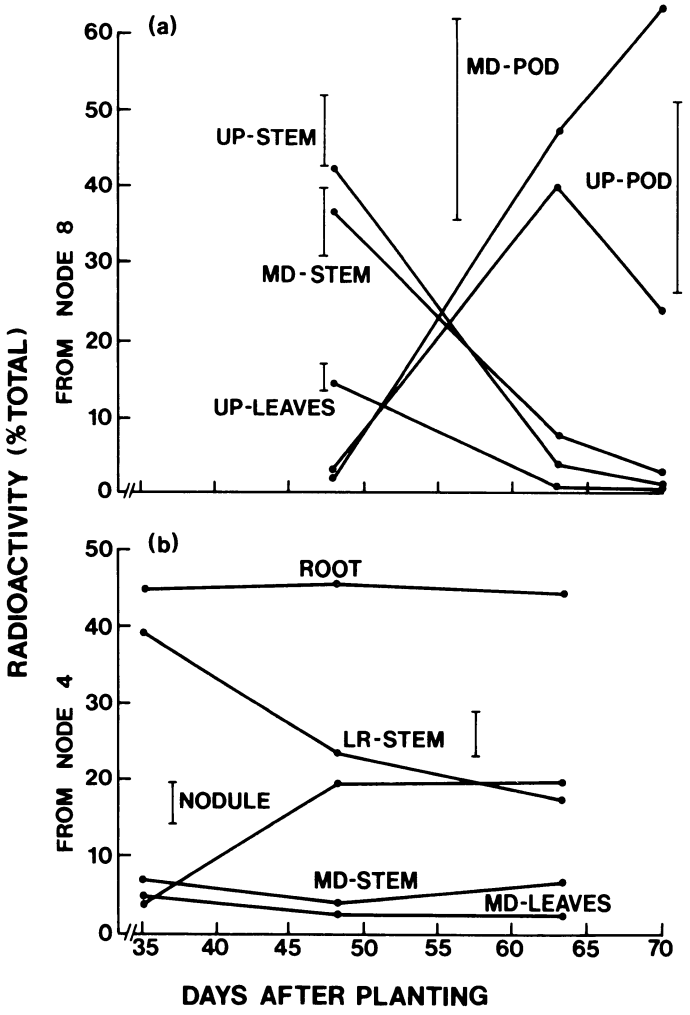

Fig. 1. Distribution percentage of ^{14}C-activity from the leaf at node 8 (a) or 4 (b) in various parts of bean plants. Vertical bars – LSD 5% (No bar – not significant). Plant parts not shown contained less than 3% of the ^{14}C-activity. LR, MD, and UP correspond to lower, middle, and upper shoot sections, respectively.

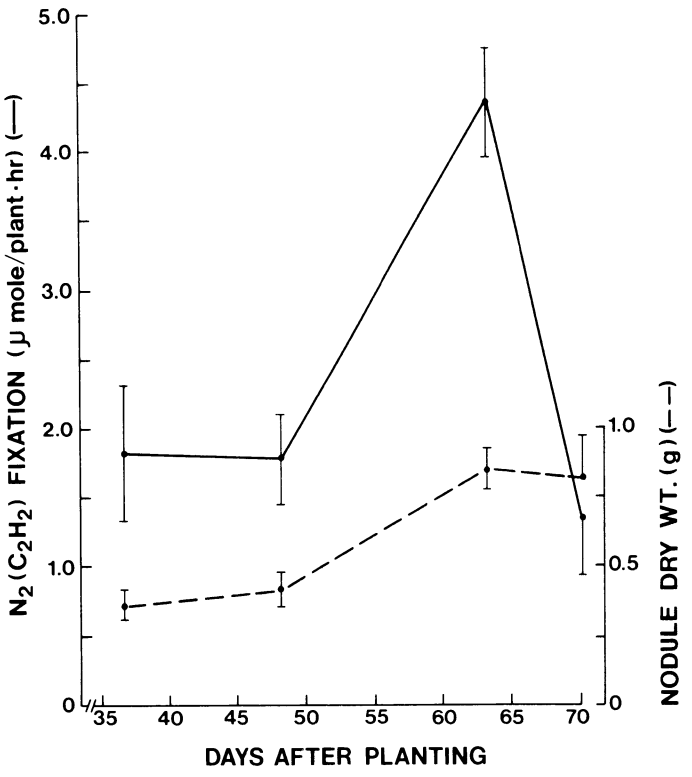

Fig. 2. Nitrogen (C_2H_2) fixation and nodule dry weight of bean plants at different growth stages. Vertical bars = SE.

however, a dramatic shift in the distribution of ^{14}C-activity from the leaf at node 8, with the developing fruits on middle and upper stem sections acquiring over 85% of the recovered ^{14}C-photosynthate (Fig. 1a). The proportion of radioactivity in both groups of pods was similar, although the dry weight of the upper pods was 63% greater than that of middle pods (Table 1). The decline of the middle and upper stem sections as sinks for ^{14}C-photosynthate reflects the rapid loss of starch from these sections following early pod-fill (Table 2).

