available at www.sciencedirect.com
ScienceDirect
journal homepage: www.elsevier.com/locate/apsoil

Biological control of *Striga hermonthica* by *Cubitermes* termite mound powder amendment in sorghum culture

Z. Andrianjaka [a,b,c,d,e], R. Bally [a,b,c,d], M. Lepage [f,g], J. Thioulouse [a,b,h,d], G. Comte [a,b,c,d], M. Kisa [e], R. Duponnois [e,*]

[a] Université de Lyon, Lyon F-69003, France
[b] Université Lyon 1, Lyon F-69003, France
[c] CNRS, UMR5557, Ecologie Microbienne, Villeurbanne F-69622, France
[d] IFR 41, Villeurbanne F-69622, France
[e] Institut de Recherche pour le Développement (IRD), UMR 113 CIRAD/INRA/IRD/AGRO-M/UM2, Laboratoire des Symbioses Tropicales et Méditerranéennes (LSTM), 34398 Montpellier, France
[f] Institut de recherche pour le développement (IRD), UR 179 SeqBio, 01 BP 182 Ouagadougou, Burkina Faso
[g] Centre National de la Recherche Scientifiques (CNRS), Laboratoire d'Ecologie, UMR 7625, Ecole Normale Supérieure, 75230 Paris Cedex 05, France
[h] CNRS, UMR5558, Laboratoire de Biométrie et Biologie Evolutive, Villeurbanne F-69622, France

ARTICLE INFO

Article history:
Received 3 April 2006
Received in revised form
28 February 2007
Accepted 1 March 2007

Keywords:
Termite
Arbuscular mycorrhiza
Striga
Sorghum
Plant protection

ABSTRACT

Striga hermonthica (Del.) Benth is an obligate root hemi-parasite of several cereals. Its effect on cereal crops is the main constraint for food production in sub-Saharan Africa. Various control methods have been already proposed, but the infestation by these parasitic plants persists. An appropriated method for *Striga* management adapted for the African farmer is very much needed. In this study, amendment of soil infested by this phytoparasite with *Cubitermes* mound powder is proposed as chemical amendment and natural microbial inoculum, to promote plant growth and reduce damage by *S. hermonthica* on sorghum (*Sorghum bicolor* L.). The influence of *Cubitermes* mound powder on the development of several microbial groups (arbuscular mycorrhizal fungi, actinomycetes, saprophytic fungi) was investigated in a pot experiment with sorghum cultured in a sandy soil infested by *S. hermonthica*. In the amended soil, sorghum growth and mycorrhizal colonization of sorghum plants were significantly greater than in the control treatment. Mycorrhizal colonization was negatively correlated with the number of emerged *Striga* plants per pot and positively correlated with sorghum growth. The relationship with substrate-induced respiration (SIR) responses showed that amended soil was characterized by its response to hydroxybutyric acid (catabolic marker of mycorrhizal colonization) and non-amended soil by its response to phenylalanine. We noted that the number of emerged *Striga* plants in amended pots was significantly decreased. Since *Cubitermes* mound suspensions did not affect *Striga* seed germination under axenic conditions, it suggests that the amendment with *Cubitermes* powder reduces *S. hermonthica* infestation indirectly, i.e. via its effect on the indigenous soil microflora. Overall, it appears that management of *Cubitermes* mounds is a promising strategy to consider for effective protection of sorghum from *Striga* infestation.

* *Corresponding author*. Present address: Laboratoire Commun de Microbiologie IRD/ISRA/UCAD, Centre de Recherche de Bel-Air, BP 1386, CP 18524 Dakar, Senegal. Tel.: +221 849 33 22; fax: +221 849 33 02.
E-mail address: robin.duponnois@ird.sn (R. Duponnois).
0929-1393/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.apsoil.2007.03.001

1. Introduction

Striga hermonthica (Del.) Benth. (Scrophulariacea), also known as witchweed, is a serious pest for cereals in the savannah region of Africa (Olivier, 1995). This obligate root hemi-parasite, native to savannah ecosystems, can cause important yield losses in cereals such as maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L.) and millet (*Pennisetum typhoides* L.) (Parker, 1991). Lenné (2000) asserted that this species is the largest biological constraint on food production in sub-Saharan Africa and crop losses due to *Striga* were estimated to more than 7 billions US$.

The life cycle of *Striga* is mainly dependent on that of its host. *Striga* produces thousands of minute, dust-like seeds that can remain viable in the soil for over a decade (Bebawi et al., 1984). Germination of seeds is induced by exudates of many plants, including both the above-mentioned cereals and so-called trap crops (Bouwmeester et al., 2003). The latter plants stimulate *Striga* germination but without becoming infected by the root hemi-parasite, as subsequent attachment and haustorial formation can only take place on true hosts (Parkinson et al., 1987; Ejeta and Butler, 1993; Olivier, 1995).

Approximately 75% of the overall *Striga* damage to the host is made during its subterranean stage of development (Bebawi et al., 1984; Parker and Riches, 1993). Although it has been demonstrated that up to 35% of the plant carbon demands are met by its autotrophic host (Pageau et al., 1998), this source-sink relations between *Striga* and its host does not fully explain the damage done by the parasite. Rank et al. (2004) demonstrated that *Striga* exerts a potent phytotoxic effect on the host. This phytotoxic effect is evident in the fact that the damage that *Striga* does to the cereal crops can reach maximum before the parasite emerges above ground. Managing *Striga* below ground is therefore a crucial for successful *Striga* management.

The control of *Striga* is difficult to achieve because of its high fecundity. In addition, seed germination is asynchronous (Worsham and Egley, 1990). Therefore, management of *Striga* infestation needs an integrated approach including host plant resistance, cultural practices, and chemical and biological treatments. Among all the components of this integrated *Striga* management, biological control could be used in Africa by the resource-poor farmers if it gives a demonstrable crop-yield benefit within one growing season (Ahonsi et al., 2002). In this context, it has recently been demonstrated that certain soil microorganisms can inhibit or suppress germination (Parker and Riches, 1993; Berner et al., 1995; Miché et al., 2000; Ahonsi et al., 2002). Indeed, some studies (Bouillant et al., 1997; Ahonsi et al., 2002) have shown that certain soil-borne saprophytic bacteria have a potential to control *S. hermonthica* by inhibiting seed germination. However, the survival of inoculated bacteria was usually not sustained in subsequent crops and it implied repeated application for every cropping season (Ahonsi et al., 2002). Another strategy is to use natural compounds (i.e. organic matter inputs) (Sauerborn et al., 2003) or N fertilization (Khan et al., 2002).

