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Effect of diammonium phosphate application on strigolactone production and *Striga hermonthica* infection in three sorghum cultivars

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Summary

Striga hermonthica infection poses a major constraint to sorghum production in sub-Saharan Africa, and low soil fertility aggravates the *S. hermonthica* problem. Under mineral nutrient deficiency, the sorghum host secretes large quantities of strigolactones, signalling molecules, into the rhizosphere. These induce *S. hermonthica* seed germination and subsequent infection of the host roots. In a combination of field and glasshouse experiments, we analysed the effect of microdose applied diammonium hydrogen phosphate (DAP) fertiliser on production of strigolactones, *S. hermonthica* infection and yield of three different African sorghum genotypes (CGM-19/1-1, Lina-3, DouaG). The sorghum cultivars all produced the strigolactones sorgomol and 5-deoxystrigol, albeit in different quantity and ratio. Without fertiliser, high *S. hermonthica* infection and emergence occurred under both glasshouse and field conditions. DAP application reduced secretion of sorgomol and 5-deoxystrigol and reduced *S. hermonthica* germination (66–70%), emergence (49–73%) and dry biomass (90–96%) under glasshouse conditions. Under field conditions, DAP microdosing reduced *S. hermonthica* emergence by 40–84% and increased sorghum grain yield by 47–142%. Thus DAP application reduced secretion of strigolactones into the rhizosphere and *S. hermonthica* parasitism both under controlled and field conditions. Microdosing of DAP may prove to be an efficient and cost effective option to reduce *S. hermonthica* damage in sorghum in sub-Saharan Africa, particularly in combination with other control options, such as intercropping, use of organic fertiliser and hand pulling of *S. hermonthica* at flowering to achieve integrated *S. hermonthica* management.

Keywords: strigolactones, purple witchweed, diammonium hydrogen phosphate, *Sorghum bicolor*.

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Introduction

Sorghum (*Sorghum bicolor* L.) is an important food and feed crop in sub-Saharan Africa (SSA). At present, this subsistence crop is grown on about 24 million hectares in Africa, accounting for 60% of the 40 million hectares of sorghum grown globally (FAO, 2009). However, total sorghum production in Africa during 2009 was only 22 million tonnes, which is about 37% of the worldwide production. The average yield of sorghum in most African countries is about 0.9 t ha^{-1}, which is substantially lower than the world average yield of 1.4 t ha^{-1}. The current sorghum production per unit area is not sufficient to meet the demand for human consumption,

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animal feed, fuel and building material requirements of a rapidly growing African population. Drought, bird attacks, insect pests, diseases and parasitic weeds are major constraints to sorghum productivity in SSA, of which the parasitic weeds *Striga hermonthica* (Del.) Benth and *Striga asiatica* (L.) Kuntze are among the most important biotic constraints (Haussmann *et al*., 1998; Khan *et al*., 2006, 2008; Guo *et al*., 2011). Degraded soils, nutrient depletion and low soil fertility are recognised as major abiotic factors responsible for low sorghum production in SSA (Palé *et al*., 2009). It is generally observed that phosphorus (P) and nitrogen (N) are the most limiting nutrients for sorghum production in Africa (Bationo & Mokwunye, 1991). The use of fertiliser in sorghum by African farmers is limited as a result of poor accessibility and availability and high fertiliser prices (Bekunda *et al*., 1997; Bagayoko *et al*., 2000, 2011; Dembele *et al*., 2000). Insufficient application of fertiliser limits sorghum productivity by reduced growth and development, but also by increased *S. hermonthica* infestation. The corollary is that fertiliser application has been shown to suppress *S. hermonthica* infection and improve growth and productivity of the host (Gacheru & Rao, 2001; Oswald & Ransom, 2001).

Germination of *S. hermonthica* seeds is triggered by the presence of signalling molecules, strigolactones, in the rhizosphere (Bouwmeester *et al*., 2003, 2007), which are secreted by the roots of host and non-host plants (Yoneyama *et al*., 2007; Lopez-Raez *et al*., 2008; Jamil *et al*., 2011). When mineral nutrients become deficient in the soil solution, arbuscular mycorrhizal (AM) fungi may develop symbiotic interactions with plants mediated by strigolactones (Bouwmeester *et al*., 2007). AM fungi facilitate the uptake of minerals in return for plant-produced carbon. Indeed, it has been reported that root exudates from plants grown under nutrient-limited conditions were very active in inducing hyphal branching, whereas root exudates collected from plants grown under high phosphorous (P) conditions did not stimulate branching (Lopez-Raez *et al*., 2008). Increased secretion of strigolactones by roots of host plants under P- and nitrogen (N)-deficient conditions has been reported (Yoneyama *et al*., 2007; Lopez-Raez *et al*., 2008), suggesting that application of fertilisers that contain both N and P, such as NPK and diammonium hydrogen phosphate (DAP), could be useful in reducing *S. hermonthica* infection indirectly by inhibiting strigolactone secretion (Jamil *et al*., 2012).

