Utilization of Net Photosynthesis for Nitrogen Fixation and Protein Production in an Annual Legume

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ABSTRACT

The economy of C and N in nodulated cowpea (Vigna unguiculata [L.] Walp.) was described in terms of fixation of CO\textsubscript{2} and N\textsubscript{2}, respiratory losses of C, and the production of dry matter and protein.

Net daytime gain of C by the shoot (net photosynthesis) rose to a maximum at flowering and then declined sharply due to abscission of leaves. Maximum N fixation occurred 10 days prior to maximum net photosynthesis. Shedding of nodules reduced fixation to zero by midfruiting. Fifty per cent of the plant's N and 37% of its net photosynthate were assimilated before flowering; 39% of plant N was incorporated into seed dry matter.

Respiration of nodules and roots utilized 24% of the C from net photosynthate assimilated over the growth cycle; night respiration of shoots, 20%; dry matter production in seeds, 17%; and dry matter production in other plant parts, 39%. The proportion of net photosynthate translocated to the nodulated root decreased from 41 to 14% during growth. Developing fruits were major competitors for translocate. Nodules consumed 9% of the C from the plant's total net photosynthate, 43% of which was respirate, 6% made into dry matter, and 51% returned to the shoot with N fixation products.

For every 1 g N fixed, net photosynthate equivalent to 6.8 g carbohydrate was consumed by nodules, 25.7 g carbohydrate by the nodulated root. Translocate was used most efficiently for N fixation in late vegetative growth when nodules were most active and their carbohydrate supply still adequate.

During vegetative growth and early flowering (0 to 78 days after sowing) cowpeas consumed 17.2 g net photosynthate (as carbohydrate) for every gram of protein synthesized in its shoot. The comparable conversion in seed production was 32.5 g net photosynthate/g seed protein or 6.6 g/g seed dry matter.

MATERIALS AND METHODS

Plant Material. Plants of cowpea (Vigna unguiculata [L.] Walp. cv. Caloona) inoculated with an effective Rhizobium were grown in sand culture in a naturally lit, temperature-controlled glasshouse during summer in Perth, Western Australia. Day temperatures were 30 to 35 C, night temperatures 19 to 22 C, conditions close to the optimum for the species (16). Plants were well watered and received adequate N-free mineral nutrient solution throughout growth.

Plant Harvest and Analysis. Fresh and dry wt were determined for leaflets, shoot axes (stem + petioles), roots, nodules and reproductive parts (flowers + immature fruits, pods and seeds) harvested from samples of 20 plants collected at intervals during growth. Dry matter C was estimated by dichromate digestion (13); total N, ethanol-soluble, and ethanol-insoluble N by Kjeldahl analyses. Leaflet area was determined by photocopying leaflets on standard document copying paper and weighing the cut-out images.

Xylem Sap Collection and Analysis. Xylem exudate was collected as bleeding sap from the cut base of stems at regular intervals throughout the life cycle. It was analyzed for amides and amino acids on a Beckman 118 amino acid analyzer operating in the "physiological fluids" mode using low temperatures and lithium buffers. Allantoin and allantoic acid, major nitrogenous solutes of xylem (unpublished data), were separated by TLC using butanolic-acetic acid-water, 12:3:5 as solvent. The ureide spots were reacted with Ehrlich's reagent, and the yellow colors estimated directly on the chromatograms using an integrating densitometer (Chromoscan, Joyce Lubel, Middlesex).

Respiration Measurement. Respiration of intact nodulated roots was monitored continuously by passing humid, CO\textsubscript{2}-free air through the rooting medium of 12 plants, two root systems in each of six containers of 1.5-liter capacity. Respired CO\textsubscript{2} in the effluent stream was absorbed in a series of Pettenkofer tubes containing KOH (9). After precipitating carbonate with BaCl\textsubscript{2}, change in normality of the KOH was estimated by titration with HCl. Rate of gas flow to the enclosed root systems was adjusted such that CO\textsubscript{2} evolution from root and nodules built up in the rooting medium to a level within the range 0.09 to 0.15% v/v. CO\textsubscript{2} concentrations were gauged to be similar to that encountered in a soil situation (9).