Recently, termite mound materials, through their effects on ''indigenous'' soil microbial communities, have been proven to promote plant growth and increase nutrient supplies from soil organic and inorganic amendments (Duponnois et al., 2005). In addition, termite mound materials can be used as natural microbial inoculants. In that study, different termite mound materials belonging to different termite feeding groups were tested, and preliminary results indicate that *Cubitermes* mound powder had the most promising effect. *Cubitermes* spp., which belong to the soil-feeding termite group, feed on mineral soil particles mixed with organic matter. Their feces are then incorporated into the mound, enriching the surrounding soil with C and N (Black and Okwakol, 1997).

Epigeal termite mounds are generally considered to be islands of higher concentration of nutrients in tropical areas (Spain and Okello-Oloya, 1985). In addition, soil microbial activity and diversity is highly modified by these soil-feeding termites (Fall et al., 1999; Brauman et al., 2000). Considering its potential to enhance plant growth, its nutrient content, and its effect on microflora composition, it appears that *Cubitermes* powder may be useful as natural amendment/inoculant to control plant parasites such as *Striga* in low-input agriculture.

The goal of this study was to determine whether *Cubitermes* mound material, used as a chemical amendment and microbial inoculum, can promote plant growth and reduce *S. hermonthica* parasitism through effects of the mound material on soil microorganisms. Thus, we investigated the influence of a *Cubitermes* mound powder on the development of several key microbial groups (arbuscular mycorrhizal fungi, actinomycetes, saprophytic fungi) known to play an important role in ecological processes (Garbaye, 1991; Hooker and Black, 1995), in a pot experiment with sorghum cultured in a sandy soil highly infested by *S. hermonthica*.

2. Materials and methods

2.1. Epigeal mound sampling

Five *Cubitermes* mounds were sampled in a shrubby savanna located 50 km north of Ouagadougou (Burkina Faso), near the village of Yaktenga. These soils are shallow and rich in gravels above a hard-pan level. Large hydromorphic spots (called ''bowé'') characterize the landscape, and they are intertwined with deeper soils. Mushroom-shaped *Cubitermes* mounds are located predominantly on the shallow soils. Termite mound materials (about 5 kg) were pooled, crushed, and passed through a 2-mm sieve in order to obtain a fine powder.

2.2. Chemical and microbiological characteristics of the *Cubitermes* mound powder

In order to determine the initial composition of *Cubitermes* mound powder, chemical and microbial analyses were carried out (Table 1). The methods used follow the techniques of Duponnois et al. (2005). Briefly, NH_4^+ and NO_3^- contents were measured according to the method of Bremner (1965) and available P content using the method of Olsen et al. (1954). Ergosterol content (fungal biomass index) was determined using the Grant and West (1986) method and microbial biomass using the fumigation-extraction method (Amato and Ladd, 1988). Enumeration of colony forming units (CFU) was performed on Difco™ Actinomycete Isolation Agar for the

Please cite this article in press as: Andrianjaka, Z. et al., Biological control of *Striga hermonthica* by *Cubitermes* termite mound powder amendment in sorghum culture, Appl. Soil Ecol. (2007), doi:10.1016/j.apsoil.2007.03.001

Table 1 – Biological and chemical characteristics of *Cubitermes* mound powder

NH_4^+ ($\mu g\ N\ g^{-1}$ of dry mound powder)	40.9
NO_3^- ($\mu g\ N\ g^{-1}$ of dry mound powder)	206.9
Available P ($\mu g\ g^{-1}$ of dry mound powder)	7.7
Microbial biomass ($\mu g\ C\ g^{-1}$ of dry mound powder)	17.0
Actinomycetes ($\times 10^2$ CFU g^{-1} of dry mound powder)	37.3
Ergosterol ($\mu g\ g^{-1}$ of dry mound powder)	1.38

actinomycetes and on Dichloran–Rose-Bengal–Chloramphenicol (DRBC) Agar for the saprophytic fungi.

2.3. *In vitro screening of Cubitermes mound powder as inhibitor of S. hermonthica seed germination in axenic experiment*

The effect of *Cubitermes* mound powder on *Striga* germination was investigated in an axenic experiment. Seeds of *S. hermonthica* were collected from parasitic plants growing in a sorghum field at Kambouinsé (20 km from Ouagadougou, Burkina Faso). This field was heavily infested by *S. hermonthica*, with >10 *Striga* shoots m^{-2}. The seeds were surface-sterilized for 5 min in 70% ethanol, and then for 10 min in an aqueous 1% NaOCl solution. Seeds were rinsed >5 times with sterile water, and approximately 100 seeds were kept on sterile and moistened Whatman glass fiber paper disks (1 cm diameter; Whatman Int., Maidstone, England) in sterile Petri dishes.

Plates were then placed in the dark at 30 °C in an incubator for at least 14 days. Synthetic germination stimulant (Strigol GR-24) must be added to induce *Striga* seed germination (Johnsson et al., 1976; Bouillant et al., 1997). A 1 L stock was made by dissolving 100 mg of GR-24 in 10 mL of acetone, and by diluting it with sterile distilled water. The stock was kept at 4 °C.

Cubitermes effects on the seed germination were assessed using three suspensions of *Cubitermes* mound powder (0.01; 0.1 and 1 $g\ L^{-1}$). Paper disks containing conditioned seeds were air-dried in axenic conditions, and placed into Petri dishes (5 cm diameter) containing glass-fiber disks impregnated with a mixture of GR-24 and *Cubitermes* mound suspensions (in a 9:1, v:v ratio). One mixture represents one treatment and five replicates were used per treatment. As a control, we inoculated the seeds with the synthetic germination stimulant alone. Plates were incubated for 4 days in the dark at 30 °C, and germinated *Striga* seeds were counted under 250× magnification.

