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Effect of fertilization and biocontrol application frequency on cocoa pod diseases

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Abstract

Three native and two commercial biocontrol agents (*Clonostachys rosea* and *Trichoderma* spp.) were evaluated against the cocoa diseases moniliasis, witches' broom, and black pod. Antagonists were applied either separately or as mixed inoculum in comparison with a copper fungicide and a nontreated control. Cultural control (weekly removal of diseased pods) was practiced in all treatments. Field trials were conducted on neglected cocoa farms in eastern Peru from 1998 to 2000. The fungicide treatment and *C. rosea* strain G-4 did not reduce disease. The other single-strain antagonists reduced moniliasis. Additionally, *Trichoderma longibrachiatum* and *Trichoderma stromaticum* reduced witches' broom and *Trichoderma virens* reduced black pod. With strain mixtures, yield increases of up to 15% were obtained. A mixture of four antagonists was superior to mixtures of two or three antagonists with respect to multiple disease control and yield. Biocontrol in combination with cultural control was more economical than cultural control alone; chemical control was least economical. Fertilization improved yields by 11% independent of the disease control measure and compensated for the additional costs with net returns improving by 9%. Increasing application frequencies of a mixed biocontrol inoculum improved moniliasis and witches' broom control linearly. Moniliasis exhibited a stronger response. Witches' broom was significantly lower than the nontreated control only if ten applications were administered in two-week intervals. This application frequency increased yields by 15%. It was followed by three applications adjusted to the production cycle (yield increase: 12%). Three adjusted applications were the most economical biocontrol strategy under the conditions of eastern Peru. Net returns were increased by 12%. Recommendations for technology transfer are presented.

Keywords: Antagonism; Application frequency; Biological control; Black pod; *Clonostachys rosea*; Cocoa; Copper fungicide; *Crinipellis perniciosa*; Fertilization; Moniliasis; *Moniliophthora roreri*; *Phytophthora palmivora*; *Theobroma cacao*; *Trichoderma longibrachiatum*; *Trichoderma stromaticum*; *Trichoderma virens*; Witches' broom

1. Introduction

Moniliasis, caused by *Moniliophthora roreri* (Cif. and Par.) Evans et al.; witches' broom, caused by *Crinipellis perniciosa* (Stahel) Singer; and black pod, caused by *Phytophthora palmivora* (Butl.) Butl. are the most devastating cocoa pod diseases in Peru. The incidence of moniliasis is increasing (Soberanis et al., 1999), with pod losses up to 100% reported (Evans et al., 1998). The arrival and spread of moniliasis during the early 1990s, together with a lack of effective and economical disease control, resulted in the abandonment of 16,500 ha of cocoa out of a total of 32,000 ha (Servicio Nacional de Sanidad Agraria, 2000), rendering Peru a net importer of cocoa beans (Fujimori et al., 1996). In an attempt to reverse this trend, 7500 ha are targeted for rehabilitation (Servicio Nacional de Sanidad Agraria, 2000).

Until recently, cultural disease control, which involves the regular removal of diseased pods and of vegetative brooms, was the only measure available to the small-holder farmer in eastern Peru. Weekly removal of diseased pods was most efficient and economical. Compared with removal at two-week intervals, the incidence of moniliasis was reduced by 26–41%, witches' broom by 14–57%, and black pod by 35–66%. Weekly removal of diseased pods improved yields by 31% and net returns by 32%, as compared with removal at two-

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week intervals. In comparison with traditional management, which comprises harvesting and manual weed control only but lacks disease control measures, the positive effect of cultural management was even more striking (Soberanis et al., 1999). However, since the study by Soberanis et al. (1999), cocoa prices have fallen by over 40%. Although the relative benefit of the different management strategies is quite insensitive to cocoa prices, adoption of the recommendation has plummeted because of decreasing net returns. If the national plan of rehabilitation is to be followed, supplementary disease control methods must be implemented.

Krauss and Soberanis (2001a) isolated five native mycoparasitic strains of *Clonostachys rosea* (Link: Fr.) Schroers et al. and three of *Trichoderma* spp. They were compared in combination or separately with the commercial biocontrol agent *Trichoderma virens* (Miller et al.) Arx strain GL-21 isolated from SoilGuard for their potential to control moniliasis, witches' broom, and black pod. All but one of the treatments selected for field trials reduced moniliasis, the main disease, by 15–25%. No significant reduction of witches' broom and black pod was achieved, but the performance of a combination of five *C. rosea* strains was consistently better against all three diseases simultaneously. Yields increased by 16.7% and net returns by 24% during the first two years of rehabilitation in previously abandoned farms.

In the present study, we concentrated on the improvement of neglected rather than abandoned farms in the Huallaga and Monzón valleys of eastern Peru in order to develop recommendations for each step in the rehabilitation program. The specific objectives of this study were (1) to compare biocontrol with chemical control in addition to cultural control; (2) to improve the previously reported inoculum mixture to achieve significant reduction of all three diseases simultaneously; (3) to optimize the application frequency to effect more economical biocontrol; and (4) to investigate the compatibility of fertilization with biocontrol with the aim to increase overall productivity.

