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BUNDLE SHEATH LEAKINESS IN RELATION TO DECREASED PHOTOSYNTHESIS IN MAIZE INFECTED WITH *STRIGA HERMONTHICA*

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Abstract

The significance of the leakage of CO_2 from the bundle sheath for the reduced photosynthetic rate of maize infected with the root hemiparasitic angiosperm *Striga hermonthica* was investigated in sand culture at three rates of nitrogen. The *Striga* × N interaction on the responses measured was not significant. Infected maize plants were characterized by significantly lower rates of photosynthesis, greater bundle sheath leakiness, and greater carbon isotope discrimination than the uninfected plants. However, the ratio of the intercellular to ambient concentration of CO_2 (C_i/C_a) was similar for both infected and uninfected plants. The activities of ribulose biphosphate carboxylase oxygenase (Rubisco) and phosphoenolpyruvate carboxylase (PEPCase) were similar for infected and uninfected plants. Application of nitrogen increased the rate of photosynthesis but carbon isotope discrimination and bundle sheath leakiness decreased with increased nitrogen supply. Nitrogen supply neither affected C_i/C_a nor the activities of Rubisco and PEPCase. The results suggest that the reduced growth and dry matter accumulation of *Striga*-infected maize is due to greater leakage of CO_2 from the bundle sheath cells rather than a reduction in the extractable activities of Rubisco and PEPCase.

Résumé

AFLAKPUI, G. K. S.: *Faisceau de gerbe percé par rapport à la photosynthèse diminuée en maïs infecté de* Striga hermonthica. L'importance de la fuite de CO_2 de faisceau de gerbe pour la proportion photosynthétique diminuée de maïs infecté d'angiosperme hemiparasitaire de recine *Striga hermonthica* était enquêté dans la culture de sable à trois proportions d'azote. *Striga* × interaction N sur les réactions mesurées n'était pas considérable. Les plantes de maïs infectées étaient caractérisées par des proportions de photosynthèse considérablement plus faibles, la percé de faisceau de gerbe plus élevée, et la discrimination d'isotope de carbon plus élevée que les plantes non-infectées. Cependant la proportion de la concentration intercellulaire contre la concentration ambiante de CO_2 (C_i/C_a) était semblable pour les plantes infectées et non-infectées. Les activitées de ribulose biphosphate carboxylase oxygenase (Rubisco) et phosphoenolpyruvate carboxylase (PEPCase) étaient semblables pour les plantes infectées et non-infectées. Application d'azote augmentait la proportion de photosynthèse mais la discrimination de carbon isotope et de faisceau de gerbe inétanchéité diminuiat avec l'augmentation de l'alimentation en azote. L'alimentation en azote n'affectait ni C_i/C_a ni les activités de Rubisco er PEPCase. Les résultats suggèrent que la croissance réduite et l'accumulation de matière sèche de maïs infecté de *Striga* est provoquées par la fuite considérable de CO_2 de cellules de faisceau de gerbe plutôt qu'une réduction des activités extractibles de Rubisco et PEPCase.

Introduction

When host crops are infected by the parasitic weed, *Striga*, they grow less well, yields are lower and the crops can fail completely. In previous studies, *Striga*-infected host plants showed reduced rates of photosynthesis, as measured by

the infra-red gas analyser, smaller leaf area and smaller total biomass in comparison to control plants (Press *et al.*, 1987; Press, Tuohy & Stewart, 1987; Frost *et al.*, 1997; Aflakpui, Gregory & Froud-Williams, 1998). Although the effects of *Striga hermonthica* (Del.) Benth. species on the productivity of host crops have been documented for many years, the physiological bases and mechanisms of the reduced growth and yield are not well understood. The lower productivity of infected host plants has been attributed to a transfer of carbon from host to parasite (Press *et al.* 1987), and a reduction in the rate of photosynthesis of host plant leaves (Press & Stewart, 1987; Press, Tuohy & Stewart, 1987).

