EFFECTS OF COMPOST AMENDMENT AND THE BIOCONTROL AGENT *CLONOSTACHYS ROSEA* ON THE DEVELOPMENT OF CHARCOAL ROT (*MACROPHOMINA PHASEOLINA*) ON COWPEA

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SUMMARY

*M. phaseolina* is a destructuve pathogen causing charcoal rot of cowpea and other crops in the semi-arid areas of the Sahel (north-west Africa). Chemical management is not feasible in conditions of subsistence farming, and the plurivorous nature of the fungus limits the effectiveness of some cultural methods. This study aimed at identifying the effects of composting on the survival of *M. phaseolina* and of soil application of compost alone or in combination with the biocontrol agent *Clonostachys rosea* in inoculum density of *M. phaseolina* and on cowpea production. Inside the compost heap with diseased cowpea residues, the temperature reached 52 to 60°C and completely destroyed *M. phaseolina* microcrlerotia. Addition of compost to planting holes significantly suppressed charcoal rot disease. Among the doses tested 6 tonnes of compost alone or supplemented with 50 kg NPK ha⁻¹ resulted in 28-45% lower Area-Under-the-Disease-Progress-Curves (AUDPC) and 43-66% higher cowpea production. The addition of compost combined with *C. rosea* in the planting holes reduced the AUDPC up to 4-fold and increased the grain yield 2-5-fold. The best treatment was a mixture of two *C. rosea* isolates and the compost.

**Key words**: Sahel, planting hole, biocontrol agent, *Clonostachys rosea*, disease progress curves.

INTRODUCTION

Cowpea (*Vigna unguiculata* Walp.) is the most important pulse crop in the Sahel (north-west Africa), with 12.5 Mha yr⁻¹ (Singh *et al.*, 1997). In this region, crop production is most limited by water and nutrient stress (Penning de Vries and Djiteyè, 1982), which are both key predisposing factors to infection by the fungal pathogen *Macrophomina phaseolina*, the causal agent of charcoal rot (*Cook et al.*, 1973; Rose and Barthès, 2001; Songa and Hillocks, 1996). *M. phaseolina* is a soilborne plant pathogen with a very wide host range. Its microcrlerotia, formed in senescing shoot tissues, survive well in soil (Mayek-Pérez *et al.*, 2002; Short *et al.*, 1978). Persisting droughts have led to more continuous cultivation, animal overgrazing, low biomass production and termite activity (Gisse and Hall, 2002; Gisse *et al.*, 1995; Hulme, 1992; Wæzel and Haigis, 2002). As a result, charcoal rot has become increasingly important in cowpea and other crops, including sorghum (*Sorghum vulgare*), groundnut (*Arachis hypogaea*), okra (*Hibiscus esculentus*), sesame (*Dolichos lablab*) and sorrel (*Hibiscus sabdariffa*) (Adam, 1986; Paré, 1990). The yield loss caused by charcoal rot disease has been estimated at about 10% or an annual loss of 30,000 tonnes corresponding to $146 million for Niger and Senegal (T. Adam, personal communication).

Current agricultural practices in the Sahel do not include any methods for the management of charcoal rot disease. Crop rotation is of doubtful value because of the wide host range of the pathogen and its persistence in the soil (Ndiaye *et al.*, 2008). Seed treatment with fungicides against soilborne and seedborne pathogens including *M. phaseolina* (Gamliel *et al.*, 2000; Singh *et al.*, 1990) cannot be afforded by subsistence farmers of the Sahel.

