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generate the size index for each hatchling; high (positive) scores characterized large animals and small (negative) scores characterized small turtles.

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16. We thank M. Gardiner for information on the turtle nests, L. R. Jones for assistance in gathering the data, J. R. zumBrunnen for assistance with analyses, K. Jee for the drawing, and N. Heisler for typing. Turtle eggs were collected near Crook, Colorado, under authority of permit 79-112 from the Colorado Division of Wildlife. This research was supported in part by National Science Foundation grant DEB 77-08148.

31 July 1980; revised 22 April 1981

## Carbon Flow in Plant Microbial Associations

**Abstract.** Measurement of the distribution of the photosynthesis product in the symbiotic association of a legume, a mycorrhizal fungus, and nitrogen-fixing bacteria showed that the fungus incorporated 1 percent of the photosynthesis product and respired 3 percent. The nodules of a 5-week-old plant utilized 7 to 12 percent of the photosynthesis product. The legume compensated in part for the needs of its microbial partners through increased rates of photosynthesis.

Symbiotic associations between plants and microorganisms have a major effect on plant growth and nutrient cycling. Rhizobia associated with legumes can fix 450 kg of nitrogen per hectare per year, and vesicular arbuscular (VA) mycorrhizal fungi enhance the uptake of many elements, notably phosphorus. The carbon flow to the nodules of legumes

grown in sand culture has been measured (1, 2). The dynamics and quantities of the carbon flow to the VA mycorrhiza and the interactions between the two microbial symbionts are unknown.

We used field and growth chamber studies,  $^{14}\text{C}$  and  $^{15}\text{N}$  labeling, and fungal and nodule biomass measurements to determine (i) the quantities of plant car-

bon translocated to the mycorrhizal and rhizobial symbionts of faba beans (*Vicia faba*), (ii) the extent of nitrogen fixation by rhizobia in nodules of mycorrhizal and nonmycorrhizal plants, and (iii) the effect of the carbon utilized by the microorganisms on host growth. The VA fungus *Glomus mosseae*, which we used as inoculum, had significantly increased the growth of *V. faba* and the phosphorus contents in the field at low or moderated levels of soil phosphorus (3).

The cost of the mycorrhizal infection to the plant was studied on 4- to 5-week-old *V. faba* plants growing in a mixture of soil and sand (1:1) with and without mycorrhizal and rhizobial infection. To obtain plants of similar size in the various treatments, nonmycorrhizal plants were supplemented with potassium acid phosphate ( $\text{K}_2\text{HPO}_4$ ), and nitrate nitrogen was added to nonrhizobial treatments. Carbon distribution and flow to symbionts were determined by exposing the above-ground plant parts to  $^{14}\text{CO}_2$  in a Plexiglas chamber designed so that atmosphere beneath the ground could be separated from that above ground. The  $^{14}\text{C}$  contents of plant materials, nodules, and external hyphae were determined by liquid scintillation after dry combustion and absorption of the  $^{14}\text{CO}_2$  in NaOH (4). Carbon dioxide, respired by underground portions during and after the pulse labeling, was absorbed for  $^{14}\text{CO}_2$  determination; fungal biomass was measured by microscopy (5).

