The tripartite interaction between sorghum, *Striga hermonthica*, and arbuscular mycorrhizal fungi

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The tripartite interaction between sorghum, *Striga hermonthica*, and arbuscular mycorrhizal fungi

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Abstract

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The witchweed *Striga hermonthica* is proving to be a nightmare for smallholder farmers in the Sahel and Savannah regions of Africa. The close and intricate biological association between this phytoparasite and the cereal host makes its management difficult. Damage to the host begins even before *Striga* comes out of the soil. Also, infestation and severity correlate negatively with soil fertility. Arbuscular mycorrhizal (AM) fungi have a variety of ecological functions ranging from improved uptake of relatively immobile nutrients and subsequent translocation to the host, protection of host roots from pathogens, to soil aggregation and structural stability. This thesis addresses the question whether these beneficial micro-organisms could also play a role within the *Striga*-cereal (patho)system. Mycorrhizal fungi could either enhance the fitness of the cereal, thereby compensating for the damage afflicted by *Striga*, or have a direct negative effect on *Striga*. The possibility of AM fungal effects being dependent on sorghum cultivar, inoculum density, possible AM effects through alteration of host root exudate as well as the possible role of P nutrition within the tripartite interactions were investigated.

Inoculating *Striga*-infested sorghum with AM fungal propagules either in pots or in the field resulted in a significant reduction in the performance of *Striga* in terms of numbers attached to the root systems, relative time of emergence, numbers emerged and total dry weight of the emerged *Striga* shoots at harvest of sorghum. Host-plant-dependent effects were evident. AM effects on *Striga* were more pronounced with the more *Striga*-tolerant S-35 sorghum cultivar compared to the effects observed with the *Striga*-sensitive CK60B cultivar as host plant. Inoculation with AM fungi could compensate for the damage by *Striga* in the S-35 sorghum cultivar. Germination of preconditioned *Striga* seeds after exposure to root exudates from sorghum roots colonized by AM fungi was significantly reduced. Cultivar-dependent effects were again evident, with effects being more prominent with root exudates from AM fungi colonized S-35 plants: a near nil germination of *Striga* seeds receiving root exudates of S-35 sorghum plants compared to a 50% reduction in germination of seeds upon exposure to root exudates from AM fungi colonized CK60B plants. The observed AM compensation of damage by *Striga* on host occurred independent of the inoculum density of AM fungi and was not affected by P application. The observed direct negative effects of AM fungi on *Striga* increased with higher inoculum density, but not with P application. The direct

negative effect of AM fungi on *Striga* is therefore not a consequence of the positive effect of AM fungi on P uptake or growth of sorghum. AM fungi have the potential to affect *Striga* during various stages of the life cycle: germination, attachment, emergence, and possibly subsequent growth and development. Further experimentation under controlled conditions and in farmers' fields is needed and suggestions are made for subsequent experiments. Ways of making use of these interactions in an integrated management system sought to stop the downward spiralling yields of cereal crops as a result of *Striga* infection in the African Savannah and Sahel regions are also discussed.

Key words: *Striga*, arbuscular mycorrhiza, cereals, management.

Dedicated to my parents for instilling in me the delicate balance of being satisfied without being complacent

Preface

"Nothing will ever be attempted if all possible objections must be first overcome": Samuel Johnson. This piece of work testifies this thought as by the time I set off with my proposal, many people I approached doubted whether this was workable. With the foregoing phrase in mind, I simply continued drafting my proposal at the same time ensuring that there should be the necessary allowances against fiasco, eventually.

I would like to thank my promotor, Prof. Dr. M.J. Kropff for his willingness to take on this role. His critical comments on the manuscripts and the whole structure of the thesis are highly acknowledged. With specific regard to the scientific aspects of the thesis, I owe a lot to my co-promotor Dr. T.W. Kuyper. Dr. Kuyper was instrumental in the design of experiments, data analyses and review of the manuscripts. As the virtual library (databases of current and relevant literature over the Internet) was hardly accessible in Cameroon, the visits of Dr. Kuyper to Cameroon for other PhD students and his visit purposely for my project, were very rewarding. I relied very much on current literature that Dr. Kuyper always brought with him as hard copy or as soft copy in his readily available random access memory.

The constructive criticisms of Ing. Aad van Ast from the early stages of formulating the proposal to implementation and write-up of the thesis and the discussions we had, helped me to accomplish my work successfully. Apart from the scientific aspects of the thesis, I bothered Aad with all kinds of problems I encountered in Wageningen and am very much indebted to him for his forever willingness and readiness to come to my aid throughout the research period.

The research project started off (pre- and post-proposal phases) with three key units of Wageningen University: Crop and Weed Ecology (CWE), Ecological Phyto-pathology and Soil Quality. In implementing, only Crop and Weed Ecology, and Soil Quality were actively involved. I would like to seize this opportunity to thank Prof. M. Jeger and Dr. A. Termorshuizen of the former Ecological Phytopathology Group. Prof. Jeger accepted to be my promotor even though for a rather limited period of time as other commitments precipitated his departure from Wageningen University. Dr. Termorshuizen played a key role in the acquisition of funds for the research from the Netherlands Foundation for Advancement of Tropical Research (WOTRO).

It was an uphill task for the researcher to link up efficiently with the whole team given that the bulk of the research was conducted in Cameroon where communication was not (yet) at its best whereas the other supervisors were based in the Netherlands. The timely visits of Prof. Dr. L. Brussaard to Cameroon were handy for discussions. Prof. Brussaard also formed a vital relay link in information flow from/to the other

members of the team in Wageningen.

Back to Cameroon, I acquired the starter inoculum from Dr. D. Nwaga of the Department of Plant Physiology, University of Yaoundé I, Cameroon. I wish to express my appreciation for his work on biofertilizers in Cameroon as this spared me the time to build inoculum from scratch. Eddy Leonard Mangaptché Ngonkeu was very instrumental in the preliminary bioassays that I conducted both at the Plant Biotechnology lab and at IRAD. The assistance he gave me and the hectic moments we spent out of the lab are gratefully acknowledged. I am indebted to Dr. J.A. Takow of IRAD Barombi-Kang for his assistance in the pre- and post-proposal phases of my research. Dr. Takow played a key role in the acquisition of funds from WOTRO.

Heart felt thanks go to Dr. N.A. Onguene. I was lucky that Dr. Onguene worked on a PhD at Wageningen University in the framework of the Tropenbos Cameroon Programme and, after a successful thesis defence, relocated to IRAD Nkolbisson. The lab facilities he brought with him were very useful and helpful to me in Cameroon. Dr. S. Nyasse kindly accepted me in the laboratory of phytopathology at IRAD Nkolbisson.

Mes sincères gratitudes vont au Délégué Provincial d'Agriculture pour le nord, M. Hamadou Abdoulaye, et au Chef de Base Phytosanitaire du nord à Garoua, M. Hamidou Farikou, pour leur soutien logistique inestimable à la réalisation de la présente étude.

A special thanks to Matthijn de Boer, Bebom N.K. Tite and Lester Narvaez with whom I conducted some experiments during their practical periods. Matthijn de Boer came all the way from the Netherlands to experience research and life in the tropics. We spent close to four pleasurable months together. The farmers in north Cameroon, especially, Simeon Takoua, Djongma Justin, Wabé André were curious about my field experiments, collaborated and/or participated willingly.

I was hosted by CWE during the 2×6 months stay in Wageningen. I am grateful for the warmth of the staff and personnel of CWE. In particular, I would like to thank Gon van Laar for her help in the editorial part of the thesis, Leonie van Scherrenburg, Hilde Holleman, Henriette Drenth, Sjaak Tijnagel, Peter van der Putten, Gijsbertje Berkhout and Loualidi Boutaher for their assistance in my day to day activities at CWE. It was no problem for Tom van Mourik and Jonne Rodenburg when their office, particularly the desk and PC of Tom were assigned to me during write up of my thesis. I derived much pleasure in being the room mate of Wim Westerhuis and then Johan van Nieuwburg. I would also like to reach out through CWE to the personnel of Wageningen Plant Sciences Experimental Centre (former Unifarm) who assisted me in one way or the other in the course of some pot experiments in the greenhouse.

I was entitled to a special treat from two persons in Wageningen: Ali Ormel hosted

Contents

Chapter 1

General introduction

Cericulture in north Cameroon

The West African country Cameroon spans a wide variety of climatological and agro-ecological conditions, ranging from the rain forest zone in the south and south-west to savannah in the north (Fig. 1.1). Rain-fed agriculture is the mainstay of Cameroon's economy and the variability in climate, especially rainfall, reflects the types of crops that are grown in different regions of the country. With respect to climate and its impact on vegetation and agriculture, the situation in north Cameroon is of particular interest. North Cameroon stretches from latitudes 9° to 13° N and longitudes 14° to 15° E, and comprises of the lowland Guinea Savannah between latitudes 9° and 10° N and the lowland Sudano-Sahelian Savannah between latitudes 10° and 13° N. The region is characterized by two marked seasons; a relatively short rainy season (June to September) and a dry season that lasts at least seven months. In the rainy season precipitation is low (below 900 mm annually), with 60% to 70% of the rains occurring during July and August (Brabant and Gavaud, 1985; Obale-Ebanga, 2001). In the dry season the low atmospheric humidity increases the average annual temperatures and the level of dryness. Many small streams dry up completely while many big rivers reduce considerably in volume. The vegetation turns brown and is scorched at certain places due to the very low atmospheric humidity. Not only do rivers and vegetation suffer but human beings as well. Under harsh conditions, people's lips, feet and skin crack, the days are usually very hot while the nights are relatively cold.

Luvisols, Planosols, Alfisols and clayey Vertisols represent the main agricultural soils in north Cameroon (Brabant and Gavaud, 1985; Obale-Ebanga, 2001). The Luvisols, Planosols and Alfisols have sandy to sandy loam topsoils and are generally old and highly weathered soils formed mostly in Precambrian bedrocks and in older deposits on the pediments. These soils are widely cultivated for rainy season crops and are highly susceptible to degradation of their chemical and physical properties when converted from natural savannah vegetation to cropland. Degradation is largely caused by the disaggregation of surface horizons leading to compaction and hard setting, the loss of soil organic matter, associated with inappropriate continuous cultivation (Brabant and Gavaud, 1985; Seiny-Boukar, 1990; Obale-Ebanga, 2001). The sandy to sandy loamy textured soils have predominantly low clay activity minerals and low nutrient reserves. Their organic matter content is low as well as their water-holding capacity. These properties incur a degradation of their physical properties and a
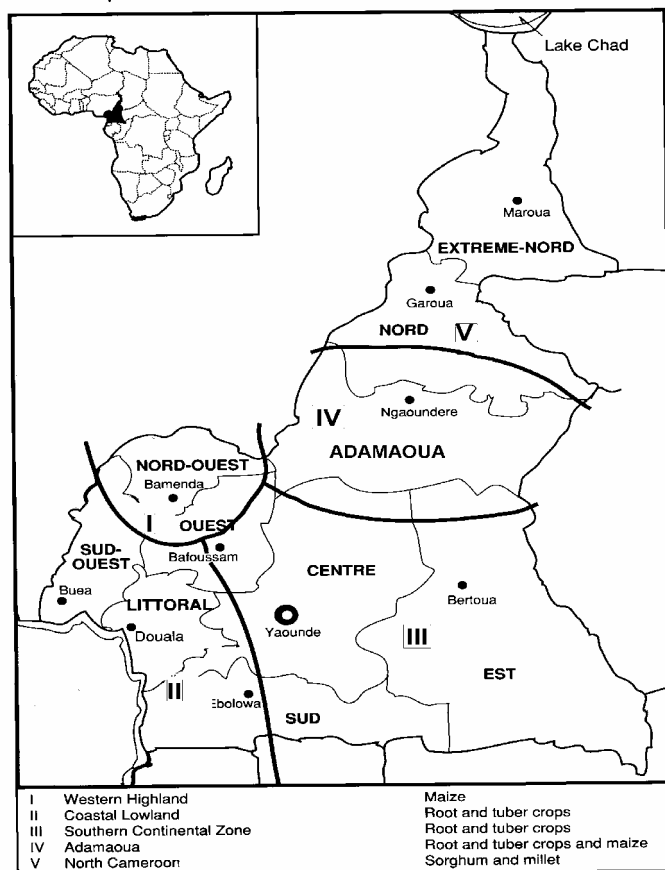
Fig. 1.1. Major agro-ecological zones and food crops of Cameroon. (Source: Cameroon National Root Crops Improvement Program, Terminal Report, 1987).

decline in chemical fertility upon continuous cultivation.

The Vertisols range from pedogenetic Pellic Vertisols, developed on relatively basic rock types in accumulative lower slope positions, to geogenetic Chromic Vertisols in recently clayey alluvial sediments. These Vertisols have a high cation exchange capacity which, depending on the clay content ranges from 15 to 30 cmol per kg soil, a high chemical fertility and a poor horizon differentiation (Brabant and Gavaud, 1985; Obale-Ebanga, 2001). A lesser degradation of physical properties and decline in chemical fertility occur in continuous cultivated clayey soils.

The soils as well as the prevailing relative humidity influence the types of crops that are grown in this region and various land use histories have been identified (Obale-Ebanga, 2001). On the less deep and sandy soils, cultivation of the cereal staples sorghum and millets, as well as groundnuts, dominate. Maize, a more demanding cereal crop in terms of nutrient uptake (Jokela and Randall, 1989) and its cultivation is restricted to the more fertile sandy loamy soils. The major cash crop grown in this

region is cotton (*Gossypium hirsutum*). Cotton is the only crop that receives heavy input of agro-chemicals. Cotton is sown at the onset of rains in June mainly on Alfisols, Planosols, Luvisols and occasionally on Vertisols. Annual rotation of cotton with rainy-season sorghum is the dominant cropping system in north Cameroon. Continuous cotton/sorghum production for about 8 to 10 years usually alternates with a period of 6 to 10 years of natural fallow constituting the cotton-based land use history which has been practised for several decades.

Sorghum

Sorghum bicolor (L.) Moench (Poaceae), a cereal originating in Africa, is widespread throughout the inter-tropical zone and its cultivation now extends well into the temperate regions. Sorghum is a major grain cereal of the tropical savannah vegetation region. It is one of the world's major food crops particularly in the semi-arid tropics of Africa and India characterized by high temperature and low rainfall. Sorghum is a main staple of the people's diet in Africa, the Middle East and Asia. It provides grain for consumption in the form of stiff or thin porridges, steam-cooked products such as couscous, or beverage for the resource-poor farmers. The leaves and stems are also used as forage for livestock, building materials and fuel for cooking. In the industrialized countries, sorghum is generally used as animal feed. Thanks to its ability to grow in some of the world's most austere environments, its versatility as a food and feed grain, and its ability to produce high yields, sorghum will continue to be one of the most precious cereal commodities (Frederiksen, 1986).

Sorghum occurs both as a weed species in Africa's Savannah ecosystems (Wood and Lenné, 2001) and as a cultivated cereal. Cultivated sorghum (Fig. 1.2) is classified into five main varieties and ten intermediate ones based on the characteristics of the spikelet and the panicle (Harlan and De Wet, 1972), viz:

- Guinea sorghum, typical for West Africa. The panicle is loose and the spikelets generally have open glumes that enclose an elliptical grain. The plants are generally tall and sensitive to daylength.
- Durra sorghum is mainly grown in East Africa, the Middle East and India. The panicle is very compact and is usually carried on a curved peduncle. The glumes are small and tightly attached to a globular grain.
- Caudatum sorghum is mainly grown in Central and East Africa. It is characterized by an asymmetric grain, which is flattened on the ventral surface and convex on the dorsal. The panicle may be very variable in shape.
- Kafir types. Small sorghum varieties mostly cultivated in southern Africa. The grain is symmetrical and the glumes are of variable size; the panicle is relatively compact and cylindrical.

- Bicolor sorghum is grown throughout Africa, but also particularly widespread in Asia. These varieties have the most primitive characteristics of all sorghums. The panicle is generally loose and the small grain is completely covered by large, closed glumes.

The ten intermediate varieties that are the most commonly grown have combinations of characteristics derived from the main varieties. Studies of enzyme diversity tend to confirm the theory of domestication of sorghum postulated by Harlan and De Wet (1972). They suggested that the domestication of sorghum occurred in two phases: in the first phase, a primitive sorghum of the *bicolor* type was domesticated on the edge of the Sahara. The modern varieties then independently developed, from the primitive type, in various parts of Africa.

Two varieties of sorghum: *babouri* and *muskwari* are prominent in north Cameroon. The two varieties (*babouri* and *muskwari*) belong to the Caudatum and Durra groups respectively, with a characteristic curving of the peduncle of the panicle. Both varieties are transplanted towards the end of the rainy season. The *muskwari* varieties are transplanted on to Vertisols with clay content of up to 40% (Chantereau and Nicou, 1994), at the beginning of the dry season while *babouri* sorghums are transplanted on to hydromorphic soils before the end of the rains. Prior to transplanting the Vertisols are not tilled. The grass vegetation that covers the Vertisols during the rainy season is

Fig. 1.2. Different sorghum crop types: caudatum (foreground) and guinea (background). Photo: Jonne Rodenburg.

slashed and burnt on site in order to prevent weed growth. The ash from the burnt plant material is a source of nutrients for the crop; no inorganic fertilizers are used.

Holes of between 20 to 25 cm deep are made using a pointed stake and two sorghum seedlings are planted in the hole, taking care that the roots make good contact with the bottom of the hole. *Muskwari* growth and yield depend on the annual rainfall, residual moisture and inherent fertility of the soils. Most *muskwari* fields have been under intensive crop production for more than 70 years and constitute the *muskwari*-based land use history (Obale-Ebanga, 2001).

Sorghum production accounts for about 17% of the estimated 115 million metric tons of annual cereal production in Africa, coming second to maize (FAO, 2002). About 450,000 metric tons of sorghum grain were produced in north Cameroon in 2002 on 360,000 ha (FAO, 2002). The average grain yield of sorghum in Africa is less than 1 metric ton ha^{-1}, well below the estimated 3 metric tons ha^{-1} in the industrialized world (FAO, 2002). A whole range of growth-reducing factors are responsible for this low grain yield. The inherent low fertility of most tropical soils is also to be blamed for the low yields. These soils are not rich in organic matter. The fact that sorghum is generally grown on a yearly basis on the same piece of land with hardly any measures to restore the fertility of the already poor soils greatly compromises the biological yield of this crop. Sorghum leaves and stems provide forage for animals. The stover is also excellent as construction material hence little or no litter is added back to the soil. Thus repeated cultivation and harvest constitute depletion of nutrients and organic matter that are already marginal. Sorghum is constantly challenged by many above- and below-ground pests and pathogens because of the range of environments in which it is cultivated. It also faces steep competition for nutrients, moisture and light from a wide range of annual and perennial weed species such as *Andropogon* spp.*, Brachiaria* spp.*, Cynodon dactylon*, *Cyperus rotundus*, *Digitaria* spp., *Echinochloa colona*, *Eleusine indica*, *Euphorbia* spp.*, Portulaca oleracea* and *Trichodesma* spp. (Holm *et al*., 1977; Ogborn, 1980). The effects of diseases and weeds and the inherent low fertility of soils on which sorghum has to strive are aggravated by the vulnerability of sorghum to damage by the obligate root hemi-parasite *Striga* especially in the major sorghum producing regions of Africa.

Striga

Among the most remarkable features of flowering plants is their adaptations to varied physical and biotic environments. A specific adaptation is parasitism, in which modified roots are used to transfer water, minerals, and a diverse collection of carbon compounds from a host to the parasite (Press and Graves, 1995). In the plant kingdom parasitism evolved independently, occurring in at least 17 different plant families.

However, there are only 8 families that include species of importance as weeds, (Parker and Riches, 1993). Two specific families: Scrophulariaceae and Orobanchaceae, nowadays often considered one family, Orobanchaceae (Olmstead *et al*., 2001), are prominent as they contain more than 45 genera and approximately 1600 parasitic species displaying a continuum of parasitic abilities ranging from facultative to obligate parasites, hemi-parasites of widely varying photosynthetic ability, and a diverse collection of heterotrophic holo-parasites (DePamphilis *et al*., 1997). Plants of the genus *Striga* are generally known as witchweed. The genus *Striga* is considered native to Africa (Raynal-Roques, 1996). This continent harbours about 83% of the total number of *Striga* species in the world (Raynal-Roques, 1991). Only a few of these species are (yet) economically important: *S. hermonthica*, *S. asiatica*, *S. gesnerioides, S. densiflora, S. euphrasioides*, *S. aspera* and *S. forbesii* (in decreasing order of economic importance). All but one (*S. gesnerioides*) of the aforementioned species attack cereals such as maize, sorghum, millet and rice, the major sources of carbohydrates in the tropics. *Striga gesnerioides* parasitizes broad-leaved crops such as cowpea and tobacco.

Because of its obligate parasitic nature, the life cycle of *Striga* spp. is conditioned by exchange of signals between the host, the abiotic environment and the parasite. Non-dormant seeds of *Striga* after being conditioned by exposure to moisture at 25 to 30 °C for 1 to 2 weeks, germinate only in the presence of an exogenous germination stimulant exuded by the roots of hosts plants, or some non-host plants (Vallance, 1950; Reid and Parker, 1979; Okonkwo, 1991; Ransom and Njoroge, 1991). Chemicals from the host root trigger the germinated *Striga* seeds to form haustoria, and to attach and penetrate the tissue of a host root in close proximity (2-3 mm). A union is hence established between the *Striga* seedling and the host with drain of water, minerals and photosynthates from the host through the haustorium to the parasite. As the *Striga* seedling grows, it develops adventitious roots from the base of the elongating stem. When these adventitious roots come into contact with host roots, secondary haustoria are formed. Drain of water, minerals and photosynthates from the host often leads to a reduction in host shoot growth, wilting and leaf chlorosis, poor economic yield (Ramaiah *et al*., 1983; Doggett, 1988; Parker, 1991). Reduction in host growth due to *Striga* spp. infecting cereals is often enormously greater than can be explained by the simple removal of resources by the parasite (Press *et al*., 1996) in contrast with *S. gesnerioides* infecting broad-leaved crops. Production of toxins by *Striga* spp. in cereals that might alter host metabolism has been suggested (Doggett, 1988; Parker and Riches, 1993). Interference of *S. hermonthica* with the host's hormone balance (Drennan and El Hiweris, 1979; Frost *et al*., 1997) and photosynthesis (Press *et al*., 1987; Press and Stewart, 1987; Graves *et al*., 1989; Cechin and Press, 1993a; Gurney

et al., 1995; Ramlan and Graves, 1996; Frost *et al.*, 1997; Van Ast *et al.*, 2000) is regarded as the most likely explanation of the *Striga* damage in cereals even though studies on a wild grass host (Watling and Press, 1998) seem to suggest that the mechanism through which *Striga* limits host growth does not directly involve host photosynthetic metabolism. Because of copious amounts of seeds, up to 200,000 (Parker and Riches, 1993), that can be shed by just a single *Striga* plant, the close biological association between *Striga* and their host plants, and yield losses linked to this weed, it is widely believed that *Striga* may have become the greatest biological constraint on food production in Africa, a more serious problem than insects, birds, or plant diseases (Ejeta *et al.*, 1992).

Yield loss assessment due to *Striga* is complicated by the fact that significant damage is inflicted before the parasite emerges. In addition, losses vary greatly according to the inherent sensitivity of the host plant, climatic conditions and the level of infestation. Nevertheless, Sauerborn (1991) estimated annual losses of more than four million metric tonnes of grain (US$ 480 million) due to *Striga* in just six West African countries. About 82% of the land in north Cameroon is arable and two-thirds of this area is infested by *Striga* (Parkinson *et al.*, 1991). Virtually all the area planted to rainy season sorghum and millet, the main staple food crops in north Cameroon, are affected by *S. hermonthica* (Ayongwa and Ngawa, 1999).

Control of *Striga hermonthica* in sorghum

Various control methods (e.g., hand-pulling, hoe-weeding, trap- and catch cropping) have been tried out with no conclusive and consistent results for the subsistence farmer. This may partly be due to the difficulty to deplete huge amounts of seeds that have accumulated and continue to accumulate in the seedbank over the years. The average size of the seedbank measured to a depth of 15 cm can be as high as 38,000 viable seeds per square metre (Smith and Webb, 1996) in heavily infested fields. Heavy infestations are typical for sorghum fields in north Cameroon. *Striga*-resistant varieties that do not require agro-chemicals would be an adequate and cheap solution to the *S. hermonthica* problem. However, if resistant varieties would demand heavy fertilization, as is the case with most of the so-called improved high-yielding varieties, adoption by resource-poor farmers will be minimal. Resistant varieties are defined (Parker and Riches, 1993) as those that show less attack, and with very few attached and/or emerged *Striga* plants. The converse of this is susceptibility. Tolerant varieties on the other hand are parasitized to the same extent as a standard variety but suffer less damage. The converse of tolerance is sensitivity. Resistant varieties are still not immune to attack by *S. hermonthica*. They, however, possess some mechanisms that allow them to limit the level of infestation, especially the number of *Striga* seeds

germinating and/or attaching to the roots. Tolerant varieties appear not to possess a mechanism to slow down the growth and development of *Striga* once attached but they are able to withstand the effects and yield reasonably well even with successful establishment of *Striga* seedlings.

Unfortunately, the development and deployment of sorghum varieties with efficient defence systems against *S. hermonthica* have not been successful to date. There are still no sorghum varieties with immunity (complete resistance) or even a high level of resistance reliable over any wide area (Parker and Riches, 1993). Durability and stability of vertical resistance, based on a single or a few genes, is challenged by the allogamy of *S. hermonthica* and the existence of geographical strains. Horizontally (polygenic) resistant or tolerant varieties of sorghum, expressed mostly by improved varieties (e.g., S-35, CS-95, CS-54 in Cameroon) or local landraces, can support high *S. hermonthica* infestations while preserving reasonable yield levels but at the same time enabling large scale production of *S. hermonthica* seeds – a potential risk not only for other non-tolerant host crops but also for the very tolerant host crops which, eventually, could be overwhelmed by the huge pressure from the enormous amounts of seeds accumulating in the seedbank.

The S. *hermonthica* problem in Cameroon in particular is aggravated by the fact that the most promising trap crops (crop plants that stimulate suicidal germination of *S. hermonthica* seeds), such as cowpea, that could help in reducing the seedbank are highly susceptible to attack by the parasitic weeds *S. gesnerioides* and *Alectra vogelii* (Anon., 1996). Many authors (e.g., Gworgwor and Weber, 1990; Singh *et al.*, 1991; Akobundu, 1991; Ejeta *et al.*, 1992) have recognized that the *Striga* problem may be too widespread and too severe to control using a single approach and have stressed the need for an integrated control approach (host plant resistance, cultural practices, and chemical treatments) in order to obtain a broad-scale *Striga* control. A more feasible approach would be the integrated resource (soil, water, solar radiation, labour, and purchased inputs) and crop management aimed at sustainability. This is especially needed because virulence of *Striga* increases with declining soil fertility and rainfall (Vogt *et al.*, 1991). Sustainable agro-ecosystems as well as natural ecosystems require balanced and functional microbial interactions in the soil. One of such interactions in savannah agro-ecosystems, that still has been scarcely studied and which could have an influence on the *Striga*-sorghum parasitic association, is the interaction between the roots of sorghum and arbuscular mycorrhizal (AM) fungi under *Striga* infection.

