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Crop Protection xxx (2010) 1–8

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

A novel, integrated method for management of witches' broom disease in Cacao in Bahia, Brazil

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A R T I C L E I N F O

Article history:
Received 21 April 2009
Received in revised form
8 February 2010
Accepted 10 February 2010

Keywords:
Biological control
Mycoparasites
Tricoderma
Integrated management

A B S T R A C T

A three-year field study was conducted in Bahia, Brazil to validate several strategies for management of witches' broom disease in cacao caused by *Moniliophthora perniciosa*. Treatments which were applied alone or in combination included applications of biological control fungus *Trichoderma stromaticum*, fungicide copper hydroxide and phytosanitary broom removal. When compared with untreated control treatments, higher pod yields and consistently lower pod losses were obtained by alternating fungicide with biocontrol application. Pod losses caused by witches' broom were also reduced by fungicide treatment or by phytosanitary broom removal when applied alone or in combination, however total pod production per tree was consistently low whenever broom removal was used as a management strategy. While application of biocontrol fungus alone was not able to reduce witches' broom on pods, it reduced vegetative broom formation and also increased the number of pod-forming flower cushions. The present study indicates that alternating fungicide copper hydroxide with biocontrol fungus *T. stromaticum* without expensive phytosanitary broom removal is not only a better disease management strategy in Bahia but also results in better yields and thereby better net economic returns.

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1. Introduction

Cacao production in Brazil has been in decline for a number of years since the outbreak in the late 1980s (Pereira et al., 1990) of witches' broom disease caused by the basidiomycete fungus *Moniliophthora perniciosa* (=*Crinipellis perniciosa* Aime and Phillips-Mora, 2005). Since the outbreak, several thousand hectares of once productive cacao farms have been abandoned in Brazil and also in other parts of Latin America (Evans and Prior, 1987; Pereira et al., 1996; Griffith et al., 2003; Hebbar, 2007). The state of Bahia accounts for nearly 60% of the total cocoa production in Brazil. Cacao is planted there under the shade of forest species and under highly humid conditions with year round rainfall (Midlej and Santos, 2007). This environment is ideal for pathogen infection, persistence and rapid spread (Rudgard & Butler, 1987; Andebrhan, 1988;). The basidiospores released by the pathogen infect all meristematic tissues of cacao, including young shoots, flower cushions and pods (Wheeler, 1985). Infected tissues also lose apical dominance and, suffer hypertrophy, resulting in broom formation. Brooms are green and photosynthesize at an early stage, but eventually die and produce basidiocarps containing infective badisiospores on the dry brooms (Wheeler, 1985).

Previous efforts to contain the rapid spread of the disease in Bahia state failed for several reasons, most importantly, the lack of genetic resistance during the initial outbreak of the disease (Pereira et al., 1996). Witches' broom has been equally destructive in neighboring countries, such as Peru, Ecuador and Colombia, where losses range from 30 to 60%, and are often compounded by the presence of another equally-destructive disease, frosty pod rot

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0261-2194/$ – see front matter © 2010 Published by Elsevier Ltd.
doi:10.1016/j.cropro.2010.02.006

Please cite this article in press as: Medeiros, F.H.V., et al., A novel, integrated method for management of witches' broom disease in Cacao in Bahia, Brazil, Crop Protection (2010), doi:10.1016/j.cropro.2010.02.006

caused by *Moniliophthora roreri* (Evans and Prior, 1987; Purdy and Schmidt, 1996; Griffith et al., 2003). Recently in Brazil, there has been a slight improvement in the total production due to deployment of disease tolerant varieties and better understanding of the disease epidemiology (Lopes et al., 2003). Grafting with resistant cultivars is accepted as the quickest method to rehabilitate older farms. Current estimates are that 30% (~150,000 ha) of the 450,000 ha under cacao production in Bahia has been grafted with new tolerant varieties. However, yields in most farms are still low (350 kg/ha) (IBGE, 2007). Reduction in disease pressure using basic good farming practices such as phytosanitation, fertilization or application of fungicides have been practiced in Brazil for several years (Pereira, 1985a; Pereira et al., 1996; Aitken, 1997). Fungicide application on cacao in Brazil was developed mainly for managing black pod disease caused by *Phytophthora* spp. (Pereira, 1985b; Laker and Ram, 1991; Aitken, 1997). Increase in costs of copper-based fungicides over the past few years and low yield (300 kg/ha) has made this expensive intervention uneconomical for the vast majority of farmers.

The Integrated Pest Management (IPM) method currently recommended to the cacao farmers by the Brazilian cacao research and extension agency CEPLAC (Comissao Executiva do Plano da Lavoura Cacaueira) include four to six applications of copper fungicides plus two to four phytosanitary broom removals per year and also application of biocontrol fungus *Trichoderma stromaticum* (Rudgard and Butler, 1987; Aitken, 1997; Almeida et al., 2003). However, the integrated approach has not been adopted widely due to high costs of materials and labor (Aitken, 1997; Midlej and Santos, 2007). Phytosanitation, unless practiced over a wide geographic area, was not effective in controlling the disease (Rudgard and Butler, 1987). Even though 95% of the brooms were removed at an experimental site in Rondonia State in Brazil, 50% of the pods were still infected (Rudgard and Butler, 1987). Therefore, there is a need, especially in low input small farms, to find alternatives, to rationalize the use of expensive chemical fungicides and to reduce the frequency of expensive, labor-intensive phytosanitary broom removal.

