

# Carbon Isotope Ratios Demonstrate Carbon Flux from C<sub>4</sub> Host to C<sub>3</sub> Parasite<sup>1</sup>

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## ABSTRACT

Carbon isotope ratios of mature leaves from the C<sub>3</sub> angiosperm root hemiparasites *Striga hermonthica* (Del.) Benth (−26.7‰) and *S. asiatica* (L.) Kuntze (−25.6‰) were more negative than their C<sub>4</sub> host, sorghum (*Sorghum bicolor* [L.] Moench cv CSH1), (−13.5‰). However, in young photosynthetically incompetent plants of *S. hermonthica* this difference was reduced to less than 1‰. Differences between the carbon isotope ratios of two C<sub>3</sub>–C<sub>3</sub> associations, *S. gesnerioides* (Willd.) Vatke—*Vigna unguiculata* (L.) Walp. and *Oryza sativa* L.—*Rhamphicarpa fistulosa* (Hochst.) Benth differed by less than 1‰. Theoretical carbon isotope ratios for mature leaves of *S. hermonthica* and *S. asiatica*, calculated from foliar gas exchange measurements, were −31.8 and −32.0‰, respectively. This difference between the measured and theoretical δ<sup>13</sup>C-values of 5 to 6‰ suggests that even in mature, photosynthetically active plants, there is substantial input of carbon from the C<sub>4</sub> host. We estimate this to be approximately 28% of the total carbon in *S. hermonthica* and 35% in *S. asiatica*. This level of carbon transfer contributes to the host's growth reductions observed in *Striga*-infected sorghum.

Although environmental factors other than those affecting C<sub>i</sub> may influence carbon isotope ratios, the magnitude of these is thought to be small (16), and experimental evidence is accruing to support the model (Eq. 1) (5, 6, 8, 10, 11, 17, 27).

*Striga hermonthica* and *Striga asiatica* are root hemiparasitic angiosperms possessing the C<sub>3</sub> pathway of carbon fixation. Their preferred host is the C<sub>4</sub> plant, sorghum, from which they obtain a large proportion of their water and inorganic solutes. Although transfer of <sup>14</sup>C-labeled metabolites has been demonstrated (15, 25), the extent of the C flux is unknown. In *Striga* the carbon isotope composition will not only reflect its own photosynthetic and environmental characteristics, but also those of the host (23).

In this paper we report the carbon isotope composition of the *S. hermonthica*-sorghum and *S. asiatica*-sorghum associations and compare them to two other host-parasite unions where both members are C<sub>3</sub> plants. Foliar gas exchange characteristics are also reported and are used to estimate theoretical carbon isotope ratios according to model of Farquhar *et al.* (Eq. 2). The data are discussed in relation to the magnitude of carbon transfer from host to parasite.

## MATERIALS AND METHODS

**Plant Material and Growth Conditions.** Four host-parasite associations were established: sorghum (*Sorghum bicolor* [L.] Moench cv CSH1)—*Striga hermonthica* (Del.) Benth (Abu Naama, Sudan); sorghum—*S. asiatica* (L.) Kuntze (Patancheru, India); *Vigna unguiculata* (L.) Walp. cv SUVITA-2—*S. gesnerioides* (Willd.) Vatke. (Niger); and *Oryza sativa* L. cv IR 36 (Philippines)—*Rhamphicarpa fistulosa* (Hochst.) Benth (Upper Volta).

Pots (15 cm diameter) were filled with 1.6 dm<sup>3</sup> of a 1:1 mixture of John Innes No. 2 compost and sand, each being inoculated with seed of one of the four parasites at a density of approximately 15 mg dm<sup>-1</sup>. Ten pots of each association were established. The host seeds were surface-sterilized and germinated for 48 h on moist glass fiber paper at 30°C before placing one into each of the corresponding parasite-inoculated pots.

All plants were grown in a glasshouse under natural light but with supplementary heating during the summer of 1986. Day and night time temperatures were maintained close to 38 and 30°C, respectively. Plants were watered twice daily.

**Carbon Isotope Composition.** For *S. hermonthica*, material was collected at three stages of development: (a) achlorophyllous below-ground stem and leaves, just prior to emergence of the plant above ground; (b) green leaves from newly emerged plants; and (c) the youngest fully developed leaves from mature plants 11 weeks from the date of sowing. At this time the youngest fully developed leaves from the other parasites and hosts were also collected. *S. gesnerioides* has small scaly leaves and, for this plant, photosynthetic stem material was included in the sample. In all cases each host was supporting at least ten parasitic plants.