The high and increasing cost of mineral fertilisers and low purchasing power of African farmers have necessitated investigating the efficacy of fertiliser application at low to very low levels. The use of very low doses of mineral fertilisers and their placement near the planting hole, a technology termed 'microdosing', have been shown to reduce application rates and thus cost of fertiliser per surface area, while still improving crop yields (Tabo *et al*., 2007). Microdosing has also been reported by farmers to reduce the negative effect of *S. hermonthica* on the host (Aune *et al*., 2007), but research into the effect of microdosing fertilisers on *S. hermonthica* infection and knowledge about the mechanism involved is lacking.

The current study aimed to elucidate the mechanism by which diammonium phosphate fertiliser application, applied through microdosing, results in reduction in *S. hermonthica* infection. To achieve this goal, we used a combination of glasshouse and field experiments with three African sorghum varieties and three doses of the commercially available fertiliser, diammonium phosphate (DAP). DAP fertiliser is widely used and popular among farmers because of its good solubility in water and relatively high percentages of P and N. The three cultivars were tested under three DAP fertiliser rates for strigolactone production and *S. hermonthica* infection in bioassays and pot trials under glasshouse conditions in the Netherlands and under field conditions in Mali.

Materials and methods

Experimental sites

The glasshouse study was carried out at Wageningen University, the Netherlands. The field trials were conducted in 2010 and 2011 at the Samanko research station of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), situated about 20 km South-West of Bamako in Mali (8°54′W and 12°54′ N; altitude: 329 m).

Seeds, soils and growth conditions

Three sorghum cultivars, CGM-19/1-1, Lina-3 and DouaG, were used. In preliminary field trials with artificial infestation of *S. hermonthica*, CGM-19/1-1 showed high emergence levels of *S. hermonthica* with low yields and was thus rated susceptible, while Lina-3 showed lower levels of *S. hermonthica* emergence and maintained good yield, thus indicating that the variety has some degree of resistance and is tolerant to *S. hermonthica*. The variety DouaG appears to be adapted to soils with low phosphorus levels (F. Rattunde, pers. comm), but its sensitivity to *S. hermonthica* is unknown. *Striga hermonthica* seeds for the pot trial were collected from a sorghum field near Cinzana in Mali (courtesy of Cheickna Diarra). *Striga hermonthica* seeds used in the germination bioassays were collected from a sorghum field in Wad Medani, Sudan (courtesy of Abdel

Gabar Babiker). The glasshouse study was carried out under controlled conditions in Wageningen University. For strigolactone collection, silver river sand and the *S. hermonthica* infection study in pots, a mixture of soil and sand (50:50) was used. The field experiment was laid out on a sandy-loam, ferruginous tropical soil with washout spots and concretions. The soil had a pH of 5.0 (pH-H_2O), organic C content of 0.29%, available phosphorus content using the Bray-1 measurement of 10.5 (mg P kg^{-1}), total nitrogen content of 204 (mg N kg^{-1}), potassium content of 66.5 mg kg^{-1} and a cation exchange capacity (CEC) of 1.8 (cmol kg^{-1}). The ICRISAT-Samanko research station is situated in a Sudanean climate, with one rainy season per year between May and October and an average annual rainfall of 950 mm, calculated over the last 10-year rainfall data. The cropping season at the site runs from mid-June to November with an average temperature of 29.1°C. Total rainfall in 2010 was exceptionally high, with 1231 mm in 67 rainy days and in the period between sowing and crop maturity and 1003 mm in 52 rainy days. Total rainfall in 2011 was slightly over the 10-year average, with 1020 mm in 51 rainy days and in the period between sowing and crop maturity and 840 mm in 40 rainy days (Figure S1).

Experimental details

The experimental details for the glasshouse study and the field study are shown in Table 1. In both pot and field trials, a full factorial combination of the three above-mentioned sorghum varieties and three fertiliser levels were laid out in a completely randomised design with four replications. The three levels of fertiliser application consisted of 0, 2 and 4 g of DAP fertiliser per plant. In the field trial, the fertiliser was applied in one hole near the plant, while in the pot study the same amount of fertiliser was distributed in three small holes around the plant. Further details of the studies conducted in the glasshouse and field are described later.

