Mycorrhiza (2009) 19:287–294
DOI 10.1007/s00572-009-0235-4

ORIGINAL PAPER

Striga seed-germination activity of root exudates and compounds present in stems of *Striga* host and nonhost (trap crop) plants is reduced due to root colonization by arbuscular mycorrhizal fungi

V. Lendzemo · T. W. Kuyper · H. Vierheilig

Received: 10 November 2008 / Accepted: 9 February 2009 / Published online: 24 February 2009

Abstract Root colonization by arbuscular mycorrhizal (AM) fungi reduces stimulation of seed germination of the plant parasite *Striga* (Orobanchaceae). This reduction can affect not only host plants for *Striga*, resulting in a lower parasite incidence, but also false hosts or trap crops, which induce suicidal *Striga* seed germination, thereby diminishing their effectiveness. In order to better understand these AM-induced effects, we tested the influence of root colonization by different AM fungi on the seed-germination activity of root exudates of the *Striga hermonthica* nonhost plants cowpea and cotton on *S. hermonthica*. We also tested the effect of AM fungi on the seed-germination activity of the *Striga gesnerioides* host plant cowpea on *S. gesnerioides*. Moreover, we studied whether mycorrhization affects the transport of seed-germination activity to above-ground plant parts. Mycorrhization not only resulted in a lower seed germination of *S. gesnerioides* in the presence of root exudates of the *S. gesnerioides* host cowpea but also seed germination of *S. hermonthica* was also lower in the presence of root exudates of the *S. hermonthica* nonhosts cowpea and cotton. Downregulation of the *Striga* seed-germination activity occurs not only in root exudates upon root colonization by different AM fungi but also in the compounds produced by stems. The lowered seed-germination activity does not appear to depend on the presence of seed germination inhibitors in the root exudates of mycorrhizal plants. The implication for *Striga* control in the field is discussed.

Keywords Arbuscular mycorrhiza · *Striga* · Strigolactones · Germination assay · Root exudates · Trap crops

V. Lendzemo
Institute of Agricultural Research for Development,
Maroua, P. O. Box 33, Maroua, Cameroon

T. W. Kuyper
Department of Soil Quality, Wageningen University,
P. O. Box 47, 6700 AA Wageningen, The Netherlands

H. Vierheilig
Institut für Pflanzenschutz, Universität für Bodenkultur Wien,
1190 Wien, Austria

H. Vierheilig (✉)
Departamento de Microbiología,
Estación Experimental de Zaidín, CSIC,
18008 Granada, Spain
e-mail: horst.vierheilig@eez.csic.es

Introduction

Cereal production in savannah regions of Africa is greatly hampered by damage due to the parasitic weed *Striga hermonthica* (Del.) Benth. (Orobanchaceae) (witchweed). This phytoparasite produces thousands of minute seeds that germinate in the presence of strigolactones (Bouwmeester et al. 2003, 2007; Steinkellner et al. 2007) exuded from the roots of the cereal hosts. After germination, the phytoparasite attaches to the roots of the host plant, forming haustoria. If a successful connection is established, *Striga* uses nutrients and carbohydrates of the host plant. *S. hermonthica* also poisons its host plant (Rank et al. 2004), resulting in host plant biomass losses that can be an order of magnitude larger than the biomass increment of *Striga*. Control of this weed has so far been extremely difficult due to the enormous seed production as well as the fact that seeds remain viable for several years. Monocropping of cereal hosts with little or no specific measures against witchweed has resulted in huge amounts of seeds accumulating in the seed bank. Strategies to deplete the *Striga* seed

bank or reduce it to tolerable levels are imperative for food security in Africa (Van Mourik et al. 2008).

One way to reduce *Striga* seed banks is the use of trap crops: crops that stimulate suicidal germination of *Striga* seeds in soil, but cannot be used as hosts because attachment and subsequent haustorial formation does not occur. Two such trap crops against *S. hermonthica* are cotton (Sauerborn et al. 2000) and cowpea (Gbèhounou and Adango 2003). Cotton and cowpea are also important (cash) crops in savannah regions of Africa (Kitch et al. 1998), including north Cameroon. Cowpea, however, is susceptible to another species of *Striga*: *Striga gesnerioides* (Dubé and Olivier 2001). To date, there are little or no quantitative data on the ability of the popular varieties of these crops in *Striga*-endemic north Cameroon to stimulate suicidal germination of *S. hermonthica* seeds.