2.4. *Greenhouse experiment*

A greenhouse experiment was conducted with sorghum to determine if *Cubitermes* mound material can be used to promote plant growth as well as reduce *S. hermonthica* parasitism. Seeds of sorghum were surface-sterilized with 1% NaOCl for 15 min and rinsed with demineralised water. They were pre-germinated for 2 days in Petri dishes on humid filter paper at 25 °C in the dark. When rootlets were 1–2 cm long, the seedlings were transplanted in soil naturally infested by *Striga*, which originated from the same sorghum field from which *Striga* seeds were collected. Before use, the soil was crushed, passed through a 2-mm sieve and carefully mixed in order to ensure the homogeneity of the *Striga* seed bank in the soil. The chemical and physical characteristics of this sandy soil were as follows: pH (H_2O) 5.6, clay 4.6%, coarse silt 0.8%, fine sand 25.5%, coarse sand 69.1%, C 2.05 g/kg, N 0.40 g/kg, C/N ratio 5.1, soluble P 4.3 mg/kg, total P 116 mg/kg. Plastic pots (5 dm^3) were filled with soil mixture, in a 1:10 (v/v) ratio with *Cubitermes* mound powder and the *Striga* infested soil. The control treatment consisted of untreated *Striga*-infested soil. Each pot received one pre-germinated seed of sorghum which was grown at ambient temperature from 15 to 40 °C with daily watering without fertilizer. There were eight replicates per treatment arranged in a completely randomized design.

The number of emerged *Striga* plants, the time of emergence and the height of sorghum plants were recorded weekly. After 14-weeks, plants were uprooted and their root systems gently washed. The sorghum roots were cleared and stained, as described by Phillips and Hayman (1970). They were placed on a slide for microscopic observation at 250× magnification (Brundrett et al., 1985). About 100 one-cm root pieces were observed per sorghum plant. The mycorrhizal colonization was expressed as [(the number of mycorrhizal root pieces)/(total number of observed root pieces) × 100]. The oven-dried weight of shoots and roots of sorghum plants was measured as well as those of *Striga* (65 °C, 1 week).

The soil from each pot was mixed thoroughly and 100 g sub-samples were taken and kept at 4 °C for further analysis. CFU enumeration of actinomycetes and saprophytic fungi was performed as described above.

2.5. *Measurement of the catabolic diversity of microbial communities in soil treatments*

Microbial functional diversity in soil treatments was assessed by measuring the patterns of *in situ* catabolic potential (ISCP) of microbial communities (Degens and Harris, 1997). Thirty-four substrates, comprising a range of amino acids, carbohydrates, organic acids and amides (Table 2), were screened for differences in SIR (substrate-induced respiration) responsiveness among soil treatments. The substrate concentrations providing optimum SIR responses were as follows: 15 mM for amino acids, 75 mM for carbohydrates, 15 mM for amides, 100 mM for carboxylic acids and 100 mM for cyclohexane (Degens and Harris, 1997). The substrates solutions (in 2-mL sterile distilled water) were added to 1.0 g dry soil in 10-mL bottles (West and Sparling, 1986). CO_2 production from basal respiratory activity in the soil samples was measured by adding 2-mL sterile distilled water to 1 g equivalent dry mass of soil. After the addition of the substrate solutions to soil samples, bottles were immediately closed and kept at 28 °C for 4 h. CO_2 fluxes from the soils were measured using an infrared gas analyzer (IRGA) (Polytron IR CO_2, Dräger™) in combination with a thermal flow meter (Heinemeyer et al., 1989). Results were expressed as $\mu g\ CO_2\ g^{-1}$ soil h^{-1}. Catabolic richness and catabolic evenness were calculated to evaluate the catabolic diversity of both soil treatments. Catabolic richness, *R*, corresponds to the number of substrates used by microorganisms in each soil treatment. Catabolic evenness, *E*, represents the variability of substrate used among the range of substrates tested, and it was calculated using the Simpson–Yule index, $E = 1/p_i^2$ with p_i = (respiration response to individual

Table 2 – List of substrates and their concentration providing optimum responses of respiration used in SIR (substrate-induced respiration) techniques

Amino acids (15 mM)
L-Phenylalanine
L-Tyrosine
L-Cystéine
L-Arginine
L-Aspargine
L-Glutamine
L-Histidine
L-Serine
L-Lysine
L-Glutamic acid
Carbohydrates (75 mM)
D-Glucose
D-Mannose
Sucrose
Amides (15 mM)
D-Glucosamine
Succinamide
N-Methyl-D-glucamine
Carboxylic acids (100 mM)
Ascorbic acid
Citric acid
DL-α-hydroxybutyric acid
Formic acid
Fumaric acid
Gallic acid
Gluconic acid
α-Ketoglutaric acid
Malic acid
Malonic acid
Oxalic acid
Quinic acid
Succinic acid
Tartaric acid
Tri-citrate
Uric acid
α-Ketobutyric acid
Cyclohexane (100 mM)
Cyclohexane

substrates)/(total respiration activity induced by all substrates for a soil treatment) (Magurran, 1988).

2.6. Statistical analysis

All data were subjected to a one-way analysis of variance and the mean values were compared using Student's *t*-test ($p < 0.05$). The percentages of mycorrhization were transformed before statistical analysis (arcsin value of square root). We used co-inertia multivariate analysis (Dray et al., 2003) to describe the relationship between two data sets: sorghum and *Striga* growth and microbial parameters (saprophytic fungi and actinomycetes infestation, mycorrhizal colonization) on one side, and SIR responses on the other side. Co-inertia analysis is a theoretical simplest multivariate analysis technique. A simple, Monte Carlo-like permutation test was then used to assess the relationship between the two data sets. Computations and graphic displays were made using the software ADE-4 (Thioulouse et al., 1997), available at http://pbil.univ-lyon1.fr/ADE-4/.

3. Results

3.1. Effect of Cubitermes mound powder on Striga seed germination in vitro

Striga seed germination was not significantly different when exposed to GR-24 alone or GR-24 supplemented with *Cubitermes* mound suspension at 0.01, 0.1 or 1 g mL^{-1}. The germination rates obtained after inoculation of these four suspensions were 55.2%, 64.6%, 64.8% and 61.4%, respectively. This means that *Cubitermes* mound material did not have a significant direct effect on *Striga* seed germination.