Recently, numerous reports have been published on the potential use of biocontrol fungi against witches' broom disease (Costa et al., 1996; Bastos, 1996a; Bastos, 1996b; Krauss and Soberanis, 2001; Bateman et al., 2005; Holmes et al., 2004; Pomella et al., 2007). Biocontrol fungi have been reported to reduce growth, sporulation and spread of cacao pathogens through direct mycoparasitism or through production of active metabolites (Bastos, 1996a; Samuels et al., 2000; Sanogo et al., 2002; Aneja et al., 2005; Degenkolb et al., 2006). Most of these studies have usually consisted of small-scale trials with *T. stromaticum* strain TVC as the active ingredient, as a part of the IPM strategy (Bastos, 1996b; Krauss and Soberanis, 2001; Hjorth et al., 2003; Hebbar, 2007; Pomella et al., 2007). Based on a recent study on diversity and spread of *T. stromaticum* (De Souza et al., 2006), two distinct genetic groups of the biocontrol fungus have been isolated from cacao farms in Bahia, one fast growing and similar to the recently introduced commercial (Tricovab) strain TVC (Group II), and the other (Group I) with no previous record of introduction but fastidious and slow growing. The presence of both the non-introduced or naturally-occurring strain (Group I) and the introduced (Group II) strain in a large area in Bahia associated specifically with the diseased witches' broom tissue (pods and brooms) has characteristics of "classical biocontrol agents". This has been defined by Holmes et al. (2004) as the introduction of a biocontrol agent to a new locale where they did not originate nor occur naturally. This is atypical or unusual for biocontrol agents of fungal pathogens. The naturally-occurring biocontrol fungus can be detected now more frequently than before in cacao farms in Bahia, and not only in areas that were sprayed but also in areas that had not been treated, indicating a possible previous introduction and natural spread of the fungus (De Souza et al., 2006). The Group I strains of *T.stromaticum*, being fastidious, are yet to be mass produced for field application. At present, the population of the naturally-occurring strains in cacao farms is considered not high enough to reduce the current disease pressure. Therefore, larger-scale production and field application has progressed only with the Group II strains of *T. stromaticum*. *T. stromaticum* isolates obtained from the recent survey from Bahia are currently being screened for their biocontrol activity on the brooms (Pomella, unpublished) and strains superior to the original type strain TVC have been obtained (Hjorth et al., 2003; De Souza et al., 2006).

This paper describes results from a three-year field study in a previously abandoned cacao field using current and new approaches for witches' broom control. The objective of this study was to identify a cost-effective and integrated method that could be recommended to cacao farmers in Bahia, Brazil. The following variables were measured (i) the effectiveness of biocontrol fungus *T. stromaticum*, fungicide copper hydroxide or their combinations to increase total pod yields (number of pods per tree) and reducing pod losses (% of pods infected) due to witches' broom disease, (ii) the effectiveness of the current practice of phytosanitary broom removal on pod losses and yields, (iii) to estimate the treatment effects on total and commercial dry bean yields, (iv) test the effectiveness of the above treatments in reducing broom formation on the branches and on the flower cushions and also stimulate flower cushion formation, the pod forming site; and finally (v) to conduct a cost analysis of the above management practices and estimate the economic returns.

2. Materials and methods

2.1. Mass production of Trichoderma

T. stromaticum Group II strain ALF56 used for the field trials was produced by slightly modifying the methodology used for large-scale commercial production of Tricovab (Pomella et al., 2007). Strain ALF 56 used in this study, isolated locally in Bahia, was recently reported to have a better ability to colonize brooms than strain TVC (CEPLAC, Brazil accession #Ts3550 isolated from Belem, Para State) which has been used as an active ingredient in the commercial formulation of Tricovab (Hjorth et al., 2003). Stock cultures stored on sterile filter paper discs at 4 °C were revived by placing them on potato sugar cane agar (PSA) medium and incubating plates at 25 °C for seven days as described previously (Hjorth et al., 2003). Before being mass produced, the mycoparasitic ability of the stored fungal preparation was tested on broom pieces as described previously (Loguercio et al., 2009). Twenty to 30 agar disks (1 cm diam) were removed from the sporulating PSA plate and transferred to 250 ml of potato sugarcane broth (PSB). The PSB broth was prepared by boiling 1 kg of peeled potatoes in 2 L of distilled water and after boiling strained using cheese cloth. To this 1 L of pre-sterilized (autoclaved twice at 121 °C for 30 min to reduce contamination) fresh sugar-cane juice was added and the volume brought up to 3 L with sterile water. The broth was distributed into 500 ml-flasks and autoclaved for 30 min at 121 °C. The culture flasks with 250 ml PSB were shaken at 200 RPM for four days at 25 °C before this culture was used for inoculating (40 ml/bag) plastic bags containing 300 g of sterile rice grains. The rice substrate bags were prepared by mixing 210 ml of distilled water and autoclaving for 60 min at 121 °C for two consecutive days. The inoculated bags were incubated for 48 h at 25 °C and agitated daily in order to aerate and stimulate sporulation. After 2–3 days of incubation and when the fungal spores were visible on the rice grains, the substrate was transferred from the bags to 50 × 30 cm

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sterile plastic trays (three bags of rice per tray) and left to sporulate further for an additional 3–4 days before being transferred to an air conditioned room maintained at 16 °C for drying. The rice substrate was incubated for approximately 7–8 days in the drying room until it had a moisture percentage of 12%. The substrate was mixed regularly for the first three days to improve aeration. After the drying step, purity, spore counts and percentage of spores able to germinate on water agar were checked. The rice substrate was now stored in a refrigerator (7 °C) until needed.