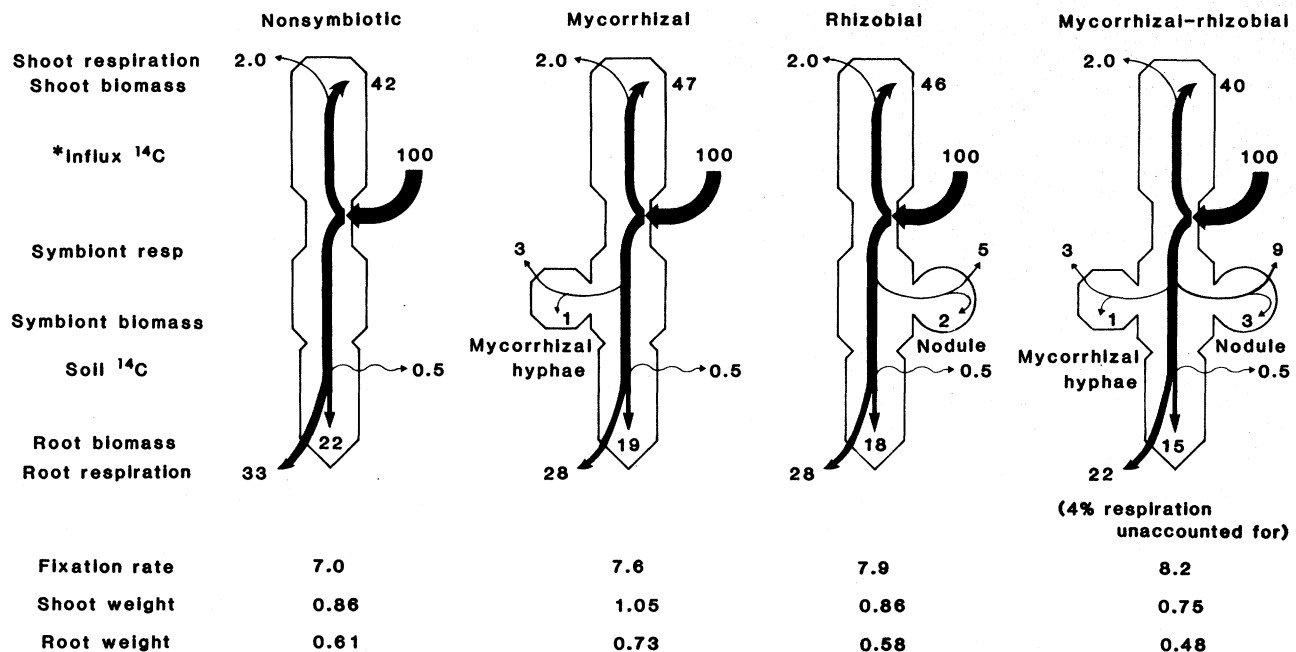


Fig. 1. The  $^{14}\text{C}$  flow to various compartments of symbiotic and nonsymbiotic faba beans (4 to 5 weeks old) after shoots were exposed above ground to  $^{14}\text{CO}_2$  under continuous light. The fixation rate is expressed as milligrams of carbon per gram of shoot per hour. The shoot weight and the root weight are expressed as grams of carbon. The carbon influx has been equalized to 100 units of carbon per gram of shoot carbon.

The plants, after exposure for 48 hours to  $^{14}\text{C}$ , were grown for an additional 96 hours in the light in a normal atmosphere to allow for  $^{14}\text{C}$  translocation, incorporation, and respiration. Symbiont respiration was calculated by attributing the difference in the evolution of carbon (milligrams of  $^{14}\text{C}$  per gram of carbon) between symbiotic and nonsymbiotic roots to symbiotic respiration. It was assumed that the symbionts of doubly infected plants had a ratio of respired carbon to biomass carbon similar to that determined for singly inoculated plants.

Plant shoots contained slightly less than half of the added label (Fig. 1). Roots accounted for 15 to 22 percent, and below-ground respiration accounted for 31 to 34 percent. The mycorrhizal fungi incorporated 1 percent and respired 3 percent of the  $^{14}\text{C}$  assimilated. Older plants with a greater weight of mycorrhizal fungi would utilize greater amounts (3). Nodules of nonmycorrhizal plants infected with *Rhizobium* incorporated 2 percent of the tracer while respiring 5 percent. Nodules of mycorrhizal hosts incorporated 3 percent of the products of photosynthesis but respired 9 percent of the  $^{14}\text{C}$  fixed. The weights of the shoots and roots of plants containing rhizobium and rhizobium plus mycorrhizal symbionts were lower than those of control plants or of plants infected with mycorrhizal fungi only. These weight differences, however, were not statistically significant. The increased  $\text{CO}_2$  assimilation in the presence of the symbionts indicates that the plant may have been able to compensate, in part, for the needs of the microbial partners. This was investigated by measuring  $^{14}\text{CO}_2$  fixation rates during an 8-hour exposure, followed by immediate harvesting of the plant materials (Table 1). The  $\text{CO}_2$  fixation rate of the mycorrhizal and rhizobial plants was 7 percent higher per unit weight of shoots than that of the control. Mycorrhizal-rhizobial plants incorporated 16 percent more  $^{14}\text{C}$  than the controls.