Variability among potential trap crops exists. Laboratory studies do not correlate positively with field data or observations (Gbèhounou and Adango 2003). One possible explanation for the discrepancy between effectiveness in vitro and in situ of the trap cropping potential of plant species relates to arbuscular mycorrhiza (AM) formation in the field. AM fungi are ubiquitous symbiotic fungi that colonize roots of more than 80% of vascular plants (Smith and Read 2008). However, the mycorrhizal symbiosis has not been taken into account in studies of trap crops for *Striga*. Usually, plants for root exudate assays are grown in pure sand (Berner and Williams 1998), or seedlings are grown in test tubes with water as medium (Gbèhounou and Adango 2003). Such plants are nonmycorrhizal, while in the field, trap crops can be mycorrhizal. Studies on the tripartite interaction between cereals (sorghum or maize), AM fungi, and *S. hermonthica* have demonstrated that levels of *S. hermonthica* seed germination can be significantly reduced with AM root colonization (Lendzemo et al. 2007; Sun et al. 2008). Lower numbers of attached and emerged *Striga* shoots in the presence of AM fungi have also been reported in laboratory and field studies (Lendzemo and Kuyper 2001; Gworgwor and Weber 2003; Lendzemo et al. 2005). Lendzemo et al. (2007) hypothesized that the formation of AM on cereals leads to a downregulation of the production and the exudation of strigolactones, germination stimulants for *Striga*, and branching stimulants for AM fungi (Akiyama and Hayashi 2006). An implication of this hypothesis is that the trapping potential of crops in the field against *Striga* would be negatively affected upon mycorrhizal formation. In this context, AM formation in cowpea would be important for the control of *S. gesnerioides* in the field but at the same time reduces the effectiveness of cowpea as a trap crop against *S. hermonthica*.

Although it has been hypothesized that mycorrhization alters root exudation of strigolactones (Lendzemo et al. 2007) and thus reduces the seed-germination activity of root exudates from mycorrhizal plants, no data are available yet on the nature of the alteration which is responsible for the altered effect of root exudates from mycorrhizal and nonmycorrhizal plants on the seed germination of *Striga* plants (Lendzemo et al. 2007; Sun et al. 2008). Recently, by mixing pea root exudates with a solution of the synthetic strigolactone analog GR-24, it could be shown that a lower seed-germination activity of the root exudates of rhizobial pea plants on *Orobanche* is at least partially due to the presence of seed germination inhibitors in those exudates (Mabrouk et al. 2007). However, it is not known yet whether root exudates of mycorrhizal plants exhibit an inhibitory effect on *Striga* seed germination or show a reduced stimulatory activity.

Recently, strigolactones have been identified as a new class of plant hormones regulating shoot branching, suggesting their presence in above-ground plant organs (Gomez-Roldan et al. 2008; Umehara et al. 2008). By testing the effect of compounds released by excised stem pieces of maize, cowpea, and soybean on *Striga* seeds, the presence of seed-germination-stimulating compounds, possibly strigolactones, in stems has been reported (Emechebe and Ahonsi 2003). As mycorrhization has been reported to alter *Striga* seed-germination activity of roots, it is tempting to speculate that similar alterations also occur in the shoot.

In the present study, the effect of mycorrhization on the seed-germination activity of root exudates of *S. hermonthica* nonhost plants (cowpea, cotton) and of the *S. gesnerioides* host plant (cowpea) was tested. Moreover, whether different AM fungi induce similar alterations of the seed-germination activity of root exudates was investigated. In order to further elucidate the nature of the alteration of the seed-germination activity of root exudates from mycorrhizal and nonmycorrhizal plants, root exudates were mixed with a GR-24 solution. We also tested whether changes of the seed-germination activity found with root exudates due to mycorrhization are also found with compounds present in stems. We integrate these results in order to understand possible mechanisms involved in the reduced seed-germination activity due to mycorrhization of true and false *Striga* host plants.

Materials and methods

Biological material

One cotton and three cowpea cultivars were used. The cotton cultivar IRMA D742 (CORAF 2005) is an improved cotton variety grown extensively in the Sahel savannah of north Cameroon. The three cowpea cultivars consisted of one spreading cultivar (VYA) and two semi-erect cultivars (BR1 and LORI). BR1 and LORI are improved cultivars

with an intermediate growth cycle, adapted and adopted in north Cameroon, whereas VYA, harvested from Maroua (Cameroon) in 2006, is a local long-cycle cultivar also adopted in north Cameroon, but which is very susceptible to the cowpea weevil *Callosobruchus maculatus*. All three cultivars are susceptible to *S. gesnerioides* (Hall et al. 2003).

Striga (*S. hermonthica*, *S. gesnerioides*) seeds were harvested from sorghum or cowpea as host in Maroua, Cameroon in October 2005. The seeds were not handled under sterile conditions. The described experiments were performed during spring 2007, when seeds had an approximate age of 18 months.

The AM fungi *Glomus mosseae* (BEG 12), *Gigaspora rosea* (BEG 9; purchased from Biorhize, Pluvault, France) and *Glomus intraradices* (DAOM 197198) were used.

Experiments were conducted either in the growth chamber (cowpea and cotton) or the greenhouse (cotton only) at BOKU Vienna in spring 2007.

Growth chamber studies

Seeds were surface-sterilized in 5% of commercial NaOCl for 3 min and subsequently rinsed thoroughly to remove the chlorine. The seeds were then pregerminated in perlite for 5 days before being transferred to 750 cm^3 pots (one seedling per pot) filled with autoclaved (for 20 min at 121°C) substrate: a mix of arable soil, fine sand (<2 mm), expanded clay in the ratio 1:1:1 *v*/*v*, and placed in a growth chamber with average day and night temperatures 16 h/23°C and 8 h/19°C: relative humidity 50% light/dark. The design was a completely randomized design with four replicate pots of each treatment. Before planting, planting holes in the mycorrhizal treatments were inoculated with 25 g each of AM fungal inoculum of *G. mosseae*. The control pots received 25 g of autoclaved inoculum and 50 ml of the filtrate of the inoculum suspension (Koide and Li 1989). To prevent water stress, the plants were watered daily with tap water, and the pots rotated twice a week to minimize positional difference effects in the growth chamber. Pots were watered with tap water on a daily basis. The plants were grown under these conditions for 21 days.