The spores were harvested from the rice substrate by suspending and agitating the rice substrate in sterile water in a 5 L blender. The suspension was then sieved (#50 mesh) to separate the spores and the mycelium from the rice grains. The spore preparation was used for field applications on the same day. The concentration of conidiospores was determined using a hemocytometer. Germination rates greater than 90% were considered ideal for field application. The spore preparations were transported to the field in polystyrene boxes and adjusted to a concentration of 10^7conidia/ml before the suspension was sprayed onto the cacao trees.

2.2. Experimental design and treatments

The experiment was carried out in 24 ha of a previously abandoned, 30 year-old commercial plot at CEPLAC experimental farm (Joaquim Bahiana) located in Itajuipe, Bahia, Brazil with approximately 1000 'Cacau Comun' (SIAL series) trees per hectare. The area was divided into three plots of approximately 5–8 ha each. In view of the persistence and possible spread (especially to the control plots) of the biocontrol agent, the given plots were assigned to the same treatment each year. Based on previous observations, and to avoid cross contamination or spread of the biocontrol agent, large plots were assigned to each treatment (broom removal, biocontrol, and untreated control plots) and split into plus or minus copper spray in a factorial design with two replicates of 25 trees each (Hjorth et al., 2003).

Before the treatments were applied in February 2004, tree height was reduced to 4 m and the branches pruned using traditional macehetes, a common tool used by cacao farmers in Brazil and world-wide. The entire experimental area was weeded with a motorized grass cutter followed by herbicidal spray (Glyphosate, Roundup, Monsanto) before and during the trial as needed. The trees were fertilized with 250 g per tree with N, P, K (20:10:10) in 2003 (late September-October) prior to the beginning of the trial and also in 2004 and 2005, but not in 2006. The field trial was carried out from 2004 to 2006, during which, disease incidence on pods and pod yields were estimated. In addition, the effects of various treatments on broom formation were also evaluated.

There were six management methods tested: (1) copper fungicide alone, (2) *T. stromaticum* alone, (3) broom removal alone, (4) broom removal with copper fungicide, (5) *T. stromaticum* alternated with copper fungicide and (6) non treated control. The copper hydroxide fungicide (Kocide WDG, Griffin LLC, Valdosta) was applied at the rate of 2 kg per hectare (or 2 g/tree) at a volume application arte of 300 ml per tree of approximately 300 L/ha. Spore suspensions (10^7conidia/ml) of *T. stromaticum* strain ALF 56 was applied at the rate of 300 ml per tree (10^9 spores/tree). A motorized mist blower (Guarany 3.5HP, São Paulo, Brazil) was used for spray application, at a volume application rate of 300 L/ha of both copper and *Trichoderma*. Separate mist blowers were used for the copper and biocontrol sprays. Broom removal was performed with machetes or longer pruning poles, depending on their height in the canopy.

Biological control agent was sprayed on trees using a motorized mist blower four times per year, from April to August. The chemical treatment was sprayed three times during the same period, 15 days after the biological treatment. In treatments where the biological agent and copper were combined, fungicides were sprayed fifteen days after the biological treatment. Three rounds of broom removal were undertaken in 2004 (on February 3, June 11 and October 15); in 2005 (on February 2 and July 14, and November 3) and in 2006 (on March 23, Aug 11 and November 15). For treatment evaluations, 25 trees from the middle of the plots were used from each replicate.

Each year, from 5 to 6 harvests, the sum of total number of pods produced, number of healthy pods, number of pods with typical witches' broom symptoms, number of pods with black pod (*Phytophthora* spp.) and other losses were recorded. The two important sources of the witches broom pathogen spores are the vegetative and flower cushion brooms formed due to pathogen infection. Since *T. stromaticum* has been reported to be an effective mycoparasite of the witches' broom pathogen, the hypothesis that it can reduce the primary pathogen inoculum source was tested here by assessing not only the number of green and dry vegetative brooms and also the flower cushion brooms on a monthly basis during 2004, 2005 and 2006 crop cycle. The flower cushions are the site of pod formation and consequently, brooms in the flower cushions also reduces the ability of the tree to set pods. The monthly numbers of active flower cushions formed were also summed from a subset of 10 trees per treatment to obtain annual total number of flower cushions per tree. The data on average monthly rainfall and average daily maximum and minimum temperatures which was recorded during the three years of the trial will not be presented here as no large variations were observed.

2.3. Economic analysis of the treatments

The input costs, total revenue and net returns were used for an economic analysis of the disease management strategies. Inputs or operational costs such as (1) labor for three rounds of broom removal was $900/ha ($300/ha/round) (1 Brazilian Real = 0.571 $US); (2) four rounds of copper fungicide application, R$850 (fungicide, R$500, fuel costs for sprayers R$180, labor r$170); (3) four biocontrol sprays, R$400 (biocontrol agent $50, fuel costs R $180, labor R$170); (4) harvesting R$100 (added to all treatments) were factored into the estimation. Fertilizer application costs were approximately R$450/ha (Material R$350 and labor R$100). Dry bean yields on a, per ha basis were estimated for each treatment by recording the average number of pods (total, healthy and diseased) produced per tree and. this was then multiplied by a factor of 0.040 kg (the total dry bean wt/pod) to obtain the dry bean weight per tree. By multiplying the dry bean yields per tree by a factor of 1000, which is the average trees planted per ha in a cacao farm, yield per ha was estimated. The current (2008) market price per kg of cocoa and the operational costs was then used to estimate total revenue and net returns.