3.2. Effect of Cubitermes mound powder on sorghum and Striga

Cubitermes mound powder added to the soil significantly increased the height of sorghum plants from 5 weeks on (Fig. 1A). *Striga* emergence started at 4 weeks, and *Cubitermes* mound powder amendment decreased the number of *Striga* plants emerged per pot from 5 weeks on (Fig. 1B, Table 3). The height of *Striga* plants was significantly decreased by amendment between 6 and 10 weeks, but from week 11 on fluctuation in the controls was particularly high and the difference between treatments was not statistically significant (Fig. 1C, Table 3).

We found no significant effects of the termite mound amendment on the shoot and root biomass of emerged *Striga* at 14 weeks (Table 3). At 14 week, the height, shoot and root biomasses and mycorrhizal colonization of sorghum plants were significantly higher in the soil amended with *Cubitermes* powder than in the control treatment (Table 4).

Table 3 – Effect of *Cubitermes* mound powder amendment on the number of *S. hermonthica* emerged, height and shoot and root biomass of *S. hermonthica* after 14-week culture

	With *Cubitermes* mound powder	Without *Cubitermes* mound powder
Height (cm)	10.9 (1.65)[a] a[b]	13.7 (2.03) a
Shoot biomass (g dry weight)	2.19 (0.66) a	2.92 (0.24) a
Root biomass (g dry weight)	0.205 (0.063) a	0.203 (0.029) a
Number of *Striga* emerged	4.2 (1.22) a	8.1 (1.61) b

[a] Standard error of the mean.
[b] Means in the same line followed by the same letter are not significantly different according to one-way analysis of variance ($p < 0.05$).

Please cite this article in press as: Andrianjaka, Z. et al., Biological control of *Striga hermonthica* by *Cubitermes* termite mound powder amendment in sorghum culture, Appl. Soil Ecol. (2007), doi:10.1016/j.apsoil.2007.03.001

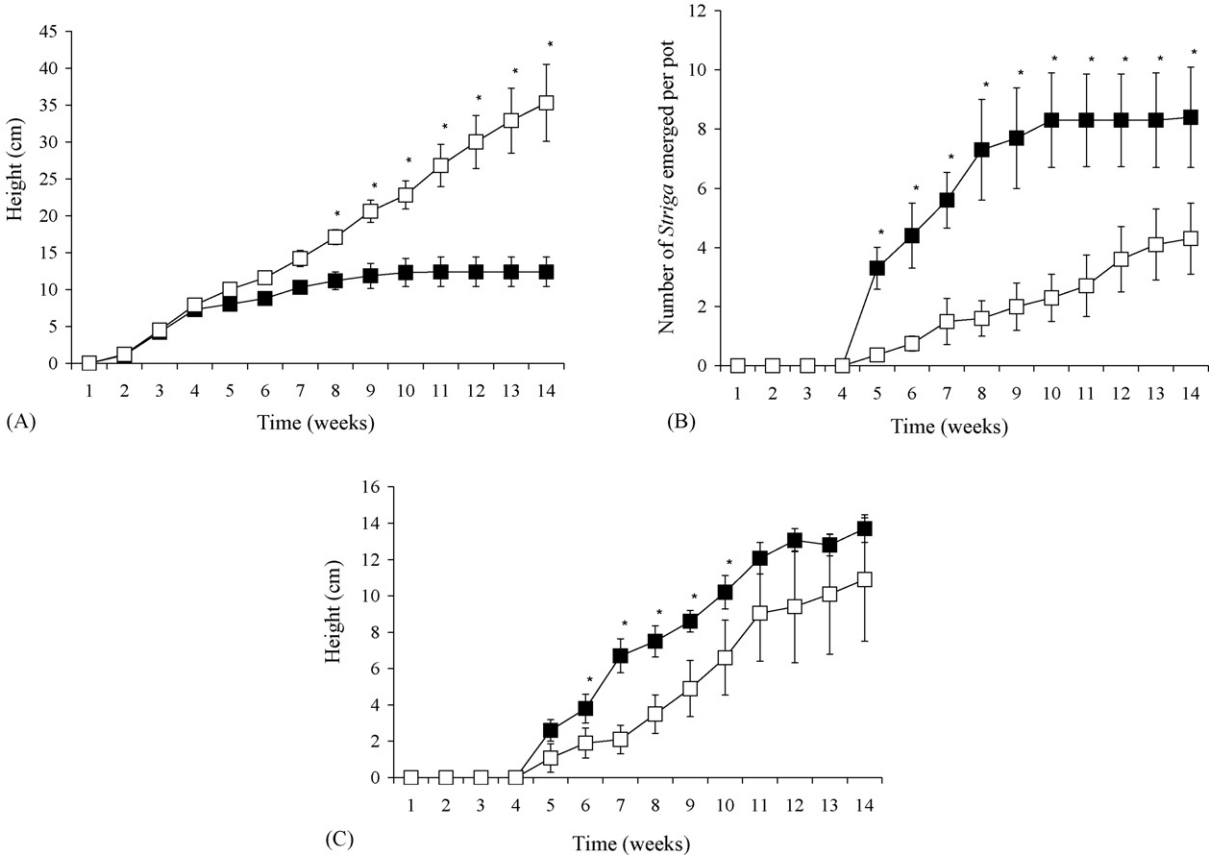

Fig. 1 – Time course changes in sorghum (A) and *Striga* (C) height and the number of *Striga* emerged (B) during the greenhouse experiment in the soils amended with *Cubitermes* mound powder (open squares) or not (closed squares).

3.3. *Effect of Cubitermes mound powder on key microbial groups*

The numbers of saprophytic fungi and actinomycetes were significantly higher in the control than in the amended soil (Table 4) but the differences were not biologically significant. Mycorrhizal colonization was negatively correlated with the number of emerged *Striga* plants per pot ($r = 0.85$; $p < 0.05$) (Fig. 2A) and positively correlated with sorghum growth ($r = 0.78$; $p < 0.05$) (Fig. 2B).

3.4. *Effect of Cubitermes powder on the catabolic potential of microbial community*

Microbial catabolic richness and evenness were not significantly different in the two treatments ($R = 33.7$ and $E = 22.9$ for the control and $R = 34.0$ and $E = 23.5$ for the *Cubitermes* treatment). The co-inertia analysis of parameters related to plant growth, saprophytic fungi, actinomycetes, *Striga* infestation and SIR responses showed that the relationship between the two data sets was significant.