Increasing the soil fertility is however a possibility (Israel *et al.*, 2005; Osunlaja, 1990). This can be done by compost amendment, which can at the same time increase the very low water holding capacity of the soil. Disease suppressive properties of compost have been reported regularly (Hoitink and Boehm, 1999; Noble and Coventry, 2005; Termorshuizen *et al.*, 2006). However, most of the reports concerned potting mixes instead of arable soils. Where significant effects on disease suppression due to organic amendments to arable soils were reported, very high amounts of compost had been applied (Coventry *et al.*, 2005). In preliminary field experiments with broadcasting of 6 tonnes ha⁻¹ compost we did not notice any effect on charcoal rot. As the application of greater quantities of compost is unrealistic due to its limited availability, we here report on the prospects of applying compost in the planting holes, using mixes of compost with soil free from *M. phaseolina*.
By doing so, the young plants, which are relatively susceptible to *M. phaseolina* (Islam et al., 2003), are not predisposed to infection. Such a locally-applied amendment corresponds to the traditional ‘Zaï’ technique practiced in Burkina Faso, where manure is applied in the seeding holes, considerably reducing the amount of manure required (Rose et al., 1993).

The effect of compost on disease suppression is thought to be through a combination of microbial competition, referred to as general suppression, and effects of specific antagonists through commensalism, hyperparasitism or induced resistance, generally referred to as specific suppression (Hoitink and Boehm, 1999). The reliability of compost in suppressing disease can be increased by combining it with a biocontrol agent (Hoitink and Boehm, 1999). In previous laboratory experiments, isolates of *Clonostachys rosea* (Link) Schroers, Samuels, Seifert & Gams (anamorph *Gliocladium verticilloides* P. d. p.) obtained from local arable soils appeared effective in reducing in vitro growth of *M. phaseolina*. *C. rosea* is a naturally occurring antagonist common in arid areas (Sutton et al., 1997). This mycoparasite has a broad host range and has been used successfully to control pathogens such as *Fusarium oxysporum* f. sp. *pisi*, *F. solani* f. sp. *pisi*, *Mycosphaerella pisiodes*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, and *Pythium* spp. (Möller et al., 2003; Xue, 2003). In a field naturally infested with a complex of soilborne pathogens, *C. rosea* increased pea emergence by 15% and yield by 15% yearly over three years (Xue, 2003). Ravnskov et al. (2006) indicated that the influence of *C. rosea* on various soil organisms depended on the presence of organic matter. Therefore, our goal was to test whether the joint application of *C. rosea* with compost in the planting hole would result in effective management of *M. phaseolina*.

Given the ubiquity of *M. phaseolina*, the survival of inoculum during the preparation of compost was tested as well. *M. phaseolina* can tolerate temperatures up to 50-52°C for 120 h in steam-sterilized soils, but the fungus is killed within 8 h at 55°C and within 10 min at 50-52°C for 120 h in steam-sterilized soils, but the fungus can tolerate temperatures up to 60°C (Paharia and Sahai, 1970).

The objectives of this research were: to determine the effect of composting on survival of *M. phaseolina* in cowpea residues, to assess the effect of compost on charcoal rot development and cowpea production, and to determine the effect of joint amendment of compost and *C. rosea* on charcoal rot severity and yield of cowpea.

**MATERIALS AND METHODS**

**Overview.** Four field experiments were carried out: in the dry (Exp. 1) and wet (Exp. 2) seasons of 2003, the wet season of 2004 (Exp. 3) and the wet season of 2005 (Exp. 4). The effect of compost amendment applied to planting holes was assessed on charcoal rot development and on the yield of cowpea in a field naturally infested with *M. phaseolina*. The biocontrol potential of six local isolates of *C. rosea* against *M. phaseolina* was tested in a laboratory and pot experiment in 2005. The potential effects of compost and two local isolates of *C. rosea* were studied in a field naturally infested with *M. phaseolina* in the wet season of 2005 (Exp. 4).

**Location and weather.** All experiments were conducted at the Regional Centre AGRHYMET, Niamey, Niger. The soil of the experimental field was sandy [sand 87%, clay 5%, organic matter 0.4%, pH 7.3, total-N 130 mg kg⁻¹ dry weight (d.w.) soil, total-P 173 mg kg⁻¹ d.w. soil, C/N 11.6]. The mean of daily temperature and relative humidity (RH) of the dry season (April-June) experiment in 2003 were respectively 33°C and 50%. The water needed for crop growth was supplied by drip irrigation. In the wet season (July-September) of 2003, the mean daily temperature was 29°C, RH 70% and rainfall 335 mm. During the experimental period (May-August) in 2004, these figures were 29°C, 70%, and 344 mm, respectively, and in 2005 (May-August) 30°C, 83%, and 531 mm, respectively.

