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Nutrition of a Developing Legume Fruit

FUNCTIONAL ECONOMY IN TERMS OF CARBON, NITROGEN, WATER[1]

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ABSTRACT

The economy of functioning of the developing fruit of white lupin (*Lupinus albus* L.) is assessed quantitatively in relation to intake and usage of carbon, nitrogen, and water. Of every 100 units of carbon imported from the parent plant, 52 are incorporated into seeds, 37 into nonmobilizable material of the pod, and the remaining 11 lost as CO_2 to the atmosphere. An illuminated fruit can make net gains of CO_2 from the atmosphere during the photoperiods of all but the last 2 weeks of its life, suggesting that it is active in assimilation of CO_2 respired from pods and seeds. This conservation activity is important to carbon economy.

Phloem supplies 98% of the fruit's carbon and 89% of its nitrogen. Most of the xylem's contribution enters early in development. Xylem and phloem supply similar sets of amino compounds, amides predominating. Ninety-six per cent of the fruit's nitrogen becomes incorporated into seeds. Sixteen per cent of the seed's nitrogen is mobilized from the senescing pod.

The transpiration ratio of the fruit is 22.5 ml per gram dry matter accumulated. Xylem supplies 60% of a fruit's total water requirement and the equivalent of two-thirds of its transpiration loss. Phloem becomes prominent as a water donor once the seeds start to fill.

The fruit exhibits a 31% conversion by weight of organic imports into food reserves of seeds. This entails an intake through vascular channels of 1756 mg sucrose and 384 mg amino compounds and an accumulation in seeds of 412 mg protein, 132 mg oil, and 110 mg perchloric acid-soluble carbohydrate.

The nutrition of a green legume fruit, like that of many other fruits, consists of three principal activities: intake of translocate through phloem, attraction through transpiration of water and solutes carried in xylem, and fixation by the fruit of CO_2 derived from the outside atmosphere or collecting in the gas space from the respiring seeds. Studies on legumes have concentrated on the first and third of these processes, especially in relation to the requirements of the developing seeds for C (4, 11).

This paper evaluates how all three activities interact quantitatively in shaping the fruit's economy of C, N, H_2O. The white lupin (*Lupinus albus*) is investigated since information is already available on phloem transport (9, 10, 12) and N metabolism (1) in its fruits.

MATERIALS AND METHODS

Plant Material. Glasshouse growth of nodulated white lupin (*L. albus* L.) in nitrogen-free sand culture was as described previously (1, 12). Field material, sown at commercial planting density, was obtained from Bakers Hill, Western Australia. The study used the three basal fruits developed from the primary inflorescence of a plant. Fruit ripening took place in spring and early summer (September to November), the normal time of ripening when grown as a seed crop in Western Australia. Age was determined by tagging at anthesis.

Harvest and Analysis of Fruit. Thirty fruits of the same age were harvested at specific intervals during the 12-week growth cycle of the fruit. Fresh weight, dry weight, C and N contents of dry matter were measured for pod and seeds. Nitrogen was determined by Kjeldahl analysis and C by dichromate digestion (14). Protein, oil, and perchloric acid-soluble polysaccharide of seeds were determined as described previously (1).

Phloem and Xylem Sap. Fruit tip phloem sap, and tracheal (xylem) sap from stem segments immediately below the fruit clusters were collected at intervals throughout the fruit's growth cycle. Sap collection techniques and analyses for organic solutes have been described (1, 9, 10).

Carbon Dioxide Exchange of Fruits. Attached fruits of glasshouse-grown plants were enclosed in the cuvette of an open gas exchange system and their CO_2 exchange monitored continuously for 48 hr. Measurements, from a sample of six fruits, were taken during each week of growth. All fruits used were situated above the leaf canopy, fully exposed to sunlight during daytime. Air flow through the cuvette (400 ml/min) was such that its CO_2 concentration did not usually change by 5% and never by more than 15% in passing over the fruit. Water circulating through an outer jacket of the cuvette achieved temperature control of its gas space to within ±2 C of ambient. The effluent gas stream was dried by condensation at 0 C and passage through magnesium perchlorate. Carbon dioxide was measured on an IR gas analyzer operating in the differential mode and calibrated using precision gas-mixing pumps (2).