Strigolactone collection

For strigolactone collection, sorghum seeds were germinated on moist filter paper at 28°C for 48 h. After germination, two seeds per pot were planted in 1.0-L pots filled with 750 mL sand during June 2010. Seven days after planting, the seedlings were thinned to one plant per pot, and DAP fertiliser was applied at 0, 2 and 4 g per plant. Each level of DAP fertiliser was applied in three small holes (5 cm deep) around the plants (at about 6 cm from the plant). The plants were irrigated with half-strength Hoagland's nutrient solution without N and P at a dose of 250 mL per pot at 48-h intervals. The plants were allowed to grow for 2 weeks in a climate chamber at 28°C (10 h)/25°C (14 h) photoperiod (supplemented with artificial light 400 μM m^{-2} s^{-1}) and 70% relative humidity. In the third week, root exudates were collected in plastic bottles by draining the pots with 1 L of nutrient solution. The collected root exudates were passed through a Grace Pure C18-Fast column (500 mg per 3 mL), and the strigolactones eluted with 4 mL of 100% acetone. These samples were purified using a Silica column (Grace Pure SPE Silica 200 mg per 3 mL). Hereto, 2 mL of the acetone eluent was transferred to a 4-mL glass vial, and the acetone evaporated in a vacuum centrifuge. After dissolving the residue in 50 μL ethyl acetate, 4 mL hexane was added. This solution was loaded on a pre-conditioned Silica column, mentioned previously. The strigolactones were eluted using 2 mL of hexane:ethyl acetate (10:90). The eluent was again dried using a vacuum centrifuge, and the residue dissolved in 200 μL of 25% acetonitrile. The samples were filtered through Minisart SRP4 0.45 μm filters (Sartorius,

Table 1 Experimental details at Wageningen University, the Netherlands and farmer's field in Mali

Parameter	Wageningen University, the Netherlands	Samanko research station, Mali
Replications	4	4
Row length	–	4 m
Row width	–	0.75 m
Plot size/pot size	1.5 L	3 × 4 m
Plant spacing	Single per pot	0.4 × 0.75 m
Sowing date	19 July 2010	18 July 2010 25 June 2011
Harvesting date	27 August 2010	19 November 2010 9 November 2011
Striga infection (Seed $plant^{-1}$)	25 mg per pot (5000)	55 mg per hill (10000)
Area harvested	1.5 L pot	14 sorghum hills (4.2 m^2) per plot
N and P-source	DAP (18% N:46% P_2O_5)	DAP (18% N:46% P_2O_5)
DAP levels	0, 2, 4 g per plant	0, 2, 4 g per hill

DAP, diammonium hydrogen phosphate.

Germany) for LC-MS/MS analysis. The remaining 2 mL of the acetone eluent of the C18 column was used for *S. hermonthica* germination bioassays, as described previously (Jamil *et al.*, 2011).

Striga hermonthica *germination bioassays*

Germination bioassays were performed as described previously (Jamil *et al.*, 2011). Briefly, clean *S. hermonthica* seeds (*c.* 50–100) were surface-sterilised with 2% sodium hypochlorite in sterile water along with 0.4% of Tween-20. After sterilisation, the *S. hermonthica* seeds were placed on 9-mm-diameter glass fibre filter paper discs. A sterilised Whatman filter paper was moistened with 3.0 mL sterilised water in a 9-cm-diameter Petri dish, and about twelve of the discs with *S. hermonthica* seeds were placed on it for pre-conditioning. After 10 days at 30°C, the discs with pre-conditioned *S. hermonthica* seeds were allowed to dry in a laminar flow cabinet. The acetone was removed from the root exudate samples using vacuum centrifugation and the residue dissolved in demineralised water. Then 50 μL of this mixture was applied on triplicate discs in a new Petri dish. To create a humid atmosphere, a moistened filter paper ring (outer diameter 9 cm, inner diameter 8 cm) was placed inside the Petri dish, and the Petri dish sealed with Parafilm. Subsequently, the *Striga* seeds were incubated in darkness at 30°C for another 48 h, and germination (seeds with radicle protruding through the seed coat) was scored with the help of a binocular microscope. GR24 at 3.3 μM and water were used as positive and negative control respectively.

Striga hermonthica *pot trial under glasshouse conditions*

Striga hermonthica emergence was studied in pots in the glasshouse at the same fertiliser levels (0, 2 and 4 g per plant) as in the field in July 2010. About 25 mg (4000) *S. hermonthica* seeds was weighed for each pot and mixed thoroughly with 1 L of the 50:50 sand and soil mixture. Plastic pots of 3.0 L volume were taken, and perforated plastic sheet was placed on the bottom of the pot. About 500 mL of soil and sand mixture without *S. hermonthica* seeds was placed in the bottom of the pot. Then 1 L of the sand and soil mixture with *S. hermonthica* seed was added. On top of this mixture, 200 mL of sand without *S. hermonthica* seeds was added. Sorghum seeds were germinated on moist rock wool at 28°C for 2 days. After germination, two seeds per pot were planted. The seedlings were thinned to one plant per pot 7 days after planting after which DAP fertiliser was applied at 0, 2 and 4 g per pot. The DAP fertiliser was applied as described previously, in three small holes (5 cm deep) around the plants (6 cm away). The sorghum seedlings were grown in the glasshouse at 28°C day (14 h) and 25°C night (10 h) with relative humidity of 70% supplemented with light (400 μM m^{-2} s^{-1}). *Striga hermonthica* emergence was recorded at 2-day intervals up to 10 weeks after planting. Then the *S. hermonthica* plants were uprooted, oven-dried at 70°C for 72 h and weighed to determine dry biomass.

