Growth and photosynthetic responses of maize and sorghum to the parasitic weed *Striga hermonthica*

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Summary

Striga hermonthica (Del.) Benth. is an angiosperm root hemiparasite which is an important weed of principally C_4 cereals in the semi-arid tropics; maize, sorghum and millet are the most important hosts. *S. hermonthica* has its greatest impact in low-input subsistence farming systems. This thesis examines the influence of *S. hermonthica* on sorghum and maize cultivars grown under both laboratory and field conditions and the influence of nitrogen (in the form of ammonium nitrate) on the association.

S. hermonthica had a marked effect on its host. Infected plants showed lower total biomass accumulation and allocated less biomass to the shoot and grain in favour of the root, compared with uninfected plants. Altered biomass partitioning resulted in higher root:shoot ratios in infected plants compared with uninfected plants. Less internode extension was observed in infected plants resulting in changes in plant architecture.

Cereals infected with *S. hermonthica* had lower rates of photosynthesis compared with uninfected plants under both laboratory and field conditions. In the early stages of the *S. hermonthica*-cereal association lower rates of photosynthesis were mainly attributed to lower stomatal conductance although the influence of the parasite on other mechanisms, such as the activity of photosystem II, could not be discounted.

The *S. hermonthica*-sorghum association was influenced by the supply of nitrogen both before and after attachment of *S. hermonthica* in laboratory grown plants. High nitrogen supply inhibited the germination and attachment of *S. hermonthica* and the effects of the parasite on growth and photosynthesis were ameliorated compared with plants grown at low nitrogen. High concentrations of nitrogen supplied after the attachment of *S. hermonthica* lowered the biomass of the parasite and alleviated the effect of *S. hermonthica* on host photosynthesis.

In the field high applications of nitrogen fertiliser at experimental sites (150-180 kg N ha^{-1}) had little effect on the level of *S. hermonthica* infection or on the growth and photosynthesis of infected and uninfected cereals. Lower doses of fertiliser (40 kg N ha^{-1}) on a farmer's field, however, did influence the association, with infected plants having higher rates of photosynthesis, growth and grain yield compared with infected plants on plots which received no added fertiliser. Possible explanations for the lack of response to nitrogen at experimental sites are discussed.

Cereal species and cultivars differed in their sensitivity to *S. hermonthica* infection. Typically *S. hermonthica* lowered host productivity and field grown plants had grain yields 21-55% lower than uninfected plants, depending on the species/cultivar used. However, the land race sorghum cultivar Ochuti, exhibited a degree of tolerance to *S. hermonthica*. Photosynthesis and grain yield were little affected by *S. hermonthica* compared with other infected cereal varieties, suggesting that an ability to maintain high rates of photosynthesis may be an important correlate of tolerance to the parasite.

Declaration

No portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification of this or any other university or institute of learning

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Publications

Parts of this thesis have appeared in the form of the following publications:

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2. Press, M.C., Gurney, A.L., Frost, D.L. and Scholes, J.D. (1996). How does the parasitic angiosperm *Striga hermonthica* influence host growth and carbon relations? In: *Advances in Parasitic Plant Research. Sixth International Parasitic Weed Symposium*, (eds. Moreno, M., Cubero, J.I., Berner, D., Joel, D., Musselman, L.J. and Parker, C.), Cordoba, Spain, pp. 303-310.

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Contents

Figures and tables

Abbreviations

A	assimilation rate
ABA	abscisic acid
A_{sat}	light saturated assimilation rate
Chl	chlorophyll
C_i	intercellular CO_2 concentration
DAP	days after planting
E	transpiration rate
GS	glutamine synthetase
gs	stomatal conductance
Hepes	4-(2-hydroxyethyl)-1-piperazine-2-ethane sulphonic acid
LAR	leaf area ratio
LWR	leaf weight ratio
NH_4NO_3	ammonium nitrate
PFD	photon flux density
PNUE	photosynthetic nitrogen use efficiency
PS II	photosystem 2
ΦPSII	a measure of the quantum efficiency of PSII electron transport
R:S	root to shoot ratio
Rubisco	ribulose 1,5-bisphosphate carboxylase/oxygenase
RuBP	ribulose 1,5-bisphosphate
SLA	specific leaf area
WUE	water use efficiency

Chapter 1

General Introduction

1.1 Parasitic plants

1.1.1 Introduction

Parasitic symbioses may be described as associations in which one organism benefits to the detriment of other members of the association (Smith and Douglas, 1987). More than 3000 species of flowering plants (approximately 1% of angiosperms) are parasitic (Kuijt, 1969), depending partially or fully on a host plant for a supply of carbon, water and nutrients (Musselman, 1980; Stewart, Graves and Press, 1989; Parker and Riches, 1993). This parasitic mode of nutrition is widespread both taxonomically, occurring in 17 families, and geographically, with parasitic angiosperms present in most habitats ranging from polar regions to the equator (Kuijt, 1969).

Parasitic angiosperms are usually classified as holoparasites or hemiparasites, according to the absence or presence of chlorophyll. Holoparasites such as *Orobanche* are devoid of chlorophyll and are unable to assimilate carbon and inorganic nitrogen, being completely dependent on the host for a heterotrophic supply of resources. Hemiparasites, such as *Striga* and *Rhinanthus*, contain chlorophyll and are capable of some autotrophic carbon assimilation (Press, Graves and Stewart, 1988; Stewart and Press, 1990; Seel *et al.*, 1992; Seel and Press, 1994). Parasitic plants are further classified according to the degree of dependency on the host. Facultative parasites (e.g. *Bartsia* and *Rhinanthus*) have the ability to set seed and survive in the absence of a host. However, this is rare in nature and growth of facultative parasites is greatly

stimulated by attachment to a host (Press *et al.*, 1993; Seel, Parsons and Press, 1993a; Seel, Cooper and Press, 1993b). Obligate parasites (e.g. *Striga*) are completely dependent on the host for survival. A distinguishing feature of parasitic plants from other higher plants is the haustorium, an organ facilitating attachment, penetration and solute transfer from the host. Plants may further be classified as root or shoot parasites, depending whether the haustorium attaches above or below ground. There are stem parasites such as the hemiparasitic mistletoes and holoparasitic dodders, and root parasites such as the hemiparasitic witchweeds (*Striga*) and holoparasitic broomrapes (*Orobanche*).

Within the 17 families, eight have genera that are agricultural weeds: Balanophoraceae, Convolvulceae, Lauraceae, Loranthaceae, Orobanchaceae, Santalaceae, Scrophulariaceae and Viscaceae (Parker and Riches, 1993). Of these families, the Scrophulariaceae and Orobanchaceae contain the root parasites *Striga* and *Orobanche*, respectively, which are of greatest agricultural importance. This chapter will describe what is known of the *Striga*-cereal association.

1.1.2 *Striga* species in agriculture

Striga is an obligate root hemiparasite of the Scrophulariaceae family. Within this genus there are 42 currently described species (Raynal-Roques, 1991; Aweke, 1992), with four regarded as serious economic pests particularly in Africa; *S. hermonthica, S. asiatica, S. aspera* and *S. gesnerioides*. *Striga* has a narrow host range restricted almost entirely to grasses (mostly C_4) notably the cereal crops maize (*Zea mays*) and

sorghum (*Sorghum bicolor*). *S. gesnerioides* is the exception as it has a broader range of dicotolydoneous hosts, usually legumes such as cowpea (*Vigna unguiculata*) (Musselman, 1980; Lane, 1989; Parker, 1991). Ecologically, *Striga* is native to savannah grasslands where it can infest several wild grasses although usually at lower densities than in agro-ecosystems (Raynal-Roques, 1987; Parker and Riches, 1993; Kuiper, Pieterse and Verkleij, 1994; Cochrane and Press, 1997). With the domestication of host cereals this genus appears to have adapted to agricultural systems dominated by cereal hosts that possess little or no resistance (Musselman, 1987). Continuous cropping and increased cultivation because of rising population pressures is resulting in the spread of *Striga*. It is suggested that 40% of the arable lands in sub-Saharan Africa are currently infested by *Striga* (Riches and Parker, 1995). The extent of yield losses caused by *Striga* infection is difficult to estimate because: i) the area under infection is not precisely known as it is difficult to survey subsistence farmers effectively, ii) infestation is not uniform both within and between areas of land and iii) the problems of creating *Striga*-free plots in areas adjacent to infested areas make grain losses difficult to quantify.

S. hermonthica is the most noxious weed of the genus (Parker and Riches, 1993). The major hosts are C_4 cereals in the semi-arid tropics; maize, sorghum and millet are the most important hosts (in decreasing order of susceptibility to the parasite) (Parker and Riches, 1993). The parasite can also infect the C_3 cereal rice (Cechin and Press, 1994a). *S. hermonthica* has its greatest impact in low-input subsistence farming systems, affecting both host growth and yield. Estimated grain yield losses typically

average 5-15%, and can be much higher under heavy infestations, even resulting in total crop failure (Doggett, 1988; Sauerborn, 1991; Parker and Riches, 1993; Riches and Parker, 1995). In addition, it is not uncommon for farmers to abandon heavily infected agricultural lands (Riches and Parker, 1995).

1.1.3 Seed germination and attachment to the host

Striga plants have a great reproductive capacity, producing large numbers of seeds in the range of 10 000 to 100 000 per plant. The small seeds (0.3 x 0.2 mm) are easily dispersed, increasing the chances of finding a susceptible host. In conditions of low relative humidity and temperature seeds can remain dormant in the soil for many years, retaining their viability (Okonkwo, 1991). Seed longevity can cause a large reservoir of seed in the soil, most of which is found in the top 30 cm (Doggett, 1984). Bebawi *et al.* (1984) reported 10% germination of *S. asiatica* seeds that had been buried in the soil for 14 years. However, seed age, prevailing environmental conditions and burial depth will all influence dormancy and seed viability (Babiker, Hamdoun and Mansi, 1987).

The life cycle of *Striga* reflects its parasitic mode of existence, depending on the host plant for chemical stimuli which control the initial stages of development. The early stages in the lifecycle involve host recognition, germination, early attachment and penetration of the host (Musselman, 1980; Stewart and Press, 1990; Worsham and Egley, 1990). It is these stages that have been targeted for the control of *Striga*.

Before *Striga* seeds can germinate they first require a period of after-ripening (usually one to several months) between shedding of the seeds and the pre-conditioning stage. Preconditioning lasts for up to several weeks under moist warm conditions (23 to 32 °C depending on the *Striga* species) before germination can occur (Okonkwo, 1991; Boone *et al.*, 1995). Dormancy is broken by the presence of chemical signals in the exudate of roots of host (and a few non-host) species. The requirement for germination stimulants ensures that a suitable host plant is present (Parker, 1991). Identification of these germination stimulants has proved difficult because of their low concentrations and instability (for reviews see Worsham and Egley, 1990; Okonkwo, 1991). The first germination stimulant, Strigol, was found in root exudates of a non-host plant, cotton (Cook, Whichard and Wall, 1972; Brooks, Beruinakatti and Powell, 1985). A very different stimulant was identified from the roots of sorghum, a host plant for *Striga*, of which the main component has been termed sorgoleone, a highly unstable compound (Chang *et al.*, 1986). However, water soluble and more water stable compounds have been reported including a strigol analogue, sorgolactone, a germination stimulant from sorghum (Hauck, Miller and Schildknecht, 1992). Perception of the stimulant by pre-conditioned *Striga* induces germination within 24 hours of exposure. Germination of *Striga* occurs at temperatures above 22 °C with optimum temperatures of 30-33 °C (Musselman, 1980; Okonkwo, 1991).

In order to survive the germinated *Striga* seeds must make contact with the host vascular system and this is achieved via the haustorium. This unique organ penetrates host tissue and forms a physiological bridge between the host and parasite, enabling

the parasite to extract nutrients and water from the host (Visser and Dörr, 1987). The *Striga* radicle initiates and develops an haustorium in response to a second series of chemical signals (xenognosins) different from those involved in germination (Lynn, Steffens and Kamat, 1981; Maiti *et al.*, 1984; Riopel and Timko, 1995). A host-derived stimulant has been isolated and identified as 2,6-dimethoxy-*p*-benzoquinone (Chang and Lynn, 1986). Observations with *Agalinus purpurea* (a related species to *Striga*) demonstrated that once initiated, haustorial development is completed outside the host within 18-24 hours (Baird and Riopel, 1984). Similar observations have been made using *in vitro* culture of *S. asiatica* (Wolf and Timko, 1991, 1992). Haustorial hairs secrete an adhesive substance that facilitates attachment to the root of the host. This is followed by rapid elongation of cells which penetrate the host cortex, possibly with the aid of hydrolytic enzymes (Nwoke and Okonkwo, 1978; Maiti *et al.*, 1984). The portion of the haustorium penetrating the host vascular tissue is referred to as the endophyte. Once the endophyte breaches the endodermis the invading cells advance to the host xylem. Apoplastic continuity is provided mainly by a series of parenchyma cells and in many host-parasite associations there is little or no direct xylem to xylem contact between the host and parasite (Fineran, 1985; Kuo, Pate and Davidson, 1989; Visser and Dörr, 1987; Pate, True and Rasins, 1991a). A recent study by Dörr (1997), however, indicated that there may be direct xylem to xylem contact between *Striga* and its cereal host. Once the parasite has established a link with the host vascular system a heterotrophic supply of water and nutrients is available and shoot development can occur. It is uncertain as to whether the haustorium has a passive or active role in solute transfer. Ultrastructure studies have indicated parenchyma cells

with a high density of cell organelles and histochemical analyses have demonstrated high enzyme activities, all of which suggest an active metabolic role for the haustorium (Visser and Dörr, 1987; Dörr, 1997).

1.2 Carbon and nitrogen relations of the host-parasite association

1.2.1 Parasite sink strength

The extent to which a parasite can compete with host sinks for carbon is dependent on the sink strength of the parasite compared with sinks of the host. Within the host plant the source of carbon may be defined as the site of photosynthetic carbon assimilation, chloroplasts, and sinks defined as all non-photosynthetic parts of the plant (Herold, 1980). Competition between host and parasite sinks will be a factor in determining the performance of both members of the association.

The strength of a parasite sink will be partly associated with the degree of dependence on the host plant. Achlorophyllous holoparasites (e.g. *Orobanche*), lack the ability to assimilate carbon and are thus a large sink being wholly dependent on a heterotrophic supply of carbon (see Section 1.1). In contrast, hemiparasites (e.g. *Rhinanthus*) are capable of autotrophic carbon gain and may represent a lesser sink compared with the holoparasites.

1.2.2 Carbon fixation

Hemiparasitic angiosperms are capable of autotrophic carbon assimilation and the rates of fixation vary both between and within species. Unattached *Rhinanthus minor*

plants showed rates of photosynthesis of 1 μmol CO_2 g^{-1} dry weight min^{-1} whereas this

increased to 22 μmol CO_2 g^{-1} dry weight min^{-1} when attached to the legume *Trifolium*

pratense (Seel *et al.*, 1993b). A similar pattern was observed in the Mediterranean

species *Bartsia trixago* which had greatly increased rates of photosynthesis when

attached to a host compared with unattached plants (Press *et al.*, 1993). However,

carbon assimilation of parasites is often at the lower range observed for C_3 plants as

observed in *Striga hermonthica* (0.5-5.0 μmol CO_2 m^{-2} s^{-1}), and coupled with high

rates of respiration autotrophic carbon gain is too low to support the parasite (Press

and Stewart, 1987; Press, Tuohy and Stewart, 1987a, 1987b; Press *et al.*, 1990;

Tuquet, Farineau and Sallé, 1990). Compared with non-parasitic C_3 plants, the genus

Striga has poorly developed palisade and mesophyll cells with few air spaces between

the spongy mesophyll cells (Tuohy, Smith and Stewart, 1986). There are a lower

number of chloroplasts (Tuquet *et al.*, 1990) and these showed low ribulose-1,5-

bisphosphatase carboxlyase/oxygenase (Rubisco) activity (Press, Shah and Stewart,

1986). Isolated chloroplasts also demonstrated reduced photosystem II activity (Sallé

et al., 1987). Holoparasites such as *Orobanche minor* can not fix CO_2 (Barker, 1997).

1.2.3 Heterotrophy in parasites

Early studies on hemiparasites had assumed that they only relied on the host for water

and inorganic solutes due to the xylem to xylem continuity between parasite and host.

However, Raven (1983) suggested that a large transfer of carbon could occur through

the xylem stream in the form of amino acids and organic acids. To determine how

much carbon is acquired by the parasite from the host it is necessary to quantify both

autotrophic and heterotrophic carbon supply. To estimate the proportion of host derived carbon, Press et $al.$ (1987c) used the discrimination of naturally occurring stable carbon isotopes (^{12}C and ^{13}C) between $Striga$ (C_3 plants) and the sorghum (C_4) host. C_4 plants are enriched in ^{13}C and have more negative $\delta^{13}C$ values in the range -22 to -35 ‰ compared with C_3 plants which have values of -10 to -18 ‰ . Using this method Press et $al.$ (1987c) demonstrated that between 28% and 35% of carbon in $S.$ $hermonthica$ and $S.$ $asiatica$ was derived from the sorghum host. However, prior to the emergence of $Striga$, the parasite had a carbon signature close to that of the host indicating all the carbon was host derived. Similar observations have been made in other parasite-host relationships for example the $S.$ $hermonthica$-millet association (Graves et $al.$, 1990). A number of studies have examined the degree of heterotrophy of hemiparasitic mistletoes, with estimated heterotrophic carbon accumulation ranging from 5% to 62% (Marshall and Ehleringer, 1990; Pate, True and Kuo, 1991b; Schulze et $al.$, 1991). Using $\delta^{13}C$ measurements, Marshall et $al.$ (1994) demonstrated that between 5 and 21% of carbon was host derived for eight mistletoe associations and Pate et $al.$ (1991b) estimated 24% of host carbon was acquired by the mistletoe $Amyema$ $linophyllum$ infecting $Casuarina$ $obesa$. The $\delta^{13}C$ measurements of the root hemiparasitic shrub $Olax$ $phyllanthi$ on two C_4 hosts, $Portulaca$ $oleracea$ and $Amaranthus$ $caudatus$, showed 30% and 19% of carbon was acquired from the host (Tennakoon and Pate, 1996). In contrast, the holoparasites obtain essentially all their carbon from the host. Holoparasites such as $Cuscuta$ $reflexa$ infecting $Lupinus$ $albus$ obtained 99.5% of its carbon from the host phloem and the remaining 0.5% from the xylem stream (Jeschke et $al.$, 1994a; 1994b; Jeschke, Baumel and Räth, 1995).

1.2.4 Nitrogen metabolism

Much emphasis is placed on the limiting role of nitrogen availability to plant communities (e.g. Lee and Stewart, 1978; Lee, Harmer and Ignacuik, 1983). Nitrate and ammonium are the most common forms of nitrogen available to higher plants. In non-parasitic plants, nitrate absorbed by the roots may be transferred in the xylem to be stored in the vacuoles of roots, shoots and storage organs, or it may be reduced to ammonia and then incorporated into amino acids. The reduction is catalysed by the enzymes nitrate reductase and nitrite reductase. Ammonia is immediately converted to organic solutes of nitrogen via the enzymes glutamine synthetase (GS) and glutamate synthase. GS exists as two isoforms in the leaf, one isoform present in the cytoplasm (GSI) and the other isoform (GSII) present in the chloroplast (e.g. Lee and Stewart, 1978; Pate, 1983; Marschner, 1993).

Parasitic plants have little access to soil nitrogen and exploit an alternative nitrogen source by directly acquiring reduced nitrogen from the host plant. Stem parasites have no access to soil nitrogen whereas root parasites potentially can acquire nitrogen from the soil, although in the case of *Striga* the root system is assumed to be vestigial or non-functional (Press, 1989). The form of nitrogen available will reflect the nitrogen metabolism of the host plant. Some root hemiparasites, such as *Striga*, have a limited ability to assimilate inorganic nitrogen because of low nitrate reductase activity (Lee and Stewart, 1978; Stewart *et al.*, 1984; Press *et al.*, 1986). McNally *et al.* (1983) and McNally and Stewart (1987) examined the assimilation of ammonia in *Striga* and

demonstrated GSI to be the major isoform with GSII comprising only 5-20% of total activity. Studies have demonstrated that some parasites are better adapted to an organic source of nitrogen. Okonkwo (1966) demonstrated that growth of *S. hermonthica* was stimulated when an organic nitrogen source (glutamine) was added to a nutrient medium containing inorganic sources of nitrogen.

1.2.5 Carbon and nitrogen transfer

The demand of the parasite for host carbon will determine the strength of the parasite sink. Alterations in biomass accumulation of the host plant suggests that parasitic angiosperms acquire carbon. Once carbon has transferred to the parasite there is no recorded backflow to the host plant and assimilates are lost to the host. A feature preventing this backflow may the biochemical isolation of carbon. Sucrose is the major translocated form of photoassimilate in the phloem of the host and its rapid conversion to hexoses can prevent reloading back to the phloem. Conversion to starch in a sink organ can lower the solute concentration and maintain an osmotic gradient for the transfer of solutes from source to sink (Herold, 1980). In parasitic plants carbohydrates are stored as polyhydric sugar alcohols (polyols) (Lewis, 1984); for example the cyclic polyol inositol and the acyclic polyol mannitol. In *Striga* the soluble carbohydrate reserves are predominantly stored in the form of mannitol (Press *et al.*, 1986). Physiologically, polyols may have an active role in the water relations of the plants by creating a lower water potential and increasing the flow of solutes from the host across the haustorium to the parasite (Stewart *et al.*, 1984). Biochemically they

may be involved in co-enzyme regulation, as well as acting as a carbohydrate store and a source of reducing power (Press, 1995a).

The acquisition of nitrogen may also be a key factor regulating the transport of solutes from the host to the parasite. In the mistletoe-host and *Striga*-host association transport processes are thought to be facilitated by high rates of transpiration in the parasite, often an order of magnitude greater than those of the host plant (Glatzel, 1983; Press *et al.*, 1987a; 1987b; Press, Graves and Stewart, 1990). Stomatal conductance in *Striga* (and other hemiparasites) shows little response to periods of darkness or water stress and thus maintains high transpiration under a range of conditions (Press *et al.*, 1987a; Shah, Smirnoff and Stewart, 1987; Smith and Stewart, 1990). In the mistletoes high transpiration rates are required to meet their nitrogen demand (Schulze and Ehleringer, 1984; Schulze, Turner and Glatzel, 1984; Ehleringer, Cook and Tieszen, 1986). High transpiration rates in the parasite coupled with low rates of photosynthesis results in low water use efficiencies (WUE). Schulze *et al.* (1984) suggested that the mistletoes regulated their WUE in response to the supply of nitrogen found in the host xylem. The regulation of transpiration as a direct response to nitrogen has not been observed in root hemiparasites (Press *et al.*, 1993; Seel *et al.*, 1993b).

Solute flow from host to parasite is also facilitated by maintenance of an osmotic gradient across the haustorium. This can be achieved by cation transfer and accumulation in the parasite. Cation accumulation occurs in all hemiparasites with

quantitative differences between host and parasite tissues and sap. In mistletoes the concentration of some elements such as potassium, nitrogen and calcium are often higher in parasite leaves compared with those of the host (Glatzel, 1983; Lamont, 1983; Schulze and Ehleringer, 1984; Ehleringer *et al.*, 1986) and this has also been observed in other hemiparasite-host associations. Rozema *et al.* (1986) demonstrated that the hemiparasite *Odontites* infecting a salt excluding plant species had a 5 to 7 fold increase in sodium, calcium and magnesium concentrations in leaves compared with the host. Similar observations have been made in the *Striga*-cereal association resulting in higher osmotic pressures in *Striga* compared with its sorghum host when supplied with a low nitrogen nutrient solution (Gworgwor and Weber, 1991).

1.3 Host responses to *Striga* infection: Growth and photosynthesis

The response of host plants to infection can vary both within and between species, ranging from little obvious response of the host to dramatic alterations in growth and yield, sometimes resulting in host death. The response of the host plant can depend on a number of biotic and abiotic factors, including: the severity of infection, the degree of dependency of the parasite on the host, the stage of development of the host when infected and prevailing environmental conditions (e.g. Graves, 1995). In the majority of cases the effects of the parasite can not be explained solely in terms of competition for water and nutrients (see Section 1.2 and Press, 1995b) as under laboratory conditions infected hosts show little sign of water or inorganic solute stress while still exhibiting lower productivity. The parasite may disrupt both physiological and metabolic functions in the host plant affecting productivity.

1.3.1 Biomass partitioning

The effect of the agricultural parasitic weed, *Striga*, on its host plant is usually severe with alterations in host performance occurring soon after parasite attachment and prior to emergence above ground (Graves, Press and Stewart, 1989). In the *S. hermonthica*-sorghum association studies have consistently demonstrated lower biomass accumulation, less internode extension and lower grain production in *Striga*-infected plants (Doggett, 1965; Press and Stewart, 1987a; Graves *et al.*, 1989; Smaling, Stin and Sloot, 1991; Cechin, 1994b). Infection with *S. hermonthica* also resulted in an altered pattern of biomass partitioning in the host with biomass preferentially allocated to the root rather than the stem (leaves are less affected), resulting in greater root:shoot ratios compared with uninfected plants (Graves *et al.*, 1989; Cechin, 1994b). An increase in respiratory tissue compared with photosynthetic tissue will increase carbon losses through respiration. Similar observations have been made in the hemiparasitic associations of *S. hermonthica-Pennisetum typhoides* (Graves *et al.*, 1990), *S. hermonthica-Oryza sativa* (Cechin, 1994b), *S. gesnerioides-Vigna unguiculata* (Graves, Smith and Stewart, 1992; Hibberd *et al.*, 1995, 1996b), *Rhinanthus minor*-host association (Seel *et al.*, 1993b) and in the holoparasite, *Orobanche aegyptiaca*-tomato association (Barker *et al.*, 1995; Barker, Scholes and Quick, 1996; Barker, 1997). It appears that biomass partitioning to tissues involved in resource capture (carbon, water and inorganic solutes) is maintained or even stimulated in preference to stem growth and reproductive output.

1.3.2 Gas exchange of infected plants

S. hermonthica influences functions of its cereal host including respiration, water and solute uptake and photosynthesis (e.g. Graves, 1995; Press, 1995a). The effect of the parasite on these functions may contribute more to lower host productivity than does a loss of resources. The lower productivity of infected plants may be accounted for by at least two ways. First, the removal of resources from the host to the parasite sink. Second, and presumed by Graves *et al.* (1989) to be more important, infected plants have lower rates of carbon fixation. Graves *et al.* (1989) constructed a carbon balance model of the *Striga*-sorghum association and showed that 80% of the loss in host productivity could be accounted for by the lower photosynthetic capacity throughout the association. The remaining 20% loss was accounted for by direct loss to the parasite.

Laboratory measurements of light saturated rates of photosynthesis of *Striga*-infected sorghum, millet and cowpea, showed that infected plants had lower rates of carbon fixation compared with uninfected plants. (Press *et al.*, 1987a, 1987b; Tuohy, Press and Stewart, 1987; Graves *et al.*, 1990, 1992; Cechin and Press, 1993a). *Striga*-induced alterations in host photosynthesis are usually detected after the emergence of the parasite and become more marked with time. The mechanistic basis of lower rates of photosynthesis in infected plants is still poorly understood. Lower rates of photosynthesis may be attributed to many changes in both physical and biochemical features of the leaf, including increased self shading of leaves because of changes in

host architecture, alterations of photosynthetic pigments, stomatal and enzyme limitations.

In the later stages of the *Striga*-host association (weeks after parasite emergence) lower rates of photosynthesis occur concomitantly with lower rates of stomatal conductance (Press *et al.*, 1987a, 1987b; Graves *et al.*, 1992). The authors suggested that stomatal limitations did not account fully for the lower rates of photosynthesis and that biochemical limitations may be imposed on the photosynthetic capacity. Graves *et al.* (1992) examined the response of photosynthesis to changes in substomatal carbon dioxide concentrations (A/Ci curves). Comparison of A/Ci curves of cowpea plants infected with *S. gesnerioides* showed a lower initial slope and final phase in infected plants, consistent with low Rubisco activity and RuBP (ribulose bisphosphate) and/or Pi regeneration (von Caemmerer and Farquhar, 1981).

Lower rates of light saturated photosynthesis in *Striga*-infected cereals have only been recorded in laboratory grown plants. A recent study found no difference in rates of photosynthesis of sorghum plants infected with *S. hermonthica* compared with control plants when grown in the field in Mali (Clark *et al.*, 1994). The authors suggested that a lower photosynthetic capacity may be a laboratory-induced occurrence.

1.4 The control of Striga

The immediate control of *Striga* presents many problems to both researchers and farmers alike. In many infested areas there is a large *Striga* seed reserve in the soil

because of the high reproductive capacity of the parasite and its ability to remain dormant for many years (see Section 1.1.3). In addition, the parasite influences the host when it is still underground thus methods employed to control parasite emergence will not have any beneficial effect on crop yield during that season. The eradication of *S. asiatica* in the USA has been achieved by fumigation with ethylene gas, which induces *Striga* germination in the absence of a host plant (Egley, Eplee and Norris, 1990; Eplee and Langston, 1991). However, fumigation is beyond the financial means of farmers in developing countries. Control methods have concentrated on improved management systems. These involve both reducing the current seed bank with the use of germination stimulants in trap crops (plants that cause the germination of *Striga* but are not a natural host, e.g. cotton), and also by preventing seed production by employing hand weeding, selective herbicides and fertiliser application.

1.4.1 The role of nitrogen in the host-parasite association

The occurrence of *Striga* in the field is often negatively correlated with soil fertility, with severe infestations often documented on soils of low nitrogen status (e.g. Pieterse and Verkleij, 1991). Early observations of *Striga* infestation stated that *Striga* was an indicator of low soil fertility (e.g. Thomas, 1943; Andrews, 1945). However, a number of later observations disputed this fact and Basinski (1955) reported heavy infestations of *Striga* in the Sudan on fertile soils. Subsequent field studies examining the influence of nitrogen fertiliser as a control method for *Striga* have often produced contrasting results. A summary of selected publications is given in Table 1.1 (also see reviews by Pieterse and Verkleij, 1991; Pieterse, 1996). A large number of studies have indicated

that applications of nitrogen fertiliser can depress *Striga* infection and ameliorate some of the detrimental effects of the parasite on growth and grain yield (Yadaraju, Hosmani and Prabhakara, 1979; Bebawi, 1981; Farina, Thomas and Channon, 1985; Hess and Ejeta, 1987; Mumera and Below, 1993). This is a contentious issue as a few studies have observed no influence of nitrogen fertilisers on the emergence of *Striga* and some have even observed higher levels of infestation with an increase in soil fertility (Osman, Raju and Peacock, 1991). Reasons for the different results may include the source of nitrogen and its time of application (see Mumera and Below, 1993), the host plant used, prevailing environmental conditions, and importantly the prior nutrient status of the soil.

Work by Cechin and Press (1993b) clearly demonstrated the role of ammonium nitrate under laboratory conditions in inhibiting both germination and subsequent attachment of *S. hermonthica* to its sorghum host. The authors showed that 3.0 mol m^{-3} ammonium nitrate reduced either the production of stimulatory compounds in the root exudate or their specific leakage from the roots. Nitrogen application also appeared to ameliorate the impact of *Striga* on host growth and photosynthesis, and the extent to which this occurred was dependent on the concentration of ammonium nitrate supplied (Cechin and Press, 1993a). The results were obtained under controlled laboratory conditions and the influence of nitrogen on gas exchange in *Striga*-infected cereals has not been examined in field grown plants. In addition, the lower biomass of *S. hermonthica* at a high nitrogen supply may have a less severe effect on the host, and the influence of nitrogen after *S. hermonthica* attachment is less well understood.

1.5 Aims of thesis

In the literature attention was initially focused on the physiological responses of sorghum to *S. hermonthica* infection under controlled laboratory conditions, whilst field observations typically examined biomass and yield production. A better understanding of the host-parasite association is required, especially in a wider range of cereal species and cultivars and also under field conditions. To date, *Striga*-induced depression of photosynthesis in laboratory grown cereals has not been observed in field grown plants. To our knowledge there have been few, if any, studies conducted in naturally infested *Striga* areas where simultaneous measurements have been made on uninfected cereals.

The use of nitrogen fertiliser has received attention with regard to its role in *Striga* control, but few studies have examined its effect on the physiological responses of the host-parasite relationship. In addition, little information is available on the effect of nitrogen on the host-parasite association both before and after attachment of the parasite.

With this in mind, the aims of this thesis are as follows:

i) To examine how *S. hermonthica* influences its cereal host during the different stages of their association. Specifically, to examine how *S. hermonthica* affects the growth, photosynthesis and nitrogen status of selected sorghum and maize cultivars and to determine the extent to which these cultivars respond differently to infection.

ii) To determine whether *S. hermonthica* can influence the gas exchange characteristics of its cereal host under field conditions. Emphasis is placed on the influence of the parasite on gas exchange throughout both the photoperiod and also the growing season.

iii) To investigate whether ammonium nitrate can influence the *S. hermonthica*-sorghum association both before and after attachment of the parasite. Attention is given to the influence of nitrogen supply on the attachment and development of *S. hermonthica*, and the interaction between nitrogen supply and *S. hermonthica* on biomass accumulation, partitioning and photosynthesis of the sorghum host.

iv) To understand the importance of the timing and dose of nitrogen fertiliser applications in the field as a potential control for *S. hermonthica* infestation.

v) To gain an understanding of the mechanistic basis of photosynthetic impairment resulting from *S. hermonthica* infection. To examine the influence of *S. hermonthica* on the relationship between photosynthesis and stomatal conductance, and photosynthesis and intercellular CO_2 concentrations. Measurements of chlorophyll fluorescence are also reported together with carbon isotope discrimination.

The approach of this thesis has been to conduct studies in both controlled environment chambers and on field grown plants in western Kenya. Laboratory studies allow investigation of specific aspects of the *S. hermonthica*-cereal association to be

conducted under precisely controlled conditions, while field studies enable the verification (or otherwise) of laboratory findings under conditions farmers are likely to experience. It is hoped that the outcome of this approach will lead to a greater understanding of the *S. hermonthica*-cereal association than could be achieved by isolated laboratory or field studies.

Table 1.1 Summary of the influence of fertiliser application in the field on the number or biomass (denoted by subscripts a and b, respectively) of emerged *Striga* plants on cereal hosts. Where possible, results from the original publications have been expressed as percentage change in Striga infestation with fertiliser application compared with unamended plots. (* denotes raw data not given).

Author	Country of study	Parasite-host association	Fertiliser	Added Nitrogen kg N ha^{-1}	*Striga* (% change)
Last (1960)	Sudan	*S. hermonthica*-sorghum	$(NH_4)_2SO_4$	90	-60%[a]
Bebawi (1981)	Sudan	*S. hermonthica*-sorghum	Urea	215	-93%[a]
Bebawi (1987)	Niger	*S. hermonthica*-sorghum	Urea	160	-80%[b]
Bebawi and Abdelaziz (1983)	Niger	*S. hermonthica*-sorghum	Urea	172	-35%[b]
Hess and Ejeta (1987)	Niger	*S. hermonthica*-sorghum	Urea	100	-55%[a]
Smaling *et al.* (1991)	Kenya	*S. hermonthica*-maize	NH_4NO_3	50-75	no effect*
Mumera and Below(1993)	Kenya	*S. hermonthica*-maize	Urea	60	-26%[a]
(data from 1989)			NH_4NO_3	60	+3%[a]
			$(NH_4)_2SO_4$	60	-5%[a]
Mumera and Below (1993)	Kenya	*S. hermonthica*-maize	As above	60	-30%[a]
(data from 1990)					
Osman *et al.* (1991)	India	*S. asiatica*-sorghum	Urea	100	+263%[b]
Farina, *et al.* (1985)	South Africa	*S. asiatica*-maize	NH_4 and NO_3	60-180	-93%[a]
Kambe (1991)	Malawi	*S. asiatica*-maize	$(NH_4)_2SO_4$	112	-53 and -78%[a]
(data from 1973 and 1974)					

Chapter 2

The effect of *S. hermonthica* on growth and photosynthesis of selected sorghum cultivars: a laboratory study.

2.1 Introduction

There are now a number of laboratory studies reporting the ways in which *S. hermonthica* influences host growth. Infection by *S. hermonthica* typically causes less internode extension in host plants, with an associated low dry matter accumulation compared with uninfected plants (Graves *et al.*, 1990; Stewart and Press, 1990; Cechin, 1994b). A common response of cereals to infection, is increased allocation of biomass to below ground organs in preference to above ground tissues resulting in an increase in root:shoot ratios (Graves *et al.*, 1990; Cechin and Press, 1994b). Previous studies have demonstrated infected plants to invest a greater proportion of biomass to roots compared with uninfected plants and allocate less biomass to stem growth and grain yield Graves *et al.*, 1992; Cechin, 1994b).

The lower productivity of *S. hermonthica*-infected cereals results from at least two processes. First, *S. hermonthica* competes with host sinks for carbon, and second, infected cereals fix less carbon than their uninfected counterparts (Press and Stewart, 1987; Tuohy *et al.*, 1987; Press, 1995a; Smith, Keys and Evans, 1995). This occurs because of changes in host architecture, affecting light capture and the ratio of photosynthetic to respiratory tissue (Graves *et al.*, 1989), and also because infected plants have lower rates of photosynthesis. Laboratory studies of *Striga*-infected cereals have reported rates of steady state photosynthesis up to 40% lower than those

of uninfected plants (Press *et al.*, 1987a; Smith *et al.*, 1995). Graves *et al.* (1989) have suggested that lower rates of canopy photosynthesis may account for as much as 80% of the carbon not gained by infected sorghum plants compared with uninfected plants. The mechanistic basis of these effects is still uncertain, although hypotheses include the production of a toxin by *S. hermonthica* (Musselman, 1987; Parker and Riches, 1993) and changes in the balance of plant growth regulators (Drennan and El Hiweris, 1979), neither of which have been proven.

Laboratory studies have typically concentrated on the responses of cereals to *Striga* infection using a single sorghum or maize cultivar within each study. Comparisons between studies may be complicated by the host plant used, as cereal species/cultivars may respond differently to *S. hermonthica* infection. This chapter aimed to examine: i) whether *S. hermonthica* exerts similar effects on four different sorghum cultivars and ii) whether different responses of the cultivars to infection are because of different levels of *Striga* infestation or because of inherent differences between the cultivars. The cultivars were grown in a controlled environment cabinet, enabling the responses of the sorghum cultivars to infection to be examined under constant conditions in the absence of environmental stresses that can occur in the field. Root observation chambers (rhizotrons) were designed so that the exact time of *S. hermonthica* attachment on the root of the host plant was observed. Under field conditions it is difficult to determine the time of attachment. In this laboratory system the time between the first *S. hermonthica* attachment and the response of growth and photosynthesis in the host plant can be quantified. Specifically this chapter reports: i)

the influence of *S. hermonthica* on the growth and biomass allocation of sorghum cultivars, including both destructive and non-destructive measurements, ii) the influence of *S. hermonthica* on gas exchange characteristics of sorghum cultivars and iii) leaf chlorophyll concentration and leaf nitrogen status of infected and uninfected sorghum plants.

2.2 Materials and Methods

2.2.1 Design of the root observation chambers (rhizotrons)

Rhizotrons were designed to observe attachment of *S. hermonthica* on cereal roots as well as the development of *S. hermonthica* throughout the period of study. Two clear perspex sheets (2 mm thick) of 30 x 40 cm were separated by solid plastic strips providing a 1 cm gap. The rhizotrons were filled with 1.2 L of sand and connected to a drip-feed nutrient system. A felt strip was secured at the bottom of the rhizotron to retain the sand but to allow free drainage. The rhizotrons were covered in black polyethylene to exclude any light from the roots.

2.2.2 Preconditioning of *S. hermonthica* seed

S. hermonthica seeds were collected from maize hosts at Kibos, western Kenya in 1993. Preconditioning of the *S. hermonthica* seed was conducted in a controlled environment cabinet (Fisons, Fi-totron PG1700), operating with a 12 hour photoperiod and a photon flux density (PFD) of 550 μmol quanta m^{-2} s^{-1} at the top of the rhizotrons. Day/night temperatures were maintained at 30/20 °C. Thirty mg of *S. hermonthica* seed was sown in each rhizotron to give approximately 2000 viable

seeds per host plant. The seed was sprinkled on to the surface of the sand in a band 5-10 cm below the top of the rhizotron. For 10 days the rhizotrons were watered at four intervals during the photoperiod to give a total volume of 50 ml of water per day. Control rhizotrons with no added *S. hermonthica* seed were established at the same time.

2.2.3 Plant material

Four cultivars of the C_4 cereal *Sorghum bicolor* (L.) Moench were used in this study and were selected for their reported traits of susceptibility to *S. hermonthica* infection (Table 2.1).

Table 2.1 Sorghum cultivars used in the experimental study and their known characteristics.

Cultivar	Characteristics
CSH-1	a known *Striga*-susceptible cultivar from India (Yaduraju *et al.*, 1979; Press *et al.*, 1987a; Cechin, 1994b)
SRN-39	a cultivar observed to have a low production of germination stimulant (Babiker and Reda, 1991; Parker, 1991; Hess, Ejeta and Butler, 1992)
Serena	a commercially available cultivar reported to show some *Striga* resistance in Tanzania (Mbwaga and Obilana, 1993)
KAT-369	a cultivar reported to be susceptible to *Striga* (J. Ransom personal communication)

The seeds were surface sterilised for 20 minutes in a 5% sodium hypochlorite solution containing two drops of the surfactant 'Tween' and were then rinsed with distilled water. The seeds were placed on 9 cm diameter discs of filter paper in a sterile petri dish and moistened with distilled water. The petri dishes were placed in the dark in an incubator at 30 °C for 48 hours.

The germinated seeds were transferred to 10 cm^3 plastic vials containing 40% full strength Long Ashton solution (Table 2.2). Three sorghum seedlings were placed in each vial and the top plugged with glass fibre filter paper. The vials were wrapped in aluminium foil to exclude light from the roots and were placed in a controlled environment cabinet under the environmental conditions described above. After 7 days a single seedling was transferred to each rhizotron with the roots evenly spread out over the surface of the sand. The rhizotrons were drip-fed with a 40% full strength Long Ashton solution four times during each photoperiod to give a total volume of 200 ml per day. Six replicate plants of each cultivar were established in the absence or presence of *S. hermonthica* and placed in the cabinet in a fully randomised design.

2.2.4 Growth analysis

Throughout the period of study non-destructive growth measurements were made until the final harvest at 77 days after planting (DAP). At 7 intervals between 21 and 62 DAP plant height was measured from the base of the stem to the youngest visible ligule on each of the six replicate plants since this is known to be a sensitive indicator of infection (Press and Stewart, 1987). Non-destructive measurements also included

the number of fully emerged sorghum leaves, the date of initial flowering of sorghum and the number of attached *S. hermonthica* per host plant.

Final biomass was determined by separating the sorghum plants into stems, leaves, roots and flower heads (where present). Individual and total leaf area of all fully expanded leaves were measured with a leaf area meter (Delta-T Devices Ltd, Cambridge, UK). Roots were separated from the sand by careful washing over a 2 mm meshed sieve after which *S. hermonthica* plants were detached from the roots at the point of tubercle attachment. The plant material was oven dried at 70 °C for 72 h prior to weighing.

Specific leaf area (SLA), the ratio of total leaf area per plant and total leaf dry weight per plant, leaf area ratio (LAR), the ratio of the total leaf area per plant and total dry weight per plant and leaf weight ratio (LWR), the ratio between total leaf dry weight per plant and total dry weight per plant, were calculated using four of the six harvested plants and the root:shoot ratio (R:S), was also calculated using the six harvested plants by using the equations in Table 2.3.

Table 2.3 The simple ratios used for instantaneous growth analysis (that derived from data collected at one harvest time only) (Pearcy *et al.*, 1989).

Parameter	Units	Instantaneous values	
SLA	$m^2\,g^{-1}$	$\dfrac{\text{leaf area}}{\text{leaf dry weight}}$	a measure of the thickness of the leaf
LAR	$m^2\,g^{-1}$	$\dfrac{\text{leaf area}}{\text{plant dry weight}}$	reflects the size of the photosynthetic surface relative to the total plant mass
LWR	-	$\dfrac{\text{leaf dry weight}}{\text{plant dry weight}}$	an index of the leafiness of the plant on a dry weight basis
R:S	-	$\dfrac{\text{root dry weight}}{\text{shoot dry weight}}$	

2.2.5 Gas exchange measurements

Rates of photosynthesis were measured on infected and uninfected sorghum plants between 22 and 63 DAP using a portable infra-red gas analyser (LCA4, Analytical Development Company (ADC), Hoddesdon, UK) with a broad leaf chamber (ADC PLC-B, 2.5 x 2.5 cm). At each measurement period one record was made per plant with 4-6 individual plants being sampled in each treatment. Measurements of gas exchange were made halfway along the length of the youngest fully expanded leaf, and were recorded after a minimum of 15 min when steady state photosynthesis had been reached. The air entering the leaf chamber was provided from a compressed air supply at a flow rate of 300 ml min^{-1} (CO_2 concentration was approximately 360 ppm). Relative humidity of the input air was adjusted to 50% by partially drying the air using

a column filled with anhydrous calcium sulphate (drierite). Leaf temperature was recorded using a thermocouple resting on the underside of the leaf. Gas exchange measurements were recorded at the growth PFD of 550 µmol quanta m^{-2} s^{-1} and a light saturating PFD of 2300 µmol quanta m^{-2} s^{-1}, supplied by a Schott KL1500T lamp. Differences between the concentration of CO_2 and H_2O vapour between the inlet and outlet gas streams were used to calculate the rates of photosynthesis and transpiration, using the equations of von Caemmerer and Farquhar (1981).

Rates of CO_2 assimilation were also measured as a function of PFD on the youngest fully expanded leaf in infected and uninfected plants (conditions as described above) at 63 DAP. Gas exchange was measured at a range of PFDs from 0-2300 µmol quanta m^{-2} s^{-1}. Leaves were placed in the leaf chamber and initially put in darkness for 10 min to record the rate of respiration. PFD was then increased step wise and measurements recorded once steady state photosynthesis was obtained. PFD in the leaf chamber was increased by interposing neutral density filters between the light source and the leaf. The response of CO_2 assimilation (A) to increases in PFD was fitted to the non-rectangular hyperbolar model, equation 2.1 (Zipperlen and Press, 1996). The equation was derived from equation 2.2 (Thornley, 1976) by solving for A and by adding a respiration term.

$$A = R_d + \frac{\alpha I + Amax - \sqrt{[(\alpha I + Amax)^2 - 4\theta\alpha Iamax]}}{2\theta} \qquad (2.1)$$

$$0 = A^2\theta - A(\alpha I + Amax) + \alpha IA_{max} \qquad (2.2)$$

A is the net carbon assimilation rate (μmol m^{-2} s^{-1}), I the irradiance (PFD) (μmol quanta m^{-2} s^{-1}), α the initial slope of the A:I curve (apparent quantum yield) (mol carbon fixed mol photon^{-1} absorbed), Rd the dark respiration rate (μmol m^{-2} s^{-1}) and θ the convexity coefficient ($0 < \theta < 1$) (dimensionless).