2.4. Statistical analysis

For cumulative annual total yield data, a Poisson regression, with Pearson adjustment for over/under-dispersion, was fitted using PROC GENMOD and all pair-wise mean comparisons were conducted SAS Institute Inc., 2005. Disease incidence was examined using the 5 or 6 month cumulative number of pods exhibiting each disease for each tree. For each of the six treatment regimes, the analysis estimated the total pods produced, incidence of pods with witches' broom symptoms and those that did not show any symptoms (healthy pods). Odds ratios were calculated for selected pairs of the 6 treatments to estimate the treatment effecting disease suppression.

The number of green, dry and flower cushion brooms were analyzed separately through the Area Under Disease Progress Curve

(AUDPC) and analyzing using analysis of variance (Proc Anova, SAS) Fry, 1978; SAS Institute Inc., 2005. Treatment means were compared using Tukey's test. The monthly numbers of active flower cushions (the site of pod formation) formed were also summed from a subset of 10 trees per treatment to obtain annual total number of flower cushions per tree and for mean comparison these figures were transformed according to $\sqrt{(x+0.5)}$ to linearize data and then submitted to analysis of variance.

3. Results

3.1. The effect of treatments on total pod yields

When compared to the various treatment combinations used in this study, alternating fungicide copper hydroxide with biocontrol fungus *T. stromaticum* yielded consistently higher number of pods (38, 43, 23 pods per tree) during the three year study (Fig. 1). The second best treatment for pod yields was when either fungicide or biocontrol fungus was applied alone without phytosaniarty broom removal. Total pod production was consistently lower and comparable to the yields in untreated control treatment whenever phytosanitary broom removal was practiced. This was true either in the presence or in the absence of fungicide application. Total pod yields were consistently lower in the control treatment during all three years of the field trials. When compared to years 2004 and 2005, in 2006 during which no fertilizer was applied the total pod yields was significantly lower across all treatments.

3.2. The effects of treatments on reducing pod losses due to witches' broom disease

The combination of copper fungicide and *T. stromaticum* application without broom removal was the most effective treatment in reducing the incidence of witches' broom on pods resulting in significantly lower percentage (20–28%) of diseased pods compared to the control (>40%) (Fig. 2). This was followed by fungicide sprays alone (22–26% diseased pods) without broom removal. The percentage of diseased pods in treatments with broom removal along with fungicide application or broom removal alone was high (43–44%) in the first year (2004) of the trial, but was lower (21–26%) in the subsequent years. The levels of pod losses during all three years (55%, 34%, 51%) in plots sprayed with *T. stromaticum* alone was high and overall comparable to the losses in the control plots (45%, 49%, 39%) (Fig. 2). As the losses due to black pod and 'other diseases' were negligible (less than 1– 2%) during the three year study, the data are not shown.

3.3. The effect of treatments on estimated total and healthy dry bean yields

As described above, the total pod yields were halved in the absence of fertilizer application in 2006 and, this was further reduced in the absence of disease management due to the witches' broom pathogen. Consequently the dry bean yield estimates (kg/ha) were higher during the first two years of the trial (Table 1). Due to higher total pod yields (38–43/tree) during 2004 and 2005, the bean yield estimated on a per ha basis with 1000 trees/ha (number of pods per tree $\times$ 0.040 kg bean dry wt./pod $\times$ 1000 trees) was highest (1520–1720 kg/ha) in the combined *T. stromaticum* and fungicide treatments. The pod loss due to witches' broom was lower ($\sim$20%) in this treatment, thus the estimated healthy bean yield per ha was consequently higher (1080 kg/ha in 2004 and 1360 kg/ha in 2005) than in other treatments (Table 1). The second best bean yield estimates in 2004 and 2005 were (1240 and 1320 kg/ha) from the plots where fungicide sprays were applied without broom removal. Due to higher pod losses, however, the total healthy dry bean yield estimates per ha in this treatment was $\sim$26% lower (840–880 kg/ha) than in the previous copper plus *T. stromaticum* combination treatment. Even without fertilizer application in 2006, both total ($\sim$900 kg/ha) and healthy bean yield ($\sim$640 kg/ha) estimates were still higher in the above two treatments when compared to control (353 kg/ha) or other treatments (Table 1).