Field study at the ICRISAT-Samanko research station in Mali

The soil for the field study was infested artificially with *S. hermonthica* seeds on 17 July in 2010 and on 24 June in 2011. Sorghum rows of 4 m length were infested with 0.5 g of *S. hermonthica* seed (about 10 000 seeds with 50% viability). *Striga hermonthica* seeds were mixed with 500 g of sand and spread out in a furrow of about 10 cm deep and 10 cm wide in a ridge. The furrow was subsequently closed with soil from the sides of the ridge. Planting distance was 0.75 m between rows and 0.4 m within the row, and individual treatment plot size was 3 m (four ridges) by 4 m (nine hills). All observations on *S. hermonthica* and sorghum were performed on the seven central plants on the two central ridges, amounting to 14 plants and 4.2 m^2. Sorghum was shown on 18 July in 2010 and on 25 June in 2011 at about 2 cm depth, and DAP fertiliser was applied on the same day, at about 5 cm from the planting hole. The three DAP fertiliser rates applied were 0, 2 and 4 g per hill, which is equal to 0, 62.5 and 125 kg per ha. Thinning to two plants per hill was performed 10 days after sowing (DAS), and a small number of missing hills were resown at this time. First weeding and ridging was performed at 25 DAS, after which all weeds but *S. hermonthica* were weeded by hand every 2 weeks. *Striga hermonthica* emergence was counted at 120 DAS, and *S. hermonthica* biomass was determined by accumulating dead and mature *S. hermonthica* plants at 90 DAS and 120 DAS and harvesting all remaining *S. hermonthica* plants at 150 DAS. The crop was harvested on 19 (124 DAS) and 9 (137 DAS) November in 2010 and 2011, respectively, and the stalk and grain yield measured after air-drying and threshing.

Statistical analysis

Data collected from the different experiments were subjected to analysis of variance (ANOVA) using GenStat Release 12 (PC/Windows XP), VSN international, UK. Multiple comparisons among treatment means [least significance difference test (LSD)] at $P < 0.05$ and linear relationships among various treatments were calculated using Fisher's analysis of variance (ANOVA).

Data from *S. hermonthica* counts at 120 DAS in the field trial were $\log_{10}$ transformed before subjecting to ANOVA.

Results

Glasshouse study at Wageningen University, the Netherlands

Two strigolactones, sorgomol and 5-deoxystrigol, were detected in all sorghum cvs (CGM-19/1-1, DouaG, Lina-3; Fig. 1). Sorgomol was identified in root exudates of sorghum and maize (Awad *et al.*, 2006; Xie *et al.*, 2009), Chinese milk vetch and white lupin (Yoneyama *et al.*, 2008) at 11.6 min with MRM channels *m/z* 339 > 242 while 5-deoxystrigol was isolated from root exudates of *Lotus japonicus* L. at 21.4 min with MRM channels *m/z* 331 > 234 (Akiyama *et al.*, 2005). The concentration of the strigolactones secreted as well as the ratio between the two strigolactones differed between the sorghum cvs (Fig. 2A and B). CGM-19/1-1 secreted particularly high levels of sorgomol and much less 5-deoxystrigol, while Lina-3 showed the reverse pattern. DouaG produced less sorgomol than CGM-19/1-1 and less 5-deoxystrigol than Lina-3. An increasing dose of DAP fertiliser strongly reduced the level of secretion of both strigolactones (Fig. 2A and B). In line with these results, application of sorghum root exudates collected from the control treatment (0 DAP) resulted in the highest *S. hermonthica* germination for all three cvs while applying DAP reduced germination by up to 66–70% (Table 2; Figure S2). Maximum *S. hermonthica* emergence (62 plants per sorghum plant in CGM-19/1-1; 45 in Lina-3 and 23 in DouaG) occurred in the control treatment, and microdosing of DAP reduced *S. hermonthica* emergence in all three cultivars (Fig. 3; Table 2). Similarly, the highest *S. hermonthica* biomass occurred in the control treatment, while applying DAP reduced biomass significantly (Table 2). The interaction between sorghum cultivar and fertiliser doses was not significant for *S. hermonthica* germination, but *S. hermonthica* emergence and dry biomass showed significant interaction. An increasing dose of DAP fertiliser

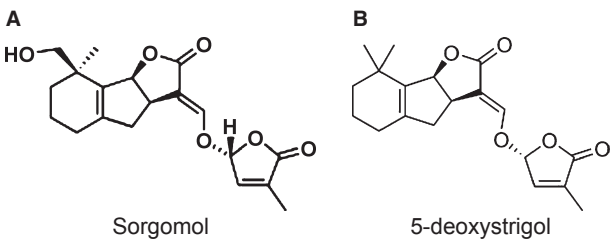

Fig. 1 Chemical structure of sorgomol (A) and 5-deoxystrigol (B), identified in root exudates of sorghum cultivars.