The carbon isotope composition of plants is widely used to distinguish between photosynthetic pathways; plants with the C<sub>3</sub> pathway of carbon assimilation have a δ<sup>13</sup>C in the range of −22 to −40‰ (mean = −28.1‰), while C<sub>4</sub> plants have a value between −9 and −19‰ (mean = −13.5‰) (12, 16). The variation within these two groups can be largely accounted for in terms of the way in which diffusion affects isotopic discrimination. Farquhar *et al.* (9) have developed a model relating carbon isotope composition to the quotient of intercellular (C<sub>i</sub>) and ambient (C<sub>a</sub>) partial pressure of CO<sub>2</sub> and C<sub>3</sub> plants.

Farquhar *et al.* (9) propose that

$$\delta^{13}\text{C} = \delta_{\text{env}} - a - (b - a) \frac{C_i}{C_a} \quad (1)$$

where δ<sub>env</sub> = isotopic composition (‰) of the CO<sub>2</sub> in the environment of a plant, a global mean of −7.8 is commonly assumed; *a* = fractionation (‰) caused by diffusion, in air *a* = −4.4; *b* = fractionation (‰) caused by carboxylation, in C<sub>3</sub> plants employing ribulose biphosphate carboxylase as the primary CO<sub>2</sub>-fixing enzyme, *b* is thought to be close to −27. Thus, substituting these values Eq. 1 becomes:

$$\delta^{13}\text{C} = -12.2 - 22.6 \frac{C_i}{C_a} \quad (2)$$

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Table I. Measured and Predicted Carbon Isotope Ratios in Host-Parasite Associations  
Means of 3 or 6 measurements are reported, together with 95% confidence limits in the case of the latter.

Species		Measured $\delta^{13}\text{C}$	Predicted $\delta^{13}\text{C}$
Host	Parasite		
<i>Sorghum bicolor</i> (C <sub>4</sub> )	<i>S. hermonthica</i> (C <sub>3</sub> ) Unemerged stem and leaves	-13.5 (-13.3 to -13.8)	
	Newly emerged leaves	-14.1	
	Mature leaves	-20.5	-31.8 (-31.6 to -32.0)
		-26.7 (-25.5 to -27.9)	
<i>Sorghum bicolor</i> (C <sub>4</sub> )	<i>S. asiatica</i> (C <sub>3</sub> ) Mature leaves	-13.7 (-13.4 to -13.9)	
		-25.6 (-25.1 to -26.0)	-32.0 (-31.4 to -32.5)
<i>Vigna unguiculata</i> (C <sub>3</sub> )	<i>S. gesnerioides</i> (C <sub>3</sub> )	-29.3	-30.0 (-29.5 to -30.7)
		-28.6	
<i>Oryza sativa</i> (C <sub>3</sub> )		-31.2	
	<i>Rhaphicarpa fistulosa</i> (C <sub>3</sub> )	-31.8	

Table II. Ambient (C<sub>a</sub>) and Intercellular (C<sub>i</sub>) Concentration of Carbon Dioxide in *S. hermonthica*, *S. asiatica*, and *V. unguiculata* Used to Calculate Predicted Carbon Isotope Ratios

Means of *n* measurements and 95% confidence limits are reported.

Species	<i>n</i>	C <sub>a</sub>	C <sub>i</sub>
		$\mu\text{l/L}$	
<i>S. hermonthica</i>	141	373 (369-377)	324 (319-330)
<i>S. asiatica</i>	35	349 (344-354)	306 (295-317)
<i>V. unguiculata</i>	25	353 (352-354)	280 (271-290)

Plant material was dried at 70°C for 48 h before being finely ground prior to carbon isotope determination.

Between 1 and 2 mg of dried material were used for carbon isotope determination (4, 14) with a Vacuum Generators Micro-mass 602C mass spectrometer. The reference gas against which samples were measured was calibrated against the National Bureau of Standards Solenhofen limestone standard. The results were recalculated relative to the PDB standard in parts per thousand (‰). Each analysis was repeated twice, and means of analyses of material from either 3 or 6 pots are reported.

**Foliar Gas Exchange Measurements.** Transient exchange rates of H<sub>2</sub>O and CO<sub>2</sub> were measured in young, fully formed leaves of *S. hermonthica*, *S. asiatica*, and *V. unguiculata* in the 7-d period prior to removal of material for carbon isotope determination. Measurements were made with a Analytical Development Company (ADC) portable infrared gas analyzer (Hoddesdon UK, model LCA2), and the partial pressure of CO<sub>2</sub> in air entering and leaving the cuvette and % RH of air in the cuvette were recorded on an ADC data logger, together with air temperature and photosynthetic photon flux density. Boundary layer resistances were calculated according to Parkinson (20), and these data were used to calculate C<sub>i</sub> according to the equations described by Von Caemmerer and Farquhar (26). For *V. unguiculata*, the leaf always filled the cuvette (2.5 × 2.5 cm), but for *S. hermonthica* and *S. asiatica* individual leaves were tagged, and the area enclosed within the cuvette was determined at the end of the experiment with a Li-Cor portable area meter (LI-3000).