Greenhouse studies

Seeds of the cotton cultivar IRMA-D742 were surface-sterilized and pregerminated in perlite, then were transferred to pots with autoclaved substrate as described above for the growth chamber experiments. Each planting hole in the mycorrhizal treatments was inoculated with 25 g of inoculum of one of the three arbuscular mycorrhizal fungi: *G. mosseae*, *G. intraradices*, or *G. rosea*. The control pots received 25 g of autoclaved inoculum. Plants were kept in the greenhouse for 3 weeks. Average day and night temperatures were 22°C for 16 h and 19°C for 8 h with day light supplemented by 400 µE s^{-1} light intensity supplied by Radium HRI-T4W/DH lamps. The design was a completely randomized design with three to four replicate pots per treatment. The pots were rotated twice a week to minimize positional difference effects on treatments. Pots were watered with tap water on a daily basis.

Exudate collection

After 21 days of growth in either the growth chamber or in the greenhouse, the root system of each plant was carefully washed free of substrate, and the entire root system was placed in 50-ml polystyrene tubes, with plants intact to collect root exudates. Distilled water was added to barely cover the root system completely in each tube. The plants were supported with hydrophilic cotton to keep them upright and to maintain the root system immersed in water. To limit influences by light on root exudates or exudation, the tubes were further wrapped with aluminum foil and left on racks under these conditions in the growth chamber or greenhouse for 24 h. Subsequently, the plants were removed and root systems were cut off, excess water was blotted with tissue paper, and root fresh weight was determined. The solution in the tubes was adjusted to 1 g root fresh weight to 10 ml volume solution with distilled water and used as fresh root exudates for germination assays with conditioned *Striga* seeds.

In order to collect cowpea stem compounds, a modification of the cut-root assay (for details see Van Mele et al. 1992) was used. The stems of three cowpea plants grown individually for 21 days were chopped into approximately 50-mm-long fragments and weighed. A 9-cm Petri dish lid was lined with two layers of filter paper (Whatman® filter paper No. 2) and then moistened with 3 ml of distilled water. A well approximately 1.5 cm deep and diameter of 1 cm made of Al foil was placed in the center of the Petri dish lid. One gram of the stem fragments of each replicate plant was placed in each Al foil well. Three 1-cm glass-fiber filter paper (GFFP) discs were placed in a triangular fashion close to each Al foil well. Distilled water (300 µl) was pipetted onto the stem fragments in the wells. In the control, 300 µl of distilled water was pipetted to an empty well surrounded by GFFP discs containing conditioned *Striga* seeds.

The mechanism through which root exudates from mycorrhizal plants show an altered *Striga* germination was investigated by using different combinations (1:1 *v*/*v*) of root exudates of the cowpea variety BR1 (mycorrhizal and nonmycorrhizal) and GR-24 (10^{-7}). To exclude a dilution effect, similar volumes of water were added to the exudates and the GR-24 solution as controls.

Conditioning of *S. hermonthica*/*S. gesnerioides* seeds

Ten milligrams of *Striga* seeds (approximately 1,500 seeds) was surface-sterilized according to the method of Matusova et al. (2004). The seeds were placed in 2% (*v*/*v*) NaOCl to which was added two drops of liquid (hand) soap for 5 min in a beaker. Subsequently, the seeds in solution were emptied on a funnel lined with folded filter paper (ø 125 mm) and rinsed thoroughly with autoclaved demineralized water to get rid of the chlorine odor. The seeds on the filter paper were dried on tissue paper. The lids of 9-cm-diameter Petri dishes were lined with two layers of 90 mm Whatman® filter paper No. 2 circles and wetted with 5 ml of distilled water. About thirty 1-cm-diameter GFFP discs (cut out with a cork borer from Whatman® GF/A filter papers) were placed on the wetted filters in each Petri dish. The surface-sterilized *Striga* seeds were carefully tapped onto the GFFP discs containing about 50 to 80 seeds per disc (Berner and Williams 1998). The Petri dishes were sealed with Parafilm, wrapped in aluminum foil, and incubated for conditioning at 28°C in the dark for 21 days.

Germination assays

For the germination bioassays with root exudates, the preconditioned seeds on the 1-cm GFFP discs were removed from the Petri dishes and dried on tissue paper for 30 min. The GFFP discs containing seeds were transferred to new lids of 9-cm Petri dishes (five discs per Petri dish), lined with a filter paper (Whatman® filter paper No. 2) ring (approximately 1-cm wide) wetted with 1 ml of distilled water. Fifty microliters of the root exudate solution was pipetted to each GFFP disc containing *Striga* seeds.

For *S. hermonthica* seeds, a positive control (50 µl of GR24 10^{-7} (0.1 mg/l)) and a negative control (50 µl of distilled water per disc) were included. GR-24 was also used in the case of *S. gesnerioides*. Because the seeds of the latter species do not always germinate in the presence of GR-24 (Berner et al. 1999), this control allowed an additional check on the seed batches used.

