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The Economy of Carbon and Nitrogen in Nitrogen-fixing Annual Legumes - Experimental Observations and Theoretical Considerations.

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ABSTRACT

Techniques are described for studying the economy of carbon and nitrogen in annual nodulated legumes. Budgets for utilization of net photosynthate are constructed for cowpea (*Vigna unguiculata* (L) Walp.) and white lupin (*Lupinus albus* L.), including expenditure in respiration and dry matter accumulation of plant parts, carbon consumption in growth, respiration and ~~nitrogen~~ export of fixed nitrogen by nodules, and the provision of recent photosynthate and earlier-fixed carbon to fruits. Sources of nitrogen to fruits are defined, and efficiencies of conversion of net photosynthate to protein of above-ground vegetative parts and of seeds are computed. Consideration is given to the timing of events associated with loss of symbiotic activity after flowering.

Literature giving estimates of the respiratory requirements of nitrogen fixation by nodules is reviewed. Rates of respiration of nodules of cowpea, white lupin and pea (*Pisum sativum* L.) are assessed from a theoretical viewpoint, basing the estimates on ATP requirements for assimilation of N_2 into nitrogenous solutes, and published values for respiration costs in plant tissues. Expressed as CO_2 output per unit of nitrogen assimilated, these estimates greatly exceed the experimentally-observed CO_2 efflux of nodules of the species. This discrepancy is examined in relation to the capacity of nodules to fix CO_2 and the uncertainty of the *in vivo* requirement of nitrogenase for ATP.

1. INTRODUCTION

Despite greatly increased attention from plant breeders and physiologists over recent decades the pulse and fodder legumes still qualify amongst the least understood of crop plants widely used in agriculture. This is partly due to the low intensity of research effort on the group, say in comparison with cereals, but it relates especially to the inherent complexities of legume functioning, particularly the legumes' capacities to fix nitrogen symbiotically and to produce forage and seeds unusually rich in protein.

Our approach over recent years has been to assemble information of a physiological, biochemical and structural nature on tissue and organ functioning of selected grain legumes with the general objective of assessing how efficiently the whole plant and its parts operate in channelling assimilatory resources into protein production. Already available is information on the following,

1) the translocatory arrangements for interchange of assimilates within the plant, and the identification of the organic and inorganic solutes moving in conducting elements of xylem and phloem (Atkins et al, 1975; Pate, 1976; Hocking & Pate, 1977),
2) the functional economy of nodule and nodulated roots, especially in relation to the efficiency of conversion of photosynthate from the shoot into amino compounds produced in nitrogen fixation (Minchin & Pate, 1973; Pate, 1976, 1977),

3) the role of vegetative parts of the shoot in the synthesis, processing and transport of assimilates destined for nourishment of fruits and seeds (Lewis & Pate, 1973; Pate *et al*, 1975; Pate, 1975, 1976), and

4) the functioning of the fruit in converting its own photosynthetic products and the translocate it receives from the parent plant into food reserves of seeds (Pate *et al*, 1977; Flinn *et al*, 1977; Atkins *et al*, 1977).

A recent extension of this approach has been to construct carbon and nitrogen budgets for the whole plant and its parts throughout growth and development, and thus obtain a picture of how assimilation of these two elements in shoot and root relates quantitatively to the growth and productivity of the plant (Herridge & Pate, 1977; Pate & Herridge, 1977). This paper summarises our progress and the conclusions we have drawn.

2. EXPERIMENTAL STUDY OF THE CARBON AND NITROGEN ECONOMY OF THE ANNUAL NODULATED LEGUME.

For our studies of the carbon and nitrogen balance of nodulated legumes, populations of plants were grown on minus nitrogen nutrient solution in sand culture. The experiments were conducted in naturally-lit glasshouses under seasonal conditions and at canopy densities equivalent to those under which the species would be normally grown as a crop plant. By use of evaporative cooling, temperatures within the glasshouse were maintained at a level close to that obtaining outdoors. Using containers of relatively large capacity (11 ℓ), and allowing only two plants per container, plant growth was not severely

restricted by volume of the rooting medium. Each container possessed a lid, with holes through which the shoots grew and through which measured amounts of water and nutrients were added. A port in the base of the container enabled excess water to be drained from the rooting medium. The basic construction of the container permitted gravimetric studies of transpiration loss from the plants, and allowed adaptation for study of the respiratory output of intact nodulated roots (see below).

The construction of a basic carbon budget for the plants required measurements of carbon gains or losses as dry matter of plant organs, and assessments of respiratory and photosynthetic exchanges of CO_2 by these same organs over specific intervals of the growth cycle. Changes in carbon, nitrogen and dry matter were studied by progressive sampling from the plant population, using harvests of at least 20 plants to reduce sampling errors due to variation between the plants (see Herridge & Pate, 1977). Other features measured were fresh weight of plant parts and area of leaflets. Root bleeding sap (xylem exudate) was collected from plants at intervals during growth and assayed for organic solutes of nitrogen.

Modification of the lidded container for measuring respiratory losses from the nodulated root was as shown in Fig.1. The base of each stem was sealed to the lid of the container with plasticine, and with the basal drainage port closed, the gas space of the root could be effectively sealed from the surrounding atmosphere. Inlet and outlet ports (Fig.1) permitted CO_2-free

air to be passed continuously through the containers, and by passing the effluent gas from the root through Pettenkoffer tubes containing KOH (see Minchin and Pate, 1973), the respired CO_2 of the nodulated root system was continuously collected. The rate of gas flow through the containers was adjusted to achieve an average CO_2 concentration around the roots matching as closely as possible the level of CO_2 in the rooting medium of similar plants not set up for root respiration studies. The root gas space of young plants showed CO_2 levels within the range 0.3 - 0.5% v/v. Levels rose to slightly in excess of 3% v/v by the time the plants had reached fruiting. The growth and nitrogen fixation of plants whose roots were enclosed for respiration was not noticeably different from that of unenclosed plants, and the final yield of dry matter and seed from plants grown in the containers was comparable with that encountered in field plot trials of the species under similar seasonal and nutritional conditions.

Two refinements of the gas flow system deserve mention. In one an infra-red gas analyzer (IRGA) is used to monitor CO_2 content of the effluent gas stream (see Minchin *et al*, 1977), thus making possible study of short-term effects on root respiration. In the other collections of CO_2 from roots are combined with pulse feeding of $^{14}CO_2$ to shoots, thereby enabling the time course of utilization of recently-fixed carbon by the nodulated root to be examined (Pate & Herridge, 1977).

Night respiration of shoots was measured using the same plants in which root respiration was being monitored. This was achieved by enclosing the shoots in plexiglass chambers of

60, 120 or 170 ℓ capacity and measuring CO_2 release from the shoot by absorption in Pettenkoffer tubes. The flow of CO_2-free air into the chamber was adjusted to achieve a concentration around the plant as close as possible to that of air (0.03%). Efflux of CO_2 was measured from dusk to dawn and the chambers removed from the shoot during the photoperiod.

Estimates of the proportional contributions of nodules and root to the respiration of the nodulated root were made by enclosing freshly detached nodules or roots in small plexiglass cuvettes and measuring their CO_2 output over the first hour following detachment using a gas sampling technique specially adapted for IRGA analysis (Atkins & Pate, 1977). Generally the estimates of respiratory output of the nodulated root made by summation of the respiration of its individual detached parts agreed reasonably well with the recorded CO_2 output of the nodulated roots of intact plants, as determined by the Pettenkoffer system (see Fig.2). Similarly, estimates of CO_2 output at night from detached parts of a shoot (eg stems + petioles, leaflets, and reproductive parts) together yielded a value for respiration close to that for the night respiration of the intact shoot.

Accordingly the "net photosynthesis" of the shoot during the day was determined indirectly as : -

Net photosynthesis. (Net C gain by shoot in photoperiod)	=	C gain as dry matter by plant parts day end night.	+	Respiratory loss of C by shoot at night.	+	Respiratory loss of C by nodulated root (day+night).

A further dimension to the carbon budget involved analysis of transport liquids of xylem. By relating the C : N ratio of xylem exudate to nitrogen increments of the shoot, estimates were made of the amount of carbon moving from root to shoot attached to products of nitrogen fixation (Minchin & Pate, 1973, 1974; Herridge & Pate, 1977).

3. THE PARTITIONING OF PHOTOSYNTHATE

As examples of our carbon balance studies we select data for two very different species of legume, one, the white lupin (*Lupinus albus* L. cv Neutra), a tall, tap-rooted species suitable for growth as a winter annual in Western Australia, the other cowpea (*Vigna unguiculata* (L.) Walp. cv Caloona), a slender-rooted tropical species, grown in Australia as a summer crop. Seeds of white lupin are high in protein (33 - 38% by weight), contain virtually no starch, and have the major fraction of their carbohydrate reserve as wall polysaccharides (Atkins *et al*, 1975); those of cowpea are starchy and of low (20%) protein content (Herridge & Pate, 1977). Both cultivars studied showed a low harvest index (seed biomass : peak vegetative biomass) in glasshouse culture, and might therefore be regarded as typical of the many species of legume requiring selection for improvement in grain yield.

The fate of net photosynthate during growth of the two species was as shown in Figs. 3 and 4, items of each budget being expressed relative to a net intake of 100 units of carbon as net photosynthate by the shoot during the photoperiod. Gas exchange studies of defoliated plants showed that fruit, stem

and petioles were at, or close to, CO_2 compensation point during the photoperiod, so these organs were pictured as relying entirely on net photosynthate of leaflets for dry matter production and maintenance of respiration at night.