Mycorrhiza

A mycorrhiza is a morphologically and physiologically distinct organ resulting from an intimate and (usually) mutually beneficial symbiosis between particular soil-

inhabiting fungi (called mycorrhizal fungi) and roots of higher plants. The benefit for the fungus is the receipt of carbohydrates from the plant, and that for the plant is the increased resource availability and nutrient uptake of the plant by the fungus in various ways. Besides foraging for mineral nutrients in the soil, several mycorrhizal fungi show an ability to take up organic nitrogen. The role of mycorrhizal fungi in the uptake of organic phosphorus has also received attention. Mycorrhizal fungi can also alleviate abiotic (heavy metals) and biotic (pathogens) stress. Mycorrhizas alter plant growth and fitness through increased resource availability and alleviation of stress. The plant and fungus co-exist over their life cycles as both have no or only limited ability for independent existence under natural conditions (Smith and Read, 1997). Various types of mycorrhizal associations have been described, based on the sitting of the fungus vis-à-vis the root surface, of which the two most important ones are: ectomycorrhizas (sheath-forming) with exclusively intercellular colonization and arbuscular mycorrhizas with both intracellular and intercellular colonization. The dominant trees of boreal and temperate zones have distinct ectomycorrhizas formed largely by basidiomycetes (Read, 1991). Their mycelium is predominantly external with a sheath around lateral roots. Hyphae also penetrate inwards between the cells of the root to form a complex intercellular system that appears as a network of hyphae in cross section, called a Hartig net. There is usually no intracellular penetration, except in senescing ectomycorrhizas and in a few so-called ectendomycorrhizas. The functional significance of ectomycorrhizal fungi for the plant is predominantly an increase in both N and P uptake (Read, 1991). Recent estimates (Brussaard *et al.*, 1997) suggest that there might well be 10,000 species of ectomycorrhizal fungi. Arbuscular mycorrhizal associations are formed between fungi of the phylum Glomeromycota (Schüßler *et al.*, 2001) and a diversity of plant life forms, including most non-woody species and tropical trees (Read, 1991). These fungi colonize the roots of up to two thirds of all plant species (Trappe, 1987). In arbuscular mycorrhizas, an internal mycelial phase with characteristic structures is present. Commonly observed internal structures are: arbuscules, vesicles, hyphal coils, and internal hyphae. Some general features of the AM-root anatomy are illustrated in Fig 1.3. Arbuscular mycorrhizal development differs not only over time but also between plant species, especially with respect to the extent of development of arbuscules, vesicles, and coils within the cortical cells. The external phase is made up of branched single hyphae that ramify through the soil, forming anastomosing networks (Read *et al.*, 1985). Worldwide, over 150 different species of AM fungi have been described (Morton and Benny, 1990). However, the AM fungal species diversity might be underestimated as the group is supposed to be truly asexual and only a limited number of spore characteristics are available for spore recognition.
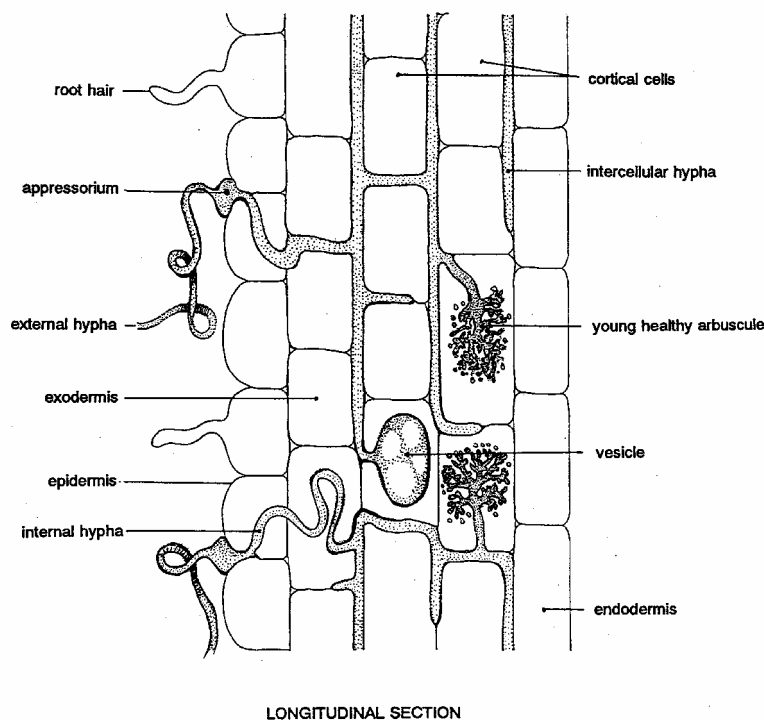
Fig. 1.3. Diagrammatic representation of AM fungal structures within the root (Brundrett *et al.*, 1994).

AM fungi and sorghum

AM fungi are known to be ubiquitous, occurring in the majority of herbaceous and graminaceous species of temperate and semi-arid grassland ecosystems as well as in many tree species of tropical and subtropical forests. Their mutualistic symbiosis with sorghum is very common. The pasture species *Sorghum sudanense* (sudangrass) is widely used as a host to maintain AM fungal pot cultures (e.g., DeMars and Boerner, 1995). There are reports of increased uptake of phosphorus, potassium, nitrogen and other nutrients from the soil (Marschner and Dell, 1994; Medeiros *et al.*, 1994; Osonubi, 1994; Isoppi *et al.*, 1995), increased growth at high soil temperatures, more efficient water utilization, and better growth in low soil moisture (Ibrahim *et al.*, 1990; Simpson and Daft, 1990; Medeiros *et al.*, 1994) by mycorrhizal sorghum plants in comparison to non-mycorrhizal plants. Significantly greater photosynthetic rates and stomatal conductance have been measured in AM fungi colonized than in non-inoculated sorghum plants. Increase in the concentration of cytokinins has been measured in plants colonized by mycorrhizal fungi (Allen *et al.*, 1980; Dixon *et al.*, 1988; Drüge and Schönbeck, 1992; Barker and Tagu, 2000). Instances whereby disease severity decreased in plants inoculated with AM fungi have been reported (Baltruschat and Schönbeck, 1975; Krishna and Bagyaraj, 1983; Perrin, 1991; Newsham *et al.*, 1995a; Kasiamdari *et al.*, 2002). Some mechanisms have been put forward to explain this phenomenon:

- Nutrient stress may weaken the plant, making it more susceptible to pathogen ingress, or more sensitive to other environmental stresses. Increased nutrient uptake with AM fungal colonization results in more vigorous plants better able to resist or tolerate root diseases. Enhanced phosphorus uptake has been shown to alter the quantity and/or quality of root exudates (Gerdemann, 1968; Ratnayke *et al.*, 1978; Hayman, 1982; Harley and Smith, 1983) that might be used by soil pathogens for spore germination and infection.

- Microbial shifts occur in the mycorrhizosphere (Meyer and Linderman, 1986; Secilia and Bagyaraj, 1987) due to alteration of root physiology and exudation. Many of those microbial shifts could influence the growth and health of plants.

- Localized morphological effects such as lignification of root cells of the endodermis have been shown to occur in AM fungal colonized roots (Dehne and Schönbeck, 1979). This might make penetration of roots by plant pathogens difficult.

- Prior occupancy of colonization sites by mycorrhizal fungi may reduce the opportunities for colonization by pathogens competing for those sites.

The tripartite interaction between sorghum, *Striga hermonthica*, and arbuscular mycorrhizal fungi

Root hemi-parasites of the Scrophulariaceae, as a whole, with respect to host plants are generalists in natural vegetation. The host range of *Rhinanthus minor,* for example, extends to at least 50 species from 18 different families (Gibson and Watkinson, 1989). Given that the vast majoriy of land plants including potential host plants of hemi-parasites are mycorrhizal (Harley and Harley, 1987; Newman and Reddell, 1987), the attachment of a hemi-parasitic plant to a host plant would lead to a tripartite system within which nutrients, water and carbohydrates flow from one associate to another (Salonen *et al.*, 2001). The flow of substances within such a tripartite system could be more complex if the root hemi-parasites are themselves colonized by AM fungi. Whether the root hemi-parasites of the family Scrophulariaceae are colonized by AM fungi or not is still not completely settled, perhaps due to the relatively few studies that have been undertaken so far on these interactions. Klein *et al.* (1991), Gworgwor (1993), and Salonen *et al.* (2001) could not detect any AM propagules in the roots of, respectively, *Striga asiatica*, *Striga hermonthica*, *Odontites vulgaris* or *Rhinanthus serotinus*. Their observations fit with those by Harley and Harley (1987) that hemi-parasitic Scrophulariaceae are non-mycorrhizal. However, Krause (1988) observed the presence of AM fungi in *Striga asiatica*. The existence of arbuscular mycorrhizal networks in soil, linking different plant species into a common mycelium may allow interplant transfer of nutrients and carbon through the mycelium. While

gross transport has indeed been demonstrated (Simard *et al*., 2002), the question remains whether net transport from one plant to another occurs in ecologically significant amounts. In the arbuscular mycorrhizal symbiosis transfer of carbon between two chlorophyllous plants seems rather unlikely (Robinson and Fitter, 1999) although it almost certainly occurs between a chlorophyllous plant and achlorophyllous members of the Gentianaceae and Corsiaceae (Bidartondo *et al*., 2002). If root hemi-parasitic and holo-parasitic Scrophulariaceae are colonized by arbuscular mycorrhizal fungi, such mycorrhiza-mediated transfer is possible. But also in the absence of such mycorrhiza-mediated transfers mycorrhizal fungi may affect the nutrient and carbon balance of the parasite through mycorrhizal effects on the host plant. In that case mycorrhizal fungi may alter the fitness of the host directly and that of the parasite indirectly. Direct fitness effects of mycorrhizal fungi on the parasite are also possible, for instance when seed germination, attachment and early growth of the parasite are affected by the presence of mycorrhizal fungi in the roots of host plants. Ultimately, it would be the balance between direct (usually negative) and indirect (usually positive) fitness effects on the parasite that determines the outcome of the tripartite interaction.

In a study on the tripartite interactions between mycorrhizal fungi, *Trifolium pratense* and *Rhinanthus serotinus* (Salonen *et al*., 2001), ectomycorrhizal *Pinus sylvestris* and *Melampyrum pratense* (Salonen *et al*., 2000), *Lolium perenne* and *Rhinanthus minor* (Davies and Graves, 1998) the performance of the parasite in terms of biomass and number of flowers, was higher when associated with mycorrhizal host both with (Salonen *et al*., 2000, 2001) or without (Davies and Graves, 1998) an improvement of the nutrient status or performance of the host as a whole by the mycorrhizal symbiosis. No such positive effects were observed on the performance of *Odontites vulgaris,* a root hemi-parasite, when associated with the host *Poa annua* and a mycorrhizal fungus (Salonen *et al*., 2001).

It is very likely that parallels of such interactions could be drawn from interactions in agro-ecosystems although the strength of the outcomes might differ from the ones observed in natural vegetation systems. A typical example of a three-way interaction in an agro-ecosystem is that among sorghum, AM fungi and *Striga hermonthica*. One could easily envisage that the sorghum × AM fungi symbiosis could have an influence on the parasitic association between *S. hermonthica* and sorghum especially if the symbiosis is established and functioning before invasion of sorghum roots by *S. hermonthica*. Such effects of AM on *Striga* parasitism could be indirect through enhanced nutrition of the mycorrhizal sorghum or by offset of hormonal imbalance due to attack by *S. hermonthica*. A more direct effect on the germination and attachment/penetration of *S. hermonthica* to sorghum roots through alteration of the

quantity or quality of sorghum root exudates is to be considered as well. Lignification of the sorghum root with mycorrhizal fungal colonization may also be a possibility. The occurrence of microbial shifts in the mycorrhizosphere could be to the favour of organisms such as the plant growth-promoting bacterium *Azospirillum brasilense* (Miché *et al*., 2000) pathogenic to *S. hermonthica.* However, given that mycorrhizal fungi (Stribley *et al*., 1980) and parasitic angiosperms including *S. hermonthica* (Graves, 1995) are both carbon sinks, dual infection of sorghum could reduce the fitness of the host by the increased carbon drain to the advantage of the parasite associate and/or the AM fungus.

Very little is known about the tripartite interactions among AM fungi, *S. hermonthica* and sorghum. Gworgwor and Weber (1992) reported prevention of *S. hermonthica* emergence in infested sorghum after inoculation with an AM fungal isolate. They observed, however, a decrease in host plant growth and dry matter production with AM fungal inoculation. Even though growth depression due to AM association is well known in some plant species (Bethlenfalvay *et al*., 1982; Buwalda and Goh, 1982; Modjo and Hendrix, 1986; Howeler *et al*., 1987), the findings of Gworgwor and Weber (1992) necessitate further investigation given the multi-functionality of arbuscular mycorrhizas (Van der Heijden and Kuyper, 2001) viz. (1) different fungi show different benefits to the same plant under the same environmental conditions; (2) the same fungus shows differential benefits to different plants under the same environmental conditions; (3) the same fungus shows differential benefits to the same plant under different environmental conditions.

Outline of the thesis
The overall objective of this thesis is to gain insight into the tripartite interactions among sorghum, *S. hermonthica* and AM fungi with emphasis on the (in)direct role of these micro-organisms in reducing *S. hermonthica* infestation and/or improving sorghum yield. To achieve this general objective, several studies were conducted at different sites: pot experiments and bioassays at Wageningen University and the Institute of Agricultural Research for Development, Yaounde, Cameroon; field experiments in north Cameroon. Chapter 2 describes results of a pot experiment conducted to find out whether AM fungi could have an effect on *Striga*-sorghum interactions. Two sorghum cultivars with contrasting sensitivity to *Striga*, the highly sensitive CK60B and the more tolerant S-35, were chosen. The sorghum cultivar S-35 revealed interesting interactions with AM fungi and *Striga* and is the subject of studies reported in Chapter 3 on the effect of amount of AM fungal inoculum on the outcomes of the tripartite interaction. In Chapter 3, an attempt is made to study a possible mechanism of AM fungal effects on *Striga*: the indirect role of AM fungal

colonization on the root exudates (germination stimulant) required by *Striga* seeds in the process of germination. The results of this preliminary study led to a more detailed study of the role of AM fungi on sorghum root exudates and germination of preconditioned *Striga* seeds, reported in Chapter 4. The role of phosphorus as well as the timing of *Striga* infection on the outcome of the tripartite interaction were studied and reported in this chapter. In Chapter 5, results of field inoculation experiments in north Cameroon are discussed. Chapter 6 discusses the results of the foregoing chapters in a broader context including the implications of the study for *Striga* management.

Chapter 2

Effects of arbuscular mycorrhizal fungi on damage by *Striga hermonthica* on two contrasting cultivars of sorghum, *Sorghum bicolor*

V.W. Lendzemo, T.W. Kuyper

Abstract

The interaction between arbuscular mycorrhizal (AM) fungi and the root hemi-parasite *Striga hermonthica* was studied in two cultivars of sorghum that are tolerant and sensitive to *Striga*. In the absence of *S. hermonthica*, AM fungi increased biomass of both cultivars of sorghum. The beneficial mycorrhizal effect on plant biomass was larger aboveground than belowground with the largest effect being noted for grain production. In the absence of AM fungi, *S. hermonthica* reduced biomass of both cultivars of sorghum to different degrees. Arbuscular mycorrhizal fungi cancelled out damage by *S. hermonthica* in the tolerant but not in the sensitive cultivar. The results are discussed in the framework of integrated sorghum management. Such management includes breeding for resistance, which could have consequences for mycorrhizal colonization, and soil management, which could affect abundance and persistence of the mycorrhizal mycelium.

Introduction

Striga hermonthica (Del.) Benth. is one of the most important agricultural weeds of cereals in the semi-arid tropics. It is an obligate root hemi-parasite, native to savannah ecosystems where wild grasses are hosts. *S. hermonthica* infestation in cereals such as maize (*Zea mays* L.), sorghum (*Sorghum bicolor* (L.) Moench) and pearl millet (*Pennisetum americanum* (L.) Beeke) can cause devastating losses of yield, and the problem is increasing (Parker, 1991). Sauerborn (1991) estimated that 21 million ha of cereal cultivation in Africa are infected by the weed, and grain production within the 44 million ha where *S. hermonthica* occurs is potentially endangered. In a large part of northern Cameroon, which is *S. hermonthica*-stricken, few fields, if any, on which cereals are grown, are not infested to varying degrees, and *S. hermonthica* is considered a bane to the subsistence farmer in this region.

Technologies for control of *S. hermonthica* have not been widely adopted. Reasons include the mismatch between technologies and the farmers' socio-economic

conditions, particularly the non-availability of economically feasible and effective technologies (Debrah, 1994). The control of *S. hermonthica* has also been very difficult because of its high fecundity. Each *S. hermonthica* plant is capable of producing from 50,000 to 500,000 minute seeds that may remain viable for more than 10 years in the soil (Bebawi *et al.*, 1984). Even at low levels of infection, damage to cereals can be substantial (Gurney *et al.*, 1999). Because of the after-ripening requirements (Worsham and Egley, 1990), the seeds of *S. hermonthica* are not preconditioned for germination at the same time. Thus, for any control measure to be effective against this weed, it should be applied persistently.

The *S. hermonthica* problem may be too widespread and too severe to control using a single approach. Management of the hemi-parasite needs an integrated approach that includes host plant resistance, cultural practices, and chemical treatments. With integrated management, it is important to understand the interaction of the host plant, sorghum, with the biotic and abiotic environment. One of the biotic interactions that could be useful in this context is that between arbuscular mycorrhizal (AM) fungi, which form mutualistic symbioses with plant roots, and cereals. The benefits that sorghum derives from AM fungi have been repeatedly described. Because of the colonization by AM fungi, sorghum shows increased growth, increased nutrient uptake, and more efficient water utilization (Medeiros *et al.*, 1994; Ortas *et al.*, 1996; Osonubi, 1994; Raju *et al.*, 1990; Ibrahim *et al.*, 1990; Simpson and Daft, 1990) compared with non-mycorrhizal plants. AM fungi have also been reported to decrease damage by soil-borne pathogens (Azcón-Aguilar and Barea, 1996). Perhaps a similar inhibitory effect of AM fungi on the growth of parasitic plants such as *S. hermonthica* on sorghum may exist.

This preliminary study investigated the hypothesis that AM fungi reduce damage by *S. hermonthica* on sorghum. It was hypothesized that (i) AM fungi increase growth and yield of sorghum, (ii) *S. hermonthica* reduces growth and yield of sorghum, (iii) AM fungi reduce sorghum damage by *S. hermonthica*, and (iv) tolerant cultivars of sorghum benefit more from this interaction between *S. hermonthica* and AM fungi than sensitive cultivars.

Materials and methods

Plant material and growth conditions
The experiment was conducted in a rain-out shelter during the months of August–October 1999, at the Biotechnology Centre, University of Yaoundé I, Cameroon. The design was a full factorial experiment with three factors, viz. arbuscular mycorrhiza (present or absent), *S. hermonthica* (present or absent) and sorghum (a sensitive or a

tolerant cultivar), and four replications. A randomized complete block design was employed in the four compartments of the rain-out shelter. Each compartment (3 m × 2 m, with raised walls to prevent rain splash, was considered as a block. Two sorghum (*S. bicolor*) cultivars were used as hosts: the highly *Striga*-sensitive inbred cultivar 'CK60B' and the *Striga*-tolerant, local cultivar 'S-35'(*Koidawa*). The AM fungal inoculum consisted of a mixture of spores in equal parts of *Gigaspora margarita* Becker and Hall (isolated from a cowpea field in North Province, Cameroon) and *Glomus clarum* Nicolson and Schenck (isolated from a cassava field in Littoral Province, Cameroon). Fungal isolates are maintained at the Biotechnology Centre, University of Yaoundé I, Cameroon (Nwaga *et al.*, 2000).

The soil used was an autoclaved mixture, in the ratio 1:1 (v/v) of black soil and coarse sand (pH (H_2O) 5.9, pH (KCl) 5.1, organic matter=0.85%, C:N ratio=10, available P (Bray II = 10 mg kg^{-1})). Seeds of *S. hermonthica* were harvested in October 1998 from mature capsules from a heavily infested field around Garoua (north province), dried, squeezed to release the seeds, and passed through a sieve with a pore size of 106 μm. Germinability of the seeds was around 80%. Ten milligrams of *S. hermonthica* seeds were thoroughly mixed with the top 6 cm soil in 5 l plastic pots of the treatments receiving *Striga*. Fifty grams of AM fungal inoculum were mixed with the top 6 cm of pot soil in the relevant treatments. Uninfested pots were established at the same time. Fifty grams of autoclaved AM inoculum were mixed with the top 6 cm of pot soils in the treatments not receiving AM fungal inoculum. Because most, if not all, micro-organisms in the soil are killed by autoclaving, microbial life excluding AM fungi was restored in all the pots as described by Hetrick *et al.* (1988). To this end, 400 g of non-sterile black soil was suspended in 4 l of distilled water, decanted and passed through filters (CAFREA No. 2) capable of retaining AM fungal propagules. The filtrate was then diluted up to 16 l with distilled water and 250 ml of the diluted filtrate added to all pots. The soil was then left to equilibrate for 2 weeks before sowing of sorghum seeds. Unless surface disinfested spores are used, AM fungal inoculum generally contains many organisms, some of which are capable of enhancing or suppressing growth. To exclude effects of these spore-associated micro-organisms, 100 g of the AM fungal inoculum was suspended in 4 l of sterile water and passed through filters as described above. One hundred millilitres of the filtrate was then added to all the pots of the non-mycorrhizal treatments.

Three sorghum seeds (after surface sterilization in 1% NaOCl for 15 min and subsequent rinsing with demineralized water) were sown per pot after the soil was mixed with AM inoculum and/or *Striga* seeds. The emerged sorghum seedlings were thinned to one per pot, 1 week after emergence. Plants were watered with tap water for the first 2 weeks, then with 100 ml per pot of Steiner Universal nutrient solution

(Steiner, 1984), but with half the amount of phosphorus to stimulate mycorrhizal fungal activity, two times per week. Average day and night temperatures in the rain-out shelter were 30 and 20 °C, respectively, and relative humidity 75%. Daylength was 12 h throughout the period of the study.

Measurements of plant performance and mycorrhizal colonization

Number of emerged *Striga* and time of emergence, relative to that of sorghum, were recorded in the course of the experiment. To measure biomass, a destructive harvest was made 80 days after emergence (DAE). Total leaf area of sorghum was measured with an area meter (LICOR 3100, Lambda Instruments Corporation, Nebraska, USA). The harvested plants were divided into panicle, leaves, stem, and roots, then oven-dried at 70 °C for 48 h before being weighed. Dry weight of *Striga* was determined in the same way.

   A random sample from each washed root system was collected before oven-drying the root system. Root fragments were cut into approximately 1 cm pieces, cleared for 10 min in 10% KOH at 121 °C in an autoclave, rinsed with water, acidified in 5% HCl for 1 min, and stained for 30 min in 0.01% acid Fuchsin dissolved in destaining solution (14:1:1 (v/v/v) lactic acid:glycerol:water). The stain was drained and the root pieces were washed thoroughly with water before destaining overnight. Percent colonization was assessed using the gridline intersection method. Mycorrhizal responsiveness (dependency – Plenchette *et al.*, 1983) was calculated by comparing biomass of mycorrhizal and non-mycorrhizal plants in the absence of *S. hermonthica*.

Statistical analysis

Data were subjected to a three-way analysis of variance with the help of the statistical package SAS (1990). Factors were AM fungi (present or absent), *Striga* (present or absent), and sorghum (tolerant or sensitive cultivar). Data on *Striga* dry weight were log-transformed. The Student-Newman-Keuls test was performed to compare mean differences among treatments. Data on panicle weight did not meet the requirements of analysis of variance, not even after data transformation. In this case, differences between means were evaluated with the Mann-Whitney *U*-test. All significance levels were set at $P < 0.05$.

Results

Biomass and growth of sorghum

Analysis of variance (Table 2.1) showed that total plant biomass was significantly affected by sorghum cultivar, mycorrhizal fungi, and *Striga*. The interaction term

between sorghum cultivar and *Striga* was also significant, whereas the other interactions were not significant. Analysis of the various plant parts generally confirmed the same trends. Dry weight of roots was significantly affected by sorghum cultivar, *Striga*, and the interaction between both factors. The factor mycorrhiza was not significant. Dry weight of stems was significantly affected by all three main factors, and also by the interactions between sorghum cultivar and *Striga*, and between mycorrhiza and *Striga*. Leaf dry weight and leaf area were significantly affected by the three main factors, the interactions sorghum cultivar × *Striga* and mycorrhiza × *Striga*, and by the three-way interaction. In the absence of mycorrhizal fungi and *Striga*, plant biomass was significantly different for both cultivars (Table 2.2).

In the absence of *Striga*, AM fungi increased sorghum biomass in both cultivars. Plant biomass of both cultivars in the presence of fungi was not significantly different. Mycorrhizal responsiveness was 0.24 for the tolerant cultivar and 0.37 for the sensitive cultivar. In the absence of AM fungi, *Striga* reduced sorghum biomass, especially of the sensitive cultivar (57% reduction, compared with 23% in the tolerant cultivar). Arbuscular mycorrhizas cancelled out damage by *S. hermonthica* (i.e., plants with arbuscular mycorrhizas plus *S. hermonthica* were not significantly different from the control) in the tolerant, but not in the sensitive cultivar.

Biomass of individual plant parts showed striking differences. Root dry weight of the sensitive cultivar was significantly negatively affected by *Striga*, both in the presence and absence of mycorrhizal fungi. In the tolerant cultivar, neither AM fungi nor *S. hermonthica* had an effect. Stem dry weight of both cultivars was (significantly) negatively affected by *S. hermonthica*. Again, AM fungi cancelled out *Striga* damage in the tolerant but not in the sensitive cultivar. Leaf dry weight was significantly

Table 2.1. *F*-values in a three-way analysis of variance of sorghum cultivar, *Striga*, and mycorrhizal fungus on dry weight of whole plant, roots, leaves, and stem, and leaf area of *S. bicolor* at 80 days after emergence[a].

Source of variation	Whole plant	Roots	Leaves	Stem	Leaf area
Mycorrhiza, M	15.65***	0.95	17.28***	11.63**	50.61***
Sorghum cultivar, C	61.24***	22.46***	10.30**	10.21**	53.73***
Striga, S	50.93***	12.47**	86.38***	58.37***	220.35***
M × C	1.14	0.00	1.02	0.24	3.02
M × S	3.95	0.26	20.23***	5.76*	29.55***
C × S	9.72**	11.86**	39.56***	6.07*	100.94***
M × C × S	3.19	2.07	5.15*	4.30	4.91*

[a] All sources of variation have DF = 1. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 2.2. Average dry weight of plants and separate plant parts (roots, stem, leaves, panicle – all plant weights in grams) in the various treatments[a].

	Tolerant cultivar		Sensitive cultivar	
	−*Striga*	+*Striga*	−*Striga*	+*Striga*
Total plant biomass (g)				
−AM	22.6 ab	17.5 bc	15.3 c	6.6 d
+AM	29.6 a	23.9 ab	24.2 ab	5.4 d
Root dry weight (g)				
−AM	3.8 a	3.4 a	2.8 a	0.8 b
+AM	3.8 a	4.1 a	3.9 a	0.4 b
Stem dry weight (g)				
−AM	7.6 bc	5.0 cd	5.9 c	2.8 d
+AM	9.4 ab	6.4 c	10.4 a	2.5 d
Leaf dry weight (g)				
−AM	3.9 c	3.8 c	4.1 c	3.0 d
+AM	4.9 b	4.1 c	5.5 a	2.5 d
Panicle dry weight (g)				
−AM	7.2 abc	5.2 bcd	2.5 de	0.0 e
+AM	11.5 a	9.2 ab	4.5 cd	0.0 e

[a] Values that are followed by a different letter indicate significant differences between treatments at $P < 0.05$ according to Student-Newman-Keuls test (Mann-Whitney U-test in the case of panicle weight).

higher in the sensitive cultivar in the presence of AM fungi and in the absence of *Striga*. In the tolerant cultivar AM fungi also increased leaf weight in the absence of *Striga*. *Striga* reduced leaf weight in the sensitive cultivar only. Total leaf area showed similar effects as leaf weight (data not shown). In the presence of *Striga* in the sensitive cultivar (irrespective of presence of AM fungi), no plants flowered and consequently panicle weight (and hence grain yield) was zero. The statistical power of the rank test was low and there were only few differences between treatments.

Emergence time and numbers of S. hermonthica
The number of *S. hermonthica* emerged 80 DAE were significantly reduced in the AM treatment in the tolerant, but not in the sensitive cultivar. More *S. hermonthica* emerged in the non-mycorrhizal treatment of the tolerant cultivar than of the sensitive cultivar (Table 2.3). No *S. hermonthica* emerged in the treatments without the parasitic plant. Mycorrhizal fungi increased emergence time of *Striga* in the tolerant but not in

Table 2.3. Number of *S. hermonthica* emerged (after 80 days), emergence time (days), and biomass of *S. hermonthica* (g)[a].

Treatment	No. of *Striga* emerged	Emergence time (days)	*Striga* biomass (g)
Tolerant cultivar			
Without mycorrhiza	16.5 a	41.5 b	1.02 a
With mycorrhiza	4.5 b	55.0 a	0.10 b
Sensitive cultivar			
Without mycorrhiza	3.8 b	44.0 b	0.28 ab
With mycorrhiza	3.5 b	44.3 b	0.33 ab

[a] Values that are followed by a different letter within a column indicate significant differences between treatments at $P < 0.05$ according to Student-Newman-Keuls test.

the sensitive cultivar (Table 2.3). In one pot with the tolerant cultivar, inoculated with AM fungi, no *Striga* emerged. Arbuscular mycorrhizal fungi significantly reduced *Striga* biomass in the tolerant but not in the sensitive cultivar (Table 2.3).

Mycorrhizal colonization

In the absence of fungal inoculum, all sorghum plants remained free of colonization by AM fungi. In the absence of *S. hermonthic*a, AM colonization of the tolerant cultivar was 81% and 69% in the presence of *S. hermonthica*. Because of the reduction in root biomass by *S. hermonthica* in the sensitive cultivar, only very few root fragments were available for investigation. All of these showed the presence of AM fungi (data not shown).