In C_4 species, phosphoenolpyruvate carboxylase (PEPCase) and ribulose biphosphate carboxylase oxygenase (Rubisco) are separated into the mesophyll and bundle sheath cells, respectively. The initial CO_2 fixation by PEPCase concentrates CO_2 in the bundle sheath cells, where it is permanently fixed by Rubisco. The high CO_2/O_2 ratio in the bundle sheath cell enhances the rate of CO_2 fixation of Rubisco by suppressing its oxygenase activity and, therefore, eliminating photorespiration. An additional consequence of the CO_2 concentrating mechanism in C_4 plants is leakage of CO_2 from the bundle sheath cells to the surrounding mesophyll where the concentration of CO_2 (C_i) is lower (Peisker & Henderson, 1992).

The photosynthetic efficiency of C_4 plants is thus reduced by the fraction of CO_2 originally fixed by PEPCase in the mesophyll that subsequently leaks out of the bundle sheath cells without being refixed by Rubisco (Peisker & Henderson, 1992). Differences in bundle sheath leakiness (Φ) among C_4 plants have been hypothesized to be partly attributable to the presence or absence of suberised lamellae in the bundle sheath cells (Hattersley, 1982). In contrast, more recent evidence by Henderson, von Caemmerer & Farquhar (1992), however, indicated that species with suberised lamellae do not have lower Φ than species without suberised lamellae. Peisker & Henderson (1992) also showed that Φ also depends on the balance between the activities of PEPCase and Rubisco.

It is also well known that plants discriminate against ^{13}C during photosynthesis; the discrimination shown by C_3 plants is larger than that shown by C_4 plants (O'Leary, 1981; 1988). According to theoretical models proposed by Farquhar (1983) and Henderson, von Caemmerer & Farquhar (1992), Φ in conjunction with the ratio of intercellular to ambient CO_2 concentration (C_i/C_a), largely determine carbon isotope discrimination (Δ) in C_4 plants. Photosynthesis, growth and yield appear to be negatively correlated with Δ in C_4 grasses (Meinzer, Plaut & Saliendra, 1994; Buchmann *et al.*, 1996). Thus environmental and genetic factors that influence partitioning of carboxylase activity between PEPCase and Rubisco should affect Φ, Δ, and photosynthetic performance in C_4 plants.

The aim of the present study was to investigate the significance of CO_2 leakage from the bundle sheath for the reduced photosynthetic rate of maize infected with *S. hermonthica*. The maize was grown at three rates of N, as N has been shown to ameliorate the deleterious effect of *S. hermonthica*. Bundle sheath leakiness (Φ) was determined from the C_i/C_a ratio and carbon isotope discrimination (Δ).

Experimental

Experimental site

The experiment was conducted in a glasshouse at The University of Reading (51° 27' N lat. and 00° 56'W long.), UK between May and Sep 97. Day and night temperatures in the glasshouse were maintained at 30/20 °C. The experimental design was a completely randomized, 3 (N rates) × 2 (*Striga* infection) factorial, replicated four times.

Conditioning Striga *seed*

Seed of *S. hermonthica* collected on a maize host in 1993 at Nyankpala, Ghana was used. A 5-g portion of *Striga* seed was added to 1 kg of sand in a polythene bag and mixed thoroughly by

shaking for 10 min. An acid-washed sand (100 g) was weighed into pots (7.5 cm diameter) and 5-g of the sand: seed mixture added (about 3500 *Striga* seed per pot). Another 95-g sand was added to cover the *Striga* seed and 50 ml water added. The pots were covered with a black polyethylene sheet and placed in a seed propagator box at 30 °C for 2 weeks to condition the *Striga* seed. After 2 weeks, during which the pots were monitored daily to ensure that the sand was moist, the content of each pot was air-dried, thoroughly mixed, and used to infect the maize plants.