The major element in the flow of carbon consisted of phloem translocation of photosynthate from leaflets to other plant parts. The small amounts of carbon carried in xylem were envisaged as being related specifically to the export of N_2-fixation products from nodules, values for the C : N ratio of the xylem sap being used to compute this coupled flow of C and N (see Table 1, and Minchin, 1973; Minchin & Pate, 1973; Pate, 1975). Mobilization of nitrogen from vegetative to reproductive parts was assumed to take place by the phloem, and the amount of carbon moved by such transport was calculated on the basis that the mobilized products had a C : N ratio similar to that of leaf protein (see Pate & Herridge, 1977).

Three phases of development were recognized in the growth cycle of the two legumes, Phase 1 spanning vegetative growth to the start of flowering, Phase 2 encompassing flowering and the early development of fruits, Phase 3 the final stage of the life cycle, dominated by the continued growth of fruits and particularly by the filling of seeds.

Phase 1 - vegetative growth (Fig.3A, Fig.4A)

Vegetative growth of cowpea spanned approximately half of the life cycle and was a time when 37% of the net photosynthate and 50% of the fixed nitrogen were assimilated. Vegetative growth of lupin occupied only one-third of the life cycle and

accomplished only 6% of the plant's total net photosynthesis and 11% of the total N fixation. Despite these differences the two species showed closely similar patterns of gross distribution of incoming photosynthate during vegetative growth. A large proportion of the fixed carbon (27 - 30%) was invested in dry matter gain of leaflets and hence in increasing the photosynthetic potential of the plant. Even larger fractions of the current photosynthate (54% in lupin, 41% in cowpea) were translocated to roots, consumption in respiration of root and nodules equalling (cowpea) or exceeding (lupin) the amounts of carbon entering dry matter of these organs. The percentage of the plant's net photosynthate utilized by nodules was higher in lupin than in cowpea, due to higher expenditure by lupin in nodule respiration, and a greater requirement for carbon in transport of fixation products from the nodules. The latter feature related to the higher C : N ratio of fixation products of lupin than cowpea (Table 1), a difference discussed at length in a later section of the paper.

Phase 2. Flowering and early fruiting (Fig.3B, Fig.4B)

This phase, 16 days long in cowpea, 44 days in lupin, was when greatest photosynthetic returns of carbon were made by the species. Lupin fixed 62% of its N, cowpea 42%, underlining the importance of this stage of growth in establishing reserves of nitrogen. The nodulated root of lupin received 49% of the plant's net photosynthate, a feature no doubt related to its tap-rooted habit and to continued growth of nodules and rootlets after flowering. The comparable figure for cowpea was 34%.

The diminishing supply of carbon to the root of cowpea resulted in a progressive widening of its top : root weight ratio (see Herridge & Pate, 1977). Nodules of lupin received 12% of the net photosynthate in this phase, nodules of cowpea 8%. These proportions were less than in Phase 1 of the life cycles. By contrast, the proportions of net photosynthate consumed in root respiration increased from Phase 1 to Phase 2, indicating higher consumption in maintenance of a larger root system, and possibly an increase in the respiratory activity of microflora feeding on decaying tissues of the roots.

The earlier flowering and faster growth of fruits of cowpea resulted in a net gain by fruits of 15% of the plant's net photosynthate *versus* 8% in the case of lupin.

Phase 3. Seed filling (Fig.3C and Fig.4C)

The species differed most markedly during this phase. Lupin maintained translocate to its nodulated root equivalent to 54% of the plant's net photosynthate, whilst in cowpea only 14% was diverted to underground organs. Nitrogen fixation continued for longer and at higher intensity in lupin than in cowpea. In lupin 27% of the total N fixed over the growth cycle was assimilated during seed fill, in cowpea only 8%. Decline in net photosynthesis through shedding of leaves started earlier and took place more rapidly in cowpea than lupin, leading to much smaller returns of fixed carbon by the former species during fruiting. Nevertheless the proportion of net photosynthate moving to reproductive organs was higher in cowpea (76%) than in lupin (38%) suggesting differences between the species in the competing power of fruits for assimilates.

4. ASSIMILATE SUPPLY AND NITROGEN FIXATION

Using the same primary data for the species, comparisons were extended to the relationships during growth between symbiotic performance and photosynthesis. Symbiotic activity (Fig. 5A, 6A) was assessed in terms of mass of nodules (g fresh wt . plant^{-1}), specific activity of nodules (mg N fixed . g fresh weight of nodules^{-1} . day^{-1}), and the relationship between these variables expressed as rate of fixation (mg N fixed ; plant^{-1} . day^{-1}). Comparable quantities for evaluating changes with time in photosynthetic activity (Fig.5B, 6B) were leaf area (dm^{2} . plant^{-1}), specific activity of leaf surface in producing net photosynthate (mg CO_2 . dm^{-2} . day^{-1}), and rate of gain of net photosynthate by the shoot (g C . shoot^{-1} . day^{-1}).

Rate of nitrogen fixation and mass of nodules per root increased parallel with increases in leaf area and in rate of production of net photosynthate by the two species, suggesting a strong measure of dependence between nodule performance and photosynthetic activity. Assimilation by leaves and nodules achieved peak specific activities well before flowering, although early losses of assimilatory efficiency were more than offset by increases in area or mass of assimilating organs. Leaf abscission and nodule senescence occurred earlier and more rapidly in cowpea than in lupin, causing a more rapid decline in assimilatory activity of the former species after flowering (see Figs 5 and 6).

The graphical representations of Fig.7 provided further detail on the timing of certain key events in the second half of

the life cycle. The parameters depicted were those deemed most relevant to declining assimilation, and each was related to a common percentage scale on which a value of 100 denoted the maximum in a specific quantity or activity attained during the growth cycle of the plant.

Several interesting features emerged : -

1) A decline in specific activity of nodules was the first evidence of degeneration of symbiotic performance in the two species. It commenced whilst nodule mass was still increasing and some time prior to the attainment of maximum net photosynthesis.

2) The quantity most closely related to declining rate of nitrogen fixation was the rate at which photosynthetically-fixed carbon was supplied to nodules. Fixation started to decline some time before the first decrease in rate of carbon supply to the whole nodulated roots, suggesting that once nodule specific activity started to decline the nodules competed less effectively with roots for translocate.

3) Declining nitrogen fixation in cowpea was correlated with increased diversion of photosynthate to fruits, implying that competition from reproductive organs might have limited the assimilate supply to nodules. This feature did not apply to lupins in which the decline in rate of supply of C to fruits occurred earlier than the decline in fixation. In fact translocation of carbon to fruits declined during much of the time when fixation rate was decreasing. The carbon budgets of lupin at this time (Fig.3C) indicated that fruits competed more with other

organs of the shoot than with the root for photosynthate. Indeed the main competitive influence for nodule functioning appeared to be the demands of the parent root for respiratory substrate.

5. NITROGEN SOURCES FOR FRUIT FILLING (Fig.8)

Although cowpea and white lupin exhibited somewhat different timings in the decline of their respective symbiotic activities, and bore evidence of different relative demands for nitrogen in filling of their seeds, the two species showed essentially similar profiles of supply of nitrogen to their fruits (Pate & Herridge, 1977; Herridge and Pate, 1977). According to the nitrogen balance sheets for this stage of development (Fig.8) fruits drew on three main sources of nitrogen, current fixation in nodules, mobilization from leaflets prior to leaf shedding, and mobilization from stem and petioles. Current fixation represented the principal source of nitrogen during early stages of fruiting; in mid-fruiting mobilization from leaflets and continued nitrogen fixation supplied substantial amounts of nitrogen; and then, in the final stage of fruit fill, mobilization from stems furnished the major (lupin) or only (cowpea) source for the fruits. Despite differences in the relative sizes and timings of the contributions from these sources, approximately 60% of the N incorporated into seeds of both species came from nodule fixation during fruiting, the remainder as mobilization from vegetative parts.

It would be interesting to know whether cultivars showing higher harvest index and yield of seed protein, would show more

efficient retrieval of nitrogen from vegetative parts to seeds than in the cultivars tested here. Earlier and more complete withdrawal of nitrogen from leaves would carry the obvious penalty of destroying photosynthetic proteins of the leaf and hence restricting the carbohydrate supply for further nitrogen fixation by root nodules. This is an aspect of legume functioning most deserving of study since it might lead to a prescription of the physiological attributes conducive to highest transfer of nitrogen to grain. A plant which would store large amounts of fixed nitrogen in its stems before flowering, and which would draw on stem nitrogen in preference to leaf nitrogen for fruit filling, would seem, at first sight, to possess features promoting a high harvest index for nitrogen. If these characteristics were combined with the capacity to maintain root and nodule integrity until the late stages of fruiting, unusually high yields of protein from seeds might be obtained.

6. MEASURING THE EFFICIENCY OF CARBON USAGE IN NODULES.

The flow profiles of Figs 3 and 4 depict the carbon consumption of nodules as comprising three basic elements, a requirement for formation and growth of the nodule, a requirement for maintaining respiration of nodule tissues and for providing energy for nitrogen fixation, and a specific requirement for exporting fixation products from the nodule as organic solutes of nitrogen. Table 2 provides data on all three aspects of the economy of nodules for the three species of legume studied so far, namely *Pisum sativum* (Minchin & Pate, 1973), *Vigna unguiculata* (Herridge & Pate, 1977) and *Lupinus albus* (Pate &

Herridge, 1977).