Discussion

The results from this preliminary study show that mycorrhizal fungi have the potential to reduce damage by *S. hermonthica* in at least one cultivar. AM fungi have been repeatedly reported to decrease damage by soil-borne fungal pathogens and root parasitic nematodes (Azcón-Aguilar and Barea, 1996; Roncadori, 1997). AM fungi have also been reported to decrease subsequent ectomycorrhizal colonization in a dual mycorrhizal plant (Van der Heijden, 2000) and systemically to suppress further mycorrhization by AM fungi in plants already colonized (Vierheilig *et al.*, 2000). Control of *S. hermonthica* by AM fungi had only been noted before by Gworgwor and Weber (1990). Their results, however, differed in a number of respects from this study. Firstly, they noted that mycorrhizal protection from *Striga* was similar for a resistant and a susceptible cultivar. Secondly, they observed that no *Striga* emerged at all in the

treatments with AM fungi. Thirdly, and most importantly, they observed that their mycorrhizal fungus (*Glomus fasciculatum* (Thaxter) Gerd. and Trappe) behaved consistently as a parasite, reducing yields of sorghum both in the presence and in the absence of *Striga*. It is not clear whether the different outcomes are because of mycorrhizal fungal species selection, cultivar choice, or experimental conditions.

Several mechanisms have been suggested by which AM fungi could decrease damage by soil-borne pathogens (Azcón-Aguilar and Barea, 1996). These mechanisms include increased nutritional status of the plant, changes in root exudates, competition for colonization sites, and mobilization of plant defence mechanisms after initial colonization by the AM fungus. Grandmaison *et al*. (1993) observed that colonization by AM fungi increased the amount of various wall-bound phenolics in roots and speculated that this binding of phenolics could be directly responsible for the resistance of AM roots to pathogenic fungi.

The genetic mechanisms of tolerance of sorghum to *Striga* are not well understood. Gurney *et al*. (1999) suggested that genetic differences between sorghum cultivars affect time of parasite attachment, with tolerant cultivars showing later attachment and later parasite emergence than sensitive cultivars. El-Hiweris (1987) reported that roots of sorghum cultivars that are tolerant to *Striga* have greater total phenolic contents than roots of sensitive cultivars. Differences in protection by mycorrhizal fungi against *Striga* in both cultivars suggests that this protective effect is plant genotype-dependent. It has been shown for several cereals that response to mycorrhizal colonization is also plant genotype-dependent and for that reason can be incorporated in breeding programmes (Smith *et al*., 1992). Mycorrhizal responsiveness was higher in the sensitive than in the tolerant cultivar. It is not clear whether lower susceptibility to *Striga* in the landrace S-35 and lower mycorrhizal responsiveness are genetically correlated. It would be worthwhile to compare sorghum cultivars that differ in *Striga* tolerance for mycorrhizal responsiveness and rate of mycorrhizal colonization.

The observations reported here do not only have implications for plant breeding but also for management of the soil ecosystem. Disturbance of the soil might affect the integrity of the mycelial network, decrease colonization on sorghum, and provide less effective protection against *Striga*. The ways in which soil disturbance affects the colonization rates of both organisms on the sorghum root system deserve further investigation in a time course study with simultaneous or sequential inoculation.

Chapter 3

Response of *Striga hermonthica* and the *Striga*-tolerant sorghum cultivar, S-35, to different levels of a mixed arbuscular mycorrhizal (AM) fungal inoculum

V.W. Lendzemo, A. van Ast and T.W. Kuyper

Abstract

The effects of various amounts of inoculum of arbuscular mycorrhizal (AM) fungi on the performance of the parasitic plant *Striga hermonthica* and of a *Striga*-tolerant cultivar of sorghum, *Sorghum bicolor*, were studied under greenhouse conditions. Performance of *Striga* was significantly negatively affected by the presence of AM fungi. The number of *Striga* plants attaching and emerging above-ground decreased with increasing amounts of inoculum. Total biomass of *Striga* was not significantly different between treatments with different amounts of inoculum. Performance of sorghum was negatively affected by *Striga* and positively by AM fungi. AM fungi compensated to a large extent the damage exerted by *Striga*. There was no additional effect of the amount of inoculum on sorghum performance, either with or without *Striga*. Root exudates of non-mycorrhizal sorghum plants stimulated *Striga* seed germination. Root exudates of mycorrhizal plants almost completely inhibited germination of *Striga*. These results suggest that AM fungi could affect *Striga* damage on sorghum in two ways, viz. (1) by increasing sorghum performance the negative effect of *Striga* could be compensated to a large extent; (2) by changing root exudation patterns *Striga* germination could be reduced. This could subsequently lead to lower attachment and emergence.

Introduction

Striga hermonthica (Del.) Benth., often known as witchweed, is an obligate root-parasitic flowering plant of the family Scrophulariaceae (Depamphilis *et al*., 1997). The species is native to Africa and is a major weed of the important food crops maize (*Zea mays* L.), sorghum (*Sorghum bicolor* (L.) Moench) and millet (*Pennisetum americanum* (L.) Beeke), the major cereal staples in the savannah and sahel regions. In a large part of north Cameroon (latitudes 9° to 13° N and longitudes 14° to 15° E), which is *S. hermonthica*-stricken, all but very few fields, if any, on which cereals are grown, are infested to varying degrees (Ayongwa and Ngawa, 1999). The traditional African cropping system of shifting cultivation consisting of prolonged fallow (more than 10 years), rotations and intercropping kept *S. hermonthica* at tolerable levels

through the decomposition of *Striga* seeds or their suicidal germination in the absence of suitable host plants (Doggett, 1984). As population pressure increased and markets developed, the demand for food production increased and land use intensified. This intensification is reflected in greater use of cereal monocropping with little or no fallow or planting with non-host crops of *Striga*. As a result, the extent and intensity of *S. hermonthica* have rapidly increased and become a threat to food production. Problems with *S. hermonthica* also become worse with declining soil fertility (Vogt *et al.*, 1991), another consequence of agricultural intensification by resource-poor farmers.

Control of *S. hermonthica* in cereals has so far proven elusive. Economically feasible and effective technologies are still to be developed (Debrah, 1994) for the cash strapped subsistence farmers in most of the *Striga*-stricken areas. The control of *S. hermonthica* has also been made very difficult due to the biology of this weed. It is very prodigious as far as seed production is concerned. The produced seeds require an after-ripening which may vary from a few weeks after shedding to more than a year (Gbèhounou, 1998) depending on the climatological conditions. With this after-ripening requirement, newly shed seeds are shielded from germination under less optimal environmental conditions. The after-ripened seeds are not capable of germination unless they are pre-conditioned for two to three weeks during which the seeds have to be exposed to moisture at suitable warm temperature (Reid and Parker, 1979; Okonkwo, 1991). Even after pre-conditioning, the seeds are germination-ready but the process can only take place in the presence of root exudates from a suitable host or non-host plant.

No panacea has so far been found for the *S. hermonthica* problem. The possibility of such a cure-all applied over a wide area seems far-fetched because of allogamy of *S. hermonthica*. Several geographical strains of this species exist (Kuiper *et al.*, 1996; Gbèhounou, 1998). The *S. hermonthica* problem is further complicated by the fact that this scourge affects mostly resource-poor farmers cultivating highly degraded soils in the savannah regions. These complexities call for an integrated approach to the problem. Such a broad-scale management of witchweed would require an approach that includes host plant breeding for resistance or tolerance, soil fertility management through organic and inorganic fertilizers, and integrated pest management through agrochemicals and soil biota. One of the soil biotic interactions that could be useful in this context is the mutualistic symbiosis between arbuscular mycorrhizal (AM) fungi and cereals. The benefits that sorghum derives from AM fungi have been repeatedly described. Due to the colonization by AM fungi, sorghum shows increased growth, increased nutrient uptake, and more efficient water utilization (Medeiros *et al.*, 1994; Osonubi, 1994; Ortas *et al.*, 1996; Raju *et al.*, 1988, 1990; Ibrahim *et al.*, 1990; Simpson and Daft, 1990) compared to non-mycorrhizal plants. AM fungi have also

been reported to decrease damage by soil pathogenic bacteria, fungi, and nematodes (Azcón-Aguilar and Barea, 1996). In a recent study on the effects of AM fungi on sorghum infested with *S. hermonthica*, we observed an inhibitory effect of AM fungi on the biomass and emergence time of *S. hermonthica* on a tolerant but not on a *Striga*-sensitive cultivar. A compensatory effect of AM fungi on the biomass of *Striga*-infected sorghum plants was also observed for the tolerant, but not for the sensitive cultivar (Chapter 2). A similar compensatory effect of a variety of AM fungal species on the sorghum-*Striga* interaction was also described by Gworgwor and Weber (2003). Jordan *et al*. (2000) reviewed the role of AM fungi in weed management. They concluded that AM fungi could stimulate 'good' weeds, however, the possibility that AM fungi could play a role in weed suppression or damage reduction by parasitic weeds was not discussed by them.

High AM fungal propagule densities reduce the length of the lag phase and increase colonization rate by the external mycelium and spread of the fungus within the roots. Under experimental conditions, the number of colonization events per unit length or mass of root increases in linear proportion to the density of the fungal inoculum (Walker and Smith, 1984; Franson and Bethenfalvay, 1989). We therefore hypothesized that the performance of *S. hermonthica* and the damage by the parasite on sorghum would decrease with increasing mycorrhizal inoculum density. As a possible mechanism by which AM fungi negatively impact *S. hermonthica* we investigated whether sorghum root colonization by AM fungi had an effect on the germination of *Striga* seeds *in vitro*. AM formation induces changes in host physiology and root exudation patterns (Azaizeh *et al*., 1995; Marschner *et al*., 1997) and, consequently, causes qualitative and/or quantitative alterations in microbial populations in the rhizosphere. For *Striga* seeds to germinate, they need secretions from the roots of the host (Fate *et al*., 1990; Hauck *et al*., 1992). Alteration of the quality and/or the quantity of the root exudates might thus affect the responsiveness of *Striga* seeds to the exudate and thus influence seed germination.

Materials and methods

Plant material and growth conditions

The first experiment was conducted in a rain-out shelter (4.10 m × 4.50 m) at the Institute of Agricultural Research for Development (IRAD) Yaoundé, Cameroon, during the months of February to April 2000. The experiment was a full factorial with two factors, viz. arbuscular mycorrhiza (0, 20, 50 and 100 g inoculum per pot) and *Striga hermonthica* (present or absent), and 5 replications. A complete randomized design was employed. The *Striga*-tolerant S-35 sorghum cultivar, also used in a

previous experiment (Chapter 2) obtained from IRAD Maroua, Cameroon, was used. The AM fungal inoculum consisted of a mixture of propagules of *Gigaspora margarita* Becker and Hall and *Glomus clarum* Nicolson and Schenck, originally obtained from the University of Yaoundé I (Nwaga *et al.*, 2000).

The soil used was an autoclaved mixture in the ratio 1:1 (v/v) of black soil (pH(H$_2$O) 5.9, pH(KCl) 5.1; organic matter 0.85%, C/N ratio 10, available P (Bray II) 10 mg kg^{-1}) and coarse sand. Ten milligrams of *S. hermonthica* seeds (approximately 2,500 germinable seeds) collected in October 1998 from *Striga*-infested sorghum fields in north Cameroon were thoroughly mixed with the top 6 cm soil in 5-liter plastic pots of the treatments receiving *Striga*. The AM fungal inoculum was mixed with the top 6 cm of pot soil in the relevant treatments. Uninfected pots were established at the same time. Fifty grams of the autoclaved AM inoculum was mixed with soils in the treatments not receiving AM fungal inoculum. Most, if not all, micro-organisms in the soil are killed by autoclaving. Microbial life excluding AM fungi was restored in all the pots as described by Hetrick *et al.* (1988). To this end, 400 g of non-sterile black soil was suspended in 4 l of distilled water, decanted and passed through filters (CAFREA No. 2) capable of retaining AM fungal propagules. The filtrate was then diluted up to 16 l with distilled water and 250 ml of the diluted filtrate added to (moistened) soil in all the pots. Unless surface disinfested spores are used, AM fungal inoculum generally contains many organisms, some of which are capable of enhancing or suppressing growth. Therefore, 100 g of the AM fungal inoculum was suspended in 5 l of sterile water and passed through filters (CAFREA No.2). One hundred ml of the filtrate was then added to all the pots that did not receive the AM fungal inoculum.

Three surface sterilized (in 1% NaOCl for 15 min.) sorghum seeds were sown per pot immediately after mixing the soil with AM inoculum and/or *Striga* seeds. The emerged seedlings were thinned to one per pot, 5 days after emergence. Plants were watered with tap water for the first 2 weeks, then with 100 ml per pot of Steiner Universal nutrient solution (Steiner, 1984), but with half the amount of phosphorus to stimulate mycorrhizal fungal activity, twice a week. Average day and night temperatures in the rain-out shelter were 32 and 22 °C respectively, and relative humidity of 65% recorded on average. Daylength was 12 hours throughout the period of the study.

Measurements of plant performance and mycorrhizal colonization
Number of emerged *Striga* was recorded in the course of the experiment. Biomass of sorghum and *Striga* and the number of attached and emerged *Striga* were determined after a destructive harvest 72 days after emergence (DAE) of sorghum. The harvested sorghum plants were divided into panicle, leaves, stems, and roots, then oven-dried at 80 °C for 48 hours before being weighed.

A random sample from each washed root system was collected before oven-drying the root system. Root fragments were cut into approximately 1-cm pieces and cleared for 10 minutes in 10% KOH at 121 °C in an autoclave, rinsed with water, then immersed in 5% HCl and stained for 30 minutes in 0.01% acid Fuchsin dissolved in destaining solution (14:1:1 lactic acid:glycerol:water). The stain was drained and the root pieces washed thoroughly with water before destaining overnight. Percent colonization was assessed using the gridline intersection method (Giovannetti and Mosse, 1980).

Collection of root exudates

Two-liter pots were filled with autoclaved sand and with 20 g, 50 g, 100 g or without additions of AM soil inoculum at the time of sowing of sorghum. Three surface-sterilized (in 1% NaOCl for 15 minutes) sorghum S-35 seeds were sown per pot and thinned to one, after emergence. The pots were placed in the rain-out shelter of IRAD and the plants allowed to grow for 40 days to ensure colonization of roots by the AM fungal propagules. Each treatment was replicated four times. After mixing the AM inoculum with the sand, 100 ml of 1/3 full strength ($3\times$ dilution) Steiner Universal nutrient solution was added to all the pots. Forty days after planting of sorghum, the roots of the plants in the fourth replicate pots were harvested, washed and stained for AM fungal colonization. Sand was then carefully washed off the roots of the other three replicates of each treatment. These seedlings, supported with non-absorbent cotton, were placed in 50 ml glass test tubes with their root systems completely immersed in 20 ml sterile distilled water. The roots were kept away from light by wrapping the test tubes in aluminium foil. The seedlings were allowed to grow under these conditions for five days. Thereafter, the seedlings were discarded and the solution in the test tubes used as fresh germination stimulant. The effect of the root exudate on the germination of *Striga* seeds was compared with the effect of the synthetic germination stimulant GR-24, an analogue of sorgolactone, the natural germination stimulant exuded by sorghum roots. The GR-24 solution was prepared as follows: to 1 mg of GR-24 powder, in a 10 ml flask, was added 1 ml 95% ethanol to dissolve the powder. After dissolving the GR-24, distilled water was added to the solution up to the 10 ml level. This solution (100 µl) was pipetted into a 50 ml flask and filled up to the 50 ml mark with distilled water and well mixed giving 0.2 mg l^{-1} of GR-24 and 0.2% ethanol.

Germination test

About 10 mg of *S. hermonthica* seeds harvested from sorghum fields in Garoua, Cameroon, in October 1998, were placed in a 50 ml flask and surface sterilized for 5

min. with a 1% solution of commercial sodium hypochlorite (NaOCl). Subsequently, the seeds were thoroughly rinsed with sterile distilled water on top of a funnel lined with filter paper. For conditioning, about 100 seeds were transferred to glass microfibre filter paper discs (1 cm diameter) moistened with 100 µl of distilled water. On top of the glass microfibre filter paper disc containing the *Striga* seeds, was placed another disc wetted with 100 µl of distilled water as well. Two layers of filter paper (90 mm diameter) were placed in a Petri dish (90 mm diameter) and moistened with 5 ml of distilled water to prevent the seeds from drying up during conditioning. Five discs were placed in each Petri dish. The dishes were sealed with parafilm, wrapped in aluminium foil and subsequently kept in an incubator at 28 ± 2 °C for two weeks.

After conditioning, the filter paper discs containing the seeds were dried for 1 hour at room temperature and placed in the middle of Petri dishes lined (only on the edge) with a double layer of filter paper moistened with 2 ml of distilled water. To each cluster of seeds on the filter paper discs, 200 µl of the solution containing root exudate of each test plant (3 test plants per treatment) was applied. The cluster of seeds on the filter plates in the control treatments received 200 µl of the GR-24 solution. The dishes were sealed with parafilm, wrapped in aluminium foil and incubated at 28 ± 2 °C for five days. At the end of the incubation period, each Petri dish was opened and the germinated and non-germinated seeds counted under a dissecting microscope. Seeds were considered germinated when germ tubes were clearly visible.

Statistical analysis
Data on sorghum biomass and mycorrhizal colonization were subjected to GLM procedures using the GenStat® for Windows (6th edition) statistical package with amount of inoculum and *Striga* as factors. Data on *Striga* biomass and the numbers of attached and emerged *Striga* were subjected to a one-way analysis of variance. Data were tested for normality, and if necessary, transformed to a natural logarithm. The means of parameters that showed significant differences in the analysis of variance were separated at $P < 0.05$ by the Least Significant Difference (LSD) test. As it was possible that the ANOVA effects were solely due to the presence or absence of mycorrhiza, and not to the amount of inoculum applied, we repeated the analyses without the non-mycorrhizal controls. Because the seeds in the Petri dishes cannot be considered independent replicates (as germinating seeds might stimulate subsequent seed germination through the production of ethylene) Fisher's test of independence, which is based on exact probabilities, was applied to test for an effect of exudates on *Striga* seed germination (Sokal and Rohlf, 1995).

Results

Performance of Striga

One-way analysis of variance indicated a significant effect of the amount of inoculum on biomass of *Striga*, number of *Striga* attached, and number of *Striga* emerged. Inoculation with AM fungi significantly reduced the biomass of *Striga* compared to the uninoculated *Striga*-infested treatments. No significant differences in *Striga* biomass were observed among the AM inoculum levels. The numbers of attached and emerged *Striga* decreased with increasing inoculum levels. The 20 g AM inoculum level was not significantly different from the non-mycorrhizal control, but both the 50 g and 100 g AM were significantly lower than the non-mycorrhizal control (Table 3.1).

Performance of sorghum

In the presence of *Striga* a significant yield reduction was noted (Table 3.2). This yield reduction was evident for total biomass, and biomass of stems, leaves and panicles. Root biomass showed the opposite pattern, being significantly higher in most cases in the presence of *Striga*. Biomass of *Striga*-infested plants was almost 25% lower than of non-infested plants, while panicle weight (grain yield) was reduced by 55%. Root:shoot ratio of infected plants more than doubled as a consequence of infection by *Striga*. Inoculation of sorghum with the AM inoculum, in the absence of *Striga*, significantly increased the total biomass and panicle weight (Table 3.2). There was no additional effect of the amount of AM fungal inoculum. Mycorrhizal responsiveness was ca 0.25 (averaged over the three inoculum treatments). Total biomass of plants without *Striga* and mycorrhizal inoculum was not significantly different from that of

Table 3.1. Performance of *Striga* on sorghum inoculated with different amount of AM inoculum. Values that are followed by a different letter in a column indicate significant differences between treatments at $P < 0.05$ with the least significance difference test. ANOVA results: [**] $P < 0.01$.

Amount of inoculum	*Striga* dry weight (mg)	*Striga* attached (#)	*Striga* emerged (#)
0 gram	483 b	203 c	36 c
20 gram	166 b	147 bc	21 bc
50 gram	53 a	95 ab	6 ab
100 gram	95 a	41 a	3 a
ANOVA (1-way)	**	**	**

Table 3.2. Total biomass (dry weight), dry weight of separate plant parts (roots, stem, leaves, panicle), root:shoot ratio and mycorrhizal colonization in the various treatments. S−: without *Striga*; S+: with *Striga*; AM 0, 20, 50, 100: amount of inoculum added 0 (non-mycorrhizal control), 20, 50, or 100 gram, respectively. Values of total biomass and dry weight of root and stem are back-transformed.

Treatment	Biomass (g)[a]	Root (g)	Stem (g)	Leaf (g)	Panicle (g)	Root: Shoot	Colonization (%)
S−; AM 0	44.5 ab	13.1 a	15.2 cd	4.9 ab	6.6 b	0.42 a	0.7 a
S−; AM 20	63.4 d	14.3 ab	31.5 e	4.8 ab	10.9 c	0.32 a	32.4 bc
S−; AM 50	60.9 d	19.0 cd	23.9 de	5.1 b	10.9 c	0.47 a	36.6 bc
S−; AM 100	54.2 cd	15.4 abc	20.3 d	5.1 b	11.5 c	0.40 a	29.4 bc
S+; AM 0	36.7 a	18.7 cd	7.8 a	3.7 ab	3.4 a	1.05 c	3.7 a
S+; AM 20	44.6 b	21.5 d	9.6 a	3.4 a	5.3 ab	0.93 bc	42.8 c
S+; AM 50	43.7 ab	18.2 bcd	14.0 bc	3.6 a	5.2 ab	0.74 b	31.1 bc
S+; AM 100	49.7 bc	20.4 d	10.5 ab	4.0 ab	4.1 ab	0.87 bc	27.2 b
ANOVA (2-factor)							
Striga, S	***	***	***	**	**	***	n.s
Mycorrhiza, M	***	n.s.	**	n.s	*	n.s	***
S × M	n.s.	n.s	*	n.s	n.s	*	n.s

[a] Values that are followed by a different letter in a column indicate significant differences between treatments at P < 0.05 with the least significant difference test. Significance of ANOVA: n.s. not significant; * $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P < 0.001$.

plants with *Striga* and mycorrhizal inoculum. Again, there was no additional effect of the amount of AM inoculum. Panicle weight of plants without *Striga* and mycorrhizal fungi was still higher (around 35%) than that of plants with *Striga* and mycorrhizal inoculum.

Mycorrhizal colonization

In the absence of fungal inoculum the sorghum plants remained largely free of colonization by AM fungi. In the presence of fungal inoculum, a significant difference in the fractional sorghum root colonization was observed 72 DAE only between the 20 g inoculum level and the 100 g level (Table 3.2). Mycorrhizal colonization 40 DAE in the second experiment increased with increasing amounts of inoculum: 5%, 29%, 39% and 48%, respectively, for the 0 g, 20 g, 50 g and 100 g treatments.

Table 3.3. Percentage of *Striga hermonthica* seeds germinating after exposure to the synthetic germination stimulant GR-24, or to sorghum root exudates obtained with 20 g, 50 g, 100 g or without AM inoculation. The difference between high germination (> 50%) at 0 M and low (< 5%) to zero germination with 20, 50, and 100 gram is statistically significant. (Fisher's exact test of independence, $P = 0.009$).

Treatment	Germination (%)
GR-24	78
0 gram	81
20 gram	3
50 gram	0
100 gram	0

Germination of Striga *seeds*

Sorghum roots that were not or hardly colonized by AM fungi induced *Striga* germination to a degree comparable to that of GR-24, the synthetic *Striga*-germination stimulant. Sorghum roots in the mycorrhizal treatments very strongly suppressed germination of *Striga* seeds (Table 3.3). The chance that exudates of all 9 plants inoculated with AM fungi resulted in low germination of *Striga* (< 5%) and exudates on 3 plants without AM fungi in high germination (> 50%) was low (P = 0.009; Fisher's exact test).

Discussion

In this study we confirmed the earlier observation that the presence of AM fungi could, to a substantial amount, compensate for the negative effect of parasitism by *Striga*. A comparison between non-mycorrhizal and non-infected plants with mycorrhizal and infected plants indicated that they were of similar biomass, however panicle weight (and ultimately grain yield) were still lower. In the earlier study panicle weight of the non-mycorrhizal non-infected plants was not-significantly different from that of the mycorrhizal and infected plants (Chapter 2). The slightly different outcomes in both studies may be related to the time the experiment was harvested and/or set up. The previous experiment was conducted in August-October and the present study in February-April (with on average somewhat lower air humidity), and with plants harvested one week earlier than in the previous experiments. However, the trends in the two studies are similar. A compensatory effect of AM fungi on *Striga* damage on sorghum has also been described by Gworgwor and Weber (2003).

Weight loss of sorghum due to *Striga* infection was much larger than the biomass of *Striga*. On average *Striga* biomass was less than 10% of the weight reduction in

sorghum. This is less than the 24% biomass reduction of sorghum attributed to *Striga* biomass reported by Van Ast *et al.* (2000). A possible explanation could be due to stronger intraspecific competition within *Striga* as we observed much higher numbers of *Striga* seedlings attached to sorghum than Van Ast *et al.* (2000). Intraspecific competition in *Striga* seedlings has a much larger impact on biomass gain of *Striga* than on the amount of phytotoxic substances produced by the parasites.

It seems likely that this compensatory effect of AM fungi on damage by *Striga* is a function of the generally improved nutritional status, especially the higher phosphorus content as a consequence of mycorrhizal colonization. Azcón-Aguilar and Barea (1996) mentioned increased plant nutritional status following upon mycorrhizal colonization as an important mechanism of plant protection against pathogens. However, hormonal effects may also have played a role. Plants that are infected by *Striga* show lower levels of indole-3-acetic acid (Press *et al.*, 1999), whereas mycorrhizal colonization increases root IAA content (Torelli *et al.*, 2000). Clearly, effects of *Striga* and AM fungi on the hormonal status of the cereal plant merits further research.

Colonization by AM fungi also reduced the number of attached and emerged *Striga* and the total *Striga* biomass. A decrease in the number of seedlings that attach and emerge could be due to changes in the rhizosphere as a consequence upon colonization by AM fungi. The observation that exudates of roots that were colonized by AM fungi almost completely suppressed germination of *Striga* seeds, lends support to rhizosphere changes as a further mechanism by which *Striga* damage could be reduced. Upon colonization by AM fungi the quality and quantity of root exudates substantially change. Marschner *et al.* (1997) observed a 50%-78% decrease in carbon compounds exuded in the rhizosphere in mycorrhizal pepper (*Capsicum annuum*) compared to non-mycorrhizal plants when the mycorrhizal fungus *Glomus intraradices* (fractional root colonization being 34%) was used. An isolate of *Glomus deserticola* showed only 8% fractional root colonization and no effects on root exudates were observed. They also concluded that mycorrhizal colonization simplified the composition of the rhizosphere soil solution. From data in Gworgwor and Weber (2003) it is also clear that the number of *Striga* emerged was lowest in mycorrhizal treatments with the highest colonization levels.

Norman and Hooker (2000) observed that exudates of non-mycorrhizal strawberry roots stimulated sporulation of the pathogenic oomycete *Phytophthora fragariae* to a much stronger extent (number of sporangia per Petri dish 6-10 times higher after 72 hours) than exudates of roots colonized by *Glomus etunicatum* and *G. monosporum*. These effects occurred both *in vitro* and *in vivo*. Such inhibitory effects by AM fungi on the performance of other root-colonizing organisms could be part of a general

mechanisms whereby AM fungi maintain a competitive advantage for root occupation, even against subsequent colonization by AM fungi (Pinior *et al.*, 1999; Vierheilig *et al.*, 2003). Although the major effects of mycorrhizal colonization on root exudates are a decline and simplification of exudates, the possibility that exudates of mycorrhizal plants contain substances that specifically inhibit *Striga* should for the time being not be excluded. Whereas the composition of root exudates of non-mycorrhizal sorghum has been described by Czarnota *et al.* (2003), nothing is known about possible qualitative and quantitative changes upon mycorrhizal colonization. Further research is needed to characterize composition of the exudates of mycorrhizal and non-mycorrhizal sorghum plants. Subsequent work should also separate the direct effects of changes in the exudates and changes in the rhizosphere populations due to mycorrhization. Changes in bacterial populations in the mycorrhizosphere could affect abundance of the plant growth-promoting rhizobacterium *Azospirillum brasilense*, which has been reported to inhibit seed germination of *Striga* (Miché *et al.*, 2000).

While germination of *Striga* seeds was almost completely inhibited by exudates of mycorrhizal roots, we did not observe a similar effect in experiment 1. This could have been caused by the timing of the establishment of the AM symbiosis compared to that of *Striga* germination. A complete inhibition of germination would only occur if mycorrhiza-affected changes occur rapidly enough compared to *Striga* germination. For *Striga* seeds to germinate they need to be conditioned for about two to three weeks at suitably warm temperature and moisture (Reid and Parker, 1979; Okonkwo, 1991) and subsequently exposed to germination stimulant (root exudates). Furthermore, experiment 2 was executed under homogeneous conditions in a Petri dish, where all seeds of *Striga* were exposed to similar concentrations of the exudates. In soil, root exudate concentrations will show a high variability, depending on the rate of production and consumption of exudates in the rhizosphere, the degree to which exudates are adsorbed to organic matter, and the degree to which roots are colonized. It is very difficult if not impossible to have a root system in a pot, which is colonized by AM propagules one hundred per cent. Thus under such circumstances, the roots that escape colonization will very likely encounter *Striga* seeds and get infected by them, unless the effects of AM colonization are systemic.