The Petri dishes were sealed with Parafilm and incubated in the dark at 28°C for 48 h. Seeds were subsequently observed under a binocular (stereo) microscope for germination or not. Seeds were considered germinated when the radicle came out of the seed coat.

AM fungal colonization

To visualize AM fungal colonization, root samples were chopped into approximately 1-cm-length fragments and cleared in 5% KOH for 3 min in a water bath at 90°C. After clearing, the root samples were rinsed with tap water and stained by boiling for 3 min in a 5% ink (Shaeffer; jetblack) in household vinegar solution (Vierheilig et al. 1998). The percentage of root colonization was determined according to the method of Newman (1966).

Statistical analyses

The percentages of mycorrhization and of germinated *Striga* seeds were transformed (arcsine value of square root) before statistical analyses. Data were subjected to ANOVA or GLM procedures with the help of the statistical analysis package SAS (SAS 1999-2001) and mean values separated with the Student–Newman–Keuls test.

Results

In all bioassays, the (water) control did not result in germination of *Striga* seeds. Seeds of *S. hermonthica* germinated in the presence of GR-24, whereas no germination of *S. gesnerioides* seeds was observed with GR-24. Roots of plants in the nonmycorrhizal treatments were not colonized by AM fungi irrespective of the plant species, whereas AM-inoculated plants always showed AM root colonization (Tables 1, 2, 3, and 4).

Root exudates of all three cultivars of cowpea in the mycorrhizal condition significantly reduced seed germination of *S. hermonthica* and *S. gesnerioides* compared to

Table 1 In vitro germination (%) of *Striga* seeds upon exposure to root exudates of cowpea (BR1, LORI, VYA)

Treatment	*Striga hermonthica* *	*Striga gesnerioides**	AM colonization (%)
BR1 (−AM)	12.2	26.9a	
BR1 (+AM)	1.1	3.1cd	32
LORI (−AM)	9.8	24.0a	
LORI (+AM)	4.6	14.2ab	15
VYA (−AM)	5.8	13.1bc	
VYA (+AM)	2.2	12.6bc	20
ANOVA (2-factor)			
Cowpea (C)	ns	ns	
Mycorrhiza (M)	–**	–**	
C×M	ns	*	

Germination in the presence of GR24 (10^{-7}): *S. hermonthica* 28.9%; *S. gesnerioides* 0%. Water control 0%. Root exudates of four plants per treatment were collected and tested individually. Values that are followed by a different letter in a column indicate significant differences between treatments at $P<0.05$ with Student–Newman–Keuls test. Significance of ANOVA: ns, not significant

−AM root exudates from nonmycorrhizal seedlings; *+AM* root exudates collected from seedlings that were colonized by *Glomus mosseae*

*$P<0.05$; **$P<0.01$

Table 2 In vitro germination (%) of *Striga hermonthica* seeds in the presence of root exudates from cowpea BR1 (+AM: root exudates collected from mycorrhizal seedlings; −AM root exudates collected from non mycorrhizal seedlings), GR24 10^{-7}, water, or their mixtures

Stimulant	Germination (%)
+AM and GR24 (mixture1:1 *v*/*v*)	88.2a
−AM and GR24 (mixture1:1 *v*/*v*)	81.9b
GR24 only (no dilution)	31.7c
GR24 and water (dilution 1:1 *v*/*v*)	28.7c
−AM and water (dilution 1:1 *v*/*v*)	16.3d
−AM only (no dilution)	13.1d
+AM and water (dilution 1:1 *v*/*v*)	0e
+AM only (no dilution)	0e
Water only	0e

Values are means of untransformed values (n=4). Means with the same letter in a column are not significantly different (P<0.05)

exudates from the nonmycorrhizal plants. The reduction upon mycorrhization was largest for cowpea cultivar BR1 for both *Striga* species (Table 1).

In the dilution assays, no *S. hermonthica* seeds germinated in the presence of water, whereas in the nondiluted GR-24 treatment (10^{-7}) and the diluted GR-24 treatment (GR24 10^{-7} and water; dilution 1:1 *v*/*v*), seed germination was similar (31.7% and 28.7%, respectively; Table 2). Diluting (1:1 *v*/*v*) the root exudates from mycorrhizal or nonmycorrhizal cowpea variety BR1 with distilled water did not change the effect on *S. hermonthica* seed germination compared to the undiluted exudate. In the presence of root exudates from mycorrhizal cowpea, *S. hermonthica* seed germination again was lower compared to the nonmycorrhizal treatment. Adding an equivalent volume of GR-24 to root exudates of the mycorrhizal or the nonmycorrhizal treatment resulted in a significant increase in *Striga* seed germination, with germination values twice those of the treatment with GR-24 alone (Table 2).