Growing the host crop

The content of each 7.5-cm pot (200 g sand: seed mixture) was added to 1.3 kg washed river sand in 20-cm diameter pots and covered with 500 g sand to give a total of 2 kg sand per pot. Seed of maize (*Zea mays* L. cv. Okomasa) was surface sterilized with 1 per cent sodium hypochlorite, washed with water and planted at a depth of about 2.5 cm. Two seeds were sown in each pot and, subsequently, thinned to one after expansion of the first leaf. At the same time, seeds were also planted in 2 kg sand uninfected with *Striga*. Plants were watered with 200 ml full strength, nitrogen-free Long Ashton nutrient solution three times a week; on other days the plants were watered with tap water. Nitrogen was applied at 22.2, 66.6 and 133.2 mg per pot (equivalent to 20, 60, 120 kg N ha^{-1}) as NH_4NO_3 dissolved in water at the three leaf stage (9 DAP). The rates of N were chosen for their agronomic significance.

Measurements

Photosynthesis. Photosynthesis was measured in maize at 28, 42, 56, 63 and 70 DAP. A portable infra-red gas analyser (LCA-3, Analytical Development Company, Hoddesdon, UK) was used to make instantaneous measurements on the youngest fully expanded leaves of both infected and uninfected maize plants. All measurements were made in the morning between 09.00 h and 11.30 h at ambient CO_2 concentrations (approximately 350 μmol^{-1}). The leaf cuvette had an area of 625 mm^2 (ADC PLC) with a fitted halogen lamp to give a photosynthetically active radiation (PAR) of about 750-950 mol m^{-2} s^{-1}. The concentration of CO_2 entering and leaving the cuvettee was recorded with an ADC data logger. The data were later used to compute CO_2 exchange rates on a leaf area basis, stomatal conductance and internal CO_2 concentration using the equations described by von Caemmerer & Farquhar (1981).

Leaf chlorophyll concentration. Chlorophyll was extracted from the 0.05 g of fresh fully expanded leaves used for gas exchange measurements. Leaves were ground with 10 ml of 80 per cent acetone with a mortar and pestle. The mixtures were centrifuged at 12,000 g for 5 min. The concentration of chlorophyll was determined by measuring the absorption of supernatant with a single-beam spectrophotometer (CECIL CE 1020, Cambridge, UK). Extractable chlorophyll (mg g^{-1}) was estimated using the formulae of Inskeep & Bloom (1985). The equations for quantifying Chl *a*, Chl *b* or total Chl in acetone were given as:

$$\text{Chl } a = 12.63\, A_{664.5} - 2.52\, A_{647} \quad (1)$$

$$\text{Chl } b = 20.47\, A_{647} - 4.73\, A_{664.5} \quad (2)$$

$$\text{Total Chl} = 17.95\, A_{647} + 7.90\, A_{664.5} \quad (3)$$

where A_{647} = absorbance at 647 nm (maximum for Chl *b*); $A_{664.5}$ = absorbance at 664.5 nm (maximum for Chl *a*).

Leaf sampling for assays of carboxylase and Δ

The youngest fully expanded leaf blades were excised and portions immediately frozen in liquid nitrogen. The frozen samples were stored at -80 °C for between 1 and 3 months until the carboxylase assays were carried out. The other portion of the leaf was dried at 80 °C for the determination of Δ.

Enzyme activities

Assays of Rubisco and PEPCase were based on slightly modified methods reported by Crafts-Brandner & Poneleit (1987) and Crafts-Brandner *et al.* (1984), respectively. Standard linear curves of enzyme activities carried out with enzymes

obtained commercially (Sigma) and leaf extracts, and a quench curve for ^{14}C were generated prior to determining activities of the enzymes.

PEPCase extraction and assay

Fresh-frozen leaf samples (0.05 g) were ground in 5 ml of ice-cold extraction buffer [50 m*M* Tris-HC1 (*p*H 8.0), 10 m*M* $MgC1_2$, 0.1 m*M* EDTA, 10 m*M* $NaHCO_3$, 5 m*M* isoascorbate, 5 m*M* DTT (dithiothreitol), 1% (w/v) casein, and 2% (w/v) PEG-3350] with a mortar and pestle, and clarified by centrifugation for 20 min at 20,000 g at 0-4 °C, and the supernatant used for enzyme assays.