While a comparison between non-mycorrhizal and mycorrhizal plants yielded highly significant effects on both sorghum and *Striga*, the effect of the amount of inoculum on sorghum performance was only very limited. Various explanations can be suggested for this lack of effect. First, the amount of inoculum added might in all cases be sufficiently high to reach the maximum effect. Second, we harvested plants 72 days after addition of mycorrhizal inoculum, and that time period might have been sufficiently long to mask any effects of initial differences in inoculum density.

Differential speed of colonization might have been possible among the treatments as evidenced by the roots harvested and stained under the root exudate studies, where colonization after 40 days increased with the amount of inoculum applied. McGee *et al.* (1999) observed arbuscular mycorrhizas in cotton to be rapidly initiated from 100 propagules and a delay of initiation and spread from fewer propagules but with all but one propagule densities subsequently reaching a plateau. On the other hand, there was a distinct effect of the amount of inoculum of the numbers of *Striga* attached and emerged. Mycorrhizal inoculum decreased the numbers of *Striga* attached and emerged, but not completely so. Especially in the treatment of 20 g mycorrhizal inoculum added, the number of *Striga* plants was high. This would still be a threat to current and/or future cereal crops if these *Striga* plants were allowed to seed. It is therefore imperative that mycorrhizal management and/or inoculation be part of an integrated approach to control *Striga* and not be considered as a panacea.

Chapter 4

Reduced performance of *Striga hermonthica in vitro* and *in vivo*: the role of arbuscular mycorrhizal fungi

Abstract

Two sorghum cultivars: the *Striga*-tolerant S-35 and the highly *Striga*-susceptible CK60B were grown in the presence or absence of the root hemi-parasite *Striga hermonthica*, with or without arbuscular mycorrhizal (AM) fungal inoculation, and with or without phosphorus addition in the medium. Two destructive harvests were made at 24 days after sowing of sorghum (DAS) and 45 DAS. Root exudates were collected at each harvest time and tested for effects on germination of preconditioned *S. hermonthica* seeds. Root exudates from AM fungal inoculated sorghum plants inhibited germination of preconditioned *S. hermonthica* seeds at both harvests. The magnitude of this effect was sorghum-cultivar-dependent. A significant (85% or more) reduction in the germination of *S. hermonthica* seeds upon exposure to root exudates from AM inoculated S-35 plants was observed at both harvest times whereas a significant (50%) reduction was observed with root exudates from AM inoculated CK60B plants only at 45 DAS. With AM inoculation, the number of parasite seedlings attached and/or emerged on sorghum was reduced. Again, this reduction was more pronounced with AM inoculated S-35 plants compared to AM inoculated CK60B plants. There was no evidence of any phosphorus mediated effect on parameters studied. The negative effects of *Striga* upon host inoculation with AM fungi can be explained in part by AM mediated effects on the quality and/or quantity of root exudation.

Introduction

Sorghum is the fifth largest food grain crop in the world after wheat, rice, maize and barley (Chantereau and Nicou, 1994). In savannah regions of Africa where sorghum is one of the major grain cereal staples, it is particularly overwhelmed by *Striga hermonthica* infestation. *Striga* spp. are root hemi-parasites belonging to the family Scrophulariaceae. North Cameroon (latitudes 9° to 13° N and longitudes 14° to 15° E) lies within the savannah belt spanning West Africa, and *S. hermonthica* is endemic in that part of the country. Efforts to combat this phytoparasite had no effect to date. Reasons for the rather limited success in *Striga* control include the prodigious seed production by this weed and the fact that most of the proposed effective control methods (e.g., injection of ethylene in the soil to stimulate suicidal germination of *Striga* seeds) are not within the reach of the bulk of farmers practising subsistence agriculture.

There are strong indications that the symbiosis between the roots of sorghum and arbuscular mycorrhizal (AM) fungi can influence the *S. hermonthica* – sorghum interactions (Chapters 2 and 3; Gworgwor and Weber, 2003). The underlying mechanism for the observed poor performance of *Striga* on cereal crops upon AM fungi colonization is still not known. Preliminary investigations suggest involvement of AM fungi in various stages of the life cycle of sorghum and *Striga*. AM fungal colonization of sorghum roots may induce changes in the root exudates of the host (Chapter 3) leading to lower germination and consequently a reduced attachment and emergence of *Striga*. Changes in sorghum root cells (e.g., lignification) may delay *Striga* attachment and emergence, allowing the sorghum plants to escape to some extent from the most damaging impact in the seedling stage (Chapter 2). Increased nutrient uptake as a result of mycorrhizal colonization may allow sorghum plants to more easily compensate for the damage (carbon loss) as afflicted by *Striga* (Chapter 2; Gworgwor and Weber, 2003).

The role of root exudates in influencing the activities of rhizosphere organisms is diverse. Roots of mycotrophic plants exude substances that enhance mycorrhizal growth and branching around roots, thereby stimulating colonization by AM fungi (Tawaraya *et al*., 1998). This effect is regulated by phosphorus supply (Ratnayake *et al*., 1978; Schwab *et al*., 1983), as plants with a higher phosphorus status show lower levels of exudation and lower colonization levels (Lu *et al*., 1994; Olsson *et al*., 2002; Azcón *et al*., 2003). Upon colonization, however, mycorrhizal plants exude substances that inhibit further mycorrhizal colonization. This regulatory effect, which probably increases fitness of the first colonizing fungus, has not only been observed against subsequent colonization by AM fungi (Pinior *et al*., 1999; Gryndler *et al*., 2003; Vierheilig *et al*., 2003), but also against rhizobia (Catford *et al*., 2003) and pathogens (Norman and Hooker, 2000). It may also be effective against parasitic plants such as *Striga*. For *Striga* seeds to germinate, they need secretions from the roots of the host (Fate *et al*., 1990; Stewart and Press, 1990; Hauck *et al*., 1992). For the germinated seeds to attach, they need signals in the root exudates to induce haustorial formation. The haustorium is a unique infection structure that provides a physiological bridge between host and parasite, facilitating the transfer of host-derived water and solutes to the developing parasite through direct host-parasite, xylem-xylem continuity (Dörr, 1997). A range of phenolic compounds have been identified as signalling molecules for *Striga* seed germination or haustorial development (Albrecht *et al*., 1999). Eventual alteration of the quality and/or quantity of root exudate of a *Striga* host, following N- or P-addition might also affect the germination and/or attachment with a consequence on the performance of *Striga*.

Formation of AM induces changes in host physiology that can be decisive for root

exudation patterns (Azaizeh *et al*., 1995; Marschner *et al*., 1997). Generally speaking, colonization of AM fungi leads to decline (quantitative effects) and simplification (qualitative effects) of the composition of the exudates, which may feed back to microbial populations in the rhizosphere. Timing of the colonization of roots by various rhizosphere organisms may be an important factor that regulates subsequent colonization events. Privileged access of the first colonizing AM fungus over other AM fungi or rhizobia is stronger if the time between the different colonization events is longer (Catford *et al*., 2003). Van der Heijden (2000) reported that on the dual mycorrhizal plant *Salix repens* simultaneous inoculation of an ectomycorrhizal and AM fungus did not result in mutual inhibition, whereas sequential inoculation of *Glomus mosseae* followed by an ectomycorrhizal fungus resulted in a significant suppression of the ectomycorrhizal fungus. The timing of AM colonization of roots and *Striga* germination in the vicinity of roots may therefore also be expected to affect the degree to which AM fungi may reduce *Striga* performance.

In pot experiments with AM fungal inoculation affecting *Striga* performance negatively (Chapter 2), *S. hermonthica* seeds and AM fungal propagules were mixed simultaneously with the soil in the pots prior to sowing of sorghum seeds. *Striga* seeds, before they can germinate, require preconditioning at a suitable temperature and sufficient moisture. Germination of *Striga* seeds increases with the duration of the preconditioning up to three weeks after which it decreases (Reid and Parker, 1979). Mycorrhizal propagules (spores, hyphae, colonized root fragments) require sufficient moisture and the right temperature to germinate or resume growth. Spores of some AM fungi are dormant when first formed but after periods of storage, dormancy is overcome. The spores then become quiescent and capable of germinating rapidly and fairly synchronously under conditions of appropriate moisture and temperature (Smith and Read, 1997). In reported pot experiments on the interaction AM fungi-sorghum-*Striga,* it is very likely that whereas the *Striga* seeds were preconditioning during the first few weeks after set up, AM fungal propagules colonized roots and established a functional connection.

The objectives of this study were twofold: (i) to investigate the possibility that root exudates from AM fungi-colonized sorghum plants influence germination of preconditioned *S. hermonthica* seeds due to modification of the quantity or quality of root exudates, and (ii) whether preconditioning *Striga* seeds prior to infestation would favour *Striga* infection and improve its performance vis-à-vis AM fungi. We hypothesized that:

- Fewer preconditioned *Striga* seeds germinate upon exposure to root exudates from sorghum roots colonized by AM fungi.
- Increasing P in medium negatively affects AM fungal root colonization, thus

mitigating effects of root exudates on germination of *Striga* seeds *in vitro*.
- Increasing P availability for non-mycorrhizal plants would reduce the amount of exudates and hence negatively impact *Striga* seed germination.
- The root exudate effects are sorghum genotype dependent.

Materials and methods

Plant material and growth conditions

The experiment was conducted in Wageningen University during the months of July-August 2003 in a greenhouse with simulated tropical conditions. Average weekly temperatures in the greenhouse varied between 25 °C to 36 °C during the day and from 23 °C to 35 °C at night. Since the experiment was conducted during the summer months, daylength was regulated at 12 h using black screens. Relative humidity varied between 53% and 68% during the day and 68% to 71% at night. The medium used in growing sorghum, was a mixture in the ratio 1:3 of arable soil and coarse sand. This soil/sand mix was gamma-radiated at 10 kGy. The arable soil had a pH-KCl of 6.7, organic matter content of 1.3% and total N content of 0.05%. Plants were grown in 2-litre black plastic pots filled with 2 kg of the sterilized soil/sand mixture, with drainage holes in the bottom. The design was a full factorial scheme with the three factors being AM fungal inoculum (0 and 50 g per pot), P (0 and 30 kg P ha^{-1} in the form of milled triple superphosphate) and *S. hermonthica* (0 and 5 mg (1000) germinable seeds per pot). In all, 8 treatments per sorghum cultivar (S-35 or CK60B) replicated 5 times in a randomized complete block design. Two destructive harvests were done: 24 days after sowing (DAS) and 45 DAS.

The *S. hermonthica* seeds used were harvested from plants growing on sorghum in Mali (Africa) in 1998. The AM fungal soil inoculum was a mixture of propagules of *Glomus clarum* Nicolson and Schenck and *Gigaspora margarita* Becker and Hall (Chapter 2). Prior to set up, the *S. hermonthica* seeds were mixed with quartz sand (50 g) in aluminum trays, and kept moist for 10 days in the greenhouse for the seeds to precondition. A similar amount of quartz sand with no *S. hermonthica* seeds was kept moist in the greenhouse for 10 days prior to set up. The sand/*S. hermonthica* seeds mix, inoculum of AM fungi, P fertilizer or their combinations were mixed with the top 6 cm soil in the pots in the relevant treatments at set up. The control treatments were established at the same time: moist quartz sand was mixed with soil in the upper 6 cm of the pots that did not receive *Striga* seeds, whereas 50 g of gamma-radiated soil inoculum of AM fungi was mixed through the soil in pots that did not receive inoculum of AM fungi. Three pregerminated sorghum seeds were sown per pot and thinned to 1 per pot just after emergence. Plants were watered with tap water in the

course of the experiment. No fertilizer was applied apart from triple superphosphate addition in the relevant treatments at the start of the experiment. The pots were observed on a daily basis for *Striga* emergence and the number of shoots recorded. At each harvest, soil was carefully washed off the roots and observed for attached *Striga*. The number of attached *Striga* on the root systems was recorded.

Subsequently, seedlings were placed in flat-bottom flasks with sufficient amount (70 ml at first harvest and 250 ml during second harvest) of demineralized water to keep the whole root system immersed. The roots were kept away from light by wrapping aluminum foil around the flasks. The plants were kept under these conditions for 36 h before the solution in the flasks was collected and used as fresh germination stimulant.

Germination test

Striga seeds were surface sterilized in 2% sodium hypochlorite containing 0.02% (v/v) Tween 20 for 5 minutes, subsequently rinsed with sterile demineralized water, and dried for 30 minutes in a laminar air flow cabinet. For preconditioning, 80-100 surface sterilized *Striga* seeds were picked up on moistened (with 50 µl of sterile demineralized water) glass microfibre filter paper (GMFP) discs (Ø 1 cm), placed in sterile Petri dishes lined with one layer of Whatman filter paper (Ø 90 mm). To keep the seeds moist during preconditioning, the filter paper was wetted with 2.7 ml of sterile demineralized water. The Petri dishes were sealed with parafilm, wrapped in aluminum foil and subsequently kept in an incubator at 30 °C for 21 days. After preconditioning, the GMFP discs containing *Striga* seeds were dried for 20 minutes in a laminar air flow cabinet and 5 GMFP discs placed in the middle of each Petri dish lined with a ring (1.0-1.5 cm wide) of filter paper. The filter paper ring was moistened with 0.9 ml of sterile demineralized water. To each cluster of seeds on a GMFP disc, 50 µl of the root exudate was applied. Some extra GMFP discs containing preconditioned *Striga* seeds received applications of 50 µl of GR-24 (0.1 mg l^{-1}), an artificial germination stimulant (Zwanenburg *et al.*, 1994) solution to ascertain germinability of seeds while some others receiving 50 µl sterile demineralized water and served as negative control. All Petri dishes were sealed with parafilm, wrapped in aluminium foil and incubated at 30 °C for five days. Germinated and non-germinated seeds were counted under a dissecting microscope (× 50 magnification). Seeds were considered germinated when germ tubes were clearly visible.

Fractional root colonization and biomass determination

After root exudates were collected, approximately 2 g (fresh weight) of roots were cut and chopped into roughly 1-cm fragments, stained in 0.01% Trypan blue in lacto-

glycerol (Kormanik and McGraw, 1982) and evaluated for mycorrhizal colonization with the gridline-intersect method (Giovannetti and Mosse, 1980). After collection of root exudates, the plant material from the various treatments was divided into shoots (stem and leaves) and roots then dried at 80 °C for 24 h in an oven.

Root exudate analyses

Four root exudate samples (S-35 and CK60B with or without AM fungal inoculation) were analysed for possible qualitative and/or quantitative modifications upon AM fungi colonization, by high performance liquid chromatography (HPLC). Two milliliters of each root exudate sample was freeze dried, taken up in 2 ml of 75% aqueous methanol and filtered through a 0.2 µm polytetra-fluorethylene (PTFE) filter. HPLC separation of the methanolic filtrate was performed on a reversed phase C_{18} column (Luna 150 × 4.6 mm; Phenomenex) at 40 °C using a Waters 600E Multisolvent Delivery System (Waters Corp., Etten-Leur, The Netherlands) and a photodiode array detector (Waters 996) to record spectra (240-600 nm) of compounds eluting from the column on-line. The following gradient of acetonitril in 0.1% formic acid was used (at a flow rate of 1.0 ml per min): 5%-25% linear in 30 min, then 25%-30% in 5 min and 30%-50% in 2 min, followed by a 3 min washing with 50% acetonitril in 0.1% formic acid. After washing, the eluent composition was brought to the initial condition in 2 min, and the column was equilibrated for 6 min before next injection.

Data analysis

In some treatments of the S-35 cultivar, no *Striga* attached to root systems. Such data did not meet assumptions of ANOVA. Mann-Whitney *U*-test and Kruskal-Wallis test (Hoshmand, 1998) were used to test for significance of mycorrhiza or phosphorus effects on number of attached or emerged *Striga* as well as *in vitro* germination of preconditioned *Striga* seeds. Data on percentage root length colonized by AM fungi, and sorghum biomass were subjected to ANOVA procedures with the help of the statistical package SAS® System for Windows (8th edition). Prior to ANOVA procedures data were transformed: square root transformation, $\sqrt{x+0.5}$ for percentage root colonized at 24 DAS or $\sqrt{x}$ for data on sorghum biomass at all harvest dates; arcsine $\sqrt{x}$ transformation for percentage root colonized at 45 DAS.

Results

Sorghum root colonization by AM fungi

In treatments that did not receive inoculum of AM fungi, sorghum roots remained free of AM fungal colonization. Colonization levels at 24 DAS varied between 3.5% and

4.8%, and at 45 DAS between 28% and 49%. Colonization levels were similar between the treatments irrespective of P addition or cultivar differences.

Germination of preconditioned S. hermonthica *seeds on exposure to root exudates*
Root exudates collected from sorghum plants grown in the absence of AM fungi, when applied to preconditioned *Striga* seeds induced very high germination of the *Striga* seeds. With exposure of preconditioned *Striga* seeds to root exudates from plants inoculated with AM fungi, germination was largely suppressed. Irrespective of AM fungi inoculation, the time of harvest or sorghum cultivar, there was no significant influence of P fertilization on root exudate stimulation or inhibition of *Striga* seed germination (Table 4.1).

The magnitude of the inhibition of seed germination upon exposure to exudates from mycorrhizal plants appeared to be sorghum cultivar-dependent. With root exudates from AM fungi inoculated S-35 plants, germination of *Striga* seeds was about 15% of germination obtained with root exudates from non-inoculated plants. In contrast, germination of *Striga* seeds after exposure to root exudates from AM inoculated CK60B plants was 50% of germination in the non-inoculated CK60B plants (Table 4.1). Mann-Whitney *U*-test showed this suppressive effect of root exudates of the cultivar S-35 to be significant at both harvests unlike the root exudates of CK60B

Table 4.1. Percentage germination of *S. hermonthica* seeds after exposure to GR-24 (0.1 mg l^{-1}) or to sorghum root exudates obtained from plants grown for 24 days (harvest 1) or 45 (harvest 2) days with AM fungal inoculation (M− = no inoculation; M+ = addition of 50 g per pot) and/or P (P0 = no addition of P fertilizer; P30 = addition of the equivalence of 30 kg ha^{-1} of triple superphosphate). Values are means of five replicates. Mann-Whitney *U*-test: n.s. not signifiant ($P > 0.05$); [**] $P < 0.01$.

| | S-35 | | CK60B | |
Treatment	Harvest 1	Harvest 2	Harvest 1	Harvest 2
GR-24	52.6	61.7	52.6	61.7
M− P0	13.3	48.5	6.2	41.1
M− P30	12.3	49.8	5.7	52.3
M+ P0	1.6	2.5	1.3	29.1
M+ P30	1.4	0.9	4.0	26.4
	Mann-Whitney U-*test*			
Mycorrhiza	[**]	[**]	n.s.	[**]
Phosphorus	n.s.	n.s.	n.s.	n.s.

that showed significant effects at second harvest only (Table 4.1). No *Striga* seeds germinated on exposure to sterile demineralized water.

Effects of AM fungi inoculation on attached and/or emerged Striga

No *Striga* seedlings were observed on the root systems of sorghum at first harvest 24 DAS in all the treatments. By 45 DAS higher numbers of *Striga* had attached and/or emerged on treatments that did not receive inoculum of AM fungi (Table 4.2). In the AM fungi inoculated treatments, there was a significant reduction in both the numbers emerged and the numbers attached but still below ground. This reduction in numbers was much larger for the sorghum cultivar S-35 than for CK60B. Almost no (> 90% reduction) *Striga* were attached to the root systems of treatments of S-35 that received AM fungi inoculum compared to about 50% reduction in number of attached *Striga* in CK60B cultivar (Table 4.2). There was no apparent phosphorus effect on the number of attached and/or emerged *Striga* irrespective of sorghum cultivar (Table 4.2).

   With AM fungi inoculation a delay of 3 days in the time of emergence of first *Striga* shoots was observed in the S-35 sorghum cultivar only, although this was not significantly different from treatments with no AM fungi inoculation as shown by ANOVA.

Effect of AM inoculation on host performance

In treatments with no AM fungal inoculation total dry weight of sorghum was, on average, higher during the two harvests. With AM fungi inoculation, total dry weight of sorghum was, on average, lower at both harvests irrespective of P addition, *Striga* infection or sorghum cultivar (Table 4.3).

Table 4.2. Effect of AM fungal inoculation and P addition on the number of *Striga* attached or emerged on sorghum at 45 DAS[a]. For explanation of parameters see Table 4.1.

	S-35		CK60B	
	Attached	Emerged	Attached	Emerged
M− P0	8.2a	2.8a	18.6a	11.2a
M− P30	7.6a	3.0a	16.6a	6.6a
M+ P0	0.4b	0.0b	8.8b	5.4b
M+ P30	0.6b	0.2b	7.8b	6.2b

[a] Values are means *n=5*. In each column means followed by the same letter are not significantly different (Kruskal-Wallis test, $P < 0.05$).

Table 4.3. Effect of *Striga* (S− = without infestation; S+ = infested with approximately 1000 germinable *Striga* seeds per pot), AM fungi (M− = no inoculation; M+ = addition of 50 g per pot) and phosphorus (P0 = no addition; P30 = addition of equivalent of 30 kg ha^{-1} P per pot in the form of triple superphosphate) on the biomass (g) of sorghum.

	Harvest 1 (24 DAS)		Harvest 2 (45 DAS)	
	CK60B	S-35	CK60B	S-35
S− M− P0	1.16 (1.3)	1.98 (3.9)	3.04 (9.2)	4.12 (17.0)
S− M− P30	1.36 (1.8)	2.08 (4.3)	3.68 (13.5)	1.06 (16.5)
S− M+ P0	1.26 (1.6)	1.84 (3.4)	3.10 (9.6)	3.60 (13.0)
S− M+ P30	1.06 (1.1)	1.96 (3.8)	3.14 (9.8)	3.48 (12.1)
S+ M− P0	1.24 (1.5)	1.96 (3.8)	3.30 (10.9)	4.94 (24.4)
S+ M− P30	1.22 (1.5)	1.96 (3.8)	3.14 (9.8)	4.62 (21.3)
S+ M+ P0	1.16 (1.3)	1.94 (3.8)	2.40 (5.8)	3.46 (12.0)
S+ M+ P30	1.22 (1.5)	1.94 (3.8)	2.86 (8.2)	3.84 (14.7)
SED[a]	0.11	0.10	0.20	0.28
ANOVA[b]				
S	n.s.	n.s.	**	*
AM	n.s.	n.s.	***	***
P	n.s.	n.s.	*	n.s.
S×AM	n.s.	n.s.	n.s.	*
S×P	n.s.	n.s.	n.s.	n.s.
AM×P	n.s.	n.s.	n.s.	n.s.
S×AM×P	*	n.s.	**	n.s.

[a] Standard error of difference. Values are means, *n=5*. Data were square root-transformed. Means of untransformed data are given in parentheses.

[b] *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; n.s. not significant.

Analysis of root exudates

HPLC chromatographic profiles of the root exudates with or without AM fungal inoculation revealed possible simplification of the root exudates upon AM fungal colonization. At almost all the retention times, lower peaks were observed for the samples from AM fungal colonized roots irrespective of sorghum cultivar (Fig. 4.1 and Fig. 4.2). The total amount of phenolic compounds was also lower with AM colonization irrespective of sorghum cultivar (Fig. 4.1 and Fig. 4.2).
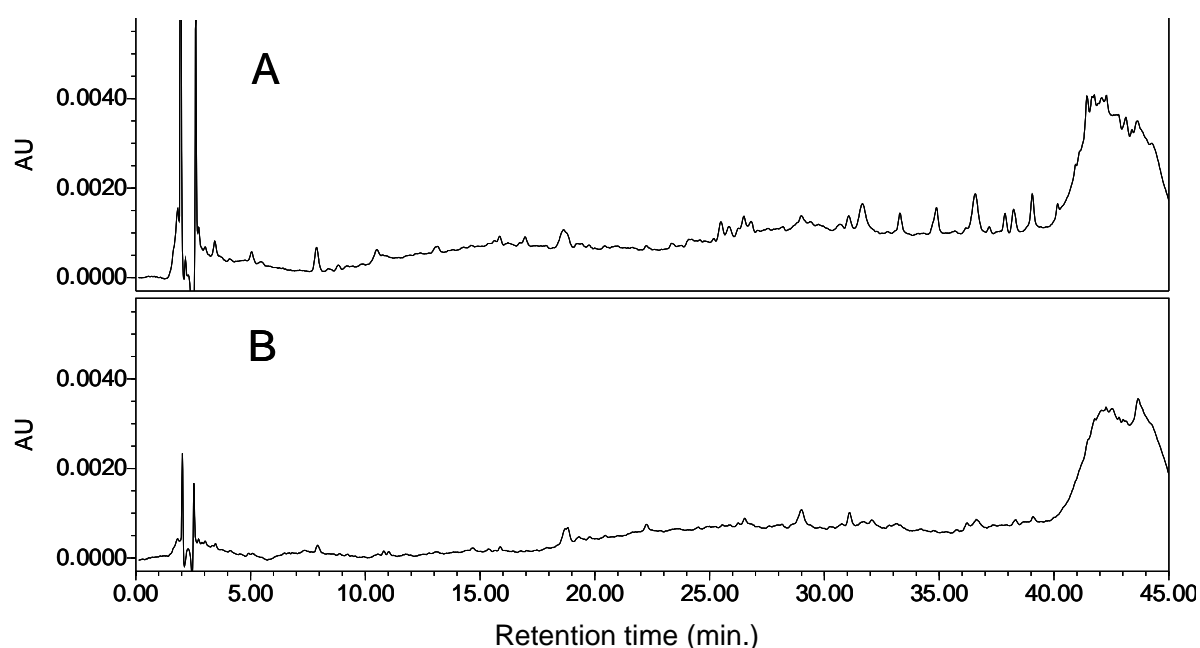
Fig. 4.1. HPLC chromatogram (recorded at 280 nm) of methanolic extracts of sorghum CK60B root exudates (A) without AM fungal inoculation and (B) with AM fungal inoculation. AU = absorption units.
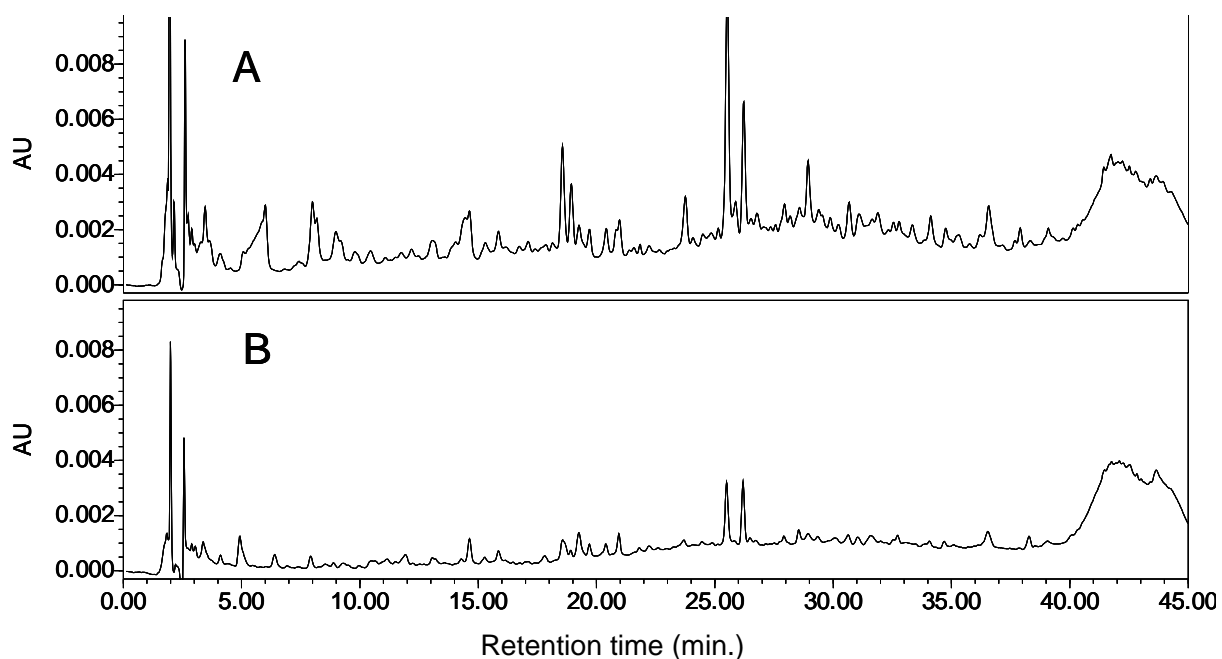

Fig. 4.2. HPLC chromatogram (recorded at 280 nm) of methanolic extracts of sorghum S-35 root exudates (A) without AM fungal inoculation and (B) with AM fungal inoculation. AU = absorption units.