Table 4 – Effect of *Cubitermes* mound powder amendment on the growth of sorghum plants, mycorrhizal colonization and CFU enumeration of saprophytic fungi and actinomycetes after 14-weeks culture

	With *Cubitermes* mound powder	Without *Cubitermes* mound powder
Height (cm)	35.2 (5.2)[a] a[b]	12.4 (1.99) b
Shoot biomass (g dry weight)	6.09 (1.07) a	1.24 (0.26) b
Root biomass (g dry weight)	2.46 (0.58) a	0.57 (0.12) b
Mycorrhizal colonization (%)	45.7 (1.57) a	32.1 (1.89) b
Saprophytic fungi ($\times 10^3$ CFU g^{-1} of soil)	6.2 (0.89) a	8.8 (0.52) b
Actinomycetes ($\times 10^3$ CFU g^{-1} of soil)	5.7 (0.85) a	13.6 (0.51) b

[a] Standard error of the mean.
[b] Means in the same line followed by the same letter are not significantly different according to one-way analysis of variance ($p < 0.05$).

Please cite this article in press as: Andrianjaka, Z. et al., Biological control of *Striga hermonthica* by *Cubitermes* termite mound powder amendment in sorghum culture, Appl. Soil Ecol. (2007), doi:10.1016/j.apsoil.2007.03.001

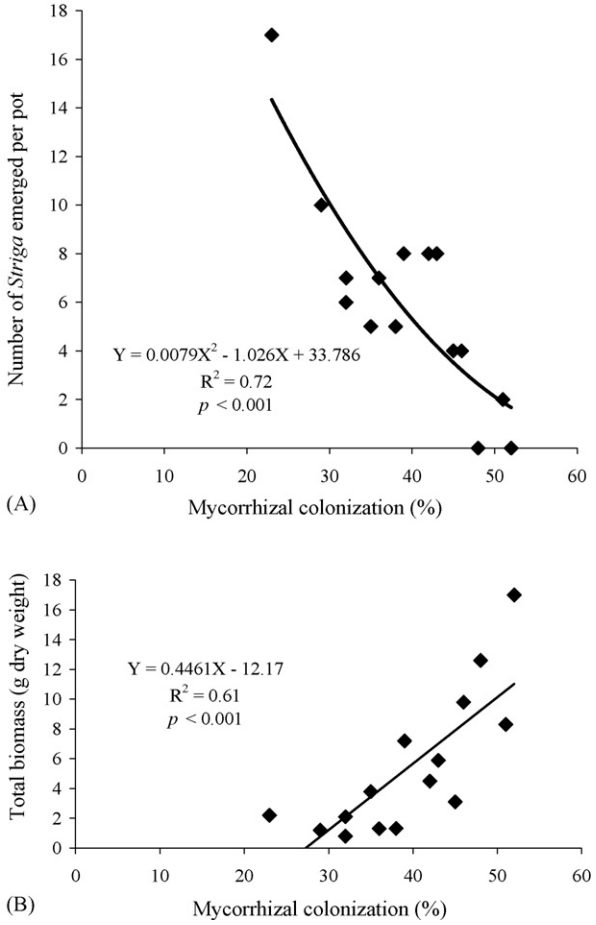

Fig. 2 – Correlations between mycorrhizal colonization and *Striga* development and sorghum growth (A: number of emerged *Striga*; B: total biomass of *Striga* plants).

There was a strong relationship between plant growth and microbial parameters and SIR responses (Monte Carlo permutation; $p < 10^{-3}$). However, there was also a strong relationship between plant growth and *Cubitermes* mound powder amendment (Fig. 3). The factor map of co-inertia analysis for plant growth and microbial variables (Fig. 3A) showed the formation of two clusters: one with sorghum growth and mycorrhizal colonization parameters and the other with parameters related to saprophytic fungi, actinomycetes and *Striga* infestation. We noted a clear distinction between amended (Am) and non-amended (NAm) soil samples. The interpretation of whole factor map showed that sorghum growth was clearly enhanced in the amended soil (right part of the figure), while *Striga* was favored in the non-amended soil (left part of the figure). The non-amended soil was more colonized by actinomycetes while mycorrhizal colonization was larger in the amended soil. We can also distinguish the two soil treatments based on SIR responses. Indeed, the amended soil was characterized by its response to phenylalanine and the non-amended soil by its response to hydroxybutyric acid (Fig. 3C and D).

4. Discussion

From this study, we conclude that *Cubitermes* mound amendment has the potential to decrease damage done by *S. hermonthica* and to significantly increase sorghum growth. Although amendment did not affect directly *Striga* seed germination, it resulted in fewer emerged *Striga* plants and decreased the negative parasitic effect of this plant on sorghum growth. This effect of *Cubitermes* mound powder against *Striga* development can be explained in two main ways: (i) mound powder amendment may induce some soil chemical modifications (e.g. increase of soil N content) unfavorable to *Striga* development and/or (ii) mound powder amendment may modify the structure and functioning of the soil microflora which subsequently has negative effects on *Striga*.

It is generally assumed that nitrogen has a negative impact on *Striga* performance only at high levels (>60 kg ha^{-1}) (Khan et al., 2002). In our study, *Cubitermes* mound powder supplied nitrogen at a rate equivalent to only 0.07 kg ha^{-1}, far lower than the N level at which N addition is efficient against *Striga*. The effect of phosphorus on *Striga* infestation is rather inconsistent in the literature. It has been reported that phosphorus addition reduced germination of parasitic plants *Rhinanthus minor* and *Orobranche minor* (Davies and Graves, 2000; Yoneyama et al., 2001). On the contrary, Lendzemo et al. (in press) did not record any effect of P addition on the levels of seed germination stimulants that were produced. Hence, the impact of *Cubitermes* mound powder amendment against *Striga* was unlikely to be due to soil N and P enrichment.

