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CARBON ECONOMY OF SOYBEAN–*RHIZOBIUM–GLOMUS* ASSOCIATIONS

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SUMMARY

Carbon uptake and allocation in plants that were largely dependent on microbial symbionts for N and P was compared to that in plants given inorganic fertilizer. Soybeans (*Glycine max* L. Merr.) were grown in sterilized soil and were either left uninoculated, or were inoculated with *Rhizobium japonicum* (Kirschner), or both *R. japonicum* and *Glomus fasciculatum* (Thaxter *sensu* Gerd.). Uninoculated plants were given N and/or P fertilizer at rates required to produce plants similar in size to inoculated plants. Carbon flows to plant parts, root nodules and vesicular–arbuscular mycorrhizas were measured in six- and nine-week-old plants by determining the distributions of ^{14}C after pulse labelling with $^{14}CO_2$.

Root nodules in non-mycorrhizal plants utilized 9% of total photosynthate; this was increased to 12% in nodulated, mycorrhizal plants. Mycorrhizas used 17% of the total photosynthate of six-week-old plants; this fell to 8% after nine weeks. Rates of $^{14}CO_2$ fixation in leaves of nodulated or nodulated plus mycorrhizal plants were up to 52% higher than in plants without microbial symbionts. Part of the increase was due to higher specific leaf area in plants colonized by symbionts, but other factors such as source–sink relationships, starch mobilization and leaf P concentrations were also involved in the host-plant adaptations to the C demand of the microbial endophytes.

Key words: Carbon-14, *Glycine max*, mycorrhiza, nitrogen fixation, photosynthesis.

INTRODUCTION

Rhizobium and vesicular–arbuscular (VA) mycorrhizal fungi derive carbon (C) compounds from their host plants. Because of the potential effects on plant yield, the energy requirements of the *Rhizobium* symbiosis and N_2 fixation have been studied (Atkins, Herridge & Pate, 1978; Ryle, Powell & Gordon, 1979; Warembourg, 1983). Attention has also been directed to the measurement of the energy costs of VA mycorrhizal symbiosis and of the tripartite symbioses involving legumes, *Rhizobium* and VA mycorrhizal fungi. Pang & Paul (1980) found additional allocation of C to mycorrhizal roots of *Vicia faba*, equivalent to about 10% of total photosynthate. Kucey & Paul (1982) found that N_2-fixing nodules of non-mycorrhizal *V. faba* utilized 6% of total photosynthate and those of mycorrhizal plants 12%. The mycorrhizas respired or incorporated about 4% of total photosynthate in both nodulated and singly-infected plants. Kucey & Paul also found that the C-fixation rate (g C g^{-1} shoot d. wt h^{-1}) was increased in plants supporting microbial symbionts to 'compensate' for the additional C utilization, such that similar plant growth rates were maintained. Non-symbiotic controls were given N and/or P fertilizer at rates designed to produce equal plant growth in

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all treatments. Photosynthetic 'compensation' was a necessary consequence of equal plant growth rates, since both the root nodules and the mycorrhizas used plant C.

Mycorrhizal *Allium porrum* translocated 7% more C to the roots than did non-mycorrhizal plants of similar size (Snellgrove *et al.*, 1982). The increased C allocation to the roots was associated with a decrease in specific leaf mass (g dm^{-2} d. wt) and increased leaf hydration. They suggested that this adaptation could enable the mycorrhizal plant to maintain a greater photosynthetic capacity without increasing plant C requirements. Large increases in rates of photosynthesis and transpiration were found in mycorrhizal *Bouteloua gracilis* and attributed to decreased leaf diffusive resistance (Allen *et al.*, 1980).

The significance of symbiotic associations together with the need to gain more information concerning the ability of the host plant to adapt to the needs of its microbial partners led to this work. We examined the individual and interactive effects of *Rhizobium* and VA mycorrhizal symbiosis on the C economy of soybeans and investigated the host–plant adaptations involved in the 'photosynthetic compensation' for the C costs of these symbioses.

Materials and Methods

Soil

A medium-textured, slightly acid (pH 5·6) loam of the Josephine series (mesic Typic Haploxerult) was sieved (1 cm mesh) and sterilized with ethylene oxide (12 h, 45 °C) to eliminate indigenous VA mycorrhizal fungal propagules and rhizobia. The soil was pre-incubated for three weeks to allow for the elimination of ethylene glycol formed during sterilization, then limed (10 g $CaCO_3$ kg^{-1} soil) to pH 6·9–7·1 to alleviate manganese toxicity found in plants grown in this soil at pH 5·6. Bicarbonate-extractable P was 4 μg g^{-1} soil, and total P was 0·31 mg g^{-1} soil.

Plants

Soybean (*Glycine max* L. Merr. cv Amsoy 71) seeds were surface sterilized in 0·2% $HgCl_2$ and germinated at 27 °C for 2 d. Three seeds were planted in each 1·5 dm^3 pot containing 1·25 kg soil, and the treatments were replicated four times at each harvest date. After one week, the seedlings were thinned to one per pot. Plants were grown in a growth chamber with day and night temperatures of 30 and 25 °C respectively, and the relative humidity varied between 70 and 90%. The photoperiod was 16 h and PAR varied from 550 to 450 μmol m^{-2} s^{-1} from the centre to the edge of the platform. Pots were rotated to minimize positional effects.