**Microorganisms.** To study the survival of *M. phaseolina* during composting, naturally infected cowpea stems densely infested with *M. phaseolina* microsclerotia were collected in the field. To monitor the viability of sclerotia in the composting pit and to evaluate colonization of cowpea tissue with *M. phaseolina*, 150 mg (d.w.) of the milled tissues were mixed with 100 ml of Semi Selective for *Macrophomina* (SSM) medium and poured into 10 Petri dishes. The SSM medium was prepared as follows: 1.5 ml of 0.525% sodium hypochlorite, 1 ml of 0.5% chloramphenicol dissolved in 95% alcohol, and 10 ml of 2.25% quintozene (PCNB) were added to 100 ml of a potato dextrose agar (PDA) maintained at 55°C in a water bath. The medium was then poured into 10 Petri dishes and incubated for 7-10 days at 33°C, and the colonies of *M. phaseolina* were counted. For the dual culture tests with *C. rosea*, inoculum of *M. phaseolina* isolate GM3 (highly virulent on cowpea, originally isolated from millet root and stored at 5°C on PDA) was used. For the pot experiment with *C. rosea*, inoculum of *M. phaseolina* was prepared by soaking millet (*Pennisetum glaucum*) cv. HKP for 24 h in distilled water, and autoclaving in a 250 ml flask (121°C for 30 min) 50 g of the soaked grains. After cooling, the grains were inoculated with *M. phaseolina* by placing six 5-mm discs from a 3-day-old culture on PDA and the flasks were incubated for 15 days at 30°C. After oven-drying at 37-40°C for 5 days the colonized grains were milled, placed in plastic bags, sealed, and kept at 4°C until use. Isolates of *C. rosea* (Table 1) were obtained from cowpea rhizosphere soil and roots or from rhizosphere soil of sorghum and stored at 4°C. Isolations were made
from soil by serial dilution, and from roots by plating 150 mg milled dry roots on PDA as described above. Isolates were grown on PDA for two weeks at 30°C, and spore suspensions were prepared by washing the culture with sterile water.

Compost preparation. A compost pit 3 m long, 1.5 m wide and 0.20 m deep was dug under a mango tree. After having humidified the bottom, a 3 cm clay layer was laid and then a 5-cm layer of cow manure and a 20 cm layer of 90% millet and 10% fonio (*Digitaria exilis*) straw (cut into pieces about 10 cm long) were placed in the pit. The mixture was well moistened, compacted, flattened, and dusted with 500 g of ash. New layers of manure and straw were then added in the same way up to 0.80 m height. The heap thus obtained was covered with millet stalks. Every 15 days the mixture was turned over, moistened and compacted. Temperature in the heap was recorded daily at 14 h in the centre and at three other spots of the heap 40-60 cm deep, using a thermometer (WWT LF91, Sartorius and Schott, The Netherlands). The temperature of the pit was taken as the mean of the four records. The compost was considered to be mature when the temperature of the heap remained constant after turning over (Yacouba *et al.*, 2001).

Chemical analysis of the compost. Total N and total P content of compost samples was determined according to by Novozamsky *et al.* (1983, 1984). In short, 300 mg air-dried, finely ground compost material was digested using a mixture of H$_2$SO$_4$, Se, salicylic acid, and H$_2$O$_2$. After digestion, N and P were measured using segmented-flow analysis spectrometry. Bioavailable nutrients were determined as described by Houba *et al.* (2000). Air-dried, ground compost samples were extracted for 2 h in 0.01 M CaCl$_2$ using a 1:10 extraction ratio (w/v). In the extract NO$_3$-N, NH$_4$-N, total soluble N (Nt) and PO$_4$-P were determined using segmented-flow analysis spectrometry. Na and K were determined using Flame Atomic Emission Spectrometry (Flame-AES).