The third best treatment in terms of total bean yields (1200–1320 kg/ha), was with *T. stromaticum* spray alone and this was quite similar to those obtained with copper fungicide treatment alone. However the estimated total healthy dry bean yield per ha was lower (520–720 kg/ha) than the above two treatment

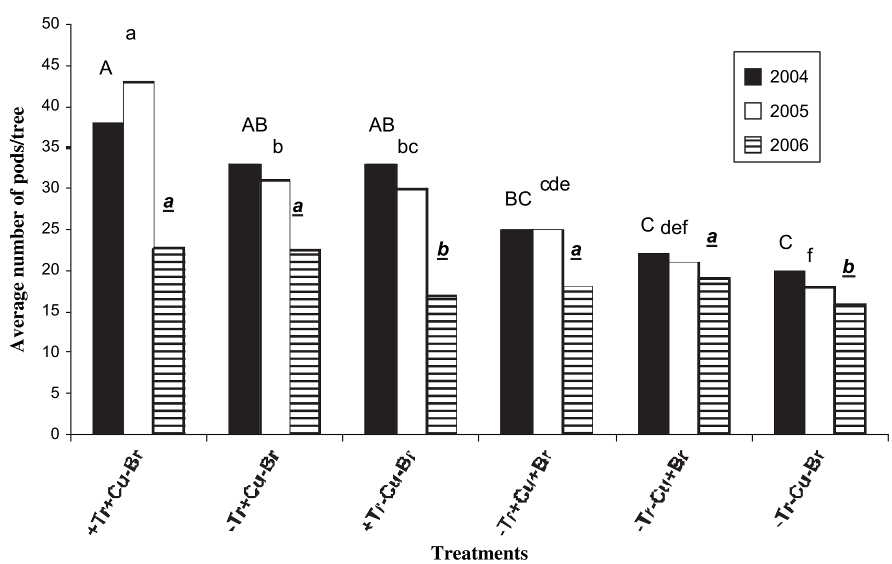

Fig. 1. Effect of various treatments on total pod yields per tree in field trials conducted in Bahia, Brazil. Bars show the mean number of pods per tree per year. Treatments are: Br: phytosanitary broom removal; Ts: application of *Trichoderma stromaticum*; Cu: application of copper fungicide (Kocide) and the control represented by untreated plants. Means followed by the same letter within the years 2004 (capital letters), 2005 (lowercase letters) and 2006 (lowercase, bold and italics) are not significantly different from each other according to Tukey's test ($p \leq 0.05$).

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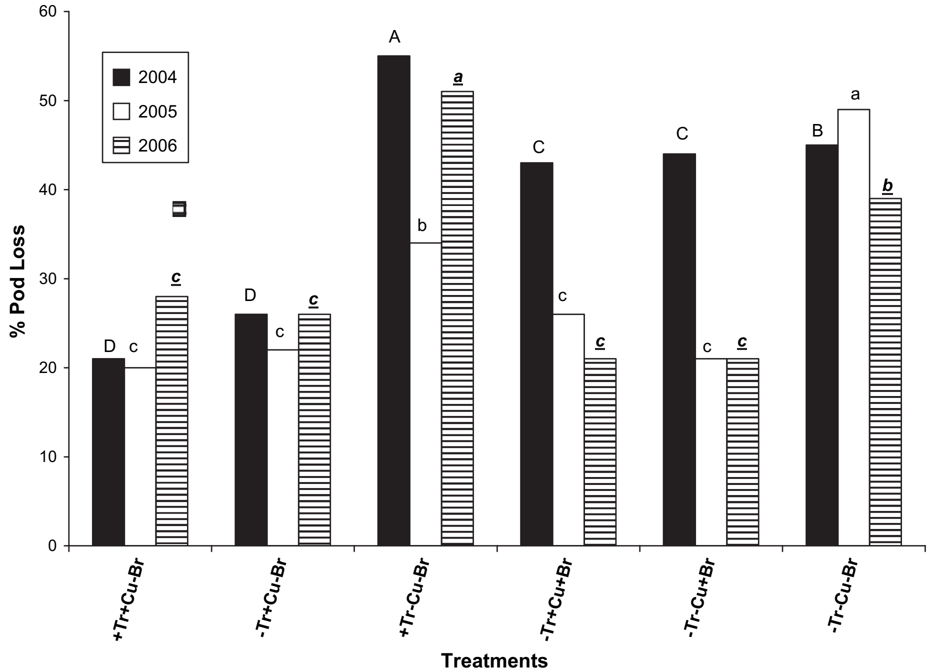

Fig. 2. Effect of various treatments on percentage (%) of diseased pods in field trials conducted in Bahia, Brazil. Bars show the percentage of diseased pods per tree per year. Treatments are: Br: phytosanitary broom removal; Tr: application of *T. stromaticum*; Cu: application of copper hydroxide (Kocide). Means followed by the same letter within the years 2004 (capital letters), 2005 (lowercase letters) and 2006 (lowercase, bold and italics) are not significantly different from each other according to Tukey's test ($p \leq 0.05$).

combinations due to higher pod losses (34–55%). In plots where phytosanitary broom removal treatment was applied along with or without fungicide application, the total bean yield estimates (840-1000 kg/ha) and healthy bean yields (480 to 720 kg/ha) in 2004 and 2005 were also lower than in the combinations described above. Compared to all the above treatments, the untreated control plots had the lowest estimated total bean yield (720–880 kg/ha) and also the lowest yield of healthy beans (320 kg/ha), which is the figure normally obtained in farms in Bahia (18). Although the estimated total and healthy bean yields were lower in 2006 than in years 2004 and 2005, the treatments ranked similar to the previous years with yields being the lowest in the control treatment and higher in the fungicide alone or combined fungicide-biocontrol treatments without phytosanitation. (Table 1).