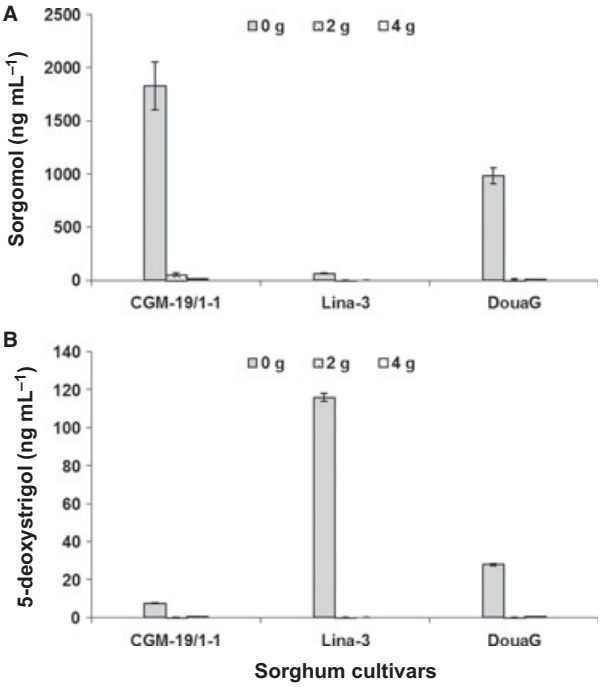

Fig. 2 Effect of microdosing of diammonium phosphate on secretion of sorgomol (A) and 5-deoxystrigol (B) in three sorghum cultivars. The purified root exudates were analysed using MRM-LC-MS (see Materials and methods). Bars represent means of amount of individual strigolactones (ng mL^{-1}) as determined by MRM-LC-MS in triplicate ± SE (n = 3).

significantly reduced all the *S. hermonthica* infection parameters, which was strikingly similar for the three cultivars (Table 2). Shoot dry weight and total dry biomass increased with application of DAP, while root weight and root:shoot ratio showed reverse trends and decreased with increasing dose of DAP (Table 3). Root:shoot ratio decreased from 3.6 to 0.9 for cv. Lina-3 and decreased from 6.9 to 0.6 for cv. DouaG with increasing DAP fertilisation, with a significant interaction between DAP fertilisation and variety.

Striga hermonthica *emergence and biomass and sorghum stalk and grain yield at ICRISAT Samanko research station, Mali*

ANOVA did not show any significant interaction between sorghum cultivar and DAP fertiliser application for any of the parameters from the field experiments. Therefore, only the mean values of the main effects of DAP fertiliser and sorghum cultivar are shown. Sorghum cultivar affected *S. hermonthica* emergence and dry biomass only in 2010, with cv. CGM-19/1-1 having significantly higher *S. hermonthica* numbers and biomass than Lina-3 and DouaG. No effects of cultivar on *S. hermonthica* parameters were found in 2011. Cultivar only affected stalk weight significantly, with DouaG

Table 2 *Striga hermonthica* germination, emergence and dry biomass as influenced by diammonium hydrogen phosphate (DAP) microdosing in three sorghum cultivars in the pot trial under glasshouse conditions at Wageningen UR, the Netherlands

DAP (g)	Germination (%)	Emergence (no.)	Dry biomass (g)
CGM-19/1-1			
0	45 ± 3.1†	62 ± 3.5†	6.0 ± 0.3†
2	29 ± 1.3	26 ± 3.3	2.0 ± 0.4
4	14 ± 1.9	17 ± 1.2	0.2 ± 0.03
Lina-3			
0	35 ± 5.2†	45 ± 4.8†	5.2 ± 0.3†
2	21 ± 2.5	26 ± 2.9	1.6 ± 0.8
4	11 ± 1.3	14 ± 2.5	0.3 ± 0.1
DouaG			
0	38 ± 4.3†	23 ± 1.5†	3.1 ± 0.5†
2	20 ± 4.1	18 ± 1.0	1.1 ± 0.1
4	13 ± 1.6	12 ± 1.4	0.3 ± 0.1
Cultivar (*P*)	NS	<0.001	<0.001
DAP (*P*)	<0.001	<0.001	0.001
Cultivar × DAP (*P*)	NS	<0.001	<0.01
Linear	(−ve)***	(−ve)***	(−ve)***
SEM‡	2.0	2.2	0.3
LSD 5%§	5.6	4.5	0.6
CV (%)	27	20	33

LSD, least significance difference.
†Means ± standard error $n = 4$.
‡Standard error of difference of means.
§Least significant differences of means at $P = 0.05$ by ANOVA test.
$^{**}P < 0.01$; $^{***}P < 0.001$.