The mound material amendment in our study significantly increased sorghum growth and decreased the emergence of *Striga* plants. These effects were significantly correlated with the extent of the arbuscular mycorrhizal (AM) symbiosis. Among soil micro-organisms, arbuscular mycorrhizal fungi have been found to be essential components of sustainable soil–plant systems (Smith and Read, 1997; Van der Hejden et al., 1998; Schreiner et al., 2003). It has been previously demonstrated that AM fungi increase sorghum growth, nutrient uptake, and water utilization (Medeiros et al., 1994; Osnubi, 1994). AM fungi may also reduce damage by soil-borne pathogens (Azcon-Aguilar and Barea, 1996) and can retard *Striga* development (Lendzemo and Kuyper, 2001). Indeed, the AM association can led to lower *Striga* germination, attachment, and emergence (Lendzemo and Kuyper, 2001).

Certain plant growth-promoting rhizobacteria, in particularly *Azospirillum brasilense*, can also act against *Striga* germination via bacterial exudates. Indeed, *A. brasilense* strains have been isolated from the rhizosphere of sorghum undamaged by *Striga* plants in an African soil highly infested by these parasites (Kabir et al., 1996). Recent studies have demonstrated that compound(s) released by *A. brasilense* inhibit seed germination and radicle growth of *Striga* (Bouillant et al., 1997; Miché et al., 2000; Dadon et al., 2004). Therefore, we expect that under natural conditions these bacteria could have a major impact both on parasite germination and on sorghum growth.

In the present study, the sorghum plants were not inoculated with AM fungi and yet the development of the native AM fungi community was increased by the mound

Please cite this article in press as: Andrianjaka, Z. et al., Biological control of *Striga hermonthica* by *Cubitermes* termite mound powder amendment in sorghum culture, Appl. Soil Ecol. (2007), doi:10.1016/j.apsoil.2007.03.001

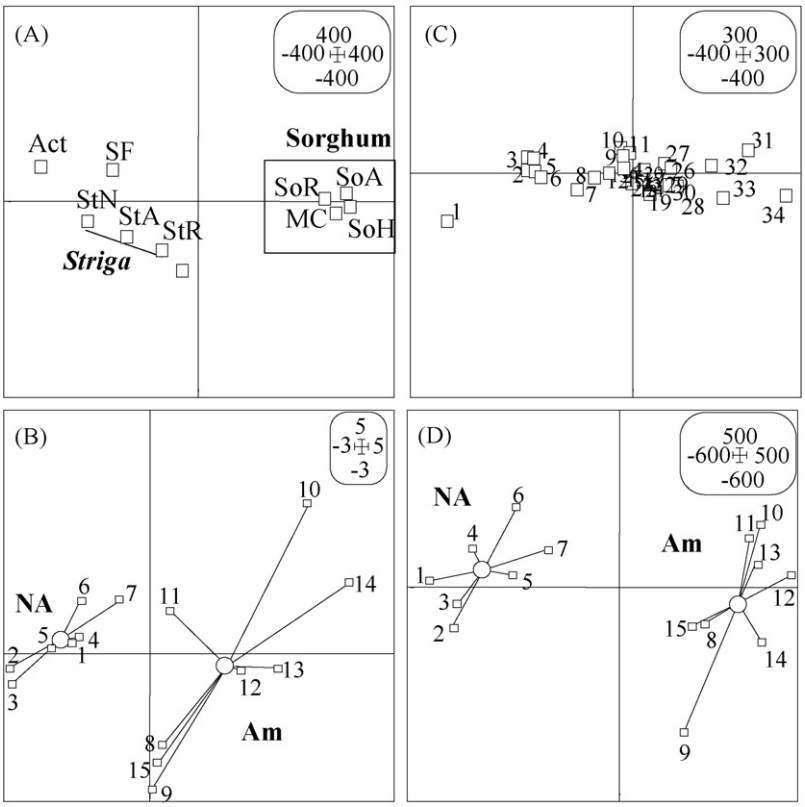

Fig. 3 – Co-inertia analysis of the SIR responses of the soils amended with *Cubitermes* mound powder (experimental treatment) or non-amended (control treatment) and sorghum growth, *Striga* growth and microbiological variables. (A) Factor map of sorghum and *Striga* growth (SF: saprophytic fungi; Act: actinomycetes; MC: mycorrhizal colonization; StN: number of emerged *Striga* per pot; StA: *Striga* aerial biomass; StR: *Striga* root biomass; SoA: sorghum aerial biomass; SoR: sorghum root biomass; SoH: sorghum height). (B) Factor map of sorghum and *Striga* growth and microbial variables soil samples (Am: *Cubitermes* mound powder amendment; Nam: non-amended soil). (C) Factor map of SIR responses (1: phenylalanine; 2: ketobutyric acid; 3: formic acid; 4: ascorbic acid; 5: asparagine; 6: sucrose; 7: oxalic acid; 8: glucose; 9: malic acid; 10: histidine; 11: citric acid; 12: mannose; 13: glutamine; 14: uric acid; 15: cyclohexane; 16: succinic acid; 17: gallic acid; 18: Na-citrate; 19: fumaric acid; 20: cystein; 21: glucosamine; 22: glucamine; 23: glutamic acid; 24: lysine; 25: tartaric acid; 26: gluconic acid; 27: serine; 28: quinic acid; 29: ketoglutaric acid; 30: malonic acid; 31: arginine; 32: succinamide; 33: tyrosine; 34: hydroxy-butyric acid). (D) Factor map of SIR responses soil samples (for the legend, see Fig. 3B).

powder amendment. Epigeal mounds built by soil-feeding termites are considered as islands of higher microbial diversity and activity in tropical areas (Brauman et al., 2000). It could be that the mound powder used in this study contained AM fungi and/or mycorrhiza helper bacteria (MHB) (Duponnois and Garbaye, 1991), bacteria that have the ability to enhance AM symbiosis formation (Paula et al., 1992; Von Alten et al., 1993). In addition, the number of actinomycetes was slightly lower in the amended soil, and it has been demonstrated that this microbial group could be antagonistic to mycorrhizal development. For instance, it has been previously found that antibiotic production by *Streptomyces* isolates may be responsible for an antagonistic effect against *Glomus fasciculatum* (Krishna et al., 1982). The positive influence of *Cubitermes* mound amendment on mycorrhizal formation could be a result of their indirect impact on mycorrhizal-antagonistic micro-organisms.