3.4. Estimate of net returns and economic analysis of the management methods

Economic analysis of the above management methods were conducted based on estimated healthy bean yields and input or operational costs and a complex set of results were obtained. Using the current market prices of 1 kg of cocoa beans, total revenue and net returns were calculated (Table 2). Inputs such as (1) labor for three rounds of broom removal, R$ 900/ha (R$300/ha/round) (1 Brazilian Real = to 0.571 US$); (2) four applications of copper fungicide, R$850 (fungicide, R$ 500; fuel costs for sprayers R$ 180; labor, R$170); (3) four biocontrol sprays, R$400 (Biocontrol agent R$50; fuel costs R$180; labor R$170); (4)

Table 1
Estimated annual yield per ha of cacao beans in field trials in Bahia, Brazil.

Treatment/years	Total beans (kg/ha)[a]			Healthy beans (kg/ha)[b]		
	2004	2005	2006	2004	2005	2006
Copper fungicide (Cu)	1320	1240	904	840	880	656
Trichoderma stromaticum (Ts)	1320	1200	675	525	720	316
Broom removal (Br)	880	840	764	480	640	599
Br + Cu	1000	1000	723	520	720	553
Ts + Cu	1520	1720	909	1080	1360	636
No treatment	800	720	630	320	320	353

[a] Total bean yields/ha = total pods/tree × 0.040 kg bean weight per pod × 1000 trees/ha.
[b] Healthy bean yield/ha = healthy pods/tree × 0.040 kg bean weight per pod × 1000 trees/ha.

Table 2
Projected economic returns from various interventions based on cacao bean yield data collected in the three-year field study.

Variables	Year	Treatments[a]					
		Ts + Cu	Cu	Ts	Br	Br + Cu	No treatment
Estimated yield[b]	2004	1080	840	520	480	520	320
	2005	1360	880	720	640	720	320
	2006	636	656	316	599	553	353
Operational[c] $R costs/year/ha	2004	1800	1400	950	1450	2300	550
	2005	1800	1400	950	1450	2300	550
	2006	1350	950	500	1000	1850	100
Revenue[d] ($R/ha)	2004	3348	2604	1612	1488	1612	992
	2005	4216	2728	2232	1984	2232	992
	2006	1971	2033	979	1857	1714	1094
Net returns[e] ($R/ha)	2004	1548	1204	622	38	−−688	442
	2005	2306	1328	1282	34	−68	442
	2006	621	1083	479	857	−136	994

[a] Treatments: Br: broom removal; Ts: application of *T. stromaticum*; Cu: application of copper fungicide (Kocide).
[b] Healthy bean yield/ha = healthy pods/tree × 0.040 kg bean weight per pod × 1000 trees/ha.
[c] Operational costs: the following costs were used for the economic analysis and (1) labor for three rounds of broom removal, R$ 900/ha (R$300/ha/round); (2) four applications of copper fungicide, R$850 (fungicide, R$ 500, fuel costs for sprayers R$ 180, labor, R$ 170); (3) four biocontrol sprays, R$ 400 (Biocontrol agent R$50, fuel costs R180, labor R$ 170); (4) harvesting costs R$ 100; (5) fertilizer costs $R 450 (material R$ 350, labor R$ 100). Note: fertilizer was not applied in 2006.
[d] Total revenue calculated based on current price of $R 3.1/kg dry beans (US ~$2/kg).
[e] Net returns calculated by subtracting operational costs from the total revenue. 1 Brazilian real = 0.571 $US.

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harvesting R$100. (added to all treatments) and (5) fertilizer costs R$ 450 (material R$ 350/ha and labor $100) were factored into the estimations (Table 2) (Aitken, 1997; Almeida et al., 2003; Midlej and Santos, 2007).

In the first two (2004 and 2005) years of the trial, when the plots were fertilized, the total net returns estimates per ha in the broom removal combined with copper fungicide application treatment with an average yield between 500 and 700 kg/ha, was the lowest (negative returns), followed by the broom removal treatments. The net returns in the control plots, with lower average yields of only 300 kg/ha was better than the above two interventions for all three years. Even, when the yields were lower in 2006, the net returns in the control and the broom removal treatments were much higher than the combined treatment broom removal and copper fungicide . The most expensive intervention was broom removal, followed by fungicide application, fertilization, and biocontrol application, in that order. Application of broom removal along with copper did not improve pod production or bean yield high enough, to make these interventions cost-effective. At least three years of continuous broom removal was needed to reduce losses and improve net returns.

However, application of copper or *T. stromaticum* alone resulted in much higher net returns during the same period, especially in conjunction with fertilizer application. This was due to higher bean yields, lower losses and lower implementation (phytosaniation) cost in the copper fungicide treatment and higher bean yields and lower implementation (phytosaniation and fungicide application) costs in the biocontrol treatment. By year two, both these interventions were similar in terms of net returns. The net returns, in copper fungicide treatment without broom removal was consistent for all three years.

The better overall net returns were in 2004 and 2005 when *T. stromaticum* was applied in combination with copper without the expensive phytosanitary broom removal. When fertilizer application is a part of the management method, there seems to be a synergistic effect of the combined *T. stromaticum* and copper fungicide treatment, even though there was still a 20–28% loss in production due to witches' broom.

3.5. Effects of treatments on broom formation

As expected, the number of dry, green and flower cushion brooms per tree was highest in control trees that did not receive any treatment. When compared to untreated control treatment, application of biocontrol fungus, copper fungicide or broom removal on its own significantly reduced dry, green and flower cushion brooms (Table 3). However, when copper was combined with biocontrol agent or broom removal, there was no further reduction in broom numbers compared to copper application alone. For reasons that cannot be explained, only the flower cushion broom numbers in *T. stromaticum* treatment was significantly lower in the absence of copper fungicide rather than in its presence.