2.2.6 Foliar chlorophyll concentration

At 7 intervals from 21 to 62 DAP non-destructive measurements of foliar chlorophyll concentration were made on the youngest fully expanded leaf using a chlorophyll meter (SPAD-502, Minolta Camera Co Ltd, Osaka, Japan). Measurements were made to coincide with measurements of gas exchange. Six measurements were made halfway along the leaf either side of the midrib. Readings from the meter were calibrated against the chlorophyll concentration of leaves an area basis as determined by extraction in acetone (Schaper and Chacko, 1991; Piekielek and Fox, 1992). The calibration curve was constructed by growing the four sorghum cultivars as described in Section 2.2.3 but by providing the plants with a Long Ashton solution containing nitrogen at concentrations of between 0.5 and 4 mol m^{-3} ammonium nitrate. Discs of 2 cm^2 were removed from the youngest fully emerged leaf and immediately ground using a pre chilled pestle and mortar with 1 ml 80 % (v/v) acetone in low light. The suspension was transferred to a polyethylene centrifuge tube. The pestle and mortar were rinsed twice with 1 ml of 80 % acetone and each time the wash was added to the centrifuge tube. The extracts were centrifuged for 3 min at 3000 x g (Centaur MSE, Fisons, UK) to remove particulate matter. The suspension was transferred to a test tube and made up to 5 ml with the extraction medium. One ml of the solution was

development by 77 DAP, while cultivars KAT-369 and Serena which had initiated flowering by this date had heads that were significantly smaller being 7.2% and 8.3% of the dry weight of heads in uninfected plants ($p < 0.001$). Infected plants allocated a greater proportion of the total biomass to the root and less to stems (Table 2.5). For example in the cultivar CSH-1, uninfected plants allocated 54.6% of total plant biomass to roots whereas infected plants allocated 72.1%, an increase of 17.5%. In contrast, the proportion of biomass allocated to the stem was 11.1% lower in infected plants compared with uninfected controls. The proportion of biomass allocated to the developing flowers was also lower in infected plants compared with uninfected controls but biomass allocation to the leaves was unaffected by *S. hermonthica*.

S. hermonthica had a severe effect on the morphology of infected plants which was evident at the final harvest. Sorghum growth was altered by the presence of the parasite with infected hosts being stunted, as indicated by the height to the youngest ligule. The negative effect of the parasite on stem growth and the increase in biomass allocation to the roots, resulted in greater root:shoot ratios compared with uninfected plants (Table 2.6). However, this was not significant for KAT-369 because of a much lower root biomass compared with uninfected plants. The LWR and LAR of sorghum plants were not affected by *S. hermonthica* except for KAT-369. Infection of this cultivar resulted in greater LWR and LAR reflecting a lower total plant dry weight accompanied by no significant changes in leaf weight or leaf area. SLA was not affected by *S. hermonthica* infection in any of the four cultivars.

S. hermonthica infection caused a lower canopy size in cultivar CSH-1 relative to the controls, reducing the area for carbon assimilation (Table 2.6). Total leaf area in infected plants was 55.8% of the uninfected plants. Infection of CSH-1 did not result in lower numbers of fully expanded leaves compared with the controls but lowered the area of individual leaves below those of a similar age in the control plants (Figure 2.2A). The difference in total leaf area was due to less leaf expansion as opposed to leaf initiation. The change in leaf expansion was not uniform throughout the canopy (Figure 2.2A). Leaves in position 8 to 11 did not show significantly lower leaf areas but leaves 12 to 20 were significantly smaller than those of the controls ($p < 0.01$). In contrast to CSH-1, infection of Serena resulted in no change in total leaf area (Table 2.6) but changes within the canopy were also observed. (Figure 2.2B). Where lower leaf areas were observed in infected CSH-1 plants, infection of Serena resulted in an increase of leaf area of leaves in position 14 to 16 compared with uninfected controls. The total leaf area of cultivars SRN-39 and KAT-369 showed no response to infection and less dramatic changes within the canopy structure (Figure 2.2C and D) compared with cultivars CSH-1 and Serena. Lower rates of leaf initiation were observed in cultivars SRN-39 and KAT-369. SRN-39 showed a decrease in leaf area at position 16 whereas the area of individual leaves within the canopy of KAT-369 showed no response to infection.

S. hermonthica infection also caused a change in the dry weight of individual leaves within the canopy of cultivars CSH-1 and KAT-369. Lower leaf dry weights in infected CSH-1 hosts were observed from leaf number 12 as was seen with the

changes in leaf area (Figure 2.3A). Infected KAT-369 plants showed a significant reduction in weight of leaf number 11 and 12 (Figure 2.3D). Leaf dry weight in both Serena and SRN-39 showed no significant response to *S. hermonthica* infection (Figure 2.3B and C). The different changes observed in leaf area and leaf weight, within the canopy of *S. hermonthica*-infected Serena and SRN-39, imply that although a change in SLA is not observed in the youngest leaves, differences between infected and uninfected plants arise within the canopy.

2.3.3 Gas exchange

Infection by *S. hermonthica* on the photosynthesis in all four cultivars was similar at the two measurement PFDs (growth and light saturating PFD), thus data are reported for the light saturating PFD only (2300 µmol quanta m^{-2} s^{-1}) (Table 2.7).

At 63 DAP uninfected plants of CSH-1, Serena and SRN-39 had similar rates of photosynthesis, with KAT-369 showing slightly lower rates. However, a marked effect of *S. hermonthica* was observed on the rates of gas exchange for cultivars CSH-1 and Serena. Infection of SRN-39 and KAT-369 had no significant effect on gas exchange. Rates of photosynthesis of infected CSH-1 and Serena plants were 58.7% and 76.7% of those in uninfected plants, respectively. The lower rates of photosynthesis were accompanied by significantly lower rates of transpiration and stomatal conductance. Infected CSH-1 plants had rates of transpiration and stomatal conductance 62.2% and 45.6%, respectively, of uninfected controls, while infected Serena plants were again less severely affected with rates 76.0% and 51.3% of uninfected controls, respectively.

During the measurements from 20 to 38 DAP (data not shown) for cultivars CSH-1 and Serena, rates of photosynthesis were comparable in both infected and uninfected plants. From 38 DAP, 7 days after observed *S. hermonthica* attachment, infected plants showed lower rates of photosynthesis compared with uninfected plants in these two cultivars.

S. hermonthica influenced the way in which cultivars CSH-1 and Serena responded to PFD (Figure 2.4A and B). Both the curvature of the line and the light saturated rates of photosynthesis were affected (Table 2.8). Infection resulted in saturated rates of photosynthesis 57.4% and 77.4% of those of uninfected plants of CSH-1 and Serena, respectively, with saturation occurring at a lower PFD compared with uninfected plants. The quantum yield of CO_2 fixation of infected CSH-1 plants was 66.6% of uninfected plants, but the quantum yield of infected plants of Serena was unaffected when compared with controls. Light response curves of SRN-39 and KAT-369 were unaffected by infection (Figure 2.4C and D).

2.3.4 Foliar chlorophyll and total nitrogen concentration

Foliar chlorophyll concentration of the youngest fully expanded leaf was greater in the presence of *S. hermonthica* but only significantly so for CSH-1 (Table 2.9). Between 21 and 50 DAP the chlorophyll concentration did not vary between uninfected and infected plants in all of the four cultivars (Figure 2.5). After this period there was a decline in the chlorophyll concentration of the uninfected plants of CSH-1 and KAT-

369 with the infected plants maintaining a constant level. Infected plants of SRN-39 responded differently to infection with a steady increase in chlorophyll concentration compared with controls. In contrast, chlorophyll concentration of Serena showed little response to infection. At the end of the measurement period, 62 DAP, an increase in chlorophyll concentration of 19%, 4% 15% and 20% was recorded in cultivars CSH-1, Serena, SRN-39 and KAT-369, respectively compared with uninfected plants.

Total nitrogen concentration expressed per unit leaf weight, was greatly affected by *S. hermonthica* infection (Table 2.9). At the time of harvesting, infected plants of CSH-1, Serena, SRN-39 and KAT-369 showed nitrogen concentrations 16.9%, 21.8%, 16.7% and 54%, respectively, higher than uninfected controls, although the difference was not significant in the case of SRN-39. The lower rates of photosynthesis and higher nitrogen concentrations in infected CSH-1 and Serena plants resulted in lower photosynthetic nitrogen use efficiency (PNUE) compared with uninfected plants. A slight lowering of photosynthesis in infected plants of SRN-39 (although not significant) also resulted in lower PNUE compared with controls. High nitrogen concentrations in infected KAT-369 plants together with no changes in photosynthesis resulted in slightly lower PNUE compared with controls.
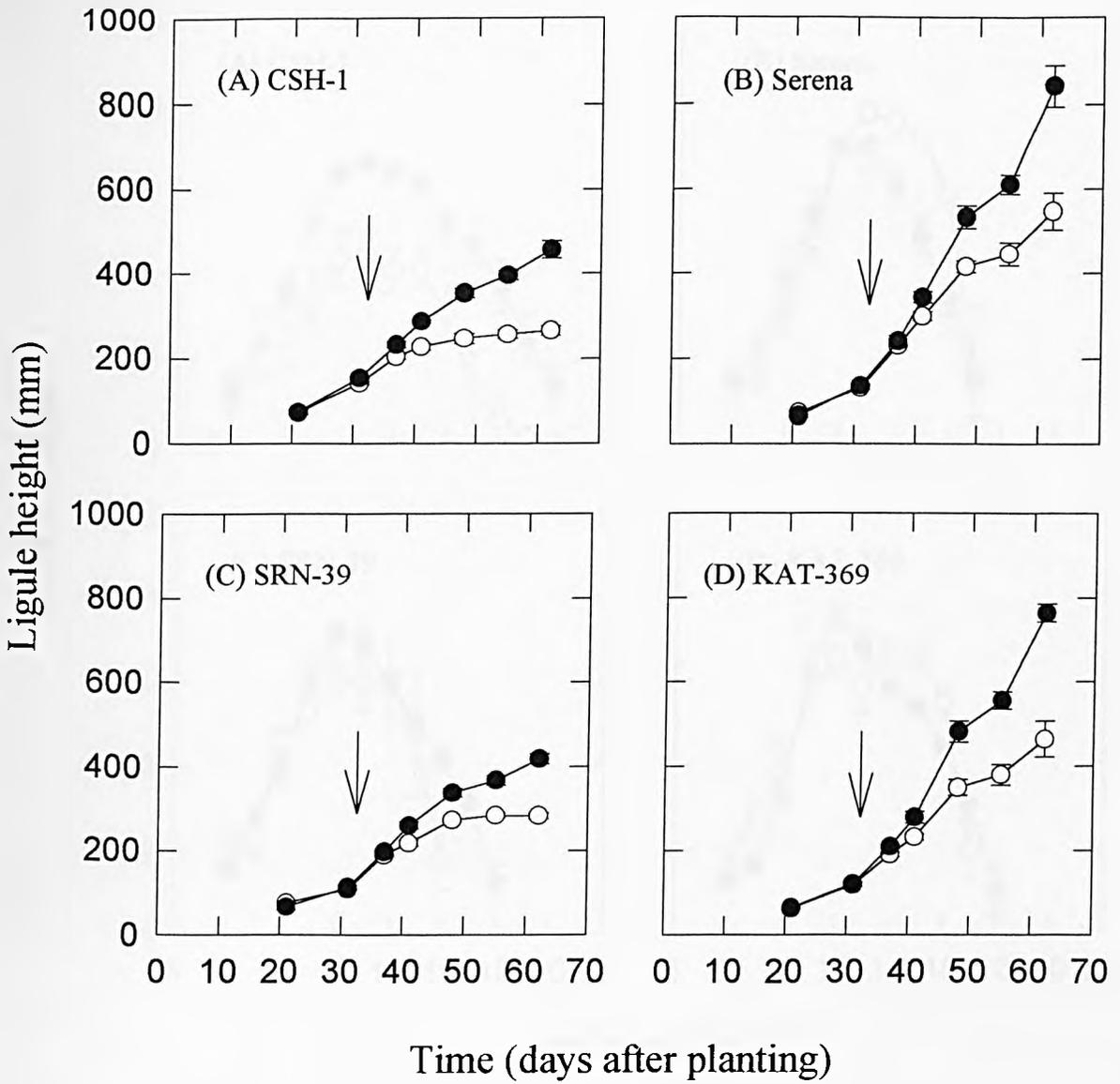
Figure 2.1 Distance from the base of the stem to the youngest ligule of sorghum cultivars CSH-1, Serena, SRN-39 and KAT-369. Plants were grown in the absence (closed symbols) or presence (open symbols) of *S. hermonthica*. Means and standard errors of six measurements are reported and analysed using analysis of variance procedures (see text). The first visible *S. hermonthica* attached to the host roots is denoted by the arrow.
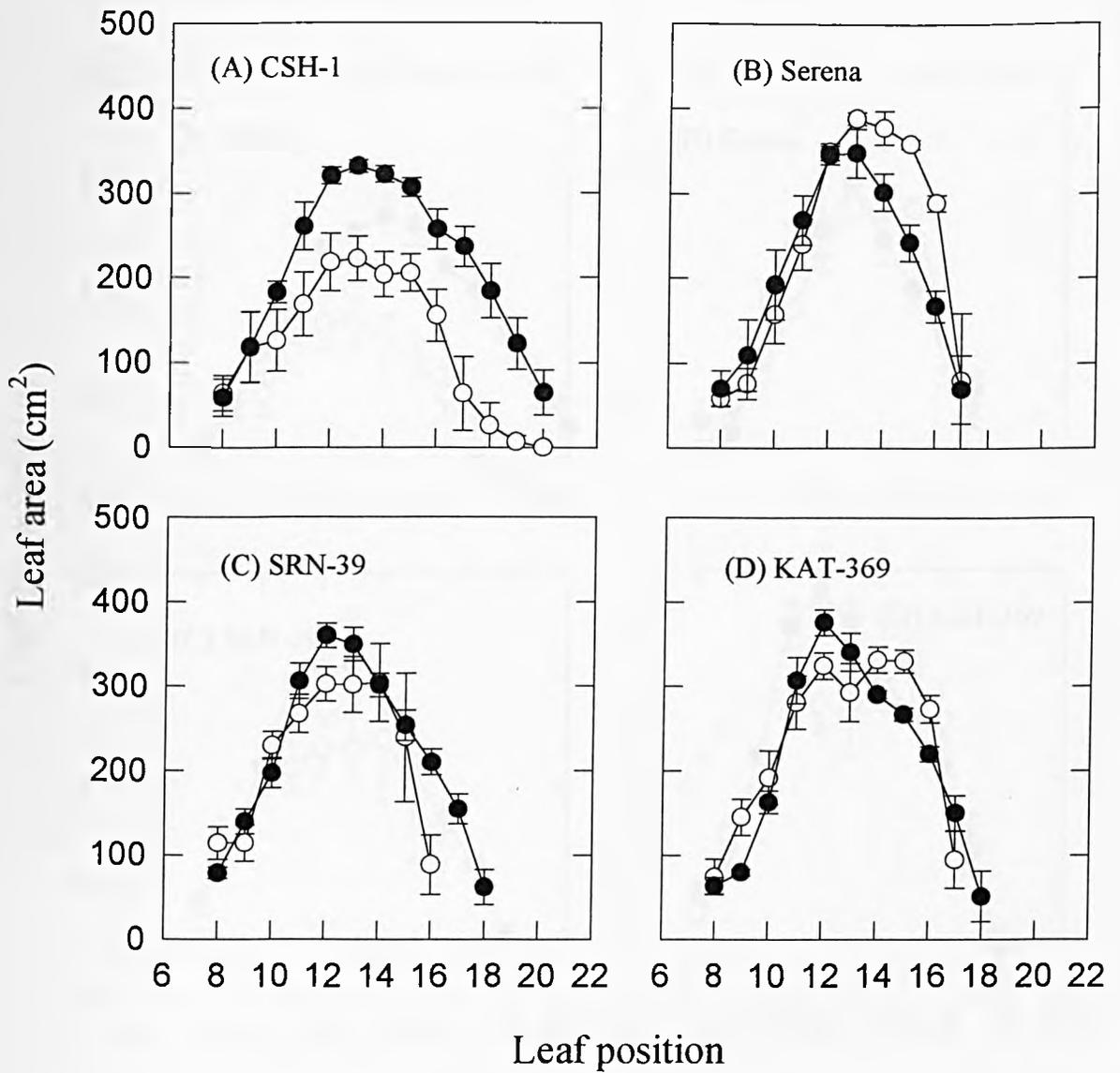
Figure 2.2 Leaf area (cm^2) in sorghum cultivars CSH-1, Serena, SRN-39 and KAT-369, measured from the sixth emerged leaf to the youngest fully expanded leaf at 77 DAP. Plants were grown in the absence (closed symbols) or presence (open symbols) of *S. hermonthica*. Means and standard errors of four measurements are reported and analysed using analysis of variance procedures for the effect of the parasite (see text).
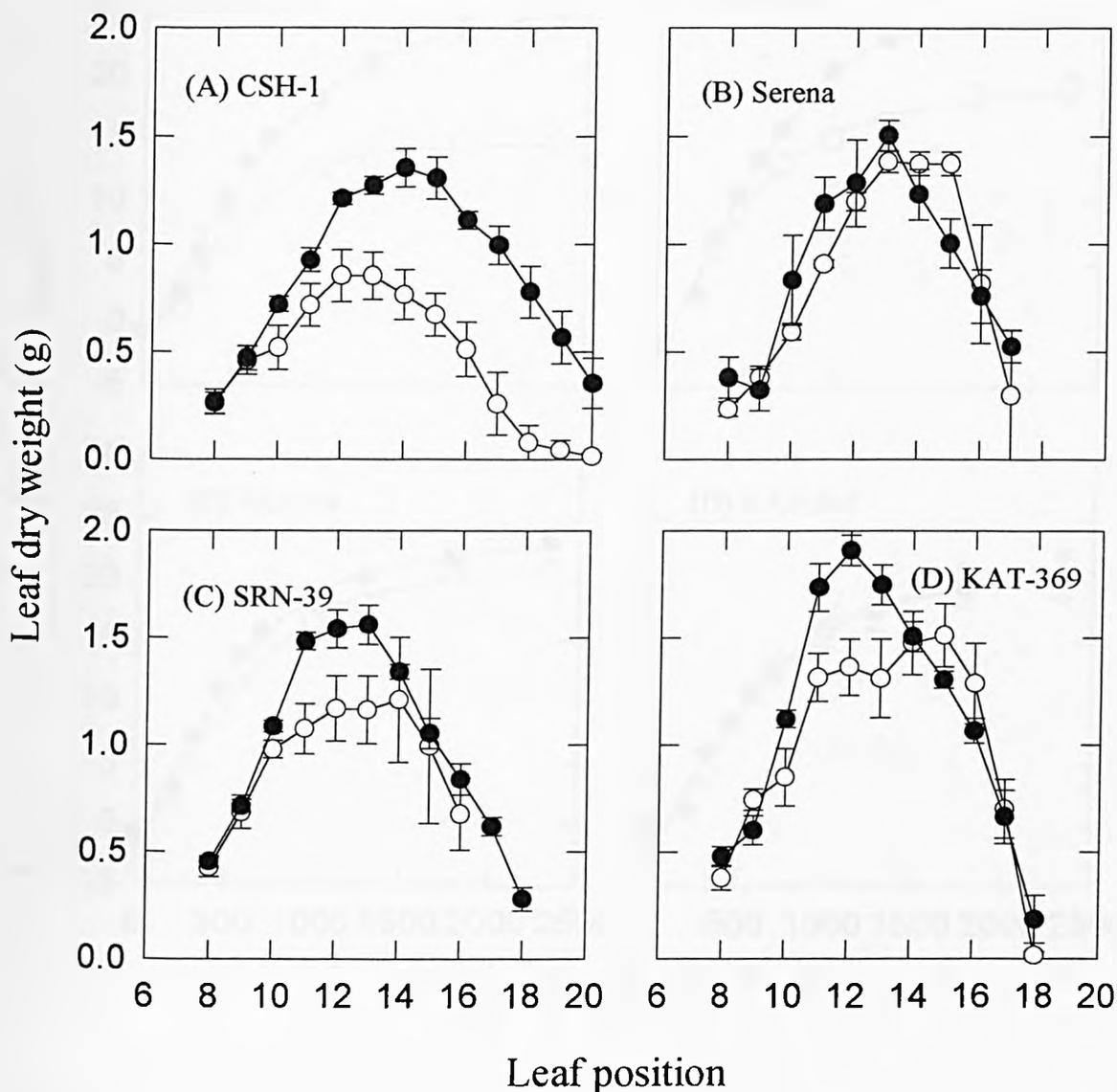
Figure 2.3 Leaf weight (g) in sorghum cultivars CSH-1, Serena, SRN-39 and KAT-369, measured from the sixth emerged leaf to the youngest fully expanded leaf at 77 DAP. Plants were grown in the absence (closed symbols) or presence (open symbols) of *S. hermonthica*. Means and standard errors of four measurements are reported and analysed using analysis of variance procedures for the effect of the parasite (see text).
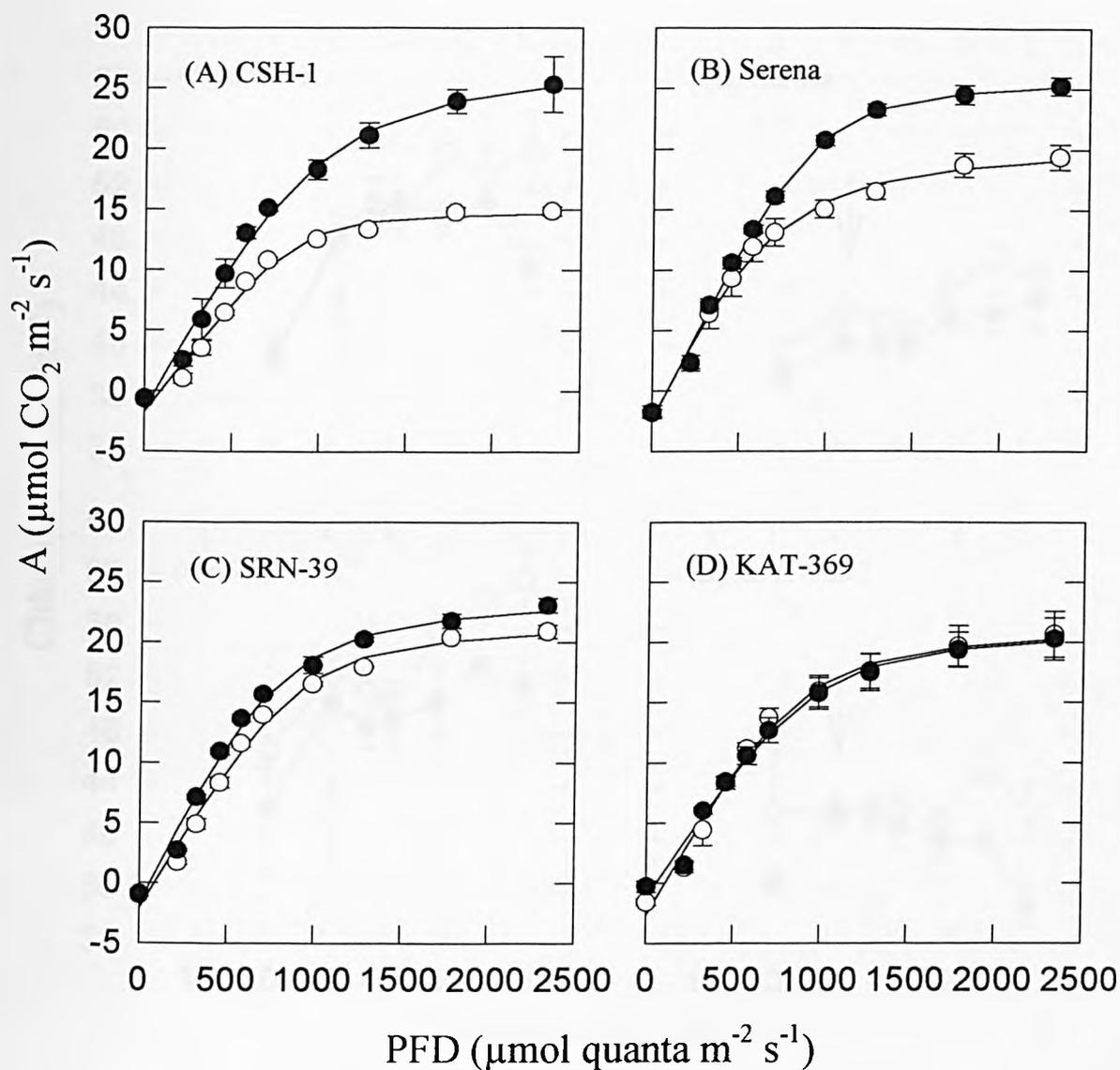
Figure 2.4 The response of photosynthesis to PFD in sorghum cultivars CSH-1, Serena, SRN-39 and KAT-369, grown in the absence (closed symbols) or presence (open symbols) of *S. hermonthica*. The points represent the mean value from four plants while the lines denote the mean of the best fitting curve using equation (2.1). The statistical analysis of the model parameters were tested using analysis of variance procedures followed by Tukey's multiple comparison tests (see text).
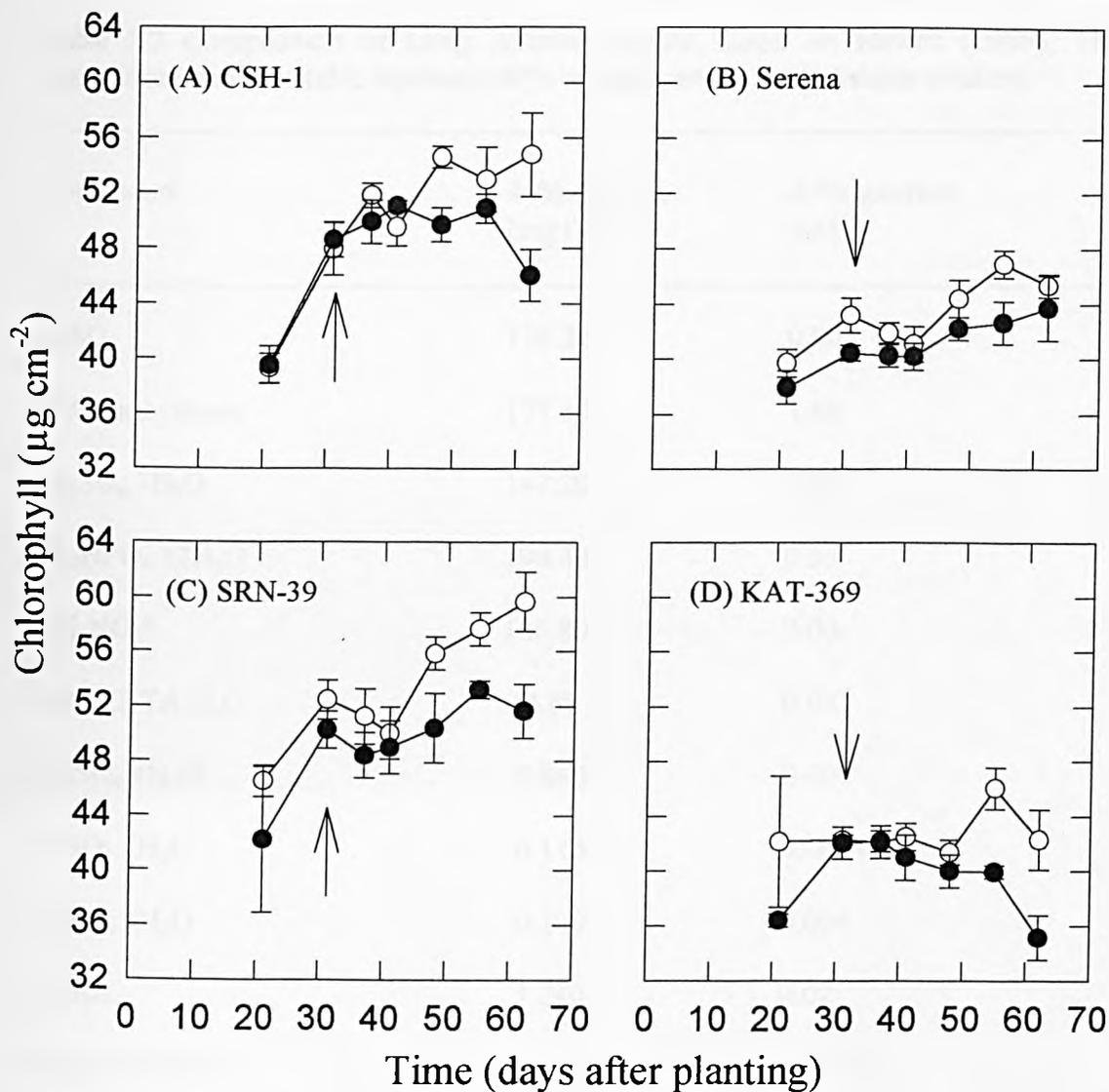
Figure 2.5 Chlorophyll concentration of the youngest fully expanded leaf of sorghum cultivars CSH-1, Serena, SRN-39 and KAT-369, grown in the absence (closed symbols) or presence (open symbols) of *S. hermonthica*. Means and standard errors of six measurements are reported and analysed using analysis of variance procedures (see text). The first visible *S. hermonthica* attached to the host roots is denoted by the arrow.

Table 2.2 Composition of Long Ashton solution based on Hewitt (1966). The concentrations in the table represent 40% of full strength Long Ashton solution.

Compound	40% solution (mg l^{-1})	40% solution mM
K_2SO_4	139.20	0.80
$CaCl_2$ anhydrous	177.60	1.60
$MgSO_4.7H_2O$	147.20	0.60
$Na_2HPO_4.12H_2O$	190.40	0.53
NH_4NO_3*	160.80	2.00
$FeNaEDTA.H_2O$	14.68	0.04
$MnSO_4.4H_2O$	0.890	0.004
$ZnSO_4.7H_2O$	0.116	0.0004
$CuSO_4.5H_2O$	0.100	0.004
H_3BO_3	1.240	0.02
$Na_2 MoO_4.2H_2O$	0.048	0.0002
NaCl	2.340	0.04

* The concentration of NH_4NO_3 was varied as required.

Table 2.4 The number and final biomass of attached *S. hermonthica* plants per sorghum host plant at 77 DAP. Means and standard errors of six measurements are reported. Kruscal-Wallis and analysis of variance procedures show the differences between cultivars to be non-significant (p > 0.05).

Cultivar	Number of *S. hermonthica* plants	Dry weight of *S. hermonthica* (g)
CSH-1	16.83 ± 3.11	0.240 ± 0.097
Serena	12.66 ± 1.82	0.113 ± 0.047
SRN-39	16.33 ± 2.33	0.181 ± 0.035
KAT-369	9.66 ± 2.45	0.138 ± 0.097

Table 2.5 Dry weight partitioning of sorghum plants in the absence (-) or presence (+) of *S. hermonthica* at 77 DAP. Means and standard errors of six plants are reported and the percentage of the total plant biomass is reported in parenthesis. The original data were analysed using two way analysis of variance followed by Tukey's multiple comparison tests. Means not sharing a common superscripted letter in the same column are significantly different (p < 0.05).

Cultivar	Striga	Total plant biomass (g)	Root biomass (g)	Stem biomass (g)	Leaf biomass (g)	Flower biomass (g)
CSH-1	-	78.44 ± 5.50^b	42.81 ± 3.06^{bc} (54.6%)	19.72 ± 1.370^{de} (25.1%)	13.02 ± 1.050^c (16.6%)	2.883 ± 0.376^c (3.7%)
	+	46.08 ± 4.10^a	33.21 ± 3.13^{abc} (72.1%)	6.453 ± 0.558^a (14.0%)	6.425 ± 0.709^a (13.9%)	0.000 ± 0.000^a (0%)
Serena	-	59.44 ± 2.99^a	27.05 ± 1.71^a (45.5%)	20.27 ± 1.390^e (34.1%)	9.975 ± 0.487^{bc} (16.8%)	2.132 ± 0.582^c (3.6%)
	+	53.82 ± 1.44^a	33.14 ± 0.76^{abc} (61.6%)	12.22 ± 1.420^b (22.7%)	8.282 ± 0.360^{ab} (15.4%)	0.178 ± 0.178^b (0.3%)
SRN-39	-	59.30 ± 3.47^a	30.74 ± 1.94^a (51.8%)	14.91 ± 1.370^{cd} (25.2%)	11.07 ± 0.579^{bc} (18.7%)	2.572 ± 0.317^c (4.3%)
	+	45.99 ± 3.16^a	29.47 ± 2.20^a (64.1%)	7.938 ± 0.724^{ab} (17.2%)	8.583 ± 0.917^{ab} (18.7%)	0.000 ± 0.000^a (0%)
KAT-369	-	85.49 ± 2.84^b	43.92 ± 2.24^c (51.4%)	24.77 ± 0.936^e (28.9%)	13.08 ± 0.471^c (15.3%)	3.707 ± 0.536^c (4.4%)
	+	56.25 ± 6.78^a	32.26 ± 4.79^{ab} (57.4%)	12.61 ± 1.770^{bc} (22.4%)	11.36 ± 0.134^{bc} (20.2%)	0.026 ± 0.026^b (.04%)

Table 2.6 LWR, LAR, SLA, root:shoot ratio and total leaf area of sorghum plants grown in the absence (-) or presence (+) of *S. hermonthica* at 77 DAP. Means and standard errors of four-six replicates are reported and analysed using analysis of variance followed by Tukey's multiple comparison tests. Means not sharing a common superscripted letter in the same column are significantly different (p < 0.05).

Cultivar	*Striga* infection	root:shoot ratio	LWR $(g\ g^{-1})$	LAR $(cm^2\ g^{-1})$	SLA $(cm^2\ g^{-1})$	Total leaf area (cm^2)
CSH-1	-	1.201 ± 0.033^{ab}	0.166 ± 0.006^{ab}	38.27 ± 2.08^{bc}	252.96 ± 8.87^{b}	2754.40 ± 133.39^{c}
	+	2.624 ± 0.213^{d}	0.140 ± 0.012^{a}	30.09 ± 3.05^{ab}	263.20 ± 20.2^{b}	1539.12 ± 307.62^{a}
Serena	-	0.848 ± 0.067^{a}	0.168 ± 0.006^{ab}	36.88 ± 2.31^{abc}	228.50 ± 5.55^{ab}	2118.00 ± 62.89^{ab}
	+	1.630 ± 0.112^{bc}	0.151 ± 0.009^{a}	42.34 ± 3.29^{c}	267.89 ± 9.13^{b}	2338.03 ± 114.83^{bc}
SRN-39	-	1.091 ± 0.066^{ab}	0.180 ± 0.005^{ab}	44.78 ± 3.98^{c}	250.60 ± 25.4^{b}	2412.39 ± 127.18^{bc}
	+	1.863 ± 0.197^{c}	0.184 ± 0.015^{ab}	42.05 ± 3.37^{c}	256.20 ± 20.1^{b}	1977.45 ± 225.20^{ab}
KAT-369	-	1.065 ± 0.068^{a}	0.152 ± 0.003^{a}	26.88 ± 0.42^{a}	188.54 ± 3.76^{a}	2310.39 ± 105.54^{bc}
	+	1.379 ± 0.202^{abc}	0.207 ± 0.019^{b}	39.76 ± 4.13^{bc}	220.80 ± 17.8^{ab}	2375.25 ± 109.61^{bc}

Table 2.7 Photosynthesis (A), transpiration (E) and stomatal conductance (gs) in sorghum plants measured at 63 DAP at light saturation, grown in the absence or presence of *S. hermonthica*. Means and standard errors of four measurements are reported and the data analysed using a two way analysis of variance followed by Tukey's multiple comparison tests. Means not sharing a common superscripted letter in the same column are significantly different ($p < 0.05$).

Cultivar	*Striga*	A (μmol CO_2 m^{-2} s^{-1})	E (mmol H_2O m^{-2} s^{-1})	gs (mol H_2O m^{-2} s^{-1})
CSH-1	-	25.33 ± 2.29^c	1.56 ± 0.312^{bc}	0.313 ± 0.037^d
	+	14.88 ± 0.36^a	0.97 ± 0.064^a	0.143 ± 0.027^{ab}
Serena	-	25.26 ± 0.73^c	1.75 ± 0.131^c	0.273 ± 0.026^{cd}
	+	19.38 ± 1.04^b	1.33 ± 0.152^b	0.140 ± 0.035^{ab}
SRN-39	-	23.06 ± 0.59^{bc}	1.32 ± 0.048^b	0.193 ± 0.026^{bc}
	+	20.91 ± 0.56^b	1.32 ± 0.003^b	0.130 ± 0.036^b
KAT-369	-	20.29 ± 1.76^b	1.26 ± 0.165^{ab}	0.083 ± 0.026^a
	+	20.68 ± 1.89^b	1.29 ± 0.101^{ab}	0.076 ± 0.016^a

Table 2.8 Analysis of light response curves (A_{sat}, light saturated photosynthesis, α, apparant quantum yield) for sorghum cultivars grown in the absence or presence of *S. hermonthica*. The parameter values given are the best fit to the non-linear model (equation 2.1). Means and standard errors of four curves from each treatment are given and the data analysed using two way analysis of variance followed by Tukey's multiple comparison tests. Means not sharing a common superscripted letter in the same column are significantly different ($p < 0.05$).

Cultivar	*Striga*	A_{sat} (μmol CO_2 m^{-2} s^{-1})	α (dimensionless)
CSH-1	-	29.43 ± 2.78^{c}	0.027 ± 0.002^{b}
	+	16.90 ± 0.61^{a}	0.018 ± 0.001^{a}
Serena	-	28.94 ± 1.90^{c}	0.029 ± 0.002^{b}
	+	22.41 ± 1.39^{b}	0.029 ± 0.005^{b}
SRN-39	-	25.80 ± 0.92^{bc}	0.029 ± 0.001^{b}
	+	23.82 ± 0.74^{b}	0.024 ± 0.001^{ab}
KAT-369	-	25.15 ± 1.31^{bc}	0.027 ± 0.001^{b}
	+	24.30 ± 2.62^{bc}	0.024 ± 0.001^{ab}

Table 2.9 Chlorophyll concentration (measured 62 DAP), nitrogen concentration and photosynthetic nitrogen use efficiencies (PNUE) (measured 63 DAP) of sorghum plants grown in the absence or presence of *S. hermonthica*. Means and standard errors of four-six replicates are reported and analysed using two way analysis of variance procedures followed by Tukey's multiple comparison tests. Means not sharing a common superscripted letter in the same column are significantly different ($p < 0.05$). PNUE is calculated from the mean photosynthesis and mean nitrogen data.

Cultivar	*Striga*	Chlorophyll ($\mu g \ cm^{-2}$)	Nitrogen ($mg \ g^{-1}$)	PNUE ($\mu mol \ CO_2 \ mol \ N \ s^{-1}$)
CSH-1	-	46.05 ± 1.87^{bc}	32.03 ± 1.42^{bc}	319.86
	+	54.79 ± 3.04^{d}	37.47 ± 1.09^{d}	228.58
Serena	-	43.75 ± 2.38^{bc}	28.03 ± 0.13^{b}	314.35
	+	45.38 ± 0.82^{bc}	34.16 ± 1.90^{cd}	278.23
SRN-39	-	51.65 ± 1.95^{cd}	29.33 ± 2.21^{bc}	332.35
	+	59.53 ± 2.20^{d}	34.24 ± 1.69^{cd}	256.77
KAT-369	-	35.14 ± 1.62^{a}	21.11 ± 0.18^{a}	308.36
	+	42.29 ± 2.18^{ab}	32.52 ± 1.66^{bcd}	277.90

2.4 Discussion

S. hermonthica had a marked effect on the performance of infected sorghum plants, altering growth, biomass partitioning and gas exchange characteristics compared with uninfected plants. However, the extent to which the parasite influenced host performance was cultivar dependent. There are few, if any, cultivars of sorghum that demonstrate true resistance to *S. hermonthica*, although some cultivars do show a degree of tolerance (Parker and Riches, 1993). This may be defined as an ability to perform relatively well in the presence of the parasite. This study examined four cultivars in a closely controlled environment and it is apparent that the performance of sorghum can be impaired shortly after *S. hermonthica* attachment. It is interesting to note that attachment of the parasite occurred at the same time in all four cultivars and there were no significant differences in the numbers or biomass of attached *S. hermonthica* by 77 DAP. The different responses of the four cultivars to infection must therefore be due to inherent traits and not a consequence of differential levels of *S. hermonthica* infection or times of attachment.

The effect of *S. hermonthica* on the growth of sorghum.

Infection by *S. hermonthica* altered the growth and allocation of biomass in a similar manner in all four cultivars although CSH-1 was the most severely affected. Within 10 days of observed *S. hermonthica* attachment to the roots of the host, significantly lower ligule heights were observed and the difference between infected and uninfected plants increased over time. This is consistent with earlier studies of the *S. hermonthica*-cereal association (Cechin and Press, 1993a, 1994b; Taylor, Martin and

Seel, 1996). Uninfected plants of CSH-1 and SRN-39 were smaller in height than either KAT-369 or Serena, but the difference in internode extension between infected and uninfected plants was similar in all the cultivars. Thus the effect of *S. hermonthica* on ligule height was not affected by inherent height differences between cultivars. At the final harvest *S. hermonthica* had lowered total biomass accumulation of infected sorghum plants. These data were not significant for SRN-39 and Serena which may reflect large variation within treatments and a small sample size used. Low biomass accumulation has been observed in other host-parasite relationships including *O. aegyptiaca*-tobacco and *S. gesnerioides*-cowpea associations where infected plants accumulated 30% and 25% less biomass compared with uninfected plants (Graves *et al.*, 1992: Hibberd *et al.*, 1996a, 1996b).

Parasite induced changes in the biomass partitioning within the host.

The allocation of biomass to various plant tissues and organs can be an important determinant of the regulation of growth. Infection by *S. hermonthica* altered the pattern of assimilate allocation resulting in an alteration of dry weight partitioning between above and below ground tissues. The increased allocation of dry matter to the roots of infected plants in preference to the shoot caused an increase in the root:shoot ratio. This indicates an increase in the proportion of respiratory to photosynthetic tissue thus increasing the sink:source ratio above that of uninfected plants. Similar alterations of biomass allocation have been observed in previous studies of the *Striga*-sorghum and *Orobanche*-tomato relationship (Graves *et al.*, 1990; Cechin and Press, 1993a; Barker *et al.*, 1996).

Lower carbon partitioning to the above ground fraction of infected plants was disproportional between the tissues and also between the cultivars. Stems and flowering heads were the most severely affected whilst allocation of biomass to leaves was generally unaffected with the exception of CSH-1, where infected plants had 49% of the leaf biomass of uninfected plants. Reproductive output was dramatically impaired by infection with lower head dry weights compared with uninfected plants. Cultivar differences again arise as there is a cessation, or at least a delay, in the onset of flowering in CSH-1 and SRN-39. Even though leaf biomass was affected in CSH-1 with infection, it is interesting to note that the effect of *S. hermonthica* on leaf area and biomass was not uniform throughout the canopy. *S. hermonthica* caused less leaf expansion and biomass accumulation from leaf 10 onwards while leaves 6 through to 9 were unaffected even though attachment was observed at this time. Lower leaf biomass accumulation and leaf expansion in infected plants became more marked as each subsequent leaf developed. In the cultivars where total leaf biomass was unaffected by infection, some leaves did show significantly lower rates of expansion but this effect was limited to a few of the older leaves with the upper canopy unaffected.

Source-sink relations in the host parasite association

In cowpea infected with *S. gesnerioides* (Hibberd *et al.*, 1996b) and in tomato infected with *O. aegyptiaca* (Barker *et al.*, 1996), the lower biomass of the host compared with uninfected plants can be largely accounted for by the source-sink relations within the

host-parasite system. The large biomass of the parasite suggests that it acts primarily as a carbon and nitrogen sink, decreasing the source-sink ratio of infected plants. This decrease in the ratio of photosynthetic to respiratory tissue will further reduce the availability of carbon to the host plant. In the *Striga*-sorghum association the relationship is not so simple. As with the *Orobanche*-tomato relationship, changes are observed in the root:shoot ratio of infected sorghum, resulting in a higher proportion of respiratory to photosynthetic tissue (see above). The larger proportion of tissue allocated to the root is a primary sink for carbon accumulation and the high transpiration rates of the attached parasites will increase the flux of carbon (and nutrients) through the haustoria (Graves *et al.*, 1989; Press, Smith and Stewart, 1991). The difference in biomass between infected and uninfected plants cannot be accounted for by the biomass of the attached parasite. However this approach is rather simplistic since it takes no account of the rates of carbon gain and loss from components of the association. Rates of respiration of *S. hermonthica* could be one cause of carbon loss although in this study it is unlikely that the very low *Striga* biomass could cause such a carbon loss through respiration. Graves *et al.* (1989) constructed a carbon balance model for the *S. hermonthica*-sorghum association using the cultivar CSH-1, where only 20% of the predicted loss in host production could be accounted for by a direct loss of carbon to the parasite.

S. hermonthica-induced changes in carbon assimilation of sorghum

The loss of productivity in infected sorghum plants may be partially accounted for by a parasite induced lowering of photosynthesis. By 42 DAP, when a stunting of the main

stem had occurred, lower rates of photosynthesis were observed in infected plants of CSH-1 and Serena compared with uninfected controls. A parasite induced lowering of host photosynthesis has been observed in the laboratory with a number of *Striga*-cereal associations (Press *et al.*, 1987a; Graves *et al.*, 1990; Cechin and Press, 1993a; Smith *et al.*, 1995). However, infection in cultivars SRN-39 and KAT-369 had no deleterious effect on the rates of photosynthesis throughout the entire experimental period, although the parasite lowered growth in these cultivars. In this study only the youngest fully expanded leaves were measured and it is possible that infection resulted in changes of photosynthesis lower in the canopy. In addition to the changes in growth and biomass allocation, *S. hermonthica* altered the architecture of host plants. A stunting of internode elongation was observed, resulting in a close packing of leaves which is likely to have increased self shading within the canopy. Light saturated rates of photosynthesis were different between infected and uninfected sorghum plants but maximum rates occurred at a PFD of over 2000 μmol quanta m^{-2} s^{-1} regardless of infection. Shading within the infected plant canopy will have an effect on the flux of photosynthetic radiation received by the older leaves and could further lower canopy photosynthesis. The *Orobanche aegyptiaca*-tomato association (Barker *et al.*, 1996) showed infected plants invested a higher percentage of their biomass into leaves in an attempt to compensate for reduced net photosynthesis and so increased LAR and LWR. Higher SLA in these infected plants indicates changes in leaf morphology or chemical composition (Lambers and Poorter, 1992). Infected plants appear to form thinner leaves to increase leaf area available for light interception. In contrast to this

association, no increased investment into leaf production was observed in the *S. hermonthica*-sorghum relationship.