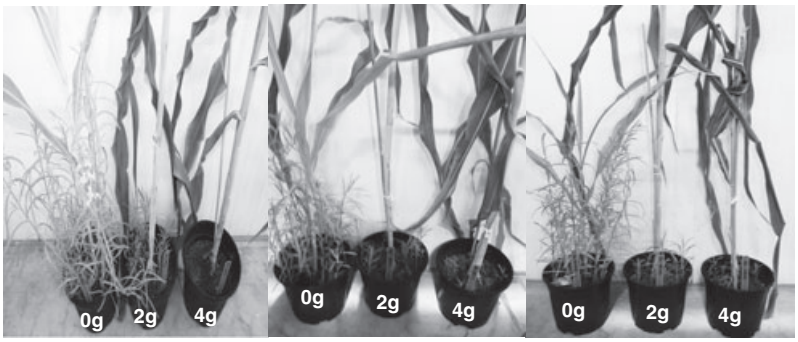

Fig. 3 Effect of diammonium phosphate microdosing on *Striga hermonthica* emergence at Wageningen University, the Netherlands. The emergence counted on individual sorghum host plant (cvs CGM-19/1-1, Lina-3 and DouaG) at 0 g diammonium hydrogen phosphate (DAP) (62, 45, 23), 2 g DAP (26, 26, 18), 4 g DAP (17, 14, 12).

having highest stalk yield and CGM-19/1-1 having the lowest stalk yield. Sorghum grain yield was not significantly different between cultivars in any of the 2 years.

ANOVA showed significant effects of DAP fertiliser application in 2010 and 2011 for *S. hermonthica* biomass and sorghum stalk and grain yield, while DAP fertiliser application only significantly affected *S. hermonthica* emergence in 2010 although in 2011 there was a similar negative trend (Table 4). The control treatment (without DAP) resulted in maximum *S. hermonthica* emergence of 9.9 and 12.1 plants m^{-2} and dry biomass of 17.5 and 9.7 g m^{-2} in 2010 and 2011 respectively. On average, over all cultivars, *S. hermonthica* emergence was reduced by 67% and 54% in 2010 and 2011, respectively, when comparing the application of 4 g of DAP fertiliser with the control treatment. Application of 2 g of DAP fertiliser per planting hole resulted in 26% and 55% reduction in 2010 and 2011 respectively. *Striga hermonthica* biomass followed the same trend with maximum reductions of 78% and 55% in 2010 and 2011 respectively.

Diammonium hydrogen phosphate application affected sorghum grain and stalk yield significantly, and both showed the same positive trend with the DAP rate. Compared with the control treatment, sorghum grain yield increased from 1.2 to 1.9 t ha^{-1}, by 60% and 50% with the application of 2 g DAP per hill and by 70% and

Table 3 Plant biomass (shoot/root) in various sorghum cultivars in response to diammonium hydrogen phosphate (DAP) microdosing in the pot trial under glasshouse conditions at WUR, Wageningen

DAP (g)	Shoot dry biomass (g)	Root dry biomass (g)	Total dry biomass (g)	Root:shoot ratio
CGM-19/1-1				
0	5.4 ± 0.3†	19.6 ± 0.6†	25.0 ± 1.0†	3.6
2	11.4 ± 0.4	18.8 ± 1.4	30.2 ± 1.8	1.6
4	18.2 ± 0.7	16.8 ± 1.4	35.0 ± 2.2	0.9
Lina-3				
0	4.1 ± 0.2†	24.2 ± 0.5†	28.3 ± 0.7†	5.9
2	18.5 ± 0.3	20.4 ± 1.0	38.9 ± 1.3	1.1
4	20.4 ± 0.4	20.6 ± 0.9	41.0 ± 1.3	1.0
DouaG				
0	2.7 ± 0.2†	18.5 ± 1.0†	21.2 ± 1.2†	6.9
2	11.8 ± 0.6	15.8 ± 1.1	27.6 ± 1.7	1.3
4	20.7 ± 0.7	11.8 ± 0.9	32.5 ± 1.6	0.6
Cultivar (*P*)	<0.01	<0.01	<0.001	<0.001
DAP (*P*)	<0.001	<0.001	<0.001	<0.001
Cultivar × DAP (*P*)	<0.001	NS	NS	<0.001
Linear	(+ve)***	(−ve)**	(+ve)***	(−ve)***
SEM‡	1.0	1.4	1.8	0.4
LSD 5%§	2.0	2.8	3.7	0.8

LSD, least significance difference.
†Means ± standard error $n = 4$.
‡Standard error of difference of means.
§Least significant differences of means at $P = 0.05$ by ANOVA test.
$^{**}P < 0.01$; $^{***}P < 0.001$.