Nutrient solutions

Pots were watered three times per week with a modified Johnson's nutrient solution (Pacovsky, Paul & Bethlenfalvay, 1986) with or without N and P. Once each week the pots were flushed with distilled water. Plants inoculated with *Rhizobium* received an N-free solution and those with VAM, a P-free solution. Uninoculated soybeans that were harvested at six weeks had received nutrient solutions containing 4 mM N (as NH_4NO_3) and 0·4 mM P (as KH_2PO_4), while those harvested at nine weeks had received nutrient solutions containing 3 mM N and 0·2 mM P. These concentrations had previously been found to compensate for the

effects of the microbial endophytes on plant growth, such that plants of all treatments were of similar size and developmental stage when carbon flow experiments were performed at six and nine weeks (Pacovsky *et al.*, 1985). No single set of nutrient concentrations allowed both six- and nine-week-old uninoculated plants to 'match' the growth of those with microsymbionts.

Microbial endophytes

Glomus fasciculatum (Thaxter *sensu* Gerd.), obtained from Abbott Laboratories (Long Grove, Il. 60047), was cultured on sorghum (*Sorghum bicolor* (L.) Moench cv G766) in the Josephine soil. Plants not receiving P fertilizer were inoculated with 60 cm^3 of soil containing 300–350 spores of *G. fasciculatum*. Other pots were watered at planting with a leachate of the VA mycorrhizal inoculum sieved (0·43 μm) free of VA mycorrhizal propagules. *R. japonicum* (strain USDA 61A118) cultures were used to inoculate plants given N-free nutrient solutions.

Carbon flow measurements

Plants were pulse-chase labelled with $^{14}CO_2$ after six or nine weeks of growth. An apparatus similar to that used by Pang & Paul (1980) separated above and below ground atmospheres, provided control of $^{14}CO_2$ concentration in the above-ground portion, and allowed the separate collection of CO_2 evolved below ground in each pot. The above-ground atmosphere was contained by a canopy fabricated from thin plastic film (Propafilm C, Imperial Chemical Industries) and was common to all plants in the chamber.

One week before labelling, the soil–root system of each plant was enclosed in a modified pot with a plastic lid fitted with ports for gas circulation and watering. The aperture for the plant stem was sealed with an acrylic latex caulking compound (DAP Inc., Dayton, OH) and the system tested for gas leaks by introducing a small positive pressure (approx. 10 kPa). The plants were placed in the labelling chamber and allowed to equilibrate to the chamber conditions (30 °C day and 25 °C night, 16 h daylength, PAR 450 μmol m^{-2} s^{-1}). A flow (100 cm^3 min^{-1}) of CO_2-free air was passed through the headspace of each pot for 24 h before labelling to bring the soil atmosphere into equilibrium with the gas stream. The above-ground atmosphere in the chamber was purged of $^{12}CO_2$ during the 8-h dark period prior to labelling, by recirculating the gas through 0·5 M NaOH.

Labelling with $^{14}CO_2$ (350 ± 20 μl l^{-1}, specific activity 7·4 MBq g^{-1} C) was begun at the start of the photoperiod and was continued for 16 h in the light. The $^{14}CO_2$ was generated by the gradual addition of $NaH^{14}CO_3$ from a burette to excess lactic acid. This was controlled by a solenoid valve operated from a ratemeter with an adjustable set point. The ratemeter measured the output of a Geiger Muller tube inserted in the gas stream of the recirculating above ground atmosphere. The consumption of $NaH^{14}CO_3$ solution and radioactivity of the above ground atmosphere were recorded. The CO_2 concentration was checked at 1 h intervals by gas chromatography.

At the end of the photoperiod, $^{14}CO_2$ was removed from the atmosphere within the chamber. Subsequent $^{14}CO_2$, evolved by dark respiration in the plant shoots, was collected by recirculating the gas stream through 0·5 M NaOH solution. In the following photoperiods the plastic canopy was removed to expose the plants to normal atmospheric $^{12}CO_2$ in the light, but replaced at night to collect respired $^{14}CO_2$.

The soil–root system of each plant was continuously purged with CO_2-free air, and CO_2 evolved in respiration below ground in each pot was trapped in 0·2 M NaOH solution. The plants were watered twice each day at rates previously determined to maintain soil moisture content between 15 and 23 % (50 and 80 % of water holding capacity).

Acetylene reduction assay for nitrogenase

The pots were disconnected from the gas flow system after the 16 h labelling and a 67 h chase period (11 h of final photoperiod). Serum caps were fitted to the gas ports for each pot and 35 cm^3 (approx. 7 kPa) acetylene injected. Duplicate 1 cm^3 gas samples were removed after 30, 60 and 90 min and ethylene production was measured by gas chromatography.

Harvest

Plants were harvested 80 h after cessation of labelling. Leaves were separated from stem and petioles and fresh weights recorded. Leaf area was determined using a Li-Cor L-1500 leaf area meter (Lincoln, NB 68504) and plant parts were immediately transferred to a 60 °C drying oven. Roots were shaken free of loose soil and washed in tap water on a sieve, and the washings collected. Nodules were picked from the roots and dried. The washed roots were cut into 1 cm lengths, and a weighed sub-sample removed for determination of mycorrhizal infection. The remaining roots were dried at 60 °C. All dried plant material was ground to < 40 mesh.