Discussion

The observation that exudates from sorghum roots colonized by AM fungi suppressed germination of *Striga* seeds, lends support to rhizosphere changes as a mechanism by which *Striga* damage could be reduced (Chapter 3). Upon colonization by AM fungi the quality and quantity of root exudates substantially change. While root exudates of non-mycorrhizal sorghum have already been described by Czarnota *et al.* (2003), no data are available that indicate potential changes in the exudate composition of sorghum consequent on AM fungal colonization. Marschner *et al.* (1997) observed a 50%-78% decrease in carbon compounds exuded in the rhizosphere in mycorrhizal pepper (*Capsicum annuum*) compared to non-mycorrhizal plants when the mycorrhizal fungus *Glomus intraradices* was used. They also concluded that mycorrhizal colonization simplified the composition of the rhizosphere soil solution. Both a direct simplification of the root exudate composition and a simplification due to changes in the rhizosphere community composition with its specific metabolites could have occurred. Evidence for simplification of the root exudate composition is visible with the low peaks obtained in the chromatographic profiles of root exudates from AM fungal colonized plants. Given that root exudates in our study were not sterilized, subsequent work should attempt to separate the direct effects of changes in the exudates and changes in the rhizosphere populations due to mycorrhization. Bacteria or micro-organisms intimately associated with roots could persist on the roots after roots are removed and subsequently washed before being placed in the flasks as in the present study. Changes in the bacterial communities in the rhizosphere consequent on AM fungal colonization have been described by Marschner *et al.* (2001), Wamberg *et al.* (2003), and Marschner and Baumann (2003). Such community wide changes may also affect the germination of *Striga* seeds as shown for the plant growth-promoting rhizobacterium *Azospirillum brasilense* (Miché *et al.*, 2000). Sood (2003) also showed that plant growth-promoting rhizobacteria prefer mycorrhizal over non-mycorrhizal roots, which again may feed back to the performance of *Striga* seeds.

It is possible that a simplification of root exudates occurred with AM fungal inoculation in our experiment. Strigol, an analogue of sorgolactone, has been observed to stimulate germination of preconditioned *Striga* seeds at concentrations as low as 10^{-16} M (Heather *et al.*, 1974; Hsiao *et al.*, 1981). Unfortunately, neither strigol nor sorgolactone were identified by Czarnota *et al.* (2003). A priori, one would not suspect that only a quantitative effect on a substance like strigol that the phytoparasite could detect below picomolar concentrations would have such dramatic effects (more than 90% reduction) on *Striga* germination. This suggests the importance of qualitative changes as well (complete repression of either the production of strigol or production of a toxin or a substance that immobilizes strigol). Serial dilution of root exudates

from AM fungal inoculated plants of both sorghum cultivars and effects of dilution on germination of preconditioned *Striga* seeds, are consistent with the hypothesis that both modes of action are operative. Fig. 4.3 could suggest that AM fungi on S-35 produce a toxin, whose effectiveness decreases upon serial dilutions, whereas AM fungi on CK60B reduce the amounts of the germination stimulant, thereby lowering germination upon subsequent dilutions. However, it may be premature to link the magnitude of the effect on *Striga* germination with the magnitude of the quantitative changes in exudation. If the hypothesis that germinating *Striga* seeds in Petri dishes affect germination of neighbouring seed were to hold, then the apparent activity of strigol even after several dilutions might simply be due to this cascading effect and not the high activity of strigol. Even though it is known that *Striga* seeds are responsive to germination stimulants only when they are able to synthesize ethylene (Logan and Stewart, 1991; Babiker *et al.*, 1993; Mohamed *et al.*, 2001; Sugimoto *et al.*, 2003) and that exogenous ethylene (Egley and Dale, 1970; Eplee, 1975) or ethylene-generating compounds (Egley and Dale, 1970; Chancellor *et al.*, 1971) induce *Striga* seed germination and have been applied in the field to induce suicidal germination of *Striga*
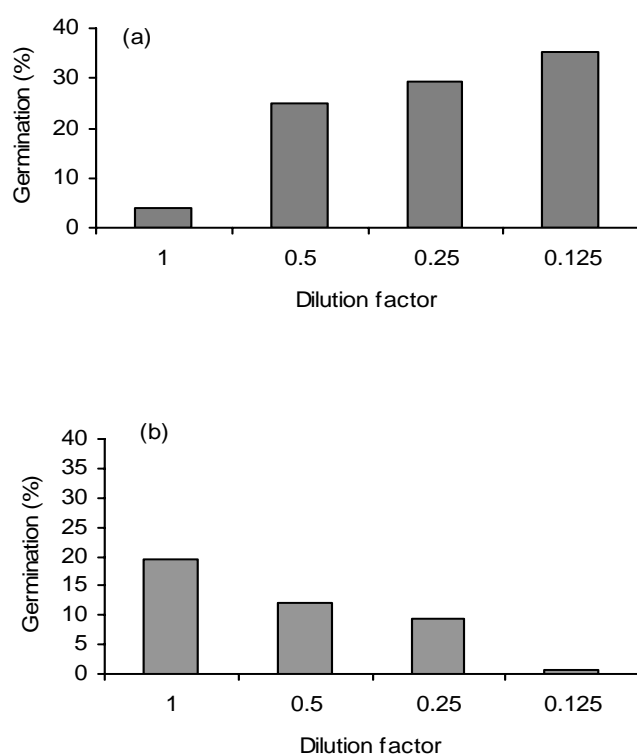

Fig. 4.3. Effect of diluting (with demineralized water) root exudates from AM inoculated sorghum roots (a) S-35 and (b) CK60B on the germination of preconditioned *Striga* seeds.

seeds, no studies have reported the possibility of germinating *Striga* seeds stimulating neighbouring seeds at least *in vitro*. *Striga* seed mortality under field conditions has been observed to vary with seed density in nylon gauze bags (Van Mourik *et al*., 2003), with the highest mortality recorded for bags containing pure *Striga* seeds compared to mortality in bags with *Striga* seed-sand mixture (low density and less seed to seed contact); an evidence for seed to seed influence. In the field the eventuality of *Striga* seeds affecting kin seed germination might seem remote but could be important in 'closed systems' such as sealed Petri dishes (Gbèhounou and Adango, 2003) and microtitre plates (Gurney *et al*., 2003) where all seeds are exposed to similar exudate concentrations. Consequently, it is unlikely that similar reductions of germination due to AM fungi would occur in the field. However, even some reduction in the field will contribute to lower attachment and emergence, as observed in the field (Chapter 5) and could, therefore, be important as part of a strategy to manage *Striga*.

Changes in root exudation after colonization by AM fungi would be an important mechanism in the tripartite interaction between sorghum, *Striga* and AM fungi if mycorrhiza-affected changes occur rapidly enough before *Striga* germination and attachment. Hart and Reader (2002) studied colonization strategies of 21 AM fungal isolates. Four species of *Glomus* and one species of *Acaulospora* already colonized leek (*Allium porrum*) within one week. In a preliminary study on the rate of colonization of roots of the sorghum cultivars S-35 and CK60B by AM fungi, roots were colonized already at 7 days after inoculation (Lendzemo, unpublished observations). The inhibitory effect on *Striga* germination was observed 25 DAS so the changes in root exudation must have taken place in 3-4 weeks. For *Striga* seeds to germinate they need to be preconditioned for about two to three weeks at suitably warm temperature and moisture (Reid and Parker, 1979; Okonkwo, 1991) and subsequently exposed to germination stimulant. While this suggests that mycorrhizal colonization may be sufficiently rapid to be relevant under field conditions, agricultural practices that increase the rate and/or extent of mycorrhizal colonization could amplify the effect. Next to timing, the extent of mycorrhizal colonization could be an important factor. It would be difficult to have a root system, in the pot or field, colonized one hundred per cent by AM fungi. The roots that escape colonization will very likely encounter *Striga* seeds and get infected, unless the effects of AM colonization are systemic and rapid. There is some evidence suggesting that systemic resistance against pathogens may be induced by AM fungi (Cordier *et al*., 1996; Pozo *et al*., 2002). Support for the importance of timing by giving AM fungi an advantageous position for root colonization is provided by Van Delft *et al*. (2000), who observed that no till plots, where intact mycorrhizal networks may allow a more

rapid colonization of sorghum seedlings, showed a lower emergence of *Striga*. However, these authors did not investigate the possible importance of a mycorrhizal connection in the sorghum-*Striga* interaction.

The effects of root exudates upon AM colonization of roots, as observed in this study are unlikely to be due to an improved phosphorus status of the plant. With P addition neither the root exudate effects on *Striga* seed germination nor the effects of AM colonization on the number of attaching and/or emerging *Striga* were affected. Root colonization levels by AM fungi were similar irrespective of P application at both harvests. It is possible that sorghum growth in the pots was nitrogen limited and that the equivalence of 30 kg ha^{-1} of P fertilizer applied per pot was below the level that influences colonization and development of the fungal isolates. Alternatively, the inoculum potential of AM fungi used in this study might have been high. Olsen *et al.* (1999) observed that percentage colonization of roots of *Capsicum annuum* at a high inoculum density (about 1000 non-dormant infective propagules/g air-dry soil) was not affected by P application.

Any influence of root exudates on germination of *Striga* seeds *in vitro* or negative effects on *Striga* germination, attachment and/or emergence in the pots, as direct effects of P in the absence of AM fungal inoculation were not to be expected as observed in this study. Phosphorus fertilization appears to have a negative effect on *Striga* only with simultaneously applied N fertilizer (Gacheru and Rao, 2001) in contrast with other root hemi-parasites of the same family Scrophulariaceae that are negatively impacted with P fertilization (Davies and Graves, 2000).

At both harvests, the performance of the two sorghum cultivars was reduced with AM fungi inoculation irrespective of P addition or *Striga* infection. Transient growth depression at the early stages of plant growth following AM colonization is often observed in mycorrhiza and this might have been the case especially at 24 DAS. It is also possible that the observed growth depression with AM fungi colonization was a result of suboptimal lighting within the greenhouse, high potential of the inoculum used and/or pot size. Light is essential for mycorrhizas especially during the early stages of establishment of the fungus within the root system. During colonization of roots by AM fungi, the benefits are unidirectional: the fungus derives carbon from the host to establish a functional network of extraradical hyphae in the soil. Suboptimal light conditions (especially low photosynthetic active radiation (PAR) levels) would mean suboptimal photosynthesis and insufficient carbon to satisfy the needs of the host and the surplus demand by the fungus. Also, growth response due to mycorrhiza decreases when experiments are executed in smaller pots (Bååth and Hayman, 1984). Plant roots under such conditions, have access to available nutrients and cannot derive much additional benefit from extraradical hyphae of the fungus for nutrient uptake.

While germination of *Striga* seeds was almost completely inhibited by exudates of mycorrhizal roots for the sorghum cultivar S-35, we did not observe a similar large effect for CK60B despite the fact that the levels of colonization were similar for both cultivars. Sorghum cultivars have been bred for resistance against or tolerance of *Striga*, and such breeding may indirectly have affected production of exudates, both in the mycorrhizal and non-mycorrhizal conditions. It would be interesting to test several sorghum cultivars with differential sensitivities to *Striga* infection, for effects of their root exudates on *Striga* seeds upon AM fungal colonization.

Chapter 5

Field inoculation with arbuscular mycorrhizal fungi reduces *Striga hermonthica* performance on cereal crops and has the potential to increase cereal production

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Abstract

The cereal crops maize and sorghum were grown in the field in north Cameroon, Africa, during the cropping seasons (June to October) of 2000 for maize, and 2001 and 2002 for sorghum. Both cereals were grown uninfested or infested with seeds of the root hemi-parasite *Striga hermonthica,* and without or with inoculation with a mixed soil inoculum of arbuscular mycorrhizal (AM) fungi (*Glomus clarum* and *Gigaspora margarita*). Infection of maize by *S. hermonthica* resulted in a significant reduction in cob yield of 20%. The effects of *S. hermonthica* on sorghum in the absence of AM inoculation were relatively marginal in 2001: infestation with *S. hermonthica* led to a reduction of 7% of the panicle yield compared to a significant reduction by 26% in 2002. With AM fungal inoculation, a significant reduction (30 % and more than 50% on maize and sorghum, respectively) in the number of *S. hermonthica* shoots was noted. Dry weight of *Striga* followed the same pattern (40% reduction on maize, and 46% and 63% reduction on sorghum in 2001 and 2002, respectively) after AM fungal inoculation. Reduction in the number of *S. hermonthica* shoots with AM fungi inoculation resulted in a significant interaction effect of *S. hermonthica* and AM fungi on yield parameters of sorghum in the cropping season of 2002. No significant yield increase was noted for maize or sorghum in 2001. The results are discussed in the context of managing mycorrhizas as a component of integrated management of *S. hermonthica* on cereals aimed at sustainability.

Introduction

Witchweed, *Striga hermonthica* (Del.) Benth. (Scrophulariaceae), seriously affects cereal production in north Cameroon in the heart of the African Savannah. This weed is parasitic in nature and highly prolific. An individual *Striga* plant produces thousands of tiny dust-like seeds that can remain dormant in the soil for 15-20 years (Ramaiah *et al*., 1983). Germination, attachment and haustorial formation are all dependent on *Striga* seeds receiving chemical cues from host roots (Stewart and Press, 1990). Sorghum (*Sorghum bicolor* (L.) Moench), millet (*Pennisetum americanum* (L.) Beeke), and maize (*Zea mays* L.), the major staple food crops in north Cameroon are

hosts of *S. hermonthica* and suffer to various degrees from infection by this weed.

The system of shifting cultivation characterized by continuous cultivation of the same piece of land for 2-7 years followed by fallowing for more than 10 years, during which the soil could regain its natural fertility, ensured some degree of ecological sustainability in the past. Anthropogenic factors, mainly the continuous surge in human population and the land tenure system, have forced farmers to abandon the practice of shifting cultivation and adopt a more permanent cropping system characterized by intensive tillage with little or no organic or inorganic fertilizer application, and short fallows (less than 10 years) when practiced. Very few farmers own land in north Cameroon and as a result most farmers have little or no vested interest in basic soil fertility management, when and where possible, on land leased to them for a limited period of time. With continuous cultivation of cereal hosts, and no specific control measures against *Striga*, huge amounts of *Striga* seeds have accumulated (and continue to accumulate) in the soil seedbank as evidenced by the large numbers of *Striga* shoots that carpet cereal fields during the months of October-November even after the crops are harvested (Lendzemo, *unpublished*).

Sustainable agricultural systems, in terms of enhanced crop production with simultaneous reduction of adverse effects on the environment, are those that rely more on the natural capacity of the soil to generate and maintain a favourable soil structure, to supply the plant with nutrients in sufficient quantities at the right time (synchronization) and the right place (synlocalization), and to prevent or suppress soil-borne pests and diseases (Brussaard, 1997). The search for sustainable agricultural systems is not a unique preoccupation for agricultural systems in the industrialized world characterized by heavy reliance on agro-chemicals (high input vs high output), but applies as well to agricultural systems in the developing world characterized by a negative balance between input and output nutrient flows – the soil being actually mined in the latter. Shifting cultivation used to be sustainable to some extent and reduced effects of pathogens and pests including *Striga*. With the abandonment of this practice, other sustainable agricultural systems have to be sought. In sustainable agricultural systems the soil biota (plant roots and soil organisms) play an important role. The contribution of arbuscular mycorrhizal (AM) fungi within such systems cannot be overemphasized. These fungi play key ecological roles in, for example, nutrient acquisition (Smith and Read, 1997), disease prevention (Newsham *et al.*, 1995a), and soil aggregate formation (Miller and Jastrow, 2000; Rillig *et al.*, 2002). As far as the tripartite interaction between AM fungi, sorghum and *Striga hermonthica* is concerned, AM fungi have been found to have a significant influence on the outcome under controlled conditions. On the one hand, *Striga* performance in the presence of AM fungi was negatively impacted, with reduced and/or delayed germination (Chapter

4), attachment (Chapter 3), and emergence (Chapter 2; Gworgwor and Weber, 2003). On the other hand, AM fungi had a direct positive effect on the yield of the sorghum (Chapter 2; Gworgwor and Weber, 2003). Sustainable systems targeting *Striga* management on cereals in general or sorghum in particular might have to take into account the management of this symbiotic interaction.

Due to the complex biotic interactions in the field, which might be affected indirectly or directly by the abiotic conditions, results from greenhouse studies and particularly such studies using sterile plant growing media, can hardly be projected to field conditions *a priori*. It is necessary to evaluate such systems under field conditions before any meaningful conclusions or generalisations can be made. The need to evaluate the results of studies under controlled conditions is even more warranted in the particular case of mycorrhizal fungi given the multi-functionality of this symbiosis (Van der Heijden and Kuyper, 2001) viz: (1) different fungi show different benefits to the same plant under the same environmental conditions; (2) the same fungus shows differential benefits to different plants under the same environmental conditions; (3) the same fungus shows differential benefits to the same plant under different environmental conditions.

The objective of this study was thus to attempt to validate the results of the tripartite interactions among sorghum, AM fungi, and *S. hermonthica* obtained under controlled conditions, in the field. Whether similar outcomes could be observed in the field on maize infested with *Striga* was also investigated. The key hypotheses were:

- Infestation and subsequent infection of maize or sorghum cultivars by *S. hermonthica* in the field reduces the performance of the cereal crop.
- Inoculation of maize or sorghum in the field with AM fungal inoculum increases crop performance and grain yield.
- The presence of AM fungi reduces the number of *Striga* shoots emerging and the biomass of the emerged *Striga* shoots, and delays the relative time of emergence.
- Colonization of roots of maize and sorghum by AM fungi, and derived benefits from the symbiosis compensates for damage by *S. hermonthica*.

Materials and methods

Field experiment in 2000

The experiment was conducted at IRAD field station (9°16'394" N, 13°29'524" E) in Garoua, north Cameroon, during the cropping season (June-October) of 2000. The field used had been under fallow for more than 10 years after which cotton was grown (as first crop after fallowing) during the cropping season of 1999. Cotton is a trap crop for *Striga* (it induces suicidal germination of *Striga* seeds in the soil). Fallowing land

for several years is known to negatively affect *Striga* seeds in the soil. Very few, if any, *Striga* seeds were thus to be expected in the seedbank thus making room for artificial infestation with *Striga* seeds and the possibility to have appropriate control (*Striga*-free) treatments for comparison. The soil had a pH-KCl of 5.3, organic carbon of 1.52%, P Bray I of 7 ppm and total N content of 0.14%. The experiment was laid out as a full factorial, randomized complete block design. The factors were: maize cultivar (the *Striga*-sensitive CMS8501 and the *Striga*-tolerant Advance NCRE (Thé, personal communication); S. *hermonthica* seeds (with/without) and AM fungal inoculum (with/without addition). All 8 treatments were replicated four times. Each plot consisted of 4 rows, 3 m long and 80 cm apart. Within the rows, maize seeds were sown every 50 cm. The AM inoculum was a soil mixture of propagules of *Glomus clarum* Nicolson and Schenck and *Gigaspora margarita* Becker and Hall originally obtained from the University of Yaoundé I in Cameroon (Nwaga *et al*., 2000). Fifty grams of this soil inoculum was applied in each sowing hole of the relevant experimental treatments at time of sowing of maize. A total of 35 kg of AM fungal inoculum was required for the experiment. The *Striga* seeds, harvested in October 1999 from *Striga* shoots growing on sorghum in north Cameroon, were mixed with finely sieved, through 180 µm sieve, sand to give an infestation density of about 1000 germinable seeds (Berner *et al*., 1997) per sowing hole. The sowing holes of the relevant plots were infested with the *Striga* seed/sand mixture just before maize seeds (4 per hole) were sown. Emerged maize seedlings were thinned to two, one week after emergence giving a total of 54 maize plants per plot. The equivalence of about 100 kg ha^{-1} of NPK (16:13:10) was applied 15 days after sowing (DAS) of maize. At 46 DAS, urea at the rate of 30 kg ha^{-1} was applied. Plots were hand-weeded of all weeds except *Striga* at 45 DAS and 70 DAS. The time of emergence of the first *Striga* shoots in the relevant plots was recorded. Following emergence of first *Striga* shoots the two central rows were observed at weekly intervals for *Striga* shoots and numbers emerged recorded. At harvest, the two central rows of maize were harvested to measure grain and stover yields. The maize ear moisture content, useful in calculating maize yield, at the time of harvest was measured using the moisture tester INSTO$^{®}$ Auburn, IL. U.S.A.

The *Striga* shoots beneath the plants in the central rows were harvested, dried and weighed. Root samples were collected at random from five plants of the two central rows at harvest after careful digging with a pickaxe. These root samples were bulked, chopped into small pieces of about 1 cm in length and stored in 50% ethanol. About 2-3 g of the roots were subsampled and stained following a modified procedure of Kormanik and McGraw (1982). The major modifications were that, firstly, a cold staining procedure was employed and secondly the concentration of KOH, for clearing

roots, was diluted from the recommended 10% to 2.5%. The stained roots were examined for mycorrhizal colonization using the gridline intersection method (Giovannetti and Mosse, 1980). At the time of collection of root samples, about 50 g soil samples were collected as well from those sowing holes. AM fungal spores were extracted from 25 g sub samples by the wet-sieving technique (Daniels and Skipper, 1982).

Field experiments in 2001 and 2002

A *Striga*-free piece of land also left fallow for more than 10 years and at the same location as the maize experiment above was used for setting up the experiments during two cropping seasons (2001 and 2002). Cotton was grown during the cropping season of 2000, as the first crop after fallowing. The soil had a pH-KCl of 6.4, organic carbon of 0.41%, P Bray I of 9 ppm and total N content of 0.008%. The design and layout were similar to the maize experiment above except for cultivar selection. Only one cultivar was used: S-35 which was reported to show some tolerance to attack by *S. hermonthica* (Parker and Riches, 1993; Gupta and Lagoke, 2000), and widely adopted in the far north province of Cameroon (Yapi *et al*., 1999) was employed. The length of the plots was 5 m and a total of about 18 kg of the AM fungal inoculum was applied during each cropping season. All other practices such as application and amount of AM fungal inoculum or *Striga* seeds per sowing hole, manner of weeding, harvesting of roots and staining, collection of soil samples and extraction of AM fungal spores were similar to maize experiment. No fertilizer was applied.

Data analyses

Data on crop yield parameters and *Striga* biomass were subjected to analysis of variance (ANOVA) procedures for a randomized complete block design. Data on maize yield did not meet ANOVA requirements (normality, homogeneity of variances) even after transformation; therefore the non-parametric Mann-Whitney U test was used to test for significance of the main effects only. Data on the fractional root colonization by AM fungi and number of spores were respectively arcsine- or square root-transformed (Sokal and Rohlf, 1995) before subjecting to ANOVA procedures. The means of parameters for which significant treatment effects were found, were separated using the Least Significant Difference (LSD) test (*P < 0.05*). The emergence of *Striga* shoots over time on sorghum was described using the beta growth function (Yin *et al*., 2003). The beta growth function is an empirical equation for the sigmoid pattern of determinate growth. It can also be used to describe the pattern of *Striga* emergence over time. Compared to the logistic equation the beta function has the advantages of being asymmetrical around time and being zero at $t = 0$. In this case, it

calculates number of *Striga* emerged (*w*) in dependence of time, using three parameters: t_m, the time at which the maximum emergence rate of *Striga* shoots is obtained; t_e, the time at which maximum number (w_{max}) of *Striga* emerge. The parameters were estimated with the help of regression analyses procedures, SAS ® System for Windows (8th edition).

Results

Influence of S. hermonthica *on maize and sorghum*
Uninfested maize plants yielded significantly higher than the *Striga*-infected plants (Table 5.1). With *Striga* infection of maize, a 20% reduction in cob dry weight was observed (Table 5.1). Panicle dry weight of sorghum at harvest was higher without *Striga* in both seasons but not significantly (Table 5.2 and Table 5.3). Infection of sorghum by *Striga* in the absence of AM fungal inoculation resulted in 7% reduction of panicle dry weight in 2001 (Table 5.2) and 26% reduction in 2002 (Table 5.3).

The average number of *Striga* plants emerging per square meter and the *Striga* dry weight significantly differed between maize cultivars. Fewer shoots and less dry weight of *Striga* were observed on plots planted with the *Striga*-sensitive cultivar CMS-8501 compared to those with the *Striga*-tolerant cultivar Advance NCRE (Table 5.4).

Striga emergence on sorghum differed with years. In 2002, more *Striga* shoots emerged on sorghum per square meter compared to 2001 (Table 5.5). Biomass of emerged *Striga* in sorghum fields followed a similar pattern to that of the number emerged: the dry weight of *Striga* harvested from the two central rows of sorghum was significantly higher in 2002 compared with 2001 (Fig. 5.1). Cereal biomass was 25% higher in 2002 than in 2001 (Table 5.2 and Table 5.3). Less than one *Striga* plant, on average, emerged per square meter in the control plots in 2002.

Influence of AM fungal inoculation on crop performance and AM fungal propagules
There was no significant effect of AM inoculation on the yield parameters of either sorghum or maize yield. Similar AM fungal spore numbers and level of AM fungal colonization of roots were observed for all the treatments irrespective of genotype or AM inoculation (Tables 5.1-5.3). The overall level of AM fungal colonization of sorghum roots was higher in 2001 compared to 2002 (Tables 5.2 and 5.3).

Table 5.1. Effect of *S. hermonthica* infestation (S− = no infestation and S+ = approximately 1000 germinable seeds per sowing hole) and AM fungal inoculation (AM− = without AM fungi addition; AM+ = addition of 50 g per sowing hole) on maize cob yield, percent root colonization by AM fungal propagules, spores per gram of soil in the field (season 2000) at maturity of maize cultivars (CV1 = Advance NCRE and CV2 = CMS-8501). M-W U (Mann-Whitney *U*-test) and ANOVA results: n.s. not significant, $P > 0.05$; [*] $P < 0.05$; [**] $P < 0.01$; [***] $P < 0.001$.

Treatment	Cob yield [a] (kg ha^{-1})	Colonization [a] (%)	Spores [a] (g^{-1})
CV1 AM− S−	2475	26.2(19.5)	7.2(51.8)a
CV1 AM− S+	1914	28.9(23.3)	6.2(38.4)b
CV1 AM+ S−	2336	25.0(17.9)	6.0(36.0)b
CV1 AM+ S+	1827	32.4(28.7)	6.3(39.7)b
CV2 AM− S−	2269	29.0(23.5)	7.2(51.8)a
CV2 AM− S+	1922	30.0(25.0)	6.2(38.4)b
CV2 AM+ S−	2304	31.6(27.4)	6.5(42.2)ab
CV2 AM+ S+	1766	34.9(32.7)	6.7(44.9)ab
	M-W U	ANOVA	ANOVA
CV	n.s.	n.s.	[*]
AM	n.s.	n.s.	n.s.
S	[**]	n.s.	n.s.
CV×AM		n.s.	[***]
CV×S		n.s.	[**]
AM×S		n.s.	n.s.
AM×CV×S		n.s.	[***]

[a] Values are means. Data on percent colonization and spore numbers were, respectively, arcsine- or square root-transformed. Values of untransformed data are in parentheses. In each column, values with same letter are not significantly different according to LSD ($P < 0.05$) test.

Influence of AM fungal inoculation on performance of S. hermonthica *and consequence on host performance*

The relative time of emergence of first *Striga* shoots on maize (data not shown) and sorghum did not differ between the treatments but differed between the years for sorghum. Whereas *Striga* shoots were observed on sorghum as from 60 DAS in 2001, *Striga* shoots were observed on sorghum at 40 DAS in the cropping season of 2002 (Figs 5.2-5.3).

Table 5.2. Effect of *S. hermonthica* infestation (S− = no infestation and S+ = approximately 1000 germinable seeds per sowing hole) and arbuscular-mycorrhizal (AM) fungal inoculation (AM− = no addition and AM+ = 50 g soil inoculum per sowing hole) in the field (year 2001) on the stover yield (leaves + stem), panicle dry weight, dry weight of 1000 grains, percentage sorghum root colonized by AM fungi, and number of AM fungal spores g^{-1} soil sample at harvest during the cropping season 2001 in north Cameroon. Sorghum cultivar was S-35.

Treatment	Stover [a] (kg ha^{-1})	Panicle [a] (kg ha^{-1})	1000 grain [a] (g)	Colonization [a] (%)	Spores [a] (g^{-1})
AM− S−	3875	1687	29.62	48.8 (56.6)	2.1 (4.4)
AM− S+	3750	1562	28.52	48.5 (56.1)	2.8 (7.8)
AM+ S−	4500	1736	29.55	55.5 (67.9)	2.5 (6.2)
AM+ S+	3156	1593	29.20	48.5 (56.1)	2.4 (5.8)
ANOVA results					
S	n.s.	n.s.	n.s.	n.s.	n.s.
AM	n.s.	n.s.	n.s.	n.s.	n.s.
S×AM	n.s.	n.s.	n.s.	n.s.	n.s.

[a] Some data were transformed: arcsine transformation for percent root colonized and square root transformation for data on spore numbers. In parentheses are values of untransformed data. For explanation of ANOVA results see Table 5.1.

Table 5.3. Effect of *S. hermonthica* infestation and AM fungal inoculation on sorghum cv S-35 in the field during the cropping season 2002. For explanation of the various parameters see Table 5.2.

Treatments	Panicle [a] (kg ha^{-1})	1000 grain [a] (g)	Colonization [a] (%)
AM− S−	2337a	26.26	35.9 (34.4)
AM− S+	1714b	24.96	37.4 (36.9)
AM+ S−	2004ab	25.72	40.0 (41.4)
AM+ S+	2208a	24.28	40.8 (42.7)
ANOVA results			
S	n.s.	n.s.	n.s.
AM	n.s.	n.s.	n.s.
S×AM	**	n.s.	n.s.