The concentration of various forms of N, P and K of the compost used in this study are shown in Table 2. Besides these nutrients, the sodium and organic matter contents are also listed.

Effect of composting on the survival of microsclerotia of *M. phaseolina* in cowpea. Cowpea stems naturally infected with *M. phaseolina* were cut in 3-5 cm long pieces, and well mixed. Fifteen nylon stocking bags containing 5 g of these cuttings were placed on each of the three layers of the compost heap. In addition, 45 bags of cuttings were exposed to ambient conditions next to the compost pit on top of the soil in the shade. Each week, three bags were withdrawn from the compost pit, the cuttings dried at 40°C in an oven for 15 days, ground in a mill (Type MM2, Retsch, GmbH and Co, Germany) and assayed for viable sclerotia using the SSM medium for *M. phaseolina* described above. In the same way, samples of bags exposed to ambient air were analyzed weekly for viable sclerotia.

Biocontrol potential of *C. rosea* against *M. phaseolina*. In vitro experiment. Six isolates of the antagonist were tested for their ability to inhibit the growth of *M. phaseolina* on PDA. Two 6-mm mycelial discs of actively growing cultures of *M. phaseolina* and *C. rosea* were placed side by side (1 cm apart) in the centre of three 9-cm diameter Petri dishes and the plates incubated at 30°C for 7 days (each treatment was repeated three times). The effect of the bioagents was determined by calculating the ratio of growth of the pathogen in the presence and absence of *C. rosea*.

Pot experiment. The two isolates of *C. rosea* that performed best in the in vitro assay were tested for their ability to reduce infection of cowpea by *M. phaseolina* in a

**Table 1.** Isolates of *Clonostachys rosea* used in this study.

<table>
<thead>
<tr>
<th>Lab code</th>
<th>Abbreviation</th>
<th>Origin</th>
<th>Year of isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PiRNC1</td>
<td>Cr1</td>
<td>cowpea root</td>
<td>2004</td>
</tr>
<tr>
<td>PiRNC2</td>
<td>Cr2</td>
<td>cowpea root</td>
<td>2004</td>
</tr>
<tr>
<td>PiRNC3</td>
<td>Cr3</td>
<td>cowpea root</td>
<td>2004</td>
</tr>
<tr>
<td>PiRNC4</td>
<td>Cr4</td>
<td>cowpea root</td>
<td>2004</td>
</tr>
<tr>
<td>UARhS1</td>
<td>Cr5</td>
<td>cowpea rhizosphere soil</td>
<td>2005</td>
</tr>
<tr>
<td>PiRhS2SO</td>
<td>Cr6</td>
<td>cowpea rhizosphere soil</td>
<td>2005</td>
</tr>
</tbody>
</table>

**Table 2.** Nutrient content (mg kg$^{-1}$ d.w.) and organic matter content (%) of the compost. Means of two determinations on three samples each.

<table>
<thead>
<tr>
<th>N-N03</th>
<th>N-NH4</th>
<th>N-organic</th>
<th>P-PO4</th>
<th>Na</th>
<th>K</th>
<th>N-total</th>
<th>P-total</th>
<th>Organic matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>152.4</td>
<td>13.5</td>
<td>386.1</td>
<td>53.6</td>
<td>1100</td>
<td>4827</td>
<td>10419</td>
<td>2491</td>
<td>22.3</td>
</tr>
</tbody>
</table>
pot experiment. The *M. phaseolina*-millet inoculum and spore suspensions of isolates Cr1 and Cr2 of *C. rosea* were added to sterilized soil at a density of 5% (w/w; Mayek-Pérez et al., 2001) and 10^8 CFU g^-1 dry weight soil, respectively, and well mixed. Pots (1000 ml) were filled with the infested soil, watered, placed in the screenhouse overnight and one surface-disinfested cowpea seed cv. Mouride was sown per pot. Treatments were *M. phaseolina* overnight and one surface-disinfested cowpea seed soil, respectively, and well mixed. Pots (1000 ml) were phaseolina units of 0, 52, 104, and 121 kg NPK ha^-1, respectively. by drip irrigation. In 2005 one insecticide application