Gas exchange measurements on field-grown, attached fruits used a closed, temperature-controlled cuvette of 1-liter volume. The water jacket around the cuvette effectively prevented significant temperature changes after transfer from sunlight to darkness. A small fan circulated the cuvette's atmosphere. One-ml samples were taken from the gas space at 5-min intervals over the 15-min period following enclosure.

Transpiration Measurements. A Grieve-Went electric hygrometer apparatus (6) was used to measure rates of water vapor loss from attached fruits (glasshouse material). Changes in relative humidity were measured within a 10-sec period after enclosure of the fruit, thus minimizing complications due to changes in temperature or stomatal condition. Transpiration recordings were taken from the same 30 fruits during two 24-hr cycles of each week of their life. The brief periods of enclosure necessary for the transpiration measurements did not appear to affect growth and development of the fruits. Results using the hygrometer technique were compared with measurements of short term (2-5 min) water loss from similarly aged, detached fruits using a torsion balance (13). There was good agreement between the methods, as had been shown previously (6) for other material.

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RESULTS

Growth, and Carbon and Nitrogen Balance of Developing Fruit, Pod and Seed. A first phase of growth (0–4 weeks after anthesis) is one of pod expansion, primarily through uptake of H_2O. Intake of C and N is low at this stage and seed growth negligible. A second phase (4–8 weeks) shows a high rate of utilization of C and N by the fruit and rapid growth of its seeds. The third, final phase (8–12 weeks) coincides with laying down of storage reserves in seeds (1). Intake of C and N is maintained at a high level until the pod and seed commence to dehydrate. Ninety-six per cent of the fruit's N is eventually contained in the seeds; mobilization of N from pod tissues during the last 4 weeks of fruit development is estimated to supply 16% of the seed's total N requirement.

These features suggest that the growth pattern of the lupin fruit is broadly similar to that of other legume fruits (Fig. 1) (5, 8).

Organic Solute Transport to the Fruit. Amounts and concentrations of the major organic compounds of phloem and xylem are shown in Figure 2. The compounds listed are estimated to represent 95% of the C and virtually all of the N transported to the fruit through vascular channels. Phloem is by far the more concentrated source of solutes and the importance of its sucrose and amides as general nutrient sources to the fruit has been established (1, 12). Phloem sap becomes less concentrated in sucrose but more concentrated in amino compounds as fruit development proceeds (Fig. 2A), effecting a change of from 19.6:1 to 10.5:1 in the C/N (w/w) ratio of the translocate. Early, when the ratio is high, there is a large C requirement for constructing the structural framework of the fruit; later, when the ratio is lower, synthesis of storage protein creates especially high demands for N.

Sugar is absent in xylem fluids serving the fruit. Xylem amino compounds reach maximum concentration 5 weeks after anthesis, and then decline (Fig. 2B). Xylem carries a very similar spectrum of nitrogenous solutes to phloem, and the amino acid balance of these channels varies little during fruit development (Fig. 2, C and D) (12). The low C/N (w/w) ratio of xylem fluid (1.96–2.06:1) reflects the high amide content. The substantial concentration difference for N in xylem and phloem should be noted. The significance of this has been discussed (7).

Water Budget and Carbon Dioxide Exchange of the Fruit. Transpiration, and changes in fruit tissue water are shown in Figure 3, A and B, respectively. More water is incorporated into fruit tissue than is transpired over the first 4 weeks of growth, but transpiration then becomes by far the larger item. Maximum water usage occurs over the period 6 to 8 weeks. Water intake then declines abruptly and an increasing proportion of the transpired water comes from dehydration of pod and seed.

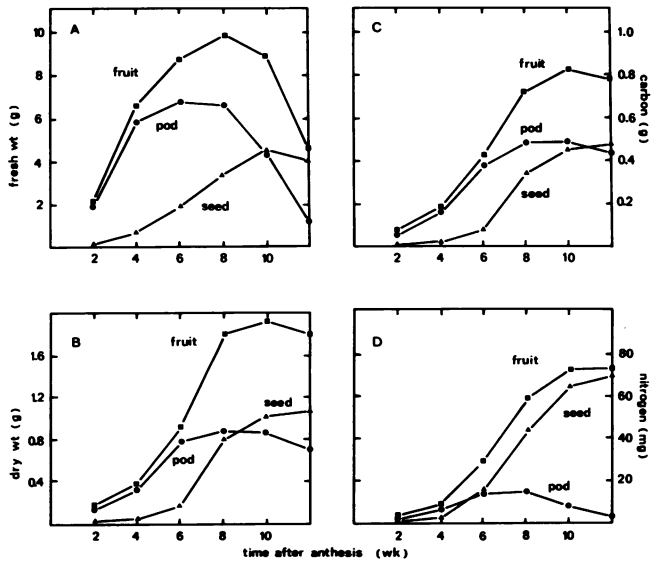

FIG. 1. Changes during development of the *L. albus* fruit. A: Fresh weight; B: dry weight; C: carbon content; D: nitrogen content.