In each species the carbon used in export of fixed nitrogen comprised the largest item (46 - 52%) of the nodule's budget; respiratory losses amounted to 36 - 39% of the carbon consumed, and the remaining 9 - 16% was used for nodule growth. Expressed in terms of gram atoms of C consumed per gram atom of N fixed (bottom section of Table 2) nodules of cowpea turned out to be considerably more economical in terms of carbon usage (3.2 g atom C . g atom N fixed^{-1}) than those of either lupin (5.1 of same units) or garden pea (4.8 of same units). The use of ureides in export of nitrogen was the major factor in the better economy of cowpea nodules, but the low value for CO_2 output per unit of nitrogen fixed also contributed to the efficiency of the species. Nodules of cowpea had lower proportions of their volume as bacteroid-containing tissue than nodules of pea or lupin, and this may have had some bearing on the apparent differences in respiratory efficiency in nitrogen fixation. Cowpea nodules also showed higher specific activity for nitrogen fixation than did lupin (Figs 5A and 6A), average and maximum specific activities of cowpea nodules being 6.3 and 8.4 mg N fixed . g fresh weight nodules^{-1} . day^{-1} respectively, *versus* 5.1 and 7.1 of the same units respectively for lupin.

The literature records a number of attempts to assess the cost in terms of carbon of nitrogen fixation by legume nodules. Table 3 summarizes information from such studies including, for comparative purposes, the data already described here for cowpea, pea and lupin.

One experimental approach (Table 3, item 1) was to study dry weight differences between nodulated plants and non-nodulated plants supplied with an 'equivalent' amount of combined nitrogen, usually in the form of nitrate. Any reduction in dry matter production by the nodulated plant in comparison with the non-nodulated was then taken to represent the 'extra' cost in terms of photosynthate of fixing molecular nitrogen as opposed to assimilating the combined form of nitrogen. Expressed in these terms the values obtained varied from 0.37 to 4.7 g atom C respired . g atom N fixed^{-1} for subterranean clover (Gibson, 1966) to 5.9 to 12.1 (same units) for soybean (Allam, 1931).

A second approach (Table 3, item 2) was to compare the respiratory output of nodulated and non-nodulated root systems of intact plants and from this determine what extra component, if any, of the respiration was attributable to nodules. In this manner, Bond (1941) estimated that 25% of the CO_2 efflux of the underground organs of a soybean plant was due to nodular respiration. From this he calculated a respiratory efficiency for nodules of 8.9 g atom C . g atom N fixed^{-1}. A variation on this approach, used by Minchin and Pate (1973) on *Pisum sativum*, was to compare CO_2 efflux from nodulated roots of intact plants with that of roots of similarly aged non-nodulated plants supplied with nitrate. When data were expressed in terms of CO_2 output per unit of N assimilated little difference was found in the respiratory outputs of the two classes of plants. In this instance non-nodulated roots must have borne the cost of reducing much of the nitrate absorbed from the rooting medium since

roots of *Pisum* possess an active nitrate reductase system (see Oghoghorie & Pate, 1971).

A third class of estimation (Table 3, item 3), also on *Pisum sativum*, involved measurement of the respiratory output of detached nodulated roots under conditions of varying nitrogen fixing capacity, as estimated by C_2H_2 reduction (Mahon, 1977). It was concluded that the component of respiration linked specifically to the N fixation process of nodules cost the equivalent of 7.9 g atom C.g atom N fixed^{-1} (Mahon, 1977).

Fourthly data used for costing nitrogen fixation were obtained by measurements of respiration of detached nodules or freshly excised segments of nodulated root (see Table 3, item 4).

The validity of each of the approaches mentioned above must be questioned. Comparisons based on measurements from nodulated and non-nodulated plants assume strict comparability in all respects save those relating to the assimilation of nitrogen. This condition is rarely if ever achieved. For instance root systems assimilating nitrate are usually larger than their nodulated counterparts and the morphology and physiology of their shoots may also differ quite radically (see Bouma, 1970; Minchin & Pate, 1973). Indeed unless combined nitrogen is applied at the same rate as that at which symbiotic counterparts are fixing, and unless the application of combined nitrogen is delayed until N fixation has started, it is almost inevitable that very different patterns of growth will result in the two sets of plants. Furthermore a proportion of the nitrogen absorbed by nitrate-fed plants is likely to be reduced photosynthetically in

the shoots at essentially no cost in terms of ATP and reductant from respiration. It is then patently unfair to compare carbon economies of nodulated and non-nodulated roots in terms of total nitrogen assimilation.

Direct measurements of respiratory output from detached nodules or segments of nodulated roots suffer the obvious disadvantage that wounding effects, starvation effects, or loss of nitrogenase activity following detachment of the nodule are likely to complicate the issue. Also, almost invariably, the respiration studies are made in air, in which any capacity which the root nodules might have to fix carbon dioxide would not be effectively displayed. This is considered in detail later on.

Finally, in connection with our own studies (Table 3, item 5) several criticisms apply. Firstly, the use of rooting media containing organic matter is precluded for respiration studies on intact root systems since rhizosphere micro-organisms might decompose components of the organic matter and thus contribute 'extra' CO_2 to that released from carbon of plant origin. This rules out studies simulating plant performance in soil. Secondly, problems of roots becoming pot bound during growth will apply, just as in any pot culture work. This applies particularly to a tap-rooted species such as lupin, in which roots may penetrate for over 1.5 metres and exploit a soil volume of 100 - 200 litres, compared with a volume of only 11 litres in the containers used for respiration study. Thirdly, the system fails to distinguish between CO_2 released from living tissues and that resulting from microbial decomposition of root leachates

or decaying root tissues. Judging from the very low content of non-living organic matter present in the rooting medium of fruiting plants, decomposition of dead tissues or root and nodule must be very effective under the conditions of the gas flow system. Consequently a substantial contribution of CO_2 from micro-organisms is to be expected. Of course when using silica sand as potting medium this CO_2 must have been derived from photosynthetically-fixed carbon, albeit largely that synthesized at an earlier time in growth.

The most important criticism of our respiration studies relates to the technique for estimating the separate contribution of nodules and roots to the respiratory output of the whole part as measured by the gas flow : Pettenkoffer system. As mentioned earlier, this is accomplished by measuring the respiration of freshly-detached nodules and supporting roots, and using the masses and specific activities of respiration of these organs to apportion the CO_2 efflux of the whole nodulated root system between nodule and root components. The requisite measurements of respiration of detached nodules and root carry the inherent disadvantages mentioned earlier, although the sum of the CO_2 output from nodule and root in most instances matched reasonably well with the efflux from the whole nodulated root.

7. THEORETICAL RESPIRATION COSTS FOR NITROGEN FIXATION IN LEGUME NODULES.

Studies feeding $^{15}N_2$ to detached nodules or isolated bacteroids (see Bergersen, 1971; Dilworth, 1974), suggest that

ammonia is the first stable product of nitrogen fixation. The stoichiometry of ATP utilization during reduction of nitrogen has not been clearly defined but from published values for isolated nitrogenase 4 ATP molecules per $2e^-$ is regarded as an average estimate (Dilworth, 1974; Dixon, 1975; Burris, 1976). The well-documented requirement of nodules for oxygen, the values of 1 or slightly greater than 1 for the respiratory quotient of detached nodules (Allison *et al*, 1940; Bergersen, 1971) and the presence of tricarboxylic acid (TCA) cycle enzymes with an autoxidizable cytochrome system in bacteroids (Bergersen, 1971), beat evidence that oxidative phosphorylation might be the principal source of ATP for the nitrogenase.

Ammonia produced in nitrogen fixation is assimilated into organic compounds by specific ammonia assimilatory enzymes (Miflin and Lea, 1977). The significant levels of NADH-dependent glutamate synthase/glutamine synthetase in extracts of nodules of an amide-producing plant (*Lupinus* (Robertson *et al*, 1975)) and ureide-producing plants (*Vigna*, *Phaseolus* (Atkins *et al*, 1978 (in press))) suggests that there may be a common route for ammonia assimilation regardless of the classes of secondary products which may subsequently form.

The situation in amide-producing legumes, such as *Lupinus*, *Vicia*, *Pisum*, and *Trifolium* (see Pate, 1977), is depicted in Fig. 9. Amino acids are shown as forming by amino transferase reactions with keto acids, glutamine directly from glutamine synthetase, and asparagine by a glutamine -, ATP - dependent asparagine synthetase, as demonstrated for extracts of lupin

nodules by Scott, *et al* (1976).

The picture is much less clear for ureide-producing legumes, although $^{15}N_2$ feeding studies prove that allantoin and allantoic acid are formed in nitrogen fixation (Matsumoto *et al*, 1977; Atkins *et al*, 1978). These compounds might form as in animal tissues by aerobic degradation of purines, (Reinbothe & Mothes, 1962). Alternatively ureide synthesis might be accomplished from glyoxylate and urea as suggested by Bollard (1959). These hypothetical pathways are illustrated in Fig. 10. The purine-based pathway pictures two carbon atoms of the purine ring coming from glycine and two from formyl groups (labelled "C1" in Fig.10). One nitrogen atom is provided from glycine, two by transfer of the amide-N of glutamine and the fourth donated from aspartate. The regeneration of aspartate could be achieved by reversal of aspartase, although there is no evidence of such a system in nodules. It is shown in Fig. 10 as an amino transfer to oxalacetate formed from fumarate via malate and generating NADH. Xanthine oxidase, uricase and allantoinase, enzymes capable of aerobic breakdown of the purines, have been demonstrated in nodule extracts of soybean (*Glycine max*)(Tajima & Yamamoto, 1975) cowpea (Atkins, *et al*, 1978) and *Phaseolus vulgaris* (Atkins & Rainbird, unpublished).

The alternative route for ureide synthesis (Fig.10) utilizes the well-known reactions of urea synthesis from arginine, and the hypothetical condensation of urea with glyoxylate by reversal of the degradative purine pathway. Enzymes active in this condensation have yet to be demonstrated, although ^{14}C

labelling studies on fungi and higher plants (see Bollard, 1959) indicate that urea and glyoxylate might be precursors of allantoin.