Although lower leaves can supply appreciable amounts of photosynthate to fruits developing higher in the bean plant (13, 17), no such movement was found in this study. The lack of a contribution to pods from the node-4 leaf possibly resulted from an abundance of starch in the stem, senescence of lower leaves, and continued root growth which diverted photosynthate downward.

At mid pod-fill the pods of the middle and upper shoot sections together contained over 90% of the ^{14}C-photosynthate from the node-8 leaf. The concentration of starch in the middle stem section, from which the node-8 leaf originated, declined 42% over the 7 days preceding the harvest at the mid pod-fill stage. The amount of starch lost from the middle stem was equal to 45% of the gain in dry matter by the pods over this period. Presumably a large share of this remobilized starch was translocated to developing fruit, as was current photosynthate.

The results show that although the 2 leaves dosed with $^{14}CO_2$ were separated by only 3 nodes, the distribution of ^{14}C-photosynthate from these source leaves was very different. Since a majority of ^{14}C-photosynthate from the node-4 leaf moved to the root and nodules, these organs were also undoubtedly major sinks for assimilate translocated from leaves below node 4. As senescence of the lower leaves progressed, the more distal leaves would be expected to contribute a larger share of photosynthate to the lower portions of the plant. However, less than 1% of total radioactivity from the node-8 leaf was recovered in the lower stem, roots, and nodules at mid pod-fill, even though most of the lower leaves had abscised or were senescent. The dry weight of lower leaves decreased over 80% between the preflowering and mid pod-fill. Movement of ^{14}C-photosynthate from the leaf at node 8 to the lower stem and below was probably restricted because of a large demand for assimilate by developing fruit, in addition to an apparent excess of carbohydrate in the lower stem as evidenced by its continued accumulation of starch (Table 2). Presumably photosynthate translocated from a leaf at some node between 4 and 8 was partitioned more evenly between upper and lower parts of the plant.

Nitrogen fixation. The rate of N_2 (C_2H_2) reduction remained relatively unchanged at about 1.8 μmole/plant per hr until after flowering (day 48) before increasing to 4.3 μmole/plant per hr at early pod-fill (Fig. 2). This rate is about 30% of the maximum reported for larger field-grown plants of this cultivar (9). The rate of N_2 fixation rapidly decreased to the lowest value at mid pod-fill. The postflowering peak in N_2 fixation and the subsequent rapid decline is consistent with other reports of the seasonal profile of fixation by bean (7, 8, 18). Mean nodule dry weight was essentially the same at early and

mid pod-fill (Table 1), and the loss in N_2 fixation (Fig. 2) probably resulted from the increased proportion of senescent (i.e., green) nodules observed at the last harvest. Specific nodule activity (μmole C_2H_2 reduced/g nodule dry wt per hr) ranged from 5.8 on day 35 to 3.3 at mid pod-fill, but because of large variations the values are not significantly different.

The greater movement of photosynthate to developing bean fruit (Fig. 1a) was concurrent with decreased photosynthesis of the lower leaves. This loss of photosynthate activity was shown by Bethlenfalvay and Phillips (5) and is implied in the present study by the yellowing and abscission of lower leaves. Both diminished photosynthesis and more active sinks in the upper part of the plant could restrict the availability of photosynthate to roots and nodules and reduce the rate of N_2 fixation. The concentration of soluble sugars in nodules did decrease 64% between early and mid pod-fill, whereas that of starch failed to show a significant change (Table 2). Starch may not be readily available for nitrogenase activity (11), although transferring bean plants to low light caused a total disappearance of starch in nodules within 5 days (4). As N_2 fixation decreased after early pod-fill, the concentration of starch plus sugars in the root decreased 38% while dry matter accumulation (i.e., growth) continued. Antoniw and Sprent (3, 4) also found continued gain in dry weight of bean roots as nodule senescence increased and the rate of N_2 fixation rapidly declined. Nodules may be less able to compete with root tissues for substrate as the supply of photosynthate decreases. A limitation of photosynthate apparently does not affect the specific nodule activity of healthy (i.e., pink) bean nodules, but rather reduces N_2 fixation by restricting the production of nodule tissue (4). In this study, the large amount of starch stored in stem suggests that the rapid loss of N_2 (C_2H_2) fixation and increased nodule senescence may not have resulted from insufficient carbohydrates. However, it is conceivable that leaves export other substances which are critical to the continued growth and function of nodules.

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