Analyses

Total CO_2 collected in NaOH traps was measured, after precipitation of carbonate with $BaCl_2$, by back titration with HCl to pH 7. Carbon-14 in liquid samples (NaOH traps and root washings) was determined by liquid scintillation counting on 1 ml aliquots mixed with 10 ml Scintiverse 11 (Fisher Scientific, Pittsburg, PA).

Ground plant samples (5 mg) were digested in scintillation vials with 1 ml hyamine hydroxide (1 M in methanol) at 60 °C for 24 h, the digests were partially decolorized with H_2O_2 (100 μl, 30 %) and 0·5 ml glacial acetic acid was added to suppress chemoluminescence (Fuchs & de Vries, 1970). Scintiverse 11 (10 ml) was added and the samples stored in the dark for 24 h before scintillation counting. Total carbon was released from soils by wet digestion (Nelson & Sommers, 1982), collected in 0·2 M NaOH, and ^{14}C determined.

Total N in plant samples was determined after Kjeldahl digestion, ammonia was measured (QuikChem method no. 904218) using a flow injection autoanalyser (Lachat QuikChem Systems, Madison, WS). Inorganic P (Pi) was extracted from ground plant material by shaking in 0·5 M H_2SO_4 for 16 h. Total P was measured by the ammonium molybdate method (QuikChem method no. 110518) after perchloric acid digestion of unextracted material.

For measurement of leaf starch and ethanol-soluble sugars, ground plant material was extracted five times with ethanol (80 % at 80 °C). The residue was hydrolysed in 5 % perchloric acid and sugars estimated by the anthrone method (McCready *et al.*, 1950).

Percentage mycorrhizal infection was estimated by the line intercept method after staining root segments with trypan blue (Phillips & Hayman, 1970). Intraradicle fungal biomass was also assessed by the chitin content of roots after

extraction, alkaline hydrolysis and determination of glucosamine (Bethlenfalvay, Pacovsky & Brown, 1981). Samples of extraradicle mycorrhizal fungal hyphae were picked from the root surface, weighed, digested in hyamine hydroxide and their radioactivity determined by scintillation counting.

Results

The objective of growing plants of all treatments to equal weight was not completely achieved. At six weeks the dry weights of fertilizer-compensated, non-symbiotic plants (controls) were up to 18% greater than of plants with symbionts; at week 9, the differences were not statistically significant (Table 1). Nodule weights were increased in mycorrhizal plants, by 38% at six weeks and by 86% at nine weeks, compared to those of plants with *Rhizobium* alone. Intraradicle mycorrhizal fungal biomass was similar to that of nodule weight in six-week-old plants, comprising 6·6% of root mass (68% infection). At nine weeks, intraradicle mycorrhizal fungal biomass was 7·9% of root mass (76% infection) and equivalent to 65% of nodule weight.

Leaf composition and C uptake

Specific leaf area (leaf area/leaf dry weight) was increased by 10% in six-week-old nodulated plants and by 24% in plants with both symbionts (Table 2). The smaller differences in specific leaf area at week 9 were not statistically significant. In nodulated, non-mycorrhizal plants, the ratio of total leaf area to plant dry weight (leaf area ratio) was unchanged in comparison to controls. Thus leaf area was maintained, but leaf mass reduced. The leaf area ratio of mycorrhizal, nodulated plants was increased in proporton to the increase in specific leaf area. That is, the ratio of leaf to plant mass was maintained, resulting in greater leaf area per unit plant dry weight in comparison to fertilizer-compensated plants.

The starch content of leaves of mycorrhizal, nodulated plants was reduced to 50% of that in control plants at six weeks (Table 2). These leaves also contained higher concentrations of N, P and Pi, particularly at six weeks, where total P and Pi concentrations were approx. 75% higher than in leaves of fertilizer-compensated plants (Table 2). Concentrations of ethanol-soluble sugars were higher at six weeks than at nine weeks in all treatments, and the leaves of dually-infected plants contained 20% more ethanol-soluble sugars than did control or nodulated plants at six weeks.

Specific ^{14}C uptake rates were greater in plants supporting microbial symbionts than in fertilizer-compensated plants (Table 2). The rate of ^{14}C uptake g^{-1} leaf in six-week-old plants was increased by 24% in the presence of *Rhizobium* and by 52% in nodulated, mycorrhizal plants. Nodulation increased the rate of ^{14}C uptake g^{-1} leaf by 16% in nine-week-old plants. The addition of mycorrhiza to nodulated plants increased the rate by 23% relative to non-symbiotic plants, but the effect of mycorrhizal infection was not significant. Differences in specific leaf area could account for about half the increase in the rate of ^{14}C uptake in both nodulated and dually-infected plants (Table 2).

Carbon allocation

Below-ground CO_2. Amounts of $^{14}CO_2$ released by plant and microbial respiration below ground reached maxima at the end of the 16 h labelling period.

Table 1. *Dry weights (g) of plant parts, nodules and mycorrhizal fungi*

Component	Six weeks			Nine weeks		
	Nitrogen/ phosphorus	*Rhizobium*/ phosphorus	*Rhizobium*/ *Glomus*	Nitrogen/ phosphorus	*Rhizobium*/ phosphorus	*Rhizobium*/ *Glomus*
Leaves	3·18^{a}	2·66^{b}	2·56^{b}	5·80^{x}	4·22^{y}	5·69^{x}
Stem + petioles	2·95^{a}	2·90^{a}	2·34^{b}	6·12^{x}	4·95^{x}	5·59^{x}
Roots	2·29^{a}	1·92^{b}	1·75^{c}	4·17^{x}	3·79^{x}	3·37^{x}
Nodules	—	0·10^{a}	0·14^{b}	—	0·22^{x}	0·41^{y}
Intraradicle fungal biomass	—	—	0·12^{a}	—	—	0·27^{x}
Total	8·42^{a}	7·58^{ab}	6·91^{b}	16·10^{x}	13·19^{x}	15·32^{x}

Means within rows without common superscripted letters differ significantly at $P = 0{\cdot}05$.