PEPCase activities were determined by incorporating $H^{14}CO_3^-$ into acid stable (^{14}C) malate. To initiate the PEPCase reaction, 200 μl of original enzyme extract was thoroughly mixed with 800 μl of an assay medium [100 m*M* Tris-HC1 (*p*H 8.0), 5 m*M* $MgC1_2$, 0.1 m*M* EDTA, 0.2 m*M* NADH, 5 m*M* PEP, 2.5 m*M* DTT, 10 m*M* NaH^{14}CO3 (1.0 μCi/assay) and 5 units of malate dehydrogenase]. Assays were run for 1.5 min at 30 °C, terminated by adding 100 μl of 6 N acetic acid, dried at 60 °C (in a water bath placed in a fume cupboard), to remove the remaining free CO_2. Radioactivity (^{14}C) was measured by liquid scintillation counting (Wallac 1409 Counter, Milton Keynes, UK) after adding 0.5 ml water and 5 ml of scintillation fluid. Reaction mixtures without enzyme extracts were used as controls in each set of assays.

Rubisco extraction and assay

Fresh-frozen leaf samples (0.05 g) were ground in 5 ml of ice-cold extraction buffer [50 mM Tris-HC1 (*p*H 8.0, 10 m *M* M_gC1_2, 0.1 m *M* EDTA, 10 m*M* $NaHCO_3$, 5 m*M* isoascorbate and 5 m*M* DTT] with a mortar and pestle, and clarified by centrifugation for 20 min at 20,000 *g* at 0-4 °C, and the supernatant used for enzyme assays.

To initiate the Rubisco reaction, 200 μ1 of original enzyme extract was mixed with 800 μl of an assay mixture [50 m*M* Tris-HC1 (*p*H 8.0), 5 m*M* $MgC1_2$, 0.4 m*M* ribulose 1,5-bis P, 0.05 m*M* EDTA and 10 m*M* NaH$^{14}CO_3$ (1.0 μCi/assay)]. Reactions were terminated after 4 min by adding 0.1 ml 6 N acetic acid. Samples were dried directly in the vials at 60 °C, the residue taken up in 0.5 ml water and 5 ml of scintillation fluid added before counting.

Determination of Δ and Φ

The oven-dried halves of the youngest fully expanded leaves collected as described above were finely ground. Sub-samples were combusted and the relative abundance of ^{13}C and ^{12}C in the CO_2 produced was analysed by mass spectrometry using an isotope ratio mass spectrometer [Roboprep CN Biological Sample Converter (Europa Scientific, Crewe, UK)]. Stable carbon isotope composition was expressed as the $^{13}C : {}^{12}C$ ratio relative to that of the Pee Dee Belemnite standard. Isotopic discrimination (Δ) was calculated as (Rajinth *et al.*, 1995):

$$\Delta = \delta_a - \delta_p / 1 + \delta p \qquad (4)$$

where δ_p is the isotopic composition of the plant material and δ_a is the isotopic composition of the air. The $\delta\ ^{13}C$ value of the air in the glasshouse was assumed as -8.1‰ (Meinzer, Plaut & Saliendra, 1994). Bundle sheath leakiness to CO_2, (Φ), was estimated from foliar Δ values and measurements of C_i/C_a during gas exchange measurement as follows (Rajinth *et al.*, 1995):

$$\Phi = [\Delta - a + (a - b_4)C_i/C_a]/[(b_3 - s)C_i/C_a] \qquad (5)$$

where *a* is the fractionation during diffusion of CO_2 in air (4.4‰), b_4 is the combined fractionation during dissolution of CO_2 to bicarbonate and subsequent fixation by PEPcase (-5.2‰ at 30 °C), b_3 is the fractionation during carboxylation by Rubisco (29‰), *s* is the fractionation during leakage of CO_2 from the bundle sheath to the mesophyll (1.8‰) and C_i/C_a is the ratio of intercellular to ambient concentration of CO_2.