Gas exchange measurements (Fig. 3C) demonstrate that a fruit fully exposed to light can effect daytime gains of C from its outside atmosphere from week 1 until its pod turns yellow and dehydrates 10 weeks after anthesis. During the early phase of growth (0–4 weeks), daily gains through photosynthesis are roughly in balance with respiration losses at night. Over the next phase of growth (4–8 weeks), nightly losses increase markedly, presumably due to increased respiration of the rapidly enlarging seeds. Net photosynthesis of the fruit remains high and constant during this stage, but increasingly large net deficits of CO_2 are recorded. The final phase of growth (8–12 weeks) witnesses a declining rate of photosynthetic activity, but a continuance of night respiration at near-maximum rate. (Fig. 1C). Net weekly losses of CO_2 are therefore very high, equivalent, in fact, to 53% of the total import of C by the fruit during this stage (Table I).

Integration of the CO_2 exchange data (Fig. 3C) for the 12-week period of fruit development shows a net photoperiod gain of 63 mg C/fruit and a net nightly loss of 161 mg C. Assuming that the 8 to 12 C temperature differential promoted, on average, a rate of daytime respiration twice that at night, gross photosynthetic fixation over the fruit's life would be 63 + (2 × 161) = 385 mg C. This is a highly significant return relative to the 787 mg C incorporated into dry matter by the fruit. (Table I).

The data of Figure 3C refer to fruits exposed to full daylight above the leaf canopy. In the field situation, the first formed fruits of *L. albus* are usually shaded by foliage of the axillary branches and consequently operate well below compensation point during daytime. The gas exchange data of Table II bear evidence of this. When estimates of gross CO_2 fixation rates of fully illuminated and poorly illuminated fruits are compared (right-hand column, Table II), the significance of fruit photosynthesis in conserving respiratory products becomes evident.

The internal gas space of lupin fruits contains high levels of CO_2 (1.2–1.6% by volume) suggesting that the fruit wall acts as an effective barrier to gaseous exchange with the outside atmosphere. The physiological implications of these observations are being investigated.

Assessment of the Relative Importance of Xylem and Phloem in Delivering Carbon, Nitrogen, and Water to the Fruit. The basic approach to these estimates (Table I) is to assume that the fruit is nourished by mass flow in xylem and phloem, and that these channels furnish assimilates in the proportions and concentrations illustrated in Figure 1. Calculations are then made to find what mixture of xylem and phloem streams would be required to fulfil precisely the recorded intake of C and H_2O over a specific growth interval, the "goodness of fit" of the approach being then evident from how closely the N delivered by this mixture matches the actual increment of N recorded in the fruit over the same period of time (compare item 4 with the sum of items 12 and 13, Table I). A calculation covering the complete 12-week growth cycle (column marked Total, Table I) shows that a phloem-xylem mixture averaging 4:6 by volume is required to meet the fruit's C requirements of 885 mg C and water intake of 43.4 ml, and in so doing accounts for 97% of the fruit's intake of 72.5 mg of N. As might be expected, the fit of data is less perfect when smaller inputs and shorter time intervals are considered (Table I).

The estimates suggest that xylem and phloem supply water continuously during fruit development, the xylem contribution amounting to the equivalent of two-thirds of the total water lost in transpiration (items 5 and 9, Table I). Phloem is clearly the dominant donor of C and N to the fruit, and it becomes progressively more important in supplying water once the seeds start to