2. Materials and methods

2.1. Fungal material

Plant pathogens and their mycoparasites were isolated in the surroundings of Tingo María, Peru, using the pre-colonized plate baiting technique as previously described (Krauss and Soberanis, 2001a). Three native antagonists were used in this study: two strains of *Clonostachys rosea* (G-4 and G-7 = IMI 382480) and one strain of *Trichoderma longibrachiatum* Rifai (IMI 382482). These local isolates were compared with commercial biocontrol strains *T. virens* strain GL-21 isolated from SoilGuard, (Grace Biopesticides, Columbia, MD, USA) and *Trichoderma stromaticum* Samuels et al. isolated from Tricovab (CEPLAC, Brazil). The latter (previously called *Trichoderma viride* Pers.) has successfully been used against witches' broom (Bastos, 1996). Reference strains were submitted to the International Mycological Institute, CABI Bioscience, UK, for identification. Fungal stock cultures were kept on potato dextrose agar (PDA) slants and under water.

Inoculum for field trials was prepared using a two-step liquid/solid-state fermentation adapted after Hebbar and Lumsden (1999). Conidial suspensions of mycoparasites were obtained by flooding PDA plates with sterile distilled water (SDW) and filtering it through Whatman filter paper No. 2 to remove hyphal fragments. The suspensions were subsequently used to inoculate 250 ml Erlenmeyer flasks containing 100 ml of SDW with 3% molasses and 0.5% brewers yeast. The flasks were incubated on an orbital shaker (Newbrunswick Scientific, Model G-33, Edison, N.J., USA) at 100 rpm for 5 days. Polypropylene bags were filled with 500 g rice and 100 ml tap water and autoclaved. After cooling to room temperature, they were inoculated with 50 ml of the liquid fermenter. The bags were incubated for 10 days at ambient temperature (ca 24 °C) under fluorescent light.

For quality control, 10% of rice bags were randomly sampled. The solid rice substrate (1 g) was extracted in SDW (24 ml with 1% Tween 80). The conidial concentration was measured using a haemocytometer. All antagonists yielded concentrations of $\geqslant 10^7$ cfu ml^{-1} after 15 days in the two-step liquid/solid-state fermentation. Suitable dilutions were plated onto PDA and incubated for 8–9 h for *T. virens* and *T. longibrachiatum*, or 10–11 h for *C. rosea* and *T. stromaticum*. Germination was checked under a compound microscope counting at least 100 spores on each of five replicate plates. Only inoculum with 100% germination was used in field trials.

In the field, antagonists were applied at a concentration of 10^7 cfu ml^{-1} and a rate of 3.13×10^{12} cfu ha^{-1}. When strain mixtures were used, they had equal proportions totaling 10^7 cfu ml^{-1}.

2.2. Field trials

A preliminary screen of effectiveness against *M. roreri* was done by the use of seedling bioassays (Krauss and Soberanis, 2001a). Three field trials, each with three farms, were conducted in the 1998/1999 season (one trial) and the 1999/2000 season (two trials), which lasted from November/December to September/October and were within the typical meteorological range. Details on the experimental area were previously given (Krauss and Soberanis, 2001a,b;

Soberanis et al., 1999). Fields were located in Afilador, Castillo Grande, Pendencia, and Bella within the same region where mycoparasites had been isolated. All plots had a minimum size of 0.5 ha and were at least 4 km apart. Tree spacing was 4 m $\times$ 4 m. All fields were under mixed shade (25–50%) dominated by *Inga edulis*. They contained a mixture of cocoa hybrids, dominated by Forastero-types, of 10–15 years of age under low-input management. This type of management consists of regular harvesting as well as occasional manual weed control and cultural disease control. No reliable information regarding the frequency and thoroughness of crop husbandry prior to the installation of trials could be obtained. Some trees in Bella had been rejuvenated by grafting in 1994, but these were not used in the trial.

In the crop year 1998/1999, treatments consisted of the five antagonists applied individually four times, in monthly intervals, starting at the peak of flowering in December 1998. For comparison, a copper fungicide (Akuprox Q, Dipagro, Lima, Peru) was used at the recommended dosage of a.i. 3 g l^{-1}. Nonsprayed plots served as the control.

In the crop year 1999/2000, the fertilization trial and the application frequency trial were conducted. For the fertilization trial, the fungicide treatment and an absolute control were compared with four mixed biocontrol inocula: (1) *T. longibrachiatum* + *T. stromaticum*; (2) *T. longibrachiatum* + *T. virens*; (3) *T. longibrachiatum* + *T. stromaticum* + *T. virens*; and (4) *C. rosea* strain G-7 + *T. longibrachiatum* + *T. stromaticum* + *T. virens*. Ten applications were administered every two weeks starting at flowering. Fertilization consisted of applying 500 g *Guano de Isla* and 200 g KCl (both Ministry of Agriculture, Proabono, Peru) to the foot of one-half of the experimental plants at the peak of flowering. At the end of this trial, a soil sample consisting of five subsamples of 200 g each (total 1 kg) was collected in the 10–30 cm soil layer under the canopy of cocoa trees in each field and submitted to the Soil Science Laboratory of the Universidad Nacional Agraria de la Selva (UNAS) for analysis.