Table 2. *Composition and ^{14}C uptake rates of leaves of* Glycine max–Rhizobium japonicum–Glomus fasciculatum *associations*

Leaf properties	Six weeks			Nine weeks		
	Nitrogen/ phosphorus	*Rhizobium*/ phosphorus	*Rhizobium*/ *Glomus*	Nitrogen/ phosphorus	*Rhizobium*/ phosphorus	*Rhizobium*/ *Glomus*
Specific leaf area ($dm^2\ g^{-1}$ leaf d. wt)	1·64^{a}	1·80^{a}	2·04^{b}	1·69^{x}	1·84^{x}	1·82^{x}
Leaf area ratio ($dm^2\ g^{-1}$ plant d. wt)	0·62^{a}	0·63^{a}	0·78^{b}	0·61^{x}	0·59^{x}	0·68^{y}
Hydration (% H_2O)	73·5^{a}	73·7^{a}	75·6^{a}	72·6^{x}	73·1^{x}	73·6^{x}
Starch (%)	16·0^{a}	16·8^{a}	7·7^{b}	16·3^{x}	15·9^{x}	15·7^{x}
EtOH soluble sugars (%)	9·6^{a}	9·8^{a}	11·8^{b}	7·5^{x}	7·0^{x}	7·3^{x}
Total N (%)	2·28^{a}	2·10^{a}	2·78^{b}	2·08^{x}	2·30^{y}	2·56^{z}
Total P ($\mu g\ g^{-1}$)	866^{a}	888^{a}	1583^{b}	812^{x}	1042^{xy}	1056^{y}
Total Pi ($\mu g\ g^{-1}$)	267^{a}	289^{a}	472^{b}	410^{x}	416^{x}	584^{y}
^{14}C uptake						
(mg $^{14}C\ g^{-1}$ leaf h^{-1})	2·10^{a}	2·60^{b}	3·20^{c}	1·83^{x}	2·13^{y}	2·25^{y}
(mg $^{14}C\ dm^{-2}$ leaf h^{-1})	1·28^{a}	1·44^{ab}	1·57^{b}	1·08^{x}	1·16^{xy}	1·23^{y}

Means for single dates within a row without common superscripted letters differ significantly at $P = 0{\cdot}05$.

The subsequent rates of $^{14}CO_2$ evolution fluctuated diurnally, in phase with a diurnal variation in total CO_2 evolution, probably due to the effect of the day–night temperature cycle, but showed an overall decline until harvest. The rates of $^{14}CO_2$ evolution from dually-infected plants at six and nine weeks and exponential decay curves fitted to the data are shown in Figure 1. The continued evolution of $^{14}CO_2$ would have increased the proportion of ^{14}C released by respiration if collection had been continued beyond 80 to 83 h. Extrapolation of the decay curves indicates that a further 3 to 4 % of the ^{14}C would have been mineralized by 200 h. The specific activity of the CO_2 reached maxima about 8 h later than the maximum absolute rates of $^{14}CO_2$ efflux.

Above ground CO_2. The release of $^{14}CO_2$ by respiration of the plant shoots in the dark declined rapidly so that by the third dark period after labelling (64 to 72 h) the $^{14}CO_2$ collected was less than 10 % of that evolved in the first dark period. Since