Our costing of the synthesis of nitrogenous compounds generated in nodules (Table 4) has assumed that the requirements of synthesis for ATP and reducing power are as shown in Figs 9 and 10. Two further assumptions are made : -

1. ATP → AMP ≡ 2 ATP → 2 ADP

2. NADH + H^+ → NAD^+ ≡ 3 ATP

(ie the $P/2e^-$ ratio of oxidative phosphorylation is 3)

Proceeding on this basis the amino-N of amino acids and the amino-N of amides are seen to each cost the equivalent of 4 mol ATP per mol NH_3 incorporated, the amide-N of glutamine 1 ATP, and the amide-N of asparagine 3 ATP. The cost of ureide synthesis is estimated as 3 mol ATP per mol NH_3 incorporated in ureide N, regardless of whether the purine or urea/glyoxylate pathway is being utilized. However, it should be noted that certain hypothetical reactions of the urea/glyoxylate pathway can not be costed so that the ATP requirements of this pathway may well have been underestimated.

The ATP requirement in synthesis of the nitrogenous compounds formed in nitrogen fixation of a species can then be calculated on the assumption that solutes are generated in the proportions evident from analysis of xylem sap (Table 1). Relevant data for *Vigna*, *Lupinus* and *Pisum* are illustrated as item A of Table 4. The overall costs of assimilation of ammonia into organic solutes turn out to be very similar despite

major differences in composition of fixation products of the species. However, the use of ureides for assimilating NH_3 and transporting nitrogen gives a slightly better economy in terms of ATP consumption, than were asparagine used for these purposes (Table 4A).

Item B of Table 4 provides an estimate of the amount of CO_2 likely to be generated in assimilation of N_2 into amino compounds. A requirement is assumed of 3 e^- and 6 ATP molecules per molecule of NH_3 produced by nitrogenase (Figs 9 and 10), so that 3.5 mol CO_2 would be evolved per mol NH_3 reduced, if ATP and reductant were provided by sugar oxidation via the Embden-Myerhof glycolytic pathway followed by TCA cycle metabolism and oxidative phosphorylation. A similar source of ATP is assumed to be used assimilating ammonia into amino compounds, and this is costed for each species in terms of CO_2 output using the ATP requirements indicated in item A of Table 4. The total CO_2 efflux from the nodule associated specifically with nitrogen fixation (lowest entry, Table 4B) is then computed by addition of the requirements for nitrogenase and for ammonia assimilation.

A further loss of CO_2 might be associated with the formation of keto acids or any other precursors of the nitrogenous solutes shown in Fig.9 and 10. Assuming that these compounds were generated within the nodule, rather than being provided as translocate from the shoot, oxidation of sugar in the nodule would be required to generate these carbon skeletons. However, since this would contribute energy for assimilation of nitrogen it is not considered to represent an additional item in the

respiratory budget of N assimilation.

A final element in the nodule's respiration relates to growth and maintenance. Here we use values suggested for higher plant tissues by Penning de Vries et *al* (1974), and Penning de Vries (1975), namely 0.2 g CO_2 evolved per 1 g dry matter synthesized in growth, and 30 mg glucose respired . g dry matter^{-1} day^{-1} in maintenance respiration (see Table 4C).

The total costs of nitrogen fixation in terms of CO_2 efflux are shown in Table 4D as the sum of items for nitrogenase activity, for NH_3 assimilation, and for respiration in growth and maintenance of the nodule. The values obtained turn out to be very similar for the three species, 4.64 molecules CO_2 . per atom nitrogen fixed in *Vigna*, 4.78 (same units) in *Lupinus*, and 4.74 in *Pisum*. The nitrogenase requirement of 3.5 molecules CO_2 per atom fixed is in each case the largest item of the nodule's respiration budget, amounting to 73 - 75% of the estimated output of CO_2. Assimilation of ammonia into amino compounds, is the next largest item (equivalent to 22 - 24% of the nodule's CO_2 output), whilst the respiratory requirements for maintenance and growth of the nodule account for only 3% of the estimated net output of CO_2.

8. THE DISCREPANCY BETWEEN THEORETICAL AND OBSERVED CO_2 OUTPUT FROM LEGUME NODULES.

For each of the species we have studied observed rates of CO_2 efflux from nodules are considerably less than that expected from theoretical considerations (cf data of Tables 2 and 4). Thus, for *Vigna unguiculata* the experimentally-obtained value is

1.3 molecules CO_2 per atom N fixed, compared with a theoretical value of 4.6 of the same units. For *Lupinus albus* comparable values are 2.0 (observed) and 4.8 (theoretical), and for *Pisum sativum* 1.7 (observed) and 4.7 (theoretical). Thus, either the theoretical assumptions have overestimated the energy requirements of nitrogen fixation, or the observed gaseous efflux from the nodule grossly underestimates the actual release of CO_2 in respiratory metabolism of nodule tissues.

Probably the least certain component of the theoretical estimates is the ATP requirement for nitrogenase (Dixon, 1975). Indeed the measured requirements of the isolated enzyme for ATP may well be a gross overestimate of *in vivo* consumption. This would be the case were the structural configuration necessary for function of the enzyme system in the living bacterial cell to be maintained without continuous stabilization involving ATP hydrolysis (Thorneley & Eady, 1973; Dilworth, 1974).

The cost in terms of C utilization might also be reduced were some of the ATP required for nitrogen assimilation to be generated by a mechanism other than decarboxylation of TCA cycle acids. According to Dixon (1972) nodules whose bacteroids show hydrogenase activity exhibit an oxygen requirement 35% lower than those lacking hydrogenase. The oxidation of hydrogen produced by nitrogenase might therefore be an effective mechanism for ATP synthesis in the nodule (Dixon, 1972).

As mentioned earlier in connection with the carbon budgets, evidence exists that nodules are capable of CO_2 fixation (Minchin

& Pate, 1973; Lawrie & Wheeler, 1975; Christeller et al, 1977). The large phosphoenolpyruvate (PEP) carboxylase activity of nodules (Christeller et al, 1977), and the observation that their pyruvate kinase is inhibited by NH_4^+ (Peterson & Evans, 1977), suggests that CO_2 fixation might be related to nitrogen fixation by providing oxalacetate. This would effect an anaplerotic input for simultaneous generation of α-keto acids and reductant by the TCA cycle.

The extent to which fixation of CO_2 might occur is indicated for nodules of *Lupinus angustifolius* by the studies of Christeller *et al* (1977). Their data, based on rates of C_2H_2 reduction and $^{14}CO_2$ fixation, suggest a maximum rate for nodules of the species of 0.8 molecules CO_2 per atom N fixed. If the fixed CO_2 were derived entirely from tissue respiration and at a rate stoichiometric with the TCA cycle's catalytic requirement for oxalacetate, 1 molecule of CO_2 would be conserved for each molecule of amide synthesized (ie 0.5 mol CO_2 . mol NH_3^{-1}). In addition to this involvement oxalacetate might be used for synthesis of malate. Our studies using *Lupinus albus* show that xylem bleeding sap of detached nodules contains malate at approximately 20 mM, in a proportion roughly equivalent to 1 molecule malate per 3.3 molecules of amino compounds exported from the nodule. This finding, and the observation that xylem sap of nodulated roots of *Pisum sativum* has its aspartate, asparagine and malate labelled with ^{14}C after $^{14}CO_2$ has been fed to the gas space of the root system (Pate, unpublished data), supports the hypothesis that fixation of CO_2 into C_4 compounds might be an important aspect of nodule

economy, at least in those legumes exporting amides and amino acids from their nodules. The implications of malate synthesis in terms of the ionic balance of the nodule and its exported products, remains to be evaluated.

Finally, there is the possibility that some of the CO_2 released from nodule tissues might leave the nodule through the xylem as dissolved carbon dioxide or bicarbonate. An earlier publication (Minchin & Pate, 1973) found this to be insignificant in the overall CO_2 loss of nodules of *Pisum sativum*. Our more recent observations on the levels of CO_2 and HCO_3^- in freshly collected xylem exudate from nodulated roots substantiate this conclusion for *Lupinus albus*.

To summarize, there exists a puzzling anomaly between observed and theoretically-predicted rates of CO_2 efflux from nodules. The discrepancy would be considerably reduced were nodules to engage in conservation of respired CO_2 by means of their PEP carboxylase system, and were mechanisms other than sugar oxidation to provide ATP for nitrogenase function. It is clear that it will not be possible to appreciate fully the basic strategies of nodule functioning until the ATP requirement for functioning of nitrogenase has been understood.

9. THE PHOTOSYNTHETIC COST OF PROTEIN PRODUCTION IN THE ANNUAL LEGUME.

The value of legume crops stems principally from the protein which they produce in seed or above-ground vegetative biomass, so it is of considerable interest to assess how efficiently they form these classes of protein from net photosynthate.

The studies presented here allow these assessments to be made for pot-grown plants of *Lupinus albus* and *Vigna unguiculata* under closed canopy conditions in glasshouse culture. They therefore are likely to provide some general indication of the conversion ratings likely to obtain under comparable seasonal and nutritional conditions as a field crop. Since the cultivars studied are used as green manure or forage crops as well as for grain production, it seems appropriate to frame the calculations in terms of protein synthesized in above ground vegetative parts by the time of harvest as a green crop, or at plant maturity on the basis of protein accumulated by seeds. Relevant data are found in Table 5.

By the time of peak content of nitrogen in vegetative parts (79 days in cowpea, 95 days in lupin) cowpea had expended 17.2 g net photosynthate (expressed as carbohydrate) per gram of protein accumulated in above ground parts, white lupin 24.7 g carbohydrate per gram protein. By plant maturity the conversion efficiencies to seed protein were 32.5 g net photosynthate . g protein^{-1} for cowpea, 31.0 g . g^{-1} for lupin. It would be interesting to see how other cultivars and species perform in these respects, esperially those cultivars selected for high yield of seed.