Table 4 Mean *Striga hermonthica* emergence and dry biomass and sorghum stalk and grain yield under three levels of diammonium hydrogen phosphate (DAP) fertiliser and three sorghum cultivars under field conditions at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)-Samanko in Mali during 2010 and 2011

	Striga emergence (no. m^{-2})*		Striga dry biomass (g m^{-2})		Sorghum stalk yield (t ha^{-1})		Sorghum grain yield (t ha^{-1})	
	2010	2011	2010	2011	2010	2011	2010	2011
Cultivar								
CGM-19/1-1	12.5^{a}	7.4	17.4^{a}	4.9	6.7^{a}	8.6^{a}	1.5	2.7
Lina-3	5.3^{b}	7.4	8.8^{b}	4.2	9.2^{b}	10.6^{b}	1.9	2.2
DouaG	2.7^{b}	8.2	6.2^{b}	5.1	10.0^{b}	13.0^{c}	1.8	2.5
DAP								
Control	9.9^{a}	12.1	17.5^{a}	9.7^{a}	6.3^{a}	8.2^{a}	1.2^{a}	1.9^{a}
2 g DAP per hill	7.3^{ab}	5.4	10.2^{ab}	2.4^{b}	9.3^{b}	11.6^{b}	2.0^{b}	2.8^{b}
4 g DAP per hill	3.2^{b}	5.6	4.6^{b}	2.1^{b}	10.3^{b}	12.5^{b}	2.1^{b}	2.8^{b}
Cultivar (*P*)	<0.001	NS	0.031	NS	0.004	<0.001	NS	NS
DAP (*P*)	0.01	NS	0.016	0.032	<0.001	<0.001	<0.001	0.003
Cultivar × DAP (*P*)	NS	NS	NS	NS	NS	NS	NS	NS
SEM‡	2.29	4.23	4.12	3.04	0.89	0.86	0.19	0.27
LSD 5%§	4.72	8.73	8.51	6.27	1.83	1.77	0.39	0.57

§Least significant differences of means at $P = 0.05$ by ANOVA test.
*ANOVA and LSD test were performed on log-transformed data for emerged *Striga* numbers. The table, however, shows the untransformed values.
‡Standard error of difference of means.

47% with the application of 4 g DAP per hill in 2010 and 2011 respectively.

Discussion

Parasitic plants generally prevail on nutrient deficient soils, and many studies have reported a decrease in *S. hermonthica* infection upon application of N and P (Gworgwor & Weber, 1991; Kim *et al.*, 1997; Adagba *et al.*, 2002). A direct or indirect relationship between the presence of mineral nutrients and *S. hermonthica* infection has been suggested in previous studies (Cechin & Press, 1993; Gacheru & Rao, 2001; Showemimo *et al.*, 2002; Pageau *et al.*, 2003; Kamara *et al.*, 2007). The

germination of *S. hermonthica* seed is associated with the secretion of germination stimulants by host plants. The secretion ultimately depends upon the nutrient status of the soil (Jamil *et al.*, 2011). It has been demonstrated that under N and P deficiency, host plants secrete high amounts of germination stimulants into the rhizosphere, while supply of sufficient N and P reduces this secretion (Ayongwa *et al.*, 2006; Yoneyama *et al.*, 2007; Lopez-Raez *et al.*, 2008; Jamil *et al.*, 2012). Our previous studies showed that the effect of N was less pronounced than the effect of P on strigolactones secretion. As DAP fertiliser contains 18% N and 46% P_2O_5, high availability of P in DAP might lead to less production of strigolactones. However, direct suppressing effect of N on *Striga* spp. cannot be neglected (Simier *et al.*, 2006).

The three sorghum cultivars secreted the highest amount of strigolactones in the treatment without DAP fertiliser, and the secretion decreased with increasing rates of application of DAP fertiliser (Fig. 2). The secretion of these strigolactones in the treatment without DAP fertiliser caused maximum germination and emergence of *S. hermonthica* in the pot and in the field. The sorghum cv. DouaG showed some resistance with less strigolactone production and *S. hermonthica* infection, while cv. CGM-19/1-1 appeared highly susceptible with high sorgomol secretion and *S. hermonthica* infection in the control treatment. Low germination stimulant–producing sorghum cultivars have been reported to exhibit resistance to *S. hermonthica* in the field and have been tested and adopted in many African countries where they were found effective against *S. hermonthica* (Hess *et al.*, 1992; Ejeta, 2005, 2007).