For *Striga* species, measurements were made during the middle of the photoperiod, during which light (700-950  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) did not limit photosynthesis (22). For *V. unguiculata*, supplementary lighting was provided by a Thorn 400 W MBIF metal halide lamp, and measurements were made at between 1050 and

1100  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Between 25 and 141 measurements were made for each species.

## RESULTS

Unemerged *S. hermonthica* stem and leaf tissue had a  $\delta^{13}\text{C}$  value of -14.1‰ (Table I), a value comparable with that obtained for its C<sub>4</sub> host, sorghum (-13.5‰). This value for sorghum is consistent with those reported elsewhere for C<sub>4</sub> grasses (13). After emergence of *S. hermonthica*, the  $\delta^{13}\text{C}$  value decreased to -20.5‰ and in mature leaves was -26.7‰. The  $\delta^{13}\text{C}$  value for mature leaves of *S. asiatica*, -25.6‰, was comparable with that of *S. hermonthica*. The  $\delta^{13}\text{C}$  values for mature leaves of both C<sub>3</sub> *Striga* species were considerably lower than those of their C<sub>4</sub> host. In contrast, the *Vigna unguiculata*-*S. gesnerioides* and *Oryza sativa*-*Rhaphicarpa fistulosa* associations had carbon isotope ratios that differed by less than 1‰ (Table I).

Eq. 2 was used to calculate theoretical carbon isotope ratios based on foliar gas exchange measurements (Table II). Estimated  $\delta^{13}\text{C}$ -values for mature leaves of *S. hermonthica* and *S. asiatica* were -31.8 and -32.0‰, respectively: 5.1‰ more negative than the measured value for *S. hermonthica* and 6.4‰ more negative than that of *S. asiatica* (Table I). In contrast, the measured and estimated  $\delta^{13}\text{C}$ -values for *V. unguiculata* were in close agreement, being -29.3 and -30.0‰, respectively.

This discrepancy between measured and estimated  $\delta^{13}\text{C}$  for *S. hermonthica* and *S. asiatica* can be interpreted with respect to input of carbon from the C<sub>4</sub> host. Using these data (Table I), we estimate sorghum would have to provide 28 and 35% of the total carbon in mature leaves of *S. hermonthica* and *S. asiatica*, respectively.

## DISCUSSION

The change in the  $\delta^{13}\text{C}$ -value of *S. hermonthica* with development illustrates some increase in the ability of the plant to provide its own carbon. The large difference between *S. hermonthica* pre- and post-emergence cannot be explained in terms of inter-organ variation since, with the possible exception of seed, these are usually less than 2‰ (16, 18). It is possible that some of the carbon in newly emerged *S. hermonthica* leaves could have been imported from the below-ground parts of the plant. However, it is unlikely that juvenile carbon made a significant contribution to the leaves formed in mature plants, since these are initiated above ground and the ratio of above to below ground

dry matter was large (approximately 25:1).

The difference between theoretical and measured carbon isotope ratios suggests that mature *S. hermonthica* and *S. asiatica* plants are still receiving a substantial proportion of their carbon from the  $\text{C}_4$  host. Although *Striga* is capable of independent photosynthesis, high rates of night time respiration result in a negligible net carbon gain (22). While the parasite can provide enough carbon for maintenance, an additional input in the order of 28 or 35% will provide a significant source of carbon for growth.

It seems unlikely that these data could be explained in terms of an unusual isotopic discrimination by ribulose biphosphate carboxylase in *Striga* ( $b$  in Eq. 1). Although Farquhar *et al.* (9) suggest that  $b$  lies close to  $-27$ , estimates have ranged from this value to  $-39$  (1, 7, 24, 28). In order to account for the measured  $\delta^{13}\text{C}$  in terms of the gas exchange data,  $b$  would have to equal  $-21.1$  and  $-19.7$  for *S. hermonthica* and *S. asiatica*, respectively.

Our findings suggest that as *Striga* develops it exerts a reduced demand on the host for photosynthate. However, the difference between theoretical and actual carbon isotope ratios demonstrates that substantial carbon transfer is still occurring when the parasite is fully active photosynthetically. Although precise quantification is limited by the extent of the model and a knowledge of contributions from respiration, export and import, we suggest that mature *S. hermonthica* and *S. asiatica* plants obtain about a third of their carbon from their host. This contrasts with the view held from earlier studies on *Striga* (2, 3). This loss of host photosynthate, coupled with the pathogenic effect that *Striga* has on photosynthesis in sorghum (22), probably account for the massive growth reductions observed in sorghum parasitized by *Striga* (19, 21).

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