It is perhaps surprising to find for these two legumes efficiencies of only 4 to 5.8% for conversion of net photosynthate to vegetative protein, and only 3% for the comparable conversion to seed protein. However, at present there are no strictly comparable studies on other crop plants, legume or

non-legume, so it is not possible to say how representative the values obtained are for cultivated plants as a whole, let alone how close they might be to the maximum attainable by the most efficient of our protein yielding crops. The decision on whether or not to expand world usage of legumes for protein production might well rest on the basis of such comparative measurements.

The present data suggest that symbiotic nitrogen fixation is not, of its own, a particularly costly item in the utilization of photosynthate by pulse legumes. An understanding of their effectiveness in producing protein requires assessment of all aspects of plant performance, especially, it would seem, the respiratory losses of plant parts and the processes involved in the mobilization of nitrogenous solutes to fruits and seeds. These studies and the relevant comparisons with non-leguminous species should provide interesting case histories of the economy of functioning of crop plants.

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TABLE 1. Composition of nitrogenous fraction of xylem sap from three grain legumes[1]

	Vigna unguiculata	*Lupinus albus*	*Pisum sativum*
		% of N	
amino acids	10	21	12
glutamine	10	12	15
asparagine	10	67	63
ureides[2]	70	ND	10
		g atom . g atom^{-1}	
C : N of xylem sap[3]	1.5	2.5	2.2

[1] Average composition from analyses of samples of root bleeding sap collected over the growth cycle.

[2] Allantoin + allantoic acid.

[3] Calculated using ureides = 1C : 1 N; asparagine = 2C : 1 N; glutamine = 2.5 C : 1 N; amino acids = 4C : 1 N (the value for aspartate, the major xylem amino acid in the three species)

TABLE 2. Functional economy of carbon and nitrogen in nodules of three grain legumes.

Species	*Vigna unguiculata*	*Lupinus albus*	*Pisum sativum*
Period of growth		days	
	0-78[1]	0-94[1]	21-30[2]
		$mg.plant^{-1}$	
N fixed	726	788	27.3
N exported	705	761	25.5
		$mg.plant^{-1}$	
C used in export of N[3]	969	1789	53.2
C lost as CO_2 in nodule respiration.	789	1372	40.4
C incorporated into nodule mass.	253	298	18.4
Total C requirement of nodule	2011	3459	112.0
		g atom . $g\ atom^{-1}$	
Total C required/N fixed	3.23	5.12	4.78
C exported/N exported	1.60	2.74	2.43
C respired/N fixed	1.27	2.02	1.73

1. Spans vegetative growth and early fruiting.
2. The 9-day period in vegetative growth immediately before flower initiation.
3. Estimated from the amount of N exported and the C : N ratio of xylem sap (Table 1).

TABLE 3. Studies which have estimated the respiratory cost of N_2 fixation in legume nodules.

Method	Species	g atom C respired . g atom N fixed^{-1}	Reference
1. Estimated by dry weight difference between nodulated and non-nodulated plants.	*Vicia faba*	2.61[1]	Christiansen-Weniger (1923)
	Medicago sativa	3.36[1]	"
	Trifolium subterraneum	1.4 - 4.7[1,2]	
		0.37[1,3]	Gibson (1966)
	Glycine max	5.9 - 12.1[1]	Allam (1931)
2. Estimated by difference in respiration (CO_2 output) of the root systems of nodulated and non-nodulated plants.	*Glycine max*	8.9[1]	Bond (1941)
3. Estimated by the difference in respiration (CO_2 output) of detached nodulated roots, under conditions of varying C_2H_2 reduction.	*Pisum sativum*	7.9[1]	Mahon (1977)
4. Direct measurements of respiration using detached nodules or detached root segments bearing nodules.	*Glycine max*	2.5[4]	Tjepkema (1971)
	Glycine max	22.6[5]	Bergersen (1971)
5. Direct measurements of respiration (CO_2 output) by intact nodulated root systems over extended periods of growth, and estimation of the separate respiratory contributions from root and nodule by measurements of CO_2 output on freshly detached nodules and roots.	*Pisum sativum*	1.73	Minchin and Pate (1973)
	Vigna unguiculata	1.27[6]	Herridge and Pate (1977)
	Lupinus albus	2.02[6]	Pate and Herridge (1977)

1. Assumes 40% C in dry matter or carbohydrate.
2. Cost of establishment of nodules
3. Cost of maintenance of nodules
4. Calculated from O_2 uptake, assuming an R.Q. of 1.
5. pO_2 = 185.2 mm Hg.
6. Calculated as in Table 2.

TABLE 4. Theoretical estimates of ATP consumption and CO_2 production by nitrogen-fixing nodules of three grain legumes

	Vigna	*Lupinus*	*Pisum*
A. ATP requirements for synthesis of organic solutes of nitrogen from NH_3 [1]	mol ATP . mol NH_3^{-1}		
amino acids	0.40	0.84	0.48
glutamine-amino	0.20	0.24	0.30
-amido	0.05	0.06	0.08
asparagine-amino	0.20	1.34	1.26
-amido	0.15	1.01	0.95
ureide	2.10	-	0.30
Total cost for NH_3 assimilation	3.10	3.49	3.37
B. CO_2 output associated with nitrogen assimilation [2]	mol CO_2 . mol NH_3^{-1}		
N_2 reduction to NH_3	3.50	3.50	3.50
NH_3 incorporation into organic N solutes.	1.03	1.16	1.12
Total N assimilation cost	4.53	4.66	4.62
C. CO_2 output for growth and maintenance	mol CO_2 . mol NH_3^{-1}		
C loss as CO_2 in nodule formation [3]	0.06	0.06	0.11
C loss as CO_2 in nodule maintenance [4]	0.05	0.06	0.01
Total nodule cost	0.11	0.12	0.12
D. Total CO_2 output	mol CO_2 . mol NH_3^{-1}		
Items B + C	4.64	4.78	4.74

1. Assumes amino compounds are formed in proportions suggested from xylem sap analysis (Table 1) and synthetic pathways as in Figs. 9 and 10.
2. Calculated as 3 ATP per CO_2, assuming that the P/2e$^-$ ratio of oxidative phosphorylation is 3.
3. Calculated using 0.2 g CO_2 produced . g dry wt synthesised^{-1} (Penning de Vries et al, 1974).
4. Calculated using 30 mg glucose . g dry matter^{-1} . day^{-1} (from (Penning de Vries, 1975).

TABLE 5. Costs in terms of net photosynthate of protein production in cowpea (*Vigna unguiculata* (L) Walp. cv Caloona) and white lupin (*Lupinus albus* L. cv Neutra)[1]

	Vigna	*Lupinus*
(A) Synthesis of protein in above-ground vegetative parts[2]		
Production of net photosynthate (g carbohydrate . plant^{-1})	50.1	66.7
Protein accumulated in shoot (g . plant^{-1})	2.91	2.70
Net photosynthate consumed per unit of protein synthesized (g carbohydrate . g protein^{-1})	17.2	24.7
(B) Synthesis of seed protein[3]		
Production of net photosynthate (g carbohydrate . plant^{-1})	61.8	103.6
Protein accumulated in seed (g . plant^{-1})	1.90	3.34
Net photosynthate consumed per unit of seed protein synthesized (g carbohydrate . g protein^{-1})	32.5	31.0

1 Data for cowpea from Herridge & Pate (1977) and for white lupin from Pate & Herridge (1977).

2 Measured over the period from germination to the time of maximum nitrogen content in above-ground vegetative parts (ie at 79 days in *Vigna*, 95 days in *Lupinus*).

3 Production of net photosynthate calculated for complete growth cycle, seed protein measured as amounts present in plants at full maturity (ie 120 days after germination in cowpea, 135 days in white lupin).

Figure 1. The gas flow-Pettenkoffer system used to measure CO_2 release from intact nodulated root systems of legumes.
1. Pump; 2. Soda lime "carbosorb" towers to remove CO_2;
3. 20-litre gas mixing chamber; 4. Main gassing lines;
5. Pressure release line; 6. Needle valve flow controllers;
7. Needle valve fine flow controllers;
8. Perforated inlet tube to rooting chamber;
9. 11-litre metal container with a "gas tight" lid;
10. Silica sand rooting medium, free of organic matter;
11. Coarse gravel to aid drainage;. 12. Drainage port;
13. Plasticine seal at stem/lid junction. 14. Gas outlet;
15. Pettenkoffer tubes containing KOH to absorb respired CO_2;
16. Gas line to a reference container, without plants;
17. Inlet port for application of water and nutrients.

Figure 2. CO_2 efflux of intact nodulated root systems (●—●) and the sum of separate measurements of CO_2 efflux from freshly detached nodules and their supporting root (histogram). *Lupinus albus* L. cv Neutra plants during vegetative development.

Figure 3. Flow diagram depicting the input, transport and utilisation of net photosynthate for three phases in the development of nodulated plants of *Lupinus albus* L. cv Neutra. Items in the budget are expressed relative to a net intake of 100 units C by the shoot.

Figure 4. Flow diagram depicting the input, transport and utilisation of net photosynthate for three phases in the development of nodulated plants of *Vigna unguiculata* (L) Walp. cv Caloona. Items in the budget are expressed relative to a net intake of 100 units C by the shoot.

Figure 5. Components of nitrogen fixation in nodules (A) and photosynthetic performance of leaves (B) during growth and development of *Lupinus albus* L. cv Neutra plants.