The mechanisms causing lower rates of photosynthesis are poorly understood. In this study, lower rates of photosynthesis were accompanied by lower rates of stomatal conductance and transpiration, as has been observed in previous laboratory studies (Press *et al.*, 1987a; Press and Stewart, 1987). From this study it is not possible to determine whether lower stomatal conductance was a primary cause of lower rates of photosynthesis or if it occurred as a consequence of changes in photosynthesis (Wong, Cowan and Farquhar, 1979). The response of photosynthesis to changes in PFD provides information on the efficiency of light utilisation. In this study lower rates of light saturated photosynthesis occurred in infected plants of CSH-1 and Serena. This was accompanied with a decrease in the convexity of the light response curve and in the case of CSH-1, a significantly lower apparent quantum yield of carbon fixation. These observations for infected plants are often the consequence of photoinhibition (Leverenz *et al.*, 1990; Ramlan and Graves, 1996), caused by leaves being exposed to high PFD in excess of that which can be used for photosynthesis (see e.g. Bolar-Nordenkampf and Öquist, 1993; Öquist, Chow and Anderson, 1992). The decrease in efficiency of photosynthesis in saturating light may result as a consequence of photoinhibition or more specifically, damage to photosystem II (PSII) and/or the dissipation of energy through photoprotective mechanisms (Demmig and Bjorkman, 1987; Baker and Horten, 1988; Öquist, 1992). Infection by *S. hermonthica* lowers the rate of carbon assimilation and may increase the sensitivity of host plants to

photoinhibition providing an additional loss of photosynthetic capacity. The effect of *S. hermonthica* on light saturated photosynthesis and chlorophyll fluorescence and will be addressed in a later chapter.

The effects of *S. hermonthica* on foliar chlorophyll and nitrogen concentrations.
The chlorophyll and nitrogen concentrations of uninfected sorghum plants were similar between all four cultivars. Infection by *S. hermonthica* caused an increase in chlorophyll concentration compared with uninfected plants in the upper canopy leaves, although this was only significant in the cultivar CSH-1. The trend towards an increase in foliar chlorophyll concentration would indicate greater nitrogen being partitioned into the chlorophyll of infected plants. At the low levels of *S. hermonthica* infection observed in this study the parasite may cause a sink dependent stimulation of nitrogen uptake and assimilation. An increase in nitrogen in leaves of infected plants has also been demonstrated in the *Cuscuta reflexa*-castor bean association (Jeschke and Hilpert, 1997; Jeschke, Baig and Hilpert, 1997) and in the *S. gesnerioides*-cowpea association (Hibberd *et al.*, 1996b). A strong relationship has been observed in C₃ plants between foliar nitrogen concentration and light saturated rates of photosynthesis (Evans, 1989) because of the large proportion of nitrogen in leaves which is invested in photosynthetic apparatus. However, the youngest fully expanded leaves in *S. hermonthica* infected CSH-1 and Serena plants had lower rates of photosynthesis despite increased nitrogen concentrations in the leaves compared with uninfected plants. Although the nitrogen concentrations were higher in these plants, bulk nutrient analysis of leaf tissue does not determine any differences in compartmentation of

nitrogen within the leaf. This raises the possibility that *S. hermonthica* may influence the partitioning of nitrogen within the photosynthetic components and the nitrogen may be rendered unavailable for photosynthesis.

Conclusions

This study has demonstrated that *S. hermonthica* can exert severe effects on its sorghum host, resulting in less biomass accumulation and lower rates of photosynthesis compared with uninfected plants in some cultivars. The level of *S. hermonthica* infection was similar in all cultivars thus the extent to which these processes are affected is dependent on the sorghum cultivar. Reductions in growth cannot simply be attributed to a change in the source-sink relationship of the host-parasite association or a reduction in carbon fixation, but may involve changes in plant growth regulators or other aspects of metabolism.

The results obtained in this study have been observed under controlled laboratory conditions. The aim of the next chapter is to determine whether the effects of *S. hermonthica* on photosynthesis and biomass accumulation observed in the laboratory also occur under field conditions. It is of particular interest to observe whether cultivar differences arise in both sorghum and maize cereals and whether this is due to differential levels of infection or whether any cultivars show inherent tolerance to *S. hermonthica*. To date, measurements of diurnal and canopy photosynthesis are limited under laboratory conditions, thus measurements in the field will increase our knowledge of the effect of the parasite on host photosynthesis. Field studies allow

measurements of grain production and the primary aim will be to identify cultivars that

can maintain grain yields in the presence of *S. hermonthica*.

Chapter 3

The effect of *S. hermonthica* on growth and photosynthesis of selected maize and sorghum hosts: a field study

3.1 Introduction

Chapter 2 clearly demonstrated that *S. hermonthica* had a marked influence on the growth and biomass accumulation of its sorghum hosts and could lower the rates of gas exchange compared with uninfected controls. The extent to which *S. hermonthica* influenced its host was dependent on the sorghum cultivar with a range of responses being observed.

There are a number of studies reporting the ways in which *Striga* influences host growth, although those which have incorporated uninfected control plants have been largely laboratory- rather than field-based (Musselman, 1987; Parker and Riches, 1993; Cechin, 1994b). To our knowledge there are no published studies in Africa where *Striga*-free plots have been established in *Striga*-contaminated areas. It is well known that under laboratory conditions infection of maize and sorghum cultivars by *S. hermonthica* results in lower rates of photosynthesis although results shown in Chapter 2 demonstrate that this may not occur in all cereals. However there are few (if any) rigorous studies of photosynthesis in the field of *Striga*-infected cereals. One field study in Mali failed to demonstrate any deleterious influence of *S. hermonthica* on photosynthesis (Clark *et al.*, 1994), which is perhaps surprising in the light of data

collected from laboratory studies, and raises the possibility that changes in photosynthesis in the presence of *S. hermonthica* may not occur in the field.

In an attempt to resolve this enigma, a field study was conducted in Kenya. Eight hosts were examined, three maize cultivars and five sorghum cultivars (see Section 3.2.1). In order to examine cultivar responses to infestation it was important to compare infected plants with uninfected controls, and so an essential component of the study was to establish *Striga*-free plots. The primary aim was to determine the influence of *S. hermonthica* on cereal photosynthesis during the first 14 weeks of growth and to observe whether cultivar differences were apparent in the field in the presence of *S. hermonthica*. Where possible, the cultivars examined in the laboratory were grown in the field. To determine the effect of *S. hermonthica* on the host, non-destructive measurements of growth (ligule height) and chlorophyll were also made. On selected cultivars detailed measurements of diurnal photosynthesis were recorded, estimates of canopy photosynthesis together with destructive measurements of plant biomass. Because of the importance of photosynthesis for final grain production, the relationship between these parameters was also examined.

3.2 Materials and Methods

3.2.1 Experimental design and plant material

A two way factorial design was employed using the C_4 cereals *Zea mays* (L.) and *Sorghum bicolor* (L.) Moench grown in either the presence or absence of *S. hermonthica*. Three maize cultivars and five sorghum cultivars were studied and were

selected for both their commercial availability to local farmers and reported traits of

susceptibility to *S. hermonthica* (Table 3.1).

Table 3.1 Maize and sorghum cultivars used in the experimental study and their known characteristics.

Maize Cultivars	Characteristics
Nyamula	a local yellow maize reported to show some resistance to *Striga* attack by farmers in Western Kenya (Frost, 1995)
H511	a late maturing commercially available hybrid
Katumani	an early maturing commercially available cultivar

Sorghum Cultivars	Characteristics
CSH-1	a *Striga*-susceptible cultivar from India (Yaduraju *et al.*, 1979; Press *et al.*, 1987a; Cechin, 1994b)
Serena	a commercially available cultivar reported to show some *Striga* resistance in Tanzania (Mbwaga and Obilana, 1993)
SRN-39	a cultivar observed to have a low production of germination stimulant (Babiker and Reda, 1991; Parker, 1991; Hess *et al.*, 1992)
Seredo	a commercially available cultivar supporting high numbers of *Striga* (Babiker and Reda, 1991; Haussman *et al.*, 1996)
Ochuti	a local red sorghum reported to show some *Striga* resistance (Uchuti in Kijaluo) by local farmers (Frost, 1995)

3.2.2 Experimental plots

The study was conducted at the Kenya Agricultural Research Institute at Kibos, near

Kisumu in western Kenya (latitude 0°4'S; longitude 34°48'E; altitude 1214 m).

Forty eight experimental plots, each covering 15 m^2 were established in March 1996 prior to the start of the long rain season. The entire area had previously been planted with maize and had shown moderate levels of *S. hermonthica* infestation. In order that plants could be grown in the absence of *S. hermonthica*, the entire area had been fumigated with methyl bromide gas (at a rate of 500 kg ha^{-1}), in early February, to kill all the *Striga* seeds in the soil seed bank. This technique has also been used to control *Orobanche ramosa* (Emiroglu, Nemli and Küçüközden, 1987). Eplee bags (3 x 3 cm fine mesh (<0.2 mm) nylon bags) containing 200 *S. hermonthica* seeds were placed between 10 and 20 cm depth in the soil prior to fumigation. Germination tests were carried out after fumigation using GR-24 (Gbèhounou, Pieterse and Verkleij, 1996) and these showed a 100% seed kill.

Each treatment was replicated three times within the study area. Within each 15 m^2 plot the cereals were grown with an inter-row spacing of 0.6 m and were sown at 0.5 distances along each row. Only the central four rows were used for measurements, with the outer rows comprising the guard rows and with safety margins of 0.5 m between each plot. At the time of planting the cereals (20th March 1996) 24 of the 48 plots were artificially infested with *S. hermonthica* seed collected in Kibos in 1994 from a maize host. The seed was mixed with finely sieved sand and sown to give an infection density of 2000 seeds per host plant. The seeds were dug to a depth of 10-15 cm around the planting hole.

All plants received a dressing of calcium ammonium nitrate and tri-super phosphate, applied at a rate of 40 kg P ha^{-1} and 40 kg N ha^{-1} respectively. The fertilisers were placed in a hole adjacent to the seed at the time of planting. An insecticide (carbofuran) was applied at the time of planting at a rate of 2 g per plant to protect against early attacks of stem borers, and the plants were again treated 22 DAP. The plots were hand weeded for all weeds other than *S. hermonthica* at 46 DAP.

In summary, three maize and five sorghum cultivars were examined in the absence or presence of *S. hermonthica*. Each plot was replicated three times within the study area providing 120 individual plants per treatment (excluding guard rows).

3.2.3 Growth measurements

At eight to ten intervals between 21 and 86 DAP plant height was measured from the base of the stem to the youngest visible ligule on 15 tagged individuals for each treatment. At 92 DAP the maize cultivars H511 and Katumani and the sorghum cultivars CSH-1 and Ochuti were harvested. Plants were separated into stems and leaves and total biomass was determined for eight individuals, the measurements being made on plants other than those tagged for height measurements. Plant material was oven dried for 76 h at 70 °C before being weighed. Final yields were determined on the tagged plants at 108 DAP by threshing the grain from the head and oven drying at 70 °C for 72 h. The maize cultivar Katumani was harvested at 96 DAP due it maturing earlier than the other cultivars. The emergence of *S. hermonthica* was recorded around the base of the stem of the tagged plants throughout the study. When the final biomass

measurements were taken on selected cultivars at 80 DAP the *S. hermonthica* plants were harvested at soil level discarding below ground biomass.

3.2.4 Gas exchange measurements

At seven intervals between 20 and 91 DAP instantaneous (60-120 s) rates of gas exchange were measured on maize and sorghum plants on 12 individual plants for each treatment. Measurements were made using a portable infra-red gas analyser (LCA-4 ADC, Hoddesdon, UK) and twelve measurements were made per treatment. Measurements were made halfway along the length of the youngest fully expanded leaf at ambient CO_2 concentrations (380 ppm) and relative humidity (50%). All measurements were recorded between 10.30 and 14.00, when the PFD was in excess of 2000 μmol quanta m^{-2} s^{-1}. The leaf cuvette had an area of 625 mm^2 (ADC PCL-B), and a flow rate of 300 ml min^{-1} was used. Differences between the concentration of CO_2 and H_2O vapour between the inlet and outlet gas streams were used to calculate rates of photosynthesis and transpiration, using the equations described by von Caemmerer and Farquhar (1981).

In addition, diurnal gas exchange measurements were made on the infected and uninfected maize cultivars H511 and Katumani and on the sorghum cultivars CSH-1 and Ochuti. Measurements were made between 48 and 55 DAP when *S. hermonthica* was clearly visible above ground. Four individual plants from each treatment were studied at approximately 60 min intervals during a 12 h period from sunrise to sunset. Gas exchange measurements for attached *S. hermonthica* plants were recorded using

the upper most leaves. Stomatal conductance measurements were simultaneously recorded with gas exchange using a porometer (Delta T devices, Cambridge, UK) which was recalibrated throughout the day to take into account the changes in temperature and relative humidity.

In the four cultivars named above, gas exchange was recorded within the plant canopy. The light environment naturally declined throughout the canopy from PFDs of above 2000 μmol quanta m^{-2} s^{-1} in the uppermost canopy to approximately 800 μmol quanta m^{-2} s^{-1} in the lower canopy. However, due to the large spacing between each plant, PFD was often sufficient to light saturate photosynthetic rates of the lower leaves. Leaves in positions 8, 10 and 12 were measured at 2000 quanta μmol m^{-2} s^{-1} (to determine the potential saturating rates of gas exchange), from the time of full expansion until 91 DAP or senescence. For clarity, data are reported for 91 DAP only and compared with rates of photosynthesis in the youngest fully expanded leaf in position 20, 18, 16 and 20 for H511, Katumani, CSH-1 and Ochuti, respectively. An infra-red gas analyser was used to make these measurements as described above.

3.2.5 Foliar chlorophyll concentration

At 10 intervals from 19 to 89 DAP non-destructive measurements of foliar chlorophyll content were made on the youngest fully expanded leaf using a chlorophyll meter (SPAD-502, Minolta, UK). Six measurements were halfway along the leaf either side of the midrib. Readings from the meter were calibrated against the chlorophyll content

of leaves on an area basis as determined by extraction in acetone as described in Chapter 2 Section 2.2.6.

3.2.6 Foliar nitrogen concentration

Leaf tissue samples were taken from all infected and uninfected cultivars at 91 DAP for analysis of total nitrogen. Four measurements from each treatment were made. Analysis of tissue nitrogen was made as described in Chapter 2 Section 2.2.7.

3.2.7 Statistical analysis

The response of each maize and sorghum cultivar to *S. hermonthica* infection was analysed using one-way analysis of variance procedure for a randomised block design (Minitab version 10.2). Cultivars were not be directly compared statistically due to the different rates of growth and maturation of the cereals. Cultivar differences in the numbers of emerged *S. hermonthica* per host plant were analysed using a non-parametric Kruscal-Wallis test followed by multiple comparison procedures (Zar, 1984). Regression analysis was determined on Minitab (version 10.2) and a one way analysis of variance procedure carried out on the regression line.

3.3 Results

3.3.1 *S. hermonthica* infection

Throughout the entire experimental period there was no *S. hermonthica* emergence on any of the control plots. Emergence of *S. hermonthica* first occurred at 41 DAP on all the maize cultivars and four of the sorghum cultivars, with the exception of the local

land race, Ochuti (data not shown). Ochuti showed a slight delay in *S. hermonthica* emergence by 7 days, with emergence being observed at 48 DAP. At 86 DAP when the final *S. hermonthica* counts were made (Table 3.2), maize and sorghum cultivars generally supported similar number of *S. hermonthica*. Within the maize cultivars, H511 supported the highest number of parasites (20 parasites/host) compared with the cultivars Katumani and Nyamula (16 parasites/host), although Kruscal-Wallis analysis showed this data to be not significant ($H = 3.15$, $P = 0.208$). The numbers of emerged parasites differed between sorghum hosts, with CSH-1 supporting significantly more parasites than any of the other sorghum cultivars (30.6 parasites/host) ($H = 22.67$, $P < 0.001$). SRN-39 supported the lowest number of parasites (12.3 parasites/host) having 59.8% less parasites compared with CSH-1.

3.3.2 Cereal growth

Table 3.2 shows a summary of the growth characteristics of both maize and sorghum cultivars at the time of the final measurement. It is clear from this data that the response of cereals to infection by *S. hermonthica* differed greatly between the cultivars.

Grain yield was greater in uninfected maize compared to sorghum, but in the presence of *S. hermonthica* grain yield was generally lowest in maize hosts with both H511 and Katumani having 54% lower grain yields when infected with *S. hermonthica* compared with uninfected plants. Infected plants of Nyamula also showed low grain yields although yields were 20% greater than infected H511 or Katumani even though numbers of emerged *S. hermonthica* were similar between all maize cultivars. In

contrast, the yield response to infection in sorghum cultivars covered a larger range with the greatest difference between infected and uninfected plants being observed in CSH-1, with infected plants having 49.9% less grain compared with uninfected plants. The smallest effect of *S. hermonthica* was observed in SRN-39 and Ochuti with infected plants showing grain yields 21.2% and 22.4% below that of uninfected plants, respectively. Interestingly CSH-1 showed the highest numbers of emerged parasites and infected plants had the lowest grain yield.

Large differences were observed in the distance from the base of the stem to the youngest ligule between infected and uninfected cereals and the degree of stunting of the main stem reflected the grain yield. The final ligule heights of uninfected cereals differed greatly between the cultivars (Table 3.2). In the maize cultivars studied, uninfected plants of Katumani were much shorter than Nyamula or H511, and within the sorghum cultivars, CSH-1 and SRN-39 were shorter than Serena and Seredo, which in turn were shorter than Ochuti. However, the extent to which the parasite caused stunting appeared to be unrelated to whether the genotype was a 'tall' or 'dwarf' variety. For example the effect of *S. hermonthica* was greatest in the dwarf sorghum cultivar CSH-1, showing 22.9% less internode extension compared with controls, whereas in the second shortest cultivar, SRN-39, the effect of *S. hermonthica* was least severe, with infected hosts having only 9.9% less internode extension compared with uninfected plants.

Figure 3.1 shows the effect of *S. hermonthica* on ligule height of two selected maize and sorghum cultivars in greater detail between 21 and 86 DAP. The effect of *S. hermonthica* on ligule height in the maize cultivars, H511 and Katumani, and sorghum cultivar, CSH-1, was significant by 34 DAP (p < 0.01), before the first parasites emerged. At each subsequent time point these differences became more marked and by 86 DAP internode extension was greatly affected compared with uninfected controls as seen in Table 3.2. The difference between infected and uninfected plants in Ochuti was not apparent until 48 DAP, at the time of *S. hermonthica* emergence. In the remaining maize cultivar, Nyamula, (graph not shown) an identical pattern was observed compared with the response of the other maize cultivars. In the remaining sorghum cultivars changes in ligule height with infection were significant by 48 DAP, 7 days after parasite emergence.

Infection by *S. hermonthica* had a severe effect on the chlorophyll concentration in the youngest fully expanded leaf in all cereals (Table 3.2). By 89 DAP infected maize cultivars showed a foliar chlorophyll concentrations between 30.6% and 37.6% below those of uninfected plants. Sorghum cultivars, CSH-1 and Seredo, were most affected by infection with plants having chlorophyll concentration 50.5% and 41.1% below uninfected plants. Ochuti was the least affected with infected plants having 17.1% lower chlorophyll concentrations than uninfected controls. A detailed examination of the decline in leaf chlorophyll of selected cereals (Figure 3.2) shows that lower chlorophyll contents in infected plants are apparent around the time of *S. hermonthica* emergence. This is true for all cereals with significant reductions being observed

between 47 and 54 DAP (p < 0.001). The sorghum cultivar Ochuti does not show such severe loss of chlorophyll and significant reductions are only observed at 75 DAP, 27 days after parasite emergence.

S. hermonthica had a marked effect on the above ground dry weight partitioning (Table 3.3). Infection resulted in less stem biomass accumulation compared with uninfected plants, reflecting stem height data. Maize cultivars H511 and Katumani had stem dry weights 61.7% and 53.4% of uninfected controls, respectively. The stem biomass of infected CSH-1 was 54.1% of uninfected plants but Ochuti was not significantly affected. This contrasts with the ligule height data where significantly less internode extension occurred with infection for this cultivar. *S. hermonthica* infection did not affect leaf dry weight in the four cultivars examined. The dry weight of above ground material of *S. hermonthica* was similar for both maize cultivars but the sorghum cultivar CSH-1 supported 74.4% more tissue compared with Ochuti.

3.3.3 Instantaneous gas exchange

A marked effect of *S. hermonthica* was observed on the rates of carbon assimilation of maize and sorghum (Figure 3.3) reflecting the lower ligule heights and grain yields reported above. For maize cultivars both uninfected and infected plants show an initial rise in the rate of photosynthesis between 21 and 40 DAP, after which rates gradually declined. The decline in photosynthesis was greater in infected plants than in uninfected plants and differences between the treatments were significant by 48 DAP in Katumani (p < 0.01) and by 57 DAP in H511 and Nyamula (P < 0.05), eight days

after *S. hermonthica* emergence. For sorghum cultivars a similar pattern was observed with the differences between infected and uninfected plants increasing with time. Lower rates of photosynthesis were significant by 47 DAP in CSH-1 ($p < 0.001$) and Serena ($p < 0.05$), by 54 DAP in SRN-39 and Seredo ($P < 0.001$). However, lower rates of photosynthesis in infected Ochuti plants were only significant at the final measurements made at 91 DAP ($p < 0.001$).

By the final measurements at 91 DAP (Table 3.4) parasite-induced lowering of photosynthesis in maize cultivars was most severe in the cultivar Katumani, with infected plants having rates of photosynthesis 79.8% below uninfected controls. Nyamula was least affected with infected plants having rates 30.7% below uninfected plants. Infection of sorghum cultivars resulted in a range of responses with CSH-1 being the most severely affected (as seen with the grain and ligule height data) with rates of photosynthesis 54.7% below uninfected controls. Ochuti was the least affected cultivar with infection resulting in rates of photosynthesis only 12% below uninfected plants. Lower rates of photosynthesis were accompanied by lower rates of transpiration and stomatal conductance with the exception of Ochuti where no significant differences between infected and uninfected plants were observed (Table 3.4). The gas exchange data was used to calculate values of instantaneous water use efficiency (WUE) . No significant changes were observed in water use efficiency with infection with the exception of Katumani and CSH-1 where larger changes in photosynthesis than transpiration occurred resulting in WUE 56.5% and 24.0% lower than uninfected plants, respectively.

3.3.4 Diurnal gas exchange

The extent to which *S. hermonthica* influenced photosynthesis in CSH-1 varied through the day (Figure 3.4). During the initial rise in photon flux density between 6.00 and 9.00 there were no significant differences between infected and uninfected plants. By 10.00 plants infected with *S. hermonthica* had significantly lower rates of photosynthesis, with this pattern remaining until 16.00, after which rates declined rapidly in both infected and uninfected plants. Stomatal conductance followed a similar pattern to rates of photosynthesis as did transpiration although the difference between the two sets of plants was not significant until after midday. A similar diurnal pattern was observed in cultivars H511 and Katumani (Table 3.5) However throughout the entire period no differences between infected and uninfected plants of Ochuti were observed. During periods of high PFD and temperature, lower rates of photosynthesis were recorded in the afternoon compared with those recorded in the morning period in all cultivars (excluding Ochuti). For example, in H511, Katumani and CSH-1 at 11.30, infected plants showed rates of photosynthesis 18.5%, 45.9% and 30.2% below their controls respectively. At 14.00 at a similar PFD and temperature to those recorded at 11.30, rates of photosynthesis were 25.4%, 47.9% and 41.0% below their controls, respectively.

Diurnal measurements of the gas exchange characteristics of attached *S. hermonthica* (Table 3.5) are steady throughout the day with slight increases in all variables during the afternoon period. Throughout the day rates of photosynthesis are greatly lower

than their cereal hosts, the maximum rate recorded was 10.68 μmol m^{-2} s^{-1} on a maize host showing a rate of photosynthesis of 27.83 μmol m^{-2} s^{-1}. Rates of transpiration however are higher than both their host plant and that of uninfected plants. In the afternoon period (14.00) rates exceed that of the host by over 50%. Stomatal conductance of the parasite generally exceeds that of its hosts but does not exceed uninfected controls.

3.3.5 Gas exchange within the cereal canopy

S. hermonthica has a large effect on rates of photosynthesis within the lower canopy in three of the four cereals measured (Figure 3.5). In cultivars H511, Katumani and CSH-1 leaves in position 8, 10 and 12 showed significantly lower rates of photosynthesis compared with equivalent leaves of uninfected plants ($p < 0.01$). Greater differences in the rates of photosynthesis between infected and uninfected plants are observed lower in the canopy when compared with the youngest fully emerged leaf at 91 DAP. In contrast to these cultivars, infected Ochuti plants do not show lower rates of photosynthesis in the lower canopy compared with uninfected controls and in leaves in positions 10 and 12 there is a stimulation of photosynthesis by 6.8% and 31.7% above those of uninfected leaves. Thus despite lower rates in the youngest fully emerged leaf, the lower canopy does not show such depressions.

3.3.6 Foliar nitrogen concentration and photosynthetic nitrogen use efficiency

Infection by *S. hermonthica* in maize cultivars had no effect on the specific leaf area (SLA) or leaf nitrogen content measured at 91 DAP (Table 3.6). The mean rate of

photosynthesis and mean nitrogen content on an area basis was used to calculate the photosynthetic nitrogen use efficiency (PNUE). Lower rates of photosynthesis in infected plants together with no changes in nitrogen content resulted in lower PNUE in infected plants compared to uninfected controls. In contrast to the maize cultivars, SLA was significantly lower in the sorghum cultivar CSH-1 but unaffected in the other four cultivars. There was a trend for nitrogen concentrations to be lower in infected plants compared with uninfected controls however this was only significant for cultivars Serena and SRN-39. PNUE was lower in all infected plants compared with controls with the exception of Ochuti.

3.3.7 The relationship between photosynthesis and grain yield

For the majority of maize and sorghum cultivars there was a positive relationship between photosynthesis and grain yield (Figure 3.6) with analysis of variance showing the regression lines to be significant (Table 3.7). Maize cultivars H511 and Katumani showed a good relationship between the variables but in the cultivar less severely affected by *S. hermonthica*, Nyamula, the relationship was not as strong. A similar pattern is observed in the sorghum cultivars. The cultivars severely affected by infection; CSH-1, Seredo and Serena, showed a good relationship between photosynthesis and grain yield, with high values of r^2, however cultivars SRN-39 and Ochuti, which are less responsive to infection, showed poor regressions. In Ochuti the regression line does not significantly differ from zero.
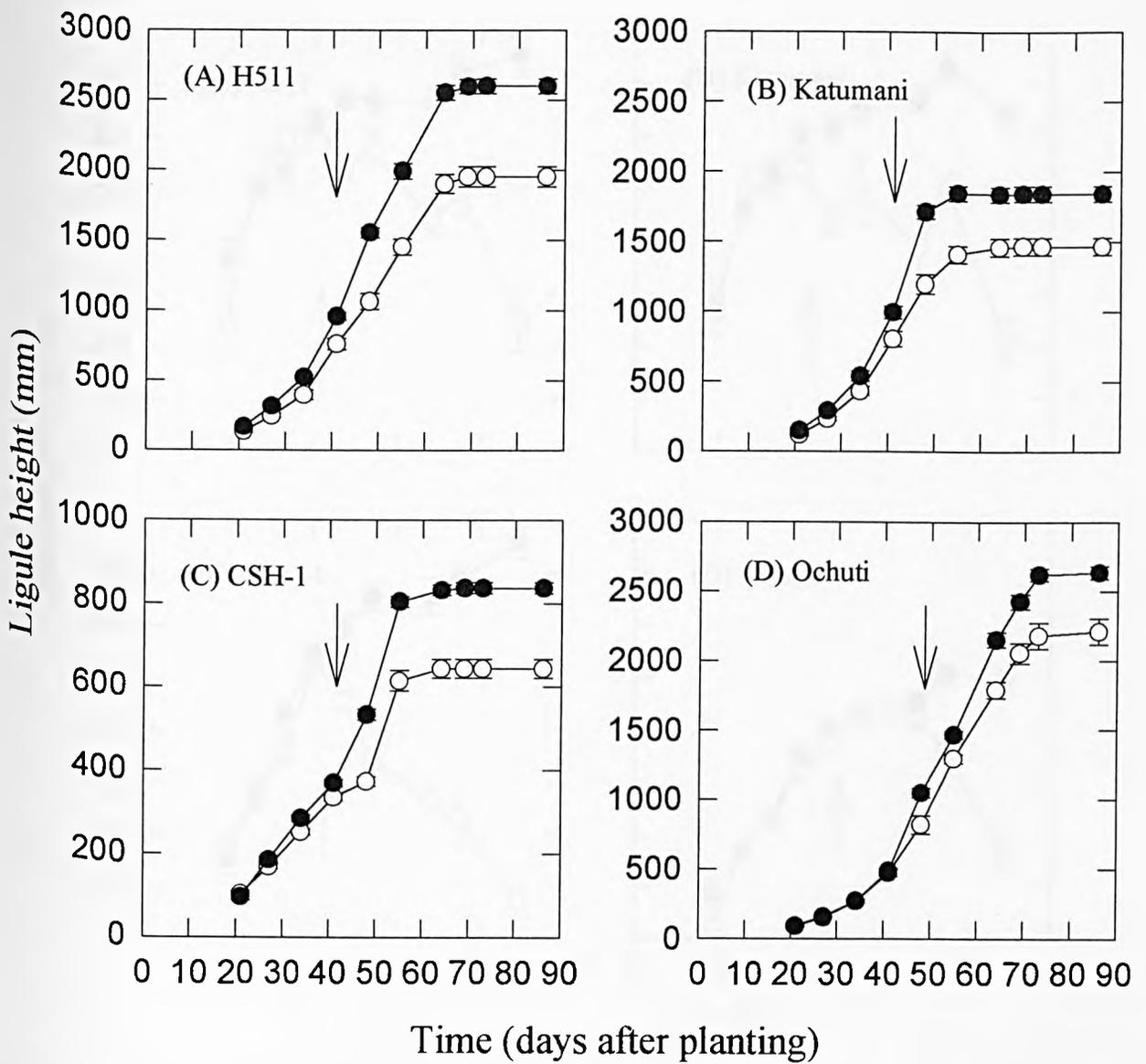
Figure 3.1 Distance from the base of the stem to the youngest ligule (mm) of selected maize cultivars (graphs (A) and (B)), and sorghum cultivars (graphs (C) and (D)), (see Table 3.1 for summary of all cultivars). Note the difference in scale use for CSH-1. Plants were grown in the absence (closed symbols) or presence (open symbols) of *S. hermonthica*. Means and standard errors of 12 measurements are reported and the data analysed using analysis of variance procedures (see text and Table 3.1). The timing of the first emergence of *S. hermonthica* is denoted by the arrow.
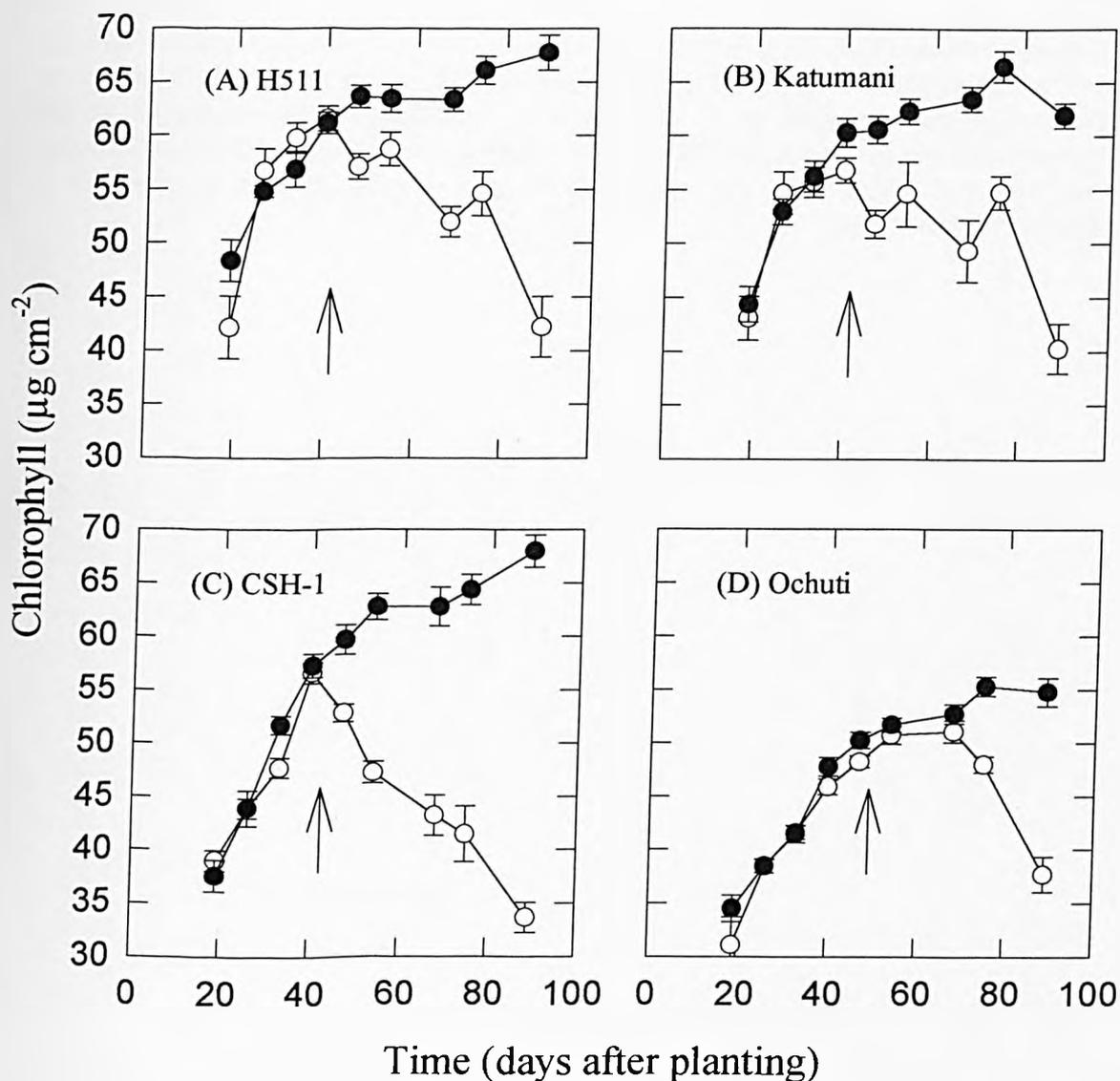
Figure 3.2 Chlorophyll concentration (μg cm^{-2}) of the youngest fully expanded leaf of selected maize cultivars (graphs (A) and (B)), and sorghum cultivars (graphs (C) and (D)), (see Table 3.1 for summary of all cultivars). Plants were grown in the absence (closed symbols) or presence (open symbols) of *S. hermonthica*. Means and standard errors of 12 measurements are reported and the data analysed using analysis of variance procedures (see text and Table 3.1). The timing of the first emergence of *S. hermonthica* is denoted by the arrow.

Figure 3.3 Rates of photosynthesis, A, at 2000 μmol m^{-2} s^{-1} for maize (graphs (A)-(C)) and sorghum (graphs (D)-(H)) cultivars grown in the absence (closed symbols) or presence (open symbols) of *S. hermonthica*. Means and standard errors of 12 measurements are reported and the data analysed using analysis of variance procedures (see text and Table 3.4). The timing of the first emergence of *S.hermonthica* is denoted by the arrow.

Figure axes: y-axis labeled A (μmol CO_2 m^{-2} s^{-1}); x-axis labeled Time (days after planting).

(A) H511

(B) Katumani

(C) Nyamula

A ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)

(D) CSH-1

(E) Seredo

(F) Serena

(G) SRN-39

(H) Ochuti
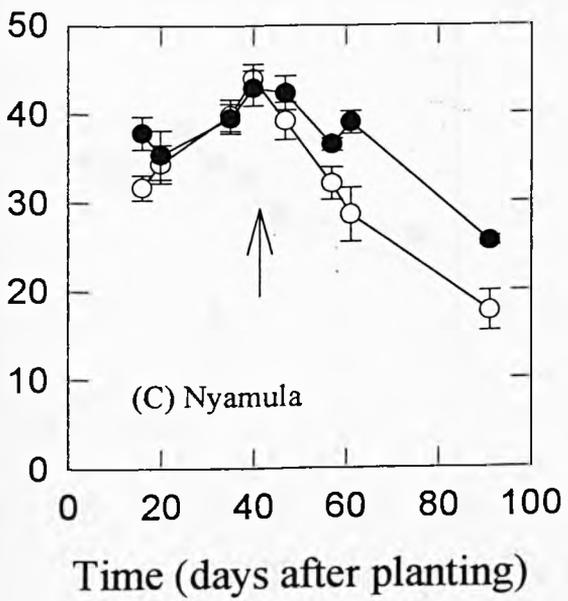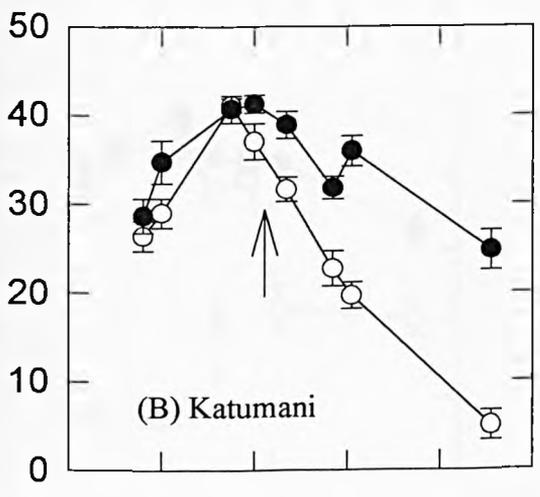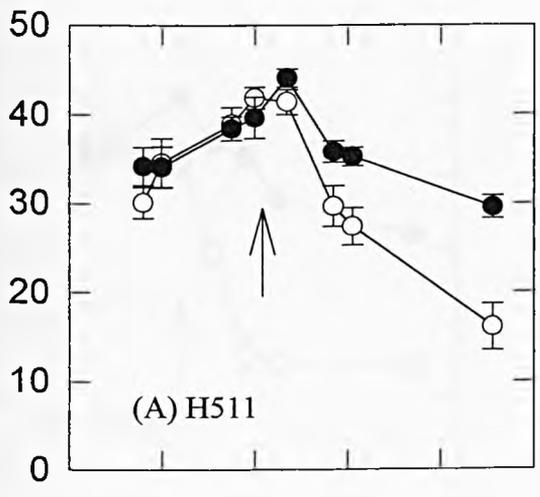
Time (days after planting)
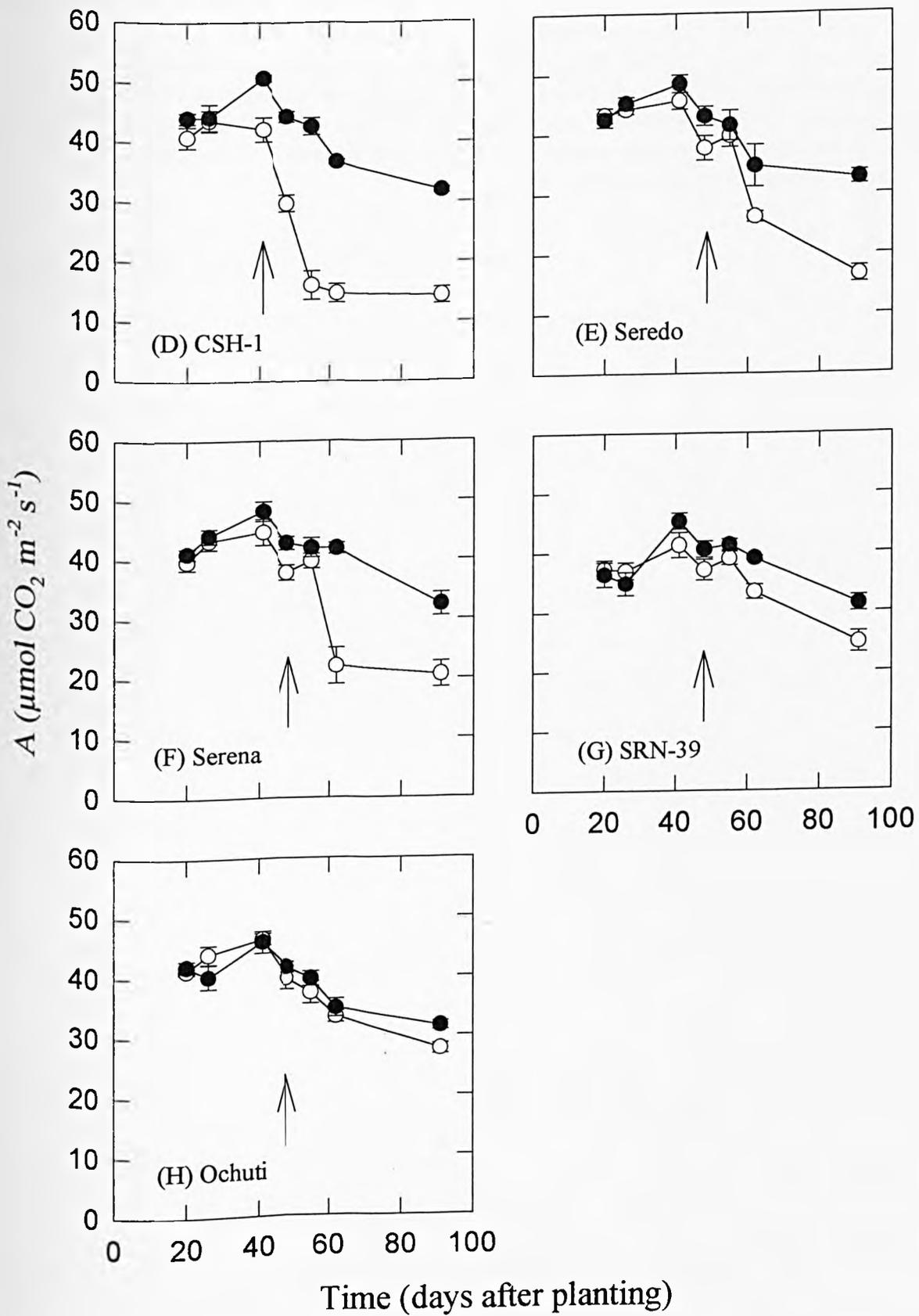
Figure 3.4 Rates of photosynthesis (A), transpiration (E), stomatal conductance (gs), photon flux density (PFD, closed diamond) and temperature (open diamond) for the sorghum cultivar CSH-1, grown in the absence (closed symbols) or presence (open symbols) of *S. hermonthica* and measured during a 12 hour period. Means and standard errors of 4 measurements are reported and the data analysed using analysis of variance procedures (see Table 3.5).