Table 3 In vitro germination (%) of *Striga hermonthica* seeds in the presence of root exudates from cotton cultivar IRMA D742 seedlings colonized by different AM fungal species

Species	Germination (%)	Colonization (%)
Water	0c	
GR24 (10^{-7})	28.9a	
Control (noncolonized plant)	31.8a	0c
Gigaspora rosea	9.5b	16b
Glomus intraradices	24.7a	14b
Glomus mosseae	20.6ab	24a

Values are means (arcsine square root transformed) of three or four replicates due to some plants that did not survive. In parentheses are means of percent root colonization by AM fungal species. Means with the same letter in a column are not significantly different (P<0.05)

Table 4 Effect of stem exudates of the cowpea cultivars VYA and BR1 colonized by *Glomus mosseae* (+AM) or not (−AM) on the in vitro germination of *Striga hermonthica* seeds

Treatment	Germination (%)
VYA (−AM)	60.2
VYa (+AM)	41.3
BR1 (−AM)	40.0
BR1 (+AM)	27.0
ANOVA[a] (2-factor)	
Cowpea (C)	–**
Mycorrhiza (M)	–**
C×M	ns

AM colonization of VYA roots=41%; AM colonization of BR1 roots=19%. Germination in the presence of GR24 (10^{-7})=45.1%. Values are means of three replicate plants tested individually

**P<0.01

[a] Significance of ANOVA: ns, not significant

When comparing the root exudates of cotton plants colonized by different AM fungi, in general, it was observed that root exudates from mycorrhizal cotton plants induced a lower germination of *S. hermonthica* seeds than root exudates from nonmycorrhizal control plants (Table 3). The reduction was largest when plants were colonized by *G. rosea* and smallest when plants were colonized by *G. intraradices*. The lower seed-germination activity did not seem to correlate with AM colonization because colonization by *G. rosea* (16%) was similar to that of *G. intraradices* (14%). Root colonization of plants colonized by *G. mosseae* was the largest (24%), whereas the decrease of seed-germination activity was intermediate (Table 3).

Compounds present in stems of mycorrhizal cowpea plants also showed a lower germination activity on *S. hermonthica* seeds than compounds present in stems of nonmycorrhizal cowpeas (Table 4). The effects varied with the cowpea cultivar. The cultivar VYA stimulated *Striga* germination more than cultivar BR1, irrespective of mycorrhizal condition. In this experiment, mycorrhizal colonization of VYA was larger than that of BR1.

Discussion

Monocropping of cereal hosts of *S. hermonthica* with little or no specific measures against this witchweed results in the accumulation of huge seed banks in the soil. Among the suggested methods in combination with others, to reduce *Striga* seed banks, is the use of trap crops such as cotton (Sauerborn et al. 2000) and cowpea (Gbèhounou and Adango 2003) in rotation or association with cereals. An efficient trap crop stimulates the germination of as many *S. hermonthica* seeds as possible in the soil. However, as

shown by Van Mourik et al. (2008), crop rotations cannot prevent the build-up of a huge seedbank of *S. hermonthica*. Whereas intercropping could be effective as a way of reducing the *S. hermonthica* seedbank, farmers' practices (late sowing of cowpea at relatively low densities) generally compromise the effectiveness of this potential control strategy. However, the extent to which crop rotations and intercropping affect mycorrhizal colonization rates of cowpea and cereals was not investigated by these authors.

Cotton and cowpea are both trap crops for *S. hermonthica*, but cowpea also is a host for *S. gesnerioides*. In our experiments, root exudates of cotton and cowpea cultivars from Cameroon reduced *S. hermonthica* seed germination when plants were colonized by AM fungi compared to nonmycorrhizal plants. Interestingly, we found a similar pattern with seeds of *S. gesnerioides* and root exudates of its host cowpea. Root exudates of cowpea equally reduced *S. gesnerioides* seed germination in the mycorrhizal condition compared to the nonmycorrhizal controls. The largest reduction after mycorrhization was observed in cowpea cultivar BR1, for both *S. hermonthica* and *S. gesnerioides*. Whether differential effects of cowpea cultivars were due to different levels of mycorrhizal colonization (also largest in BR1) or to genetic differences between the cultivars cannot, at present, be assessed.

Our observations confirm and expand previous results with root exudates of mycorrhizal sorghum (Lendzemo et al. 2007) and maize (Sun et al. 2008) on germination of *S. hermonthica*. In our study, we could show that the reduction of seed-stimulating activity due to mycorrhization also occurs in the trap crops cotton and cowpea, which are both false hosts for *S. hermonthica.* AM root colonization of trap crops could therefore affect their trap cropping potential. It is likely that this effect explains previous contradictory observations on the effectiveness of trap crops. Gbèhounou and Adango (2003) reported a lack of correlation between the trap cropping potential of legumes in vitro and their performance in situ. Whereas in vitro root exudates were collected from nonmycorrhizal plants, in situ plants were grown in natural soils and thus were most probably mycorrhizal.

Although the reduction of *S. hermonthica* seed germination with AM fungal colonization might appear to be a trade-off between the trap cropping potential and the potential benefits of AM, mycotrophic leguminous trap crops would enable propagules of AM fungi to accrue in the soil and possibly result in earlier colonization of the subsequent cereal crop in rotation, with consequent negative effects on *Striga* germination and emergence under field conditions (Lendzemo et al. 2005). Considering the lack of selectivity of many AM fungi, this beneficial effect on mycorrhization in the field could contribute to the effectiveness of trap crops in rotations (Van Mourik et al. 2008).