Figure 6. Components of nitrogen fixation in nodules (A) and photosynthetic performance of leaves (B) during growth and development of *Vigna unguiculata* (L) Walp. cv Caloona plants.

Figure 7. Temporal relationships between various parameters of nodule functioning and the carbon economy of leaves, fruits and roots during later stages of the growth cycle of (A) *Lupinus albus* L. cv Neutra and (B) Vigna unguiculata (L) Walp. cv Caloona. The parameters are related to a common percentage scale on which a value of 100 represents the maximum observed during the life of the plant. Abbreviations: -
N fixation - rate of accumulation of fixed N;
net Ps - rate of net gain of C by shoot in day;
nodule SA - rate of N fixation per unit fresh wt. of nodules;
C to fruits, C to nodules, C to nod roots - rate of supply to these organs of C of net photosynthate.

Figure 8. Sources of nitrogen for developing fruits of (A) *Lupinus albus* L. cv Neutra and (B) *Vigna unguiculata* (L) Walp. cv Caloona.

Figure 9. Probable metabolic pathways for the formation of amino acids, and amides in nitrogen fixation of legume root nodules. The scheme indicates the requirements for reductant and ATP in reduction of nitrogen to ammonia, and in the assimilation of ammonia into specific amino compounds.

Figure 10. Alternative metabolic pathways for the formation of ureides (allantoin and allantoic acid) in nitrogen fixation of legume root nodules. The schemes indicate requirements for reductant and ATP in reduction of nitrogen to ammonia, and in the incorporation of ammonia in ureide synthesis.

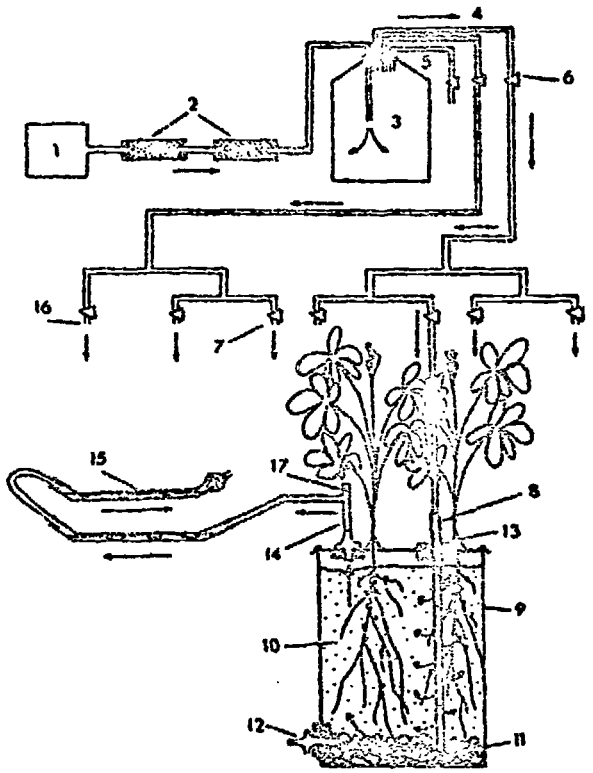

Figure 1. The gas flow-Pettenkoffer system used to measure CO_2 release from intact nodulated root systems of legumes.
1. Pump; 2. Soda lime "carbosorb" towers to remove CO_2; 3. 20-litre gas mixing chamber; 4. Main gassing lines; 5. Pressure release line; 6. Needle valve flow controllers; 7. Needle valve fine flow controllers; 8. Perforated inlet tube to rooting chamber; 9. 11-litre metal container with a "gas tight" lid; 10. Silica sand rooting medium, free of organic matter; 11. Coarse gravel to aid drainage; 12. Drainage port; 13. Plasticine seal at stem/lid junction; 14. Gas outlet; 15. Pettenkoffer tubes containing KOH to absorb respired CO_2; 16. Gas line to a reference container, without plants; 17. Inlet port for application of water and nutrients.

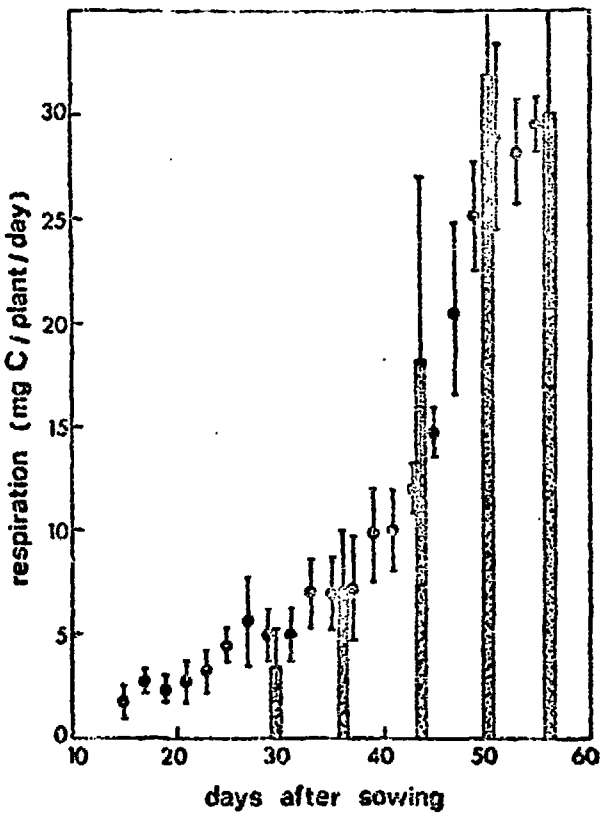

Figure 2. CO_2 efflux of intact nodulated root systems (●——●) and the sum of separate measurements of CO_2 efflux from freshly detached nodules and their supporting root (histogram). *Lupinus albus* L. cv Neutra plants during vegetative development.

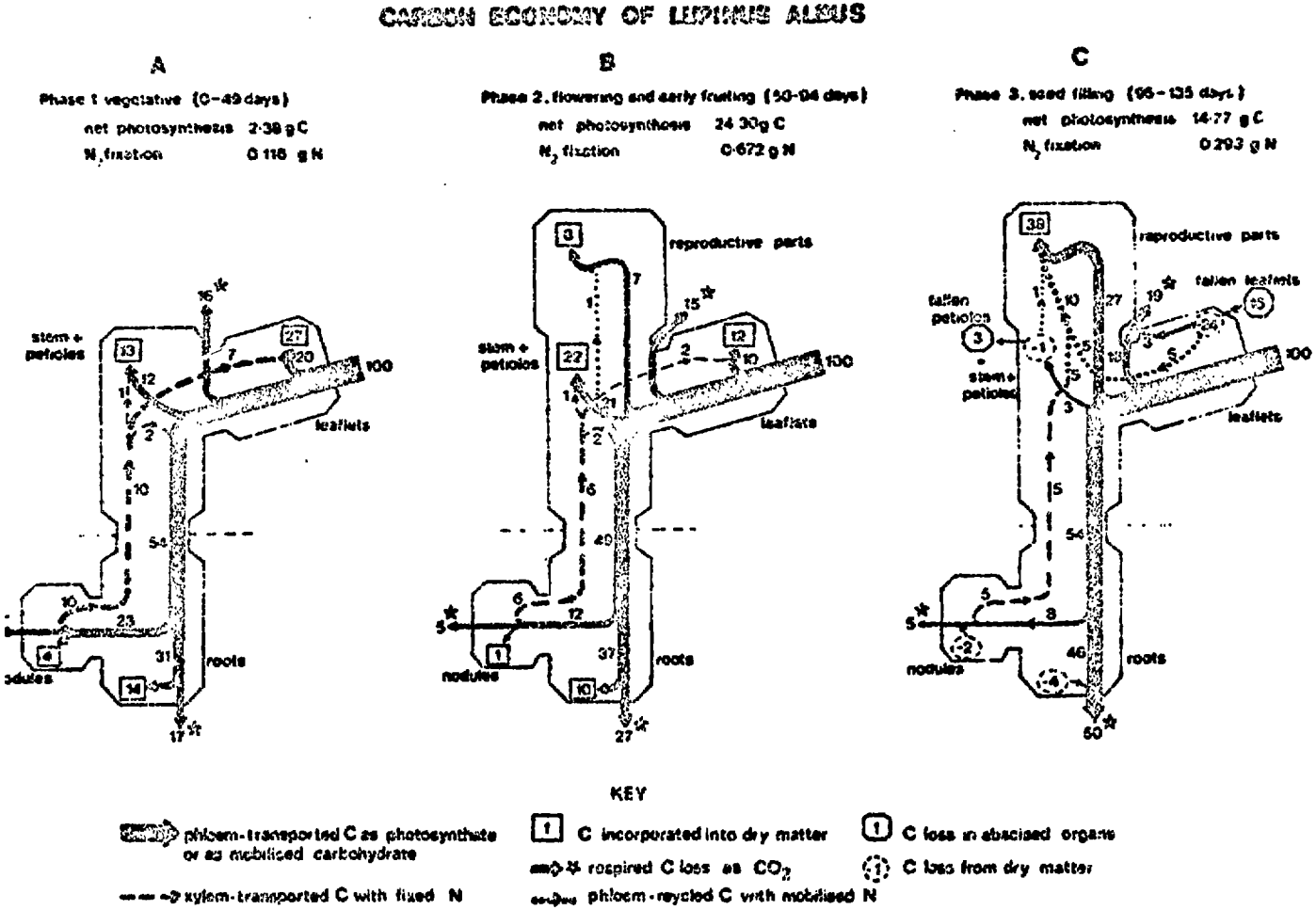

Figure 3. Flow diagram depicting the input, transport and utilisation of net photosynthate for three phases in the development of nodulated plants of *Lupinus albus* L. cv Neutra. Items in the budget are expressed relative to a net intake of 100 units C by the shoot.