When needed, plots were weeded with a hoe or watered day during 3 days by a drip irrigation system before soil moisture depth of 15 mm in the seeding line) once a was irrigated with 83 liters of water (corresponding to a used to reduce infections of young plantlets. Each plot was sterilized sand and filling seeding holes. Sterilized sand was been steam sterilized for 8 h 3-4 days prior to planting. experiment confirmed existing knowledge (Ravnskov et al., 2006) that the biocontrol had no effect in the absence of organic matter. Treatments were applied in holes of 20 cm diameter and 15 cm depth. Each plot consisted of 4 rows of 10 hills. Inter-row distance was 60 cm and between-hill distance 30 cm. The total plot size was 7.2 m². Compost (0, 54, 108, or 108 g + 1 g N, P_2O_5, K_2O for treatments c0, c3, c6, and c6+, respectively) was mixed with 2.5 kg of soil per hole, that had been steam sterilized for 8 h 3-4 days prior to planting. In experiment 4 with *C. rosea*, the antagonist inoculum was mixed with the compost prior to mixing with sterilized sand and filling seeding holes. Sterilized sand was used to reduce infections of young plantlets. Each plot was irrigated with 83 liters of water (corresponding to a soil moisture depth of 15 mm in the seeding line) once a day during 3 days by a drip irrigation system before cowpea cv. Mouride was planted (one seed per hole). When needed, plots were weeded with a hoe or watered by drip irrigation. In 2005 one insecticide application

with 12 g ha^-1 deltamethrin (Decis 12 EC, Aventis) was carried out against aphids (*Aphis craccivora*) at the flowering stage. Observations were made every 14 days from germination to harvest. Incidence, disease intensity, and time to death were recorded. Disease incidence was measured as the proportion of plants that were dead or severely wilted at each rating. The area under the disease progress curve (AUDPC) was used to measure disease progression. The standardized AUDPC was calculated following Shaner and Finney (1977):

\[
AUDPC = \sum_{i=1}^{n} \frac{(x_i + x_{i+1})}{2} (t_i - t_{i+1})
\]

in which \( n \) is the number of evaluation times, \( x_i \) is the disease intensity at each evaluation time, and \( (t_i - t_{i+1}) \) is the time duration.

Germination rate and plant survival, pod, grain and hay dry weight 75-90 days after planting were also recorded.

**Effect of compost amendments on soil inoculum of *M. phaseolina* and cowpea production.** 

**Field experiments.** The four field experiments were conducted in a naturally infested soil in a completely randomized block design with three replications. For the 2003 and 2004 trials the treatments consisted of four fertilization rates: 0 ton ha^-1 (code c0), 3 tonnes ha^-1 (c3), 6 tonnes ha^-1 (c6) and 6 tonnes ha^-1 + 50 kg (N, P_2O_5, K_2O) (15:15:15) ha^-1 (c6+), which corresponds to fertilizer units of 0, 52, 104, and 121 kg NPK ha^-1, respectively. In 2005 the treatments consisted of 3 tonnes ha^-1 of compost mixed with *C. rosea* isolates Cr1 or Cr2 (each at 10^8 CFU g^-1 d.w. compost) or the combination of Cr1 and Cr2 (both at a density of 10^8 CFU g^-1 d.w. compost). The control consisted of amendment with compost without inoculum of *C. rosea*, as a preliminary experiment confirmed existing knowledge (Ravnskov et al., 2006) that the biocontrol had no effect in the absence of organic matter. Treatments were applied in holes of 20 cm diameter and 15 cm depth. Each plot consisted of 4 rows of 10 hills. Inter-row distance was 60 cm and between-hill distance 30 cm. The total plot size was 7.2 m². Compost (0, 54, 108, or 108 g + 1 g N, P_2O_5, K_2O for treatments c0, c3, c6, and c6+, respectively) was mixed with 2.5 kg of soil per hole, that had been steam sterilized for 8 h 3-4 days prior to planting. In experiment 4 with *C. rosea*, the antagonist inoculum was mixed with the compost prior to mixing with sterilized sand and filling seeding holes. Sterilized sand was used to reduce infections of young plantlets. Each plot was irrigated with 83 liters of water (corresponding to a soil moisture depth of 15 mm in the seeding line) once a day during 3 days by a drip irrigation system before cowpea cv. Mouride was planted (one seed per hole). When needed, plots were weeded with a hoe or watered by drip irrigation. In 2005 one insecticide application