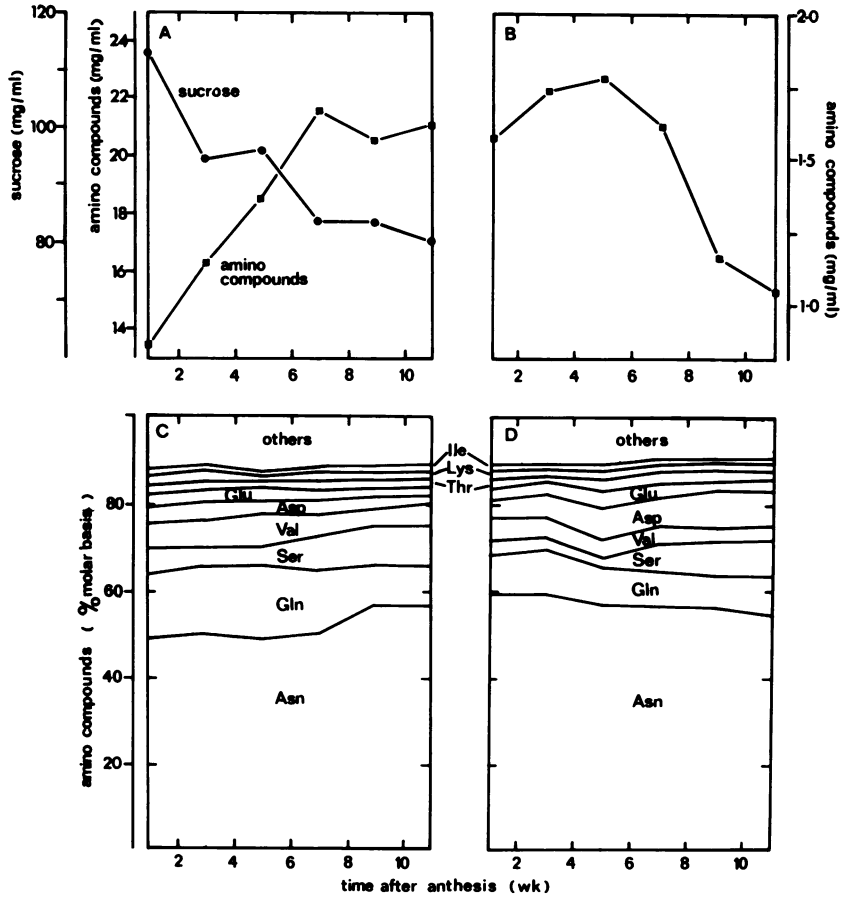

FIG. 2. Changes during fruit development of *L. albus* (white lupin) in the major organic solutes of conducting channels serving the fruit. A: Solutes, fruit tip phloem sap; B: amino compounds, xylem (tracheal sap); C: composition amino fraction phloem sap; D: composition amino fraction xylem sap.

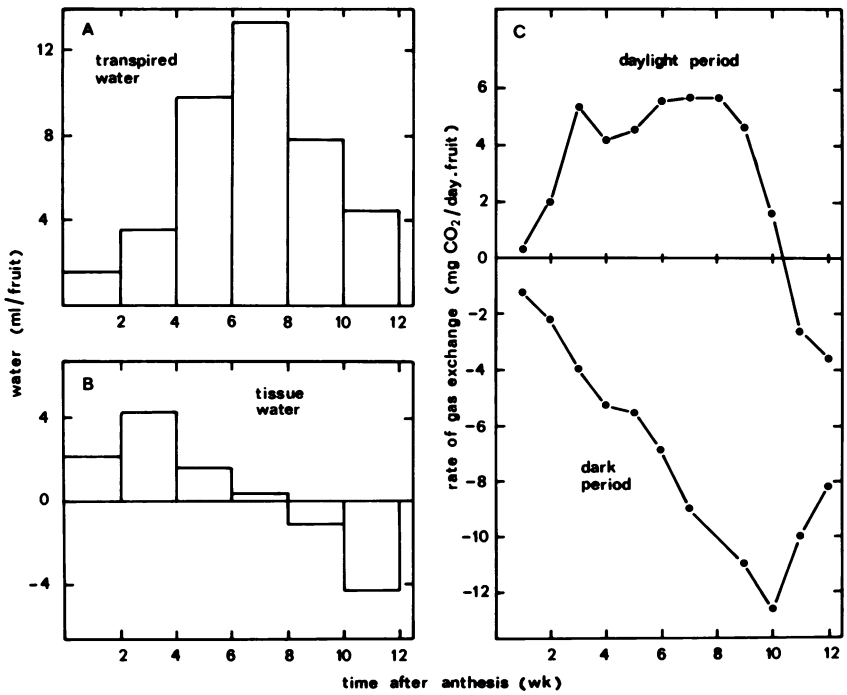

FIG. 3. Water economy and carbon dioxide exchange of developing fruits of *L. albus* (white lupin). A: Transpiration loss; B: changes in tissue water; C: carbon dioxide exchange (natural day length, spring, Western Australia). All measrements refer to glasshouse-grown, attached fruits, fully exposed above the leaf canopy.