Respiration of intact shoots at night was measured on the same 12 plants whose root respiration was currently being measured. The shoot of each plant was enclosed in a Perspex cylinder, through which CO\textsubscript{2}-free air of ambient humidity was passed. CO\textsubscript{2} output from the effluent stream was measured using Pettenkofer tubes, as for root respiration. CO\textsubscript{2} levels in the shoot environment were maintained close to atmospheric (0.03% CO\textsubscript{2} v/v) by adjustment of flow rate of the gas stream relative to the rate of CO\textsubscript{2} evolution by the enclosed shoot. Measurements were made from dusk to dawn, and the cylinders removed during daytime. Growth and N accumulation of plants

Understanding the physiological and biochemical relationships between photosynthesis and N fixation is fundamental to an appreciation of how nodulated legumes operate as producers of organically bound N. Studies (2-4, 7) have described the changing parameters of symbiotic activity over the growth cycle of the host legume, the functional economy of nodule and nodulated root in converting translocate to amino compounds (9, 12), the translocatory arrangements for interchange of assimilates among plant organs (10, 11), and, in the case of grain legumes, the efficiency with which fruits utilize assimilates from the plant in synthesis of food reserves of seeds (13). This paper views these activities within the general context of whole plant functioning, especially in relation to the utilization of assimilatory resources in protein production.

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enclosed from respiration measurements were not significantly different from those of unenclosed plants of similar age (see also refs. 8 and 9).

Estimates of the separate contributions of nodules, roots, shoot axes, leaflets, and reproductive parts to respiration of shoot or nodulated root were made by enclosing known weights of freshly detached organs in Perspex containers containing air at ambient temperature, and withdrawing 1-ml samples of the enclosed gas at 5-min intervals over a 30-min period for analysis of CO₂ build-up using an IR gas analyzer (URAS I, Hartmann & Braun, West Germany) (1). Measurements of detached-organ respiration at intervals over a night (shoot parts) or over a full 24-hr cycle (root and nodules) allowed estimates of daily output of CO₂ to be obtained. The pooled results for CO₂ output from the freshly detached parts of a shoot or nodulated root agreed well (to within ±15%) with data for CO₂ evolution from comparable attached whole shoots or root systems using the Pettenkoffer gas flow system. For example, over a 42-day period of vegetative growth, Pettenkoffer analyses showed an evolution of 465 mg C as CO₂ from the rooting medium surrounding the nodulated root of the intact plant. Studies of detached-organ respiration over the same period gave estimated outputs of 89 mg C for detached nodules and 410 mg C for the parent root—a total respiratory output for nodules and root of 499 mg C. There was no evidence of wounding effects on respiration of freshly detached organs.

RESULTS

GROWTH, ACCUMULATION OF C, N, AND DRY MATTER

Changes in these parameters were as shown in Figure 1. Three principal stages in the growth cycle were recognized.

Phase 1 (0-61 Days). Vegetative Growth. This extended to the start of flowering and included the lag phase of seedling establishment and half of the log phase of growth. High proportions of the assimilated C and N were invested in dry matter of leaflets, stems and petioles, roots, and nodules. The level of N in dry matter was high (2.6-3.1%) during this phase.

Phase 2 (62-78 Days). Flowering and Early Fruiting. This included the second half of the log phase of growth and the main period of flowering. Particularly large increases occurred in the C of stem + petioles, and N of leaflets (Fig. 1, B and C). By the end of the phase maxima were attained in root and nodule dry wt, and in C, N, and dry wt of vegetative parts of the shoot. Percentage N in dry matter fell from 2.8 to 2.1 during this phase, suggesting that N fixation was not keeping pace with net gains of C.

Phase 3 (79-120 Days). Seed Filling. This phase was dominated by the growth and filling of fruits. Abscission of leaves (leaflets and petioles) and shedding of nodules occurred from 79 to 92 days. All vegetative parts lost C, N, and dry wt. Mobilization of N from leaflets prior to abscission occurred with 31% efficiency and from the shoot axis during senescence with 32% efficiency. Assuming 100% efficiency in transfer of N, mobilization from leaflets would have supplied 25% of the fruit’s requirements for N. Retrieval of N from the shoot axis, occurring somewhat later (Fig. 1C), would have met 19% of the fruit’s needs. The remaining 56% of the fruit’s N was likely to have been derived from N fixation after flowering, but possibly also from N mobilized from senescent roots (Fig. 1C). Nitrogen content of dry matter increased from 2.1 to 2.6% during this phase. Since little N fixation was taking place, the increased N content of dry matter indicated that retrieval of N from leaves prior to abscission was providing N at a relatively faster rate than net photosynthesis was adding C to the plant.