E (mmol H₂O m⁻² s⁻¹), gs (mol H₂O m⁻² s⁻¹), A (μmol CO₂ m⁻² s⁻¹), PFD (μmol quanta m⁻² s⁻¹), Leaf temperature (°C) plotted against Time of day (24 h).
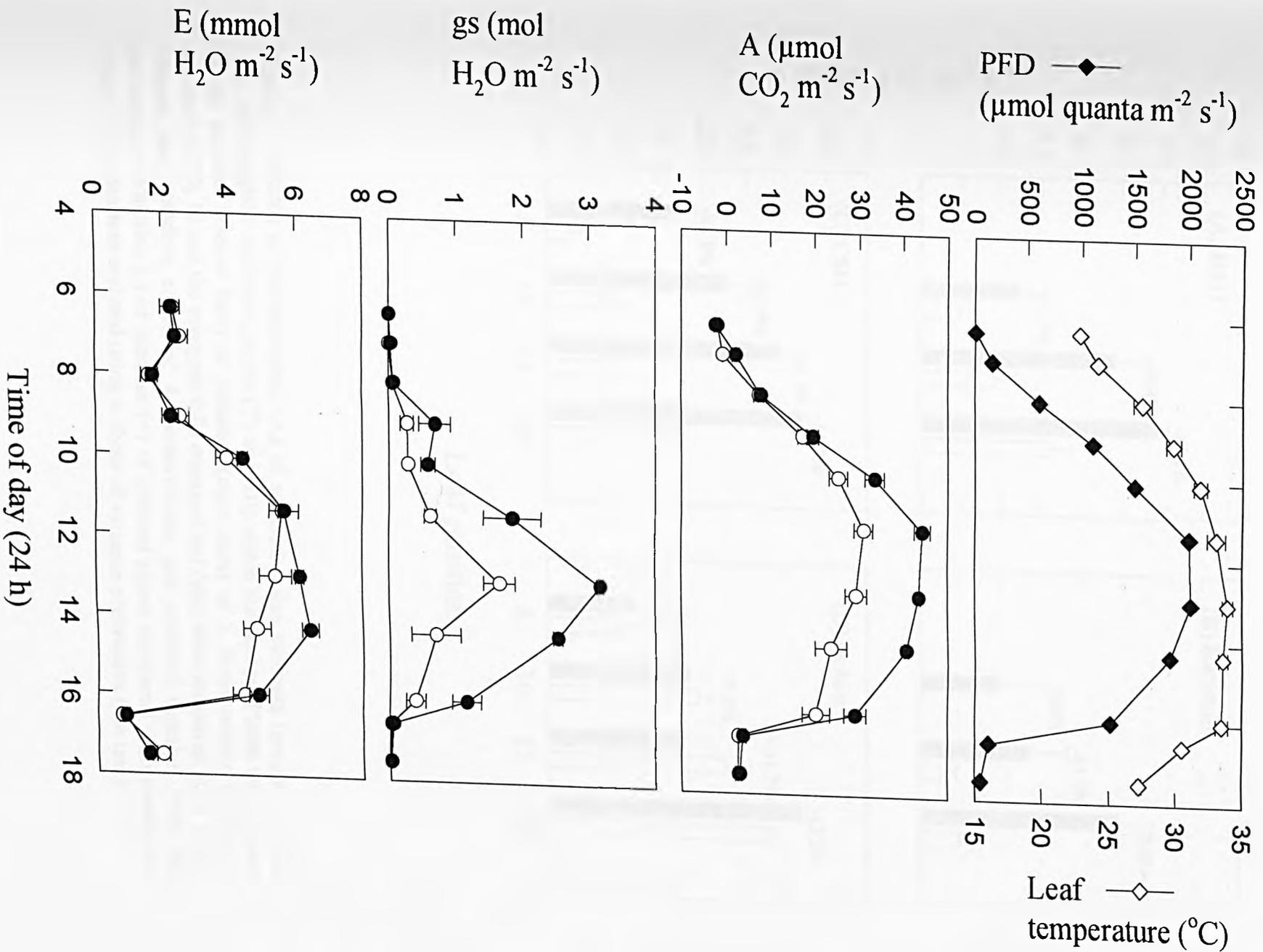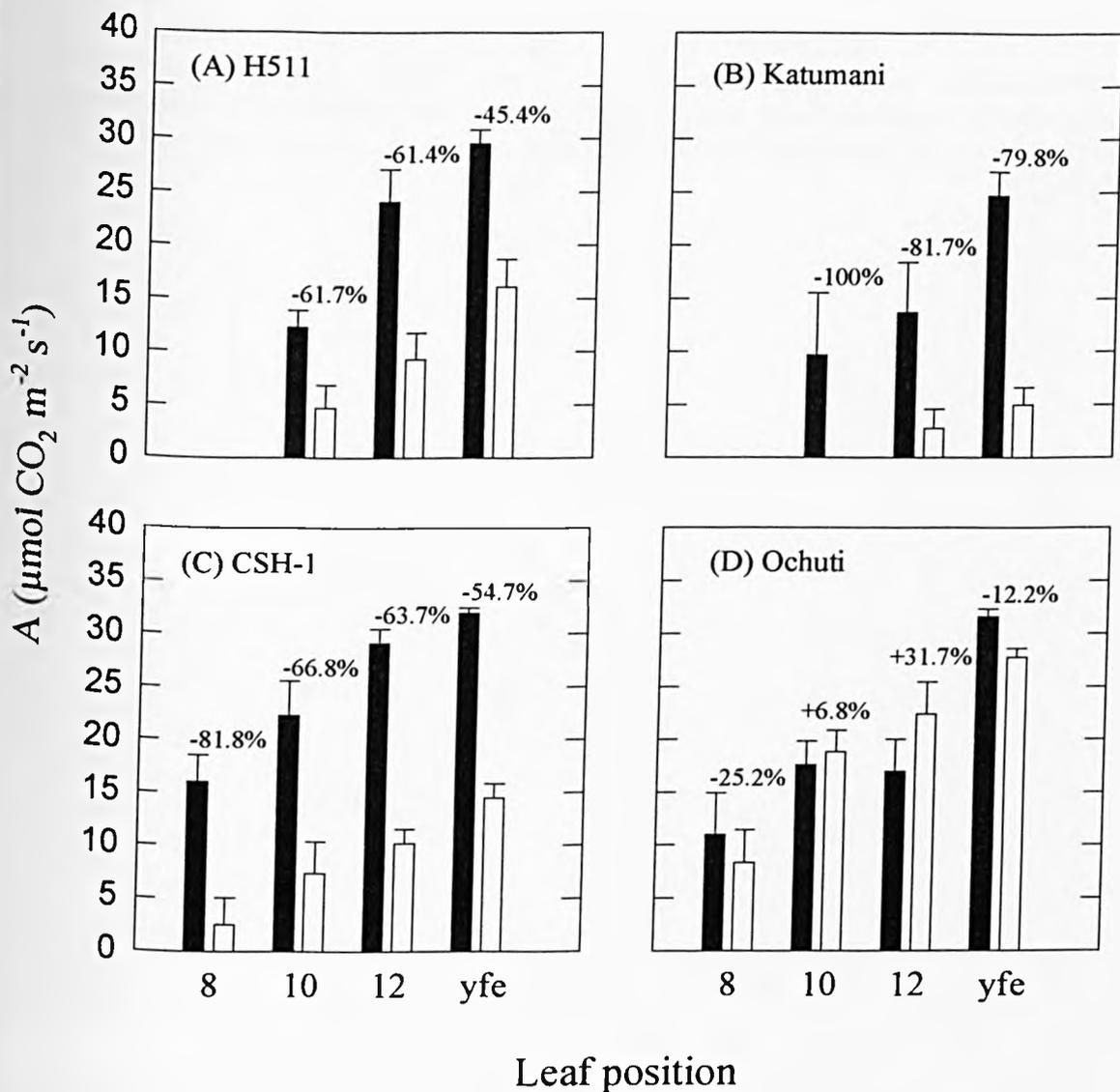
Figure 3.5 Rates of photosynthesis (A) of selected maize cultivars (graphs (A) and (B)) and sorghum cultivars (graphs (C) and (D)) within the canopy. Plants were grown in the absence (closed bars) or presence (open bars) of *S. hermonthica*. Leaves in position 8, 10, 12 and the youngest fully emerged leaf (yfe) were measured at 91 DAP. Means and standard errors of 4 measurements are reported together with the percentage reduction (-) or increase (+) of infected plants compared with uninfected plants. The data was analysed using analysis of variance procedures (see text).

Figure 3.6 The relationship between grain yield and final measurements of photosynthesis, A, for maize (graphs (A)-(C)) and sorghum (graphs (D)-(H)) plants grown in the absence (closed symbols) or presence (open symbols) of *S. hermonthica*. All values are for individual measurements. For regression equations and analysis see Table 3.7

Grain yield (g)

(A) H511

(B) Katumani

(C) Nyamula

A (μmol CO_2 m^{-2} s^{-1})

Grain yield (g)
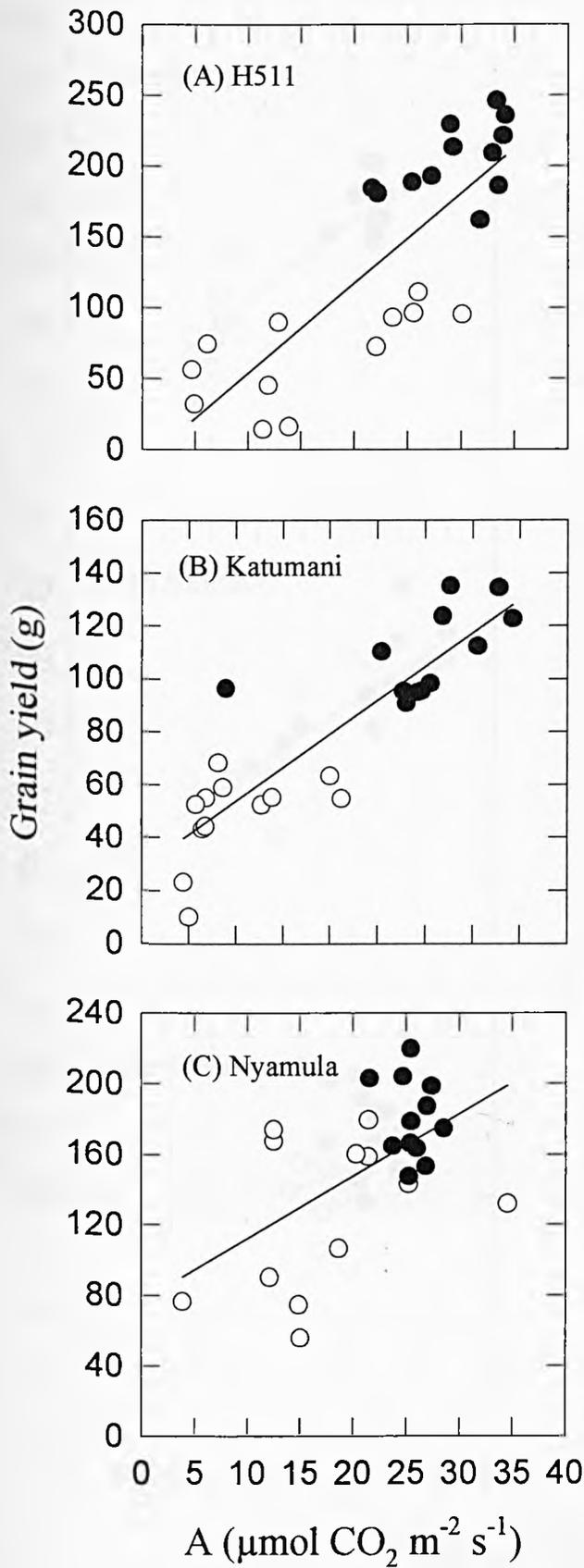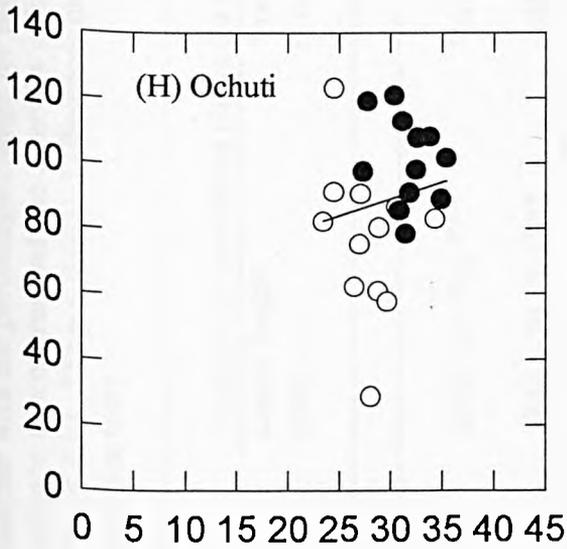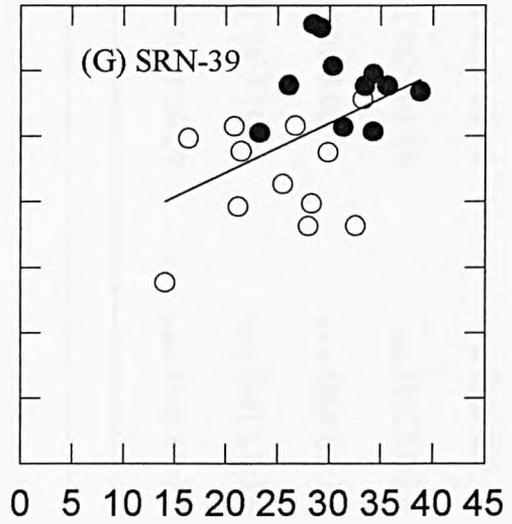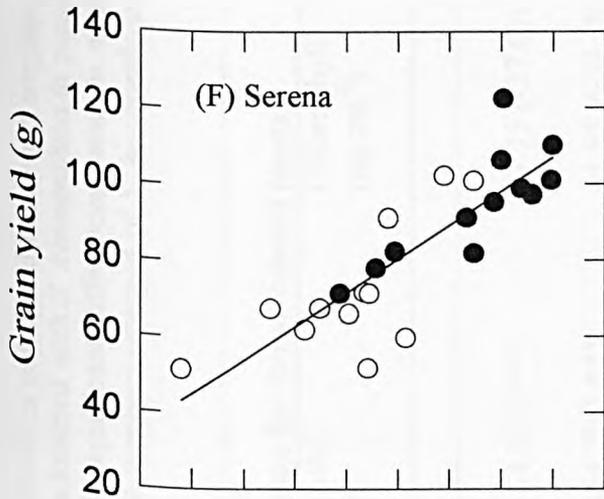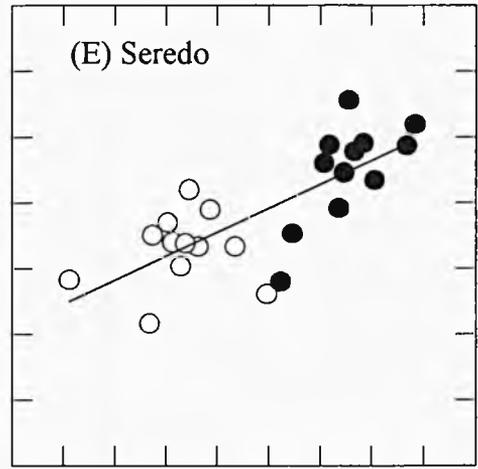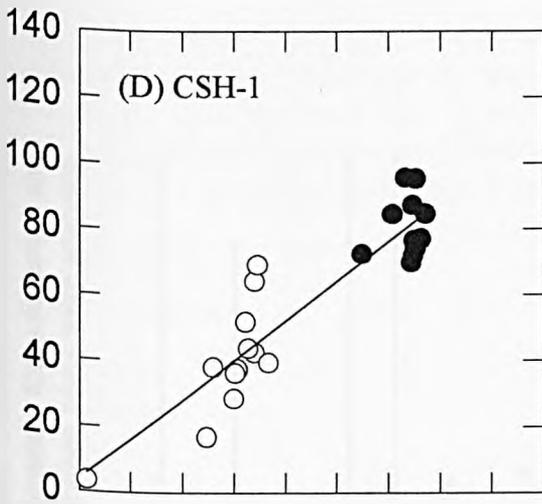
A (μmol CO_2 m^{-2} s^{-1})

Table 3.2 *Summary of growth characteristics and chlorophyll concentrations of uninfected maize and sorghum cultivars. Mean values of 12-15 replicates are reported, together with the percentage change for plants infected with S. hermonthica in parenthesis. The data was analysed using one way analysis of variance procedures and the asterisks indicate significant differences between measurements for uninfected and infected plants (* p < 0.05, ** p < 0.01, *** p < 0.001). Differences in the numbers emerged of S. hermonthica between hosts were analysed using Kruscal-Wallis procedures (see text).*

| Cultivar | Number of *Striga* per host | Variable for uninfected plants (% change for *Striga*-infected plants) | | |
		Ligule height (mm)	Grain yield (t ha^{-1})	Chlorophyll (μg cm^{-2})
Maize				
H511	20.0 ± 1.82	2602(-24.9%) ***	7.62 (-54.6%) ***	67.75 (-37.6%) ***
Katumani	16.5 ± 1.97	1844 (-20.2%)***	4.40 (-54.2%) ***	61.95 (-34.9%) ***
Nyamula	16.3 ± 2.12	2552 (-16.1%)***	7.20 (-33.3%) ***	66.11 (-30.6%) ***
Sorghum				
CSH-1	30.6 ± 3.33	836 (-22.9%) ***	3.35 (-49.9%) ***	67.99 (-50.5%) ***
Seredo	16.5 ± 1.42	1342 (-16.2%)***	3.68 (-27.1%) ***	56.61 (-41.1%) ***
Serena	15.8 ± 1.35	1254 (-9.19%)*	3.87 (-30.4%) ***	56.06 (-36.2%) ***
SRN-39	12.3 ± 1.68	968 (-13.3%) ***	4.40 (-21.2%) **	65.19 (-34.5%) ***
Ochuti	14.6 ± 1.9	2634 (-16.1%)***	3.91 (-22.4%) *	54.80 (-17.1%) ***

Table 3.3 Dry weight partitioning of maize and sorghum plants grown in the absence (-) or presence (+) of *S. hermonthica* at 92 DAP. Dry weight of *S. hermonthica* is also shown. Means and standard errors of four plants are reported and the data was analysed using analysis of variance procedures. Asterisks indicate significant differences between measurements for uninfected and infected plants (* $p < 0.05$, ** $p < 0.001$).

Cultivar	*Striga*	Stem (g)	Leaves (g)	*Striga* (g)
Maize				
H511	-	167.1 ± 17.4	48.85 ± 7.29	-
	+	103.1 ± 3.89 **	37.55 ± 2.50 ns	4.27 ± 0.61
Katumani	-	106.3 ± 17.3	27.75 ± 4.49	-
	+	56.7 ± 7.70 *	16.88 ± 4.27 ns	5.68 ± 1.68
Sorghum				
CSH-1	-	78.25 ± 2.77	19.80 ± 1.07	-
	+	42.30 ± 10.6 *	15.65 ± 1.70 ns	7.82 ± 3.15
Ochuti	-	127.0 ± 10.1	34.78 ± 1.10	-
	+	112.4 ± 21.1 ns	33.78 ± 5.04 ns	2.00 ± 0.72

Table 3.4 Photosynthesis, transpiration, stomatal conductance and water use efficiency (photosynthesis / transpiration) of uninfected maize and sorghum plants. Mean values of 12 measurements for each parameter are reported, together with the percentage change for plants infected with S. hermonthica, in parenthesis, at 91 DAP. The data was analysed using analysis of variance procedures and the asterisks indicate significant differences between measurements for uninfected and infected plants (ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Cultivar	Photosynthesis ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	Transpiration ($mmol\ H_2O\ m^{-2}\ s^{-1}$)	Stomatal conductance ($mol\ H_2O\ m^{-2}\ s^{-1}$)	Water use efficiency ($\mu mol\ CO_2\ mmol^{-1}\ H_2O$)
Maize				
H511	29.55 (-45.5%) ***	4.14 (-47.7%) ***	0.139 (-64.0%) ***	7.203 (-3.6%) ns
Katumani	24.71 (-79.8%) ***	3.61 (-64.5%) ***	0.105 (-76.2%) ***	6.695 (-56.5%) ***
Nyamula	25.55 (-30.7%) **	3.60 (-23.5%) *	0.112 (-29.5%) *	7.219 (-4.2%) ns
Sorghum				
CSH-1	31.95 (-54.7%) ***	5.81 (-44.4%) ***	0.216 (-62.2%) ***	5.670 (-24.0%) *
Seredo	32.69 (-49.4%) ***	5.77 (-42.4%) ***	0.222 (-61.7%) ***	5.723 (-11.8%) ns
Serena	32.48 (-36.4%) ***	5.95 (- 25.8%) ***	0.235 (-46.8%) ***	5.455 (-10.3%) ns
SRN-39	31.37 (-20.8%) ***	5.98 (-19.1%) *	0.241 (-34.4%) ***	5.290 (-3.6%) ns
Ochuti	31.61 (-12.2%) *	4.86 (-9.6%) ns	0.165 (-5.0%) ns	6.724 (-4.2%) ns

Table 3.5 Rates of photosynthesis (A, μmol CO_2 m^{-2} s^{-1}), transpiration (E, mmol H_2O m^{-2} s^{-1}), stomatal conductance (gs, mol H_2O m^{-2} s^{-1}) for maize and sorghum cultivars grown in the absence (-) or presence (+) of *S. hermonthica* and measured during a 12 hour period between 48 and 55 DAP. Data are reported for 8.00, 11.30, 14.00 and 16.30 hours only, and the mean PFD (μmol quanta m^{-2} s^{-1}) and leaf air temperature ($^{\circ}$C) at that time is given. Gas exchange data are also reported for attached *S. hermonthica*. Means only of 4 measurements are reported and the treatment effects (infection) analysed using analysis of variance procedures (ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Parameter	Cultivar/ species	Treatment	Time of day (24 h)			
			8.00	11.30	14.00	16.30
			PFD / temperature			
			239 / 20	1627 / 30	1668 / 31	592 / 30
A	H511	-	3.06	34.17	35.11	22.89
	H511	+	2.72 ns	27.83 *	26.19 *	15.87ns
	Striga		0.20	10.68	8.79	5.78
E	H511	-	2.34	2.66	5.67	3.22
	H511	+	2.08 ns	2.63 ns	4.64 ns	2.71 ns
	Striga		4.11	4.88	8.05	5.19
gs	H511	-	0.68	0.70	1.32	1.34
	H511	+	0.61ns	0.48 **	0.47 *	0.25 **
	Striga		0.65	0.51	0.61	0.36

Parameter	Cultivar/ Species	Treatment	Time of day (24 h)			
			8.00	11.30	14.00	16.30
			PFD / temperature			
			375 / 23	1968 / 30	1986 / 31	159 / 28
A	Katumani	-	7.20	36.98	27.99	7.83
	Katumani	+	5.27 ns	20.00 **	14.58 ***	8.07ns
	Striga		-1.84	9.50	8.17	0.57
E	Katumani	-	2.96	2.88	3.10	0.63
	Katumani	+	2.61 ns	2.16 *	2.29 *	0.57 ns
	Striga		5.26	5.86	4.02	1.82
gs	Katumani	-	1.01	0.24	0.18	0.08
	Katumani	+	0.73 ns	0.23 ns	0.09 *	0.04 **
	Striga		1.32	0.54	0.90	0.52

Parameter	Cultivar/species	Treatment	Time of day (24 h)			
			8.00	11.30	14.00	16.30
				PFD / temperature		
			310 / 27	2000 / 33	2020 / 33	130 / 30
A	CSH-1	-	7.69	44.34	40.61	29.07
	CSH-1	+	7.53 ns	30.97 **	23.95 **	20.41ns
	Striga		4.73	7.01	4.45	5.63
E	CSH-1	-	1.71	5.63	6.41	4.84
	CSH-1	+	1.59 ns	5.75 ns	4.80 *	4.14 ns
	Striga		4.59	9.26	7.41	7.47
gs	CSH-1	-	0.07	1.85	2.53	1.16
	CSH-1	+	0.06 ns	0.62 ns	0.69 *	0.38 ns
	Striga		0.52	1.17	1.36	0.31

Parameter	Cultivar/species	Treatment	Time of day (24 h)			
			8.00	11.30	14.00	16.30
				PFD / temperature		
			239 / 23	1627 / 31	1668 / 31	592 / 26
A	Ochuti	-	9.91	37.62	31.35	8.33
	Ochuti	+	10.63 ns	41.72 ns	33.14 ns	10.37ns
	Striga		0.02	3.83	10.03	1.19
E	Ochuti	-	2.34	3.91	3.73	0.53
	Ochuti	+	1.97 ns	5.31 ns	4.17 ns	0.30 ns
	Striga		5.81	4.41	9.43	2.09
gs	Ochuti	-	0.08	0.99	2.77	0.08
	Ochuti	+	0.09 ns	0.17 ns	2.36 ns	0.06 ns
	Striga		1.22	0.47	1.41	0.22

Table 3.6 SLA, foliar nitrogen concentrations (N), photosynthesis (A) and photosynthetic nitrogen use efficiency (PNUE) of sorghum and maize plants grown in the absence (-) or presence (+) of *S. hermonthica* at the final measurements 91 DAP. Means of 8 measurements for SLA and N and 12 measurements for A are reported. PNUE is expressed as a mean figure. Data were analysed using analysis of variance procedures for treatment effects (infection) (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Cultivar	*Striga*	SLA $(cm^2\ g^{-1})$	N $(mg\ g^{-1})$	A $(\mu mol\ CO_2\ m^{-2}\ s^{-1})$	PNUE $(\mu mol\ CO_2\ mol\ mol\ N^{-1}\ s^{-1})$
Maize					
H511	-	171.47	27.17	29.55	260.8
	+	161.21 ns	25.26 ns	16.11***	142.4
Katumani	-	147.30	22.37	24.71	226.1
	+	159.25 ns	22.84 ns	4.97 ***	48.40
Nyamula	-	161.17	27.45	25.55	207.3
	+	176.02 ns	24.17 ns	17.69 **	179.2
Sorghum					
CSH-1	-	143.59	33.34	31.95	192.4
	+	124.36 *	29.56 ns	14.48 ***	84.94
Seredo	-	168.00	29.34	32.69	256.4
	+	161.99 ns	26.56 ns	16.55 ***	140.5
Serena	-	174.20	32.41	32.48	239.1
	+	144.27 ns	26.22 *	20.65 ***	156.1
SRN-39	-	143.42	29.84	31.37	204.5
	+	163.20 ns	26.30 *	24.85 ***	189.1
Ochuti	-	171.22	29.77	31.61	251.2
	+	173.00 ns	24.65 ns	27.74 *	261.6

Table 3.7 The relationship between photosynthesis at 91 DAP and final grain yield in maize and sorghum cultivars. Calculated values from the regression analysis are from individual measurements. F-values and p-values from the analysis of variance are also reported.

Cultivar	Regression equation	r^2	Significance of the regression	
Maize				
H511	$y = -9.0 + 6.32x$	0.66	$F = 42.93$	$p \leq 0.001$
Katumani	$y = 41.2 + 2.53x$	0.77	$F = 71.82$	$p \leq 0.001$
Nyamula	$y = 76.6 + 3.54x$	0.31	$F = 9.76$	$p \leq 0.05$
Sorghum				
CSH-1	$y = 4.0 + 2.41x$	0.83	$F = 109.19$	$p \leq 0.001$
Seredo	$y = 49.1 + 1.46x$	0.57	$F = 28.97$	$p \leq 0.001$
Serena	$y = 36.2 + 1.77x$	0.71	$F = 54.05$	$p \leq 0.023$
SRN-39	$y = 58.9 + 1.51x$	0.22	$F = 5.98$	$p \leq 0.023$
Ochuti	$y = 58.2 + 1.03x$	0.02	$F = 0.54$	$p \leq 0.469$

3.4 Discussion

The deleterious effects of *S. hermonthica* on the growth and photosynthesis of its sorghum hosts have been documented in laboratory studies (Press and Stewart, 1987; Graves *et al.*, 1989; Cechin and Press 1993a). In Chapter 2 it was demonstrated that the extent to which *S. hermonthica* reduced growth and photosynthesis was cultivar specific and that the difference in growth between infected and uninfected cereals was not necessarily a consequence of lower rates of carbon fixation.

Does *S. hermonthica* lower rates of photosynthesis of maize and sorghum hosts in the field ?

This study demonstrated that *S. hermonthica* lowers the rate of photosynthesis and growth in field-grown maize and sorghum cultivars, supporting laboratory findings for other cultivars of these two crops (Press *et al.*, 1987b; Graves *et al.*, 1989; Smith *et al.*, 1995). Subsequent grain yields were also lowered with infected plants yielding between 21 and 54% below uninfected plants, responses often observed under field conditions (Bebawi, 1981; Doggett, 1988; Parker and Riches, 1993; Clark *et al.*, 1994). In contrast to the findings in Chapter 2, lower rates of photosynthesis were observed in all cultivars with infection, including SRN-39, which previously showed no photosynthetic response under laboratory conditions. In the field-grown cereals the degree of response to infection by *S. hermonthica* was also cultivar specific. Infection of the maize cultivars resulted in lower rates of photosynthesis and growth compared with uninfected plants. However, the local land race, Nyamula, showed less of a deleterious response to infection with higher rates of photosynthesis, growth and grain

production in the presence of *S. hermonthica* compared with infected plants of H511 and Katumani. The early maturing cultivar, Katumani, was as severely affected as the late maturing variety H511, indicating that the effect of *S. hermonthica* is similar irrespective of the growth period. Within the sorghum cultivars a range of responses were observed, with the greatest differences in growth and photosynthesis between infected and uninfected plants observed in cultivars CSH-1 and Seredo. Grain yields of CSH-1 were 50% below those of uninfected plants. However from the study it appears that two of the sorghum cultivars, SRN-39 and Ochuti, show some tolerance to infection, as they could maintain higher rates of photosynthesis in the presence of the parasite compared with infected plants of the other cultivars and produce an acceptable grain yield. Although this study has only examined three maize and five sorghum cultivars, it does provide evidence that there may be a relationship between tolerance to the parasite and the ability to maintain high rates of photosynthesis.

Prior to this study a parasite-induced lowering of photosynthesis had only been observed under laboratory conditions (e.g. Cechin, 1994b) and the data presented here contrast with those of an earlier study of a *Striga-* susceptible sorghum cultivar in Mali (Clark *et al.*, 1994), where lower rates of photosynthesis in *S. hermonthica*-infected sorghum plants were not observed. It is possible that the difference between these findings and those of Clark *et al.* (1994) may be explained by the emergence time of *S. hermonthica* above ground, as indeed may the response of the cultivar Ochuti in this study. Clark *et al.* (1994) report a late emergence of *S. hermonthica*, 54 DAP, with measurements of gas exchange being reported at two subsequent time points, 67 and

79 DAP. Emergence time of *S. hermonthica* above-ground varies, and has been reported as early as 21 DAP in Sudan (Bebawi, 1981), although in our study emergence occurred at 41 DAP in all cultivars except Ochuti. *Striga* exerts much more severe effects on the host when it attaches to young plants, and delaying attachment has been shown to reduce its deleterious effects on both host growth and photosynthesis (Cechin and Press, 1993c). Thus it is suggested that the findings of Clark *et al.*, (1994) may be confounded by late attachment of the parasite, or that the cultivar used may show some tolerance to infection.

The limited photosynthetic response in infected Ochuti plants, may also be due to a delayed *S. hermonthica* emergence, as emergence occurred 7 days later when compared with the other cultivars, indicating later parasite attachment. The response of photosynthesis to infection in SRN-39 was also less severe compared with the other cultivars. SRN-39 is reported to produce low concentrations of germination stimulants from the roots thus reducing *S. hermonthica* germination and subsequent attachment (Babiker and Reda, 1991; Parker, 1991; Hess *et al.*, 1992). A low number of attached parasites could have less of a detrimental effect on the host. However in this study emergence of *S. hermonthica* was not significantly different to three of the remaining four cultivars and emergence also occurred at 41 DAP, thus SRN-39 may also have an ability to maintain photosynthesis in the presence of *S. hermonthica* as well as Ochuti. The number of emerged parasites may not reflect the actual number of attached parasites, which is not possible to observe in the field. However the laboratory study in Chapter 2 shows that SRN-39 did not have lower numbers of

attached parasites than the other cultivars, two of which were also used in this study (CSH-1 and Serena). The disparity in the response of photosynthesis to infection in SRN-39 between the field and the laboratory may be explained by the growth conditions. The slightly lower rates of photosynthesis in infected SRN-39 plants in the field may be caused by not only the parasite but in addition the host will experience other environmental stresses, for example water stress, which may contribute parasite-induced lowering of photosynthesis. In the laboratory the plants will be free of some of these limitations and the effects of *S. hermonthica* may be lessened.

During the twelve hour photoperiod the sorghum and maize cultivars experienced changes in environmental conditions, particularly in terms of PFD and temperature. Rates of photosynthesis for uninfected plants increased with an increase in PFD and temperature, as is typically observed in C_4 cereals (see e.g. Hay and Walker, 1989; Lawlor, 1993; Pereira, 1995). The diurnal measurements made on maize cultivars H511 and Katumani and on the sorghum cultivar CSH-1, clearly showed that the photosynthetic capacity of infected plants was significantly lower than that of uninfected plants at high PFD and temperature. Interestingly, lower rates of photosynthesis were observed in the afternoon compared with the morning at comparable PFDs and temperatures (Table 3.5). This was associated with a change in stomatal conductance although lower rates of photosynthesis were observed before any detectable change in stomatal conductance. This indicates that lower stomatal conductance may be responding to lower rates of carbon fixation (Wong *et al.*, 1979), or that it contributes to the lower fixation.

S. hermonthica had low rates of photosynthesis throughout the day but had rates of transpiration far exceeding those of the host, as has been observed in previous laboratory studies (Press *et al.*, 1987b; Shah *et al.*, 1987). This will facilitate solute acquisition from the host and this process may subject the host to water stress (Stewart and Press, 1990). Under field conditions water stress would be greater in the afternoon and may account for the greater stomatal closure and further lower rates of photosynthesis below that of uninfected plants. In the event that infected plants were experiencing greater water stress compared with control plants, they may be more susceptible to photoinhibition. If high leaf temperatures are imposed on water deficits in a high light environment (as often found under field conditions), plants with closed stomata are unable to dissipate absorbed solar radiation resulting in photoinhibition and reduced carbon assimilation (Baker and Horton, 1988; Pereira, 1995). Photosynthesis of Ochuti showed no response to infection throughout the day. Instantaneous measurements of photosynthesis in infected Ochuti plants showed lower rates of photosynthesis but these changes were only apparent towards the end of the growing period after the diurnal measurements were recorded.

Can cultivars which maintain high rates of photosynthesis in the presence of *S. hermonthica* maintain high grain yields?

Crop biomass and grain production are seldom closely linked with rates of carbon fixation because of involvement of different processes and time scales. In addition there are problems in methodology. However, a number of previous studies in maize

and sorghum lines have identified positive correlations between photosynthesis prior to flowering and grain production (Vietor and Musgrave, 1979; Peng, Krieg and Girma, 1991). In this study there is a positive relationship between rates of instantaneous gas exchange and grain yield in all of the *Striga*-susceptible cultivars. It appears that infected plants which have higher rates of photosynthesis produce more grain, for example Ochuti. There is still a greater variation in grain yield in infected plants compared with uninfected plants that cannot be explained by changes in instantaneous gas exchange but overall it does appear that high rates of photosynthesis may contribute towards acceptable yields in the presence of *S. hermonthica*.

Instantaneous rates of photosynthesis on single leaves will underestimate whole plant photosynthesis due to changes not only through the day as observed in the diurnal measurements, but also because of different rates of photosynthesis within the canopy attributable to both leaf age and light attenuation. In the four cultivars measured, the difference between rates of photosynthesis in infected and uninfected plants in the upper canopy reflected differences in photosynthesis within the lower canopy. Larger photosynthetic differences between infected and uninfected plants were observed in the older leaves but their overall contribution to plant daily carbon fixation will be lower than the younger uppermost leaves. These older leaves were measured at high PFD to determine their maximum potential for carbon fixation, but due to a stunting of infected plant stems, increased self shading will occur and could further reduce photosynthesis. Lower canopy photosynthesis in Ochuti is not affected by infection and in some leaves there is an elevation of photosynthesis above that of uninfected

plants. A similar stimulatory effect on rates of photosynthesis has been observed in the young leaves of *S. hermonthica*-infected sorghum at very low levels of infection (Cechin, 1994b; Cechin and Press, 1993a).

Parasite induced changes in growth and biomass partitioning

The ligule height data showed that *S. hermonthica* negatively influenced the vegetative growth of the maize and sorghum cultivars before the emergence of the parasite, thus giving an early warning of infection. As with the laboratory experiment, the influence of the parasite on ligule height was unaffected by whether or not the cultivars were tall or dwarf. Measurements of plant biomass of selected cultivars showed infected plants to have a lower stem biomass compared with uninfected plants. However the stem biomass of infected Ochuti plants was not significantly different from uninfected plants and so does not correspond to measured maximum ligule heights where less internode extension is observed. It may be that ligule height in this cultivar is not as sensitive an indicator for biomass as in the other cultivars studied and that infected plants have thicker stems. Infection does not alter the biomass partitioning to the leaves in any of the cultivars. SLA is also unaffected by infection thus the photosynthetic area of infected plants is not lowered. Above-ground biomass of *S. hermonthica* reflected the number of emerged parasites. There was a similar parasite biomass between maize cultivars but the sorghum cultivar CSH-1 had a greater *S. hermonthica* biomass compared with Ochuti. Because of the higher *S. hermonthica* biomass supported by CSH-1 compared with other hosts, it is not clear as to whether the large responses of growth and photosynthesis are because of this level of infection and whether less

severe responses would have occurred at lower infection. The large parasite biomass (and numbers of emerged parasites) on this dwarf cultivar may cause a great sink on the host and explain why CSH-1 was the most severely affected sorghum cultivar when compared with the taller cultivars supporting less *S. hermonthica* biomass, for example Ochuti.

The effect of *S. hermonthica* on foliar chlorophyll and nitrogen contents

Infection by *S. hermonthica* resulted in chlorophyll contents below those of uninfected plants in all maize and sorghum cultivars. The decline in chlorophyll concentration from the time of *S. hermonthica* emergence is coupled with lower rates of photosynthesis. Typically associated with the degradation of chloroplasts is a loss of photosynthetic enzymes and reduced electron transport which all contribute to lower carbon fixation (Hay and Walker, 1989). Nitrogen content was not affected in maize plants but there was a trend for infected sorghum plants to have lower nitrogen concentrations per unit leaf weight. Nitrogen uptake in plants is dependent on energy, required for active uptake and assimilation. More specifically, nitrogen utilisation is dependent on photosynthesis for the provision of energy and carbon skeletons for the incorporation of nitrogen into organic molecules (Ullrich, 1992; Marschner, 1993). The lower nitrogen concentrations in infected plants could be a consequence of decreased nitrogen assimilation or competition with the parasite for the nutrient. Less nitrogen uptake could occur in infected plants: first, because lower rates of photosynthesis in infected plants could lower the energy available for uptake and second, because of lower root biomass which could reduce the area for nitrogen

uptake. However, greater root:shoot ratios in infected plants (see Chapter 2) indicate that the area for uptake may not be limiting. *S. hermonthica* may also affect the partitioning of nitrogen into leaf chlorophyll in some cultivars.

S. hermonthica induced lowering of chlorophyll concentrations in the field contrast with some laboratory studies, where an increase in chlorophyll concentration has been observed in *S. hermonthica*-infected plants (Chapter 2; Smith *et al.*, 1995). The contrasting results may be explained by the degree of *S. hermonthica* infestation and differences between laboratory and field growth conditions. The low biomass of attached *S. hermonthica* plants in the laboratory study (Chapter 2) may cause a sink dependent stimulation of nitrate uptake. In the field greater levels of infestation may result in a larger nitrogen demand by the parasites and cause mobilisation of nitrogen from host pools, lowering leaf nitrogen concentrations compared with uninfected plants. In addition, the plants in the field were of an older age when the samples were taken (91 DAP) compared with the laboratory study (63 DAP). Foliar nitrogen and chlorophyll concentrations naturally decline with age (Hay and Walker, 1989) and the difference between the two studies may be accounted for by the difference in plant age when the samples were taken.

Interactions with other nutrients may also affect nitrogen concentrations for example phosphorus. Symptoms of phosphorous deficient plants include less leaf expansion and leaf initiation, an increase in chlorophyll concentration (Marschner, 1993), lower nitrate assimilation (Rufty, Mackown and Israel, 1990) and low phosphorus

concentrations in the chloroplast stroma can lower rates of photosynthesis (Fredeen, Raab and Madhusudana, 1990; Marschner, 1993). Many of these symptoms are observed with infection thus *S. hermonthica* may also affect phosphorus uptake or partitioning within the plant. Although soil samples are not available, field grown plants were not likely to be nutrient limiting. Fumigation of the soil is likely to have released microbial-bound nutrients resulting in a nutrient flush and there was a high fertiliser input. Quantification of nitrogen and phosphorous assimilation of infected and uninfected plants and the interaction of nutrients would be of interest.

Conclusions

This study demonstrates that *S. hermonthica*-infected sorghum and maize cereals do show lower rates of photosynthesis in the field, supporting laboratory studies. Sorghum cultivars SRN-39 and Ochuti show some tolerance to the presence of *S. hermonthica* as the parasite has less of a detrimental effect, with higher rates of photosynthesis and grain yield compared with infected plants of the other cultivars studied. There may be a relationship between tolerance to the parasite and an ability to maintain high rates of photosynthesis when infected.

The photosynthesis and growth data demonstrate that under laboratory and field conditions the host plants are responding to infection in a similar manner although there are slight difference in the degree of response. An explanation for these differences between the studies may be due to the prevailing environmental conditions such as light, temperature and water supply. In addition there may be differences in the

nutrient status of the cereals between the studies, especially the nitrogen status of infected plants. Laboratory and field studies have demonstrated a role of nitrogen in the host-parasite association as nitrogen appears to alleviate some of the detrimental effects of *S. hermonthica* infection. There have been many reports of the effect of nitrogen fertiliser on both the germination and attachment of *S. hermonthica* and a subsequent increase in host biomass and yield (see e.g. Parker and Riches, 1993; Press and Graves, 1995). However, there is limited information on the importance of nitrogen after attachment of the parasite. The next chapter aims to examine the role of nitrogen fertilisation in the *S. hermonthica-Sorghum bicolor* association, by controlling both the concentration of nitrogen and its application before and after *S. hermonthica* attachment.

Chapter 4

The influence of nitrogen on the *S. hermonthica*-sorghum association before and after attachment of the parasite

4.1 Introduction

Chapters 2 and 3 demonstrated that *S. hermonthica* infection of sorghum and maize cultivars resulted in an alteration of growth and photosynthesis of the host plant when compared with uninfected plants. Infection lowered the rate of instantaneous gas exchange and lowered plant biomass accumulation. A lower biomass partitioning to the stem and an associated increase in biomass partitioning to the root, increased the root:shoot ratio compared with uninfected plants. The extent to which *S. hermonthica* alters growth and photosynthesis is in part dependent on the host genotype (see Chapters 2 and 3), however, the carbon budget of the host-parasite association will also be dependent on the carbon and nutrient supply. The degree of infestation and response of the host to infection has been negatively correlated with an increase in nitrogen supply and in turn the nutritional status of the host (see e.g. Pieterse and Verkleij, 1991).

Laboratory studies have shown that this may be explained by the effect of nitrogen on the germination and attachment of *S. hermonthica* to the roots of the host. After a period of after-ripening and preconditioning (see Introduction), *S. hermonthica* seeds will complete germination in the presence of a stimulatory compound, occurring in the root exudate of host plants, together with those of a few non-host plants (see e.g. Musselman, 1980; Worsham, 1987; Okonkwo, 1991; Boone *et al.*, 1995). A second

series of stimulants thought to be present on the surface of the root, induces haustorial formation (see e.g. Musselman, 1980; Riopel and Timko, 1995). If successful attachment and penetration of the haustoria on the root of the host occurs, the parasite will develop. These stages in the lifecycle of *S. hermonthica* represent possible targets for the control of the parasite. Laboratory studies have shown that nitrogen plays a central role in the inhibition of germination and attachment of *S. hermonthica* (Pieterse and Verkleij, 1991; Cechin and Press, 1993b) and is thought to act by reducing the production of stimulatory compounds by the roots of the host.

Infected cereals grown under high nitrogen conditions (provided by the application of nitrogen fertiliser) often have increased grain yields and biomass accumulation compared with infected cereals grown in poor nutrient soils This increase in production is often associated with a decrease in the numbers of attached *S. hermonthica* (Bebawi, 1981, 1987; Farina *et al.*, 1985; Hess and Ejeta, 1987; Raju *et al.*, 1990; Agbobli, 1991; Osman *et al.*, 1991). An understanding of the role of nitrogen in the amelioration of the effects of *S. hermonthica* on its host, is of fundamental importance if it is to be employed as an effective control strategy. However, the extent to which increased performance of the infected host is attributable to the lower numbers of attached parasites is uncertain since nitrogen may also play a role in alleviating the detrimental effects of the parasite after *S. hermonthica* attachment. Plant growth and yield are dependent upon rates of photosynthesis, which in turn can be dramatically affected by nitrogen availability. The carbon economy and nitrogen economy of a plant are closely related, and there is a

positive correlation between light saturated rates of photosynthesis and foliar nitrogen concentrations (Field and Mooney, 1986; Evans, 1989). This greater nitrogen supply would influence the photosynthesis activity and hence carbon accumulation of both host and parasite.

This chapter aims to address the role of ammonium nitrate on both pre- and post-attachment phases of the *S. hermonthica-Sorghum bicolor* association. Plants were supplied with nutrient solution containing low or high nitrogen supply to determine the effects on nitrogen on the early phase of *S. hermonthica* attachment and subsequent growth of the host. Rhizotrons (described in Chapter 2) allow the exact timing of *S. hermonthica* attachment to be observed so a number of plants can receive an increase in nitrogen supply after substantial parasite attachment. This allows the concentration of nitrogen to be controlled both before *S. hermonthica* germination and more importantly it can be increased after *S. hermonthica* attachment to examine the subsequent role of nitrogen on the host-parasite relationship. Chapters 2 and 3 demonstrated ligule height, biomass partitioning and photosynthesis of sorghum plants to be sensitive indicators of infection, thus this chapter reports: i) the response of growth and allometry to nitrogen supply in infected and uninfected plants, ii) the influence of nitrogen on photosynthesis at growth and light saturating irradiances in infected and uninfected plants, iii) the effect of nitrogen supply on the biomass and numbers of attached *S. hermonthica* and iv) the response of foliar nitrogen and chlorophyll concentrations to nitrogen supply in infected and uninfected plants.

4.2 Materials and Methods

4.2.1 Plant material and growth conditions

Sorghum cultivar CSH-1 was used for this study and infected with *Striga hermonthica* seeds, collected from maize hosts at Kibos, western Kenya in 1993. Sterilisation and germination procedures of the sorghum seeds were carried out as described in Chapter 2 Section 2.2.3. Plants were grown in controlled environment cabinets (Fisons, Fitotron PG1700), operating with a 12 hour photoperiod and a PFD of 550 µmol quanta $m^{-2} s^{-1}$ at plant height. Day/night temperatures were maintained at 30/20 °C. Ambient CO_2 (350 ppm) and relative humidity (50%) were maintained throughout the study.

4.2.2 Experimental design

Rhizotrons were designed to allow an observation of the cereal roots and the development of *S. hermonthica* throughout the period of study as described in Chapter 2 Section 2.2.1. Preconditioning of the *S. hermonthica* seed and infection of the rhizotrons were conducted as described in Chapter 2 Section 2.2.2.

Germinated sorghum seeds were transferred to 10 ml plastic vials (see Chapter 2 Section 2.2.3) containing 40% full strength Long Ashton nutrient solution with 20% ammonium nitrate. After 7 days a single seedling was transferred to each rhizotron with the roots evenly spread out over the surface of the sand. Ninety six rhizotrons were established, 48 of which were infected with *S. hermonthica*. The rhizotrons were drip-fed with a 40% full strength Long Ashton solution four times during each photoperiod to give a total volume of 200 ml per day. Sixty four of the rhizotrons

were supplied with Long Ashton solution containing 1.0 mol m^{-3} ammonium nitrate (2.0 mol m^{-3} nitrogen) and the remaining 32 were supplied with 3.5 mol m^{-3} ammonium nitrate (7.0 mol m^{-3} nitrogen). At 37 DAP, 32 of the rhizotrons being supplied with 1.0 mol m^{-3} ammonium nitrate had the nitrogen concentration increased to 3.5 mol m^{-3} ammonium nitrate for the remaining duration of the study. This time was selected because by this stage germination and attachment of the *Striga* plants was largely completed.

The rhizotrons were placed in two controlled environment cabinets (see 4.2.1) in a fully randomised design and the chambers were exchanged between cabinets at 7 day intervals to avoid possible growth differences.

4.2.3 Growth measurements

At regular intervals from 26 to 58 DAP, the number of developing *S. hermonthica* plants per host plant was observed through the perspex sheets on the entire surface root system.

In addition, both destructive and non-destructive growth measurements were made. At 7 intervals between 14 and 58 days after planting (DAP) plant height was measured from the base of the stem to the youngest visible ligule on all of the plants.

Destructive measurements of plant biomass were determined at 37, 51 and 62 DAP on 4-6 individual plants at each harvest. The sorghum plants were separated into stems,

leaves, roots and tillers (where present). Roots were separated from the sand by careful washing over a 2 mm meshed sieve after which the *S. hermonthica* were dissected from the roots at the point of tubercle attachment. The plant material was oven dried at 70 °C for 72 h prior to weighing. Total leaf area was calculated by measuring sections of fully expanded leaves using a leaf area meter (Delta- T Devices Ltd, Cambridge, UK) which were then oven dried and weighed.

Specific leaf area (SLA), leaf area ratio (LAR) and leaf weight ratio (LWR), were each calculated using the six harvested plants at 62 DAP. The root:shoot ratio (R:S) was calculated at each harvest. The variables were calculated by using the equations in Chapter 2 Table 2.3.