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AUDPC = \sum_{i=1}^{n} \frac{(x_i + x_{i+1})}{2} (t_i - t_{i+1})
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Germination rate and plant survival, pod, grain and hay dry weight 75-90 days after planting were also recorded.

**Statistics.** The computer program Genstat for Windows, 8th Edition (IACR-Rothamsted, UK) was used for statistical analyses of the parameters studied. All data were subjected to analysis of variance (ANOVA) following a randomized complete block design. The treatment means were compared using Duncan’s Multiple Range Test at \( P = 0.05 \). Where needed, data were transformed by log (x+1) before statistical analysis.

**RESULTS**

**Temperature kinetics in the compost pit and microsclerotia viability.** The highest temperature measured during composting was 64°C, 8 days after preparation of the pit. The mean daily temperature in the pit exceeded 50°C for 22 days and 55°C for 13 days (data not shown). The mean weekly temperature in the compost pit was 46, 56, and 52°C in the first, second, third, and fourth week, respectively (Fig. 1).

The density of sclerotia in cowpea residues before composting was high (2691 sclerotia g^-1 d.w.). During composting, the density of viable microsclerotia decreased from week 3 onwards and the pathogen was undetectable from week 9 onwards. No significant changes were noticed in the number of microsclerotia in crop residues exposed to ambient conditions (Fig. 1).

**Disease development and cowpea yield in 2003 and 2004.** In the dry season of 2003, disease developed most prominently, as expressed by the relatively high AUDPC levels and the low yields (Fig. 2). Plants in the control plots were significantly \( P < 0.021 \) more damaged than in the compost-amended plots except for AUDPC at 3 tonnes ha^-1. Compost amendment consistently led to
higher yields. The disease suppressive effect was significant \((P = 0.003)\) at 3 tonnes ha\(^{-1}\) in 2004 and for the other two experiments at 6 tonnes ha\(^{-1}\) compost. At this last amendment level, AUDPC was 28, 56 and 45% lower than the control for the 2003 dry, 2003 wet and 2004 experiment, respectively. Yields were 66, 43 and 59% higher than the control, respectively. The effect of additional NPK was more evident for the yield than for the AUDPC over the three years of the study (Fig. 2).

Effect of joint amendment of compost and *Clonostachys rosea* on charcoal rot development and on cowpea production. Among the tested isolates of *C. rosea* in dual culture, Cr1 and Cr2 showed the strongest antagonistic activity; these isolates completely overgrew the colonies of *M. phaseolina* within 7 days (Table 3). No inhibition zones between radial growths of the two fungi were observed.

In the pot experiment, the two isolates of *C. rosea* strongly and significantly suppressed *M. phaseolina*, resulting in healthy plants and low microsclerotial densities (Table 4). Seedling emergence increased from 44\% for the treatment with *M. phaseolina* alone to 89\% for either Cr1 or Cr2 co-inoculated with *M. phaseolina*. Combining isolates Cr1 and Cr2 resulted in 98\% emergence and the least infection (29 microsclerotia g\(^{-1}\) tissue) (Table 3).

In the field experiments, there was significantly more disease in the plots amended with compost alone than in the plots amended with both the compost and the *C. rosea* isolates Cr1 and Cr1Cr2 (Fig. 3). Plant yield was significantly increased by the *C. rosea* treatments but Cr1 performed better than Cr2 and joint inoculation of Cr1 and Cr2 resulted in the highest yields (Fig. 3).