Table I. Carbon, Nitrogen and Water Budgets of the Developing Fruit of Lupinus albus (white lupin) and Estimates of Delivery of these Materials by Xylem and Phloem

Amounts per fruit	Fruit Age(weeks) 0-2	2-4	4-6	6-8	8-10	10-12	TOTAL
1. Carbon increment as dry matter (mg)	65.5	86.7	257.2	309.5	102.3	-34.5	786.7
2. Carbon lost as CO_2 to atmosphere (mg)	-2.2	-0.7	-3.5	-14.0	-31.0	-45.2	-96.6
3. Total carbon requirement (mg)	67.7	87.4	260.7	323.5	133.3	10.7	883.3
4. Nitrogen increment as dry matter (mg)	4.0	5.9	18.5	30.0	13.1	1.0	72.5
5. Water loss in transpiration (ml)	1.57	3.67	9.82	13.23	7.85	4.47	40.61
6. Increment-loss in tissue water (ml)	2.05	4.20	1.55	0.35	-1.16	-4.22	2.77
7. Total water input (ml)[1]	3.62	7.87	11.37	13.58	6.69	0.25	43.38
8. Water intake by phloem (ml)	1.13	1.69	4.87	6.92	2.98	0.24	17.51
9. Water intake by xylem (ml)	2.49	6.18	6.50	6.66	3.71	0.01	25.87
10. Carbon intake by phleom (mg)	66.30	83.40	256.30	320.00	131.80	10.70	868.50
11. Carbon intake by xylem (mg)	1.37	3.96	4.36	3.53	1.52	0.00	14.74
12. Nitrogen intake by phloem (mg)	2.94	4.72	16.11	26.63	11.05	0.91	62.36
13. Nitrogen intake by xylem (mg)	0.70	2.01	2.17	2.37	0.75	0.00	8.00

[1]Estimates (items 7-13) assume that phloem and xylem operate as mass flow systems and furnish assimilates in proportions and concentrations given in Fig. 2. The estimates specify the mixtures of xylem and phloem streams which fulfill precisely the recorded carbon and water inputs of the fruit. The goodness of fit of the data is apparent from how closely the amounts of nitrogen delivered by these mixtures (items 12 and 13) match the recorded nitrogen increments of the fruit (item 4) during the specified intervals of the growth cycle.

Table II. Gas Exchange of Attached 9-week Fruits of Field-Grown White Lupin (Lupinus albus).

	CO_2 Evolution Light	Dark[2]	Gross Photosynthesis[1]
	μg CO_2/fruit·hr		
Fully exposed above canopy[3]	7 ± 27[4]	723 ± 48	716
Submerged within canopy	607 ± 60	1137 ± 44	530

[1]CO_2 evolution in the dark minus CO_2 evolution in the light (10.00 - 12.00 hr)

[2]Exchange immediately following measurement in light

[3]Receiving full sunlight during experiment

[4]Mean values ± standard error of 20-25 fruits

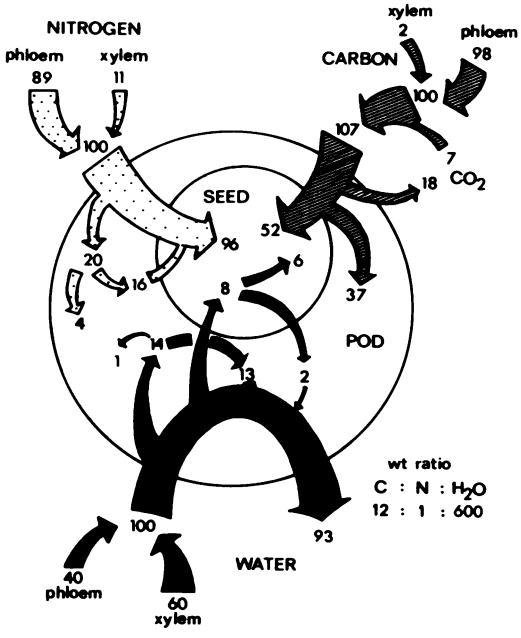

FIG. 4. Economy of carbon, nitrogen, and water over the growth cycle of a fruit of white lupin (*L. albus*). Xylem and phloem deliveries are expressed relative to a net intake of 100 units of a specific commodity. Net photosynthesis, respiration loss, transpiration, dehydration losses in ripening, and nitrogen mobilization from pod to seed are depicted. The relationship by weight between the imports is shown.