Statistical analyses

All data were subjected to analysis of variance and nitrogen effect on all measured responses partitioned into linear and quadratic components

TABLE 1
Leaf chlorophyll concentration of maize influenced by Striga hermonthica *infection and N supply*
SE is from a 2 × 3 factorial experiment with 4 replicates

	Days after planting				
	28	*42*	*56*	*63*	*70*
	Leaf chlorophyll concentration (mg g^{-1} fresh wt)				
Infected	1.21	1.22	0.76	0.67	0.65
Uninfected	1.08	1.08	0.65	0.60	0.54
SE	0.08	0.08	0.09	0.027	0.05
N rate (kg ha^{-1})					
20	0.84	0.85	0.62	0.54	0.51
60	1.16	1.15	0.71	0.67	0.58
120	1.45	1.44	0.78	0.73	0.66
SE	0.10	0.11	0.12	0.03	0.06
Contrasts					
Striga	NS	NS	NS	*	*
Nitrogen					
Linear	**	**	NS	NS	*
Quadratic	NS	NS	NS	NS	NS
Striga × N	NS	NS	NS	NS	NS

SE's are for means in each column; **,* - contrasts for means in each column differ at $P < 0.01$ and 0.05, respectively; NS - not significant

using orthogonal polynomials with SAS (GLM Procedure).

Results

Leaf chlorophyll

The concentration of chlorophyll, averaged across all N treatments, in the youngest fully expanded leaves of infected and uninfected maize was similar at the first three sampling times although there was a higher concentration of chlorophyll in infected maize (Table 1). At the last two sampling times, the concentration of chlorophyll in infected maize was greater than in uninfected maize. Nitrogen supply increased chlorophyll concentration averaged across infection levels at most sampling times (Table 1). The concentration of chlorophyll in leaves of both infected and uninfected maize decreased with time. Similarly, the effect of N on chlorophyll decreased with time. Unlike the chlorophyll concentration, the chlorophyll *a : b* ratio was affected neither by *Striga* infection nor by N supply at any sampling time (data not shown).

Photosynthesis

The rate of net photosynthesis of the youngest fully expanded leaves, averaged across all N treatments, for infected and uninfected plants is shown in Table 2. The rate of photosynthesis was lower for infected plants than for uninfected plants at all sampling times ($P < 0.001$). Stomatal conductance and the intercellular concentration

TABLE 2
The rate of photosynthesis of maize as influenced by Striga hermonthica *infection and N supply*
SE is from a 2 × 3 factorial experiment with 4 replicates

	Days after planting				
	28	*42*	*56*	*63*	*70*
	Rate of net photosynthesis (μmol $m^{-2}s^{-1}$)				
Infected	7.71	8.64	5.78	3.65	2.89
Uninfected	11.09	12.28	7.60	4.92	4.11
SE	0.27	0.35	0.25	0.22	0.19
N rate (kg ha^{-1})					
20	6.38	5.91	4.94	2.72	1.85
60	8.21	9.43	6.82	4.57	3.70
120	13.61	16.03	8.30	5.57	4.95
SE	0.38	0.49	0.35	0.31	0.27
Contrasts					
Striga	***	***	***	***	***
Nitrogen					
Linear	***	***	***	***	***
Quadratic	NS	NS	NS	NS	NS
Striga × N	NS	NS	NS	NS	NS

SE' s are for means in each column; *** - contrasts for means in each column differ at $P < 0.01$ and 0.05, respectively; NS - not significant

of CO_2 were similar for infected and uninfected maize (data not shown). Increased N supply also increased the rate of photosynthesis, averaged across infection levels at all sampling times (Table 2). At all N rates, the rate of photosynthesis decreased with time.