**DISCUSSION**

In moist conditions both high (> 50°C) and low (< 5°C) temperatures have been reported to adversely affect the survival and growth of *M. phaseolina* (Papavizas, 1977; Sheik and Ghaffar, 1987). In the present
study, composting was effective in destroying microsclerotia in plant debris. Temperatures in the compost heap remained high (46-60°C) during the first 4 weeks of composting, which likely caused the sharp reduction in microsclerotia. Indeed, Sheik and Ghaffar (1987) reported that in wet soil, viability of sclerotia of *M. phaseolina* was destroyed at constant temperatures > 55°C for 1 day or for 2 h per day for 14 days. Turning the compost every 15 days allowed the exposure of all parts of the compost heap to lethal temperatures. High moisture content (Dhingra and Sinclair, 1975; Sharma et al., 1995; Sheik and Ghaffar, 1987), release of volatile toxic compounds during composting (Lodha et al., 1997), and high levels of microbial populations are other factors that likely reduced the viability of the microsclerotia. The release of volatile toxic compounds such as ammonia has also been reported during the decomposition of pearl millet debris (Sharma et al., 1995), which was the main component of the compost heap.

Charcoal rot development, expressed by AUDPC, was more severe during the dry than the wet season, which likely was due to the high temperatures (33°C) and low relative humidity (50%), which both predispose hosts to infection by *M. phaseolina* (Mihail, 1989). Compost amendment significantly suppressed charcoal rot disease. Six tonnes of compost alone or amended with 50 kg ha⁻¹ NPK improved cowpea production by 60% and sup-

<table>
<thead>
<tr>
<th>C. rosea</th>
<th>M. phaseolina</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolate</td>
<td>Diameter of colony (cm)</td>
</tr>
<tr>
<td>Cr1</td>
<td>8.3a</td>
</tr>
<tr>
<td>Cr2</td>
<td>6.5b</td>
</tr>
<tr>
<td>Cr3</td>
<td>6.3b</td>
</tr>
<tr>
<td>Cr4</td>
<td>4.4c</td>
</tr>
<tr>
<td>Cr5</td>
<td>3.3c</td>
</tr>
<tr>
<td>Cr6</td>
<td>5.9b</td>
</tr>
</tbody>
</table>

Figures followed by the same letter are not significantly different at *P* < 0.05. In control Petri dishes the mean diameter of the colonies of *M. phaseolina* was 8.5 cm and that of *C. rosea* 9 cm 7 days after incubation at 30°C.

![Table 3. Colony diameter of *Clonostachys rosea* and *Macrophomina phaseolina* plated side by side (1 cm apart) in the centre of a 9-cm diam Petri dish, and extent of growth inhibition of *M. phaseolina*. Data are means of three replications.](image)

**Table 4.** Effect of *Clonostachys rosea* (two strains, Cr1 and Cr2, alone or in combination) on infection of cowpea seedlings by *Macrophomina phaseolina*. Data are means of three replications.

<table>
<thead>
<tr>
<th>M. phaseolina</th>
<th>C. rosea</th>
<th>Seeding stand (%)</th>
<th>Seeding d.w. (g plant⁻¹)</th>
<th>Root d.w. (g plant⁻¹)</th>
<th>Microsclerotia g⁻¹ d.w. root</th>
<th>Microsclerotia g⁻¹ d.w. stem tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>-</td>
<td>100a</td>
<td>0.84a</td>
<td>0.30a</td>
<td>0e</td>
<td>0a</td>
</tr>
<tr>
<td>-</td>
<td>Cr1Cr2</td>
<td>100a</td>
<td>0.86a</td>
<td>0.27a</td>
<td>0e</td>
<td>0a</td>
</tr>
<tr>
<td>+</td>
<td></td>
<td>44b</td>
<td>0.10c</td>
<td>0.08b</td>
<td>330a</td>
<td>176b</td>
</tr>
<tr>
<td>+</td>
<td>Cr1</td>
<td>89a</td>
<td>0.85a</td>
<td>0.20a</td>
<td>43c</td>
<td>0a</td>
</tr>
<tr>
<td>+</td>
<td>Cr2</td>
<td>89a</td>
<td>0.43b</td>
<td>0.20a</td>
<td>90b</td>
<td>0a</td>
</tr>
<tr>
<td>+</td>
<td>Cr1Cr2</td>
<td>98a</td>
<td>0.85a</td>
<td>0.22a</td>
<td>294d</td>
<td>0a</td>
</tr>
</tbody>
</table>