4.2.4 Gas exchange measurements

Rates of photosynthesis were measured on infected and uninfected sorghum plants between 26 and 54 DAP (data shown for 54 DAP only), using a portable infra-red gas analyser (LCA4, Analytical Development Company (ADC), Hoddesdon, UK) as described in Chapter 2 Section 2.2.5. Gas exchange measurements were recorded at the growth PFD of 550 μmol quanta m^{-2} s^{-1} and a light saturating PFD of 2000 μmol quanta m^{-2} s^{-1}, supplied by a Schott KL1500T lamp.

4.2.5 Foliar chlorophyll concentration

At 7 intervals from 14 to 56 DAP, non-destructive measurements of foliar chlorophyll concentration were made on the youngest fully expanded leaf using a chlorophyll

meter (SPAD-502, Minolta Camera Co Ltd, Osaka, Japan) to coincide with measurements of gas exchange. Six measurements were made halfway along the leaf either side of the midrib. Readings from the meter were calibrated against the chlorophyll concentration of leaves on an area basis as determined by extraction in acetone (Schaper and Chacko, 1991; Piekielek and Fox, 1992) (see Chapter 2 Section 2.2.6).

4.2.6 Foliar nitrogen concentration

Leaf tissue samples were taken from the sorghum plants 54 DAP for analysis of total nitrogen. 4 cm^2 discs were taken from the youngest fully expanded leaf and dried at 70 °C for 72 hours and weighed. Total nitrogen was analysed as described in Chapter 2 Section 2.2.7.

4.2.7 Statistical analysis

The effect of the nitrogen treatments on the attachment of *S. hermonthica* was analysed using non-parametric Kruscall-Wallis procedures (Minitab statistical package, version 10.2) followed by non-parametric multiple comparison procedures (Zar, 1984). Treatment effects (the response of sorghum to *S. hermonthica* infection and the different responses between the nitrogen treatments) were analysed using two-way analysis of variance procedures for a randomised block design (Minitab, 10.2). Where different sample sizes were used, for example between harvests or within measurements at one time point because of plant death, general linear model was

employed. Tukey's multiple comparison tests were carried out on the original data using the appropriate analysis for both equal and unequal sample sizes (Zar, 1984).

4.3 Results

4.3.1 *S. hermonthica* attachment

Throughout the experimental period there was no parasite emergence above the surface of the sand in the rhizotrons. Attachment was first observed on sorghum plants grown at 1.0 mol m^{-3} ammonium nitrate at 26 DAP (Figure 4.1). There was a marked increase in the number of attached parasites with time and by 56 DAP sorghum plants supported 40 parasites per host. Sorghum plants initially grown at 1.0 mol m^{-3} ammonium nitrate and then supplied with 3.5 mol m^{-3} ammonium nitrate from 37 DAP, showed no further attachment of *S. hermonthica* to the host roots after this increase in ammonium nitrate supply. Instead a decrease in the numbers of attached *S. hermonthica* was observed over time. By 56 DAP these sorghum plants supported 18 parasites per host, 45% below that of plants grown at 1.0 mol m^{-3} ammonium nitrate. Sorghum plants grown at 3.5 mol m^{-3} ammonium nitrate throughout the study showed *S. hermonthica* attachment by 33 DAP, but the number of parasites did not exceed 1 or 2 per plant.

The biomass of *S. hermonthica* at 37, 51 and 62 DAP (Figure 4.2) showed a similar pattern to the numbers of attached *S. hermonthica* observed through the perspex sheets (Figure 4.1). Significant differences existed between the biomass of *S. hermonthica* on sorghum plants grown at 1.0 and 3.5 mol m^{-3} ammonium nitrate at the

first harvest and this difference increased with each successive harvest. Sorghum plants that received an increase in ammonium nitrate supply from 37 DAP, showed no difference in the biomass of *S. hermonthica* at the second harvest, fourteen days after the increase in ammonium nitrate, compared with plants grown at 1.0 mol m^{-3} ammonium nitrate. At the final harvest, 62 DAP, the biomass of *S. hermonthica* was significantly different between the three ammonium nitrate treatments with plants grown at 1.0-3.5 and 3.5 mol m^{-3} ammonium nitrate supporting 62.2% and 99.2% less *S. hermonthica* plants, respectively, compared with plants grown at 1.0 mol m^{-3} ammonium nitrate. There was no significant relationship between the number and biomass of *S. hermonthica* plants (Figure 4.3). A large increase in the number of *S. hermonthica* plants at 1.0 and 1.0-3.5 mol m^{-3} ammonium nitrate resulted in a small increase in *S. hermonthica* biomass with the regression lines not differing significantly from zero. At 3.5 mol m^{-3} ammonium nitrate there were few attached parasites but even at this low level of infection, the slope did not differ significantly with the other nitrogen treatments. However, there was a significant effect of ammonium nitrate on *S. hermonthica* biomass with a lower mean biomass at 1.0-3.5 mol m^{-3} ammonium nitrate compared with 1.0 mol m^{-3} ammonium nitrate. *S. hermonthica* biomass at 3.5 mol m^{-3} ammonium nitrate was lower compared with all other treatments for any given number of attached parasites. The calculated mean biomass of each parasite (mean weight / mean number) decreased with an increase in ammonium nitrate supply to the host plant. Mean parasite weights of 4.05, 3.23 and 1.24 mg were found in treatments of 1.0, 1.0-3.5 and 3.5 mol m^{-3} ammonium nitrate, respectively.

4.3.2 Sorghum growth and biomass accumulation

Large differences were observed between the height to the youngest ligule on infected and uninfected sorghum plants grown at 1.0 mol m^{-3} and 1.0 increased to 3.5 mol m^{-3} ammonium nitrate (Figure 4.4). By 43 DAP the height differences were significant between infected and uninfected plants ($p < 0.05$) and at each subsequent time point these differences became more marked. By 56 DAP the ligule heights of infected sorghum hosts at 1.0 mol m^{-3} and 1.0 increased to 3.5 mol m^{-3} ammonium nitrate were 12.0% and 13.7% below uninfected plants, respectively. Throughout the measurement period the maximum ligule heights between infected and uninfected plants grown at 3.5 mol m^{-3} ammonium nitrate did not differ significantly.

Total plant dry weight and dry weight partitioning in sorghum plants was affected by the supply of ammonium nitrate and the presence of *S. hermonthica*. At the first harvest, 37 DAP (Figure 4.5A), plant dry weights were similar for uninfected and infected plants at each ammonium nitrate treatment. Plants supplied with 3.5 mol m^{-3} ammonium nitrate had a significantly greater biomass accumulation compared with plants supplied with 1.0 mol m^{-3} ammonium nitrate, with greater root, stem, leaf and tiller biomass. At the second harvest, 51 DAP (Figure 4.5B), total biomass in infected plants was still unaffected by *S. hermonthica* at 1.0 and 3.5 mol m^{-3} ammonium nitrate. However, a marked difference in biomass accumulation was observed between infected and uninfected sorghum plants where the ammonium nitrate supply had been increased from 1.0 to 3.5 mol m^{-3} ammonium nitrate. This difference in biomass appeared to be due to the stimulation of biomass accumulation in the roots, stem and

leaves of the uninfected plants with the increase in ammonium nitrate. At the last harvest, 62 DAP (Figure 4.5C), infection by *S. hermonthica* resulted in less biomass accumulation compared with uninfected hosts when grown at 1.0 and 1.0 increased to 3.5 mol m^{-3} ammonium nitrate. Total biomass accumulation in sorghum plants grown at 3.5 mol m^{-3} ammonium nitrate was not affected by the presence of *S. hermonthica* and plants were significantly larger compared with those subjected to the other ammonium nitrate treatments.

A closer examination of the components of the infected plants (Table 4.1), revealed that there was an effect of both the ammonium nitrate supply and the presence of *S. hermonthica* on the dry weight partitioning within the host at 62 DAP. Infection by *S. hermonthica* resulted in significantly less biomass being partitioned into the stem compared with uninfected plants in all of the ammonium nitrate treatments. Uninfected plants grown at 3.5 mol m^{-3} ammonium nitrate had significantly greater stem biomass compared with plants grown at 1.0 mol m^{-3} ammonium nitrate. The effect of *S. hermonthica* on stem biomass accumulation was partially alleviated at this ammonium nitrate supply with the stem biomass exceeding that of infected plants at the two other ammonium nitrate treatments. The effect of *S hermonthica* on leaf biomass followed the same trend as stem biomass with less biomass being partitioned into the leaves with infection at all ammonium nitrate treatments. Again, nitrogen supplied as 3.5 mol m^{-3} ammonium nitrate resulted in greater leaf biomass accumulation compared with the other ammonium nitrate treatments. Infected plants at 3.5 mol m^{-3} ammonium nitrate had a greater leaf biomass than infected plants at the other ammonium nitrate

treatments. Tiller production was unaffected by infection but was stimulated by a high ammonium nitrate supply.

The negative effect of *S. hermonthica* on stem growth and the increase in biomass allocation to the root resulted in an alteration of the allometry of infected plants (Figure 4.6). Early harvests at 37 and 51 DAP showed no significant change in the root:shoot ratios in the presence of *S. hermonthica* at any ammonium nitrate supply. By 62 DAP, greater root:shoot ratios were observed in infected plants compared with uninfected plants grown at 1.0 mol m^{-3} ammonium nitrate. Infected plants grown at 3.5 mol m^{-3} ammonium nitrate showed no significant change in the root:shoot ratio compared with uninfected plants. At this higher ammonium nitrate supply both infected and uninfected plants allocated a greater proportion of biomass into above ground components resulting in lower root:shoot ratios compared with plants grown at 1.0 mol m^{-3} ammonium nitrate. Where the nitrogen supply was increased from 1.0 to 3.5 mol m^{-3} ammonium nitrate infected plants showed no significant change in the root:shoot ratio compared with uninfected plants. Infected plants had a significantly lower root:shoot ratio compared with infected plants grown at 1.0 mol m^{-3} ammonium nitrate because of a greater proportion of biomass allocated to the shoots.

The LWR, LAR and SLA were unaffected by the presence of *S. hermonthica* or the supply of ammonium nitrate (Table 4.2), with the exception of the LWR of infected plants that received 1.0 increased to 3.5 mol m^{-3} ammonium nitrate. An increase in the leaf dry weight with no change in root biomass resulted in an increase in LWR above

that of infected plants at 1.0 and 3.5 mol m^{-3} ammonium nitrate. The LAR of these plants was also increased although this data was only significant at $p < 0.1$.

4.3.3 Foliar nitrogen and chlorophyll concentration

Figure 4.7 shows the chlorophyll concentration of the youngest fully expanded leaf of infected and uninfected sorghum plants. At 1.0 mol m^{-3} ammonium nitrate (Figure 4.7A), between 12 and 42 DAP the chlorophyll concentration did not vary between uninfected and infected plants. After this period there was a continued increase in the chlorophyll concentration of the infected plants with the uninfected plants maintaining a constant level. By 56 DAP infected plants had chlorophyll concentrations 15.3% above those of control plants. The chlorophyll concentration of plants grown initially at 1.0 mol m^{-3} ammonium nitrate and then increased to 3.5 mol m^{-3} ammonium nitrate showed no response to infection (Figure 4.7B). After 37 DAP when the ammonium nitrate supply was increased, the chlorophyll concentration also increased and by 56 DAP contents were similar to plants grown at high ammonium nitrate (Figure 4.7C). In plants grown at 3.5 mol m^{-3} ammonium nitrate (Figure 4.7C) the presence of *S. hermonthica* had no effect on leaf chlorophyll compared with controls. Throughout the study the chlorophyll concentrations of these plants exceeded that of plants grown at a lower ammonium nitrate supply.

Foliar nitrogen concentrations were significantly lower in plants supplied with 1.0 mol m^{-3} ammonium nitrate compared with plants supplied with 3.5 mol m^{-3} ammonium nitrate and also 1.0 increased to 3.5 mol m^{-3} ammonium nitrate (Table 4.3). At these

two high ammonium nitrate treatments, infection had no effect on foliar nitrogen. Infected plants grown at 1.0 mol m^{-3} ammonium nitrate had 29.9% more nitrogen compared with uninfected plants.

4.3.4 Gas exchange

Rates of steady state photosynthesis measured at 2000 μmol quanta m^{-2} s^{-1} were approximately double those measured at 500 μmol quanta m^{-2} s^{-1} (Table 4.3). Ammonium nitrate supply had no effect on the rate of photosynthesis in uninfected plants measured at both PFDs. Significantly lower rates of photosynthesis were observed in infected plants grown at 1.0 mol m^{-3} ammonium nitrate with rates being 32.2% and 25.6% below those of control plants at 500 and 2000 μmol quanta m^{-2} s^{-1}, respectively. This was mirrored by lower rates of transpiration and stomatal conductance (data not shown) as previously observed in Chapters 2 and 3. *S. hermonthica* had no significant effect on photosynthesis at the higher ammonium nitrate treatments accompanied with no differences in transpiration and stomatal conductance between infected and uninfected plants (data not shown). Greater differences between infected and uninfected plants grown at 1.0 mol m^{-3} ammonium nitrate were observed when photosynthesis was expressed on a nitrogen and chlorophyll basis (expressed using rates of photosynthesis recorded at 2000 μmol quanta m^{-2} s^{-1}). This is because of a marked lowering of photosynthesis in infected plants coupled with an increase in chlorophyll and nitrogen concentrations.
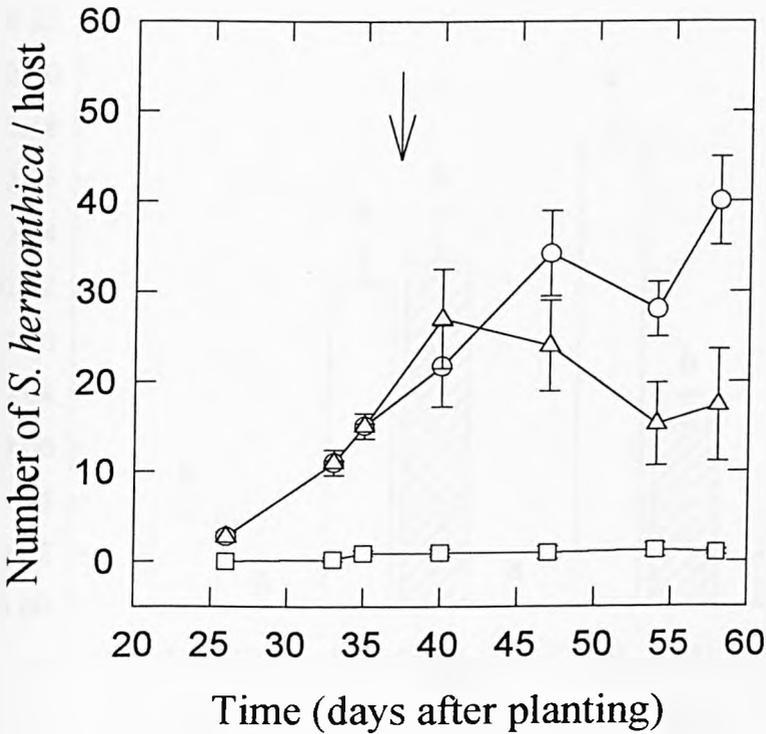
Figure 4.1 The number of attached *S. hermonthica* on sorghum plants from 26 to 58 DAP. Sorghum plants were grown in Long Ashton nutrient solution containing 1.0 (circles), 1.0-3.5 (triangles) and 3.5 (squares) mol m^{-3} ammonium nitrate. Means and standard errors of 4-16 replicates are reported and the data analysed using Kruscal-Wallis procedures (see text). The change in nitrogen concentration from 1.0 to 3.5 mol m^{-3} ammonium nitrate is denoted by the arrow.
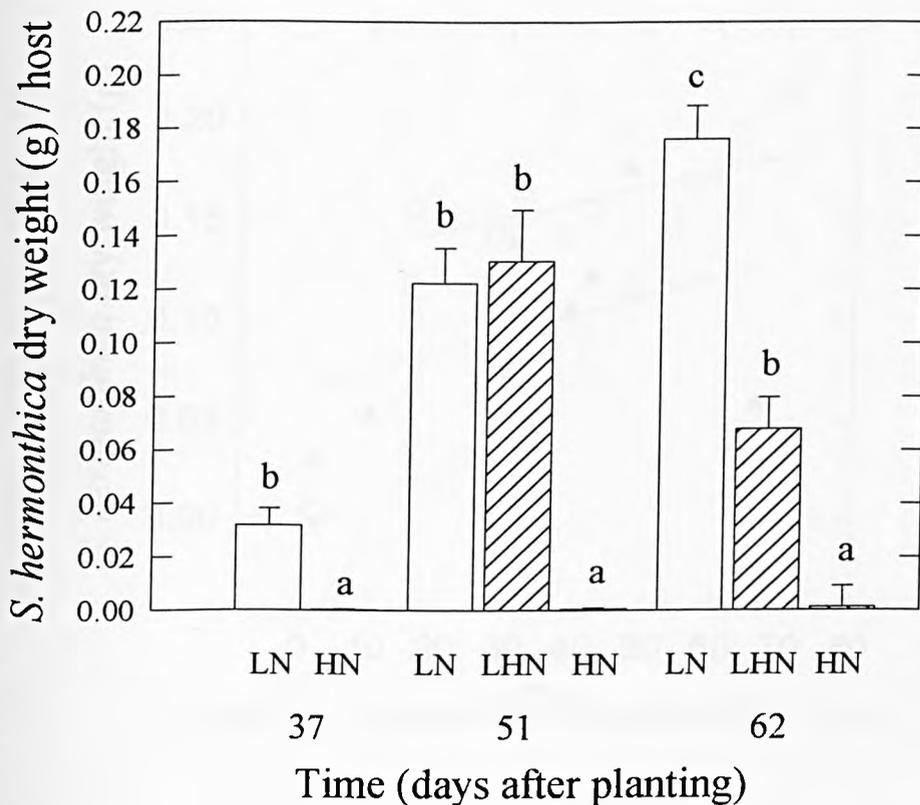
Figure 4.2 Dry weight of *S. hermonthica* on sorghum plants at 37, 51 and 62 DAP. Plants were grown in Long Ashton nutrient solution containing 1.0 (LN), 1.0-3.5 (LHN) and 3.5 (HN) mol m^{-3} ammonium nitrate. Means and standard errors of 4-6 plants are reported and analysed using analysis of variance procedures followed by Tukey's multiple comparison tests. Bars not sharing the same letter at each harvest are significantly different (p < 0.05).
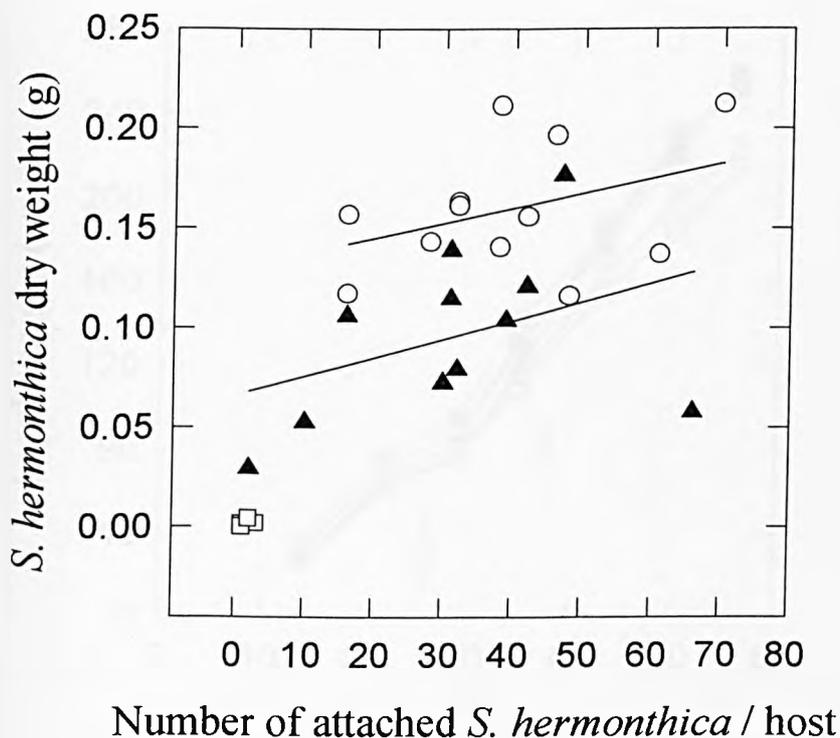
Figure 4.3 The relationship between the number of attached *S. hermonthica* and *S. hermonthica* dry weight (g) per host plant at 51 and 62 DAP (data presented together). Plants were grown in Long Ashton solution containing 1.0 (open circles), 1.0-3.5 (closed triangles) and 3.5 (open squares) mol m^{-3} ammonium nitrate. The regression equations are 1.0 mol m^{-3}, y = 0.130 + 0.0007x, (r^2 = 0.14), 1.0-3.5 mol m^{-3}, y = 0.066 + 0.0009x, (r^2 = 0.16), 3.5 mol m^{-3}, y = 0 + 0.0010, (r^2 = 0.33). Analysis of covariance procedures showed the slopes of the regression lines to not differ significantly (p > 0.8) but nitrogen had a significant effect on the y-intercept (p < 0.001).
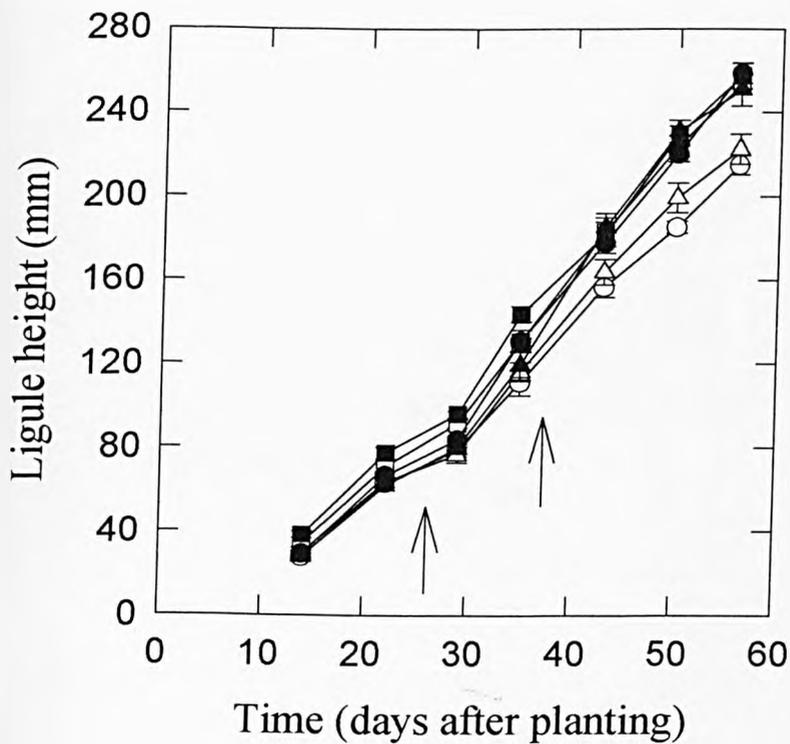
Figure 4.4 Distance from the base of the stem to the youngest ligule of sorghum plants grown in the absence (closed symbols) or presence (open symbols) of *S. hermonthica* and in Long Ashton nutrient solution containing 1.0 (circles), 1.0-3.5 (triangles) and 3.5 (squares) mol m^{-3} ammonium nitrate. Means and standard errors of 6-8 replicates are reported and the data analysed using analysis of variance procedures (see text). The first arrow denotes the first attached *S. hermonthica* and the second arrow denotes the change in ammonium nitrate concentration from 1.0 to 3.5 mol m^{-3} ammonium nitrate.

Figure 4.5 Dry weight partitioning in sorghum plants in the absence (-) or presence (+) of *S. hermonthica* at 37, 51 and 62 DAP. Plants were grown in Long Ashton nutrient solution containing 1.0 (LN), 1.0-3.5 (LHN) and 3.5 (HN) mol m^{-3} ammonium nitrate. R, S, L and T indicate root, stem, leaf and tiller dry matter. Means of 4-6 plants are given and the data analysed using analysis of variance procedures (see text).

(A) 37 DAP

(B) 51 DAP

(C) 62 DAP

T
L
S
R

LN

LHN

HN

Treatment

Dry weight (g)
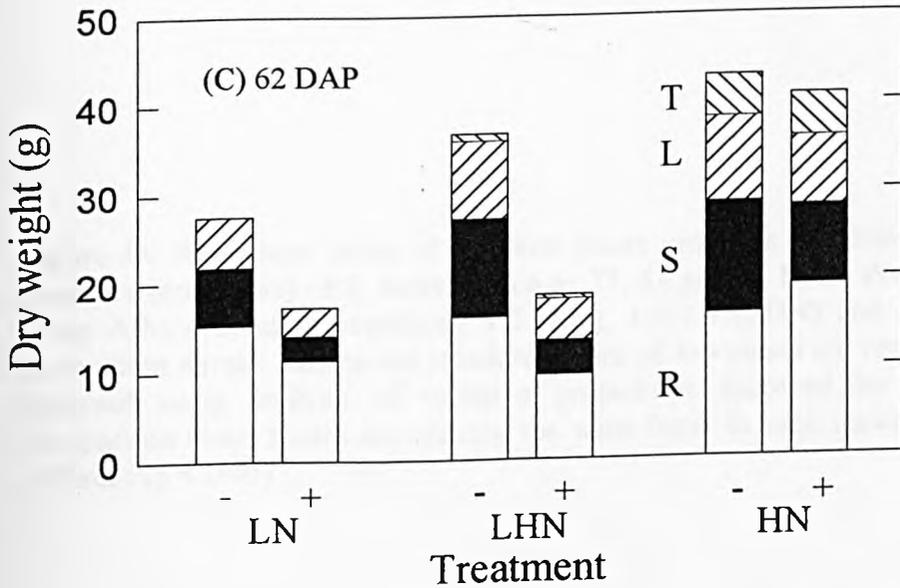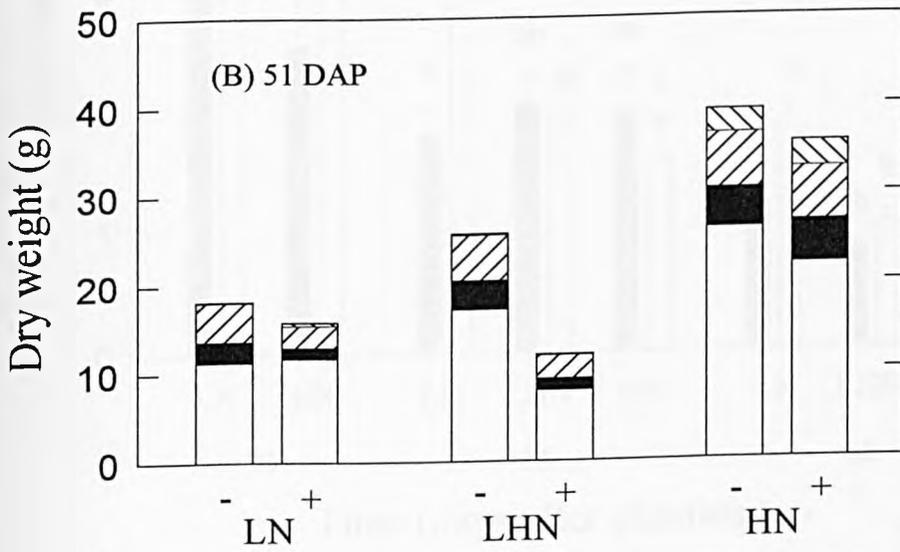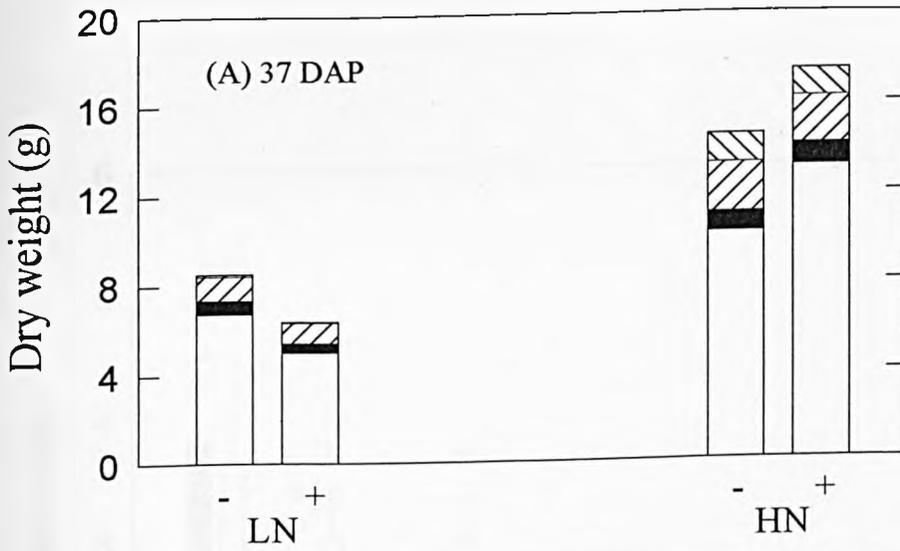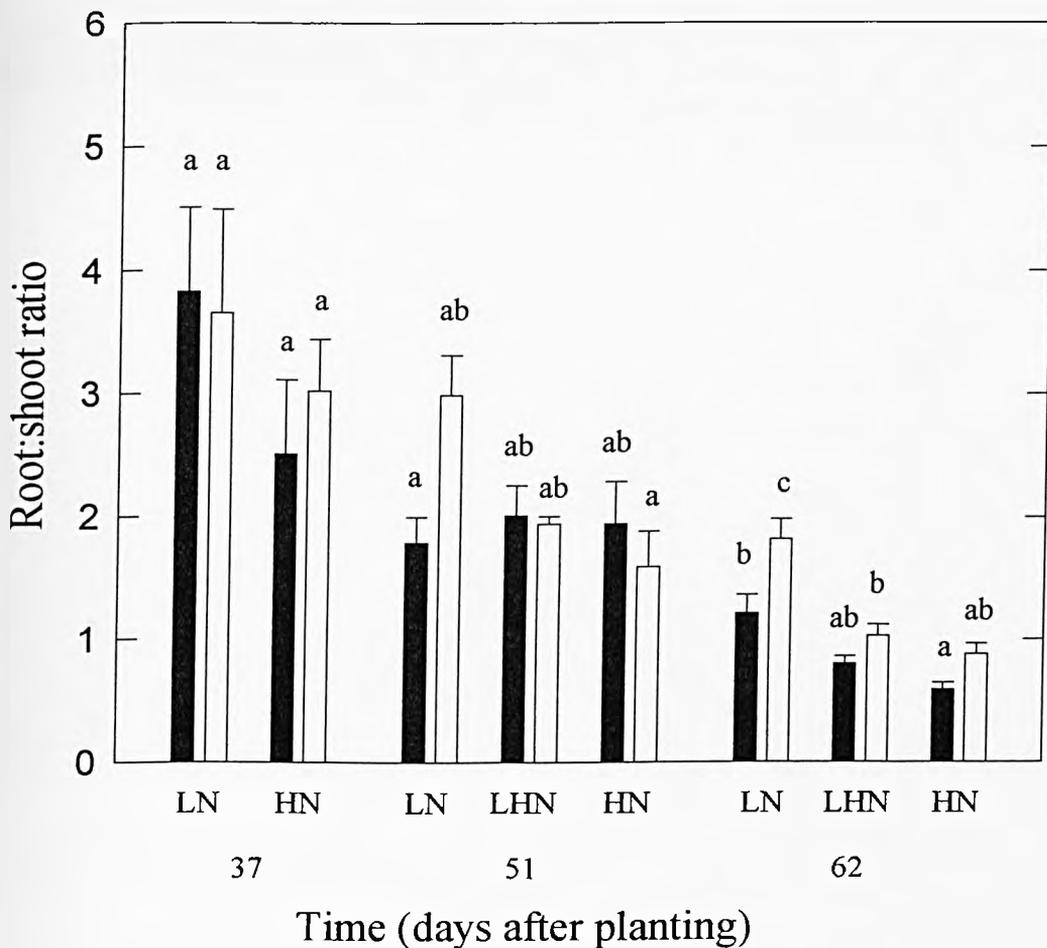
Figure 4.6 Root:shoot ratios of sorghum plants grown in the absence (filled bars) or presence (open bars) of *S. hermonthica* at 37, 51 and 62 DAP. Plants were grown in Long Ashton solution containing 1.0 (LN), 1.0-3.5 (LHN) and 3.5 (HN) mol m^{-3} ammonium nitrate. Means and standard errors of 4-6 plants are reported and the data analysed using analysis of variance procedures followed by Tukey's multiple comparison tests. Means not sharing the same letter in each harvest are significantly different (p < 0.05).

Figure 4.7 Chlorophyll concentration in the youngest fully expanded leaf of sorghum plants grown in the absence (closed symbols) or presence (open symbols) of *S. hermonthica* and in Long Ashton nutrient solution containing 1.0, (circles), 1.0-3.5 (triangles) and 3.5 (squares) mol m^{-3} ammonium nitrate. Means and standard errors of 6-8 replicates are reported and the data analysed using analysis of variance procedures (see text). The first arrow denotes the first attached *S. hermonthica* and the second arrow (Figure B) denotes the change in ammonium nitrate concentration from 1.0 to 3.5 mol m^{-3} ammonium nitrate.

Chlorophyll content ($\mu g\ cm^{-2}$)

(A) 1 mol m^{-3} NH$_4$NO$_3$

(B) 1 - 3.5 mol m^{-3} NH$_4$NO$_3$

(C) 3.5 mol m^{-3} NH$_4$NO$_3$

Time (days after planting)
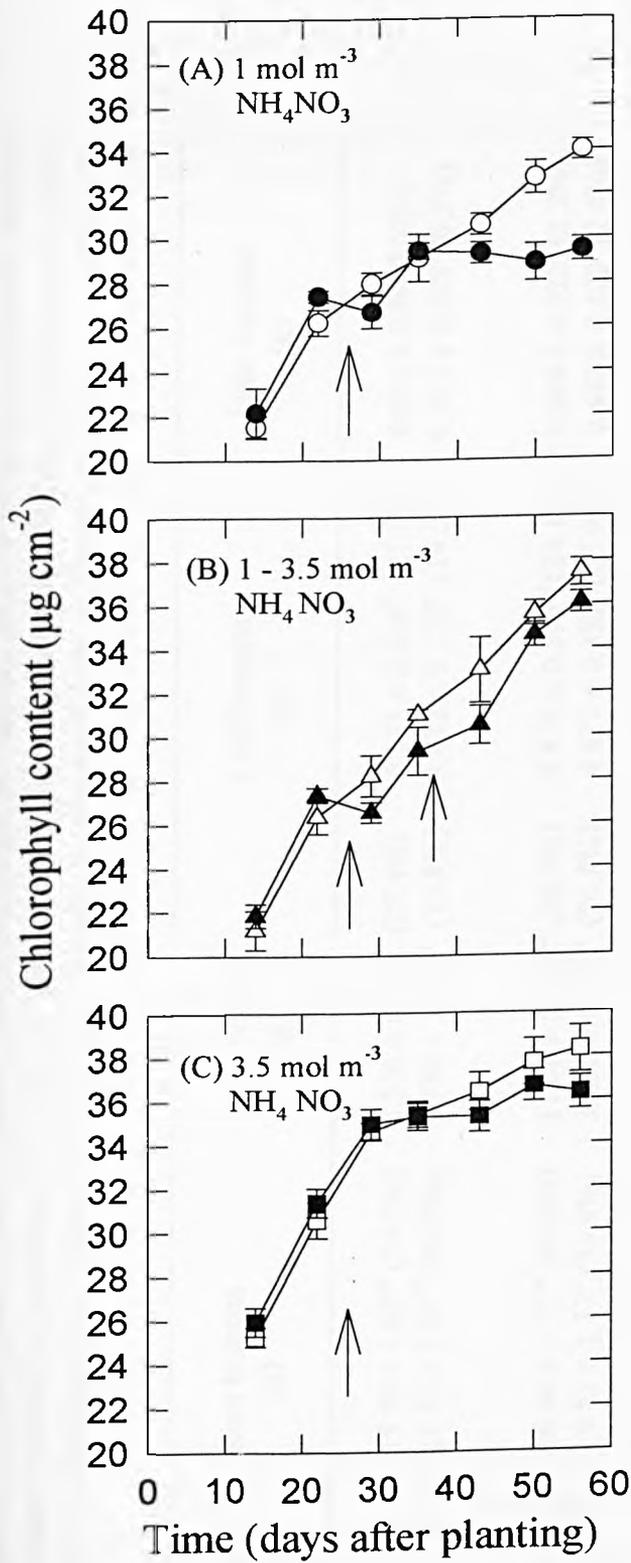
Table 4.1 Dry weight partitioning of sorghum plants in the absence (-) or presence (+) of *S. hermonthica* at 62 DAP. Plants were grown in Long Ashton solution containing 1.0, 1.0-3.5 and 3.5 mol m^{-3} ammonium nitrate. Means and standard errors of 4-6 plants are reported and the percentage of the total biomass reported in parenthesis. The original data was analysed using two way analysis of variance procedures followed by Tukey's multiple comparison tests. Means not sharing a common superscripted letter in the same column are significantly different ($p < 0.05$).

NH$_4$NO$_3$ (mol m^{-3})	*Striga*	Total plant biomass (g)	Root biomass (g)	Stem biomass (g)	Leaf biomass (g)	Tiller biomass (g)
1.0	-	27.98 ± 1.90^{bc}	15.29 ± 1.02^{ab} (54.7%)	6.556 ± 0.718^{b} (23.4%)	6.132 ± 0.236^{b} (21.9%)	0.000 ± 0.000^{a} (0%)
	+	17.56 ± 1.13^{a}	11.31 ± 1.01^{ab} (64.4%)	2.780 ± 1.010^{a} (15.8%)	3.432 ± 0.151^{a} (19.5%)	0.033 ± 0.029^{a} (0.2%)
1.0 - 3.5	-	36.84 ± 3.11^{cd}	16.06 ± 1.12^{bc} (43.6%)	11.08 ± 2.060^{bc} (30.1%)	8.873 ± 0.570^{cd} (24.1%)	0.818 ± 0.525^{a} (2.2%)
	+	18.67 ± 1.76^{ab}	9.51 ± 1.17^{a} (50.9%)	3.880 ± 0.306^{a} (20.8%)	4.817 ± 0.353^{a} (25.9%)	0.443 ± 0.100^{a} (2.4%)
3.5	-	43.49 ± 1.73^{d}	16.10 ± 1.28^{bc} (37.0%)	12.57 ± 1.280^{c} (29.0%)	10.16 ± 0.195^{d} (23.4%)	4.644 ± 0.487^{b} (10.6%)
	+	41.09 ± 3.66^{d}	19.21 ± 2.34^{c} (46.8%)	8.94 ± 2.340^{b} (21.8%)	8.090 ± 0.529^{cb} (19.7%)	4.838 ± 0.755^{b} (11.7%)

Table 4.2 LWR, LAR and SLA of sorghum plants grown in the absence (-) or presence (+) of *S. hermonthica* at 62 DAP. Plants were grown in Long Ashton nutrient solution containing 1.0, 1.0-3.5 and 3.5 mol m^{-3} ammonium nitrate. Means and standard errors of 4-6 plants are given and the data analysed using analysis of variance procedures followed by Tukey's multiple comparison tests. Means with different superscripted letters in the same column are significantly different (p < 0.05).

Ammonium nitrate (mol m^{-3})	*Striga*	SLA (cm^2 g^{-1})	LWR (g g^{-1})	LAR (cm^2 g^{-1})
1.0	-	336.3 ± 4.1[b]	0.221 ± 0.007[a]	66.81 ± 2.81[a]
	+	323.5 ± 5.6[ab]	0.198 ± 0.012[a]	58.48 ± 4.22[a]
1.0 - 3.5	-	323.6 ± 6.1[ab]	0.243 ± 0.009[ab]	78.05 ± 4.56[a]
	+	288.8 ± 14.2[a]	0.262 ± 0.012[b]	73.67 ± 5.75[a]
3.5	-	303.1 ± 10.1[ab]	0.235 ± 0.009[ab]	67.34 ± 4.84[a]
	+	308.2 ± 6.1[ab]	0.201 ± 0.018[a]	59.28 ± 6.08[a]

Table 4.3 Rates of photosynthesis (A) at 550 and 2000 μmol quanta m^{-2} s^{-1} of sorghum plants in the absence (-) or presence (+) of *S. hermonthica* at 56 DAP. Rates of photosynthesis at 2000 μmol m^{-2} s^{-1} are also expressed per unit nitrogen and chlorophyll. Plants were grown in Long Ashton solution containing 1.0, 1.0-3.5 and 3.5 mol m^{-3} ammonium nitrate. Means and standard errors of 4-8 plants are reported and the data analysed using analysis of variance procedures followed by Tukey's multiple comparison tests. Means not sharing the same superscripted letter within each column are significantly different ($p < 0.05$).

| Ammonium nitrate (mol m^{-3}) | *Striga* | Measurement PFD (μmol quanta m^{-2} s^{-1}) | | Nitrogen (mg g^{-1}) | PNUE (μmol CO_2 mol N s^{-1}) | A (μmol CO_2 mg chl h^{-1}) |
		550 A (μmol CO_2 m^{-2} s^{-1})	2000 A (μmol CO_2 m^{-2} s^{-1})			
1.0	-	13.58 ± 0.277^c	24.88 ± 1.31^b	25.48 ± 1.28^a	432.9 ± 45.3^b	30.76 ± 1.44^c
	+	9.22 ± 0.933^a	18.52 ± 1.69^a	33.12 ± 1.30^b	224.6 ± 29.8^a	19.58 ± 1.87^a
1.0 - 3.5	-	12.95 ± 0.670^{bc}	27.87 ± 1.14^b	40.50 ± 2.30^c	303.8 ± 29.5^a	24.96 ± 1.02^{bc}
	+	11.10 ± 0.923^{ab}	22.95 ± 1.40^{ab}	42.99 ± 2.21^c	234.2 ± 29.1^a	22.76 ± 2.74^{ab}
3.5	-	12.14 ± 0.375^{bc}	24.76 ± 0.77^b	41.77 ± 1.15^c	245.6 ± 13.0^a	27.94 ± 0.66^{bc}
	+	12.11 ± 0.605^{bc}	23.79 ± 2.23^{ab}	44.04 ± 0.97^c	253.1 ± 32.4^a	22.76 ± 1.82^{ab}

4.4 Discussion

There are many studies reporting the influence of nitrogen on the *Striga*-cereal association (see e.g. Pieterse and Verkleij, 1991) although the role of nitrogen in this relationship is poorly understood. It is evident from many laboratory studies that high nitrogen concentrations inhibit germination and attachment of *Striga* to its cereal host (Parker, 1984; Raju *et al.*, 1990; Cechin and Press, 1993b; Cechin, 1994b), but the effects of nitrogen post attachment are less certain. This study strongly supports a role for nitrogen supplied as ammonium nitrate at an early stage in the lifecycle of *S. hermonthica* but it also suggests that nitrogen plays a role in ameliorating the effects of the parasite on its cereal host, post attachment of *S. hermonthica*.

The effect of nitrogen on the germination and attachment of *S. hermonthica*

The successful attachment and subsequent development of *S. hermonthica* on the roots of sorghum is dependent on the concentration of ammonium nitrate supplied to the host plant. Attachment of *S. hermonthica* was lower at 3.5 mol m^{-3} ammonium nitrate compared with 1.0 mol m^{-3} ammonium nitrate. A negative effect of nitrogen on *Striga* germination and attachment using sorghum as a host has been reported for *S. asiatica* (Raju *et al.*, 1990) and for *S. hermonthica* (Cechin and Press, 1993b). Raju *et al.* (1990) suggested that ammonium nitrate may affect the germination stimulant post exudation from the roots. However, Cechin and Press (1993b) demonstrated that ammonium nitrate had an effect on the production of stimulatory compounds in the root exudate or its specific leakage from the host roots, as no effect was observed on the stability of active components in the root exudate or the perception of these

compounds by *S. hermonthica* seed. The role of nitrogen in the inhibition of haustorial formation and attachment to the root of the host is still uncertain. For initiation of the haustorium the radicle must establish surface contact with the host root. *In vitro* studies demonstrated that nitrogen supplied as urea or ammonium sulphate significantly decreased the radicle length of *S. hermonthica* (Pesch and Pieterse, 1982). While ammonium nitrate may indeed have a similar effect on radicle length (although this could not be observed in this study), the constraints of the rhizotrons resulted in *S. hermonthica* seeds being placed directly on or next to host roots. Any change in radicle length would have a minimal effect on its contact with the host root. Haustorial initiation is thought to occur as a response to a chemical stimulus present on the surface of the host root (Riopel *et al.*, 1990; Riopel, 1995). Ammonium nitrate may also affect the production of these active components in a manner similar to that found in germination. In the rhizotrons a number of plants received 1.0 mol m^{-3} increased to 3.5 mol m^{-3} ammonium nitrate. After the supply of ammonium nitrate was increased no further attachment of *S. hermonthica* was observed. In addition, a number of seeds that had formed primary haustoria either failed to develop into *S. hermonthica* plants or the primary haustoria degenerated, indicating unsuccessful haustorial penetration. Cell wall degrading enzymes allow the invading haustoria to penetrate the host root (Maiti *et al.*, 1984; Riopel, 1995) and it may be that enzyme function is impaired at high ammonium nitrate concentrations. In addition, increased physical barriers such as strengthened cell walls may increase the resistance of host cells to the invading haustoria and this may be influenced by high nitrogen supply.

Does ammonium nitrate have a direct or indirect effect on the development of *S. hermonthica* after attachment?

In addition to the effects of ammonium nitrate on germination and attachment, the development of *S. hermonthica* on the roots of sorghum was also affected by the concentration of ammonium nitrate and some *S. hermonthica* plants which were already attached died. High numbers of *S. hermonthica* attached to the sorghum root at 1.0 mol m^{-3} ammonium nitrate and this was reflected by a high parasite biomass accumulation, indicating successful parasitism. At 3.5 mol m^{-3} ammonium nitrate the total biomass of *S. hermonthica* was low, which would be expected because of the low numbers of attached parasites. However, the mean individual weight of each parasite was lower at high ammonium nitrate supply indicating that increased supply of nitrogen had a detrimental effect on the development of the parasite. Plants that received an increase in ammonium nitrate supply after *S. hermonthica* attachment, had larger parasites compared with plants grown at 3.5 mol m^{-3} ammonium nitrate throughout the study. This indicates that an early transfer of solutes from the host to the parasite is essential for parasite development. However, the parasites were still smaller than those growing under a low external nitrogen supply, demonstrating that nitrogen is having a continued effect on the development of *S. hermonthica* post-attachment.

The question arises: does ammonium nitrate have a direct or indirect on the development of the parasite? First, a direct effect of increased ammonium nitrate supply would require that the parasite was actively involved in the assimilation of

nitrogen, independent to that of the host plant. *S. hermonthica* plants normally have a poorly developed root system lacking root caps and hairs (Musselman, 1980), thus limiting the volume of soil exploited. The absence of root hairs will not affect active nitrate uptake but could affect uptake nutrient uptake dependent on diffusion, such as ammonium ions (Marschner, 1993). Root development of *S. hermonthica* under field conditions can be substantial (field observations, data not reported and G. Odhiambo, personal communication). Under the growth conditions in this study the vestigial roots showed poor development or were not produced at all (no qualitative data available), suggesting that nitrogen acquisition must be directly from the host plant.