[a] Values in the tables are means. For transformed (arcsine transformation) data, means of untransformed data are given in parentheses. Within a column values followed by a different letter are significantly different according to the Least Significant Difference (LSD) test ($P < 0.05$).

Table 5.4. Number and dry weight of emerged *Striga* shoots as affected by maize cultivar (CV1 = reported *Striga*-tolerant Advanced NCRE; CV2 = *Striga*-sensitive CMS-8501) and or AM fungal inoculation (AM− = no addition of AM fungal inoculum in sowing holes of maize; AM+ inoculation with 50 g per sowing hole).

Treatment	Emerged *Striga* [a] (m^{-2})	Dry weight *Striga* [a] $(g\ m^{-2})$
CV1 AM−	3.2 (10.2)a	9.5a
CV1 AM+	2.6 (6.8)b	5.2b
CV2 AM−	2.3 (5.3)b	5.4b
CV2 AM+	1.8 (3.2)c	4.2c
ANOVA results		
CV	***	*
AM	*	*
CV×AM	n.s.	n.s.

[a] Values are means *n=4*. Data on emerged *Striga* were square root- transformed. Means of untransformed data are in parentheses. For explanation on ANOVA results see Table 5.1.

Table 5.5. Estimated parameter values (with standard error in parentheses) of the beta growth function for emergence of *S. hermonthica* on sorghum without or with AM fungal inoculation in the field. Max. emerged = maximum number of *Striga* emerged m^{-2} per cropping season, time rate highest = approximate time, days after sowing sorghum (DAS), of highest *Striga* emergence rate, time at max. emerged = time (DAS) at which maximum number of *Striga* emerged in the various treatments.

Treatment	Without AM addition[*]	With AM addition[*]
2001		
Max. emerged	3.7 (0.3)a	1.8 (0.2)b
Time rate highest	75.0 (0.0)b	81.7 (5.1)a
Time at max. emergence	86.9 (14.4)a	101.3 (11.8)a
R^2	0.78	0.71
2002		
Max. emerged	16.2 (0.9)a	6.5 (0.5)b
Time rate highest	70.0 (1.7)b	73.5 (2.1)a
Time at max. emergence	82.9 (3.8)a	81.9 (4.4)a
R^2	0.93	0.88

[*] In a row within each cropping season, values followed by different letters are significantly different.
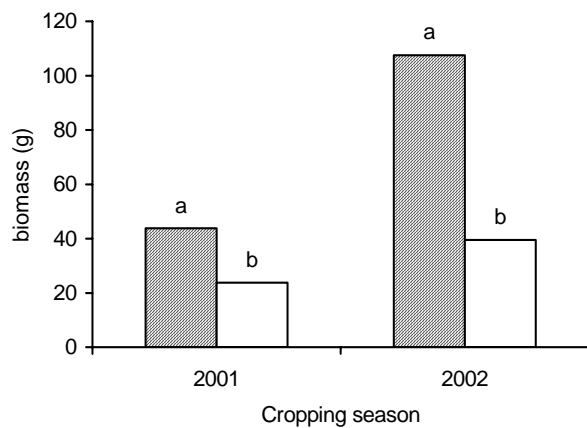
Fig. 5.1. Average (n=4) total biomass of emerged *Striga* shoots without (shaded) or with (not shaded) AM fungal inoculation on sorghum. Columns with different letters within each cropping season are significantly different ($P < 0.05$).
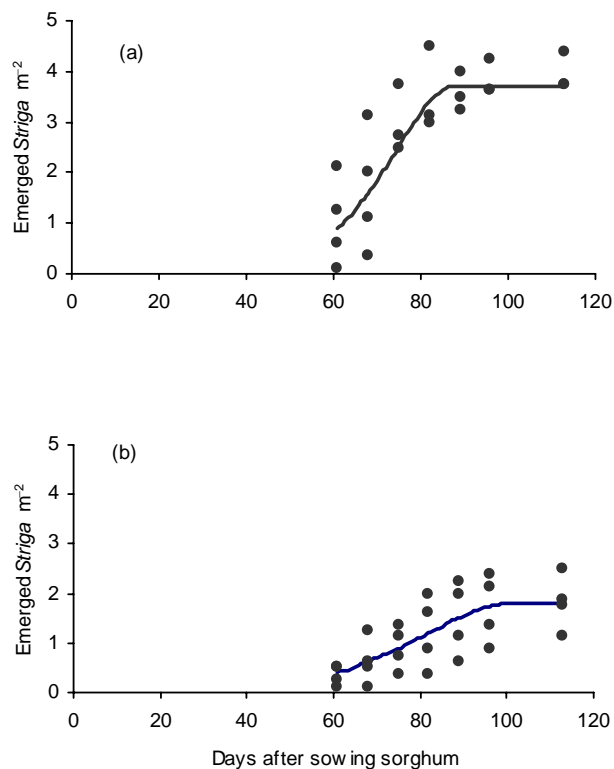
Fig 5.2. Emergence pattern of *S. hermonthica* on sorghum in the field in 2001(a) without AM inoculation and (b) with AM inoculation. Observed (points) and those described (predicted) by the beta growth function (curve). Estimated parameter values are shown in Table 5.5. Dixon's test (Sokal and Rohlf, 1995) for detection of outliers identified two outliers in (a) and those were dropped.

Fig 5.3. Emergence pattern of *S. hermonthica* in the field in 2002 (a) without AM inoculation and (b) with AM inoculation. Observed (points) and those described by the beta growth function (curve). Estimated parameter values are shown in Table 5.5.
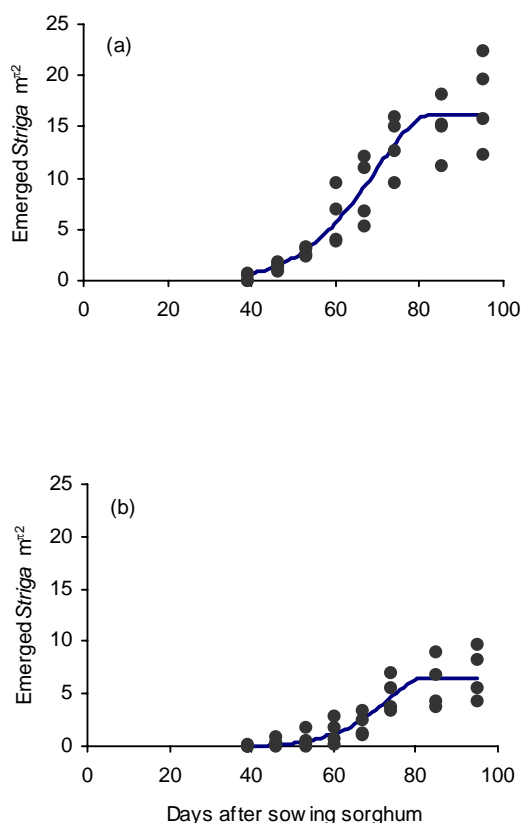
In the absence of AM fungal inoculation, relatively higher numbers of *S. hermonthica* shoots emerged per square meter on maize in 2000 and on sorghum in both 2001 and 2002. A significant reduction in number of emerged *Striga* shoots was observed with AM inoculation: more than 30% reduction on maize (Table 5.4), more than 50% and 60% reduction on sorghum in 2001 and 2002, respectively (Table 5.5). In the absence of AM fungal inoculation the highest rate of *Striga* emergence was observed in sorghum fields relatively earlier in the course of the growing season. Time (t_m) at which highest rate of *Striga* emergence occurred on sorghum was significantly delayed with AM fungal inoculation both in 2001 and 2002 (Table 5.5). A similar trend to that of the number of emerged *Striga* was observed for the total biomass of *Striga* shoots on cereals in all the three years of observations. In the absence of AM fungal inoculation, the dry weight of *Striga* shoots emerging per square meter on maize (Table 5.4) or sorghum (Fig. 5.1) in the two central rows of the plots, was significantly higher.

Discussion

Experimental set up

Very few studies have been undertaken in the field with appropriate control treatments (*Striga*-free plots) in the *Striga*-stricken regions of Africa. Very often, fumigation with methyl bromide (Gurney *et al*., 1999) is used or ethylene gas (Eplee, 1975) is injected into the soil, to free the plots of *Striga* seeds. Non-target soil biota including AM fungi can be affected by either fumigation with methyl bromide (An *et al*., 1993; Bendavid-Val *et al*., 1997) or disinfestation with ethylene (Geil *et al*., 2001; Guinel and Geil, 2002). In this study, plots that were not infested with *Striga* remained free of emerged *Striga* shoots throughout the duration of the maize experiment and the first sorghum crop, an indication that fallowing had eliminated the *Striga* seedbank. However, the root systems were not examined for possible attached *Striga* shoots that did not emerge. Some scattered *Striga* shoots emerged in the control plots in 2002. The origin of such contamination was very likely wind dispersed seeds from the *Striga*-infested plots in 2001. Wind dispersal of *Striga* seeds, although not important for long-distance dispersal of seeds (Berner *et al*., 1994), would have been important in this short distance plot-to-plot dispersal. Fallow fields are ideal for field studies on effect of *Striga*, since plots can be artificially infested or not with *Striga* seeds and effects compared and contrasted. One possible limitation is the fact that organic matter and nutrient levels will be higher in those fields compared to the bulk of farmers' fields. Mycorrhizal inoculum potential will likely also be higher after fallowing. Extrapolating results from such studies might therefore not be without complications. We also note that, given the widespread occurrence of the *Striga* problem and the persistence of *Striga* seeds in the soil, artificial infestation of *Striga* free plots might raise serious ethical questions. Such problems can be overcome in fallow fields of agricultural research institutes, but far less so in farmers' fields.

Effect of Striga *and AM fungi on cereal yield*

A significant reduction in crop yield parameters by *S. hermonthica* infection was evident for maize and for sorghum grown in 2002. The relatively weak reduction in biomass and panicle yield of sorghum in the presence of *Striga* in 2001 might be explained by within-field variability, climatic factors, and delay in the time of *Striga* emergence, and the infestation level. The first *Striga* shoots emerged on sorghum at about 60 DAS whereas in 2002 *Striga* shoots were observed at 40 DAS. Early emergence of *S. hermonthica* correlates with early attachment of *Striga* seedlings to the roots (Gurney *et al*., 1999) which in turn is a function of the *Striga* seed density (A. Van Ast, unpublished observations), and host plant characteristics such as root

architecture (Van Delft, 1997; Kim and Adetimirin, 2001). Early attachments result in severe damage to the host under controlled conditions (Cechin and Press; 1993b, Gurney *et al*., 1999) or in the field (Weber *et al*., 1995; Abayo *et al*., 1996). By the time *Striga* seeds were preconditioned in 2001, given that infestation with *Striga* was in less than 5 cm-deep sowing holes, sorghum seeds very likely germinated and escaped early *Striga* attachments. *Striga hermonthica* seeds germinated and attached at a later stage on roots developing superficially, when the host, a tolerant cultivar for that matter, could relatively put up with the *Striga* infection. The experiments were conducted on the same site, the same treatments assigned to the same plots in both years. The *Striga* plants in 2001 were harvested at the end of the cropping season and this after some *Striga* seeds would have been shed on the soil. Hoeing prior to set up in 2002 would have dispersed *Striga* seeds through the soil and beyond the sowing holes of 2001. To ensure uniformity, all the plots that received AM fungal inoculum and/ or *Striga* seeds again received AM fungal inoculum and/or *Striga* seeds in 2002. The increase in amounts of *Striga* seeds, from artificial infestation and mixing through, at hoeing, of seeds shed on soil surface in 2001, very likely increased the chances of early *Striga* attachments and an increase in number of emerged shoots in 2002.

The fact that on average sorghum yield parameters were higher in 2002 suggests the influence of abiotic factors on the *Striga*-sorghum interaction between the years. Soil moisture could have had a significant role in the outcomes of the experiments. Rainfall was more erratic in 2001 than 2002 as reflected by the sowing dates: July 18 in 2001 and July 3 in 2002. The significant reduction in maize cob yield with *Striga* infection from artificial infestation on a field that had been fallowed for several years again reiterates the relative sensitivity of maize to *Striga* infection in comparison with other cereals. Compared to the cereals sorghum or millet that have been grown from time immemorial in the African Savannah region and co-evolving with *Striga*, maize is a recently introduced crop in Africa and possesses a weak defence against this phytoparasite (Kim, 1994). First *Striga* shoots appeared on maize at about the same time (40 DAS) as on sorghum in 2002. Maize cultivars have been bred for responsiveness to fertilizer and develop relatively superficial roots. The chances for the roots to encounter *Striga* seeds throughout the growing season are higher compared to sorghum or millet. The latter develop deeper root systems and can avoid infection. In naturally infested fields, the bulk of *Striga* seeds are found in the top 5 cm of the soil under zero tillage or in the plough layer (0-20 cm) with tillage (Van Delft *et al*., 1997) and avoidance of *Striga* seeds as well as delay in the onset of *Striga* attachment through deep planting have been shown to be effective against *Striga* infection of maize in the field (Van Delft *et al*., 2000). The fact that no host genotype-dependent effects of *Striga* were observed on the maize cultivars deserves further investigation.

Both cultivars had similar levels of yield reduction even though Advance NCRE had more *Striga* shoots emerging suggesting that it might be better classified as tolerant than as resistant.

Effect of AM fungi on Striga *performance and consequence on host yield*

AM fungal inoculation reduced the number and biomass of *Striga* shoots emerging on the two cereal crops during all the three cropping seasons, in agreement with the outcomes of previous studies under controlled conditions (Chapter 2; Chapter 3; Gworgwor and Weber, 2003). Interestingly, the reduction in numbers of *Striga* and biomass consequent on AM fungal inoculation did not result in a concomitant increase in grain yield of the cereals. The lack of a significant effect of AM fungal inoculation on cereal yield parameters combined with the absence of significant differences in spore numbers or colonization levels indicates the quantity and/or quality of the indigenous AM fungi that influence cereal yield through improved (P) nutrition was already sufficient. Long-term fallowing of the plots used in this experiment might have helped in building up sufficient amounts of inoculum in the soil. Also, cotton is heavily mycorrhizal (Rich and Bird; 1974, Nehl *et al.*, 1999) and was grown in the plots prior to the experiment. Although inoculum in the soil may seem to have been sufficient in terms of final colonization levels and growth enhancement of the cereal, the fact that addition of inoculum had a suppressive effect on *Striga* is an indication that boosting the colonization rate may still have beneficial effects in managing *Striga*. Arbuscular mycorrhizal symbiosis is multifunctional and may enhance the fitness of plants through other mechanisms than increased nutrient uptake (Newsham *et al.*, 1995b). Root colonization by AM fungi initiated from different AM fungal propagule densities eventually reaches a plateau even though the rate of spread of the fungus may differ with propagule density (McGee *et al.*, 1999).

Implications for management of farmers' fields

The observations in this study indicate that AM fungi may play an important role within the *S. hermonthica*/cereal pathosystem in the field in line with previous observations under controlled conditions. One of the greatest challenges, however, is how the conditions in the plots and the management practices used in this study reflect the situation in the bulk of farmers' fields in north Cameroon. The experiments in the present study were set up in fields left fallow, earlier, for more than a decade. Such long fallow periods are the exception rather than the rule in north Cameroon. Inoculation of farmers fields with AM fungi, as in this study, does not seem feasible for the foreseeable future even though some studies (e.g., Salami and Osonubi, 2002) claim this to be a feasible option for the resource-poor farmers in Africa. Selective

hand weeding, and consequently less soil disturbance was employed in this study, as opposed to the regular hoe weeding or animal traction undertaken by the bulk of farmers. Soil disturbance through tillage has been shown to reduce the viability of some AM fungi (McGonigle *et al.*, 1990; Kabir *et al.*, 1997; McGee *et al.*, 1997; Merryweather and Fitter, 1998). Tillage can also have a negative effect on mycorrhization through the disruption of established networks of extramatrical hyphae in the soil (Smith and Read, 1997). The importance of mycorrhizal networks during early crop growth (Miller, 2000) and enhancement of nodulation in legumes (Goss and De Varennes, 2002) has been demonstrated. Managing AM fungi in *Striga*-infested farmers' fields would be the ideal. The bulk of farmers practice continuous cropping of cereals and the chance that effective (against *Striga*) indigenous AM fungal species or strains are not present at all, or not in sufficient amounts in farmers' fields cannot be discarded. Continuous cropping of cereals may have altered the AM fungal composition in those fields. Even though host specificity or selectivity is not common with AM fungi, continuous cropping of the same hosts might favour the proliferation of AM fungal species or strains well adapted to the specific circumstances in the field. Agricultural practices show differential effects on development of AM fungi (Boddington and Dodd, 2000) and AM effects have been linked to the yield decline associated with continuous cropping of the same crop (Johnson *et al.*, 1992). Significantly higher numbers of *Striga* shoots have been observed to emerge on sorghum in the field with tillage compared to the no till plots (Hess and Ejeta, 1987; Van Delft, 1997). Intact mycorrhizal networks or their disruption through tillage, early in the season, might partly relate to such observed reduced *Striga* pressure and deserve further investigation. The fact that AM fungal inoculation reduced *Striga* pressure on cereals with no visible effects, raises the question whether managing AM fungi for *Striga* would be an option worth pursuing. It is possible that the reduced *Striga* pressure as result of AM fungal inoculation was still beyond the threshold for either cereal crop. A plot of host crop yield against the number of attached or emerged *Striga* (A. Van Ast, unpublished) or biomass of *Striga* shoots (Gurney *et al.*, 1999) yields a decay curve. Management of AM fungi for *Striga* control as with any other soil (fertility) management strategy should be long term. Combining AM fungi management with other compatible *Striga* control options will certainly result in reduced *Striga* pressure as well as increased yields in long term.

Chapter 6

General discussion

The tripartite interaction between sorghum, *Striga hermonthica* and arbuscular mycorrhizal fungi

Very few studies on the *Striga*-host parasitic association have focused on the possible role of soil biotic interactions within the *Striga*-host pathosystem. This is surprising as observational and experimental data have already provided good evidence for an important role of soil biotic interactions. This evidence includes:

- The observation that a significant proportion of *Striga* seeds dies off each year in the field (Gbèhounou *et al.*, 1996) even though some seeds may stay viable in the soil for as long as 14 years (Bebawi *et al.*, 1984).
- A negative correlation between incidence and severity of this phytoparasite or its effects, with soil fertility (Samaké, 2003).
- Observations that in pasteurised soils more *S. hermonthica* attach on host compared to the unpasteurised soils (Berner *et al.*, 1996).

Within the soil biological framework, attention has been given to pathogenic effects of bacteria (e.g., Miché *et al.,* 2000) and fungi (e.g., Ahmed *et al.*, 2001) on *Striga* seed germination, but the role of mutualistic organisms in *Striga* management and control has been very largely neglected. This thesis was set up to study the specific impact of arbuscular mycorrhizal fungi on the *Striga*-host interaction. These fungi are known to play various key ecological roles in nature and might as well be affecting parasitism of cereals or other hosts by *Striga*. Both pot experiments under controlled conditions and field experiments were carried out. From the observations reported in the foregoing chapters, it can be concluded that:

- AM fungi can affect *Striga* during various stages of its life cycle, viz. germination, haustoria formation and attachment; emergence, growth and reproduction (Fig. 6.1).
- AM fungi can enhance the fitness of the cereal host and thereby allow the host to better withstand *Striga* damage.
- The extent of the influence by AM fungi depends on the AM fungal inoculum density.
- The influence of AM fungi depends on the cereal host cultivar.
- The effects of AM fungi on *Striga* can be observed under field conditions.
- The effects of the AM fungi cannot be mimicked by phosphorus application.
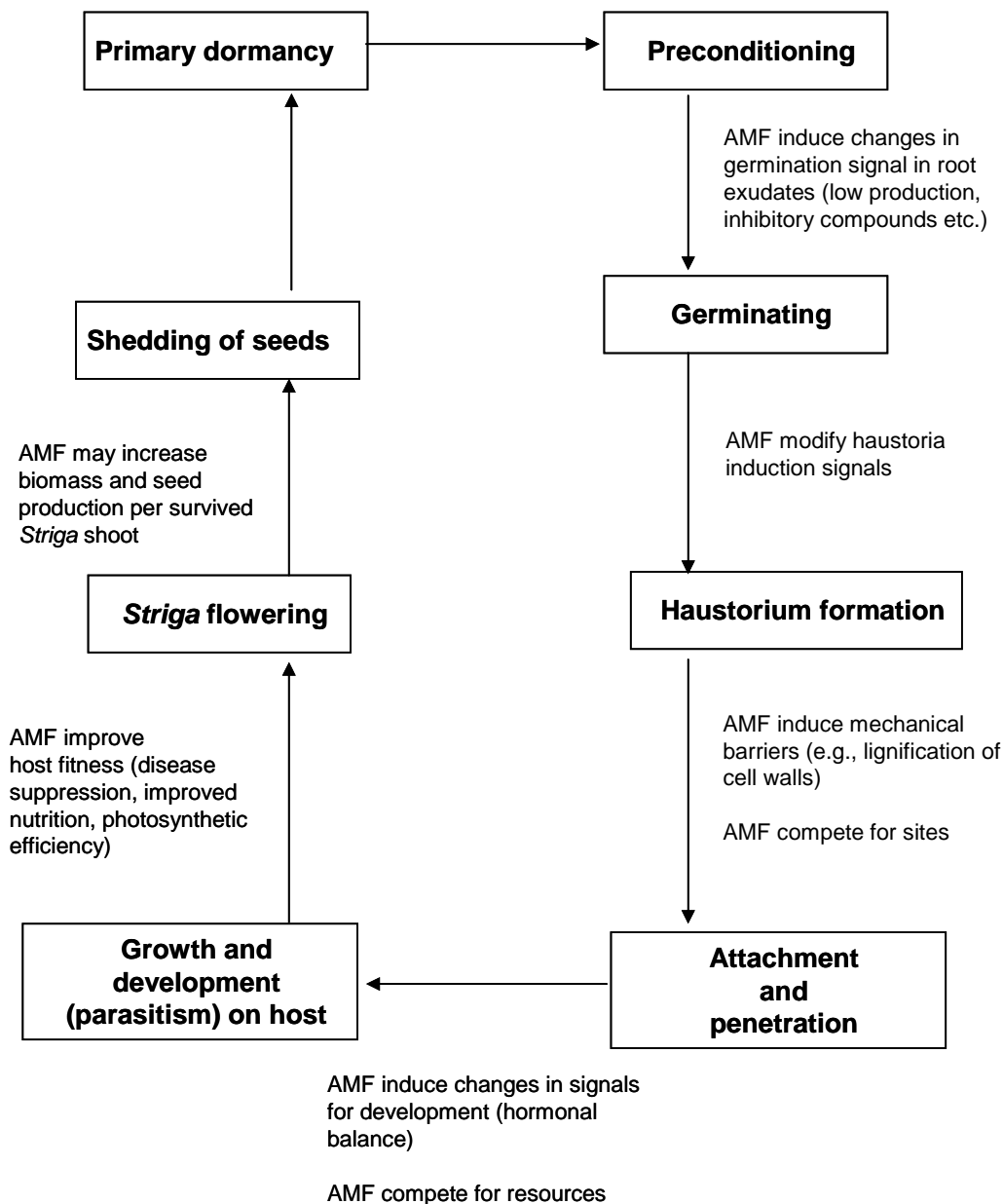
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┌─────────────────────┐                    ┌─────────────────────┐
│  Primary dormancy   │ ─────────────────▶ │   Preconditioning   │
└─────────────────────┘                    └─────────────────────┘
            ▲                                          │
            │                               AMF induce changes in
            │                               germination signal in root
            │                               exudates (low production,
            │                               inhibitory compounds etc.)
            │                                          │
            │                                          ▼
┌─────────────────────┐                    ┌─────────────────────┐
│  Shedding of seeds  │                    │     Germinating     │
└─────────────────────┘                    └─────────────────────┘
            ▲                                          │
  AMF may increase                          AMF modify haustoria
  biomass and seed                          induction signals
  production per survived                               │
  Striga shoot                                          ▼
┌─────────────────────┐                    ┌─────────────────────┐
│   Striga flowering  │                    │ Haustorium formation│
└─────────────────────┘                    └─────────────────────┘
            ▲                                          │
  AMF improve                               AMF induce mechanical
  host fitness (disease                     barriers (e.g., lignification of
  suppression, improved                     cell walls)
  nutrition, photosynthetic
  efficiency)                               AMF compete for sites
            │                                          │
            │                                          ▼
┌─────────────────────┐          ┌─────────────────────┐
│   Growth and        │ ◀─────── │    Attachment       │
│   development       │          │    and              │
│ (parasitism) on host│          │    penetration      │
└─────────────────────┘          └─────────────────────┘
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AMF induce changes in signals
for development (hormonal
balance)

AMF compete for resources

Fig. 6.1. Schematic life cycle of *Striga hermonthica* showing stages at which arbuscular mycorrizal fungi (AMF) might influence performance of the parasite.

AM influence on the life cycle of *S. hermonthica*: evidence and implications

Germination

The root exudate studies indicated that with AM fungal colonization of sorghum roots, the *Striga* germination signals in sorghum root exudate might be affected leading to interference with the stimulatory function of the root exudates on germination of preconditioned *Striga* seeds (Chapter 3 and Chapter 4). A wide range of (un)related

compounds in sorghum root exudates, that stimulate *Striga* seed germination has been observed. These compounds range from less to more stable, hydrophilic to hydrophobic, with one or the other compound reported, based on correlation with host sensitivity to *Striga*, to be the major germination stimulant in the root exudates of sorghum (Fate *et al.*, 1990; Olivier and Leroux, 1992). However, interactions between compounds (identified or unidentified) that stimulate germination of *Striga* seeds or not cannot be ruled out and it might be treacherous to claim with a degree of certainty that one compound or the other is the major or minor germination stimulant in sorghum or host root exudate. The interesting thing is that AM fungi affect the root exudation patterns of sorghum with a negative impact on *Striga hermonthica* seed germination *in vitro* even though we may not have a clue as to the specificity of the (inter)actions involved. It would be interesting to study how widespread AM induced effects on root exudation in relation to *Striga*, occur among AM fungal species and hosts of *Striga* spp. Germination assays with root exudates of the various cultivars and *Striga* seeds from various provenances and ages should be included as these factors are reported to influence *Striga* seed germination (Gbèhounou and Adango, 2003). Such studies could be related to the sensitivity of these cultivars to *Striga*. AM effects on root exudation patterns of potential trap crops of *Striga*, consequences for *Striga* seed germination *in vitro* and in the field should be studied. This could help to explain, in part, some of the observed enigmatic discrepancies of high stimulation of *Striga* seeds by some trap crops *in vitro* and their poor performance as trap crops against *Striga* in the field (Gbèhounou and Adango, 2003). With germination assays, however, the potential for seed effects on *Striga* seed germination in 'closed systems' such as sealed Petri dishes should be borne in mind and such effects have to be tested and separated where possible.

Haustoria formation and attachment of Striga *seedlings*

Haustoria of *Striga* are induced by chemical signals in root exudates of the host. It is possible that haustoria formation was impaired with AM formation given the significant reduction in number of *Striga* seedlings attaching to sorghum roots upon mycorrhizal colonization (Chapters 3 and 4). It is also possible that with successful germination those seeds were all able to develop haustoria, attach and form a functional connection with the host system. Considering a delay in emergence of *Striga* shoots as observed in the lab (Chapter 2) and in the field (Chapter 5), the first hypothesis seems more likely. Root observation chamber (rhizotron) experiments (Frost *et al.*, 1997) to study the fate of *Striga* seeds that germinate upon exposure to root exudates of AM colonized hosts would come in handy. Rhizotrons would allow observation of host roots inoculated with spores of AM fungi and *Striga* seeds. Events

from germination to seedling growth and development can be monitored in a non-destructive manner. Specific host cultivar interactions can easily be revealed with root chamber experiments: whether reported *Striga*-sensitive, tolerant, resistant hosts upon AM fungal colonization, influence similar or different stages in *Striga* life cycle and to varying degrees. Some of our results suggest that AM fungi × sorghum cultivar interaction effects on *Striga* do exist (Chapter 2 and Chapter 4). Rhizotrons could also aid in time-course, split-root studies in which the time or site of *Striga* infection and AM fungal colonization are varied. Such studies could reveal AM induced systemic effects on *Striga* as has been observed for some pathosystems involving pathogenic fungi in the presence of AM fungi (Pozo *et al.*, 2002). If cultivar × AM interaction effects on *Striga* are confirmed, field validation of results on sensitivity/susceptibility, tolerance, resistance to *Striga*, could be more complicated. The outcome of such studies would be affected by the species composition of the AMF community (presence or absence of effective (against *Striga*) AM fungal species) at the various experimental sites. AM fungal species-specific effects on *Striga* have been reported (Gworgwor and Weber, 2003), but it is not clear whether extent of colonization (or colonization rate) is the sole predictor of the negative impact of AM fungi on *Striga*.