*Observed 7 days after the onset of the experiment. *Observed 45 days after the onset of the experiment. *Figures followed by the same letter are not significantly different.
pressed charcoal rot significantly by 52% per season during three consecutive seasons. These doses probably reduced the number of root infections and subsequently led to reduction in wilt expression and plant mortality.

Compost-amended sandy soils hold more water than non-amended soils which in turn can reduce the *M. phaseolina* population or its infection on the host plant due to enhanced antagonism or site competition. For chickpea, Husain and Ghaffar (1995) reported that wilting of plants occurred within 60 days at 10-20% MHC and 10-15 days later at 40-50% MHC, although root colonization by *M. phaseolina* was greater at 40-50% MHC than at 10-20% MHC. In our study, it was evident that application of compost increased the soil MHC and consequently reduced the drought stress of cowpea plants. In the dry season, water uptake by roots of infected plants did not compensate for water loss by plant transpiration. Consequently, plants faced moisture stress, which predisposed them to infection by *M. phaseolina*. In preliminary experiments it appeared that broadcasting 6 tonnes ha⁻¹ compost before sowing did not affect plant yield or disease severity at all. Generally, application of compost at higher rates leads to more disease suppression (Blok et al., 2000).

To prevent early seedling infection, we used sterilized soil mixed with compost in the seeding hole. This is similar to the so-called ‘virgin soil technique’ introduced by Ko (1982). This method implies the use of pathogen-free soil around the plant. In the Sahelian environment this could be obtained by the use of steam-sterilized soil, of any other soil free of the pathogen, for example after solarization of a small soil surface (since smallholder farmers cannot afford plastic for treatment of large fields; Ndiaye et al., 2007), or of non-cropped dune sand. Although steam-sterilized soil was used, high infection by *M. phaseolina* was observed in the non-amended controls, indicating the importance of the compost addition.

Several mechanisms of antagonism of pathogens by *C. rosea*, including mycoparasitism, nutrient competition, and antibiotic have been suggested (Sutton et al., 1997). Characterization of the antagonistic action of local isolates of the biocontrol agent showed that the isolates did not form an inhibition zone in dual culture with the pathogens. The antagonist acted predominantly by entwining (and parasitizing) the hyphae of *M. phaseolina* and probably also by competition for space and nutrients. These results are in accordance with the findings of Xue (2003) who showed that the antagonism of *C. rosea* isolate ACM941 was a result of mycoparasitism.

In the field, joint amendment of 3 tonnes compost and *C. rosea* Cr1 or Cr1G2 was as effective as application of 6 tonnes compost + 50 kg NPK to increase yield and to reduce disease development of cowpea. According to Ravnskov et al. (2006), in addition to its antagonistic activity, *C. rosea* isolate IK726 had a growth-promoting effect on tomato due mainly to increased P-solubilisation. Therefore, the high yield of cowpea observed in the field experiment could be a result of a reduced cowpea infection by *M. phaseolina* and improved nutrient uptake by the plants.

Our results indicate that in Sahelian sandy soil, good control and substantial increase of cowpea yield can be achieved by soil amendment with 6 tonnes compost ha⁻¹. An even greater yield increase is achieved by soil amendment with 6 tonnes compost and 50 kg NPK ha⁻¹ or 3 tonnes compost augmented by *C. rosea*. Two local isolates of *C. rosea* have good potential as biocontrol agents for *M. phaseolina*. The best control of the disease was obtained when the two isolates were combined. Further work is underway to test the effect of these isolates as seed treatment on the development of charcoal rot disease and production of cowpea.

REFERENCES