Symbiotic nitrogen-fixation rates were increased by mycorrhizal infection because of an increase in nodule weight (88 mg of nodules per gram of root for rhizobial roots compared to 144 mg of nodules per gram of root for doubly infected plants). Nodular tissue on alfalfa roots has been found to increase after inoculation with mycorrhizal fungi (6). These fungi are thought to exert their effect primarily by increasing phosphorus uptake. Since some phosphorus was added to the nonmycorrhizal treatments in this experiment, other nutrients also may have been involved (7). The extra carbon

Table 1. The  $^{14}\text{CO}_2$  fixation (milligrams per gram of shoot carbon per hour) and  $^{15}\text{N}_2$  fixation (milligrams per gram of nodule) by symbiotic and nonsymbiotic 4-week-old faba beans.

	$^{14}\text{CO}_2$ fixation (mg $\text{g}^{-1}$ shoot carbon $\text{hour}^{-1}$ )	$^{15}\text{N}_2$ fixed	
		Total (mg)	Rate (mg $\text{g}^{-1}$ of nodule)
Control	17.4		
Mycorrhizal	18.8*		
Rhizobial	18.2†	0.78	16.2
Mycorrhizal-rhizobial	20.2*	1.06†	15.8

\* $P < .05$ . † $P < .10$ .

required in the presence of the mycorrhiza was offset to a large extent by higher nitrogen and carbon dioxide fixation rates (Table 1). Discussion of the carbon requirement for nitrogen fixation and other nutrient uptake by symbiotic associations is somewhat academic if the possibility that the plant can have altered photosynthetic rates in the presence of the symbionts is not considered.

Our estimates of carbon flow through root-microbial systems in soil were based on the premise that the symbionts did not significantly alter root respiration without altering root weight. Increased plant cytoplasm in fungal-infected root cells (8) and increased respiration of nodular tissue in the presence of bacteroids (9) have been noted. In our study, 4 percent of the respiration was unaccounted for in the presence of the two

microbial symbionts, indicating some interaction between the symbionts. This, however, would not affect our data on the incorporation of  $^{14}\text{C}$  into symbiont tissue, on total underground respiration, or on the relative rates of photosynthesis. The physiological interaction of host, fungi, and bacteria controls the response of the plants to microbial infection. An understanding of the various interactions and nutrient flows in such symbiotic associations should make feasible the selection, genetic manipulation, and management of each or all of the three components.

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10. Research was conducted under the auspices of a Natural Sciences and Engineering Research Council of Canada grant at the University of Saskatchewan, Saskatoon, Canada.

31 December 1980; revised 30 March 1981

## *Ureaplasma urealyticum* Incriminated in Perinatal Morbidity and Mortality

Abstract. *Perinatal morbidity and mortality are associated with colonization of the chorionic surface of the placenta by Ureaplasma urealyticum or Mycoplasma hominis or both. These organisms are more strongly associated with unfavorable gestational outcome than group B streptococci. Chlamydia trachomatis does not appear to be important in the etiology of reproductive casualties. The mechanisms linking the mycoplasmas to perinatal disorders and death are not clear but merit investigation.*

The causes of perinatal morbidity and mortality in humans are not clearly defined. Nebulous concepts such as "small for dates infants," "low birth weight," and "placental insufficiency" are often invoked, but are nonspecific or elusive as to etiology. Premature birth is the most common antecedent of infant death, and premature labor remains unexplained. Because the placenta is the active interface between mother and fetus, it is the appropriate organ to study

for clues to the causes of abnormal pregnancies.

Ureaplasmas in the female genitourinary tract have been related to low birth weight (1), infertility (2, 3), and spontaneous abortion (4, 5). Some investigators have found these organisms to be a greater threat to gestational outcome when isolated from the endometrium than from the cervix (6). We report that colonization of the chorionic surface of the placenta by *Ureaplasma urealyticum*