The majority of plants rely on nitrate and ammonium as the major source of inorganic nitrogen. Nitrate assimilation is mediated by the enzymes nitrate reductase and nitrite reductase. A number of root hemiparasites have a low capacity to assimilate nitrate ions because of limited nitrate reductase activity (Lee and Stewart, 1978; Stewart *et al.*, 1984) and the enzyme is found in extremely low amounts in *Striga* (Press *et al.*, 1986). The assimilation of ammonium is mediated by the enzymes glutamine synthetase (GS) and glutamate synthase. In the leaves GS normally exists as two isoforms, one form present in the cytoplasm (GSI) and the other form present in the chloroplast (GSII). McNally *et al.* (1983) and McNally and Stewart (1987) demonstrated GSI to be the major isoform in *Striga* with GSII comprising only 5-20% of total activity. Photorespiratory ammonia can be assimilated via GSII and there is evidence to suggest a correlation between rates of photosynthesis and levels of GSII in ferns (Stewart *et al.*, 1986). Low levels of GSII observed in *S. hermonthica* (McNally

et al., 1983) reflect low rates of photosynthesis (see e.g. Press *et al.*, 1987a; Press *et al.*, 1988). However, this was not true of leafy mistletoes some of which may exhibit high rates of photosynthesis (Schulze *et al.*, 1984). McNally *et al.*, (1983, 1987) suggested that low levels of GSII may reflect the low ability of hemiparasites to assimilate nitrate as the latter stages of nitrate reduction occurs in the chloroplast reactions. The low ability of parasites to assimilate nitrate and ammonia, together with the supply of reduced nitrogen from the host plant, suggest the detrimental effect of high nitrogen concentrations on *S. hermonthica* in the rhizotrons may be mediated through the host.

Second, despite the poor ability of *S. hermonthica* to assimilate nitrogen, studies by Okonkwo (1966) and Igbinnosa, Cardwell and Okonkwo (1996) have demonstrated a direct effect of inorganic nitrogen on the growth of the parasite. To separate whether nitrogen directly effects the parasite or whether nitrogen increases host vigour and improves performance, the parasite was grown in culture. Igbinnosa *et al.* (1996) supplied *S. hermonthica* with either ammonium or nitrate as the nitrogen source. The authors demonstrated that increasing concentrations of the ammonium ion lowered shoot length and dry weight of *S. hermonthica* while nitrate increased growth. Ammonium accumulation can be toxic to plants and low GSII activity in *S. hermonthica* is likely to lead to greater accumulation of ammonium. Increased nitrate concentration may stimulate the low activity of nitrate reductase in the parasite as it is substrate inducible. However, nitrate can lead to accumulation of solutes in plant cells causing an increase in water uptake and translocation in plant tissues which may have

increased growth. Interestingly, when plants were supplied with a 1:1 ratio of ammonium and nitrate, the suppression of *S. hermonthica* growth by ammonium was overcome. In the rhizotron study, plants were supplied with ammonium nitrate in a 1:1 ratio which therefore may not have had an inhibitory effect on *S. hermonthica* and together with the limited root system for uptake again implies a host mediated effect on *S. hermonthica* growth.

Host mediated effects of nitrogen on *S. hermonthica* growth.

Sorghum plants grown at a high external supply of ammonium nitrate have an increased nitrogen status, indicated by the high foliar nitrogen concentrations compared with plants grown at low ammonium nitrate supply. The poor development of *S. hermonthica* on sorghum plants with a high nitrogen status may seem somewhat surprising in light of the view that some root hemiparasites in the Scrophulariaceae perform better on legume hosts compared with non-legume hosts (Press *et al.*, 1993; Seel *et al.*, 1993b). In addition, Schulze and Ehleringer (1984) demonstrated that mistletoes infecting nitrogen fixing plants had growth rates 7-fold greater than mistletoes infecting non-fixing hosts.

An explanation for the lower biomass accumulation of *S. hermonthica* plants growing under a high nitrogen supply may result from a combination of host responses to an increase in host nitrogen status:

i) An increase in the nitrogen status of sorghum plants may alter the osmotic gradient between the parasite and its host. In *S. hermonthica* the polyol mannitol is the

predominant osmoticum, maintaining a larger osmotic potential than the host thus facilitating the movement of water and solutes from the host (Stewart and Press, 1990). Gworgwor and Weber (1991) determined that at low nitrogen supply the osmotic pressure of S. *hermonthica* was much greater than its sorghum host, but at higher rates of nitrogen application the osmotic pressure of *S. hermonthica* was less and the osmotic pressure of the host improved. A decrease in the osmotic gradient could lower the acquisition of solutes by the parasite and result in less parasite growth. The 3.5 mol m^{-3} ammonium nitrate supplied in this study could be sufficient to cause an increase in the osmotic pressure of the host and may partly explain a decrease in *S. hermonthica* biomass with lower carbon and nitrogen accumulation.

ii) High nitrogen supply to the sorghum plants in this study resulted in an increase in biomass partitioning to the leaves compared with plants grown at low nitrogen supply, increasing the potential area for photosynthesis. Infected plants grown at 3.5 mol m^{-3} ammonium nitrate had rates of photosynthesis similar to uninfected plants and together with the greater leaf area compared with plants grown at a low nitrogen supply, there will be an increase in rates of whole plant transpiration, indicating a greater pull of water and solutes to the host plant away from the parasite.

iii) An increase in nitrogen supply to plants can cause an alteration of the reduction of nitrogen between the root and shoot components, indicated by the proportions of nitrate reductase activity and the concentrations of nitrate and reduced nitrogen in the xylem sap (Pate, 1980; Miller, 1985; Andrews, 1986b). Nitrate reductase activity occurs in both the root and shoot components of *Zea mays*. At an external nitrogen concentration of 1.0 mol m^{-3} nitrate, root nitrate assimilation dominates, but as

external nitrogen concentrations increase above 1-2 mol m^{-3} nitrate the shoots become the main site of assimilation (Andrews, 1986a). This pattern of assimilation is likely to be similar in sorghum. Nitrate reduction in the shoot in preference to the root may affect the acquisition of reduced nitrogen by *S. hermonthica* further decreasing the carbon and nitrogen gain for biomass production.

iv) Increased nitrogen supply can alter the WUE of the parasite. High transpiration rates commonly occur, facilitating solute acquisition from the host. The Mediterranean species *Bartsia trixago* maintained transpiration rates approximately double those of the host plant (Press e*t al.*, 1993). This was also demonstrated for *S. hermonthica* in the field (Chapter 3). High transpiration rates coupled with low rates of photosynthesis result in low WUE. Schulze *et al.* (1984) demonstrated that mistletoes regulated their WUE in relation to the that of the host, or more accurately, WUE is decreased in response to an increase in nitrogen in the host xylem sap. However, the regulation of transpiration as a direct response to nitrogen supply has not been observed in root hemiparasites. In addition, the *S. hermonthica* plants in the rhizotrons did not emerge above ground and even though leaflets were formed transpiration would be limited.

The response of infected and uninfected sorghum plants to an increase in nitrogen supply

The response of sorghum plants to infection was dependent on the supply of ammonium nitrate. Uninfected sorghum plants supplied with 3.5 mol m^{-3} ammonium nitrate had greater total biomass accumulation compared with plants grown at 1.0 mol m^{-3} ammonium nitrate. At the higher nitrogen supply the partitioning of biomass to the

stem and leaves increased in preference to the root, lowering the root:shoot ratio below that of plants grown at low nitrogen supply. This response has been well characterised in both C_3 and C_4 plants (see e.g. Pate, 1980; Levin, Mooney and Field, 1989; Lee *et al.*, 1992; Smolders and Merckx, 1992; Van der Werf *et al.*, 1993).

The lower biomass accumulation and change in the root:shoot ratio of infected sorghum plants at 1.0 mol m^{-3} ammonium nitrate reflects observations in Chapter 2. However, the detrimental effect of *S. hermonthica* on the host was ameliorated at a nitrogen supply of 3.5 mol m^{-3} ammonium nitrate. Studies by Cechin and Press (1993a) show that above a minimal *Striga* loading, the host is unresponsive to the density of infection, but at the rates of infection observed here and poor parasite performance (see above) it is likely that *S. hermonthica* has a low demand for host assimilate at 3.5 mol m^{-3} ammonium nitrate.

The carbon budget of Graves *et al.* (1989) suggests that lower rates of photosynthesis in infected plants contributes to the difference in biomass accumulation between infected and uninfected plants and this is observed in infected plants grown at 1.0 mol m^{-3} ammonium nitrate. In contrast, the effects of *S. hermonthica* on growth and photosynthesis are ameliorated at 3.5 mol m^{-3} ammonium nitrate. It is interesting to observe that the effect of *S. hermonthica* on host photosynthesis was also ameliorated in plants that received an increase in ammonium nitrate supply after parasite attachment. High rates of photosynthesis may be associated with high foliar nitrogen and chlorophyll concentrations indicating a greater proportion of nitrogen being

partitioned in photosynthetic apparatus at a higher ammonium nitrate supply, emphasising the importance of host nitrogen status. Infected plants that received an increase in ammonium nitrate supply after attachment appeared unable to use the extra nitrogen for growth whereas uninfected plants showed stimulated growth. However in these plants the total proportion of nitrogen allocated to the leaves of infected plants is lowered because infection has resulted in lower leaf biomass accumulation. Rates of total leaf area photosynthesis will be lower than plants grown at high nitrogen throughout the study which may explain the biomass differences between the treatments.

Conclusions

The results of this study clearly demonstrate that nitrogen supplied as ammonium nitrate can influence the *S. hermonthica*-sorghum association at both the germination and attachment phase of the parasite and also post-*S. hermonthica* attachment. However, it is evident that to ameliorate the detrimental effects of *S. hermonthica* on growth and photosynthesis, nitrogen must influence the association at all stages of the parasites lifecycle. These results have been obtained under controlled environmental conditions and the aim of the next chapter is to determine whether nitrogen fertiliser can influence the *S. hermonthica*-cereal association in the field. In addition, it aims to determine whether nitrogen fertiliser applied as a single dose at the time of planting, or as a repeated application three times in the season, is more effective in controlling levels of infestation. The response of the cereals to subsequent levels of infestation will

be examined and whether nitrogen can alleviate the effects of *S. hermonthica* in terms of biomass accumulation, photosynthesis and grain yield.

Chapter 5

The influence of timing and dose of nitrogen application on the *S. hermonthica*-host association in the field

5.1 Introduction

The occurrence of *Striga* in the field is often negatively correlated with low soil fertility in general and nitrogen status in particular (Bebawi, 1981; Pieterse and Verkleij, 1991) and *Striga* has its greatest impact in low-input subsistence farming (Doggett, 1988; Sauerborn, 1991; Parker and Riches, 1993; Riches and Parker, 1995). In natural communities root hemiparasites tend to exist in greater numbers in ecosystems where soil nitrogen availability is low, suggesting that nitrogen supply may be an important factor in determining the success of parasite interactions (Seel *et al.*, 1992), although in natural and semi-natural ecosystems this may also be controlled by community responses to infection. High nitrogen concentrations favour the performance of the host plant. Cechin and Press (1993b) demonstrated that high nitrogen supply had a negative effect on germination and attachment of *S. hermonthica* on its sorghum host.

As a consequence of nitrogen-limited soils being commonly associated with severe *Striga* infestations, attention has focused on the use of nitrogen fertiliser as a means of controlling *Striga*. Field studies examining the influence of nitrogen on *S. hermonthica* and its cereal host have often produced inconsistent results (see e.g. Pieterse, 1996). Studies have shown that increasing the supply of nitrogen fertiliser can reduce *Striga* infection and increase host yield (Bebawi, 1981, 1987; Hess and Ejeta, 1987; Mumera and Below, 1993; Odhiambo and Ransom,

1994; Ransom and Odhiambo, 1994). However, other studies have also shown a lack of response of the host-parasite association to nitrogen additions (Osman *et al.*, 1991; Smaling *et al.*, 1991) and in a few cases even an increase in *S. hermonthica* infestation was observed on high soil nitrogen concentrations (Basinski, 1955; Sallé *et al.*, 1987). No simple relationship has emerged between the amount of nitrogen applied and the degree of *Striga* infestation or enhancement of grain yield.

The aim of this chapter was to address the role of nitrogen fertiliser in the field on the *S. hermonthica*-cereal association. In the first field season, 1994, the aim was to determine whether a high rate of nitrogen addition 150 kg N ha^{-1} applied at planting would depress the level of *S. hermonthica* infestation and improve host performance. In 1995 the effects of timing of fertiliser application were investigated by applying nitrogen either as a single dose at planting (180 kg N ha^{-1}) or as three separate 60 kg applications, resulting in 180 kg N ha^{-1} in total. A third smaller study was conducted on a nearby farmer's field, away from the experimental sites, where the soil was known to be of poor fertility. A lower dose of 40 kg N ha^{-1} was applied which is the recommended fertiliser application rate for subsistence farmers (H. Frost personal communication). Specifically, growth, grain yield and photosynthetic measurements are reported, together with the abundance of the parasite.

5.2 Materials and methods

Three studies were conducted at the Kenya Agricultural Research Institute at Kibos in western Kenya (see Chapter 3 Section 3.2.2) between 1994 and 1996,

each commencing prior to the start of the long rain season. Two studies were carried out on an experimental site previously used for fertiliser trials. The first study commenced in March 1994 and the second study commenced in March 1995 on the same experimental site. A third study was conducted on a farmer's field, a few hundred metres from the experimental plots, and commenced in March 1996. Table 5.1 shows a summary of the three trials conducted.

5.2.1 Experimental design and plant material

Experiment 1

A three-way factorial design was employed using three genotypes (one maize and two sorghum), grown in the presence or absence of *S. hermonthica*, either in the absence of added nitrogen or with nitrogen supplied as 150 kg N ha^{-1}. Two cultivars of sorghum were studied, a commercially available inbred cultivar CK60, chosen because of its known susceptibility to *Striga* and because its growth is suited to the Kenyan climate, and a local land race, Ochuti, which is reported to have some tolerance to *Striga* (see Chapter 3 and Table 3.1). The third cereal was a commercially available maize cultivar, H511 (see Chapter 3 and Table 3.1).

Experiment 2

A two-way factorial design was employed, using the commercially available maize cultivar, H511 (studied in the previous season), grown in the absence or presence of *Striga hermonthica*, either in the absence of added nitrogen, or with nitrogen supplied as a single application of 180 kg N ha^{-1} or as three applications of 60 kg N ha^{-1}.

Experiment 3

The same maize cultivar, H511, was grown in a farmer's field naturally infested with *S. hermonthica* either in the absence or presence of added nitrogen supplied as a single application of 40 kg N ha^{-1}. Plants were not grown in the absence of *S. hermonthica*.

5.2.2 Experimental plots
Methyl bromide fumigation

The study area for Experiments 1 and 2 had previously been planted with maize and had shown moderate levels of *Striga* infestation. In order that the plants could be grown in the absence of *Striga* the entire area was fumigated with methyl bromide gas, at a rate of 500 kg ha^{-1}, to eradicate any *Striga* seeds in the soil bank. The first fumigation was carried out in early March 1994 and this was again repeated in early March 1995 before commencing each study. Eplee bags (3 x 3 fine mesh (< 0.2 mm) nylon bags) containing 200 *S. hermonthica* seeds were placed 10 cm under the soil prior to fumigation. Germination tests were carried out after fumigation using tetrazolium red (Grabe, 1970) and GR-24 (Gbèhounou *et al.*, 1996) and these showed a 100% seed kill.

Experiment 1

Thirty-six experimental plots, each covering 15 m^2 (occupying a total area of 770m^2, including buffer zones), were established in March 1994. Each combination of factors (twelve in total) was replicated three times within the study area. Within each 15m^2 plot the cereals were grown with an inter-row spacing of 0.6m and were grown at 0.5m distances along each row. Only the central four rows were used for measurements with the outer two rows comprising the guard rows and with safety margins of 0.5m between each plot.

At the time of planting the cereals, late March, 18 of the 36 plots were artificially infested with *S. hermonthica* seed, collected at Kibos in 1993. The seed was mixed with finely sieved sand and sown to give an infection density of approximately 2000 seeds per host plant. The seeds were dug in to a depth of 10-15 cm around the planting hole.

All plants received a dressing of tri-super phosphate, applied at a rate of 40 kg P ha^{-1}, at the time of sowing. At the same time half of the plots received nitrogen in the form of a calcium ammonium nitrate fertiliser (containing 26% N) at a dose of 150 kg ha^{-1} (equivalent to 15g per plant). The fertilisers were placed in a hole adjacent to the seed at the time of planting. An insecticide (carbofuran) was applied at the time of planting at a rate of 2g per plant to protect against early attacks of stem borers, and the plants were again treated 28 DAP. The plots were hand weeded of all weeds other than *Striga* at 24 and 34 DAP.

The study was replicated on an adjacent field naturally infected with *S. hermonthica* that had not been fumigated (all three genotypes in the presence and absence of added nitrogen were grown with the same number of plots per treatment). This precaution was taken in order to allow for any effects the soil fumigation process may have had, other than the removal of *Striga* seed. The data from this study are not presented here, since the responses of the host and parasite to all parameters reported did not differ significantly between the naturally infested and the artificially infected plots.

Experiment 2

Eighteen experimental plots were established in March 1995 as described above. Each combination of factors (six in total) was replicated three times within the

study area. At the time of planting, late March, nine of the eighteen plots were artificially infested with *S. hermonthica* seed as described above.

All plants received a dressing of tri-super phosphate, at a rate of 40 kg P ha^{-1} at the time of sowing. At the same time, the nitrogen treated plots received either 60 or 180 kg N ha^{-1} (equivalent to 6 and 18 g per plant respectively). Nitrogen was supplied in the form of calcium ammonium nitrate (as above), and was placed in a hole adjacent to the seed at the time of planting. For the plots treated with 60 kg N ha^{-1}, two further applications of 60 kg N ha^{-1} were made at 28 and 46 DAP thus both the nitrogen-treated plots received the same final amount of the nutrient. All seeds were treated with an insecticide carbofuran applied as above and the site was hand weeded for all weed other than *S. hermonthica* at 26 DAP.

Experiment 3

Six plots were established in March 1996 using a farmer's field naturally infested with *S. hermonthica* and on soils that were considered to be nutrient poor. The maize cultivar, H511, was planted in plots of 30 m^2 (twice as large as Experiments 1 and 2) and at the spacing described above. Each treatment was replicated three times within the study area. All plants received a dressing of tri-super phosphate, applied at a rate of 40 kg P ha^{-1} at the time of sowing. At the same time half of the plots received nitrogen in the form of a calcium ammonium nitrate fertiliser at a dose of 40 kg N ha^{-1} (equivalent to 4g per plant). The fertilisers were placed in a hole adjacent to the seed at the time of planting.

5.2.3 Growth measurements

Experiment 1

At eight intervals between 13 and 63 DAP plant height was measured from the base of the stem to the youngest ligule. Final yield data was determined at 120

DAP, by oven-drying the grain at 70 °C for 48 hours. Grain yield for Katumani was determined at 100 DAP due an earlier maturation compared with the other cultivars. The number of *S. hermonthica* plants emerged above-ground was recorded on the plots throughout the study.

Experiment 2

Plant height was measured as described above between 18 and 65 DAP. Final grain yield was also determined as above at 120 DAP. To build on our knowledge from the previous season, additional measurements were made. The number of *S. hermonthica* plants emerged above ground was recorded and their dry weight was determined at the end of the study period, 60 DAP. Also at 60 DAP, total stem biomass was determined, the measurements being made on other plants than those used for height measurements. Stems were oven-dried for 76 h at 70 °C before being weighed. All measurements were recorded on 5 plants in each plot resulting in 15 measurements recorded in total per treatment. SLA of the youngest fully emerged leaves was determined at 60 DAP (see Chapter 2 Table 2.2).

Experiment 3

Plant height was measured as described previously but at 65 DAP only. The number of *S. hermonthica* plants emerged above-ground was recorded also at 65 DAP. Final grain yield was determined at 120 DAP and the grain was oven-dried (as above) prior to dry weight determination. All measurements were recorded on 5 plants in each plot resulting in 15 measurements recorded in total per treatment.

5.2.4 Gas exchange measurements

Instantaneous (60-120s) rates of photosynthesis were measured on cereals between 13 and 63 DAP for Experiments 1 and 2 and at 65 DAP only for experiment 3. Measurements were made using a portable infra-red gas analyser (LCA-4

Analytical Development Company, Hoddesdon, UK). At each measurement period one record was made per plant, with 12 individual being sampled in all per treatment. Measurements were made halfway along the length of the youngest fully expanded leaf at ambient CO_2 concentrations (approximately 355 μmol m^{-2} s^{-1}) and relative humidity (45%). All measurements were made between 09.30 h and 13.00 h, when the PFD was in excess of 1800 μmol quanta m^{-2} s^{-1}. The leaf cuvette had an area of 675mm^2 (ADC PLC-B), and a flow rate of 300 ml min^{-1} was used. Differences between the concentration of CO_2 and H_2O vapour between the inlet and outlet gas streams were used to calculate rates of photosynthesis and transpiration, using the equations of von Caemmerer and Farquhar (1981).

5.2.5 Foliar nitrogen concentration

Experiments 2 and 3

Leaf tissue samples were taken from maize plants between 60 and 65 DAP for analysis of total nitrogen concentration. Approximately 30 mg of tissue was sampled from each plot and oven dried for 48 h at 70 °C. The leaf material was ground and digested in 4 ml of sulphuric acid-salicylic acid mixture using a Kjeldahl technique (see Chapter 2 Section 2.2.7). No tissue for leaf nitrogen analysis was sampled from Experiment 1.

5.2.6 Statistical analysis

Treatment effects (the response to *S. hermonthica* infection and nitrogen addition) were analysed using two way analysis of variance procedures for a randomised block design (Minitab version 10.2) followed by Tukey's multiple comparison procedures (Zar, 1984). Where nitrogen had no effect on the photosynthetic measurements (experiment 1) data were analysed using a one way analysis of variance procedure to determine the response to the presence of *S. hermonthica*.

The numbers of emerged *S. hermonthica* were analysed using non-parametric Kruscal-Wallis procedures (Minitab version 10.2).

Table 5.1 Summary of experiments carried out in 1994, 1995 and 1996, including the cereals studied (m = maize, s = sorghum), nitrogen fertiliser applied and variables measured. The crosses denote the measurements made during each season.

Field study	Cultivar/ species	Ammonium nitrate kg N ha^{-1}	Variables measured						
			No of *Striga*	Dry wt *Striga*	Ligule height	Grain yield	Cereal dry wt	Gas exchange	Leaf Nitrogen
1994	H511 (m)	0, 150	x		x	x		x	
	Ochuti (s)	0, 150	x		x	x		x	
	CK60 (s)	0, 150	x		x	x		x	
1995	H511 (m)	0, 180, 3 x 60	x	x	x	x	x	x	x
1996	H511 (m)	0, 40	x		x	x		x	x

5.3 Results

5.3.1 *S. hermonthica* infection

Throughout the entire experimental period in Experiments 1 and 2 there was no *S. hermonthica* emergence on any of the control plots and examination of the root systems from plants grown on these plots confirmed that they were free from *S. hermonthica* infection. In Experiment 1 emergence of *S. hermonthica* first occurred at 35 DAP on the artificially infested plots containing the commercial cultivars of sorghum (CK60) and maize (H511) (data not shown). The land race cultivar Ochuti showed a slight delay in *S. hermonthica* emergence by 5 days with the first emergence being observed at 40 DAP. At 63 DAP the number of emerged *S. hermonthica* plants (Table 5.2) was highest in the plots where no nitrogen fertiliser was added, although the effect of nitrogen on the number of parasites was not significant ($p > 0.05$) The number of emerged *S. hermonthica* plants differed between hosts (Table 5.2) with maize supporting the greatest number (15 parasites/host, meaned between nitrogen treatments), and with the sorghum cultivars supporting 68% (CK60) and 36% (Ochuti) of the number of parasites on maize. In Experiment 2 emergence of *S. hermonthica* on the maize cultivar, H511, occurred at 38 DAP on all infected plots, only three days after emergence in Experiment 1 (data not shown). Throughout the study the number of emerged *S. hermonthica* plants on the plots which received added nitrogen was lower than on plots which received no added nitrogen, although not statistically so (Table 5.2). Large variation in the number of emerged parasites was observed, but there was less variation in the total biomass of parasite tissue supported by each host, which was also unaffected by the nitrogen treatments (Table 5.4). In Experiment 3 nitrogen addition did not influence the number of emerged *S. hermonthica* plants on infected H511 plants on the farmer's field (Table 5.2).

In summary nitrogen addition applied as 40, 150, 180 and 3 x 60 kg N ha^{-1} in all three studies had no significant effect on the number of emerged *S. hermonthica*

plants although there was a trend towards lower numbers of emerged parasites in Experiments 1 and 2 at the higher application rates.

5.3.2 Cereal growth

In Experiment 1 large differences were observed between the distance from the base of the stem to the youngest ligule on infected and uninfected CK60 and H511 plants (Figure 5.1A and C). By 40 DAP these differences were significant (p < 0.001). At each subsequent time point these differences became more marked and by 63 DAP the ligule height of infected CK60 and H511 plants was 26.2% and 35.3%, respectively, below those of uninfected plants (Table 5.3). Application of nitrogen did not significantly influence ligule height. In contrast to CK60 and H511, no significant differences in height to the youngest ligule between infected and uninfected plants were observed for Ochuti at any time throughout the measurement period (Figure 5.1B and Table 5.3). Again, nitrogen application was also non-significant. In Experiment 2, from 32 DAP onwards, the height to the youngest ligule of maize plants infected with *S. hermonthica* was less than that for uninfected plants with differences occurring earlier than observed for Experiment 1 (Figure 5.1D). By 65 DAP the ligule heights for infected plants were between 19% and 26.5% less than those of uninfected plants (Table 5.3). Again, nitrogen addition did not affect the ligule height of either uninfected or infected plants. In addition to ligule height, stem biomass was also measured in Experiment 2. Nitrogen addition did not affect stem dry weight of uninfected plants, but did affect the stem dry weight of infected plants, despite a lack of a significant effect on plant height (Table 5.4). The addition of 180 kg N ha^{-1} resulted in a significantly greater stem biomass compared with infected plants which received no nitrogen addition.

In contrast to Experiments 1 and 2, greater responses to a lower rate of nitrogen addition were observed for maize infected with *S. hermonthica* on a farmer's field (Experiment 3), although no *Striga*-free control plots were available to determine

this level of addition on uninfected maize plants. Nitrogen did partially alleviate the effects of the parasite on the host, with plants being 36% taller (as measured to the youngest ligule) compared with infected plants that did not receive any nitrogen addition (Table 5.3).

In Experiment 1 the absence of any influence of nitrogen on ligule heights (Figure 5.1) is reflected in the final grain yield data, where again nitrogen had no significant effect (Table 5.3). Grain yield was also unaffected by the presence of *S. hermonthica* in the case of the sorghum cultivar Ochuti. The maize cultivar (H511) showed 20.6% less grain yield compared with uninfected plants although this data was not significant. The yield of the susceptible sorghum cultivar CK60 in the presence of *S. hermonthica* was 71% below that of uninfected plants (Table 5.3). In Experiment 2 greater grain loses were observed with infection compared with the previous study. Final grain yields reflected the stem biomass data and infected plants produced 31%, 22% and 35% below the yields of uninfected plants on the unamended, single nitrogen addition and triple nitrogen addition plots, respectively (Table 5.3). In contrast to Experiment 1, high nitrogen addition did had an effect on grain yield. The single nitrogen application of 180 kg N ha^{-1} resulted in the stimulation of the grain yield of infected plants (reflecting stem biomass data) above that of infected plants on the unamended or triple nitrogen addition plots. The low rate of nitrogen application on the farmer's field (Experiment 3) influenced grain in the presence of *S. hermonthica*, reflecting the growth data. Infected plants that received nitrogen yielded 45% more grain compared with infected plants on the unamended plots (Table 5.3).

5.3.3 Gas exchange

In Experiment 1 a marked effect of *S. hermonthica* was observed on the photosynthesis of the commercially available sorghum (CK60) and maize (H511) cultivars (Figure 5.2A and C). Throughout the measurement period nitrogen had

no influence on gas exchange thus data are discussed for unamended plots only. For infected CK60 plants, differences between infected and uninfected plants were apparent before 30 DAP prior to the emergence of the parasite above-ground. There was a more or less constant drop in the rate of photosynthesis throughout the period of measurement, with final rates being 44.9% lower compared with uninfected controls at 0 kg N ha^{-1} (Table 5.5). For the maize cultivar, H511, the effect of the parasite on maize photosynthesis was not apparent until about 10 days later, following the emergence of *S. hermonthica* above ground (Figure 5.2C). By 63 DAP rates of photosynthesis in infected plants were 33.5% lower than uninfected controls (Table 5.5). The land race Ochuti showed a different response to infection with *S. hermonthica* having no detrimental effect on rates of photosynthesis during the period of measurement and infected plants showed a 6.8% increase in photosynthesis, although this was not significant (Figure 5.2B, Table 5.5). Lower rates of photosynthesis in infected plants were accompanied by significantly lower rates of transpiration (Table 5.5). By 63 DAP, infected CK60 and H511 plants had rates of transpiration 28.1% and 15% lower than uninfected controls, respectively, whilst a stimulation of 15% was observed in Ochuti (Table 5.5). The gas exchange data were used to calculate values of water use efficiency (WUE). In both CK60 and H511, WUE was 21.2% and 13.3% lower, respectively, in infected plants compared with controls, whilst no significant difference in Ochuti was observed (Table 5.5). As with the growth data, nitrogen application has no significant effect on either rates of photosynthesis, transpiration or WUE.

In Experiment 2 maize plants infected with *S. hermonthica* on the unamended plots and on plots which received three applications of 60 kg N ha^{-1} had lower rates of photosynthesis than their uninfected plants and the pattern of response was similar to Experiment 1 (Figure 5.2 D). By 63 DAP rates in infected plants were 23% and 19% below uninfected plants, respectively (Table 5.6). In contrast, infected plants supplied with 180 kg N ha^{-1} showed no significant decrease in photosynthesis

compared with uninfected controls. Infected plants on all plots had significantly lower rates of transpiration compared with controls, although infected plants at 180 kg N ha^{-1} had higher transpiration rates than infected plants at 0 and 3 x 60 kg N ha^{-1}. In contrast to Experiment 1 there were no differences between the instantaneous WUE of infected and uninfected maize.

Nitrogen application on the farmer's field (Experiment 3) had a positive effect on rates of photosynthesis in infected maize plants with rates 23% higher than infected plants not treated with nitrogen (Table 5.6). This was associated with an increase in rates of transpiration and WUE, although this data was only significant at $p < 0.1$.

5.3.4 Foliar nitrogen concentration

Leaf nitrogen concentration was measured in Experiments 2 and 3. In Experiment 2 neither infection with *S. hermonthica* nor nitrogen addition affected leaf nitrogen concentration on a weight basis (Table 5.6). Neither treatment influenced specific leaf area and resulting nitrogen concentrations expressed on an area basis were also unaffected. In Experiment 2, PNUE was lower in infected plants compared with uninfected plants on the unamended and triple nitrogen addition plots. PNUE of plants in receipt of 180 kg N ha^{-1} was only slightly below uninfected plants.

Infected plants grown in the farmer's field (Experiment 3) in receipt of 40 kg N ha^{-1} had greater leaf nitrogen concentrations compared with infected plants grown on the unamended plots (Table 5.6). Higher rates of photosynthesis and higher leaf nitrogen concentrations in these plants resulted in no difference in PNUE between the two treatments. PNUE of these plants grown in low nitrogen conditions was much higher than those measured in Experiment 2.
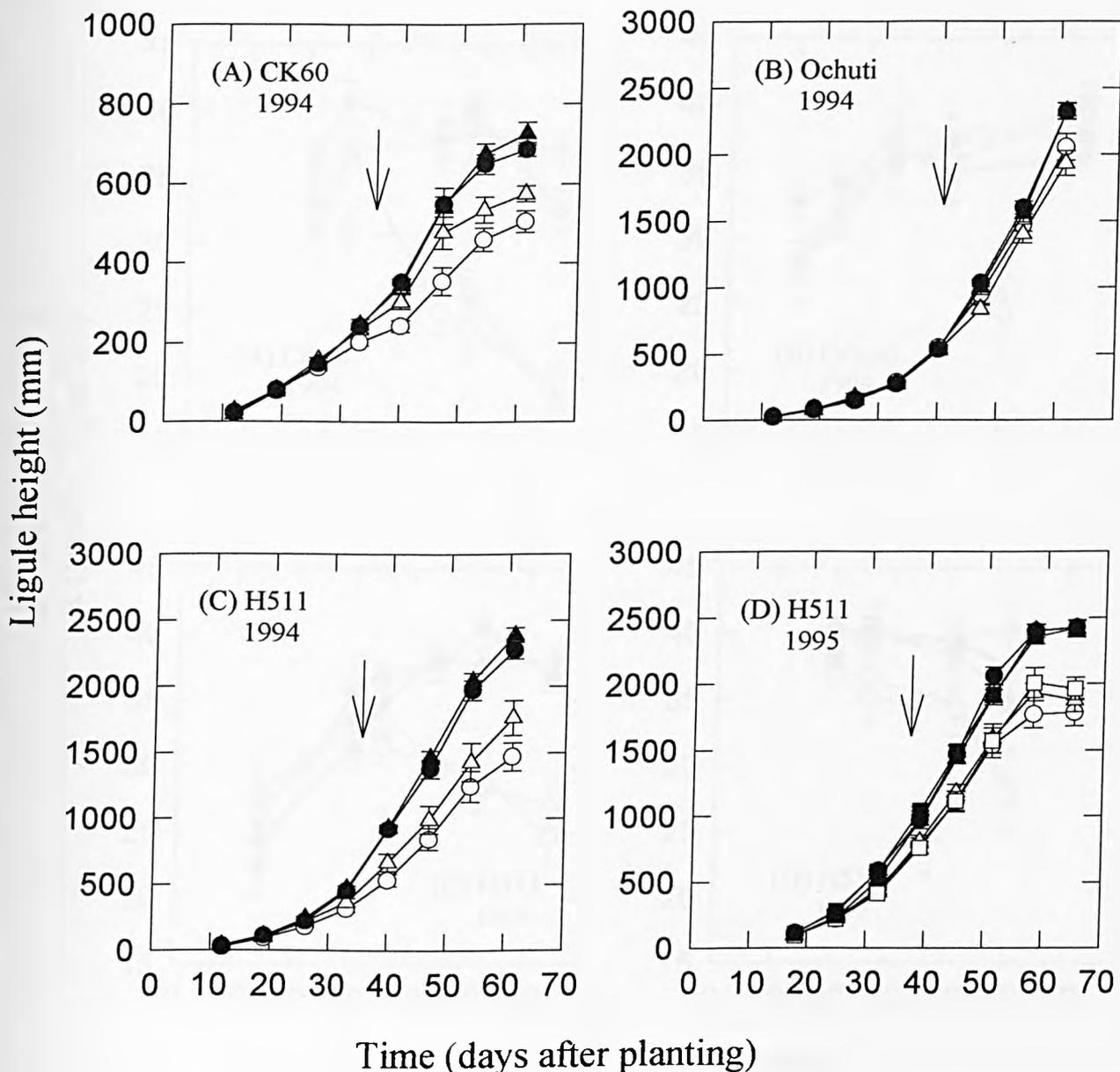
Figure 5.1 Distance from the base of the stem to the youngest ligule (mm) of sorghum and maize plants grown in the absence (closed symbols) or presence (open symbols) of *S. hermonthica*. In Experiment 1 (graphs A to C) plants were grown in the absence of added nitrogen (circles) or with a single application of 150 kg N ha^{-1} (triangles). In Experiment 2 (graph D) plants were in the absence of nitrogen (circles), or with nitrogen added as a single application of 180 kg N ha^{-1} (diamonds) or as three applications each of 60 kg N ha^{-1} (squares). The timing of the first emergence of *S. hermonthica* is denoted by the arrow.
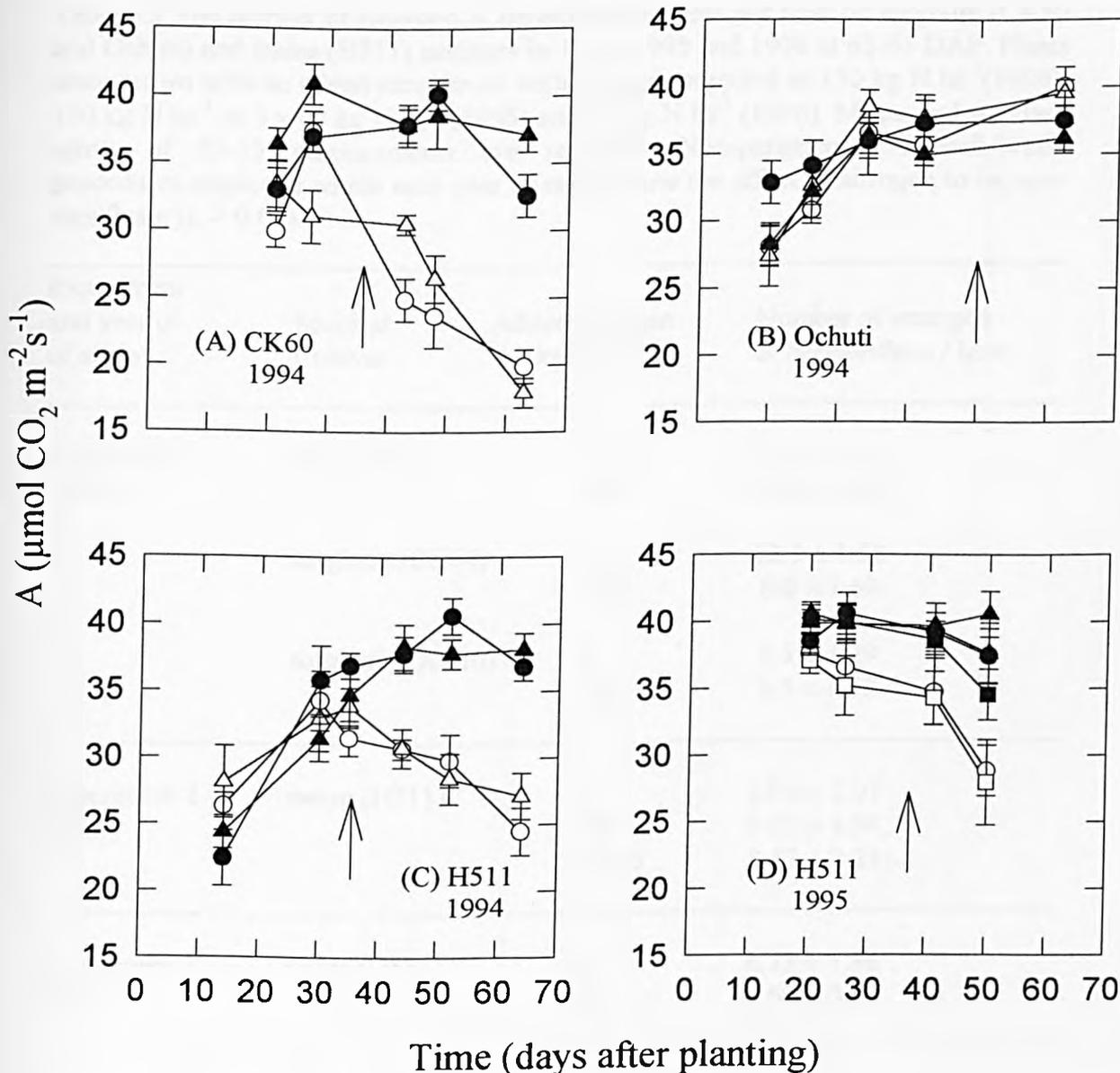
Figure 5.2 Rates of photosynthesis (A) of sorghum and maize plants grown in the absence (closed symbols) or presence (open symbols) of *S. hermonthica*. In Experiment 1 (graphs A to C) plants were grown in the absence of added nitrogen (circles) or with a single application of 150 kg N ha^{-1} (triangles). In Experiment 2 (graph D) plants were in the absence of nitrogen (circles), or with nitrogen added as a single application of 180 kg N ha^{-1} (diamonds) or as three applications each of 60 kg N ha^{-1} (squares). The timing of the first emergence of *S. hermonthica* is denoted by the arrow.

Table 5.2 The number of emerged *S. hermonthica* plants per host on sorghum (CK60 and Ochuti) and maize (H511) cultivars in 1994, 1995 and 1996 at 63-65 DAP. Plants were grown with no added nitrogen or with nitrogen supplied as 150 kg N ha^{-1}(1994), 180 kg N ha^{-1} or 3 x 60 kg N ha^{-1} (1995) and 40 kg N ha^{-1} (1996). Means and standard errors of 12-15 measurements are reported. Non-parametric Kruscall-Wallis procedures employed within each year of study show the effect of nitrogen to be non-significant (p > 0.05).

Experiment and year of of study	Species/ Cultivar	Added nitrogen kg N ha^{-1}	Number of emerged *S. hermonthica* / host
Experiment 1 (1994)	maize (H511)	0	17.5 ± 1.52
		150	13.0 ± 2.40
	sorghum (CK60)	0	12.3 ± 1.52
		150	8.6 ± 1.69
	sorghum (Ochuti)	0	5.5 ± 1.29
		150	5.5 ± 1.52
Experiment 2 (1995)	maize (H511)	0	15.0 ± 2.91
		180	9.07 ± 1.94
		3 x 60	8.47 ± 2.23
Experiment 3 (1996)	maize (H511)	0	8.73 ± 1.86
		40	8.60 ± 0.98

Table 5.3 Summary of final measurements of ligule height and grain yield for sorghum (CK60 and Ochuti) and maize (H511) cultivars grown in the absence or presence of *S. hermonthica* in 1994, 1995 and 1996. Plants were grown without the addition of nitrogen or with nitrogen supplied as 150 kg N ha^{-1}(1994), 180 kg N ha^{-1} or 3 x 60 kg N ha^{-1} (1995) and 40 kg N ha^{-1} (1996). Means and standard errors of 12-15 measurements are reported and the data analysed within each year of study using analysis of variance procedures followed by Tukey's multiple comparison tests. Variables not sharing the same superscripted letter across nitrogen treatments within each cultivar are significantly different ($p < 0.05$).

Experiment and year of of study	Species/ Cultivar	Added nitrogen kg N ha^{-1}	Ligule height (mm)		Grain yield (t ha^{-1})	
			Control	Infected	Control	Infected
Experiment 1 (1994)	maize (H511)	0	2275.0 ± 64.9^b	1472.9 ± 105.0^a	6.04 ± 0.29^b	4.79 ± 0.81^{ab}
		150	2389.9 ± 55.0^b	1765.0 ± 132.0^a	5.77 ± 0.92^b	4.69 ± 0.77^a
	sorghum (CK60)	0	685.1 ± 17.2^b	506.0 ± 27.6^a	1.76 ± 0.48^b	0.51 ± 0.48^a
		150	724.9 ± 28.7^b	575.2 ± 19.4^a	1.61 ± 0.39^b	0.76 ± 0.71^a
	sorghum (Ochuti)	0	2325.9 ± 61.3^a	2055.7 ± 99.6^a	8.25 ± 1.09^a	7.36 ± 0.36^a
		150	2324.0 ± 61.5^a	1933.5 ± 91.7^a	7.09 ± 0.35^a	5.89 ± 0.65^a
Experiment 2 (1995)	maize (H511)	0	2428.0 ± 99.9^b	1786.0 ± 99.9^a	6.29 ± 0.27^c	4.78 ± 0.19^a
		180	2419.0 ± 64.9^b	1962.4 ± 83.9^a	6.68 ± 0.31^c	5.78 ± 0.22^b
		3 x 60	2420.7 ± 60.9^b	1877.3 ± 86.9^a	7.39 ± 0.34^c	4.79 ± 0.47^a
Experiment 3 (1996)	maize (H511)	0	-	1385.7 ± 81.4^a	-	1.85 ± 0.30^a
		40	-	1881.3 ± 40.5^b	-	3.41 ± 0.73^b

Table 5.4 Stem dry weight of maize plants grown in 1995 (Experiment 2). Plants were grown in the absence (-) or presence (+) of *S. hermonthica* either without the addition of nitrogen or with nitrogen supplied as 180 kg N ha^{-1} or 3 x 60 kg N ha^{-1}. The dry weight of *S. hermonthica* plants per host is also reported (see Table 5.2 for numbers of parasites). Means and standard errors of 12-15 plants are reported and the data analysed using analysis of variance procedures followed by Tukey's multiple comparison tests. Means not sharing the same superscripted letters differ significantly ($p < 0.05$).

Parameters	*Striga*	Nitrogen application kg N ha^{-1}		
		0	180	3x60
Stem weight	-	183.5 ± 10.7 [c]	192.3 ± 8.4 [c]	183.6 ± 7.67 [c]
(g)	+	102.3 ± 7.59 [a]	135.8 ± 7.36 [b]	120.8 ± 9.01 [ab]
Striga weight (g)	+	2.10 ± 1.22 [a]	1.87 ± 0.26 [a]	2.13 ± 0.61 [a]

Table 5.5 Photosynthesis (A), transpiration (E) and water use efficiency (WUE) of uninfected sorghum (CK60 and Ochuti) and maize (H511) plants with percentage change for *S. hermonthica* infected plants in parenthesis. Measurements are from Experiment 1 (1994). Plants were grown with no added nitrogen or with nitrogen supplied at 150 kg N ha^{-1}. Means of 12 measurements are reported and analysed using two-way analysis of variance procedures. Nitrogen had no significant effect on gas exchange thus one way analysis of variance was employed to determine the effect of *S. hermonthica* (* $p < 0.05$, *** $p < 0.001$, ns $p > 0.05$).