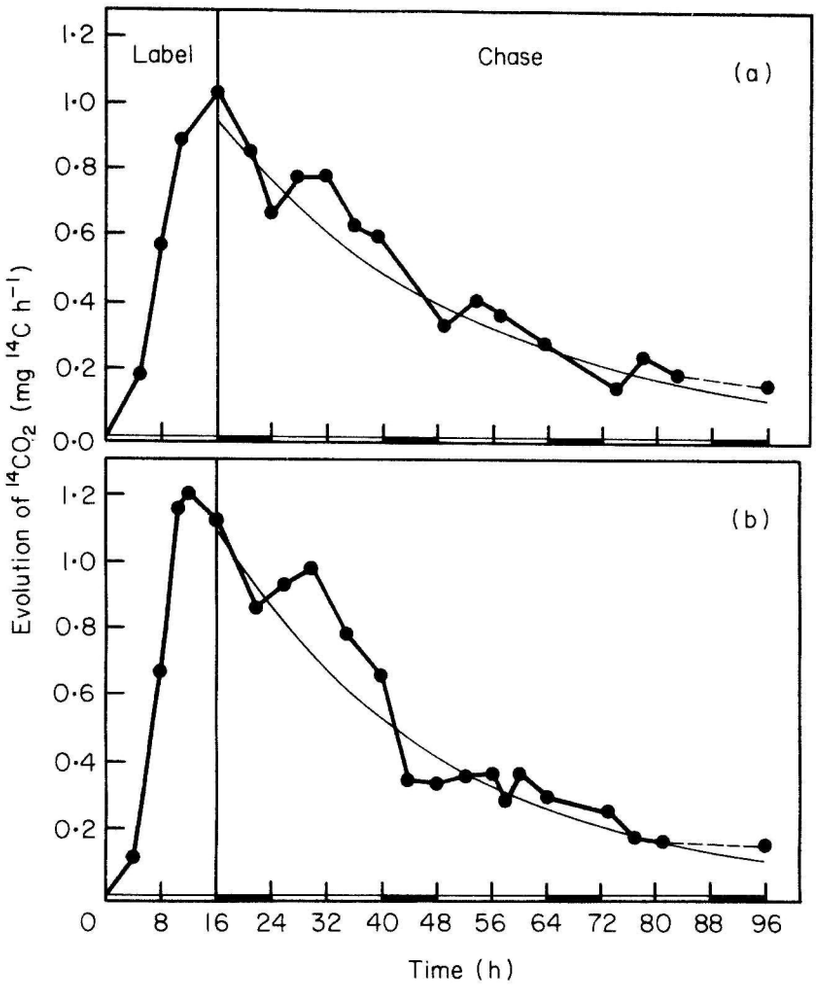

Fig. 1. Evolution of $^{14}CO_2$ by root–soil systems of *Glycine max–Rhizobium japonicum–Glomus fasciculatum* associations at (a) six and (b) nine weeks and fitted first order decay curves. (a) $y = 1{\cdot}465\ e^{-0{\cdot}0271t_{(16-83)}}$; (b) $y = 1{\cdot}781\ e^{-0{\cdot}0302t_{(16-83)}}$.

the above ground atmosphere was common to all plants, separate collection of $^{14}CO_2$ was not possible. The $^{14}CO_2$ evolved by each plant was estimated by assuming that $^{14}CO_2$ release was proportional to shoot biomass. Differences in specific activity due to differing rates of photosynthesis would lead to overestimation of $^{14}CO_2$ respiration in control plants and underestimation in those with microsymbionts (Tables 3 and 4).

Plant and microbial endophytes

The distribution of ^{14}C in fertilizer-compensated, non-symbiotic soybeans was similar at six and nine weeks. Approximately 76 % of the ^{14}C assimilated by the controls was retained or respired by the shoots, 13 % was found in the root biomass, 2 % in the soil and root washings and the remaining 9 % was evolved as $^{14}CO_2$ below ground (Tables 3 and 4).

The $^{14}CO_2$ respired by root nodules was calculated by subtraction of the $^{14}CO_2$ respired by roots without symbionts from that evolved by nodulated roots: [total − (rate per g non-nodulated × nodulated root d. wt)]. Similarly, mycorrhizal respiration was calculated by subtraction of plant root and nodule respiration from that of mycorrhizal nodulated roots: [total − (rate per g root × root d. wt + rate per g nodule × nodule d. wt)]. These calculations assume that infection by *Rhizobium* or VA mycorrhizal fungi does not affect the respiration of the plant root. Microsymbiont respiration will be overestimated if some of the $^{14}CO_2$ evolution was due to increased respiration in the host root tissue (Pate, Layzell & Atkins, 1979; Cox *et al.*, 1975).

Table 3. *^{14}C distributions (mg ^{14}C per plant) in six week old* Glycine max–Rhizobium japonicum–Glomus fasciculatum *associations after 16 h labelling with $^{14}CO_2$ and a 68 h chase period*

Component	Treatment		
	Nitrogen/ phosphorus	*Rhizobium*/ phosphorus	*Rhizobium*/ *Glomus*
Leaves	42·5	42·3[a]	40·4[a]
Stem + petioles	30·9[a]	31·2[a]	27·2[a]
Shoot respiration	10·3[a]	8·5[ab]	8·4[b]
Roots	14·3[a]	11·1[ab]	10·4[ab]
Nodules	—	1·1[a]	2·6[a]
Mycorrhiza	—	—	3·6*
Root washings + soil	1·6[a]	2·1[a]	2·4[a]
Below ground respiration			
Roots + soil	9·1[a]	7·5[ab]	6·9[b]
Nodules	—	9·0[a]	12·4[b]
Mycorrhiza	—	—	18·2
Total	107·4[a]	112·9[a]	132·5[b]

Means within a row without common superscripted letters differ significantly at $P = 0{\cdot}05$.
* ^{14}C contents of intra- and extra-radicle mycorrhizal fungal biomass assumed to be equal. Recovery of added $^{14}C = 96{\cdot}4\,\%$.

Table 4. *^{14}C distribution (mg ^{14}C per plant) in nine week old* Glycine max–Rhizobium japonicum–Glomus fasciculatum *associations after 16 h labelling with $^{14}CO_2$ and a 68 h chase period*

Component	Treatment		
	Nitrogen/ phosphorus	*Rhizobium*/ phosphorus	*Rhizobium*/ *Glomus*
Leaves	70·0[a]	53·5[b]	76·8[c]
Stem + petioles	49·0[a]	34·5[b]	50·4[a]
Shoot respiration	10·9[a]	9·7[b]	8·2[c]
Roots	23·7[a]	17·9[b]	16·9[b]
Nodules	—	2·2[a]	3·6[b]
Mycorrhiza	—	—	5·8*
Root washings + soil	2·7[a]	2·4[a]	2·7[a]
Below ground respiration			
Roots + soil	16·6[a]	15·2[a]	13·5[a]
Nodules	—	11·1[a]	20·4[a]
Mycorrhiza	—	—	9·7
Total	172·9[a]	146·4[b]	207·8[c]

Means within a row without common superscripted letters differ significantly at $P = 0{\cdot}05$.
* ^{14}C contents of intra- and extra-radicle mycorrhizal fungal biomass assumed to be equal. Recovery of added $^{14}C = 95{\cdot}3\,\%$.