Contradictory data on the germination of *S. gesnerioides* in the presence of the synthetic strigolactone analog GR-24 are available. GR-24 either stimulates germination of *S. gesnerioides* or results in a low or no stimulation (Berner et al. 1999). In our study, GR-24 clearly did not stimulate the germination of *S. gesnerioides*. No hypothesis for this differing activity of GR-24 on *S. gesnerioides* has been provided yet. Having a closer look at the data might give an explanation for these discrepancies. When the seed batch had a relatively low age (5 months), GR-24 stimulated seed germination strongly (Berner et al. 1999), whereas when seed batches were older (more than 30 months in the work of Berner et al. (1999) and 18 months in our work), GR-24 showed no or only a slight stimulatory effect. This could mean that seeds of *S. gesnerioides* lose their sensitivity to the strigolactone analog GR-24 during longer storage. However, as cowpea root exudates still stimulated the germination of all seed batches insensitive to GR-24 (Berner et al. 1999 and our results), it seems clear that these seeds are still sensitive to other seed-germination-stimulating compounds, apart from strigolactones. Further studies are needed to test this hypothesis.

Seed germination of parasitic weeds of the Orobanchaceae may not only be affected by mycorrhization. Rhizobial inoculation of legumes reduces the seed-germination activity of root exudates on *Orobanche* (Mabrouk et al. 2007). By mixing root exudates of a rhizobial pea plant with a GR-24 solution, Mabrouk et al. (2007) showed that the reduced seed-germination activity of the root exudates of rhizobial pea plants on *O. crenata* is at least partially due to the presence of seed germination inhibitors in those exudates. However, in our experiments, the combination of root exudates of nonmycorrhizal or mycorrhizal cowpea BR1 with water did not result in an increase in *Striga* seed germination, suggesting that mycorrhization did not lead to an increased production of compounds inhibitory to *Striga* seed germination. The presence of inhibitory factors should also result in reduced seed germination when mixing root exudates from mycorrhizal plants with a GR-24 solution. By mixing root exudates from mycorrhizal cowpea plants with GR-24 solution, we observed that the seed-germination activity of this combination was significantly increased compared to the GR-24 alone treatment. This effect definitely excludes an inhibitory factor to be involved in the reduced seed-germination activity of root exudates of mycorrhizal plants. Moreover, it indicates that in these root exudates, compounds are present that exhibit a strong synergistic stimulatory effect on seed germination when in combination with the strigolactone analog GR-24. As several plant-derived secondary metabolites from different chemical groups such as sorgoleone, an isoflavone, strigo-

lactones, and sesquiterpene lactones are known to stimulate seed germination of *Striga* and *Orobanche* (Bouwmeester et al. 2007), the synergistic effect observed when mixing root exudates with a solution of the strigolactone analog GR-24 might be due to the presence of other seed-germination-stimulating compounds, apart from strigolactones.

Simultaneously, the combination "root exudates from nonmycorrhizal plants plus GR-24 solution" resulted in an even higher seed germination compared to the combination "root exudates from mycorrhizal plants plus GR-24 solution". This could mean that other seed-germination-stimulating compounds, apart from strigolactones, with a synergistic stimulatory effect on seed germination in combination with the strigolactone analog GR-24 are present in root exudates of nonmycorrhizal plants at even higher levels than in root exudates of mycorrhizal plants and that mycorrhization reduces the levels of these compounds.

There are more indications that seed-germination-stimulating compounds other than strigolactones are affected by mycorrhization. Above, we mentioned that seed germination of *S. gesnerioides* was not sensitive to the strigolactone analog GR-24, but germination was stimulated by the presence of cowpea root exudates. Something similar has been observed before (Berner et al. 1999). This indicates that compounds other than strigolactones present in root exudates induce the germination of *S. gesnerioides*. As apparently strigolactones do not affect seed germination of *S. gesnerioides*, alterations of the seed-stimulation activity of root exudates from mycorrhizal plants compared to root exudates from nonmycorrhizal plants must be due to other seed-germination-stimulating compounds and not strigolactones.

Gworgwor and Weber (2003) described AM fungal species-specific effects on the *Striga* performance on sorghum. In their study, *G. mosseae* colonized sorghum roots to a larger extent than the other *Glomus* species, while it had also resulted in the largest reduction of *Striga* numbers. Their study did not, however, provide evidence for a direct link between the level of mycorrhizal colonization and the suppression of *Striga* emergence. Similarly, in our study, the level of mycorrhizal colonization and the suppression of *Striga* seed germination could not be linked. This absence of a clear link is easily explicable if the production of seed-germination-stimulating compounds is downregulated upon mycorrhizal colonization due to changes in plant P concentration because there is also no straightforward relationship between levels of colonization and increased plant P uptake. The high effectiveness of *G. mosseae* in our study (like that by Gworgwor and Weber 2003) fits with observations by Burleigh et al. (2002) who demonstrated the high efficiency of fungal P uptake (and substantial suppression of plant P transporters) by *G. mosseae*. *G. intraradices* and *G. rosea* showed similar low levels of root colonization. Interestingly, even a relatively low degree of AM root colonization by *G. rosea*, which clearly reduced seed-germination activity, could be observed, whereas no clear effect could be observed with *G. intraradices*. In the study by Gworgwor and Weber (2003), *G. intraradices* was also rather ineffective in reducing *Striga* emergence.