function as a major sink for assimilates. Xylem achieves some importance in delivering N to very young fruits (2-4 weeks old), when the sugar-N balance of the phloem stream is still relatively high (Fig. 2A) and when up to 80% of the liquid intake of the fruit takes place through xylem (Table I). Nitrogen accumulates at this time in excess of the requirements of dry matter production. It is stored in pods largely as asparagine, and in endospermic fluid, as ammonia and alanine (1). These storage pools disappear once the embryos lay down storage protein (P. J. Sharkey, unpublished).

DISCUSSION

The data presented in Figures 1 to 3 and Table I can be used to construct a scheme illustrating the economy of the white lupin fruit with respect to H_2O, C, and N (Fig. 4). Relative contributions from xylem and phloem over the developmental period are shown, and net photosynthesis, mobilization of N from senescent pods to seeds, and dehydration of fruit parts are related quantitatively to these transport activities. The budgets are based on a net intake of 100 units of a particular material through vascular connections with the parent plant. The C/N/H_2O ratio of 12:1:600 expresses the relationships by weight between these imports. The budget for C is complicated by uptake of an additional 7 units of C from the atmosphere by fruit photosynthesis. The high efficiency (80%) of mobilization of N from pod to seed during fruit ripening should be noted.

The dominant role of phloem in supplying organic solutes is evident. Xylem supplies, on average, 60% of the fruit's water, 11% of its N, but only 2% of its C. Xylem is also likely to be a vital source of those mineral elements (*e. g.* Ca) only sparingly mobile in phloem (7).

The water budget shows that 22 of the 100 units of H_2O are used in pod and seed growth, but that only 7 units ultimately remain after the pod is fully dehydrated. The transpiration ratio of the fruit is 22.5 ml/g dry matter, a water economy bettering that attributed to succulents (3). Of course, the fruit receives assimilates derived by the gaseous exchange of other photosynthetic surfaces, the transpirational penalty of this being evident as a 30-times greater loss of water from the population of leaves of a fruiting plant than from its complement of fruits.

The water economy of the legume fruit is likely to be a carefully balanced compromise, too low rates of stomatal exchange attracting too little xylem-mobile nutrients, too high a rate of ventilation detracting from the fruit's resistance to drought and its ability to accumulate and reutilize its respired CO_2. Water stress during ripening of lupin crops in Western Australia can promote premature and total defoliation, forcing the plants to ripen their seeds from previously stored assimilates and current photosynthesis of pod and stem. High efficiency in a fruit's water usage and in its conservation of respiratory products is likely to be of adaptive value in this situation. Since fruits will experience almost full illumination on a defoliated plant, photo-

synthesis by the fruit will be fully functional.

The physiology of the white lupin fruit may now be viewed in terms of its processing of incoming organic solutes into specific reserve materials of the seed. A total of 1756 mg sucrose and 384 mg amides and amino acids are imported during the fruit's life, and from these materials 412 mg protein, 132 mg oil, and 110 mg perchloric acid-soluble carbohydrate, are laid down in the seeds (1). The efficiency of conversion of imports into "useful" ergastic substances is therefore 31%, a value relating specifically to fully illuminated fruits in the optimum environment of the glasshouse. A somewhat different efficiency might be expected in the crop situation, where regimes of light, temperature, minerals, and water might be far from satisfactory.

The present study highlights the significance of the legume pod as a temporary reservoir of seed-bound solutes and as an agent of reutilization of respiratory products of the seeds. Since these functional qualities are likely to be related to pod size and thickness, the required investment of skeletal and other materials in the pod's structure can no longer be viewed as a wasteful expenditure of imported assimilates. Already, during domestication of legumes, selection for nonshattering fruits has eliminated much of the fibrous material of the pod, and it may be that fruits of modern cultivars are already close to achieving the maximum possible efficiency in conversion of imports into harvestable products. Since photosynthetic reutilization of respiratory products is so important to the fruit's economy, there might be value in selecting legume cultivars whose fruits remain green and adequately illuminated for as long as possible during development.

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