In nodulated soybeans, 1 to 1·5 % of the ^{14}C was found in the nodules and 8 % of the ^{14}C taken up by the plant was evolved as $^{14}CO_2$ by the nodules. Nodule respiration accounted for about 50 % of the below ground $^{14}CO_2$ evolution.

Allocation of ^{14}C to nodules does not include ^{14}C returned to the host as amides and ureides or any fixation of CO_2 in the nodules.

The ^{14}C retained or respired by the shoot was reduced to 58 % of total ^{14}C uptake in six-week-old plants with both rhizobial and mycorrhizal symbioses, compared to 78 % in plants without symbionts. Despite the retention of a smaller proportion of total photosynthate above ground, the specific ^{14}C incorporation rates (mg ^{14}C g^{-1} d. wt d^{-1}) in plant shoots were maintained or increased in comparison to controls (Table 5). The evolution of $^{14}CO_2$ below ground was increased from 9 to 29 % of ^{14}C uptake by the host, and the symbioses together accounted for 82 % of total below ground $^{14}CO_2$ evolution (Tables 3 and 4). At nine weeks, 66 % of the ^{14}C was retained or respired above ground, and the proportion of ^{14}C evolved as $^{14}CO_2$ below ground was reduced in comparison to six-week-old plants from 29 to 21 % of total uptake. This result was associated with a three-fold decrease in the proportion of ^{14}C evolved as $^{14}CO_2$ in mycorrhizal respiration.

Table 5. *Specific rates of ^{14}C incorporation into plant and microbial biomass*

	Six weeks			Nine weeks		
	Nitrogen/ phosphorus	*Rhizobium*/ phosphorus	*Rhizobium*/ *Glomus*	Nitrogen/ phosphorus	*Rhizobium*/ phosphorus	*Rhizobium*/ *Glomus*
	(mg ^{14}C g^{-1} d. wt d^{-1})					
Leaves	12·4^{a}	15·9^{b}	15·9^{b}	12·0^{x}	12·7^{x}	14·1^{x}
Stems + petioles	9·8^{a}	10·6^{a}	11·6^{a}	8·0^{xy}	7·3^{y}	9·5^{x}
Roots	6·3^{a}	5·5^{a}	5·4^{a}	5·7^{x}	4·5^{y}	5·3^{xy}
Plant overall	9·9^{a}	11·3^{a}	11·7^{a}	8·9^{x}	8·2^{x}	9·8^{x}
Nodules	—	11·0^{a}	18·8^{b}	—	10·0^{x}	8·7^{x}
VAM	—	—	15·6^{a}	—	—	10·9^{b}

Means within a row, for single dates, not followed by common superscripted letters, differ significantly at $P = 0{\cdot}05$.

Nodule growth, respiration and acetylene reduction

Nodule growth rates, as estimated by the specific rates of ^{14}C incorporation, were similar at both six and nine weeks in plants without VA mycorrhiza (Table 5). Mycorrhizal colonization stimulated the early development of nodules resulting in higher nodule dry weight and specific rates of ^{14}C incorporation at six weeks (Tables 1 and 5). In nine-week-old plants, nodule dry weight was 86 % greater in mycorrhizal plants than in those without mycorrhiza, but the specific rate of ^{14}C incorporation in nodules of mycorrhizal plants had fallen to 50 % of the rate at six weeks. Specific nodule activity (μmol C_2H_4 g^{-1} nodule h^{-1}) and nodule respiration rates (μmol $^{14}CO_2$ g^{-1} nodule h^{-1}) were greater at six than at nine weeks (Table 6). Ratios of nodule $^{14}CO_2$ evolution to C_2H_4 reduction (from 4·8 to 5·8:1) were close to the maximum of the range reported by Tjepkema & Winship (1980) for a variety of rhizobial and actinorrhizal symbioses.

Growth of VA mycorrhizal fungi

Intraradicle mycorrhizal fungal biomass increased from 115 mg per plant at six weeks to 266 mg per plant at nine weeks. The specific rate of ^{14}C incorporation into fungal biomass was lower at nine than at six weeks (Table 5). These rates can be expressed as instantaneous specific growth rates, 0·04 d^{-1} at six weeks and 0·03 d^{-1} at nine weeks.

Table 6. *Acetylene reduction and respiration of root nodules*

	Rhizobium		*Rhizobium* + *Glomus*	
	Six weeks	Nine weeks	Six weeks	Nine weeks
SNA (μmol C_2H_4 g^{-1} h^{-1})	62·3 ± 2·3	29·3 ± 2·6	64·9 ± 2·3	33·6 ± 1·2
Nodule respiration (μmol CO_2 g^{-1} h^{-1})	313 ± 6	171 ± 13	313* ± 17	171* ± 21
CO_2:C_2H_4 (μmol:μmol)	5 ± 1·5	5·8 ± 0·5	4·8 ± 1·4	5·1 ± 0·4
C:N† (mg:mg)	8·6	10	8·3	8·7

Values are means ± SE of four replicates.