Carbon isotope discrimination (Δ)

Infection increased the carbon isotope discrimination (Δ) of the youngest fully expand leaves, averaged across all N treatments (Table 3). Values for Δ decreased with time in the infected maize as opposed to an almost similar Δ for uninfected maize. Since successive data points represent different sets of youngest fully expanded leaves, it shows that leaves with somewhat smaller Δ values were being produced under *Striga* infection. Carbon isotope discrimination also decreased with increasing N supply at all sampling times.

Ratio of internal to ambient CO_2 concentration (C_i/C_a)

The ratio of internal to ambient CO_2 concentration (C_i/C_a), averaged across all N treatments, was similar for both infected and uninfected maize (data no shown). Similarly, N supply did not influence C_i/C_a, averaged across infection treatments.

Bundle sheath leakiness (Φ)

Bundle sheath leakiness to CO_2, averaged across all N treatments, was greater ($P < 0.0001$) for leaves of infected maize than for uninfected

maize at all sampling times (Table 4). As with Δ, Φ, averaged across infection levels, increased with decreasing N supply (Table 4) at all sampling times.

Activities of Rubisco and PEPCase

The extractable activities of PEPCase (Table 5) and Rubisco (Table 6) in the youngest fully expanded leaves, averaged across all N treatments, were similar for infected and uninfected maize. It is noteworthy that PEPCase activities at 28 DAP were greater than at other times. Also, with the exception of 28 DAP, PEPCase activity was greater in uninfected plants. With Rubisco, the activities in uninfected plants were greater than those in infected plants at all times, except at 42 DAP. N supply did not significantly affect extractable activities of Rubisco and PEPCase, averaged across infection levels, at any sampling time (data not shown). However, there was a trend towards increased activities of both PEPCase and Rubisco as N supply increased.

Discussion

Consistent with previous observations (Cechin & Press, 1994; Smith, Keys & Evans, 1995; Frost *et al.*, 1997), *Striga* infection reduced the rates of photosynthesis in maize. The data in the study show for the first time that, this reduction in photosynthesis is associated with greater leakiness of CO_2 from the mazie bundle sheath (Φ). In addition to the reduced rate of photosynthesis and greater Φ infected maize plants also had signifi-

TABLE 3

Carbon isotope discrimination of maize leaf as influenced by Striga hermonthica *infection and N supply SE is from a 2 × 3 factorial experiment with 4 replicates*

	Days after planting				
	28	*42*	*56*	*63*	*70*
	Carbon isotope discrimination ($\frac{0}{00}$)				
Infected	5.99	5.66	5.39	5.21	5.09
Uninfected	2.41	2.58	3.01	2.57	2.48
SE	0.46	0.38	0.31	0.28	0.22
N rate (kg ha^{-1})					
20	5.82	5.80	6.01	5.76	5.66
60	4.57	4.70	4.47	4.38	4.31
120	3.71	3.36	3.34	3.27	3.22
SE	0.57	0.47	0.38	0.39	0.31
Contrasts					
Striga	***	***	***	***	***
Nitrogen					
Linear	*	**	***	**	**
Quadratic	NS	NS	NS	NS	NS
Striga × N	NS	NS	NS	NS	NS

SE's are for means in each column; ***, **,* - contrasts for means in each column differ at $P < 0.001$ and 0.05, respectively; NS - not significant

TABLE 4

Bundle sheath leakiness of maize leaf as influenced by Striga hermonthica *infection and N supply. SE is from a 2 × 3 factorial experiment with 4 replicates*

	Days after planting				
	28	*42*	*56*	*63*	*70*
	Bundle sheath leakiness				
Infected	0.43	0.42	0.40	0.39	0.39
Uninfected	0.26	0.26	0.28	0.26	0.24
SE	0.012	0.011	0.009	0.010	0.012
N rate (kg ha^{-1})					
20	0.42	0.42	0.41	0.42	0.42
60	0.36	0.37	0.36	0.35	0.35
120	0.32	0.30	0.31	0.30	0.29
SE	0.014	0.015	0.011	0.013	0.015
Contrasts					
Striga	***	***	***	***	***
Nitrogen					
Linear	***	*	**	**	**
Quadratic	NS	NS	NS	NS	NS
Striga × N	NS	NS	NS	NS	NS

SE's are for means in each column; ***, **,* - contrasts for means in each column differ at $P < 0.001$ and 0.05, respectively; NS - not significant

cantly greater Δ.