A *Striga* seed-germination activity has been observed not only with root exudates but also with excised roots and stem pieces of maize, cowpea, and soybean (Emechebe and Ahonsi 2003). In the present study, the effect of stem-produced compounds of mycorrhizal and nonmycorrhizal cowpeas on *Striga* seed germination was similar to the effect with root exudates. Stem compounds of cowpea in the mycorrhizal treatment resulted in a lower germination of *S. hermonthica* seeds compared to the nonmycorrhizal treatment, indicating that in mycorrhizal plants, the levels of *Striga* seed-germination-stimulating compounds are altered not only in mycorrhizal roots but also in above-ground parts of mycorrhizal plants. These observations are of relevance in the light of the recent discovery that strigolactones act as shoot hormones that regulate shoot branching (Umehara et al. 2008; Gomez-Roldan et al. 2008). Firstly, this role as shoot branching hormones indicates the presence of strigolactones in the shoot of plants, and secondly, if strigolactones are present in the shoot and their level is altered by mycorrhization, this may contribute to alterations in the shoot branching pattern of mycorrhizal plants. However, as strigolactones have not been identified yet in above-ground parts of plants, further studies are needed to elucidate whether the observed lower *Striga* seed-germination activity of excised stems from mycorrhizal plants is due to altered levels of strigolactones or other yet un-identified *Striga* seed-germination-stimulating compounds moving to above-ground parts of plants. Moreover, it is not clear yet whether the altered *Striga* seed-germination activity in the shoot is the consequence of a lower production of seed-germination-stimulating compounds in the roots, and hence, a reduced transport of these compounds to the shoot or due to a reduced production of the seed-germination-stimulating compounds in the shoot.

To summarize, mycorrhization reduces not only the seed germination activity of root exudates of a host plant of *S. hermonthica* and *S. gesnerioides* but also of *S. hermonthica* nonhost plants that are used as trap crops. Cowpea is both a trap crop of *S. hermonthica* and a host to *S. gesnerioides*. This double role implies that although the mycorrhizal status of cowpea might negatively affect its trap cropping potential against *S. hermonthica*, mycorrhization could be helpful under infestation of cowpea by *S. gesnerioides*. The implications for management in relation to cropping strategies (intercropping, crop rotation) need further experimental testing under field conditions.

Downregulation of the *Striga* seed-germination activity exists not only in root exudates upon root colonization by different AM fungi but also in stem-produced compounds. The lowered seed-germination activity does not depend on the presence of seed germination inhibitors in the root exudates of mycorrhizal plants. The impact of mycorrhization on *Striga* seed-germination-stimulating compounds needs further investigation.

Acknowledgment This work was partially funded by a grant of the Netherlands Foundation for the Advancement of Tropical Research (WOTRO) to VL.

References

Akiyama K, Hayashi H (2006) Strigolactones: chemical signals for fungal symbionts and parasitic weeds in plant roots. Ann Bot (Lond) 97:925–931. doi:10.1093/aob/mcl063

Berner DK, Williams OA (1998) Germination stimulation of *Striga gesnerioides* seeds by hosts and nonhosts. Plant Dis 82:1242–1247. doi:10.1094/PDIS.1998.82.11.1242

Berner DK, Schaad NW, Völksch B (1999) Use of ethylene-producing bacteria for stimulation of *Striga* spp. seed germination. Biol Control 15:274–282. doi:10.1006/bcon.1999.0718

Bouwmeester HJ, Matusova R, Zhongkui S, Beale MH (2003) Secondary metabolite signalling in host-parasitic plant interactions. Curr Opin Plant Biol 6:358–364. doi:10.1016/S1369-5266(03) 00065-7

Bouwmeester HJ, Roux C, López-Ráez JA, Bécard G (2007) Rhizosphere communication of plants, parasitic plants and AM fungi. Trends Plant Sci 12:224–230. doi:10.1016/j.tplants.2007. 03.009

Burleigh SH, Cavagnaro T, Jakobsen I (2002) Functional diversity of arbuscular mycorrhizas extends to the expression of plant genes involved in P nutrition. J Exp Bot 53:1593–1601. doi:10.1093/jxb/erf013

CORAF (2005) Catalogue des variétés de cotonniers séléctionées. Lomé, Togo

Dubé MP, Olivier A (2001) Le *Striga gesnerioides* et son hôte, le niébé: interaction et méthode de lutte. Can J Bot 79:1225–1240. doi:10.1139/cjb-79-10-1225

Emechebe AM, Ahonsi MO (2003) Ability of excised root and stem pieces of maize, cowpea and soybean to cause germination of *Striga hermonthica* seeds. Crop Prot 22:347–353. doi:10.1016/S0261-2194(02) 00177-1

Gbèhounou G, Adango E (2003) Trap crops of *Striga hermonthica*: in vitro identification and effectiveness in situ. Crop Prot 22:395–404. doi:10.1016/S0261-2194(02) 00196-5

Gomez-Roldan V, Fermas S, Brewer PB, Puech-Pagès V, Dun EA, Pillot J-P, Letisse F, Matusova R, Danoun S, Portais J-C, Bouwmeester H, Bécard G, Beveridge CA, Rameau C, Rochange SF (2008) Strigolactone inhibition of shoot branching. Nature 455:189–194. doi:10.1038/nature07271