NITROGEN FIXATION

Changes during growth in rate of N fixation, nodule mass, and specific activity of nodules (mg N fixed/g fresh wt nodule/

Fig. 1. Changes during the growth of cowpea (V. unguiculata cv. Caloona) in dry weight (A), carbon (B), and nitrogen (C) of plant parts, and in N fixation rate, mass, and specific activity of root nodules (D). Data in A, B, and C are plotted cumulatively with vertical distances between successive pairs of lines representing quantities present in specific plant parts of a particular time in the life cycle. Phase 1: vegetative growth (0-61 days); phase 2: flowering and early fruiting (62-78 days); phase 3: seed filling (79-120 days).

RESPIRATORY LOSSES OF C

Rates of loss of CO₂ from nodules and roots (24-hr basis), and from intact attached shoots (night only) (Fig. 2A), were roughly proportional to fresh wt of plant parts. Maxima in CO₂ loss were at 60 to 70 days for nodules, at 80 days for the root, and at 85 days for the whole shoot. Over the period 20 to 30 days nodule respiration was a large fraction of the CO₂ output of underground parts; later, this fraction diminished markedly. From day 80 onward much of the root was dead and decaying and the bulk of the CO₂ recovered from the root environment probably resulted from bacterial decomposition rather than respiration of living root tissue.

NET PHOTOSYNTHESIS AND LEAFLET AREA

"Net photosynthesis" was defined as the net gain of C by the shoot during the photoperiod (14). The term "net photosynthetic" was used when denoting how the products of net photosynthesis were distributed and utilized for specific purposes.
within the plant. Net photosynthesis of the shoot (expressed as \( \text{g C/shoot/time interval} \) was measured indirectly for specific intervals of the growth cycle as: net gain or loss of C in dry matter of whole plant (data of Fig. 1B) + respiratory loss of C from shoot at night (Fig. 2A) + respiratory loss from nodulated root day and night (Fig. 2A). Since measurements of photosynthesis were not made, it was not possible to measure gross photosynthesis of the shoot.

**UTILIZATION AND FLOW OF C DURING GROWTH**

Flow diagrams for C were constructed for preflowering, early fruiting, and seed-filling phases of the growth cycle (Fig. 3). Items in each budget were expressed relative to the intake by the leaflets of 100 units of C through net photosynthesis. Gas exchange studies on several legumes, including *Vigna* (unpublished data) showed that stems, petioles, and fruits were maintained at or close to the CO\(_2\) compensation point when naturally illuminated during daytime, so these organs were depicted as relying on leaflets for any additional C required for dry matter gain or night respiration. The following information was included in the budgets (see Fig. 3).

**Absolute Amounts of N Fixed, Net Photosynthate Produced, and Protein Synthesized.** Relevant values were as listed in the legend to Figure 3.

**Incorporation or Loss of C in Dry Matter of Plant Parts.** Up to 79 days all organs accumulated C at the expense of leaflet photosynthesis. Losses of C in leaf abscission, root decay, and mobilization of materials to fruits occurred during the seed-filling stage. The magnitude of these losses was estimated.

**Gaseous Losses of C in Respiration of Plant Parts.** Shoot night respiration was proportioned among leaflets, shoot axis (stem and petioles), and reproductive parts on the basis of respiration rates of these organs when freshly excised from the shoot. Losses from underground parts were given for nodules and roots on a 24-hr basis.

**Cycling of C through Nodules.** The amount of C attached to fixed N returning in the xylem to the shoot was calculated on the basis of nitrogen increments of the shoot during specific intervals of growth and the C/N ratio of xylem sap (see ref. 9).

Data for sap composition were as shown in Table I. The low C/N ratio (1.3–1.7:1) reflected the high ureide content of the xylem stream.