According to the model presented in Eqn (5), Δ in C_4 plants is determined largely by Φ and C_i/C_a. In this study, C_i/C_a was similar for both infected and uninfected maize suggesting that variation in Φ rather than C_i/C_a must have been responsible for the differences observed in Δ for infected and uninfected maize. The lack of any effect of *S. hermonthica* infection and N supply on C_i/C_a is similar to the results obtained by Saliendra *et al.* (1996) for sugarcane grown under varying irrigation frequencies. Similarly, Wong, Cowan & Farquhar (1985a,b,c) also showed that C_i was unaffected by nitrogen nutrition, phosphorus nutrition, photon flux density, ambient partial pressure of CO_2 during growth, short term exposures to different photon flux densities and water stress in *Zea mays, Pennisetum purpureum, Gossypium hirsutum*, and *Phaseolus vulgaris*, thereby rendering C_i/C_a almost constant for each species.

Variation in Φ can arise from changes in the permeability of the bundle sheath cells to CO_2 and from a decrease in Rubisco activity in the bundle sheath cell relative to the C_4 cycle activity in the mesophyll. Greater Φ is a strong indication of leakage of CO_2 that is fixed initially by PEPCase in the mesophyll cells that is not then fixed by Rubisco in the bundle sheath cells. This shows that C_3 activity in the bundle sheath cells of infected maize does not match the C_4 cycle activity in the mesophyll. Smith, Keys & Evans (1995) showed an increased incorporation of ^{14}C from

TABLE 5
Extractable activity of phosphoenolpyruvate carboxylase (PEPCcase) of maize as influenced by Striga hermonthica *infection*
SE is from a 2 × 3 factorial experiment with 4 replicates

	Days after planting				
	28	*42*	*56*	*63*	*70*
	Extractable activity (μmol CO_2 g^{-1} (fr. wt) min^{-1})				
Infected	5.71	2.82	3.02	3.65	2.89
Uninfected	5.68	3.01	3.78	4.02	3.11
SE	0.45	0.38	0.69	0.32	0.36
Contrasts					
Striga	NS	NS	NS	NS	NS

SE's are for means in each column; NS- contrasts for means in each column not significant

$^{14}CO_2$ in the light into glycine and serine in leaves of infected maize. They also showed distortion of cell outlines (through electron micrographs) and a tendency for cell walls to be thinner especially in the vascular bundles and bundle sheath tissue of infected plants. From these results, Smith Keys & Evans (1995) suggested the increased radioactivity in glycine as evidence of increased photorespiratory activity. The greater Φ observed for infected maize in this study is an experimental evidence that supports the hypothesis of greater leakage of CO_2 from bundle sheath

TABLE 6
Extractable activity of ribulose biphosphate carboxylase oxygenase (Rubisco) of maize as influenced by Striga hermonthica *infection*
SE is from a 2 × 3 factorial experiment with 4 replicates

	Days after planting				
	28	*42*	*56*	*63*	*70*
	Extractable activity (μmol CO_2 g^{-1} (fr. wt) min^{-1})				
Infected	1.01	1.07	1.05	1.15	1.21
Uninfected	1.04	1.04	1.29	1.23	1.32
SE	0.053	0.09	0.16	0.15	0.19
Contrasts					
Striga	NS	NS	NS	NS	NS

SE's are for means in each column; NS- contrasts for means in each column differ at not significant

cells of infected maize proposed by Smith, Keys & Evans (1995).