Gworgwor NA, Weber HC (2003) Arbuscular mycorrhizal fungi-parasite-host interaction for the control of *Striga hermonthica* (Del.) Benth. in sorghum. *Sorghum bicolor* (L.) Moench. Mycorrhiza 13:277–281. doi:10.1007/s00572-003-0238-5

Hall AE, Cissé N, Thiaw S, Elawad HOA, Ehlers JD, Ismail AM, Fery RL, Roberts PA, Kitch LW, Murdock LL, Boukar O, Phillips RD, McWatters KH (2003) Development of cowpea cultivars and germplasm by the Bean/Cowpea CRSP. Field Crops Res 82:103–134. doi:10.1016/S0378-4290(03) 00033-9

Kitch LW, Boukar O, Endondo C, Murdock LL (1998) Farmer acceptability criteria in breeding cowpea. Exp Agric 34:475–486. doi:10.1017/S0014479798004049

Koide RT, Li MG (1989) Appropriate controls for vesicular arbuscular mycorrhizal research. New Phytol 111:35–44. doi:10.1111/j.1469-8137.1989.tb04215.x

Lendzemo VW, Kuyper TW (2001) Effects of arbuscular mycorrhizal fungi on damage by *Striga hermonthica* on two contrasting cultivars of sorghum. Agric Ecosyst Environ 87:29–35. doi:10.1016/S0167-8809(00) 00293-0

Lendzemo VW, Kuyper TW, Kropff MJ, Van Ast A (2005) Field inoculation with arbuscular mycorrhizal fungi reduces *Striga hermonthica* performance on cereal crops and has the potential to contribute to integrated *Striga* management. Field Crops Res 91:51–61. doi:10.1016/j.fcr.2004.05.003

Lendzemo VW, Kuyper TW, Matusova R, Bouwmeester HJ, Van Ast A (2007) Colonization by arbuscular mycorrhizal fungi of sorghum leads to reduced germination and subsequent attachment and emergence of *Striga hermonthica*. Plant Signal Behav 2:58–62

Mabrouk Y, Simier P, Arfaoui A, Sifi B, Delavault P, Zourgui L, Belhadj O (2007) Induction of phenolic compounds in pea (*Pisum sativum* L.) inoculated by *Rhizobium leguminosarum* and infected with *Orobanche crenata*. J Phytopathol 155:728–734. doi:10.1111/j.1439-0434.2007.01307.x

Matusova R, Van Mourik T, Bouwmeester H (2004) Changes in the sensitivity of parasitic weed seeds to germination stimulants. Seed Sci Res 14:335–344. doi:10.1079/SSR2004187

Newman EI (1966) A method of estimating the total length of root in a sample. J Appl Ecol 3:139–145. doi:10.2307/2401670

Rank C, Rasmussen LS, Jensen SR, Pierce S, Press MC, Scholes JD (2004) Cytotoxic constituents of *Alectra* and *Striga* species. Weed Res 44:265–270. doi:10.1111/j.1365-3180.2004.00398.x

SAS Software Release 8.2. 1990-2001. SAS Institute Inc., Cary, NC, USA

Sauerborn J, Kranz B, Mercer-Quarshie H (2000) Crop rotation to improve agricultural production in Sub-Saharan Africa. J Agron Crop Sci 184:67–72. doi:10.1046/j.1439-037x.2000.00368.x

Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Academic Press, London

Steinkellner S, Lendzemo V, Langer I, Schweiger P, Khaosaad T, Toussaint JP, Vierheilig H (2007) Flavonoids and strigolactones in root exudates as signals in symbiotic and pathogenic plant-fungus interactions. Molecules 12:1290–1306. doi:10.3390/12071290

Sun Z, Hans J, Walter MH, Matusova R, Beekwilder J, Verstapen FWA, Ming Z, Van Echtelt E, Strack D, Bisseling T, Bouwmester HJ (2008) Cloning and characterisation of a maize carotenoid cleavage dioxygenase (*ZmCCD1*) and its involvement in the biosynthesis of apocarotenoids with various roles in mutualistic and parasitic interactions. Planta 228:789–801. doi:10.1007/s00425-008-0781-6

Umehara M, Hanada A, Yoshida S, Akiyama K, Arite T, Takeda-Kamiya N, Magome H, Kamiya Y, Shirasu K, Yoneyama K, Kyozuka J, Yamaguchi S (2008) Inhibition of shoot branching by new terpenoid plant hormones. Nature 455:195–200. doi:10.1038/nature07272

Van Mele P, Van Damme P, Berner D (1992) A new technique to test germination response to *Striga* seeds using plants roots p. 993-999. Mededelingen. Faculteit. Landbouwwetenschappen 57(36). Universiteit van Gent

Van Mourik TA, Bianchi FJAA, Van der Werf W, Stomph TJ (2008) Long-term management of *Striga hermonthica*: strategy evaluation with a spatio-temporal population model. Weed Res 48:329–339. doi:10.1111/j.1365-3180.2008.00638.x

Vierheilig H, Coughlan AP, Wyss U, Piché Y (1998) Ink and vinegar; a simple staining technique for arbuscular mycorrhizal fungi. Appl Environ Microbiol 64:5004–5007