Striga *growth and development*

Not only did the number of emerged *Striga* plants (Chapters 2-5) and emergence rate (Chapter 5) decrease, there was also a delay in time of emergence (Chapter 2 and Chapter 4), with AM fungal inoculation. Studying events of *Striga* from seed germination to early growth and development in the presence or absence of AM fungi would answer questions as to whether delayed emergence of *Striga* could be a consequence or not of delayed haustorial formation and/or attachment, or delayed subsequent development of *Striga* after successful establishment on roots of host colonized by AM fungi. It would be interesting to study the way *Striga* performance, in terms of numbers of *Striga* flowers, capsules, seed viability, is affected by AM inoculation of the host. With AM colonization of some hosts of *Rhinanthus* spp., another hemi-parasite of the Scrophulariaceae, performance of the parasite in terms of biomass and seed production is enhanced (Davies and Graves, 1998; Salonen *et al.*, 2001). Perhaps with improved host fitness due to AM compensation for host damage by *Striga* (Chapter 2), and the few (resulting in less competition for space and/or resources) emerged *Striga* plants upon AM fungal colonization of the host, more seeds are produced compared to *Striga* seed production in the absence of AM fungal inoculation. With increasing AM fungal inoculum density fewer *Striga* shoots emerged but with higher dry weights (Chapter 3 – average dry weight of *Striga* plant (mg) in treatments with 0, 20, 50 and 100 g inoculum: 13, 8, 9, 32, respectively) and

very likely higher seed numbers per plant. Dry weight of mature *Striga* plants was found to be a good parameter for estimating *Striga* seed production on sorghum (Rodenburg *et al*., 2002). With AM fungi inoculation or management for *Striga* control, the surviving *Striga* plants could be better off, implying the need to get rid of those last survivors through some compatible control measure.

AM fungi and host sorghum

The beneficial effects of AM fungal colonization on sorghum are huge (Chapter 1). With particular relevance to *Striga* could be AM effects on hormonal balance of sorghum. *Striga*-infected sorghum plants develop high root:shoot ratios (Parker and Riches, 1993) and exceptionally high levels of ABA (Drennan and El Hiweris, 1979; Frost *et al*., 1997). The stimulatory effects of *Striga* on sorghum root growth appeared to be reduced with mycorrhizal colonization in some of our studies. Data in Chapter 3 indicate that root:shoot ratios of *Striga*-infected non-mycorrhizal plants were higher (1.05) than those of *Striga*-infected mycorrhizal plants (0.79-0.93). In a subsequent experiment (L. Narvaez and V. Lendzemo, unpublished) root:shoot ratios of *Striga*-infected non-mycorrhizal sorghum S-35, 45 days after sowing, was much higher than of the infected, mycorrhizal plants (1.59 versus 0.82). Such observations might suggest AM effects on hormonal balance. Reported (Barker and Tagu, 2000) higher levels of cytokinins in host upon AM colonization could balance the excessive ABA levels upon *Striga* infection of sorghum. ABA and cytokinins are known to act in opposite directions and may even antagonize each other in some situations (Thimann, 1992). Sorghum hormonal balance as a mechanism of AM fungal action in *Striga*-infested sorghum deserves further study.

Implications for *Striga* management

Host plant influences

It would certainly be a pretense, given the results of these studies, to view arbuscular mycorrhiza as providing the ultimate solution to the *Striga* problem. However, one thing is clear from the present studies: this mutualistic symbiosis has a significant role to play as far as *Striga hermonthica* management is concerned. More insight is still needed, though, to increase our understanding of the tripartite interaction of AM fungi, *Striga* and their host before any (broad) generalisations and recommendations can be made. Host (maize, sorghum, millet), cultivar (sensitive/susceptible, tolerant, resistant), *Striga* spp., as well as AM fungal species-specific interactions in pot experiments and experiments in farmers' fields need to be understood. AM fungal species × *Striga* interactions have been observed (Gworgwor and Weber, 2003), with a

tendency for effects of AM on *Striga* and sorghum to depend on the level of colonization of sorghum roots by the fungus. Evidence for trade-offs have been noted for resistance breeding against fungal pathogens in maize, with disease-susceptible inbreds having significantly higher levels of mycorrhizal colonization (Toth *et al.*, 1990). Specific trade-offs between breeding for resistance to *Striga* and AM fungal colonization of the roots could be revealed. Table 6.1 collates information on the response of sorghum cultivars to *Striga* and AM fungi. From that table it follows that the more *Striga*-sensitive CK60B is also more responsive to mycorrhiza than S-35, while the resistant SRN39 is similar to S-35. It would be worthwhile to test a broader array of sorghum genotypes that vary from susceptible to resistant and from sensitive to tolerant to *Striga*, for mycorrhizal responsiveness and rate of colonization.

Challenges in field experimentation

Results from the field experiments may have under- or over- estimated the mycorrhizal effects as they were conducted in fields that have been left fallow for at least ten years. Such long fallow periods are rarely, if at all, practised in most of the *Striga*-affected regions of Africa due to increased and still increasing pressure on land, as a consequence of the ever increasing human population in that region. Long fallows or

Table 6.1. Potential trade-off between *Striga* sensitivity[*] (in the absence of AM fungi) and AM fungal responsiveness[*] (in the absence of *Striga*) of different sorghum cultivars

Cultivar	Sensitivity to *Striga*	Mycorrhizal responsiveness
Set 1[a]		
S-35	−0.13	+0.13
CK60B	−0.40	+0.23
Set 2[b]		
SRN39	+0.05	+0.20
S-35	−0.00	+0.18
CK60B	−0.48	+0.25

[*] In order to have the numbers for *Striga* sensitivity and mycorrhizal responsiveness symmetrical between −1 and +1, the following formula was used: (Biomass treated plant − Biomass control plant) / (Biomass treated plant + Biomass control plant).

[a] See Chapter 2. Measurements taken 80 days after sorghum emergence (DAE).

[b] V.W. Lendzemo, unpublished. Response to *Striga* assessed after 70 DAE, mycorrhizal responsiveness after 50 DAE.

improved short fallows have a suppressive effect on *Striga* seeds in soil (Samaké, 2003) as many *Striga* seeds die off each year (Gbèhounou *et al.*, 1996). Studies on how monocropping, crop rotation, intercropping, the dominant cropping systems in savannah Africa, affect species composition and abundance of AM fungal propagules in farmers fields are needed in an attempt to understand the expansive *Striga* problem as related to biotic interactions in the soil. Soil organic matter and *Striga* dynamics (Ayongwa *et al.*, unpublished) could then be partly explained by the role of beneficial soil micro-organisms including AM fungi. Soil organic matter has been shown to correlate negatively with *Striga* infestation (Samaké, 2003) and positively with AM fungi (Gaur and Adholeya, 2002; Carvalho *et al.*, 2003). Before experimenting in farmers field, however, a balance has to be found between the potential short term risks and long-term benefits. Such experiments often require control treatments: the experimental plots need to be uniform. This might entail artificial infestation with *Striga* seeds (which may be contrary to the ethics of experimentation unless full compensation is given to the farmers for the follow-up period). In the case of *Striga*-free (control) plots, fumigation might be required, with possible negative impact on non-target biota. With some of those knowledge gaps on the tripartite interactions filled, several possibilities exist for managing AM for *Striga* control even among the cash-strapped subsistence farmers. The objective of those small holders is still not the maximization of production of any single commodity as in the modern agricultural systems of Europe and North America. They aim to attain stable reasonable yields of products to satisfy household subsistence requirements. Only surpluses, when available at all, are for sale to provide ready cash for school fees, clothing, medical expenses, etc. This leaves the farmers with little or no capital for effective but prohibitively expensive methods of pest and disease control that may have a negative influence on beneficial soil micro-organisms including AM fungi. There is, however, a developing tendency in Africa for farmers to adopt high-yielding varieties (HYV) of maize selected, screened and vulgarized by national agricultural research institutions (Talleyrand, 1992). However, given their socio-economic situation, these farmers often are unable to obtain high amounts of fertilizers to reach the potential and promised high yield levels. Rising fertilizer prices (as a consequence of structural adjustment programmes imposed by the IMF), absence of markets and failure to have control over maize prices may further put pressure on maize production systems. Adoption of sustainable management practices is still, therefore, a possibility.

Management of AM through trap cropping
Continuous cropping of cereal hosts by subsistence farmers with little or no specific measures to target this parasite is acknowledged to be one of the major causes of the

perpetuating *Striga* problem. No single control method has so far been found that can adequately address the *Striga* problem. Given the economics involved and the biology and ecology of *Striga* only an integrated management strategy aimed at long term seems the most likely way out of this vicious circle. One of the recognized components of such an integrated management system for *S. hermonthica* is the use of trap crops (crop plants that stimulate the germination of *S. hermonthica* seeds but are themselves not infected by the germinating *S. hermonthica* seeds) in rotation with cereals (Carsky *et al*., 2000, Sauerborn *et al*., 2000, Gbèhounou and Adango, 2003; Schulz *et al*., 2003). Additional benefits would be reaped from using (promiscuously) nodulating and AM responsive leguminous trap crops where and when possible. The legume crop apart from inducing suicidal germination of *S. hermonthica* seeds would, with the help of nitrogen-fixing bacteria, enrich the soil with nitrogen that may become available to subsequent crops. The leaves and haulms of the legume if ploughed into the soil would also be an additional source of nutrients for the subsequent cereal crop in rotation. Soils in the tropics are generally low in P. An adequate supply of P is necessary for nodulation in legumes and nitrogen fixation by bacteria. If effective indigenous AM fungi readily colonize the legume trap crop, the extramatrical hyphae would assist the legume in P uptake. Propagules of these AM fungi would accrue in soil. The succeeding cereal crop in rotation, hopefully, will be colonized early by accrued AM fungi propagules, and eventually benefit from the effects attributable to AM fungal colonization including a negative impact on *S. hermonthica* performance. The use of non-leguminous trap crops, e.g., cotton in rotation with cereals could still be beneficial for *S. hermonthica* control. Such trap crops apart from stimulating suicidal germination of *S. hermonthica* seeds, if mycotrophic would enhance proliferation of AM fungal propagules. Exploiting mycotrophic trap crops in improved fallows for a rather more rapid depletion of the *Striga* seedbank is another possibility. However, there could be a trade-off between AM fungal colonization of trap crops and their role of stimulating suicidal germination of *Striga* seeds if mycotrophic trap crops are found to stimulate less germination of *Striga* seeds as observed for sorghum in our studies. With evidence that a significant proportion of *Striga* seeds dies off each year and that *Striga* infestation correlates negatively with soil fertility status, cropping systems employing mycotrophic legume trap crops in rotation with cereal hosts would still be effective in suppressing *Striga* indirectly (improved host fitness through available nutrients or enhanced uptake capability with accrued AM fungal propagules).

Soil tillage for AM management
Soil disturbance through tillage has been shown to reduce the viability of some AM fungi (McGonigle *et al*., 1990; Kabir *et al*., 1997; Merryweather and Fitter, 1998).

Tillage can also have a negative effect on mycorrhization through the disruption of established networks of extramatrical hyphae in the soil (Smith and Read, 1997). The importance of mycorrhizal networks during early crop growth (Miller, 2000) and enhancement of nodulation in legumes (Goss and De Varennes, 2002) has been demonstrated. Significantly higher numbers of *Striga* shoots have been observed to emerge on sorghum in the field with tillage compared to no till plots (Hess and Ejeta, 1987; Van Delft, 1997) and could be linked to the disruption or not of mycorrhizal networks. Soil tillage practices could be a component of an integrated management strategy for *Striga* control.

AM fungi inoculum management

Inoculation of field crops with AM fungi is generally not feasible even for plantation agriculture, given inoculum production and handling costs involved. Some authors however claim adoption of AM to be the ultimate innovative 'agro-biotechnology' for the resource-poor farmers in Africa (Salami and Osonubi, 2002). The costs involved with AM fungal inoculation (excessively large quantities of inoculum required, risks of contamination with pathogens, dependency on supplier) by the resource-poor farmer would outweigh potential benefits of increase in crop production if he would adopt such a technology. However, there could be specific situations for considering field inoculation with AM fungi. In some *Striga*-endemic areas such as north Cameroon, farmers are often forced to abandon fields as a result of heavy infestation by the parasite. Under such specific circumstances, effective and efficient AM fungal species could be screened and selected for nursery inoculation (requiring small amounts) at the beginning of the rains in *Striga*-free plots, e.g., around dwellings, and later transplanted onto the heavily infested fields. Transplanting of cereals has been shown to be an effective way of controlling *Striga* in pot experiments (A. Van Ast, unpublished) as well as in the field (Oswald *et al*., 2001; Oswald and Ransom, 2002; Gbèhounou *et al*., 2004). Late infection of *Striga hermonthica* (Cechin and Press, 1993c; Gurney *et al*., 1999), as would be the case with transplanting, has less negative effects on the host. Seedlings are often observed to suffer from stress following transplanting (Oswald and Ransom, 2002). Inoculating seedlings with AM fungi before transplanting could have an additional effect of alleviating the stress of adaptation suffered by seedlings after transplanting. AM fungi for nursery inoculation could thus be screened for ability to lessen transplanting shock as well as mitigate the deleterious effects of *Striga*. A decision tree (Fig. 6.2) could aid in judging whether or not to manage or inoculate AM fungi for *Striga* control.
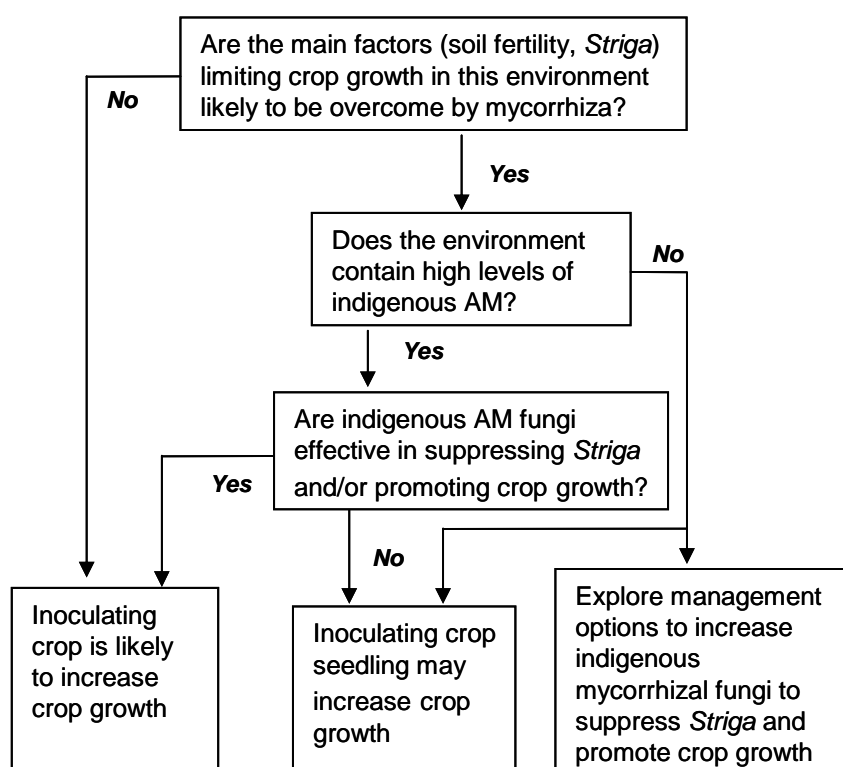
Fig. 6.2. Decision tree for use in determining under which conditions inoculation with mycorrhizal fungi or management of mycorrhizal associations is likely to be successful for *Striga* control (adapted from Dodd and Thompson, 1994; Kuyper *et al.*, 2004).

Concluding remarks

One key reality to the success of any management strategy for *Striga* control remains the level of awareness and understanding of the tricky biology and ecology of this phytoparasite by all the stakeholders, especially farmers, involved. For perceptible impact of most soil fertility management strategies, long-term, persistent application is vital. An understanding of the basic dynamics of this weed by the farmer is needed for him to continue to exercise the necessary patience and apply basic preventive, sometimes painstaking, measures. Farmers in most *Striga*-affected areas of Africa do acknowledge *Striga* is a serious constraint to cereal production but they have limited knowledge of the biology of *Striga* in relation to its effects on host crop. Getting them understand that there is, in theory, no threshold level for *Striga* as could be the case with 'ordinary' weed species, and encouraging them to take measures to minimize the number of *Striga* shoots flowering per unit area is still a challenge that has to be addressed for any management strategy for *Striga* to succeed.

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Summary

Cericulture, the cultivation of cereals, is the mainstay of farming in north Cameroon (latitudes 9° to 13° N and longitudes between 14° and 15° E), Africa where sorghum, millet and maize constitute the major sources of carbohydrate intake of the population in that part of the country. Between October and November, cereal fields in general and sorghum fields in particular, are covered by brightly pink flowers extending over wide areas depending on whether a cereal crop is/was present or not. For the uninitiated in agronomy, the beautiful scenery is captivating whereas the subsistence farmers in that part of the country ponder over why mother nature curses them with this scenery on a yearly basis. The attractive flowers are those of the parasitic angiosperm *Striga hermonthica*, a close relative of broomrape (*Orobanche*) and rattle (*Rhinanthus*). *Striga* is a cereal killer weed. Seeds of this phytoparasite germinate after receiving cues from the roots of host or some non-host in its immediate vicinity. The germinated seed then attaches and develops only on roots of a compatible host from which the *Striga* plants obtain nutrients and part of its carbon budget to the detriment of the host. Cereal yield loss is much larger than biomass gain of the attached and emerged *Striga* plants. *Striga* damage is also larger in soils with lower fertility and in soils that are more prone to drought. Low soil fertility in most *Striga*–affected areas is caused by agricultural intensification and the absence of periods of fallow which lead to losses in soil organic matter and a decline in the water holding capacity of the soil. Direct links exist between increasing population pressure (which leads to agricultural intensification), increased damage caused by *Striga* and less food security. The final outcome can be that whole villages need to be relocated while the population in the mean time is dependent on the World Food Programme of the United Nations. This series of events has also happened in north Cameroon.

Because the larger part of damage by *Striga* occurs before emergence while the hemi-parasite is still below-ground, it has turned out to be difficult for local farmers to causally link cereal yield losses with the appearance of an attractive flowering plant. Therefore, *Striga* has often been called witchweed.

A repertoire of methods to control *S. hermonthica* has been applied singly with no conclusive results or recommendations for the resource-poor farmer. Some of the methods that are effective in controlling this phytoparasite, for example, application of heavy doses of nitrogenous fertilizers or injection of ethylene gas into the soil to stimulate suicidal germination of *Striga* seeds, are prohibitively expensive for the smallholder subsistence farmer. It is becoming increasingly clear that the ecology as well as the nature of the biology of this weed warrant an integrated management

approach. Tackling the witchweed at the below-ground stages together with measures aimed at improving the soil fertility status offer an important step forward towards resolving the *Striga* problem. Under specific circumstances of low soil P, as is the general case in tropical soils, the role of arbuscular mycorrhizal (AM) fungi in the uptake and translocation of P, one of the less mobile elements in soil, has been shown to be preponderant. Almost all tropical agricultural crops are known to be dependent on and responsive to AM fungi, and sorghum is no exception. *Striga*, however, is not colonized by AM fungi. The subject of my thesis was the question whether AM fungi could indeed play a beneficial role in *Striga* management, either by benefiting the cereal or by negatively affecting *Striga*.

Chapter 2 describes the results of a pot experiment to find out whether AM fungi could have an effect on the *Striga*-sorghum interaction. Two sorghum cultivars, the *Striga*-sensitive CK60B and the *Striga*-tolerant S-35, were studied. A mixed inoculum of AM fungi, consisting of *Glomus clarum* and *Gigaspora margarita* was used. Cultivar-dependent interactions were revealed. AM fungal inoculation delayed time of emergence, reduced the number and biomass of emerged *Striga* on S-35, but not on CK60B. AM fungi could also compensate for the yield reduction of sorghum due to *Striga* in S-35, while there was no such compensation for CK60B. Interestingly, in the absence of *Striga* CK60B showed a larger responsiveness to AM fungi than S-35.

On the basis of these results with S-35 and given that this cultivar is widely adopted in the Far North Province of Cameroon because of its lower damage by *Striga*, a subsequent experiment was set up of which the results are described in Chapter 3. In this experiment inoculum density was varied in order to address the question whether inoculum density (and hence either rate or extent of mycorrhizal colonization) would additionally effect either the compensatory response of sorghum or the direct negative effect on *Striga*. This question is important, because in the mycorrhizal treatments in the experiment described in Chapter 2, some *Striga* plants still emerged. If such plants would flower and set seed, the life cycle of *Striga* would not be broken. The experiment with inoculum density indicated that at least two different mechanisms are involved in the way in which AM fungi affect the sorghum × *Striga* interaction. The compensatory response of sorghum by AM fungi was independent of inoculum density, however the number of attached and emerged *Striga* plants decreased with increasing inoculum levels. Apparently the direct negative role of AM fungi on *Striga* is density dependent. The possibility that this direct effect is due to an effect on seed germination was tested by comparing *Striga* seed germination under the influence of exudates of non-mycorrhizal and mycorrhizal sorghum plants. There was almost total suppression of germination of *Striga* seeds after exposure to root exudates from AM fungal colonized sorghum plants. While no AM density dependent effects were

observed on *Striga* seed germination, it remains possible that in these Petri dishes the concentration of the exudates was homogeneously distributed, whereas in the field it could make a difference which part of the root system is or isn't colonized by AM fungi. This possibility could explain mycorrhizal density effects in the pot experiment but not in the Petri dish.

In Chapter 4, this aspect of germination of *Striga* seeds being influenced by root exudates from AM colonized sorghum plants is explored in more detail. A new factor, phosphorus, was introduced as AM colonization improves phosphorus status of plants and that the plant's phosphorus status regulates the quality and quantity of root exudates. Both S-35 and CK60B were used. Given that in the experiments reported in Chapter 2 and Chapter 3 the AM fungal propagules and *Striga* seeds were added at the time of sowing of sorghum seeds, we also wanted to test for possible spatio-temporal effects of AM fungi within the interaction. *Striga* seeds need preconditioning of up to three weeks at sufficient moisture and temperature before they can respond to stimulus and germinate. In Chapter 4, the *Striga* seeds were preconditioned prior to mixing with soil in the pots at time of AM inoculation and sowing of sorghum seeds. Exposure of preconditioned *Striga* seeds to root exudates collected from AM inoculated S-35 plants resulted in little or no germination at the two harvest times, 24 and 45 DAS. Upon exposure to root exudates from AM inoculated CK60B plants, *Striga* seed germination was suppressed but to a significant degree only with root exudates from 45 days old plants. There was no influence of P addition on the outcomes irrespective of time of harvest or sorghum cultivar. A preliminary chemical analysis showed that mycorrhizal colonization changed exudate composition. However, it was not yet possible to come up with a reasoned hypothesis which substance(s) was (were) responsible for the observed effects. With AM fungal inoculation, the number of *Striga* attachments and emerged plants on S-35 were reduced significantly despite the fact that *Striga* seeds were somewhat favoured through prior preconditioning at the start of the set up of the experiment. Cultivar dependent effects were evident. AM fungi somewhat delayed time of emergence of *Striga* only on the S-35 cultivar in line with observations in Chapter 2. There was almost total suppression of attachment of *Striga* in the S-35 cultivar with AM fungal inoculation compared to a 50% reduction of *Striga* in CK60B. Again, P addition did not influence the outcomes of the interactions in either of the sorghum cultivars. Due to the experimental conditions (relatively low light levels and/or small pots) the AM fungi did decrease sorghum biomass. It can, therefore, be concluded that the negative impact of AM fungi on *Striga* is independent of a mycorrhizal effect on phosphorus uptake and biomass increment of sorghum.

In order to make the results of these laboratory experiments relevant for conditions in farmers' fields, they needed to be validated under field conditions. In Chapter 5, the

results of three field experiments with sorghum and maize are described. Because it is unethical to execute experiments with *Striga* addition in farmers' fields, and because treatments intended to reduce *Striga* density in heavily infected fields (such as fumigation with methylbromide or injection of ethylene) might likely affect AM fungi, we decided to set up experiments in fallow fields on a site belonging to IRAD. In those fields *Striga* seeds and/or mycorrhizal inoculum was added to planting holes. Due to large field variability (the coefficient of variation in the field was much higher than that of the laboratory experiments) only some results were significant. *Striga* addition negatively affected performance of sorghum and maize (yield reduction 7%-26%). AM fungal inoculation did not increase cereal growth, and neither was a compensatory effect by AM fungi on *Striga* damage observed. The experimental field had been fallowed for a long time. Consequently a seed bank of *Striga* was absent, but the density of the indigenous AM fungi was high, and inoculum addition did neither increase spore density nor mycorrhizal colonization levels. Lack of mycorrhizal response can, therefore, likely be explained by inoculum sufficiency. However, addition of mycorrhizal inoculum resulted in lower *Striga* emergence (reduction 30%-50%) and a lower *Striga* biomass (reduction 40%-63%). Mycorrhizal inoculation also delayed *Striga* emergence. Possibly, inoculum addition increases colonization rate (without an effect on final colonization levels) and enhances the direct negative effect on *Striga*. As mentioned, *Striga* reduction did not result in cereal yield increases.

The results are discussed in Chapter 6 by using a life cycle diagram of *Striga* in which the stages of *Striga*'s life cycle at which AM fungi could exert an influence are indicated. It is concluded that AM fungi may affect *Striga* germination through an effect on exudates. Lower attachment and emergence might be a consequence of reduced germination. However, the possibility that mycorrhizal colonization induces further changes in the root (deposits of phenolics or lignification of the root cell wall) cannot be discarded. The observation that not only the numbers of attached and emerged *Striga* plants were lower after mycorrhizal colonization, but that also emergence time was delayed, is consistent with the hypothesis that AM fungi may also be relevant at that stage of *Striga*'s life cycle. Finally, AM fungi improve the nutritional status of the host plant. This allows for better compensation of *Striga* damage. Mycorrhizal colonization may also affect the hormone balance of the host, counteracting the hormonal effects of *Striga* (through which cereals increase root biomass and very substantially decrease shoot biomass). However, larger cereal plants may be a better substrate for the smaller number of *Striga* plants that finally emerge. It remains, therefore, imperative to remove *Striga* plants in farmers' fields before witchweed can set seed. Various suggestions are given for further research towards the mechanisms by which AM fungi directly and indirectly affect *Striga*. Large

differences in exudate composition and in mycorrhizal responsiveness between different sorghum cultivars indicate genetic variation in host plants which can be used in plant breeding. It is important then to understand how breeding could at the same time affect mycorrhizal formation and responsiveness to poor soils and *Striga*.

Finally it is very important to understand the kind of management practices that farmers can apply to enhance mycorrhizal performance. Considering their socio-economic situation it would be illusory (and bad advice as well) to induce farmers to buy mycorrhizal inoculum. But farmers could easily manage their fields in ways that are favourable to AM fungi: by reducing soil disturbance through excessive tillage and weeding (which disrupts the mycelial network) and by preventing declines in soil organic matter. It is already known that such measures can contribute positively to *Striga* control, and research how these measures affect mycorrhizal performance is recommended.

Résumé

La culture des céréales constitue la base de l'agriculture au Nord-Cameroun (latitudes allant de 9º à 13º N et longitudes de 14º à 15º E). Le sorgho, le mil et le maïs sont les principales sources d'énergie pour la population locale. Chaque année entre Octobre et Novembre, les champs de céréales en général et particulièrement ceux de sorgho sont envahis par les fleurs roses sur de grandes étendues variant selon qu'une céréale y est/a été ou non. Pour les profanes, ce beau paysage est captivant alors que les pauvres paysans se demandent pourquoi la nature les maudit chaque année de cette scène. Ces fleurs attractives sont celles de l'angiosperme parasite *Striga hermonthica*, une herbe tueuse de céréales. Les graines de ce phytoparasite germent si elles sont en présence des substances chimiques spécifiques exsudées par les racines d'une plante-hôte ou non-hôte environnante. Les graines germées se fixent alors et ne se développent que sur les racines d'une plante-hôte compatible d'où elles tirent les nutriments et le carbone au détriment de cet hôte. La perte de rendement de la céréale est beaucoup plus élevée que le gain de biomasse du *Striga*. L'impact du *Striga* est aussi très élevé dans les sols peu fertiles et exposés à la sécheresse. La faible productivité des sols infestés par le *Striga* est causée par l'intensification de l'agriculture et l'absence de période de jachère qui entraînent la diminution de la matière organique et la réduction de la capacité de rétention de l'eau du sol. Il y a une relation directe entre la croissance galopante de la population (qui entraîne l'intensification de l'agriculture), les effets du *Striga* et l'insécurité alimentaire. Le résultat final étant la dépendance des populations aux programmes d'aide alimentaire ou alors la relocalisation des populations vers des zones moins infestées comme on l'observe au Cameroun.