In contrast to these results, Watling & Press (1997) reported that growth of *Sorghum bicolor* infected with *S. hermonthica* was only slightly altered under an elevated [CO_2] atmosphere. On this basis, they argued that it seems unlikely that bundle sheath leakiness alone can explain the effect of *Striga* on host growth. Probably, changes in the physical properties of the bundle sheath may be associated with increased leakiness. However, there are no data from this study to support this. A potential mechanism by which *Striga* infection can cause changes in bundle sheath may be through the transmision of toxins to the host. Consistent with the results of Press & Cechin (1994) and Smith, Keys & Evans (1995), infected and uninfected plants had similar levels of the extractable activities of Rubisco and PEPCase. Since the infected plants had lower rates of photosynthesis than the uninfected, the similarity in the levels of carboxylation could be associated with the leakage of CO_2 from the bundle sheath.

The data from this study also showed that the rate of photosynthesis was increased with increased N supply, similar to the results obtained in other previous studies (Saliendra *et al.*, 1996; Rajinth *et al.*, 1995; Cechin & Press, 1993). However, Δ and Φ increased with decreased N supply. The values of Φ obtained for uninfected maize in this tudy are within the range of 0.2 to 0.3 as reported by Henderson, von Caemmerer & Farquhar (1992). They are, however, lower than the range of 0.3-0.4 obtained for sugarcane grown under salinity (Meinzer, Plaut & Saliendra, 1994), N stress (Rajinth *et al.*, 1995) and other C_4 monocots using Δ from leaf dry matter and the mean C_i/C_a (Farquhar, 1983). In another study, the average Φ of salt-stressed *Andropogon glomeratus* plants was approximately 0.43 and exhibited siginificant diurnal variation during measurements of gas exchange and Δ (Bowman *et al.*, 1989). Buchmann *et al.* (1996) also reported values of Φ between 0.22 and 0.75 for C_4 grass species of different biochemical subtypes under varying environmental conditions. The values of Φ for infected maize thus fall in the range reported by Bowman *et al.* (1989) and Buchmann *et al.* (1996). The estimates of Δ reported here are also within the range reported by Buchmann *et al.* (1996) for several C_4 grass species and Rajinth *et al.* (1995) for sugarcane.

Increased Δ and Φ under various types of stress have been reported. Frost *et al.* (1997) reported a small but highly significant increase in discrimination against ^{13}C (shown by lower $\delta^{13}C$ values) in sorghum cultivars infected with *S. hermonthica*, a result pointing to increased Δ in *S. hermonthica* infected sorghum. Rajinth *et al.* (1995) observed increased Δ with N stress in sugarcane and commented that greater Δ values observed in sugarcane plants grown under low N availability resulted primarily from increased Φ. Saliendra *et al.* (1996) showed that both Δ and Φ of sugarcane leaves increased with water stress whilst Meinzer, Plaut & Saliendra (1994) reported increased Δ for sugarcane grown under salinity. Bowman *et al.* (1989) showed that Φ increased with increasing water and salt stress in maize and *A. glomeratus*. Their calculation showd that, in up to 55 per cent of the CO_2 which was fixed by PEPCase and transported into photosynthetic carbon reduction tissues leaked out again. In a study of several C_4 grasses, Buchmann *et al.* (1996) reported that Δ rarely remained constant but, in general, Δ values were greater in the most stressed grasses (i.e. those grown under shade and drought). Another study by Virgona & Farquhar (1996) showed that in sunflower, Δ increased under low N because of the much lower rate of photosynthesis rather than reduced stomatal conductance.

Conclusion

The reduced rates of photosynthesis and smaller biomass production of *Striga*-infected maize are due to a greater leakage of CO_2 from the bundle sheath cells as evidenced by a greater carbon isotope discrimination, rather than due to a reduction in the levels of the extractable activities of PEPCase and Rubisco. It is not clear whether the

increased bundle sheath leakiness is an effect induced directly or indirectly by *Striga*. The results from the present study show that Δ may be used as a sensitive indicator of the underlying variation of photosynthetic biochemistry in genetic programmes to select cultivars tolerant to *Striga* infection.

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