Species/ Cultivar	Added nitrogen kg N ha^{-1}	Parameter for uninfected plants at 63 DAP (% change for *Striga* infected plants)		
		A (μmol CO$_2$ m^{-2} s^{-1})	E (mmol H$_2$O m^{-2} s^{-1})	WUE (μmol CO$_2$ mmol^{-1}H$_2$O)
maize	0	36.81 (-33.5%)***	7.307 (-15.0%)*	4.899 (-13.3%)*
(H511)	150	38.07 (-28.8%)***	7.518 (-18.3%)*	5.014 (-16.9%)*
sorghum	0	32.59 (-44.9%)***	7.269 (-28.1%)***	4.824 (-21.2%)*
(CK60)	150	37.08 (-46.4%)***	7.501 (-21.7%)***	5.173 (-39.7%)*
sorghum	0	37.48 (+6.8%)ns	6.356 (+15.0%)*	5.730 (- 6.9%)ns
(Ochuti)	150	36.14 (+9.3%)ns	6.760 (+4.05%)ns	5.278 (+10.0%)ns

Table 5.6 Gas exchange measurements and foliar nitrogen concentrations for maize (H511) plants grown in 1995 (experiment 2) and 1996 (experiment 3). Maize was grown in the absence (-) or presence (+) of *S. hermonthica* either without the addition of nitrogen, or with nitrogen supplied as either 180 kg N ha^{-1} or 3 x 60 kg N ha^{-1}(1995) and 40 kg N ha^{-1} (1996). Means for 12-15 measurements are reported and the data analysed using analysis of variance procedures followed by Tukey's multiple comparison tests. Means not sharing the same superscripted letter in each column differ significantly (p<0.05). (A, photosynthesis, E, transpiration, WUE, water use efficiency, SLA, specific leaf area, N, foliar nitrogen concentration, PNUE, photosynthetic nitrogen use efficiency).

Experiment 2 (1995)

N (kg ha^{-1})	*Striga*	Amax (μmol CO$_2$ m^{-2} s^{-1})	E (mmol H$_2$O m^{-2} s^{-1})	WUE (μmol CO$_2$ mmol^{-1} H$_2$O)	SLA (cm^2 g^{-1})	N (mg g^{-1})	PNUE (μmol CO$_2$ mol N^{-1} s^{-1})
0	-	37.45^{bc}	6.04^{bc}	6.28^{a}	186.5^{a}	30.30^{a}	322
	+	28.83^{a}	4.47^{a}	6.70^{a}	177.1^{a}	28.91^{a}	248
180	-	40.56^{c}	6.79^{c}	6.02^{a}	164.6^{a}	27.57^{a}	399
	+	37.62^{bc}	5.65^{b}	6.78^{a}	173.4^{a}	26.57^{a}	344
3x60	-	34.56^{b}	6.01^{b}	5.75^{a}	173.9^{a}	26.84^{a}	313
	+	27.95^{a}	4.83^{a}	5.55^{a}	171.1^{a}	27.52^{a}	243

Experiment 3 (1996)

N (kg ha^{-1})	*Striga*	Amax (μmol m^{-2} s^{-1})	E (mmol m^{-2} s^{-1})	WUE (μmol CO$_2$ mmol^{-1} H$_2$O)	SLA (cm^2 g^{-1})	N (mg g^{-1})	PNUE (μmolCO$_2$ mol N^{-1} s^{-1})
0	+	16.30^{a}	3.14^{a}	5.09^{a}	370.0^{a}	15.06^{a}	626.0
40	+	20.05^{b}	3.44^{a}	5.74^{a}	344.6^{a}	18.52^{b}	567.3

5.4 Discussion

The deleterious effects of *S. hermonthica* on the growth and photosynthesis of cereal hosts has been well documented in laboratory studies (see e.g. Press and Stewart, 1987; Press, 1989; Cechin, 1994b; Smith *et al.*, 1995). In an earlier chapter (Chapter 3) it was demonstrated that lower rates of instantaneous carbon assimilation in *S. hermonthica*-infected cereals were observed under field conditions and that the extent to which *S. hermonthica* affected its host was cultivar specific. The addition of nitrogen as ammonium nitrate in the laboratory (Chapter 4) affected the degree of infestation and subsequent cereal growth with nitrogen playing a role in alleviating the effects of *S. hermonthica* before and after attachment.

In summary the three field trials conducted from 1994 to 1996 demonstrated that *S. hermonthica* affected the partitioning of resources within the host. Infection had a detrimental effect on photosynthesis, growth and grain yield of its sorghum (CK60) and maize (H511) hosts but no effect was observed in infected Ochuti (sorghum) plants. Application of nitrogen as calcium ammonium nitrate on the experimental plots did result in lower numbers of emerged *S. hermonthica* although differences between treatments were not significant, and nitrogen had a limited influence on alleviating the effects of the parasite. Fertiliser application on the farmer's field did significantly alleviated the effects of *S. hermonthica* on growth and photosynthesis.

Gas exchange

In Experiment 1 (1994) *S. hermonthica* influenced cereal growth and lowered rates of photosynthesis in field-grown sorghum (CK60) and maize (H511) cultivars. These studies supported the results obtained in Chapter 3 which contrasted with an earlier field study (Clark *et al.*, 1994) where lower rates of

photosynthesis in infected cereals was not observed (see Chapter 3). In contrast to the findings for the two commercially available cultivars in Experiment 1 (CK60 and H511), photosynthetic rates in the sorghum land race cultivar, Ochuti, were unaffected by the presence of *S. hermonthica*. In Chapter 3, Ochuti was shown to maintain high rates of photosynthesis and grain yield in the presence of *S. hermonthica* although data were significantly lower when compared with controls at the end of the measurement period. In Experiment 1 (1994) infected Ochuti plants showed no reduction in rates of photosynthesis, growth or grain yield supporting the hypothesis that there may be a relationship between tolerance to the parasite and the ability to maintain high rates of photosynthesis when infected.

Infected Ochuti plants in Experiment 1 showed a stimulation of photosynthetic capacity when infected with *S. hermonthica* above that of uninfected plants. *S. hermonthica* is potentially a large sink for host produced carbon. If there is a mechanistic relationship between assimilate demand and supply (Herold, 1980) then higher rates of photosynthesis might be expected to occur in host-parasite associations. In a non-limiting nitrogen environment where carbon assimilation is sink limited, the parasite provides an additional sink without having an effect on growth because carbon assimilation and amino acid synthesis could increase, compensating for any losses due to parasite acquisition. This response has been observed in a limited number of associations. Cechin and Press (1993a) observed that at low levels of *S. hermonthica* infection there was an initial increase in the light-saturated rate of photosynthesis of the sorghum host. ter Borg and van Ast (1991) reported a stimulation of growth in the *Orobanche crenata-Vicia faba* association although photosynthetic data was not reported. Cechin (1994b) suggested that in a natural community where infestation is typically at a lower range than that observed in agricultural monoculture, parasite infections may not always have such a detrimental effect on the host plant. A positive response of host plant photosynthesis has also been observed with biotrophic pathogens of leaves

such as the rust and powdery mildews although this is often a transient stimulation (Habenshaw, 1984; Murray and Walters, 1992; Scholes, 1992). This occurrence is not isolated to plant or fungal associations as the presence of feeding aphids on wheat resulted in an increase in photosynthetic activity, attributed to assimilate removal (Rabbinge *et al.*, 1981). The stimulated response of Ochuti may have been due to the removal of a sink limitation by *S. hermonthica*. The numbers of emerged parasites supported by Ochuti in 1994 were very low with mean numbers of 6 parasites / host plant. At this low level of infection *S. hermonthica* has no detrimental effect on its host and may reflect *Striga*-host associations in the natural community. It would be of great interest to study *Striga*-host interactions outside of an agricultural system.

The relationship between light saturated rates of photosynthesis and foliar nitrogen concentration is well established as nitrogen is an important constituent of the photosynthetic apparatus, including photosynthetic pigments and associated photosynthetic proteins (Evans, 1989). The nitrogen status of the leaf influences its protein and enzyme content and may directly affect carbon assimilation. Cechin and Press (1993a) reported that the nitrogen status of the host may play an important role in reducing the detrimental effects of *Striga* particularly with respect to rates of carbon dioxide fixation. In their experiments infected sorghum plants with foliar nitrogen concentrations of 11 mg g^{-1} showed lower rates of photosynthesis than infected plants with a foliar nitrogen concentration of 28 mg g^{-1} and at this higher nitrogen concentration no differences in the rate of photosynthesis were observed between infected and uninfected plants. For maize grown on the experimental plots in Experiment 2, foliar nitrogen concentrations were high (26-30 mg g^{-1}). Despite this, photosynthetic rates of infected plants were lower than those of uninfected plants on plots which received no nitrogen and on plots which received three applications of 60 kg N ha^{-1}, thus showing that *S. hermonthica* can exert an effect on photosynthesis at high nutrient status. Although nitrogen was not measured in

experiment 1, the similar rates of photosynthesis and growth indicate that plants would also have had a high nitrogen status.

Cereal growth and grain yield

The ligule height data from Experiment 1 showed that *S. hermonthica* negatively influenced the vegetative growth of H511 and CK60. Grain yield of both cereals was also lower for infected plants compared with uninfected plants, although in experiment 1 this was only so for CK60. This is perhaps surprising given the sensitivity of maize to *S. hermonthica* (Parker and Riches, 1993; see also Chapter 3). The apparent insensitivity of the maize grain yield may be explained by high yield variability within the plots and low sample size used to determine grain yield. Observations from the subsequent field season (Experiment 2) using the same maize variety support this supposition as infected plants showed significantly lower grain yields compared with uninfected plants.

The response of *S. hermonthica*-infected plants to nitrogen additions

It is perhaps surprising that in Experiment 1 nitrogen neither affected cereal growth nor photosynthesis, nor did it significantly influence the response of the cereals to *S. hermonthica*. This lack of response was also observed in the subsequent season (Experiment 2) where higher rates of nitrogen were applied. However, despite the lack of an overall response to fertiliser application, in Experiment 2 it is interesting to observe that 180 kg N ha^{-1} did have an effect on infected maize plants. The growth of infected plants as determined by ligule heights and stem biomass was stimulated above that of infected plants growing on the unamended plots and plots in receipt of 3 x 60 kg N ha^{-1}. Of particular interest is that the photosynthetic capacity of these plants was not significantly different to uninfected plants, indicating that nitrogen can ameliorate the effects of *S. hermonthica*. Infection in the field typically causes a reduction in leaf chlorophyll concentration (see Chapter

3 Section 3.3.2) possibly because of divsersion of nitrogen to the parasite, increasing protein degradation within the host leaf. However, the nitrogen status of host leaves in all treatments (Experiment 2) was similar to uninfected plants. Laboratory grown *S. hermonthica*-infected sorghum have been observed to have greater free amino acids compared with uninfected hosts (M. Adcock, personal communication) indicating that *S. hermonthica* infected cereals may have an altered ability to incorporate free amino acids into photosynthetic proteins. High rates of nitrogen supply may reduce the effects of the parasite and allow greater nitrogen partitioning into the photosynthetic apparatus thus maintaining rates of photosynthesis.

The high rates of nitrogen application used in Experiments 1 and 2 are not feasible rates for subsistence farmers. In subsistence farming systems soils are generally of a low nutrient status and so the third study was conducted on a farmer's field that was naturally infested with *S. hermonthica*. This area had received little or no fertiliser inputs and had been previously planted with maize. Fertiliser added as 40 kg N ha^{-1} is a more practical application rate in financial terms. In contrast to Experiments 1 and 2, a positive response of infected maize to this low nitrogen addition was observed, with increases in growth, grain yield and photosynthesis despite no effect of the nitrogen on *S. hermonthica* numbers. Chapter 4 demonstrated that nitrogen can exert an effect on the host-parasite association after *S. hermonthica* attachment. The response of photosynthesis and growth in Experiment 2 and 3 to nitrogen application, in the absence of a significant effect on *S. hermonthica* attachment, supports this role of nitrogen post-attachment. The lower rate of nitrogen addition in the farmer's field resulted in an increase in foliar nitrogen concentration of 23%. However even for these fertilised plants, the concentration was still substantially lower than that measured in plants grown on the experimental plots (18.5 compared with 26.6 to 30.3 mg g^{-1}), indicating that greater applications of nitrogen on nutrient poor soil may have a more marked

effect. Under these conditions of low nutrient availability maize plants have greater PNUE compared with plants grown on the experimental plots. This suggests a more effective investment of the nitrogen available (Evans, 1983; Wong *et al.*, 1985).

Why does nitrogen fertiliser have a limited effect on cereal growth and photosynthesis?

There may be a number of explanations for the lack of response to fertiliser additions. First, the experimental plots had been used previously for other studies, and in the past have received inputs of fertiliser, although there is no quantitative record of the amounts supplied. It seems likely therefore, that the availability of nitrogen may have already been high in the plots with large residual concentrations of soil nitrogen. The nitrogen concentration in maize leaf tissues reflects nutrient uptake by the plant and in turn reflects availability in the soil. High concentrations of nitrogen are measured in the uppermost leaves from the experimental plots thus suggesting that even on the unamended plots nitrogen was not limiting. The absence of any marked response of growth and photosynthesis in uninfected cereals to nitrogen addition on these plots, was probably masked by high background levels of this nutrient, supporting this hypothesis. Unfortunately, measurements of soil nitrogen are not available to confirm this supposition.

Second, the methyl bromide fumigation may have released microbially-bound nitrogen, thus adding further to the fertility status of the soil. However, the absence of any significant differences in growth and photosynthesis of infected cereals between the fumigated plots and the non-fumigated checks (see Materials and Methods Section 5.2.2) may negate this argument.

Third, the added nitrogen may not have been available to the plants. This could be the case for a number of reasons. Heavy rains in the first few weeks after planting may have leached the majority of the added nitrogen, thus rendering it unavailable to the plants. Alternatively, the nitrogen may have been bound to soil particles in such a way that it was unavailable to the plants. Wong *et al.* (1990) showed that some soils from the humid tropics are capable of retaining high concentrations of nitrate ions which are recalcitrant to leaching. Addition of nitrogen to such soils may, therefore, have little effect on plant growth. Clearly, studies of soil nutrient status are required to resolve these issues.

Fourth, unlike laboratory studies where nitrogen is often supplied throughout the growth period, in the first study all the nitrogen was supplied as a single application at sowing, and it seems unlikely that the nitrogen would have been available to the crop during most of the growth period. Yaduraju *et al.* (1979) and Mumera and Below (1993) found repeated applications of nitrogen to be far more effective in stimulating host productivity and depressing *S. hermonthica* performance than a single initial application. In the second study nitrogen applied as three 60 kg N ha^{-1} applications reduced the number of emerged parasites by 43.5% but this was not associated with any amelioration of the detrimental effects of *S. hermonthica* on the host.

In summary, the effect of *S. hermonthica* on the growth and photosynthesis of its cereal host can be partially alleviated by applications of high nitrogen fertiliser although the data obtained from experiments 1 and 2 appear to be confounded by high residual soil nitrogen. Nitrogen supplied as a single dose of 180 kg N ha^{-1} appears to be most effective in reducing the effects of *S. hermonthica*, although, because of the problems encountered it would be beneficial to repeat the study on nitrogen limited soils. However, low rates of nitrogen application of 40 kg N ha^{-1} in nutrient poor soils can improve host performance by increasing photosynthesis

and grain yield indicating that nitrogen can be used as a potential control method for *S. hermonthica*.

Chapter 6

The effect of *S. hermonthica* on photosynthesis of two sorghum cultivars: the importance of stomatal limitations and a potential role for ABA

6.1 Introduction

Little is known about the primary mechanism by which photosynthesis is affected by *S. hermonthica*. Although lower rates of CO_2 assimilation were associated with lower rates of transpiration and stomatal conductance in the host in Chapters 2 and 3 (see also Press *et al.*, 1987a; Press and Stewart, 1987), it is unclear whether stomatal conductance is a cause or a consequence of the observed effects on photosynthesis. In leaves infected by biotrophic fungal pathogens, soluble carbohydrates, particularly hexoses, often accumulate (for reviews see Whipps and Lewis, 1981; Farrar and Lewis, 1987; Scholes, 1992) leading to lower rates of photosynthesis. To date no studies have been carried out to examine the carbohydrate content of leaves from *Striga*-infected cereals.

Chapter 5 examined the effect of *S. hermonthica* on the growth and photosynthesis of susceptible cultivars of maize (H511) and sorghum (CK60) cultivars and a local Kenyan sorghum cultivar, Ochuti (reported to show tolerance to *S. hermonthica*) in the field in western Kenya. Both the *Striga*-infected maize cultivar and the sorghum cultivar (CK60) were stunted and had rates of photosynthesis 33% and 45% lower, respectively, than uninfected controls 63 days after planting. In contrast, there was

no decline in the rate of photosynthesis of infected Ochuti plants, despite the presence of *Striga* on the roots.

The aim of the work reported here was to further our understanding of the mechanisms underlying the depression of photosynthesis and growth of *Striga*-infected cereals by comparing the effect of *S. hermonthica* on two cultivars of sorghum; CSH-1 a susceptible cultivar which showed lower rates of photosynthesis when infected by *Striga* (see Chapters 2 and 3) and Ochuti, a cultivar which maintained high rates of photosynthesis in the presence of the parasite (see Chapter 5). Specifically the chapter reports the effect of *S. hermonthica* on: i) growth and biomass accumulation, ii) the nitrogen and carbohydrate content of the leaves, iii) rates of photosynthesis, transpiration and stomatal conductance throughout the time course of the interaction and iv) the kinetics of photosynthetic induction. In order to examine the hypothesis that lower rates of photosynthesis in leaves of *Striga*-infected plants are caused by a change in stomatal conductance, the effect of increasing the internal leaf concentration of CO_2 (C_i) on the rate of photosynthesis and the stable isotope composition of the leaves was examined. The abscisic acid (ABA) content of the leaves and sap of CSH-1 plants was measured as described by Frost *et al.* (1997) because of the potential importance of ABA in controlling stomatal conductance.

6.2 Materials and Methods

6.2.1 Plant material and growth conditions

Plants were grown in rhizotrons and infested with approximately 2000 seeds of *S. hermonthica* (collected from a maize host near Kisumu, western Kenya in 1993) (see Chapter 2 Section 2.2.1). The rhizotrons were transferred to racks inside a controlled environment growth cabinet (Fisons, Fi-totron PG1700), operating with a 12 h photoperiod and a photon flux density of 900 µmol quanta m^{-2} s^{-1} at plant height and a 30/20°C day/night temperature regime. Preconditioning of the *S. hermonthica* seed and infection of the rhizotrons were conducted as described in Chapter 2 Section 2.2.2.

Germinated sorghum seeds (cultivars CSH-1 and Ochuti) were transferred to plastic vials (10 cm^3) containing 40% of full strength Long Ashton solution containing 1 mol m^{-3} nitrogen in the form of ammonium nitrate (see Chapter 2 Section 2.2.3). After 7 days a single seedling was placed into each rhizotron with the roots spread out over the surface of the sand. Plants were watered with 200 ml per day of 40% full strength Long Ashton solution containing 1 mol m^{-3} nitrogen, delivered at 4 intervals during the photoperiod.

6.2.2 Growth measurements

At weekly intervals plant height was measured from the base of the stem to the ligule of the youngest fully-emerged leaf. Plants were harvested at 55 DAP and separated

into tillers, main stem shoots, main stem leaves, and roots. The latter were separated from the sand by careful washing with water, and *Striga* plants were dissected from host roots. Plant material was dried to constant weight in a forced air oven at 70 °C prior to dry weight determination. Specific leaf area (SLA) was calculated by measuring the area of a fully emerged leaf using a leaf area meter (Delta-T Devices Ltd, Cambridge, UK) and then determining the leaf dry weight (as above). Total main stem leaf area was estimated from the SLA.

6.2.3 Carbon isotope and nitrogen analysis

After drying, the midrib was removed from the youngest fully-emerged leaf and the remaining material was milled to a fine powder. $\delta^{13}C$ determination was conducted on 1 mg of dried material using a Vacuum Generators SIRA mass spectrometer attached to a Carlo Erba 1500 Series 2 CHN. An internal standard citric acid leucine (-25.9% against PDB) was used and results expressed in parts per thousand (%o) relative to the PDB standard. Foliar nitrogen concentration was analysed on the youngest fully expanded leaf as described in Chapter 2 Section 2.2.7. Five replicate measurements were carried out for each treatment.

6.2.4 Chlorophyll concentration

Leaf chlorophyll concentration was measured at weekly intervals using a Chlorophyll Meter (SPAD-502, Minolta, UK.). Readings were taken halfway along the youngest fully-emerged leaf. Readings from the meter were calibrated against the chlorophyll

content of leaves on an area basis as determined by extraction in acetone as described in Chapter 2 Section 2.2.6.

6.2.5 Measurements of CO_2 assimilation and chlorophyll fluorescence

Photosynthesis and transpiration were measured on the youngest fully expanded leaf of uninfected and *Striga*-infected plants at weekly intervals (from 30 DAP) using an infra-red gas analyser (IRGA) (LCA4 and PLC-4B leaf chamber, Analytical Development Company, Hoddesdon, UK). Measurements were carried out at ambient CO_2 (350 μmol mol^{-1}), at a temperature of 25 °C and a PFD of 900 μmol quanta m^{-2} s^{-1} (provided by a Schott KL1500T lamp).

To examine the kinetics of photosynthetic induction and the time taken for leaves to attain steady state photosynthesis, the rate of CO_2 assimilation, stomatal conductance and chlorophyll a fluorescence were measured simultaneously using an LCA4 IRGA and modulated fluorimeter (PAM-101 chlorophyll fluorimeter, Walz, Germany). A leaf was placed in the leaf cuvette and darkened for 15 min. The minimal level of chlorophyll fluorescence (Fo) was measured for 20 s after which a pulse of saturating light (6000 μmol quanta m^{-2} s^{-1}; 1 s duration) was given to determine the maximum level of fluorescence (Fm). The leaf was then irradiated with actinic light (900 μmol quanta m^{-2} s^{-1} ; growth PFD) for 20 - 30 min during which time a pulse of saturating light was given at 30 s intervals to allow photochemical quenching, ΦPSII, (Genty, Briantis and Baker, 1989) to be determined. Measurements were carried out at 40 DAP.

To determine the relationship between the rate of photosynthesis (A) and intercellular CO_2 (C_i) measurements of photosynthesis were made at saturating light (2800 µmol quanta m^{-2} s^{-1}) and varying external concentrations of CO_2 using an IRGA (Model LCA3, and PLC-3B leaf chamber, ADC, UK). CO_2-enriched air (1000 µmol CO_2 mol^{-1} gas) was supplied from a gas cylinder (BOC Special Gases Ltd, UK) and CO_2 concentration varied by means of a gas diluter (ADC, UK). Measurements were made halfway along the youngest fully-emerged leaf of infected and control plants 38 and 45 DAP respectively. Gas exchange characteristics were derived using equations described by von Caemmerer and Farquhar (1981).

6.2.6 Carbohydrate analysis

Just prior to the final harvest a leaf disc (2 cm^2) was taken from the youngest fully expanded leaf at the beginning and end of the photoperiod, and frozen in liquid nitrogen. Leaf discs were extracted in 80% buffered ethanol (50 mol m^{-3} Hepes-NaOH, 5 mol m^{-3} $MgCl_2$ pH 7.5) at 70 °C and samples analysed for glucose, fructose and sucrose using an enzyme linked assay as described in Scholes *et al.* (1994). High molecular weight fructans were extracted by incubation in water at 30 °C and measured colorimetrically (Harley and Loughman, 1966; Farrar and Farrar, 1985). Residual leaf material was homogenised in 1 cm^3 of distilled water and autoclaved. Starch was then digested with 14 units of amyloglucosidase (Sigma) and 4 units α-amylase (Boehringer) overnight. Following centrifugation 50 mm^3 of each sample was

analysed for glucose as above. Five replicate measurements were made for each species.

6.2.8 ABA content

Data are reported for ABA concentrations measured on sorghum plants grown in this study as published by Frost *et al.* (1997), using the procedure of Quarrie *et al.* (1988).

6.2.9 Statistical analysis

One way analysis of variance procedures were carried out where appropriate to determine the effect of *S. hermonthica* (Minitab version 10.2).

6.3 Results

6.3.1 *S. hermonthica* attachment

S. hermonthica plants were first observed on the roots of both sorghum cultivars at 24 DAP, 14 days after the plants were transferred to the rhizotrons. At this time the parasitic plants were less than 2 mm in size (no quantitative data available). The *S. hermonthica* plants did not emerge above the surface of the sand during the course of the experiment.

6.3.2 Growth and biomass partitioning

The height to the youngest ligule of both CSH-1 and Ochuti was significantly lower in infected plants in comparison with controls within 4 d of attachment of *S. hermonthica* to the host root system ($p < 0.01$) (Figure 6.1). Differences in height became more marked with time and by 48 DAP infected plants were 78% (CSH-1) and 69% (Ochuti) of the height of control plants (Figure 6.1). Infected plants of CSH-1 and Ochuti produced 73% and 63% of the total biomass of uninfected plants respectively (Table 6.1). *S. hermonthica* also influenced the partitioning of dry matter between the different plant parts such that the architecture of infected and uninfected plants was different. Although the dry weight of both shoot and root was lower in *Striga*-infected CSH-1 and Ochuti plants, shoot weight was affected more than root weight, with the result that root:shoot ratios were significantly greater in infected plants (Table 6.1). Total leaf area was significantly lower in both cultivars in the presence of *S. hermonthica* ($p < 0.05$). In addition, the higher SLA in infected plants indicated that leaves were thinner, although this data was not significant for CSH-1 (Table 6.1). Both sorghum cultivars supported a similar biomass of *S. hermonthica* at the time of harvesting, and the ratio of parasite:host biomass was approximately 1:100.

6.3.3 Leaf chlorophyll and nitrogen concentration

Within 4 days of *S. hermonthica* attachment the amount of chlorophyll in the leaves of infected CSH-1 and Ochuti plants was significantly greater than in control plants ($p < 0.05$) (Figure 6.2). The higher chlorophyll concentrations were maintained throughout the experiment and by 48 DAP the chlorophyll concentration of leaves from infected

CSH-1 and Ochuti plants was 16% and 47% higher than control leaves, respectively. In leaves of uninfected plants the chlorophyll concentration declined slightly as the leaves aged. Leaves of infected CSH-1 and Ochuti plants also contained higher concentrations of nitrogen when expressed on both a dry weight and area basis (Table 6.2).

6.3.4 Gas exchange

The effect of *S. hermonthica* on the rate of photosynthesis (at growth PFD: 900 μmol quanta m^{-2} s^{-1}) of the youngest fully expanded leaf of cultivars CSH-1 and Ochuti is shown in Figure 6.3. The rate of photosynthesis was lower in leaves of *Striga*-infected CSH-1 plants in comparison with control leaves from an early stage of infection and by 48 DAP the rate of photosynthesis was 25% lower in infected plants compared with uninfected plants ($p < 0.05$). The lower rates of photosynthesis were accompanied by lower rates of transpiration, and by 48 DAP rates of infected plants were 46% of those of controls (Figure 6.3). In leaves of Ochuti plants infected with *S. hermonthica* photosynthesis was initially lower in comparison with control leaves (28 DAP; $p < 0.05$) but thereafter, both the rates of photosynthesis and transpiration were similar to those of control leaves. A similar pattern of results was observed for both cultivars when photosynthesis was measured at saturating PFD (2800 μmol quanta m^{-2} s^{-1}) (data not shown). As the concentration of nitrogen per unit dry weight and per unit area was greater in leaves of infected plants of both cultivars compared with uninfected plants, PNUE was lower than in control leaves of both cultivars (Table 6.2) but this was only significantly so for CSH-1.

In order to determine whether the kinetics of photosynthetic induction were altered in leaves of *Striga*-infected plants, photosynthesis, stomatal conductance, and chlorophyll fluorescence quenching were measured during a dark to light transition in sorghum plants at 40 DAP. In both CSH-1 and Ochuti the rate of photosynthesis (measured by CO_2 assimilation or by the chlorophyll fluorescence parameter Φ_{II}) increased rapidly in leaves of control plants and a reached a steady rate after approximately 10-15 min (Figure 6.4). In both cultivars the increase in photosynthesis was accompanied by a rapid increase in stomatal conductance. In leaves of *Striga*-infected CSH-1 plants both photosynthesis and stomatal conductance increased slowly following irradiation. After 15 min the rate of photosynthesis and stomatal conductance had almost reached steady state and were lower than in control leaves (Figure 6.4). In leaves of Ochuti infected with *Striga* photosynthesis and stomatal conductance again increased slowly after irradiation. However, after a long induction time (25-30 min) they eventually reached the same values as the control leaves (Figure 6.4). There was a positive relationship between the rate of photosynthesis and the degree to which stomata were open in leaves of control and *Striga*-infected plants throughout photosynthetic induction (Figure 6.5). Comparison of the regression lines in infected and uninfected plants, shows that in CSH-1 the relationship between the variables was affected by the presence of *S. hermonthica*, with the slopes of the regression lines in infected and uninfected plants differing significantly ($p < 0.01$). In Ochuti plants, the relationship was not significantly affected by the presence of *S. hermonthica* (Figure 6.5).

To determine whether the rate of photosynthesis of *Striga*-infected plants was limited by stomatal conductance, A/C_i curves were constructed to allow a comparison of the rate of photosynthesis of leaves of control and infected plants at similar values of C_i (CO_2 concentration in the substomatal spaces). In leaves of both cultivars the initial slope of the A/Ci curve and the rate of photosynthesis at high CO_2 (> 800 ppm) was similar in leaves of control and infected plants suggesting that at this stage of infection stomatal conductance was largely limiting photosynthesis (Table 6.3). The greater discrimination against ^{13}C observed in leaf tissue from infected plants, as shown by the lower values of $\delta^{13}C$ (Table 6.3), would be consistent with a decrease in the ratio of intercellular to ambient CO_2.

In order to investigate possible causes for the decrease in stomatal conductance in leaves from infected plants the ABA content of leaves and sap from control and infected CSH-1 plants was measured when plants were 55 days old as reported in Frost *et al.* (1997). The concentration of ABA in the leaves and sap of infected plants was 36% and 52% higher than in control leaves and sap, respectively (Table 6.4).

6.3.5 Carbohydrate content

There was no effect of *Striga* infection on the amount of soluble carbohydrates or starch in leaves of CSH-1 plants in the morning or evening when compared with control leaves (Figure 6.6). In Ochuti plants infected with *Striga* the amount of soluble carbohydrates was lower than in control leaves in the morning but similar in the evening (Figure 6.6). The amount of starch in leaves from infected plants was

substantially reduced both in the morning and evening in comparison with control plants suggesting that carbohydrate reserves had been mobilised and exported from the leaf (Figure 6.6). Fructans were not detected in leaves of either CSH-1 or Ochuti.
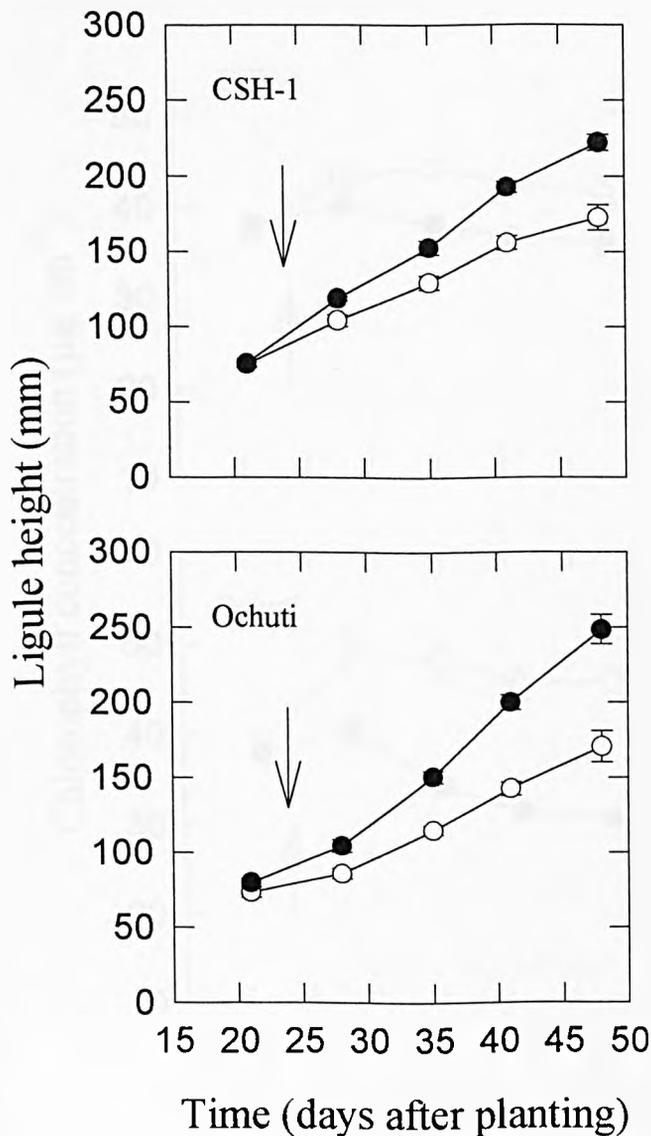
Figure 6.1 Distance from the base of the stem to the ligule of the youngest fully expanded leaf of two sorghum cultivars CSH-1 and Ochuti, grown in the absence (closed symbols) or presence (open symbols) of *S. hermonthica*. Data are means and standard errors of eight replicates and analysed using analysis of variance procedures (see text). The timing of the first visible attachment of *S. hermonthica* to the host roots is denoted by the arrow.

Figure 6.2 Leaf chlorophyll concentration (mg m^{-2}) of the youngest fully expanded leaf of two sorghum cultivars CSH-1 and Ochuti. Plants were grown in the absence (closed symbols) or presence (open symbols) of *S. hermonthica*. Means and standard errors of eight replicates are reported and analysed using analysis of variance procedures (see text). The timing of the first visible attachment of *S. hermonthica* to the host roots is denoted by the arrow.
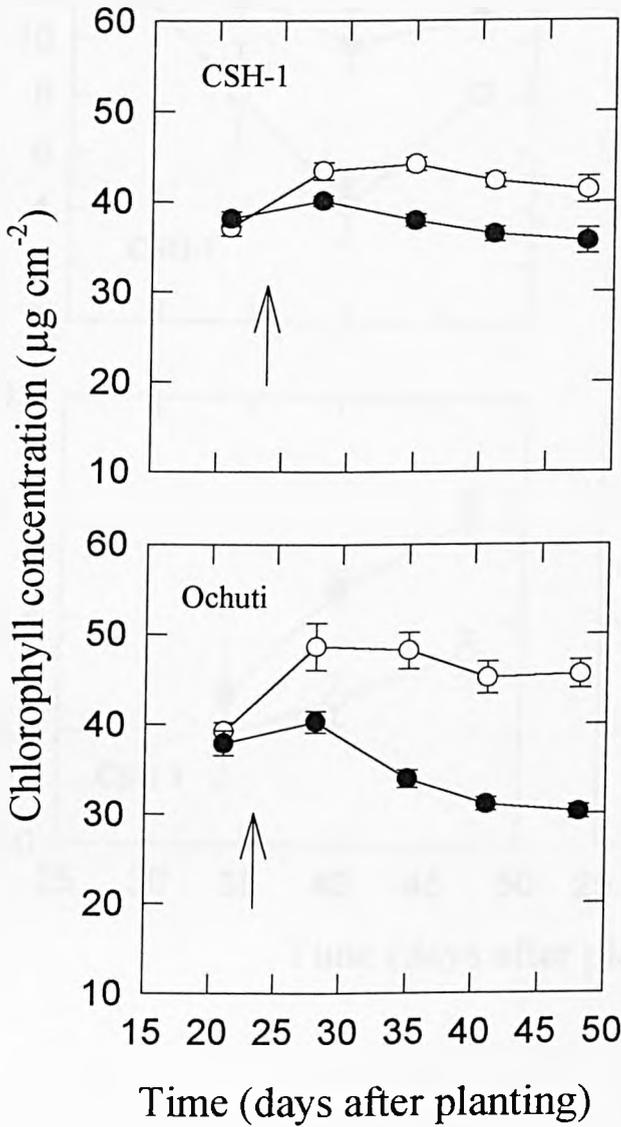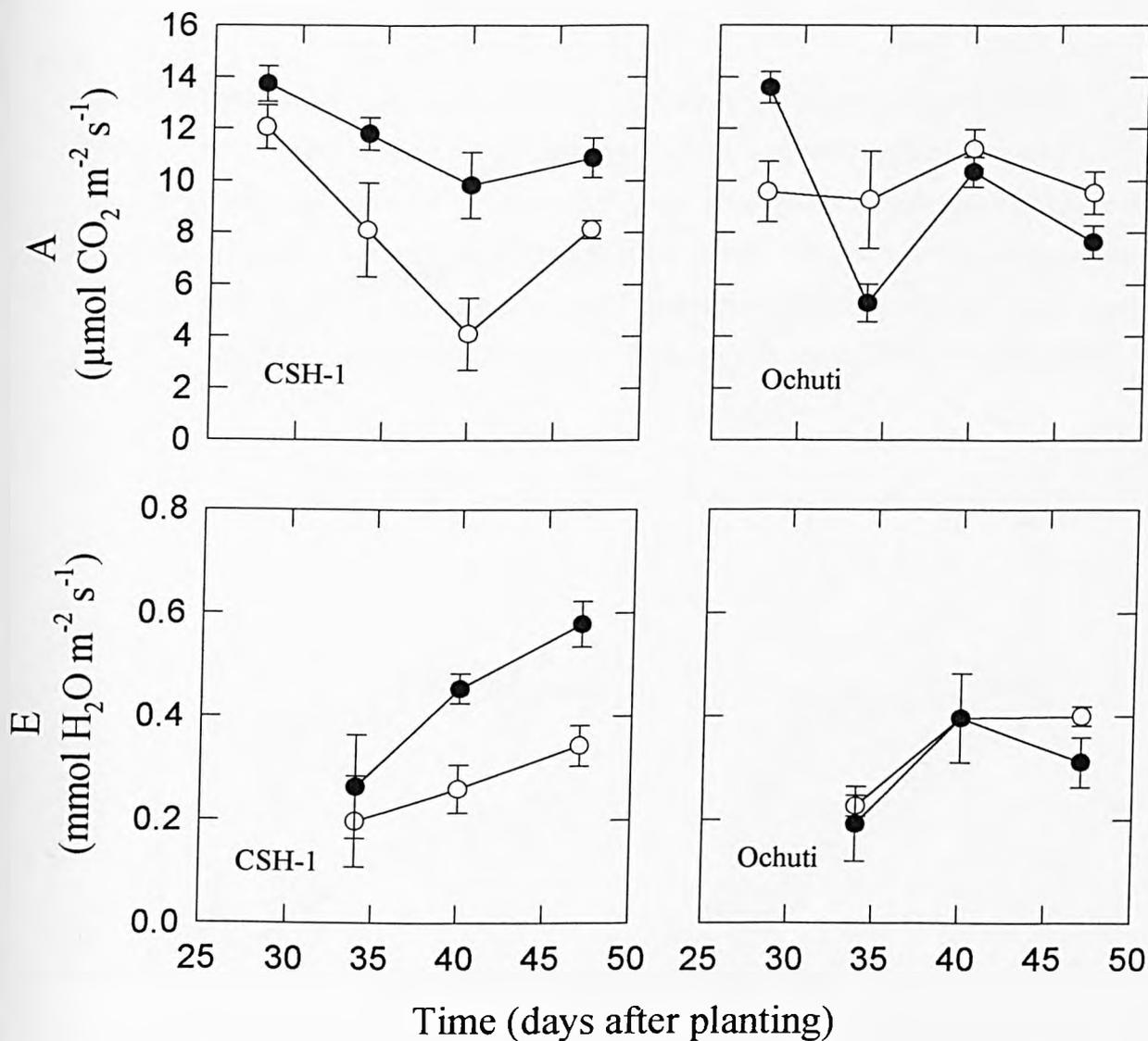
Figure 6.3 Rate of photosynthesis (A) and transpiration (E) of the youngest fully expanded leaf of two sorghum cultivars CSH-1 and Ochuti. Plants were grown in the absence (closed symbols) or presence (open symbols) of *S. hermonthica*. Means and standard errors of four replicates are reported. Measurements were made at an irradiance of 900 μmol quanta m^{-2} s^{-1}.

Figure 6.4 Photochemical quenching of chlorophyll fluorescence (Φ_{II}), rate of photosynthesis (A), and stomatal conductance (gs) of the youngest fully expanded leaf of two sorghum cultivars CSH-1 and Ochuti measured at 900 µmol quanta m^{-2} s^{-1} following a period of dark-adaptation (see text for details). Plants were grown in the absence (closed symbols) or presence (open symbols) of *S. hermonthica*. Means and standard errors of three replicates are reported. Note the difference in scale of the X-axis between the two cultivars.

CSH-1   Ochuti

Φ_{II}

A
(μmol CO_2 m^{-2} s^{-1})

gs
(mol m^{-2} s^{-1})

Time from start of illumination (min)
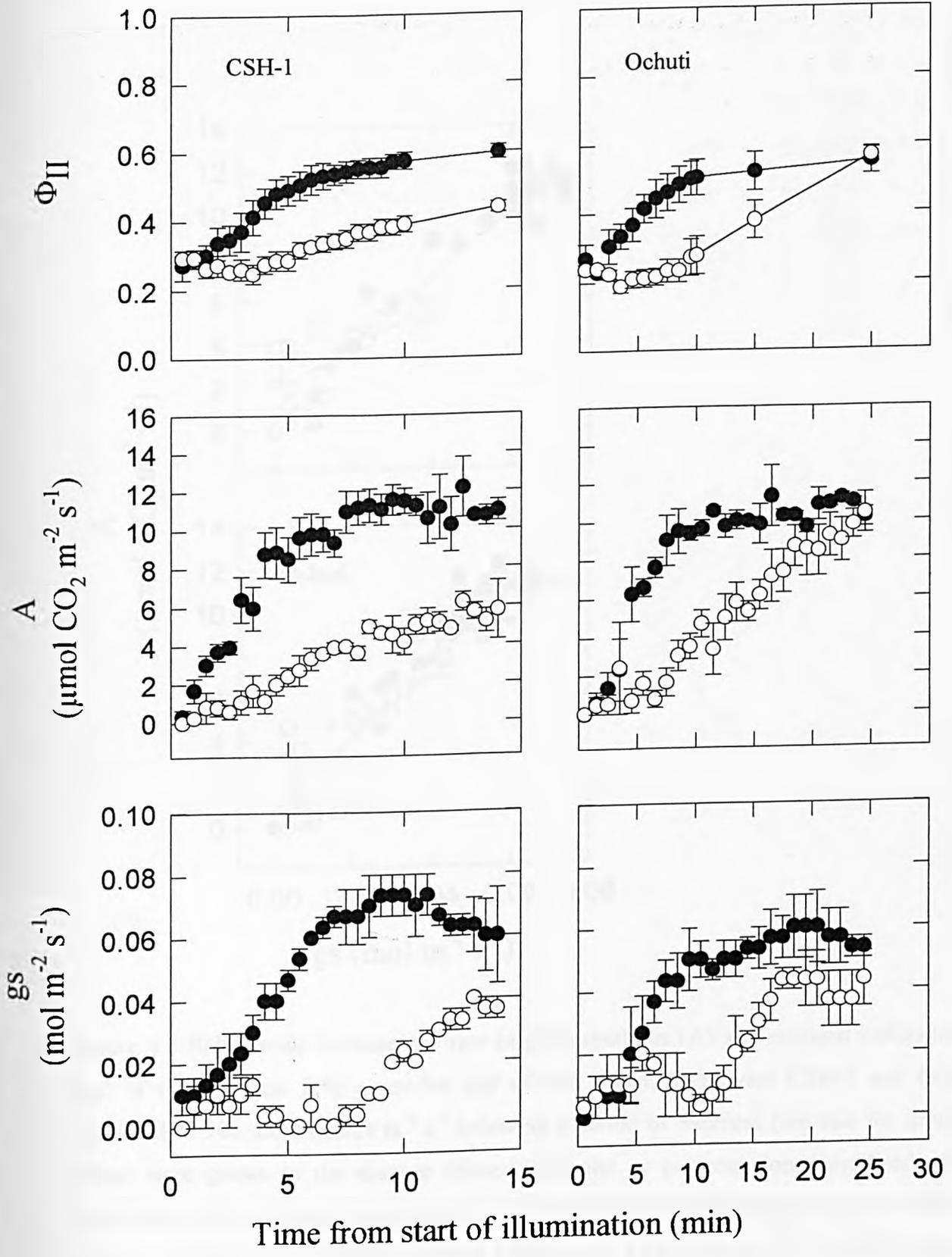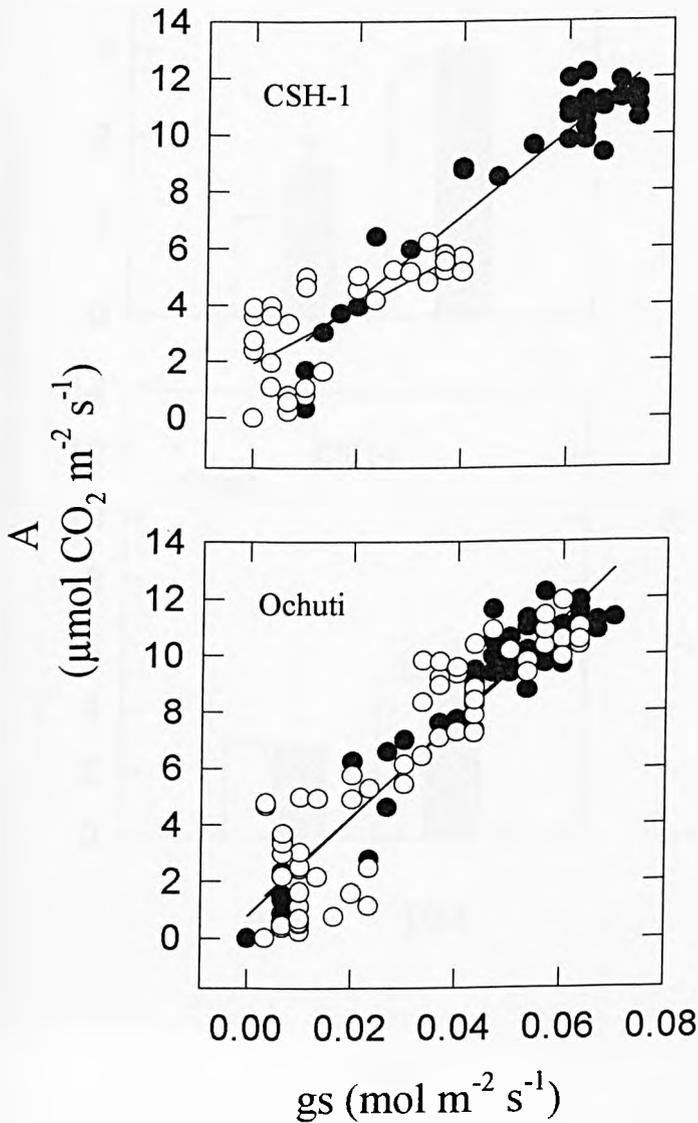
Figure 6.5 Relationship between the rate of photosynthesis (A) and stomatal conductance (gs) of the youngest fully expanded leaf of two sorghum cultivars CSH-1 and Ochuti measured at 900 μmol quanta m^{-2} s^{-1} following a period of darkness (see text for details). Plants were grown in the absence (closed symbols) or presence (open symbols) of *S. hermonthica*. Data replotted from Figure 6.4. The regression equations are control CSH-1, y = 1.31 + 147x , (r ^{2} = 0.903), infected CSH-1, y = 1.93 + 98.3x, (r^2 = 0.054), control Ochuti, y = 0.789 + 175x (r^2 = 0.915), infected Ochuti, y = 0.952 + 169x (r^2 = 0.821).
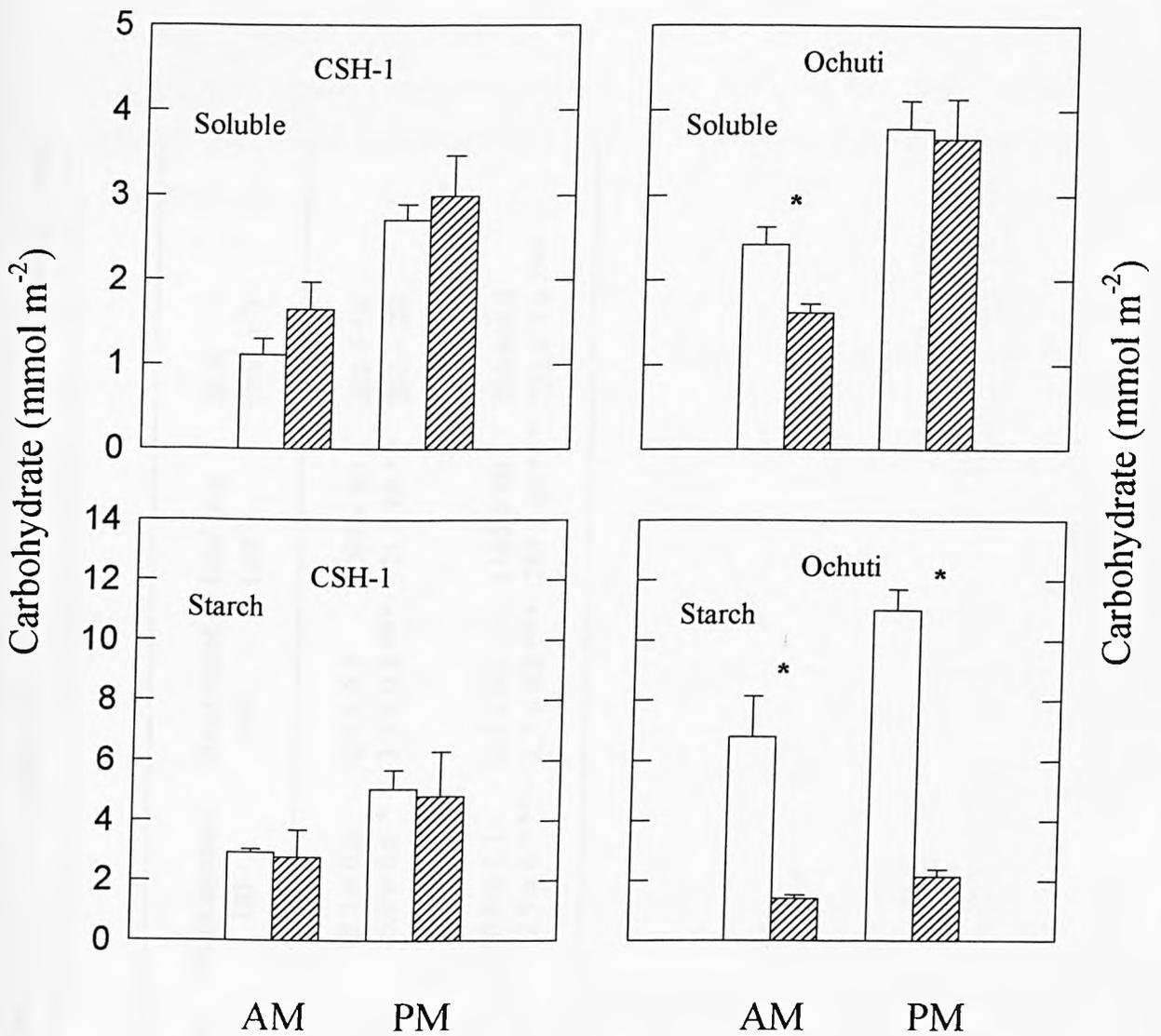
Figure 6.6 Soluble carbohydrate and starch content of the youngest fully expanded leaf of two sorghum cultivars CSH-1 and Ochuti, measured at the beginning (AM) and end (PM) of the 12 hour photoperiod at 55 DAP. Plants were grown in the absence (open bars) or presence (hatched bars) of *S. hermonthica*. Means and standard errors of five replicates are reported.