In conclusion, it appears that *Cubitermes* mound material can be used as amendment and natural microbial inoculum to promote sorghum growth and act against *Striga*. This amendment acts positively on mycorrhizal development, which could be responsible for the better sorghum growth and the inhibition of *Striga* emergence. However, more studies are necessary to identify the specific microbial factors that enhance mycorrhizal formation and to explain biological mechanisms that allow the inhibition of *Striga* development. From a practical point of view, inoculation methods have to be improved in order to optimize the potential of *Cubitermes* mound material to stimulate crop growth and control *Striga* development.

REFERENCES

Ahonsi, M.O., Berner, D.K., Emechebe, A.M., Lagoke, S.T.O., 2002. Selection of rhizobacterial strains for suppression of *Striga hermonthica* (Del.) Benth. seeds. Biol. Contr. 24, 143–152.

Amato, M., Ladd, J.M., 1988. Assay for microbial biomass based on ninhydrine-reactive nitrogen in extracts of fumigated soils. Soil Biol. Biochem. 20, 107–114.

Azcon-Aguilar, C., Barea, J.M., 1996. Arbuscular mycorrhizas and biological control of soil-borne pathogens—an overview of the mechanisms involved. Mycorrhiza 4, 457–464.

Bebawi, F.F., Eplee, R.E., Harris, C.E., Norris, R.S., 1984. Longevity of witchweed (*Striga asiatica*) seed. Weed Sci. 32, 494–497.

Berner, D.K., Kling, J.G., Singh, B.B., 1995. *Striga* research and control: a perspective from Africa. Plant Dis. 79, 652–670.

Black, H.I.J., Okwakol, M.J.N., 1997. Agricultural intensification, soil biodiversity and agrosystem function in the tropics: the roles of termites. Appl. Soil Ecol. 6, 37–53.

Bouillant, M.L., Miche, L., Ouedraogo, O., Alexandre, G., Jacoud, C., Sallé, G., Bally, R., 1997. Inhibition of *Striga* seed germination associated with sorghum growth promotion by soil bacteria. C. R. Acad. Sci. III-vie 320 (2), 159–162.

Bouwmeester, H., Matusova, R., Zhongkui, S., Beale, M.H., 2003. Secondary metabolite signaling in host-parasitic plant interactions. Curr. Opin. Plant Biol. 6, 358–364.

Brauman, A., Bignell, D.E., Tayasu, I., 2000. Soil-feeding termites: biology, microbial associations and digestive mechanisms. In: Abe, T., Bignell, D.E., Higashi, M. (Eds.), Termites: Evolution, Sociality, Symbioses, Ecology. Kluwer Academic Publishers, London, pp. 233–259.

Bremner, J.M., 1965. Inorganic forms of nitrogen. In: Black, C.A. (Eds.), Methods of Soil Analysis, Part 2, Agronomy Monographs, vol. 9. ASA and SSA, Madison, WI, pp. 1179–1237.

Brundrett, M.C., Piche, Y., Peterson, R.L., 1985. A developmental study of the early stages in vesicular-arbuscular mycorrhizal formation. Can. J. Bot. 63, 184–194.

Dadon, T., Nun, N.B., Mayer, M., 2004. A factor from *Azospirillum brasilense* inhibits germination and radicle growth of *Orobanche aegyptiaca*. Israel J. Plant Sci. 52, 83–86.

Davies, D.M., Graves, J.D., 2000. The impact of phosphorus on interactions of the hemiparasitic angiosperm *Rhinanthus minor* and its host *Lolium perenne*. Oecologia 124, 100–106.

Degens, B.P., Harris, J.A., 1997. Development of a physiological approach to measuring the catabolic diversity of soil microbial communities. Soil Biol. Biochem. 29, 1309–1320.

Dray, S., Chessel, D., Thioulouse, J., 2003. Co-inertia analysis and the linking of ecological tables. Ecology 84, 3078–3089.

Duponnois, R., Garbaye, J., 1991. Mycorrhization helper bacteria associated with the Douglas fir—*Laccaria laccata* symbiosis: effects in aseptic and in glasshouse conditions. Ann. Sci. For. 48, 239–251.

Duponnois, R., Paugy, M., Thioulouse, J., Masse, D., Lepage, M., 2005. Functional diversity of soil microbial community, rock phosphate dissolution and growth of *Acacia seyal* as influenced by grass-, litter- and soil-feeding termite nest structure amendments. Geoderma 124, 349–361.

Ejeta, G., Butler, L.G., 1993. Host parasite interactions throughout the *Striga* life cycle, and their contributions to Striga resistance. African Crop J. 1 (2), 75–80.

Fall, S., Hamelin, J., Rouland, C., Chotte, J.L., Lensi, R., Nazaret, S., Brauman, A., 1999. Microbial diversity in soil feeding termites nest in tropical arid soil. In: Proceedings of the International Meeting of the American Society for Microbiology, Chicago, IL.

Garbaye, J., 1991. Biological interactions in the mycorrhizosphere. Experentia 47, 370–375.

Grant, W.D., West, A.W., 1986. Measurement of ergosterol, diaminopimelic acid glucosamine in soil: evaluation as indicators of microbial biomass. J. Microbiol. 6, 47–53.

Heinemeyer, O., Insam, H., Kaiser, E.A., Valenzik, G., 1989. Soil microbial biomass and respiration measurements: an automated technique based on infrared gas analysis. Plant Soil 116, 77–81.

Hooker, J.E., Black, K.E., 1995. Arbuscular mycorrhizal fungi as components of sustainable soil–plant systems. Crit. Rev. Biotechnol. 15, 201–212.

Johnsson, A.W., Roseberry, G., Parker, C., 1976. A novel approach to *Striga* and *Orobranche* control using synthetic germination stimulant. Weed Res. 16, 223–227.

Kabir, M., Faure, D., Heulin, T., Achouawk, W., Bally, R., 1996. *Azospirillum* populations in soils infested by a parasitic weed (*Striga*) under sorghum cultivation in Mali, West Africa. Eur. J. Soil Biol. 32 (3), 157–163.

Khan, Z.R., Hassanali, A., Overholt, W., Khamis, T.M., Hooper, A.M., Pickett, J.A., Wadhams, L.J., Woodcock, C.M., 2002. Control of witchweed *Striga hermonthica* by intercropping with *Desmodium* spp., and the mechanism defined as allelopathic. J. Chem. Ecol. 28, 1871–1885.