Table 3
Effect of *Trichoderma stromaticum* ALF56 or phytosanitary broom removal and its combination with copper hydroxide on the dry broom, green broom and cushion broom counts per tree from 2004 to 2006.

Variables	Treatments[a]			
	Copper	Trichoderma	No treatment	Broom removal (phytosanitation)
Dry brooms	+	339 a A	176 a B	173 a A
	−	333 ab A	571a A	151 b A
Green broom	+	58 a A	28 a B	40 a A
	−	83 b A	165 a A	19 c A
Cushion broom	+	50 a A	12 b B	5 b A
	−	12 b B	106 a A	3 b A

Values of either + or − copper treatments within each variable row followed by the same lowercase letter are not significantly different from each other according to Tukey's test ($p \leq 0.05$). Number of brooms presented are average values per tree, from three years data.

[a] Treatments applied were copper fungicide, *T. stromaticum*, untreated control, and broom removal.

3.6. Effects of treatments on active flower cushion formation

In the absence of copper fungicide, the use of *T. stromaticum* stimulated significantly the formation of flower cushions, doubling (128/tree) their number compared to the control (58/tree) or broom removal (63/tree) treatments (Fig. 3). In the presence of copper fungicide, these numbers were not significantly different from each other with values of 62, 49 and 89 flower cushions per tree in the *T. stromaticum*, control and the pruning treatments respectively. Among the six treatments in this trial, application of the biocontrol fungus alone had the highest impact by increasing the number of active flower cushions per tree.

4. Discussion

Since witches' broom disease moved into Bahia State in Brazil, efforts made to contain the spread of the disease depended solely on manual phytosanitation and chemical sprays with copper based fungicides, originally developed for use against black pod disease (Pereira et al., 1996). A major constraint for managing this fast spreading disease has been lack of economically viable disease management measures that are able to improve yields and reduce the high losses. In addition, interventions, such as phytosanitation and effective chemical spraying are time-consuming and difficult when used on trees that are above 5 m in height and those cultivated on hillsides. Currently in Brazil and in most parts of Latin America, cacao is a low input crop often grown without or with low chemical inputs. However, current cacao prices are quite stable ~(~US$2000 to R$3500/ton) as a result of which farmers are more inclined to apply cost-effective IPM solutions.

The methodology described in this paper has shown that abandoned or poorly managed cacao farms can be rehabilitated and

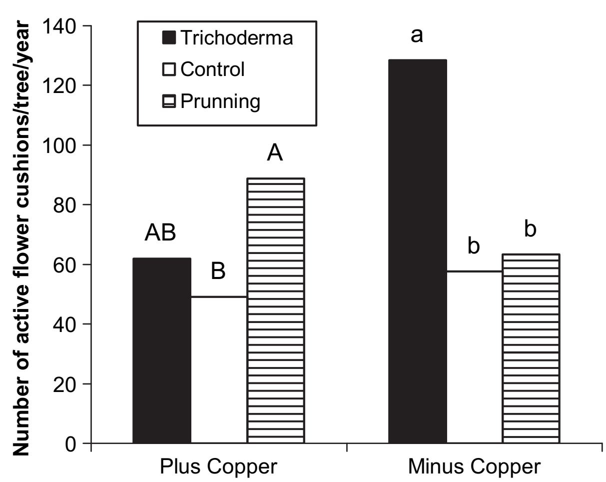

Fig. 3. Effect of copper fungicide (Kocide) on the ability of *T. stromaticum* ALF56 or phytosanitary broom removal to stimulate the number of active flower cushions per tree per year. Bars show the cumulative number of active flower cushion per tree over a year of monthly evaluations. Means followed by the same letter do not differ from each other according to Tukey's test ($p \leq 0.05$). Capital letters represent each treatment added a copper hydroxide treatment (plus copper) and small letters represent each treatment alone (minus copper). For mean comparison data was made linear by $\sqrt{(x+0.5)}$ transformation.

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become productive. This is possible if disease management is combined with other crop management strategies such as fertilizer application. The three promising interventions were (a) three copper fungicide applications alternated with four rounds of biocontrol fungus *T. stromaticum*, (b) three sprays of fungicides alone and (c) three sprays of *Trichoderma* alone. Therefore, if adopted properly, the farmer has at least three choices without using expensive phytosanitation, and which can result in an average of 30 healthy pods per tree. However, it should be pointed out again that increases in pod yields shown here are dependent on adequate fertilizer application.

Although frequent broom removal did reduce disease incidence in the second (2005) and third year (2006) of the trial, it does not seem to increase overall yields significantly even with fertilizer application. If broom removal is followed by application of fungicide, the effect on estimated total bean and healthy bean yield showed only a marginal improvement. However, when compared to the untreated control, alternating copper application with biocontrol sprays, without the use of frequent broom removal, reduced disease incidence (by 50–60%), increased total pod yield per tree and estimated total bean yield per ha (by 50–100%), and also considerably increased (100–300%) estimated healthy bean yield equivalents, the most important factor in a farmer's field. It should be pointed out again that fertilizer application should go hand in hand with the disease management treatments to obtain increased yields.