* Specific $^{14}CO_2$ evolution rates in nodules of mycorrhizal roots assumed equal to those of nodules of non-mycorrhizal roots.

† At a relative efficiency of 0·75 (Pacovsky *et al.*, 1986) the C_2H_4:N_2 conversion ratio taken as 4:1.

Measurement of extraradicle fungal biomass by the chitin method was not possible due to background interference from the soil. Bethlenfalvay *et al.* (1982) found that the ratio of extraradicle to intraradicle mycorrhizal fungal biomass in a soybean–*G. fasciculatum* association grown in an artificial potting mix decreased from about 3:1 at six weeks to 1·2:1 at nine weeks. Application of these ratios to our data, indicated that the amounts of ^{14}C in extraradicle mycorrhizal hyphae should be 5·4 mg ^{14}C per plant at six weeks and 3·5 mg ^{14}C per plant at nine weeks. These estimates exceed the total ^{14}C content of the soil and root washings at both harvest dates (Tables 3 and 4). If all the ^{14}C recovered from the soil of the mycorrhizal plants were derived from mycorrhizal fungal biomass, maximum ratios of extraradicle to intraradicle biomass would be 1·3:1 and 0·9:1 at six and nine weeks, respectively.

Phosphorus uptake

Total P uptake during the 21 d interval between harvests was increased by 29% in mycorrhizal, nodulated soybeans compared to nodulated plants without mycorrhiza. Since mycorrhizal plants had smaller roots, this represented a 44% increase in the rate of P uptake per g root (Table 7). The ratio of C utilized (in root growth and respiration) to P acquired by P-fertilized plants without mycorrhiza was about 350:1 (g atom:g atom), while the C utilization:P uptake ratio for the mycorrhizal root (lacking P fertilizer) was slightly higher at 440:1.

Table 7. *Uptake of P, and C utilization by plant roots and VA mycorrhizas*

	Nitrogen/ phosphorus	*Rhizobium*/ phosphorus	*Rhizobium*/ *Glomus*
P increment (μg) in period six to nine weeks	5226	4373	5631
Mean root d. wt (g)	3·23	2·86	2·56
P uptake rate (μg g^{-1} root d^{-1})	77	72·8	104·7
Effect of VAM (μg P g^{-1} root d^{-1})	—	—	31·9
C utilization (mg C g^{-1} root d^{-1})			
Root	9·9	9·2	9·5
Mycorrhiza	—	—	8·5
C utilized:P absorbed (g atom:g atom)			
Root	333	327	336 | 444
Mycorrhiza	—	—	690 |

Discussion

Net ^{14}C assimilation rates were increased in soybeans having either *Rhizobium* or both symbionts in comparison to uninfected, fertilizer-compensated control plants. The additional C uptake enabled plants supporting microbial symbionts to attain relative growth rates equal to or greater than those of controls, while supplying up to 29% of total photosynthate to the microbial endophytes. If the growth curve of fertilizer-compensated control plants could be made to exactly match those of plants with symbionts, the C allocation to root nodules and mycorrhizas should correspond to the increase in the rate of C assimilation. This is difficult to achieve because the effects of the symbionts on plant growth vary in time, and so plants of equal dry weight may have different relative growth rates. The specific rates of ^{14}C incorporation into macrosymbiont biomass were usually greater at the time of labelling than those of control plants, and part of the increased C assimilation was utilized in more rapid plant growth. The 52% increase in C assimilation rate g^{-1} leaf in mycorrhizal, nodulated plants at six weeks was associated with an increase in specific C incorporation rate into plant biomass of 18%, and utilization by root nodules and mycorrhizas of 12 and 17% of total photosynthate.

Mycorrhizal infection has previously been found to increase specific leaf area in leek, enabling the plant to achieve a given rate of C fixation from a smaller investment in leaf biomass (Snellgrove *et al.*, 1982). However, the total C uptake and the CO_2 assimilation rate per unit leaf area were not increased. Increased photosynthesis in mycorrhizal *Bouteloua gracilis* (Allen *et al.*, 1981) were due to decreased stomatal resistance. Stomatal density and size were unchanged and the increased CO_2 uptake was attributed to higher rates of photophosphorylation enabled by higher leaf P concentrations in mycorrhizal plants.

Our results indicate that several host-plant adaptations to the C requirements of the mycorrhizal and rhizobial symbioses occurred in soybean. Specific leaf area was increased in plants infected by *Rhizobium* alone, but leaf mass was reduced so that there was no change in the overall ratio of leaf area to plant dry weight. The effective increase in C assimilation was therefore due only to the increased rate of $^{14}CO_2$ uptake per unit leaf area, which was achieved without changes in either leaf N or P concentrations or in storage products.

It is proposed that C assimilation in fertilizer-compensated plants was 'sink limited' (Herold, 1980) due to the accumulation of photosynthetic end products which could not be used for plant growth because of nutrient, especially P, limitation. Low sink demand, due to restricted activity of plant meristems, may lead to the accumulation of sucrose-6-P in leaf mesophyll cells (Hurewitz & Janes, 1983), which binds Pi in inactive pools and reduces the exchange of Pi and triose-P across the chloroplast membrane (Giaquinta, 1980). Starch would accumulate in the chloroplast; a situation associated with decreased rates of photosynthesis (Chatterton *et al.*, 1972; Thorne & Koller, 1974). Low Pi concentration in the chloroplast may also directly affect the C-reduction cycle via the stromal ADP/ATP ratio (Walker & Robinson, 1978).