Krishna, K.R., Balakrishna, A.N., Bagyaraj, D.J., 1982. Interaction between a vesicular-arbuscular mycorrhizal fungus and *Streptomyces cinnamomeous* and their effect on finger millet. New Phytol. 92, 401–405.

Lendzemo, V.W., Kuyper, T.W., 2001. Effects of arbuscular mycorrhizal fungi on damage by *Striga hermonthica* on two contrasting cultivars of sorghum, *Sorghum bicolor*. Agr. Ecosyst. Environ. 87, 29–35.

Lendzemo, V.W., Van Ast, A., Kuyper, T.W., in press. Can arbuscular mycorrhizal fungi contribute to *Striga* management on cereals in Africa? Outl. Agric.

Lenné, J., 2000. Pests and poverty: the continuing need for crop protection research. Outl. Agric. 29, 235–250.

Magurran, A.E., 1988. Ecological Diversity and Its Measurement. Croom Helm, London.

Medeiros, C.A.B., Clark, R.B., Ellis, J.R., 1994. Growth and nutrient uptake of sorghum cultivated with vesicular-arbuscular mycorrhiza isolates at varying pH. Mycorrhiza 4, 185–191.

Miché, L., Bouillant, M.L., Rohr, R., Sallé, G., Bally, R., 2000. Physiological and cytological studies on the inhibition of *Striga* seed germination by the plant growth-promoting bacterium *Azospirillum brasilense*. Eur. J. Plant Pathol. 106, 347–351.

Olivier, A., 1995. Le *Striga*, mauvaises herbes parasites des céréals africaines: biologie et méthode de lutte. Agronomie 15, Elsevier/INRA ed., 517–525.

Olsen, S.R., Cole, C.V., Watanabe, F.S., Dean, L.A., 1954. Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate. Circular, vol. 939. U.S. Department of Agriculture, Washington, DC, p. 19.

Osnubi, O., 1994. Comparative effects of vesicular-arbuscular mycorrhizal inoculation and phosphorus fertilization on growth and uptake of maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L.) plants under drought-stress conditions. Biol. Fertil. Soils 18, 55–59.

Pageau, K., Simier, P., Naulet, N., Robins, R., Fer, A., 1998. Carbon dependency of the hemiparasite *Striga hermonthica* on *Sorghum bicolor* determined by carbon isotopic and gas exchange analyses. Austr. J. Plant Physiol. 25, 695–700.

Parker, C., 1991. Protection of crops against parasitic weeds. Crop Protect. 10, 6–22.

Parker, C., Riches, C.R., 1993. Parasitic Weeds of the World: Biology and Control. CAB International, Wallingford, UK.

Parkinson, V., Efron, Y., Bello, L., Dashiell, K., 1987. Trap crops as a cultural measure in *Striga* control in Africa. FAO Plant Protect. Bull. 35, 51–54.

Paula, M.A., Urquiaga, S., Siqueira, J.O., Döbereiner, J., 1992. Synergistic effects of vesicular-arbuscular mycorrhizal fungi and diazotrophic bacteria on nutrition and growth of sweet potato (*Ipomoea batatas*). Biol. Fertil. Soil 14, 61–68.

Phillips, J.M., Hayman, D.S., 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid 1assessment of infection. Trans. Brit. Mycol. Soc. 55, 158–161.

Please cite this article in press as: Andrianjaka, Z. et al., Biological control of *Striga hermonthica* by *Cubitermes* termite mound powder amendment in sorghum culture, Appl. Soil Ecol. (2007), doi:10.1016/j.apsoil.2007.03.001

Rank, C., Rasmussen, L.S., Jensen, S.R., Pierce, S., Press, M.C., Scholes, J.D., 2004. Cytotoxic constituents of *Alectra* and *Striga* species. Weed Res. 44 (4), 265–270.

Sauerborn, J., Kranz, B., Mercer-Quarshie, H., 2003. Organic amendments mitigate heterotrophic weed infestation in savannah agriculture. Appl. Soil Ecol. 23, 1181–1186.

Schreiner, R.P., Mihara, K.L., Mc Daniel, K.L., Benthlenfalvay, G.J., 2003. Mycorrhizal fungi influence plant and soil functions and interactions. Plant Soil 188, 199–209.

Smith, S.E., Read, D.J., 1997. Mycorrhizal Symbiosis, 2nd ed. Academic Press, London, UK.

Spain, A.V., Okello-Oloya, T., 1985. Variation in the growth of two tropical pasture plants on soils associated with the termitaria of *Amitermes laurensis* (Isoptera: Termitinae). In: Chapman, R.B. (Ed.), Proceeding of the Fourth Australasian Conference on Grassland Invertebrate Ecology, Lincoln College, Canterbury, May 13–17. Caxton Press, Caldwell, Idaho, pp. 141–145.

Thioulouse, J., Chessel, D., Dolédec, S., Olivier, J.M., 1997. ADE-4: a multivariate analysis and graphical display software. Stat. Comput. 7, 75–83.

Van der Hejden, M.G.A., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., Wiemken, A., Sanders, I.R., 1998. Mycorrhizal fungal diversity determines plant biodiversity ecosystem, variability and productivity. Nature 396, 69–72.

Von Alten, H., Linderman, A., Schönbeck, F., 1993. Stimulation of vesicular-arbuscular mycorrhiza by fungicides or rhizosphere bacteria. Mycorrhiza 2, 167–173.

West, A.W., Sparling, G.P., 1986. Modifications to the substrate-induced respiration method to permit measurements of microbial biomass in soils of differing water contents. J. Microb. Meth. 5, 177–189.

Worsham, A.D., Egley, G.H., 1990. Physiology of witchweed seed dormancy and germination. In: Sand, P.F.., Eplee, R.E., Westbrooks, R.G. (Eds.), Witchweed Research and Control in the United States. Weed Science Society of America, Champaign, USA, pp. 11–26.

Yoneyama, K., Takeuchi, Y., Yoota, T., 2001. Production of clover broomrape seed germination stimulants by red clover root requires nitrate but is inhibited by phosphate and ammonium. Physiol. Plant 112, 25–30.

Please cite this article in press as: Andrianjaka, Z. et al., Biological control of *Striga hermonthica* by *Cubitermes* termite mound powder amendment in sorghum culture, Appl. Soil Ecol. (2007), doi:10.1016/j.apsoil.2007.03.001