**Mobilization of C to Seeds with N Mobilized from Leaflets, Stem, and Petioles.** It was assumed that N in fixed form was drawn directly from the xylem by the stem and leaves. Flowers and fruits were assumed to receive their N and C supply from leaves and stem via the phloem. The experimental evidence for this came from studies on other legumes (6, 11, 13). For example, in fruits of *Lupinus albus* it was estimated that 98% of the C and 89% of the N imported by a fruit entered through phloem (13).

Protein breakdown was the presumed source of the N transferred from stem and leaf to developing fruits. Accordingly, the C mobilized with this N was computed on the basis of the C/N ratio of protein.

Several features of the C budgets proved of interest.

**Phase 1 (0–61 Days)** (Fig. 3A). Fifty per cent of the N and 37% of the net photosynthate were assimilated during this period. Fifty-three per cent of the C of net photosynthate ended up in shoot dry matter, 42% was translocated to roots, and 6% cycled through nodules—in as carbohydrate and out again as amino compounds.

The nodulated root’s consumption of C was shared almost equally between respiration and growth. The shoot axis (stem + petioles) gained only one out of every six units of C cycled through nodules; the remainder moved to the leaflets.

The high requirement of roots for current photosynthate and the coupling of leaf growth to the N and C of xylem-borne solutes suggested heavy interdependence of root and shoot at this stage.

**Phase 2 (62–78 Days)** (Fig. 3B). During this period 333 mg N were fixed for an intake of 10.8 g C. The widening shoot to root wt ratio was reflected in an increased proportion of C being retained by the shoot compared to that in preflowering stages. Reproductive parts acquired 18% of the plant’s photosynthate. Nodules ceased to gain dry matter, roots grew only slightly, and 21 of the 34 units C of net photosynthate translocated to the root were lost in respiration. Five per cent of the plant’s net photosynthate was used to transport fixed N from nodule to shoot. In contrast to phase 1, investment of newly fixed C into shoot axis (28% of net photosynthate) was greater than into leaflets (13% of photosynthate). The large sink capacity of stem and petioles was also evident in terms of intake of fixation products (Fig. 1C).
Table I. Composition of root bleeding (xylem) sap collected from cowpea plants relying solely on nodules for their nitrogen supply.

<table>
<thead>
<tr>
<th>Age of plant, N solutes as</th>
<th>N solutes as</th>
<th>C : N Weight of sap solutes</th>
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<tr>
<td>time after sowing</td>
<td>ureidesa</td>
<td>asparagine, glutamine and aspartic acidb</td>
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<tr>
<td>Days</td>
<td>2c</td>
<td>Ratio</td>
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<td>44</td>
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<td>100</td>
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a Allantoic acid plus allantoin
b Principally asparagine, glutamine and aspartic acid

c Molar basis

**Phase 3 (79–120 Days)** (Fig. 3C). Only 65 mg N were fixed during this stage despite a production of 4.8 g C of net photosynthate. The plant was close to CO₂ compensation point as respiratory losses from shoot (at night) and underground parts (day + night) consumed 96 of every 100 units C of the shoot's net photosynthate. Only 14% of the net photosynthate reached the nodulated root, providing only one-third of the C lost in respiration of underground parts.

Losses of C in abscission of leaflets and petioles were large compared with the amounts of C mobilized to fruits prior to leaf fall. Leaflets were principal recipients of N fixation products destined for fruits and seeds. The shoot axis failed to make a net gain of N from the xylem at this stage. Some 20% of the seed's requirements for C was mobilized from stem and leaves, the remainder from current photosynthesis.

**DISCUSSION**

The partitioning of net photosynthate of cowpea (cv. Caloona) for dry matter production, for maintenance of respiration, and for activities associated with the fixation of N by root nodules was as shown in Figure 4A. The main features before flowering were high allocation of photosynthate to root and nodules, large respiratory losses of photosynthetically fixed C by underground parts, high rates of accumulation of fixed N in leaflets and stem, and the cycling of a significant fraction of photosynthetically fixed C through root nodules (items marked X in Fig. 4A). Events after flowering were dominated by leaflet abscission, declining N fixation, and the monopolization of assimilates and mobilized reserves by the developing fruits. Accumulation of dry matter by stem and root declined to zero by midfruiting, and an increasing fraction of the plant’s net photosynthate became committed to the maintenance of respiration. Finally, in the closing stages of growth, dry matter increase by the fruits decreased, and substantial proportions of the reserves of nonreproductive states lost in respiration or in mobilization to fruits (Fig. 3C).