CARBON ECONOMY OF VIGNA UNGUICULATA

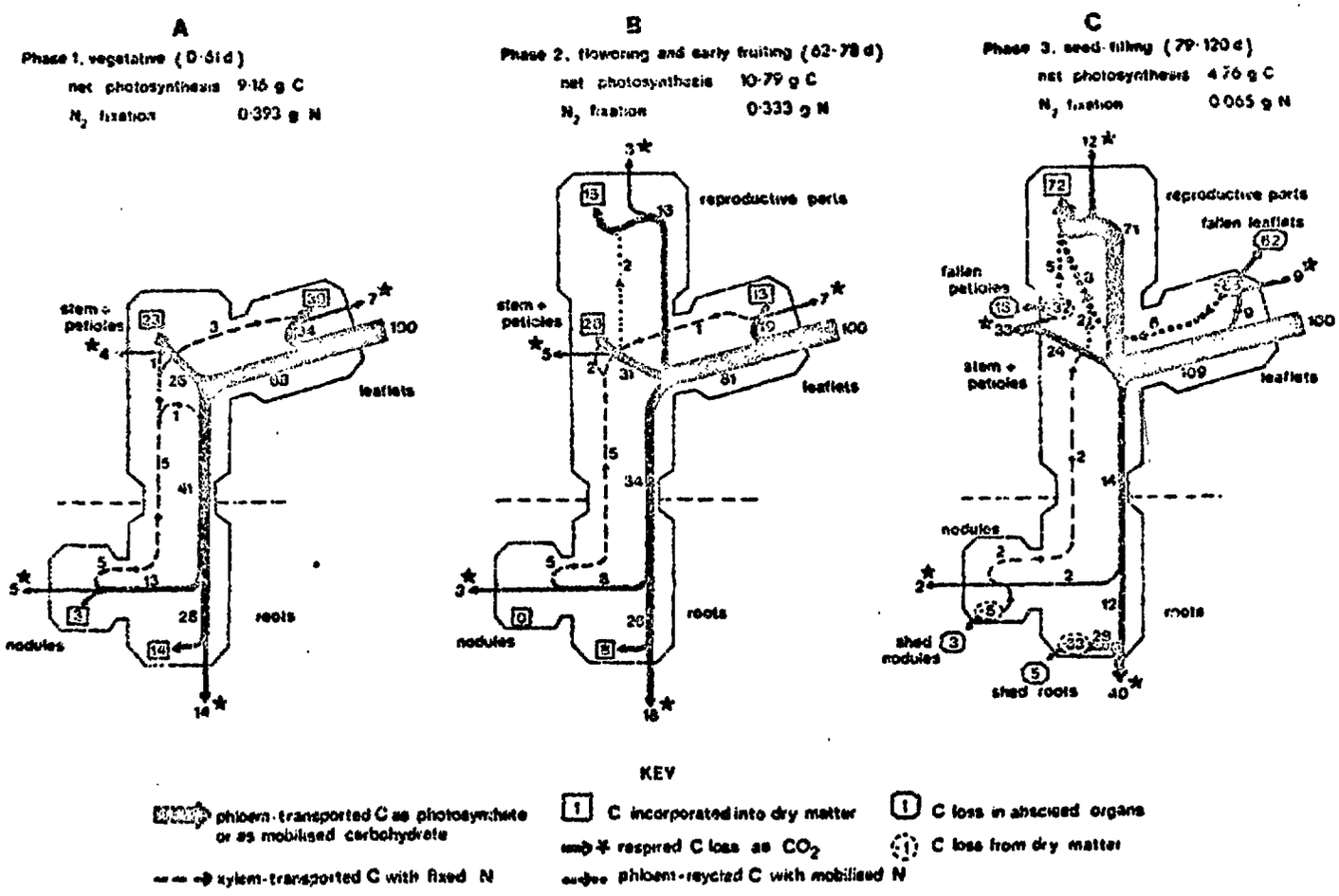

Figure 4. Flow diagram depicting the input, transport and utilisation of net photosynthate for three phases in the development of nodulated plants of *Vigna unguiculata* (L) Walp. cv Caloona. Items in the budget are expressed relative to a net intake of 100 units C by the shoot.

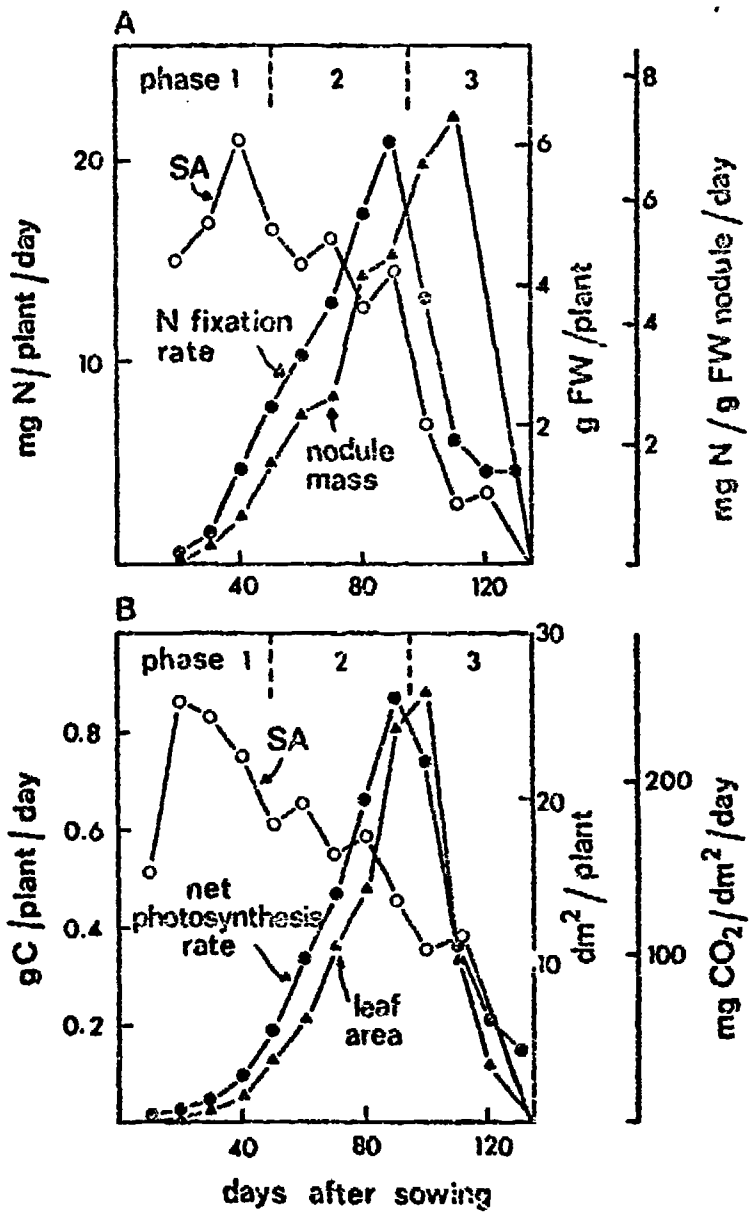

Figure 5. Components of nitrogen fixation in nodules (A) and photosynthetic performance of leaves (B) during growth and development of *Lupinus albus* L. cv Neutra plants.

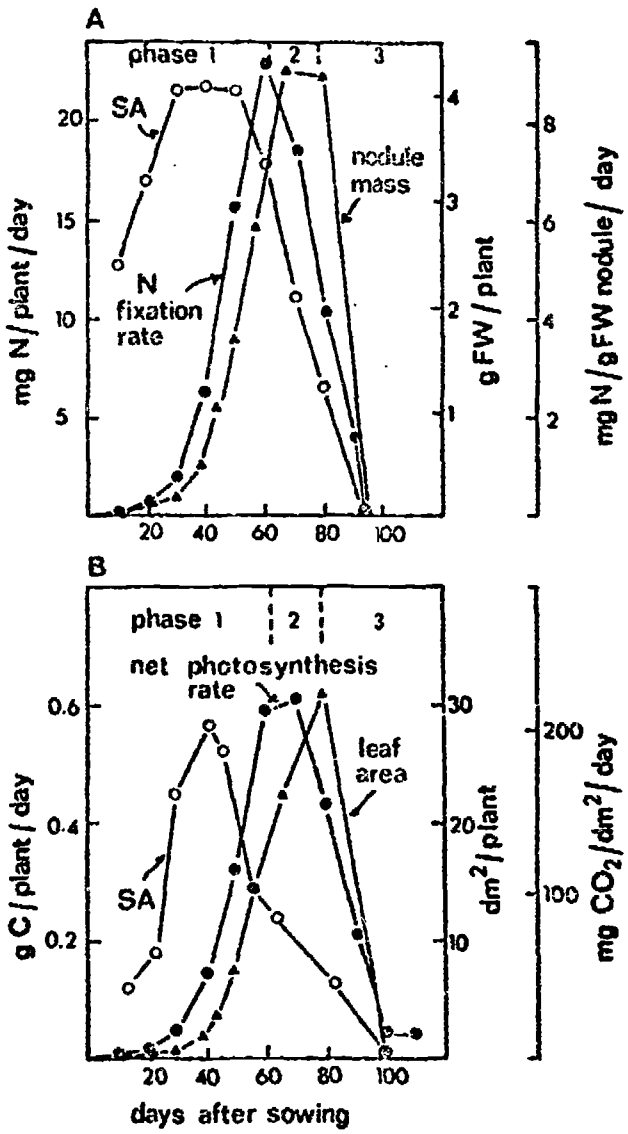

Figure 6. Components of nitrogen fixation in nodules (A) and photosynthetic performance of leaves (B) during growth and development of *Vigna unguiculata* (L) Walp. cv Caloona plants.