The results in the glasshouse pot trials are similar to the results in the field trials. In 2010, all the sorghum cvs showed statistically significant reduction in *S. hermonthica* emergence and biomass and in both years an increase in sorghum grain and stalk yield with the highest dose of 4 g DAP per hill. However, it must be noted that the intermediate dose of 2 g DAP per hill that resulted in higher sorghum grain and stalk yield also resulted in higher *S. hermonthica* emergence with the cvs DouaG and Lina-3 and higher *S. hermonthica* biomass with cv. Lina-3. There was a significant interaction between sorghum cultivar and DAP fertiliser rate for *S. hermonthica* emergence and dry biomass under glasshouse conditions, but not in the field. Although *S. hermonthica* emergence and biomass differed greatly between the most susceptible (CGM19/1-1) and the most resistant (DouaG) cultivar under the control treatment in the pot trial, this difference was not noticeable when 2 or 4 g of DAP fertiliser was applied. This suggests that DAP fertiliser effectively increases pre-emergence resistance of susceptible varieties to the level of a 'resistant' variety (a variety having much less *S. hermonthica* emergence and biomass under nutrient deficient conditions). This trend was similar (although not significant; data not shown) for the field trials.

The controlled conditions and a limiting soil volume and nutrients under glasshouse conditions probably made it possible to detect a differential reaction of the three sorghum cultivars to *S. hermonthica* as a result of different levels of DAP fertiliser, as compared with the uncontrolled and open system under field conditions. DAP microdosing may also have affected the tolerance of sorghum plants to *S. hermonthica* attack, as shown by a significant grain and stalk yield increase without a significant decrease in emerged *S. hermonthica* when comparing 0 and 2 g DAP per hill in the field trials (Table 4). This suggests that in addition to a reduction in *S. hermonthica* infection under a high rate of DAP fertiliser, a moderate rate of DAP fertiliser increases the tolerance of sorghum to *S. hermonthica* infection. The variable effect of DAP fertiliser on *S. hermonthica* reduction, but consistent effect on sorghum grain and stalk yield, indicates the importance of combining DAP fertiliser as a microdose with other control measures to develop integrated *S. hermonthica* management.

A similar study on maize in Kenya showed the same trend of *S. hermonthica* reduction with an increase in N and P fertiliser application (Jamil *et al.*, 2012), but particularly under glasshouse conditions. The results under field conditions in Kenya were less consistent, as compared with the results of the current study on sorghum in Mali, particularly in 2010. The difference in fertiliser type and mode of application of fertiliser may have caused this difference. Unlike the current study in Mali, the fertilisers used in Kenya were urea and triple super phosphate (TSP), applied as side dressing in the row. The N in urea fertiliser might be lost more quickly due to volatilisation, denitrification and leaching, as compared with the slow-releasing DAP. Similarly, P in TSP may adsorb on soil particles more quickly than the P from DAP (Tisdale, 2004). Finally, soils in West Africa are generally sandier and suffer less from acidic pH than the soils in Central Africa and East Africa (Jamil *et al.*, 2012). Hence, P availability after fertiliser application in Mali may be much better than its availability in Kenya.

The rate of DAP fertiliser application in this study is relatively high (62.5 kg per ha for 2 g per hill and 125 kg per ha for 4 g per hill), and the mode of application (microdosing) has a relatively high labour requirement (Aune *et al.*, 2007). Our current study and that of Aune *et al.* (2007) both suggest that it may be useful to study the effects still lower rates of fertiliser for its effectiveness in controlling *S. hermonthica* and

increasing yields. Nonetheless, applying DAP fertiliser through microdosing could prove effective for *S. hermonthica* control and should increase the yields of grain and stover of sorghum. Due to the low cost, as a result of the low amount of fertiliser used, and high efficiency, this technology may be attractive for poor farmers growing sorghum on infertile soils, often in fields with *S. hermonthica* infestation. Microdosing has been tested and adopted by African farmers in sorghum and pearl millet for increasing yields and as a complementary benefit, *Striga* spp. Control.

The outcomes of the present study indicate that it is possible to enhance sorghum production with DAP fertiliser in Africa. It is suggested that the use of DAP fertiliser could aid in reducing *S. hermonthica* infection due to a reduction in the secretion of strigolactones by the host plant, while at the same time improving sorghum development and yield under *S. hermonthica* infection. However, the application of DAP fertiliser should not be considered as a single option for *S. hermonthica* control, but should be combined with other options, such as intercropping and rotation with legume crops and the use of appropriate resistant cereal cultivars, to achieve integrated *S. hermonthica* and soil fertility management.

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Supporting information

Additional supporting information may be found in the online version of this article.

Figure S1 Meteorological data of field experimental site (Mali) during 2010 (A) and 2011 (B) obtained from Mali Meteorological Department.

Figure S2 Effect of diammonium phosphate microdosing on *Striga hermonthica* germination (A), emergence (B) and dry biomass (C) at Wageningen University, the Netherlands.