Given the importance of rainfall on witches' broom disease epidemiology, daily temperatures and rainfall were recorded during the three years of the trial (4, 14, 28, 29). In general the medium temperature was steady at 25 °C (25.2 ± 1.7 for 2004, 25.7 ± 1.6 for 2005 and 24.5 ± 2.1 for 2006) and total rainfall was similar in 2004 (1564.3 mm) and 2006 (1564.6 mm) but was about 25% higher in 2005 (1966.7 mm) with most of the increase in rainfall observed from April to September. The higher rainfall in 2005, however did not affect significantly the percentage of diseased pods or the total pod yields.

A highly complex set of data was obtained from the economic analysis of the management methods used in the current study. The labor and material costs in Brazil have increased over the past few years and it costs between R $285 and R $2000 (US$160–1140) per ha per year, for interventions ranging from minimal to high-input management (Midlej and Santos, 2007). Other costs such as weeding and fertilization are extra. In 2007, the cost of copper fungicides were three to four-fold more than they were in 2000. These expensive interventions have turned cacao crop economically not sustainable not only in Brazil but also in other cacao producing countries. Based on the three-year data shown in this study, the total net returns estimates in the control plots reflected the current situation in Bahia with average yields as low as 300–350 kg/ha/year (Aitken, 1997; IBGE, 2007). The overall yields and consequently the net returns in 2004 and 2005, was better when fertilizer was applied and when biocontrol fungus *T. stromaticum* was applied in combination with copper without the expensive broom removal. This management method due to higher pod yields was still profitable even though there was a 20–28% loss in production due to witches' broom. The best net returns when pod yields were lower as in 2006 was with the copper alone (R $1083) and control plots (R$994). The traditionally recommended broom removal and copper sprays had a negative return due to the costs associated with their implementation. The lower yields observed in this study (in 2006), and also in previous studies, has also shown that fertilizer application should go hand in hand with disease management strategies for obtaining economically viable or adequate pod yields (Krauss and Soberanis, 2001). Because of their high costs, fertilizer is not used regularly by small cocoa farmers. When fertilizer was not applied in 2006, the estimated dry bean yields were reduced by 25–100% depending on the treatment (Table 3). We can only speculate at present whether the sudden drop in yields in the in 2006 were only due to lower soil fertility. The above information highlights the highly complex nature of disease management in cacao, which has to take into account fluctuations in input and also commodity prices.

The current study clearly shows that *T. stromaticum* stimulates formation of active flower cushions (Fig. 3). Similar, improved flower cushion formation was reported from Cameroon with *T. asperellum* when used against black pod disease (Tondje et al., 2007a). The mechanism may be partly explained by the reduction of pathogen inoculum through direct mycoparasitism or through production of anti-fungal compounds such as nonanoic acid, cell wall degrading enzymes and polypeptides by the biocontrol agent (Bastos, 1996a; Aneja et al., 2005; Degenkolb et al., 2006). More research is needed to establish the mode of action of the biocontrol agent in the flower cushions, the important pod forming site on the tree. Although *T. stromaticum* and *T. asperellum* are tolerant to copper hydroxide, the stimulatory effect on flower cushions is not evident in the presence of copper, perhaps due to its fungistatic effect on *Trichoderma* (Veloso et al., 2002; Tondje et al., 2007b). In addition, the ability of *Trichoderma* or broom removal to reduce the number of dry, green or flower cushion brooms varies and is more evident in the absence of copper than in its presence. Despite its ability to increase the number of active flower cushions, biocontrol treatment with *T. stromaticum* does not seem to protect the pods from the witches broom disease. Thus, it is imperative to protect the additional pods formed on these flower cushions, at least for the first three months with fungicide application. In the absence of either biological control or pruning, copper significantly reduces dry, green and flower cushion broom numbers, as previously shown in field trials by Almeida et al. (2003). However, combining copper application with either biological agent or broom removal did not further reduce broom numbers. More work needs to be done on the time delay between the application of biological control agent and the copper treatment so as to minimize the fungistatic effect of copper treatments on the *T. stromaticum*.

Until more farmers in Brazil plant better disease resistant or tolerant trees, the most economical way to manage witches' broom in current susceptible varieties is large-scale adoption of the above methodology. This should include minimal phytosanitation, rational use of chemicals, biological control and also adequate fertilizer application. In the absence of biological control, the brooms which are the reservoirs of the pathogen need to be removed from the trees using expensive and labor intensive phytosanitary pruning. In addition frequent broom removal results in new flushes being formed on the trees and these are highly susceptible to pathogen infection. The semi-commercial product (Tricovab) produced by CEPLAC, is available to growers at a modest cost on a by request basis only. Recent improvements have been made to enhance the product quality by reducing particle size and moisture content of the spore preparation (see www.mycoharvester.info). Ensuring sufficient supplies of high quality biocontrol formulations will be crucial for the successful implementation of this novel management technology.

Acknowledgements

The work was supported by Fundação de Amparo a Pesquisa do Estado da Bahia (FAPESB), providing financial support for the trials and assistantship for the first author. Various additional aspects of this work have been sponsored by USDA and the cacao sustainability initiative of Mars Inc. Our sponsors support policies, programmes and projects to promote international development

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and have provided funds for this study as part of that objective. However, the opinions expressed are those of the authors alone; trade names and commercial products are described in this article solely for the purpose of providing specific information and neither the authors nor our sponsors imply recommendation or their endorsement.

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