Plusieurs stratégies de lutte ont été proposées, mais aucune stratégie unique n'a permis jusqu'à présent de contrôler complètement le parasite. Certaines méthodes de lutte telles que l'application de grandes doses d'engrais azotés ou l'injection d'éthylène dans le sol pour stimuler une germination suicidaire des graines de *S. hermonthica* sont efficaces pour le contrôle de ce phytoparasite mais ont un coût prohibitif pour les pauvres paysans. Il devient de plus en plus clair que l'écologie et le cycle biologique de *S. hermonthica* rendent son contrôle très complexe et qu'une approche intégrée doit être envisagée. S'attaquer à cette mauvaise herbe à son stade souterrain tout en ameliorant la fertilité du sol serait une étape importante vers la résolution du problème *Striga*. Dans certaines circonstances de sol à faible taux de phosphore, ce qui est généralement le cas pour les sols tropicaux, le rôle des champignons mycorhiziens à arbuscules (CMA) dans le prélèvement et la translocation du phosphore, l'un des éléments les moins mobiles dans le sol, s'est

avéré prépondérant. Ces champignons entre en association symbiotique avec les racines de la plupart des plantes tropicales, y compris le sorgho. Les racines du *S. hermonthica*, cependant, ne sont pas colonisées par les CMA. Notre étude s'est portée sur le rôle potentiel de cette symbiose dans la gestion du *S. hermonthica*.

Au Chapitre 2 nous avons mis en place un essai en pot pour voir si les CMA pouvait avoir un effet sur l'interaction de *Striga*-sorgho. Deux cultivars de sorgho, CK60B sensible au *S. hermonthica* et S-35 tolérant au *S. hermonthica* ont été étudiés. L'inoculum de CMA a été composé de propagules de deux isolats fongiques: *Glomus clarum* et *Gigaspora margarita*. Des interactions spécifiques à chaque cultivar ont été révélées. L'inoculation fongique n'a eu des effets significatifs sur la croissance de *S. hermonthica* rien que sur le cultivar S-35. C'est ainsi que le temps d'émergence a été retardé, le nombre et la biomasse des plants de *S. hermonthica* émergés ont été réduits. De même l'inoculation fongique a pu compenser la réduction du rendement chez le cultivar S-35 due au *S. hermonthica* malgré qu'il y ait eu une augmentation très significative du rendement plutôt de CK60B avec l'inoculation fongique en l'absence du parasite. Deux mécanismes d'intervention des CMA ressortent dans le Chapitre 2: un effet direct des CMA sur le *S. hermonthica* et un effet indirect par la compensation des dommages causés au sorgho par le parasite.

Suite aux résultats encourageants obtenus avec le cultivar S-35 au Chapitre 2 et étant donné que ce cultivar est largement adopté dans la province de l'Extrême-Nord du Cameroun, au Chapitre 3 nos études ont porté sur ce cultivar. Dans le Chapitre 2, avec inoculation certains plants de *S. hermonthica* ont survécu. Nous avons estimé qu'avec une augmentation de la quantité d'inoculum de CMA, le *S. hermonthica* aurait peu ou pas de possibilité de se fixer sur les racines et d'émerger. Nous avons varié la quantité d'inoculum au Chapitre 3. L'inoculation fongique a réduit d'une façon significative, le nombre de pousses de *S. hermonthica* fixées sur les racines et le nombre des plants de *S. hermonthica* émergés. L'effet a été fonction de la quantité d'inoculum de CMA, avec peu de pousses de *S. hermonthica* fixées ou émergeant suite à l'addition de la plus grande quantité d'inoculum. Nous avons aussi essayé au Chapitre 3 d'étudier le mécanisme à travers lequel les CMA influencent l'interaction *Striga*-sorgho. Plus spécifiquement, nous avons étudié l'influence de la colonisation des racines du sorgho par ces champignons sur les exsudats racinaires. Les exsudats ont été collectés de plants inoculés ou pas. Les effets de ces exsudats ont été testés sur les graines de *S. hermonthica* qui ont subit un conditionnement au préalable. On a observé une suppression quasi totale de germination des graines de *Striga* après exposition aux exsudats racinaires issus des plants colonisés par des CMA. Une fois de plus, aucun effet dépendant de densité des CMA n'a été observé.

Au Chapitre 4, nous avons mené une étude en profondeur sur cet aspect de

l'influence des exsudats racinaires sur la germination des graines de *S. hermonthica*. Un autre facteur, le phosphore, a été introduit vu son rôle régulateur dans l'exsudation racinaires, la colonisation et le fonctionnement de la symbiose mycorhizienne. Deux cultivars de sorgho (S-35 et CK60B) ont été utilisés. Etant donné qu'aux expériences rapportées aux Chapitres 2 et 3 les propagules des CMA et les graines de *Striga* ont été mélangées dans les pots au moment du semis du sorgho, nous avons estimé que les CMA seraient favorisés car les graines de *S. hermonthica* ont besoin d'une période d'imbibition de 10 à 21 jours avant de pouvoir germer. De ce fait, nous avons conditionné les graines de *S. hermonthica* avant mixage au sol dans les pots au moment de l'inoculation avec CMA et du semis des graines de sorgho. L'exposition de graines conditionnées de *Striga* aux exsudats racinaires des plants de S-35 inoculés aux CMA a résulté en peu ou aucune germination aux deux périodes de récoltes, 24 et 45 jours après semis (JAS) du sorgho. Pour le cultivar CK60B, cet effet de suppression de la germination des graines de *Striga* a été significatif rien que pour les exsudats racinaires des plants récoltés 45 JAS. Il n'y a eu aucune influence de l'addition de phosphore sur les résultats. L'inoculation fongique a, une fois de plus, eu un effet significatif sur le nombre de pousses de *S. hermonthica* fixées et les plants émergés sur le sorgho malgré que les graines de *Striga* aient été plus ou moins favorisées du fait de leur conditionnement au préalable. Les effets spécifiques à chaque cultivar étaient évidents. Les CMA n'ont différé le temps d'émergence du *Striga* que sur le cultivar S-35 en accord avec les observations du Chapitre 2. Il y avait presque suppression totale de fixation de *Striga* sur le cultivar S-35 avec l'inoculation fongique comparée à une réduction de 50% de *Striga* dans le cultivar CK60B. Une fois de plus, l'addition d'engrais phosphaté n'a eu aucune influence sur les résultats. La luminosité dans la serre et la taille de pot n'étaient pas optimales, ce qui a entraîné une baisse de la biomasse de sorgho suite à l'inoculation par les CMA. Il ressort aussi de cette étude que l'impact des mycorhizes sur le *Striga* ne dépend pas de l'effet des mycorhizes sur l'absorption du phosphore par le sorgho et de l'augmentation de sa biomasse.

Au Chapitre 5, nous avons validé les résultats obtenus sous conditions contrôlées, en milieu réel. Ces essais ont été effectués dans les champs d'expérimentation de l'Institut de Recherche Agricole pour le Développement (IRAD) Garoua. Ces champs on été mis en jachère pendant plus d'une décennie; ce qui nous a permis d'avoir des parcelles témoins car peu de graines subsistent dans le sol après tant d'années. Les poquets de sorgho ou de maïs ont été infestés avec les graines de *S. hermonthica* et/ou inoculé par un inoculum de CMA. A cause de la grande variabilité en champ, quelques résultats étaient significatifs. L'infection du sorgho et du maïs par le *S. hermonthica* a entrainé une baisse de rendement de l'ordre de 7 à 26%. L'addition du champignon n'a ni provoqué l'augmentation de la croissance de la céréale ni compensé les dégâts

causés par le *Striga* sur la plante. De même l'addition de l'inoculum n'a pas eu d'effet sur la densité des spores et la colonisation mycorhizienne. L'absence de l'effet des mycorhizes pourrait alors être expliquée par l'autosuffisance de l'inoculum local. Cependant, l'inoculation additionnel a provoqué la réduction de nombre des plants de *Striga* (30 à 50%) et de sa biomasse (40 à 63%). De même l'émergence du *Striga* a été retardée. Il est possible que l'addition de l'inoculum augmente le taux de colonisation (sans effet sur le niveau final de colonisation) et active l'impact négatif direct sur le *Striga*.

Dans le Chapitre 6, les résultats sont discutés en utilisant le diagramme du cycle biologique du *Striga* où nous avons indiqué les étapes où les CMA peuvent exercer leur influence. Nous concluons que les CMA peuvent affecter la germination du *Striga* par le biais de l'effet sur l'exsudation racinaire du sorgho. La faible fixation et le faible taux d'émergence pourraient être la conséquence de la réduction de la germination. Cependant, il ne faut pas exclure la possibilité que la colonisation mycorhizienne induise des changements au niveau des racines (accroissement du taux des phénols, lignification des parois cellulaires). Les observations faites sur la réduction du nombre des plants de *Striga* et de leur fixation après la colonisation mycorhizienne, de même que le retard causé sur son émergence sont en ligne avec l'hypothèse que les CMA peuvent influencer à ce stade du cycle biologique du *Striga*. En dehors des effets directs des CMA sur le parasite, les effets indirects à travers la plante-hôte sont possible. Le *S. hermonthica* induit chez l'hôte des changements hormonaux, notamment une baisse de taux de cytokinines. La colonisation des racines par les CMA entraîne un accroissement de taux de ces composés et pourraient rétablir l'équilibre hormonal dans la plante infecté par le *S. hermonthica*. Néanmoins, les quelques plants de *S. hermonthica* qui échappent au contrôle peuvent produire beaucoup plus de graines par plante et doivent être arrachés avant la floraison. Les différences observées au niveau de la réaction de la plante-hôte suite à l'inoculation, est une indication de la variation de contrôle génétique du phénomène au niveau de la plante-hôte qui peut être utilisée dans les programmes de sélection végétale.

En fin, il est très important de chercher à comprendre le système de gestion des sols que les paysans peuvent utiliser pour améliorer la performance des mycorhizes dans les champs infestés par le *Striga*. Envisager une inoculation à grande échelle avec des CMA, serait illusoire vu les coûts qu'engendrerait cette technique pour les paysans compte tenu de leur faible niveau socio-économique. Des pratiques moins onéreuses et d'application facile qui favorisent les CMA peuvent être utilisées par les paysans tel que la réduction de la perturbation du sol par le labour excessif et le désherbage (qui détruisent le réseau mycélien des mycorhizes) et en empêchant la diminution de la matière organique du sol.

Samenvatting

De teelt van granen is de belangrijkste vorm van landbouw in noord Kameroen (9°-13° noorderbreedte, 14°-15° oosterlengte), Afrika. De belangrijkste bron van koolhydraten voor de bevolking in dat deel van Afrika wordt gevormd door sorghum, parelgierst en maïs. Wanneer men aan het einde van het regenseizoen, in de maanden oktober en november, velden met graan bezoekt, en speciaal deze waarop sorghum wordt geteeld, valt op dat deze velden door bloemen helder roze gekleurd zijn waartussen al dan niet nog resten van het graangewas waarneembaar zijn. Voor bezoekers zonder landbouw-kundige achtergrond is deze kleurenpracht indrukwekkend. Maar voor de locale boer, wiens voedselzekerheid en inkomen afhankelijk is van de graanteelt, geldt dat de bloemen van deze planten een aanwijzing zijn dat moeder natuur hem vervloekt en van zijn jaarlijkse oogst berooft. De aantrekkelijke bloemen worden gevormd door de half-parasiet *Striga hermonthica*, een nauwe verwant van onder andere bremraap (*Orobanche*) en ratelaar (*Rhinanthus*). *Striga* is verantwoordelijk voor opbrengst-reducties en soms zelfs voor het afsterven van het graan. De zaden van *Striga* kiemen uitsluitend nadat ze de juiste signaalstoffen van de gastheerplant (of van enkele andere plantensoorten, die echter niet als gastheer kunnen dienen) in hun onmiddellijke omgeving hebben waargenomen. Het gekiemde zaadje van *Striga* hecht zich vervolgens aan de wortels van de groeiende plant wanneer die een geschikte gastheer is. De gastheer verzorgt de (half-)parasitaire plant met plantenvoedende stoffen en ook met koolstof, ondanks het feit dat *Striga* wel bladeren heeft. Het totale verlies aan productie van het graan is vele malen groter dan het gewicht van de aangehechte *Striga*-planten. Daarnaast is de schade, die deze parasitaire plant aanricht, groter in bodems die minder vruchtbaar en meer droogtegevoelig zijn. Dit betekent dat intensivering van de landbouw en het achterwege blijven van periodes van braak, leiden tot verlies aan organische stof en dus verlies aan plantenvoedende stoffen en aan vochtvasthoudend vermogen van de bodem. Daardoor leidt de toenemende bevolkingsdruk tot een alsmaar groter wordende schade door *Striga*, met als uiteindelijk gevolg dat soms hele dorpen moeten worden verplaatst en dat de bevolking in de tussentijd afhankelijk wordt van het wereldvoedselprogramma van de Verenigde Naties. Dit laatste heeft zich ook in noord Kameroen voorgedaan.

Doordat het grootste deel van de schade door *Striga* wordt aangericht in de periode dat deze nog ondergronds is, is de koppeling tussen opbrengstverlies van graan en het verschijnen van een plant met fraaie bloemen, niet onmiddellijk te leggen op basis van veldwaarnemingen. Mede daarom wordt *Striga* vaak witchweed (heksenkruid) genoemd. Een groot aantal verschillende methodes om *Striga* te beheersen is

voorgesteld en in proeven uitgevoerd. Echter, deze methoden waren lang niet altijd geschikt om aanbevolen te kunnen worden aan arme boeren. Veel methodes voor beheersing van *Striga* leverden dan ook in zulke omstandigheden weinig of geen positief resultaat op. Met name geldt dat de meest effectieve methodes om *Striga* te beheersen, zoals de toepassing van grotere doses stikstofhoudende kunstmest of het injecteren van het gas ethyleen in de bodem, waardoor de zaden van *Striga* wel kiemen maar vervolgens geen gastheer vinden om zich aan te hechten, veel te duur zijn voor boeren die niet voor de markt maar hoofdzakelijk voor hun eigen levensonderhoud produceren. Dit falen van op zichzelf succesvolle beheersingsmaatregelen heeft duidelijk gemaakt dat alleen beheersvormen, die door plaatselijke boeren geïntegreerd kunnen worden in hun teeltsystemen, kans van slagen hebben. Zulke geïntegreerde systemen maken dan gebruik van verschillende beheersingsmaatregelen die tegelijker-tijd worden uitgevoerd.

Zulke geïntegreerde maatregelen moeten zich dus richten op herstel van de bodemvruchtbaarheid, het vermogen van het graan om voldoende voedingsstoffen op te nemen, en het verhinderen dat *Striga* kiemt, zich aan het gewas hecht en daar schade aanricht. Zulke geïntegreerde maatregelen kunnen mede gebruik maken van het bodemleven, en met name van organismen welke een positief effect op gewasgroei hebben. In het bijzonder kan daarbij gedacht worden aan arbusculaire-mycorrhiza-schimmels (AM schimmels), wier rol bij nutriëntenopname, en vooral van dat van goed aan bodemdeeltjes gebonden elementen zoals fosfaat, essentieel is. Vrijwel alle tropische landbouwgewassen blijken sterk afhankelijk te zijn van, en een sterke groei-respons te vertonen op de aanwezigheid van deze AM schimmels. Het wortelstelsel van *Striga* wordt echter niet door deze schimmels gekoloniseerd. Het onderwerp van mijn proefschrift was de vraag of AM schimmels inderdaad een gunstige rol zouden kunnen spelen in de beheersing van *Striga*, hetzij door een positief effect op het graangewas, hetzij door een negatief effect op *Striga*.

In Hoofdstuk 2 worden de resultaten van een potproef beschreven waarin werd nagegaan of AM schimmels inderdaad een effect hebben op de interactie tussen sorghum en *Striga*. In deze proef werden twee rassen van sorghum gebruikt: CK60B, die bekend staat als zeer vatbaar voor *Striga*, en S-35, waarvan bekend is dat deze enige mate van weerstand heeft of tolerant is tegen *Striga*. Voor de AM schimmels werd een menginoculum, bestaande uit *Glomus clarum* en *Gigaspora margarita* gebruikt. De resultaten van deze proef waren verschillend voor beide sorghumrassen. Het inoculum van de AM schimmels had een negatief effect op *Striga* (lagere aantallen planten die opkwamen, lager gewicht van de opgekomen *Striga*-planten, uitstel van opkomstdatum) bij S-35, maar niet bij CK60B. De AM schimmels konden ook het negatieve effect van *Striga* voor een groot deel compenseren bij S-35, terwijl

er geen sprake was van schadecompensatie bij CK60B. Desondanks bleek CK60B in afwezigheid van *Striga* sterker positief te reageren op inoculatie met AM schimmels.

Op grond van de resultaten uit dit eerste experiment, en rekening houdend met het feit dat S-35 in noord Kameroen zeer regelmatig wordt gebruikt als sorghumras dat relatief minder te lijden heeft onder *Striga*, werd een vervolgexperiment uitgevoerd met alleen S-35, waarvan de resultaten worden beschreven in Hoofdstuk 3. In dit experiment werd de dichtheid van mycorrhiza-inoculum gevarieerd, zodat de vraag beantwoord kon worden of de hoeveelheid schimmelmateriaal een belangrijke extra factor is in het compenseren van de schade aan sorghum of in de rechtstreekse schade aan *Striga*. Deze vraag is van belang, doordat in de experimenten zoals beschreven in Hoofdstuk 2, nog steeds enkele *Striga*-planten in de mycorrhiza-behandeling opkwamen. Het gevaar bestaat dat deze planten alsnog bloeien en zaad vormen, zodat de levenscyclus van *Striga* in het teeltsysteem niet wordt doorbroken. Het experiment met de variatie in inoculumdichtheid leverde aanwijzingen op dat tenminste twee verschillende mechanismen betrokken zijn bij de manier waarop AM schimmels de interactie tussen *Striga* en sorghum beïnvloeden. Compensatie van het negatieve effect van *Striga* door de AM schimmels was onafhankelijk van de hoeveelheid schimmel-inoculum. Daarentegen nam het aantal aangehechte en opgekomen *Striga*-planten verder af met toenemende dosis van het schimmelinoculum. Ook het totale gewicht van *Striga*-planten nam af met toenemde hoeveelheid schimmelinoculum. Blijkbaar is er een rechtstreeks effect van de hoeveelheid mycorrhiza op *Striga*. De mogelijkheid dat dit effect veroorzaakt werd door een effect van de mycorrhizaschimmels op de kieming van *Striga* werd in een eerste experiment onderzocht. Wortelexudaten, stoffen die door wortels worden uitgescheiden in hun directe omgeving en waarin zich ook de stoffen bevinden die als kiemingsbevorderaar voor *Striga* bekend zijn, van planten met en zonder mycorrhiza werden vergeleken. Het bleek dat wortelexudaten van planten zonder mycorrhiza tot goede kieming van *Striga* zaden leidden, terwijl vrijwel geen zaden van *Striga* kiemden als ze werden blootgesteld aan exudaten van planten met arbusculaire mycorrhiza. Er was geen effect van de hoeveelheid mycorrhiza-inoculum, maar het is zeer wel mogelijk dat in een Petrischaal, waarin deze experimenten werden uitgevoerd, de concentratie van exudaten homogeen verdeeld is, terwijl het in het veld juist wel uitmaakt welk deel van de wortel al dan niet door AM schimmels wordt gekoloniseerd. Die mogelijkheid zou kunnen verklaren waardoor de dichtheid van mycorrhiza-inoculum wel een effect had op *Striga* in de potproef.

De resultaten van dit kleine proefje, waarbij bleek dat exudaten van sorghumplanten met mycorrhiza de kieming van *Striga*-zaden konden tegengaan, vormden de basis voor een uitgebreider experiment dat in Hoofdstuk 4 wordt beschreven. Zowel het vatbare ras CK60B als het tolerante ras S-35 werden gebruikt. Doordat de vorming van

mycorrhiza gewoonlijk leidt tot verhoogde opname van nutriënten, met name fosfaat, en doordat de fosfaattoestand van de plant invloed heeft op de samenstelling van de wortelexudaten, werd ook het effect van fosfaatbemesting op exudaten onderzocht. Daarnaast werd onderzocht in hoeverre het effect op zaadkieming van *Striga* afhankelijk was van de periode waarin planten met mycorrhiza hadden gegroeid. Exudaten werden verzameld van planten die 24 en 45 dagen oud waren, terwijl het eerste experiment, zoals beschreven in Hoofdstuk 3, gebruik maakte van exudaten van 40 dagen oude planten. Voor S-35 bleek dat exudaten van planten met mycorrhiza een zeer sterk remmend effect hadden op de kieming van *Striga* zaden. Het effect was even sterk bij 24 en 45 dagen oude sorghum planten. Bij CK60B bleek er wel een remmend effect van exudaten van mycorrhizaplanten van 45 dagen oud te zijn, maar niet van 24 dagen oude planten. Ook bij de planten van 45 dagen oud was het effect echter aanmerkelijk zwakker dan bij de exudaten van S-35. In alle gevallen had fosfaatbemesting geen effect. Een eerste chemische analyse van de samenstelling van de exudaten liet zien dat de totale samenstelling van de exudaten veranderde na de vorming van mycorrhiza. Het bleek echter nog niet mogelijk om een beredeneerde hypothese te formuleren welke stof(fen) voor het remmende effect verantwoordelijk was (waren). In een tweede experiment werden planten met en zonder mycorrhiza blootgesteld aan reeds geconditioneerde zaden van *Striga*. Normaliter is het nodig dat zaden van *Striga* zo'n 3 weken bij voldoende vochtigheid geconditioneerd worden, alvorens ze kunnen reageren op specifieke stoffen uit de exudaten van de gastheerplant. Door reeds geconditioneerde zaden te gebruiken werd als het ware het tijdsvoordeel van mycorrhizaschimmels met zo'n 3 weken ingekort. Desondanks werd hetzelfde negatieve effect van AM schimmels op aantallen van aangehechte en opgekomen *Striga*-planten waargenomen bij S-35 als bij de experimenten zoals beschreven in Hoofdstukken 2 en 3. Voor CK60B had mycorrhiza een veel kleiner negatief effect op de aantallen aangehechte en opgekomen *Striga* planten. Bij S-35 werd, evenals in Hoofdstuk 2, ook waargenomen dat het tijdstip van bovengronds verschijnen van *Striga* verlaat werd. Het effect was echter nu niet significant. Door de specifieke omstandigheden van het experiment (beperkte hoeveelheid licht, relatief kleine potten) bleek de aanwezigheid van mycorrhizaschimmels niet te leiden tot een positief effect op sorghum. Ook hier bleek bemesting met fosfaat geen effect op *Striga* te hebben. Op grond daarvan concludeer ik dat het negatieve effect van AM schimmels op *Striga* niet het gevolg is van een positief effect van AM op de fosfaatvoorziening en de groei van sorghum.

Voor eventuele toepassing van deze kennis in de boerenpraktijk is het nodig dat de resultaten van deze potproeven onder veldomstandigheden gevalideerd worden. Daartoe werden drie veldexperimenten uitgevoerd, die in Hoofdstuk 5 worden

beschreven. Omdat het als onethisch wordt beschouwd om rechtstreeks experimenten uit te voeren met toevoeging van *Striga* in akkers en omdat de standaardprocedures om in zwaar met *Striga* geïnfecteerde velden de dichtheid van de parasiet te reduceren (door middel van methylbromide of ethyleen) vrijwel zeker ook effecten hebben op arbusculaire mycorrhiza, werd ervoor gekozen de proef uit te voeren in proefterreinen van het landbouwkundig instituut (IRAD). In de proef werd aan veldjes hetzij zaad van *Striga* hetzij inoculum van arbusculaire mycorrhiza (hetzelfde inoculum als gebruikt in de laboratoriumexperimenten) toegevoegd. Door de grotere variatie zoals die gewoonlijk in veldproeven wordt gevonden (de variatiecoëfficiënt was in het veld veel hoger dan bij de labproeven), was slechts een deel van de uitkomsten significant. *Striga* bleek een negatief effect te hebben op de groei van sorghum en mais (een opbrengstreductie van 7%-26%). Arbusculaire mycorrhiza had echter geen positief effect op de groei van sorghum; evenmin was er sprake van compensatie van het negatieve effect van *Striga* door arbusculaire mycorrhiza. Deze proefvelden hadden geruime tijd braakgelegen, en als gevolg daarvan was er geen zaadbank van *Striga* aanwezig. Door de langdurige periode van braak was echter de dichtheid van inheemse mycorrhizaschimmels hoog en toevoeging van inoculum leidde niet tot toename van het aantal sporen in de grond of van een hogere kolonisatiegraad van het gewas. Het ontbreken van een respons op de inoculatie met mycorrhiza is daardoor waarschijnlijk verklaarbaar. Desondanks bleken in veldjes die met mycorrhiza behandeld waren minder *Striga* planten op te komen (een achteruitgang van 30%-50%) en was de totale biomassa van *Striga* eveneens lager (40%-63% lager). Ook bleek de aanwezigheid van mycorrhiza de opkomst van *Striga* te vertragen. Mogelijk leidt toevoeging van mycorrhizaschimmels tot snellere kolonisatie van de sorghumplanten en daardoor tot een rechtstreeks negatief effect op *Striga*. De teruggang in *Striga* had echter, zoals gemeld, geen effect op de opbrengst van maïs en sorghum.

In Hoofdstuk 6 vat ik de resultaten van mijn proefschrift samen aan de hand van een schema dat aangeeft in welke fase van de levenscyclus van *Striga* mycorrhiza-schimmels een direct of indirect effect kunnen hebben. Op grond van mijn experimenten concludeer ik dat AM schimmels een effect hebben op de kieming van *Striga*-zaden via een effect op wortelexudaten van gastheerplanten. Het effect van verminderde aanhechting en opkomst van *Striga* zou eventueel het gevolg kunnen zijn van de lagere kieming. Het is echter ook mogelijk dat kolonisatie door mycorrhiza-schimmels leidt tot verdere fysiologische veranderingen in de wortel (bijvoorbeeld door afzetting van fenolische verbindingen of lignine in de wortelcelwand). Het feit dat niet alleen de aantallen aangehechte en opgekomen planten lager zijn na kolonisatie door mycorrhizaschimmels, maar dat ook het tijdstip van opkomen vertraagd is, doet vermoeden dat mycorrhizaschimmels ook in die fase van de

levenscyclus van *Striga* een rol kunnen spelen. Tot slot kan mycorrhiza leiden tot sorghumplanten met een betere voedingstoestand. Daardoor zijn sorghumplanten beter opgewassen tegen *Striga*-schade. Ook kan kolonisatie door mycorrhizaschimmels de hormoonbalans van de plant beïnvloeden en de negatieve effecten van *Striga* op de hormoonbalans (als gevolg waarvan sorghum meer wortels maakt en minder boven-gronds produceert) tegengaan. Het is tegelijkertijd ook mogelijk dat zulke grotere sorghumplanten een beter substraat vormen voor het kleinere aantal *Striga*-planten dat aanhecht en opkomt. De noodzaak om in teeltsystemen de opgekomen *Striga*-planten te verwijderen blijft dus bestaan. Verschillende suggesties worden gedaan voor nader onderzoek naar de precieze mechanismen waardoor AM schimmels rechtstreeks en indirect een negatief effect op *Striga* hebben. De grote verschillen in exudaatsamen-stelling en in groeirespons op mycorrhizaschimmels van verschillende sorghumrassen geeft aan dat er blijkbaar genetische variatie is welke voor de verdere veredeling van sorghumrassen gebruikt kan worden. Daarbij is het van belang om te begrijpen hoe veredeling van graangewassen invloed kan hebben op het vermogen om mycorrhiza te vormen of om voordeel aan mycorrhiza te ontlenen.

Daarnaast is het van groot belang te begrijpen welke praktische maatregelen boeren kunnen nemen om in hun landbouwpraktijken mycorrhiza te bevorderen. Gezien de sociaal-economische omstandigheden van deze boeren is het vrijwel uitgesloten dat het kopen van mycorrhiza-inoculum een verstandige strategie is. Daarentegen zouden boeren wel in hun beheer rekening kunnen houden met maatregelen die voor mycorrhizaschimmels gunstig uitpakken zoals het voorkomen van excessieve bodemverstoring waardoor het mycorrhizanetwerk in de bodem verstoord wordt, of het tegengaan van een sterke daling van het organische-stofgehalte. Verder onderzoek naar het effect van deze maatregelen, waarvan al bekend is dat ze een bijdrage kunnen leveren aan de beheersing van *Striga*, op het functioneren van mycorrhiza wordt daarom aanbevolen.

Curriculum vitae

Venasius Wirnkar Lendzemo was born on March 3, 1968 at Kitiwum Kumbo, North West Province of Cameroon. He followed primary education at Sacred Heart School Kitiwum from 1973 to 1980; Secondary School at Government High School Kumbo from 1981 to 1985 where he obtained the GCE 'O' Level; pursued post secondary education at Cameroon College of Arts Science and Technology (CCAST) Bambili from 1986 to 1988 where he obtained the GCE 'A' level certificate. He studied for a BSc in Agronomy at University Centre Dschang where he graduated in 1991 with distinction. He then worked for the Plant Protection Department of the Ministry of Agriculture from 1992 before being transferred to the Institute of Agricultural Research for Development (IRAD) in Cameroon. In 1996, he enrolled for the MSc programme Crop Science at Wageningen University. After graduation in 1998 he got admission into the PhD programme of Wageningen University under the sandwich construction format in 1999. In the course of his PhD research in Cameroon his MSc degree earned him the rank of Research Assistant at IRAD. After obtaining his PhD, he will relocate to IRAD, Maroua Centre, Far North Province of Cameroon.

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