The timing of events likely to be associated with deterioration of symbiotic activity during later growth was as shown in Figure 4B. The first evidence of degenerative changes was a reduction in the specific activity of N fixation by nodules. This took place...
while nodule mass was still increasing (Fig. 1D) some time before flowering commenced, and several weeks before net photosynthesis had reached a maximum (Fig. 4B). The cause of the early decline in specific activity was not determined; possibly specific aging factors within the nodules were involved.

The factor which most closely synchronized with declining N fixation rate was the rate of supply of translocate to nodules from the shoot, suggesting that N fixation rate was regulated primarily by availability of carbohydrate (cf. similar conclusions for other annual legumes [4, 5, 12]). Since N fixation rate was closely matched by a rise in rate of C supply to fruits (Fig. 4B), the competitive demands of reproduction appeared to have been crucial in the curtailment of symbiosis.

The cost of N fixation in terms of consumption of net photosynthate was as detailed in Figure 4C. Consumption of C by nodules (lowest curve, Fig. 4C) fell during growth from 5 to 2 g C/g N fixed. Increase with nodule age in efficiency of utilization of C reflected an increase in the specific activity of fixation and a decrease in the utilization of C for nodule growth (Fig. 4A). The nodulated root proved to be most efficient in converting translocate to fixation products over the period 20 to 50 days (middle curve, Fig. 4C). After this its efficiency fell steadily due to nodule decay and increasing expenditure of photosynthate in root respiration. The efficiency with which the whole plant converted net photosynthate to fixation products (top curve, Fig. 4C) was determined largely by the proportion of the plant's C resource currently available to nodules. Highest returns of fixed N/unit photosynthate were made during mid-vegetative growth when nodules were at peak specific activity and still acquired a relatively high proportion of the shoot's translocate. Demand by fruits for photosynthate was held responsible for the loss of N fixation efficiency during the final phase of growth.

The study provided estimates of the efficiency of cowpea (cv. Caloona) in converting net photosynthate to plant protein. By the time of peak content of N in vegetative parts (79 days) 2.9 g protein had been formed in above-ground parts at the expense of 49.9 g net photosynthate (as carbohydrate), i.e. 17.2 g net photosynthate/g protein. Harvested at maturity, 61.8 g net photosynthate was required to produce only 1.9 g seed protein, a conversion efficiency of 32.5 g carbohydrate/g seed protein. A relatively low harvest index and a low level of protein in seed dry matter contributed to the poor performance of the cultivar in grain protein production. Other cultivars of cowpea, and other species of grain legumes might prove to be considerably more efficient in this respect.

The efficiency with which fruits converted imported assimilates into dry matter was also computed. By plant maturity "Caloona" cowpea had translocated 5.8 g C to its fruits and its seeds had accumulated 9.4 g dry matter. Assuming that the amino acid to sugar ratio of the fruit's translocate was similar to that of garden pea (Pisum sativum L.) (6), a species with similar seed composition, this amount of imported C would have been carried by 2 g amino compounds and 12.1 g sugar. Thus, in producing 9.4 g seed dry matter, the fruits would have converted 67% by weight of their organic imports into seed materials. A similar experimental approach gave a conversion rating of 69% for fruits of garden pea (unpublished data) and 50% for fruits of white lupin (Lupinus albus L.) (13). A productivity rating of 66% was suggested for the conversion parts according to changing requirements during growth for respiration and dry matter accumulation. Items marked X refer to gain as C in dry matter through acquisition of fixation products from nodules. B: Relationships in time of events associated with the decline in N fixation activity during the later stages of plant growth. (Data obtained from Figs. 1, B and D; 2, A and B; 3C.) C: Efficiency of N fixation in terms of C consumption (as photosynthate) by nodules, nodulated root, or whole plant.
efficiency of fruits of cowpea (15), based on seed composition and biochemical data for the predicted yields of protein, starch, and lipid from metabolism of glucose. It would be of value to extend studies of this kind to other grain legumes, particularly those of differing seed composition and fruiting behavior.

LITERATURE CITED
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