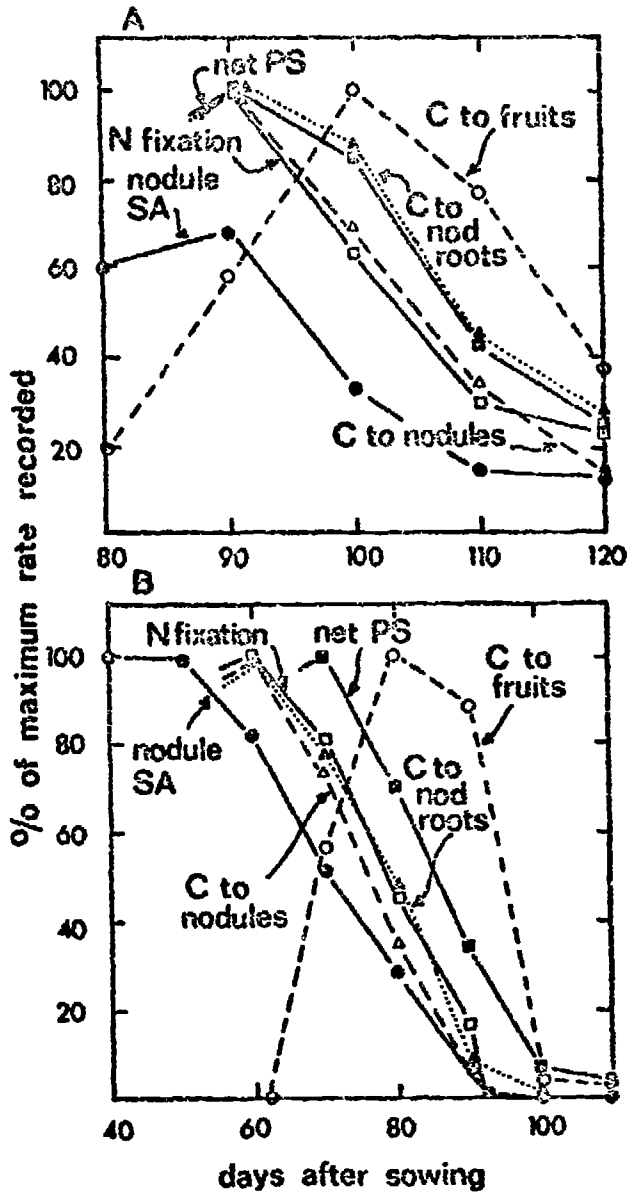

Fig.7. Temporal relationships between various parameters of nodule functioning and the carbon economy of leaves, fruits and roots during later stages of the growth cycle of (A) *Lupinus albus* L. cv Neutra and (B) *Vigna unguiculata* (L) Walp. cv Caloona. The parameters are related to a common percentage scale on which a value of 100 represents the maximum observed during the life of the plant. Abbreviations : - N fixation - rate of accumulation of fixed N; net Ps - rate of net gain of C by shoot in day; nodule SA - rate of N fixation per unit fresh wt. of nodules; C to fruits, C to nodules, C to nod roots - rate of supply to these organs of C of net photosynthate.

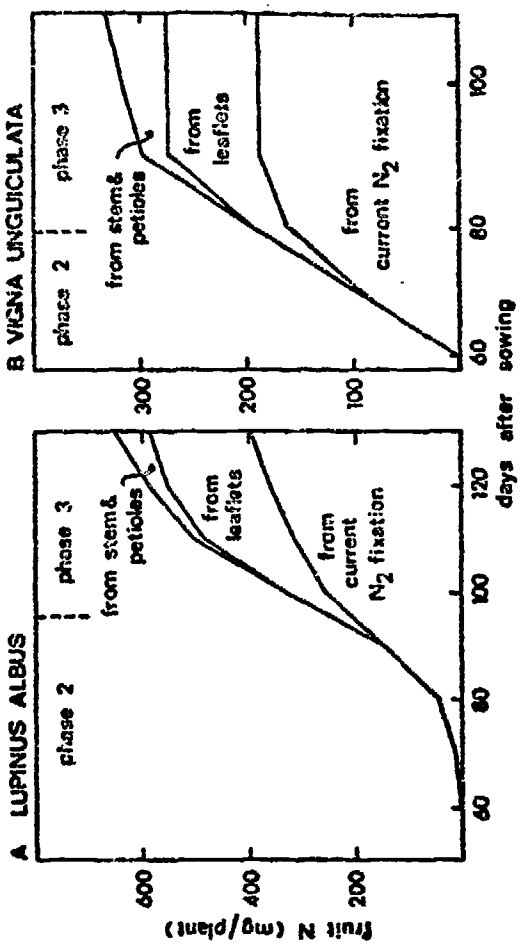

Figure 8. Sources of nitrogen for developing fruits of (A) *Lupinus albus* L. cv Neutra and (B) *Vigna unguiculata* (L) Walp. cv Caloona.

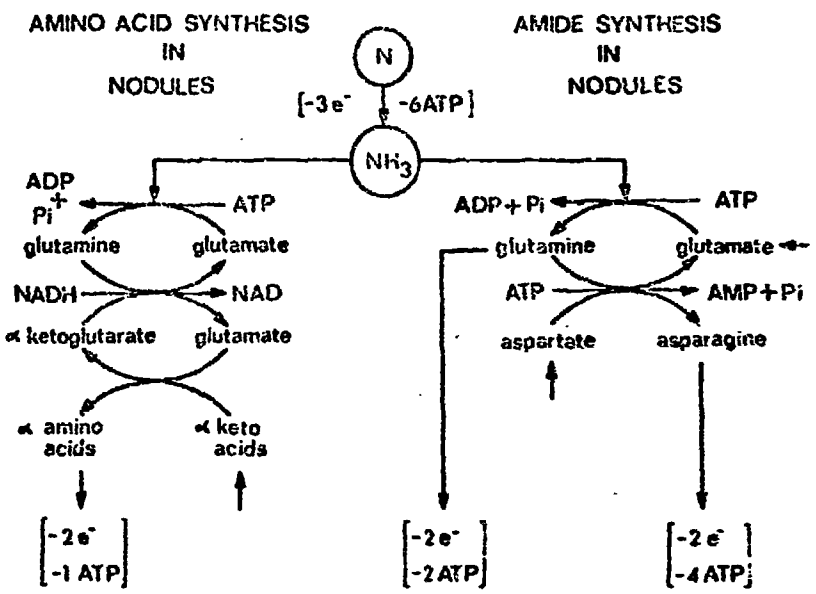

Figure 9. Probable metabolic pathways for the formation of amino acids, and amides in nitrogen fixation of legume root nodules. The scheme indicates the requirements for reductant and ATP in reduction of nitrogen to ammonia, and in the assimilation of ammonia into specific amino compounds.

TWO ALTERNATIVE PATHWAYS FOR UREIDE SYNTHESIS IN ROOT NODULES

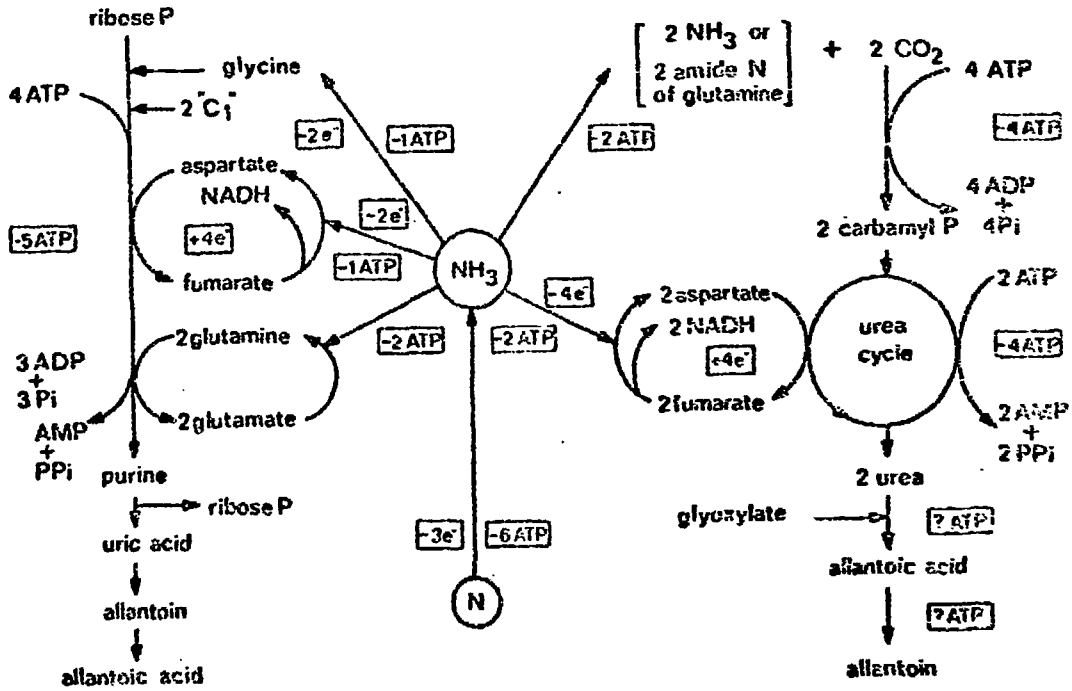

Figure 10. Alternative metabolic pathways for the formation of ureides (allantoin and allantoic acid) in nitrogen fixation of legume root nodules. The schemes indicate requirements for reductant and ATP in reduction of nitrogen to ammonia, and in the incorporation of ammonia in ureide synthesis.