Mycorrhizal, nodulated plants differed from P-fertilized nodulated plants in that increased specific leaf area was not balanced by a relative reduction in leaf mass. Thus, the ratio of leaf area to plant dry weight was increased, and C assimilation was affected by both the increased leaf area and the higher specific rate of C uptake per unit leaf area. The changes in leaf starch and water content cannot fully explain

the observed increases in specific leaf area in plants with microbial endophytes. Morphological changes either in the cellular geometry of the leaf, for example thinner laminae, or in the ratio of primary trifoliate to branch leaves also must have been involved.

The starch mobilization and enhanced C assimilation rates observed in six-week-old mycorrhizal, nodulated plants and the relatively high P concentrations in the leaves of these plants are consistent with the hypothesis that leaf P concentrations are important in controlling C assimilation and translocation (Silvius, Kremer & Lee, 1978; Priess, 1984). However, measurements of leaf Pi showed no indication of treatment effects on the ratio of Pi to total P. In addition, leaf Pi concentrations and Pi : P ratios were lower in six-week- than in nine-week-old plants, when C assimilation and translocation rates to roots were declining.

Koch & Johnson (1984) examined the distribution of ^{14}C after pulse labelling split-root citrus, in which one half of the root system was mycorrhizal. Bilateral differences in leaf P concentrations were absent, but twice as much ^{14}C-photosynthate was translocated from source leaves to mycorrhizal roots than to those without mycorrhiza. This result demonstrates that sink strength in mycorrhizal roots may affect allocation patterns independent of the effects of P or source strength.

The allocation of up to 29 % of total photosynthate to microbial symbionts shows that these can constitute a major sink for C at some stages in the development of tripartite symbiotic associations. In nine-week-old plants, the effects of the symbioses on C assimilation were smaller than at six weeks and there was a 50 % reduction in the proportion of assimilate utilized by the mycorrhizae. These effects coincided with a reduction in the apparent growth rate of nodules, the mycorrhizal fungus and probably with a decrease in the proportion of extraradicle hyphal biomass.

Models of mycorrhizal development have been concerned primarily with the spread of infection rather than the formation of biomass. The simplest form of these models (Smith & Walker, 1981; Buwalda *et al.*, 1982), is logistic with parameters which limit the maximum degree of infection. This can be adapted to describe the formation of biomass:

$$dF/dt = kF\ 1-F/nR,$$

where F is intraradicle fungal biomass, k and n are constants describing maximum specific growth rate and the maximum proportion of fungus to root, and R is root dry weight.

The data of Bethlenfalvay *et al.* (1982) suggest that the intraradicle development of *G. fasciculatum* in soybean is limited to about 10 % of root dry weight. Using initial conditions of fungal and root biomass at six weeks and $n = 0{\cdot}1$, the measured fungal biomass at nine weeks is estimated when $k = 0{\cdot}15$. The predicted increases in intraradicle fungal C of 2·4 and 3·2 mg C d^{-1} at six and nine weeks respectively exceed the ^{14}C contents of intraradicle fungal biomass by 30 % at six weeks and 12 % at nine weeks. This suggests either that the logistic model of fungal growth is inadequate, or that the estimates of intraradicle fungal biomass and specific activity are distorted by differences between internal and external fungal structures. Possible differences between the chitin content of internal and external fungal material were discussed by Hepper (1977). The specific activity of fungal structures depends on specific growth rate, thus if the growth rate of intraradicle fungus exceeded that of external hyphae, the ^{14}C content of intraradicle fungal biomass would be underestimated.

The ratio of C utilized by mycorrhizal root systems to P uptake could provide an index for comparing the 'effectiveness' of different mycorrhizal associations. Comparisons of C:P ratios of mycorrhizal and non-mycorrhizal root systems is less useful since the C cost of the plant root cannot be attributed solely to P uptake. Mycorrhizal plants grown in unfertilized soil took up P more rapidly than non-mycorrhizal plants given P-fertilizer, but the unit cost was greater. Mycotrophic growth was possible because the plant photosynthetic response was at least equivalent to the C cost of enhanced P uptake. Where increased photosynthesis could not occur, for example when light intensity was low (Bethlenfalvay & Pacovsky, 1983) or possibly when leaf area index was high (Silsbury, Smith & Oliver, 1983) zero or negative responses to mycorrhizal infection might be anticipated.

This study has demonstrated that a number of microsymbiont interactions are mediated via the plant. VA mycorrhizas increased the amount of N_2 fixation by stimulating nodule development but nitrogenase activity and specific nodule respiration were unaffected. Nutrient additions to fertilizer-compensated plants were below the levels required for optimum plant growth. If greater amounts of N and P were available to the non-symbiotic plant, sink limitations on photosynthesis would decrease and smaller photosynthetic responses to microsymbiont infection could be anticipated. Most studies, including our own, have used container-grown plants and controlled environments. Changes in specific leaf area may not have the same significance under field conditions, particularly later in the growing season due to canopy effects. However, VA mycorrhizas were found to exert their greatest effect at six weeks when P uptake and leaf development in the field are known to be maximal. Thus the laboratory results should be applicable to at least the first third of the growth cycle in the field. The response of the host plant to the microbial endophytes, through alterations in leaf morphology, sink strength and P levels is complex but must be further understood if maximum benefit from the association is to be obtained.

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