Table 6.1 Dry weight partitioning of sorghum plants grown in the absence (-) or presence (+) of *S. hermonthica*, 55 DAP. Means and standard errors are reported for eight replicate measurements and analysed using analysis of variance procedures (* p< 0.05, ** p < 0.01, *** p < 0.001).

Cultivar	*Striga*	*Striga* biomass	Total plant biomass (g)	Shoot biomass (g)	Root biomass (g)	Root:shoot ratio	Leaf area (cm^2)	SLA (cm^2 g^{-1})
CSH-1	-	-	28.0 ± 0.8	9.4 ± 0.3	18.5 ± 0.8	2.0 ± 0.1	1097 ± 80	202 ± 14
	+	0.244 ± 0.03	20.5 ± 1.1***	4.3 ± 0.4 ***	16.0 ± 0.8 *	3.7 ± 0.3 ***	721 ± 68 **	251 ± 20
Ochuti	-	-	27.3 ± 1.2	10.9 ± 0.3	16.4 ± 1.1	1.5 ± 0.1	1185 ± 40	206 ± 6.0
	+	0.217 ± 0.04	17.2 ± 0.8 ***	4.7 ± 0.3 ***	12.5 ± 0.7 **	2.7 ± 0.3 ***	753 ± 40 ***	252 ± 7.0 ***

Table 6.2 Nitrogen concentration and photosynthetic nitrogen use efficiency (PNUE) of leaves of sorghum plants grown in the absence (-) or presence (+) of *S. hermonthica.* Means and standard errors of five measurements are reported and analysed using analysis of variance procedures (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Cultivar	*Striga*	Nitrogen (g m^2)	Nitrogen (mg g^{-1})	PNUE (μmol CO$_2$ mol N s^{-1})
CSH-1	-	0.98 ± 0.05	18.9 ± 0.5	153.3 ± 5.9
	+	1.16 ± 0.09 *	25.0 ± 2.4 *	103.6 ± 10.3 *
Ochuti	-	0.81 ± 0.02	17.4 ± 0.5	129.8 ± 13.1
	+	1.2 ± 0.04 ***	30.2 ± 1.1 ***	120.0 ± 12.3

Table 6.3 The relationship between the rate of photosynthesis and intercellular CO_2 (initial gradient and Amax) and the stable isotope composition of leaves of 55 d old sorghum plants. Plants were grown in the absence (-) or presence (+) of *S. hermonthica*. Means and standard errors of five measurements are reported and analysed using analysis of variance procedures (*** $p < 0.001$).

Cultivar	*Striga*	Initial gradient (A *vs* C_i)	Amax (μmol CO_2 m^{-2} s^{-1}) (Ci $\approx$ 800 μmol mol^{-1})	δ^{13}C (‰)
CSH-1	-	0.16 ± 0.01	26.83 ± 1.47	-11.44 ± 0.03
	+	0.14 ± 0.01	26.76 ± 1.23	-12.15 ± 0.03 ***
Ochuti	-	0.15 ± 0.02	25.45 ± 1.05	-11.61 ± 0.03
	+	0.15 ± 0.01	29.64 ± 2.12	-12.12 ± 0.05 ***

Table 6.4 The effect of *S. hermonthica* on the abscisic (ABA) content of xylem sap and leaf tissue of the sorghum cultivar CSH-1. Means and standard errors of twelve measurements are reported and analysed using analysis of variance procedures (* $p < 0.05$). Data reported in Frost *et al.* (1997).

| | ABA | |
	Control	Infected
Leaf tissue (nmol g^{-1} dry weight)	33.4 ± 5.1	52.6 ± 8.4
Xylem sap (μmol m^{-3})	29.9 ± 2.4	62.2 ± 8.1 *

6.4 Discussion

How does *S. hermonthica* affect biomass partitioning and the growth of CSH-1 and Ochuti?

In this study infection by *S hermonthica* altered the growth and allocation of biomass in a similar manner in both sorghum cultivars, CSH-1 and Ochuti. A difference in the height of infected plants was observed within 4 days of visible attachment of the parasite to the host root system compared with uninfected plants and by the end of the experiment infected plants were 30% shorter. Both root and shoot biomass and total leaf area were lower than in control plants whereas the root:shoot ratio was higher. These effects of *S. hermonthica* upon the growth and biomass of its host are consistent with those reported in earlier studies (Graves *et al.*, 1990; Cechin and Press, 1993a).

Graves *et al.*, (1989) constructed a carbon balance model for the association between *S. hermonthica* and the sorghum cultivar CSH-1 and concluded that only 20% of the predicted loss in host production over the lifetime of the association could be accounted for by direct loss of carbon to the parasite, with the major limitation to productivity being a parasite-induced lowering of host photosynthesis, on a canopy scale. Whilst data presented in this study (see also Chapters 2 and 4) confirm that photosynthesis in CSH-1 was significantly lower in plants infected with *S. hermonthica* than in control plants, this was not the case for the cultivar Ochuti. Although the extent to which the parasite resulted in lower biomass accumulation and

height growth of CSH-1 and Ochuti plants was similar, the rate of steady-state photosynthesis of the youngest leaf of *Striga*-infected Ochuti was comparable with uninfected plants throughout most of the time course of the association. Although it is possible that the rate of photosynthesis was lower in older leaves of infected Ochuti plants and the architecture of these plants was different from uninfected plants, these results suggest that there may not be a simple relationship between changes in the rate of photosynthesis and alterations in the growth of infected plants. This is supported by the observation that the parasite had affected the height of the plants 4 days after attachment to the roots, before there was a measurable change in the rate of photosynthesis (CSH-1) (see Figures 6.1 and 6.3).

Drennan and El Hiweris (1979) investigated the role of plant growth regulators in determining the growth response of cultivars of *Sorghum vulgare* to *S. hermonthica*. Their study showed that xylem sap from infected plants contained lower quantities of cytokinins and gibberellins (measured by bioassay), and higher quantities of abscisic acid (ABA) than sap from uninfected plants, suggesting that perturbations in the balance of growth regulators may contribute to changes in host architecture. In this study the concentration of ABA in the sap and leaf tissue of infected CSH-1 plants was double that found in uninfected plants. In a recent study by Taylor *et al.* (1996) the ABA content of leaves from maize infected with *S. hermonthica* was higher than that of control leaves. Greater concentrations of ABA in the xylem sap reported here would be expected to reduce leaf expansion (e.g. Zhang and Davies, 1990a; Dodd and Davies, 1994), and it was apparent that the size of individual leaves was reduced in

infected plants (data not shown). ABA has also been shown to enhance the root:shoot ratio and reduce stem growth (Sloger and Caldwell, 1970; Trewavas and Jones, 1991). Clearly the effect of *S. hermonthica* on the plant growth regulator status of the host merits further investigation.

Is the decrease in stomatal conductance a cause or a consequence of the lower rates of photosynthesis in infected plants?

Although lower rates of photosynthesis are frequently recorded in leaves of *Striga*-infected plants when compared with uninfected controls, the primary cause of this phenomenon is poorly understood. Lower rates of photosynthesis are commonly associated with lower rates of transpiration and stomatal conductance (Press *et al.*, 1987b; Press and Stewart, 1987; see Chapters 2 and 3). Infection by *S. hermonthica* appears to alter the relationship between photosynthesis and stomatal conductance (Figure 6.5), but whether the latter is the cause or a consequence of other changes in photosynthetic metabolism remains controversial. Data from this study suggest that lower values of stomatal conductance are a primary cause of lower rates of photosynthesis in leaves of *Striga*-infected sorghum, for a number of reasons.

Firstly, there was a strong correlation between the rate of photosynthesis and stomatal conductance for both cultivars of sorghum. During a dark to light transition the rate of photosynthesis and stomatal conductance increased slowly in leaves of infected CSH-1 plants but never reached the same values as in control leaves (Figure 6.4). In

the cultivar Ochuti, stomata opened very slowly in leaves of infected plants, following irradiation, and this was accompanied by a slow increase in the rate of photosynthesis. When stomatal conductance equalled that of the uninfected plant, the rate of photosynthesis was also similar indicating that there were no other limitations to photosynthesis in this cultivar.

Secondly, a small but highly significant increase in discrimination against ^{13}C was observed in both sorghum cultivars in response to infection by *S. hermonthica* (Table 6.3), consistent with a decrease in the ratio of intercellular to ambient CO_2 concentration within the leaf (C_i/C_a), and hence changes in stomatal functioning (Farquhar, Ehleringer and Hubick, 1989). It is important to note however, that because of the CO_2-concentrating mechanism of the carboxylation pathway, the relationship between δ^{13}C values and C_i/C_a in C_4 plants also depends upon the proportion of carbon fixed by PEP carboxylase that subsequently leaks back out of the bundle sheath cells (Farquhar, 1983).

In order to determine whether the rate of photosynthesis of leaves of control and infected plants was similar at comparable internal concentrations of CO_2, A/C_i curves were constructed. These showed that the rate of photosynthesis was similar for a given C_i in both sorghum cultivars, consistent with a stomatal limitation to photosynthesis. However, these data contrast with those of Press *et al.* (1987a) which suggested that stomatal conductance was responding to, rather than causing, a decrease in photosynthesis since A/C_i analysis conducted on sorghum infected with *S.*

hermonthica indicated that both the maximum rate of photosynthesis at saturating CO_2 and the initial slope of the relationship was significantly reduced by the presence of the parasite. However, this response is clearly variable as a similar analysis performed by Press and Cechin (1994) showed that the maximum rate of photosynthesis was reduced by *S. hermonthica* but there was no alteration in the initial slope of the relationship. An important difference between these earlier studies and the present investigation is that in the former plants were grown in pots, and A/C_i curves constructed for older sorghum plants, once the parasite had emerged above the soil surface and become photosynthetic. Thus whilst data from this study clearly show that lower stomatal conductance is a primary cause of the decrease in photosynthesis, other effects on metabolism, particularly later during infection, cannot be ruled out. In addition, the nature and severity of effects of the parasite may be modified by both genotype (species and cultivar) and the nutritional status of the host.

Two recent studies have indicated that the extractable activities of enzymes of the photosynthetic pathway, Rubisco, PEP carboxylase and NADP-malic enzyme are unaltered in leaves of *Striga*-infected sorghum (Press and Cechin, 1994) and maize (Smith *et al.*, 1995) although in the latter study an enhanced incorporation of ^{14}C into glycine and serine suggested that there was an increase in photorespiratory metabolism. Smith *et al.* (1995) suggest that this occurred as a result of 'leaky' bundle sheath cells. However, it is also possible that an increase in photorespiration may result from the decrease in intercellular CO_2 associated with stomatal closure and this possibility is currently being investigated.

In leaves infected with biotrophic fungal pathogens soluble carbohydrates often accumulate and may contribute to the down-regulation of photosynthetic metabolism in these leaves (Scholes *et al.*, 1994; Tang, Rolfe and Scholes, 1996). There was no accumulation of carbohydrates in leaves of *Striga*-infected sorghum plants. In leaves of CSH-1 *Striga* infection had little effect on the content of soluble or storage (starch and fructans) carbohydrates even when *Striga* had emerged above ground and therefore represented a larger sink (data not shown). In contrast, in leaves from infected Ochuti plants the soluble carbohydrate content of the leaves was lower than that of controls in the morning, although carbohydrates were synthesised during the day, and there was a severe reduction in starch content (Figure 6.6). This is consistent with a mobilisation of carbohydrate reserves and increased export to the roots.

In both CSH-1 and Ochuti, the concentration of chlorophyll was higher in leaves from infected plants compared with those from uninfected plants shortly after attachment of *S. hermonthica* to the roots (Figure 6.2), The concentration of nitrogen was also greater per unit dry weight and per unit area of leaf. These results are consistent with observations in Chapters 2 and 4). As a consequence photosynthetic nitrogen use efficiency was much lower in these leaves than in those from uninfected plants.

Is ABA responsible for the lower stomatal conductance in sorghum infected with *S. hermonthica?*

At present relatively few studies have examined the role of alterations in plant growth regulators on the photosynthetic metabolism of cereals infected with *Striga*. However, in this study and in that of Drennan and El Hiweris (1979), the concentration of ABA in the xylem sap of infected plants was elevated in comparison with that of control plants and the anti-transpirational effects of ABA are well documented. Parasitic plants generally have high rates of transpiration (Stewart and Press, 1990) which may predispose hosts to water stress and thus stomatal closure. However, it seems very unlikely that lower levels of stomatal conductance resulted from severe water stress in the current study since no emergence of *S. hermonthica* above the surface of the sand in the rhizotrons occurred, and the system was maintained in a well-watered state throughout the study. It is possible that attachment of *Striga* to the roots induced localised water stress or a wounding response and there is evidence that ABA originating in plant roots can cause stomatal closure in the absence of any perturbations in leaf water potential (Zhang, Schurr and Davies, 1987; Zhang and Davies, 1990b, 1991).

In this study it is clear that the stomatal response of the two sorghum cultivars differs. Although the concentration of ABA in the sap of Ochuti was not measured, these results perhaps suggest that Ochuti may either be less 'sensitive' to changes in the concentration of ABA or that the magnitude of the changes differs. Cultivar variation in the response to exogenously applied ABA is well documented. For example, only 14 out of 34 soybean cultivars responded to ABA application, as indicated by leaf senescence, abscission and reduced stem growth (Sloger and Caldwell, 1970). For the

cultivars which were affected by ABA physiological responses and stem height inhibition were differentially modified. In addition, Quarrie (1983) has detailed genotypic variation in ABA-induced stomatal closure with plants varying from insensitive to very sensitive to ABA.

Why does *S. hermonthica* reduce the growth of Ochuti in laboratory studies but not when grown in the field?

In this study infection of the cultivar Ochuti with *S. hermonthica* resulted in smaller plants which had accumulated less biomass than their uninfected equivalents, although photosynthesis was much less affected than in the cultivar CSH-1. In contrast, when Ochuti was grown in the field in western Kenya in 1994 (Chapter 5) the height of the plants and the rate of photosynthesis of infected plants were unaffected in comparison with controls at 63 DAP (by which time *Striga* had emerged above the soil surface) and subsequent grain yield was also unaffected. A subsequent field study in 1996 showed a slight lowering of growth and photosynthesis in infected plants compared with uninfected plants, but only by the final measurements at 65 DAP. The difference in growth of infected plants in the laboratory and in the field may be due to several factors. Firstly, it is clear that the rate of photosynthesis and stomatal conductance are not severely affected in Ochuti by the presence of *S. hermonthica*, consistent with its status as a 'tolerant' host (Chapters 3 and 5). In addition, in both field studies, the time of attachment of *S. hermonthica* to the host roots is unknown and it is possible that Ochuti plants were better established at the time of attachment. The age of the sorghum plant when *S. hermonthica* attaches to the root system is an important

determinant of the host's response, with larger differences in height growth and rates of photosynthesis being observed between infected and uninfected plants with young hosts compared with older hosts (Cechin and Press, 1993c; Press and Cechin, 1994). If changes in the plant growth regulator status of the plant following infection play a fundamental role in altering growth and physiology then later attachment to the host may lessen these effects. Secondly, the nutrient status of the host is known to affect the severity of symptoms of infected plants, with plants grown under a lower nitrogen supply being more severely affected than those grown with a high nitrogen (Cechin and Press, 1993a; Chapter 4). Infected Ochuti plants grown in rhizotrons had foliar nitrogen concentrations of 30.2 mg g^{-1} (Table 6.2), similar to those of field-grown plants which had nitrogen concentrations of 24.6 mg g^{-1} (Chapter 3, Table 3.6). However, there may be other nutrients interacting with nitrogen, for example phosphorus, that may increase the effects of *S. hermonthica*. Thus the environmental conditions under which the plants are grown can significantly influence the host-parasite association, and observations of the performance of the sorghum cultivar Ochuti growing in field trials in Kenya suggest that its degree of tolerance can be variable (J. K. Ransom, personal communication).

Chapter 7

General discussion

7.1 Summary and conclusions

7.1.1 Introduction

Responses of host plants to infection by parasitic angiosperms have been studied for relatively few host-parasite associations, and the attention of researchers has concentrated on parasites that cause problems in agro-ecosystems, such as *Striga* and *Orobanche* species (see e.g. Parker and Riches, 1993; Press and Graves, 1995). Even in these associations aspects of the interaction between host and parasite are still poorly understood. The genus *Striga* is arguably the most important biotic factor constraining cereal production for small holders in semi-arid tropical Africa (Parker and Riches, 1993). Grain production of more than 44 Mha of arable land is under threat and estimated yield losses of 30-50% are common under heavy infestations (Parker, 1991; Parker and Riches, 1993). This thesis examined aspects of the *S. hermonthica*-cereal association in both laboratory and field environments, with the aim of increasing our understanding of this economically and socially important weed.

7.1.2 State of knowledge prior to thesis

Some aspects of the interaction between *Striga* and its cereal host are well understood e.g. germination and haustorial initiation and structure (Boone *et al.*, 1995; Riopel and Timko, 1995; Dörr, 1997), however, explanations for the observed effects of the parasite on host growth have proved to be elusive.

Laboratory studies of the *S. hermonthica*-sorghum association are reported by Cechin (1994b). The thesis examined the response of growth and carbon fixation in sorghum plants (cultivar CSH-1) infected with *S. hermonthica* and also the influence of nitrogen application on this relationship. The author demonstrated that *S. hermonthica* lowered the productivity of sorghum and that this was partly due to lower rates of photosynthesis in infected sorghum compared with uninfected plants. The extent to which the parasite influenced growth and photosynthesis was dependent on the concentration of nitrogen supplied as ammonium nitrate. High concentrations of nitrogen had a significant effect on the host-parasite association, ameliorating the detrimental effects of the parasite. This was partially explained by the inhibitory influence of nitrogen on germination and attachment of *S. hermonthica*, as high concentrations of nitrogen either lowered the production of stimulatory components in host roots or their rate of exudation.

7.2 Critical questions: aims of the thesis

The informative studies of Cechin (1994b) raised many important questions. The experiments were laboratory based and only examined the sorghum cultivar CSH-1, (and one C_3 cereal, rice). It was not known whether the degree of response to infection would be similar between different cereal species and cultivars. In addition, it was not known whether the physiology of cereals infected with *S. hermonthica* under field conditions would respond in a similar manner to those observed in controlled laboratory conditions. Intercultivar field studies are reported in the literature (see e.g. Ransom, *et al.*, 1991; Moreno, *et al.*, 1996), however, most either lack uninfected

controls or progress further than gross measurements of cereal production. There has been little attempt to examine the physiology of the *S. hermonthica*-cereal association under field conditions. As previously noted, in the majority of field studies uninfected plants have not been established in *Striga*-infected areas. Riches and Parker (1995) identified this issue as a factor which increases the difficulty of estimating grain losses due to *Striga* infection. A priority of this thesis was to examine how *S. hermonthica* influences its cereal host and to determine the extent to which maize and sorghum cultivars respond differently to infection.

Laboratory measurements have determined that lower rates of photosynthesis (at the whole plant level) in infected plants are one of the primary reasons for a loss of host productivity (Press and Stewart, 1987; Press *et al.*, 1987a; Cechin, 1993a). A carbon balance model for the *S. hermonthica*-sorghum association estimated that 80% of the loss of productivity of *S. hermonthica*-infected sorghum was accounted for by lower rates of canopy photosynthesis (Graves *et al.*, 1989). Despite these laboratory studies, lower rates of photosynthesis had not been observed under field conditions. A field study in Mali (Clark, 1994) failed to record lower rates of photosynthesis in infected sorghum plants compared with uninfected controls at 67 or 79 DAP (13 and 25 days after the emergence of *S. hermonthica*, respectively). These results are surprising, partly because of previous laboratory observations and also because the infected plants produced 53% less grain compared with uninfected plants. Possible explanations for the similar rates of photosynthesis between infected and uninfected plants are discussed in Chapter 3. In summary is likely that lower rates were not detected

because of: i) the late emergence of *Striga*, which may lessen the effects on the host plant (Cechin, 1993c), ii) the local sorghum studied (cultivar Tiémarifing) may respond differently to those studied in the laboratory (usually cultivar CSH-1) and iii) lower rates of photosynthesis may be experienced lower in the plant canopy. In light of these findings it was of great importance to determine whether rates of photosynthesis were lower in infected cereals grown under field conditions, whether different cereal species/cultivars responded differently to *S. hermonthica* infection and the implications of any effects on photosynthesis for grain yield. In addition, it was of importance to gain a greater understanding of the mechanism by which *S. hermonthica* lowers photosynthesis.

The negative influence of nitrogen on the germination and attachment of *Striga* has been demonstrated in laboratory studies (Parker, 1984; Raju *et al.*, 1990; Cechin, 1993b). Addition of nitrogen to the soil is considered to stimulate crop growth, alleviate the effects of *Striga* and lower *Striga* infestation. However, the beneficial effects of adding nitrogen fertiliser have not been conclusively demonstrated (see Chapter 1, Table 1.1 and reviews by Pieterse and Verkleij, 1991; Pieterse, 1996). This thesis aimed to understand more fully the role of nitrogen, supplied as ammonium nitrate in alleviating the effects of *S. hermonthica* under both laboratory and field conditions.

Complete resistance to *S. hermonthica* in sorghum and maize has not been identified. Greater progress towards identifying resistance has been made for a different

association, that between *S. gesnerioides* and cowpea (Lane *et al.*, 1993; Lane *et al.*, 1996). Partial resistance to *Striga* has been identified in a cultivar of sorghum known as the *S. asiatica* resistant (SAR) line. The SAR cultivars exude low concentrations of germination stimulants, lowering parasite germination (Hess *et al.*, 1992). The inhibition of germination has been a key target for providing parasite control whilst mechanisms or characteristics of tolerance/resistance to *Striga* after attachment of the parasite have not been identified. In this thesis a strong emphasis was placed on adopting a comparative approach by examining the effects of *S. hermonthica* on both sorghum and maize and investigating genotypes which differ in their supposed sensitivity to *S. hermonthica*.

7.3 Achievements: summary of results

7.3.1 The creation of control *Striga*-free plots

Fumigation of a naturally infested area of land with methylbromide successfully eradicated all of the *Striga* seed in the soil seed bank (Chapters 3 and 5). Methylbromide directly kills all seeds, fungal and bacterial populations of the treated soil (Russell, 1973). Ethylene gas fumigation has been used successfully in the United States for the control of *S. asiatica* (Egley *et al.*, 1990), as ethylene induces *Striga* germination in the absence of a host plant. Ethylene gas is less effective in areas of Africa because of the very large seed bank present in many infested areas. The establishment of *S. hermonthica*-free areas of land (Chapters 3 and 5) allowed a detailed comparison of infected and uninfected cereal growth and physiology under

field conditions. Importantly, the creation of control plants allowed an accurate determination of yield losses resulting from infection.

7.3.2 *S. hermonthica* impairs host photosynthesis in the field

Laboratory studies demonstrated that *S. hermonthica* adversely affected the growth of its sorghum host by competing with the host sinks for carbon and by lowering the photosynthesis of the host (Chapter 2), supporting previous studies (Press *et al.*, 1987a; Cechin, 1993a). However, for the first time lower rates of photosynthesis were observed for *S. hermonthica*-infected maize and sorghum plants grown in the field (e.g. Chapter 3), contrasting with results of Clark (1994). This indicates that the responses of laboratory grown plants to infection may have some implication for field situations.

The field data demonstrated that the typical response of sorghum and maize plants was for *Striga* infestation to lower rates of photosynthesis, although the degree of response to infection differed between cultivars. Examination of a range of sorghum and maize cultivars revealed that the extent to which photosynthesis was altered ranged from between 20 and 50% (see Chapter 3) at a similar *S. hermonthica* loading. Interestingly, the sorghum land race Ochuti, had an ability to maintain high rates of photosynthesis even when infected with *S. hermonthica*.

The field studies demonstrated a positive correlation between photosynthesis and grain yield. However, relating grain production to metabolic processes is difficult due to

differences in the time scale of the responses. Measurements of photosynthesis are usually taken on a single leaf at a particular point in time, whereas grain yield will reflect changes in the plant status with time. Despite these differences, the data indicated a relationship between the ability of infected plants to maintain high rates of photosynthesis and maintain high grain yields. At a similar level of *S. hermonthica* infestation, Ochuti demonstrated a high degree of tolerance to the presence of *S. hermonthica* (determined by grain production) compared with the other cultivars examined. However, grain yield and rates of photosynthesis of Ochuti varied between the seasons. In 1994 the yield and photosynthesis of Ochuti was not affected by *S. hermonthica* (Chapter 5 Tables 5.3 and 5.5) but in 1996 a small depression was observed with grain production and photosynthesis of infected plants being 22% and 12% below uninfected plants, respectively (Chapter 3 Tables 3.2 and 3.4). For comparison the seven other genotypes of maize and sorghum yielded between 25 and 50% of their uninfected controls and had rates of photosynthesis between 30 and 54% below uninfected plants.

The difference between the seasons may be explained by observations of photosynthetic induction and non-steady-state rates of photosynthesis in laboratory grown plants. The induction of photosynthesis after illumination of dark adapted leaves, differed between infected and uninfected Ochuti plants (Chapter 6 Figure 6.3). In the period following illumination infected plants showed rates of photosynthesis below those of uninfected plants. However, after 20-25 minutes the steady state rate of photosynthesis was equal to that of uninfected plants. The significance of this

difference in induction time may be important in the field when plants experience considerable diurnal variation in the light environment, causing infected Ochuti plants to possibly fix less carbon because of the time required to reach steady state. Differences in the PFD between field seasons may explain the inter-annual differences of Ochuti in the field.

7.3.3 Stomatal closure inhibits photosynthesis in the early stages of the *S. hermonthica*-sorghum association

Lower rates of photosynthesis of infected sorghum and maize plants grown in both the laboratory and field environments are closely correlated with lower stomatal conductance and transpiration (e.g. see Chapter 3 Table 3.4 and Chapter 6 figures 6.3 and 6.4). There is evidence that in young hosts, lower stomatal conductance was an important determinant of lower photosynthetic rates in infected plants. The response of photosynthesis of infected plants to internal carbon dioxide concentrations showed that the rate of photosynthesis was similar for a given C_i. This coupled with an increase in discrimination against ^{13}C, is consistent with a stomatal limitation to photosynthesis. (Farquhar *et al.*, 1989).

However *S. hermonthica* may influence other factors especially in the later stages of the host-parasite association. The relationship between photosynthesis and C_i was also reported for the sorghum cultivar CSH-1, by Press and Cechin (1994), and differs from the relationship observed in Chapter 6 in that lower rates of photosynthesis in infected plants were observed at high intercellular carbon dioxide concentrations. This

may reflect constraints on the regeneration of CO_2 acceptors. The difference between the results may be explained by the developmental stage of both host and parasite at the time at which the measurements were taken. Press and Cechin (1994) report data for older hosts when *S. hermonthica* had emerged above the soil, compared with measurements made on younger hosts in Chapter 6 when the parasite was below ground. In addition, Press and Cechin (1994) report foliar nitrogen concentrations in the order of 17 mg g^{-1}, whereas for the rhizotron-grown plants nitrogen concentrations were approximately double. *S. hermonthica* exerts a greater effect on host photosynthesis at lower nitrogen concentrations (see Chapter 4 and Cechin and Press, 1993a), hence, *S. hermonthica* may influence both stomatal conductance and regeneration of CO_2 acceptors depending on the developmental stage of both host and parasite and nutritional status of the host.

7.3.4 Nitrogen can alleviate the effects of *S. hermonthica* on host growth and photosynthesis.

Nitrogen fertiliser application in the field does have a role in alleviating the detrimental effects of *S. hermonthica* although the degree of response to nitrogen was dependent on the field location, which probably reflected the nitrogen status of the soil (Chapter 5). The addition of 40 kg N ha^{-1} on a farmer's field infected with *S. hermonthica* did not influence the number of emerged *S. hermonthica* plants but did alleviate the effects of the parasite on the host with grain yield and photosynthesis 45% and 23% greater than plants on the unammended plots, respectively.

The relationship between the dose and timing of fertiliser application on the *S. hermonthica*-cereal association was not conclusive and this may have been because of the soil nutrient status on the experimental sites. The foliar nitrogen concentration of the cereals were twice as great on the experimental sites at the research station compared with the farmer's field, indicating that the background nitrogen was exceedingly high, probably as a result of residual nitrogen from previous fertiliser studies. Further additions of nitrogen are unlikely to have a great effect on cereal growth. Future work involving the use of nitrogen fertilisers should involve detailed soil nutrient analysis. Interestingly, the application of 180 kg N ha^{-1} at the time of planting resulted in greater alleviation of the effects of the parasite on grain yield and photosynthesis compared with three application of 60 kg N ha^{-1} throughout the growing season (Chapter 4). A study by Mumera and Below (1993) determined that the concentration of nitrogen was more important than the timing of application, although repeated doses of fertiliser at 0 and 28 DAP were most effective in controlling *S. hermonthica*.

7.3.5 Nitrogen can influence the *S. hermonthica*-sorghum association before and after parasite attachment

Specifically designed rhizotrons were used to determine the influence of nitrogen supplied as ammonium nitrate before and after parasite attachment. High concentrations of nitrogen inhibited germination and attachment of *S. hermonthica* and the host plant showed increased vigour associated with the lower level of infection, as also reported by Cechin, (1993a, 1993b). An increase in the concentration of nitrogen

supplied to the plants following germination and attachment of the parasite resulted in partial alleviation of the effects of *S. hermonthica*, providing evidence of a further role for nitrogen in addition to its negative effect on germination and attachment. Following parasite attachment an increase from 1.0 to 3.5 mol m^{-3} ammonium nitrate resulted in greater rates of photosynthesis in infected plants compared with plants grown at low nitrogen. Some of the effects of *S. hermonthica* on biomass partitioning were also alleviated as infected plants had similar root:shoot ratios compared with uninfected plants. It is likely that the additional nitrogen was available to the host plant for investment in photosynthetic pigments and proteins and hence stimulated carbon gain. In addition, there was a host mediated effect of ammonium nitrate on the development of attached parasites, as high concentrations inhibited biomass accumulation by the parasite. Poor parasite development was likely to be the result of a reduced flow of water and solutes to the parasite resulting from an increase in osmotic potential of the host plant with an increase in nitrogen (Gworgwor and Weber, 1991). Greater partitioning of nitrogen to the leaves of infected plants at elevated ammonium nitrate supply, accompanied by high rates of photosynthesis and transpiration will maintain the flow of water to the host and away from the parasite.

7.4 How does *S. hermonthica* alter the growth and photosynthesis of its host?

7.4.1 Alterations of growth and photosynthesis in infected plants: a role for ABA?

The mechanistic basis of lower rates of photosynthesis in *S. hermonthica*-infected plants is discussed in Chapter 6, but in summary it appears to be the primary result of

stomatal limitations, at least in the younger stages of the association. The effect of *S. hermonthica* on the biomass accumulation and stomatal conductance of its host plant could result from parasite induced alterations in the concentration of ABA. Frost *et al.* (1997) demonstrated that the ABA concentration of sap and leaf tissue of infected sorghum plants (measurements were taken on plants grown in Chapter 6) was higher compared with uninfected plants. These results support earlier work by Drennan and El Hiweris (1979) and also work on ABA concentrations in the leaves of *Striga*-infected maize (Taylor *et al.*, 1996) and suggest that perturbations in the concentration of ABA may contribute to changes in host architecture. The inhibitory effects of ABA on stomatal conductance are well documented (Quarrie, 1983) and high ABA concentrations have also been shown to enhance the root:shoot ratio and reduce stem growth (Sloger and Caldwell, 1970; Trewavas and Jones, 1991). It would be of interest to determine the concentration of ABA in cultivars that show some tolerance to infection such as the sorghum cultivar Ochuti.

7.4.2 Alterations in the water and nutrient status of infected plants

In addition to the effects of ABA on growth and photosynthesis, the movement of xylem sap away from the host plant to the parasite may affect host productivity. Diversion of sap may result in a lower supply of solutes to the host and the removal of water may predispose the host to water stress adversely affecting growth and photosynthesis. Field observations demonstrated a decline in photosynthetic pigments and leaf nitrogen with infection consistent with the acquisition of nitrogenous compounds by the parasite (Chapter 3), induced degradation of existing compounds or

an alteration of nitrogen partitioning within the host. Loss of photosynthetic pigments and nitrogen can limit rates of photosynthesis (Baxter *et al.*, 1995). However, laboratory observations found increased nitrogen and chlorophyll concentrations in infected leaves even when photosynthesis was depressed. Possible explanations are discussed in Chapter 3 but the difference between studies may be attributed to the growth conditions of the plants and the age at which the leaves were sampled. Preliminary studies of the amino acid concentrations (M. Adcock personal communication) have suggested that infected sorghum plants have higher free amino acids compared with control plants and the amino acids that are altered are commonly transported to the parasite. The presence of the parasite may affect the incorporation of theses amino acids into proteins for growth. An additional supply of nitrogen may help by alleviating this limitation, stimulating host growth. The altered nutrient status of the host plant, especially the nitrogen content, merits further study.

7.5 A comparison of the *Striga-* and *Orobanche*-host association

Over 3000 species of flowering plants are parasitic (Kuijt, 1969) yet the parasitic mode of existence has been studied in very few of the parasite-host associations. Because of the agricultural importance of both *Striga* and Orobanche these parasites have received most attention and the two systems exhibit many similarities in their associations with the host. However, with an increasing number of studies examining host-parasite interactions many subtle yet important differences have become apparent.

Both *Striga* and *Orobanche* have similar effects on host growth resulting in an alteration of biomass partitioning with increased biomass allocation to the root in preference to the shoot and reproductive components. *Orobanche* associations typically result in shoot:root ratios between 60 and 90% of those of uninfected controls whereas *Striga* associations often cause more severe effects. Shoot:root ratios of *Striga*-infected cereals 18% of those of uninfected plants have been reported (Graves, 1995). Both parasites effect biomass partitioning of the host before changes in total biomass are observed (Cechin, 1994b; Barker *et al.*, 1995; 1996). In addition to changes in growth and biomass partitioning, both parasite associations alter the architecture of the host plant by stunting internode elongation, increasing self shading within the host canopy (Stewart and Press, 1990; Cechin, 1994b; Barker, 1997). Direct comparisons between the different associations should be made with caution as effects of the parasite may vary between associations depending on host age when infected (Cechin, 1993c), host species and cultivar used (Graves, 1995) and prevailing environmental conditions (see e.g. Parker and Riches, 1993).

Striga and *Orobanche* differ in their carbon relations with the host plant. The different effects of the parasite on the host may be a consequence of the degree of dependence on the host for carbon. *Striga* is a chlorophyllous root hemiparasite and can contribute towards it own carbon requirements, in contrast *Orobanche* lacks any ability for autotrophic carbon gain and receives all its nutritional requirements from the host (Musselman, 1980; Stewart and Press, 1990). Despite the lower dependence of *Striga* on host carbon compared with *Orobanche* the effects of *Striga* on its host are often

more severe, reflecting the different influences on host physiology (Press, 1995b). *Striga* receives its water and nutrients from host xylem (Dörr, 1997) and the parasite exhibits high transpiration rates and stomatal conductance, thought to increase flow to the parasite (Shah *et al.*, 1987). In contrast, assimilates are transported from the host to *Orobanche* via the phloem. Phloem has higher concentrations of carbon and nitrogen compared with xylem (Raven, 1993), and the lower rates of transpiration of *Orobanche* may reflect the high solute supply. It is unlikely that *Orobanche* exerts a significant effect on xylem tension in the host. Studies with *Orobanche* suggest that the parasite acts as an additional sink for host assimilates and alters the source-sink relationship of the host-parasite association (Barker *et al.*, 1995; Barker, 1997; Hibberd *et al.*, 1997). In the *Orobanche*-host association, total biomass of the host and the parasite is often not significantly different from that of uninfected plants (Barker, 1997). Hibberd *et al.* (1997) demonstrated that the difference in biomass between uninfected and infected tobacco was accounted for by the attached *Orobanche* (Figure 7.1, graphs A and B). Although *Striga* also acts as a sink, the difference in biomass between infected and uninfected plants can not be accounted for by diversion of resources to the parasite (Figure 7.1, graphs C and D). This is reflected by the significant effects of the parasite on host growth when the biomass of the parasite (and thus sink strength) was very small (Chapter 7).

In addition to the removal of carbon, both *Striga* and *Orobanche* can lower the rates of photosynthesis in infected plants (Press and Stewart, 1987a; Cechin, 1993a; Press, 1995b; Hibberd *et al.*, 1997), although in the *Orobanche*-host association lower rates

of photosynthesis are often observed in the older leaves and not the youngest fully expanded leaves as found in the *Striga*-host association. In contrast to the *Striga*-sorghum association where stomatal limitations appear to be the primary cause of lower photosynthesis, this may not be the case for the *Orobanche*-tomato association. Barker (1997) demonstrated lower amounts and activities of Rubisco and FBPase in infected tomato plants compared with uninfected plants. No such loss of these enzymes has been observed in the *Striga*-cereal association (Cechin, 1994b; Smith *et al.*, 1995).

Information on most parasite-host associations is limited and the greater knowledge that exists of the *Striga*- and *Orobanche*-host associations reflects their economic importance in agricultural systems. The influence of *Striga* on it natural grass hosts in the savannah grasslands has not been addressed and it would be of great interest to determine whether such detrimental effects on the host are observed outside of agro-ecosystems.

7.6 Conclusion

S. hermonthica has a marked effect on the growth and photosynthesis of its cereal host and the extent to which this occurs is dependent on the host species/cultivar. *S. hermonthica* lowered the photosynthesis of its cereal host in the field supporting laboratory observations. Knowledge of the mechanisms affecting host growth and photosynthesis are still lacking although preliminary observations suggest that plant growth regulators may have an important role in controlling stomatal conductance and

biomass allocation. Nitrogen clearly has an important influence on the *S. hermonthica*-cereal association both before and after attachment of the parasite and the next logical step would be to investigate the influence of different forms of nitrogen in both laboratory and field conditions. There are few cultivars that have proved to be tolerant or resistant to *S. hermonthica*, although the land race sorghum Ochuti, maintained growth and photosynthesis in the presence of *S. hermonthica* compared with uninfected plants and merits further study.
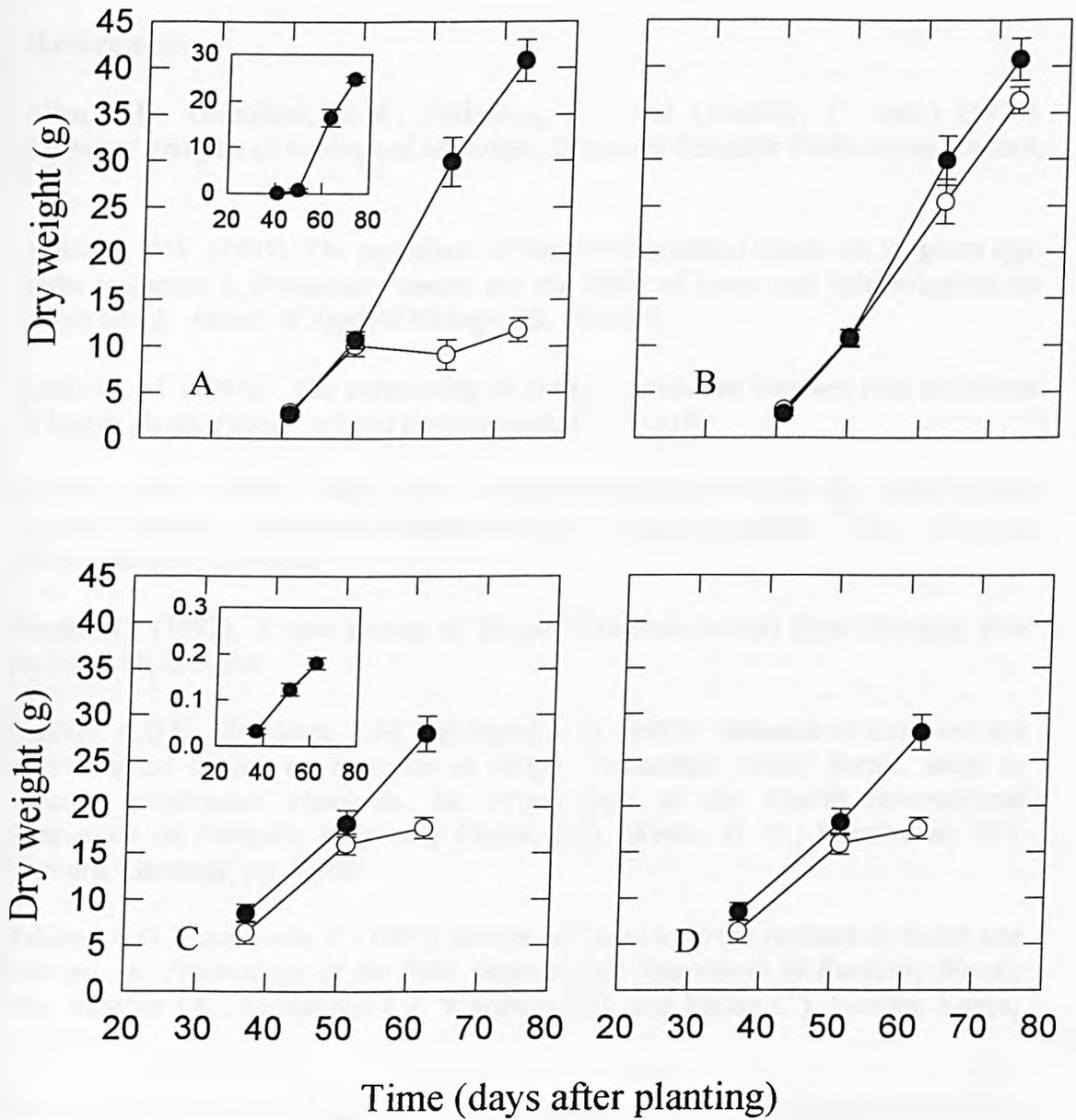
Figure 7.1A: The dry weight (g) of tobacco plants grown in the absence (closed symbols) or presence (open symbols) of *Orobanche*, graph inset shows the dry weight of attached parasites. B: The dry weight of uninfected tobacco plants (closed symbols) and the infected system (tobacco and *Orobanche*) (open symbols). C: the dry weight of sorghum plants grown in the absence or presence of *Striga*, graph inset shows the dry weight of the attached parasites (symbols as for A). D: The dry weight of uninfected sorghum plants and the infected system (sorghum plus *Striga*) (symbols as for B). Graphs A and B reproduced from Hibberd *et al.* (1997), graphs C and D data from Chapter 4.

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