For the frequency of application trial, a single mixed inoculum contained *C. rosea* strain G-7, *T. longibrachiatum*, *T. stromaticum*, and *T. virens*. Six treatments comprised different application frequencies: (1) no application (control); (2) single application in December, 1999; (3) three applications in 45-day intervals; (4) five applications in 30-day intervals; (5) ten applications in 15-day intervals, all starting in December 1999 and being continued through April, 2000 ("regular applications"); and (6) a treatment of three applications with the first one applied in December, 1999, and the remaining two in February, 2000, in 15-day intervals ("adjusted application"). The December application coincided with the flowering peak.

Cultural disease control was practiced in all trials and all treatments (including the control) and consisted of weekly removal of diseased pods as recommended for that region by Soberanis et al. (1999).

2.3. Experimental design, data recording, and statistical analysis

Field trials followed the randomized block design with three fields representing blocks. Each treatment was represented by 40 cocoa trees in 1998/1999 and 20 trees in 1999/2000. The fertilization trials had a factorial arrangement with six treatments representing one factor and the level of fertilization with the other factor. Chemical soil characteristics for fertilized and nonfertilized plots were compared by paired t tests.

The evaluation period commenced three weeks after the first application of biocontrol agent in order to avoid recording pods with a latent infection contracted prior to biocontrol application. Evaluation consisted of weekly harvesting and counting healthy, mature pods and quantifying and removing any diseased pod of at least 8 cm in length. Dead pods shorter than 8 cm were attributed to cherrelle wilt, a physiological disorder that affects young pods and cannot be distinguished with certainty from diseases at this stage (Evans, 1981). Vegetative brooms were not quantified.

The field trials yielded binomial (percentage) or Poisson-distributed (counts) data, which were analyzed using the appropriate general linear model on Genstat 5, Release 2.2. The 'RPAIR' procedure was used to allocate differences between means (Genstat 5 Committee, 1993). Linear regression was employed to analyze the effect of application frequency on disease incidence and yield. For this analysis, proportions were arcsine-transformed ($y' = \arcsin\sqrt{y}$, where y is the proportion ranging from 0 to 1), and angles were measured in grad in correspondence with the 0–100 range of percentages.

3. Results

3.1. Comparison of single-strain antagonists with fungicide

In the 1998/99 trials, the fungicide did not reduce any of the three pod diseases significantly (Table 1). Absolute yield, measured as number of healthy pods, was increased by 7%, but the percentage of healthy pods was not significantly augmented. *C. rosea* G-4 was ineffective against all three diseases, and no yield increase was observed. All the other biocontrol treatments reduced moniliasis significantly and increased yields both in terms of percentage of healthy pods and absolute yield. In addition to moniliasis, *T. longi-*

Table 1
Disease incidence (%), healthy pods (%), and numbers of healthy pods (per tree) in three biocontrol field trials in eastern Peru

Disease incidence (%)	Fungicide treatment	Biocontrol treatment					Untreated control
		Clonostachys rosea		*Trichoderma*			
	Akuprox Q	G-4	G-7	*virens*	*stromaticum*	*longibrachiatum*	
Moniliasis	22.8[cd]	24.7[d]	21.1[bc]	18.8[a]	20.1[ab]	18.9[a]	24.1[d]
Witches' broom in pods	6.3[c]	8.0[d]	7.2[cd]	6.5[c]	3.5[a]	5.1[b]	6.8[c]
Black pod	3.6[ab]	4.0[b]	3.2[ab]	2.9[a]	3.5[ab]	3.4[ab]	4.0[b]
Yield							
Healthy pods (%)	67.3[bc]	63.3[a]	68.5[c]	71.8[d]	72.9[e]	72.6[de]	65.1[ab]
Healthy pods per tree	23.3[c]	18.7[a]	23.2[c]	23.8[c]	24.2[c]	26.1[d]	21.8[b]

[a, b, c, d] Values within a row followed by the same letter do not differ at $P = 0.05$.

brachiatum and *T. stromaticum* reduced witches' broom, whereas *T. virens* reduced black pod. The native *T. longibrachiatum* led to the greatest absolute yield increase of 19.9% (Table 1).

3.2. Fertilization and biocontrol

In this factorial trial, the effect of fertilization on disease control was investigated. Table 2 shows the results of a soil analysis conducted at the end of the trials to assess whether medium-term changes in soil fertility took place. For most parameters, a consistent but small increase was noted. However, the increase was usually not significant. Only phosphorus was significantly increased ($P = 0.004$). There was a notable increase in potassium (K) in Pendencia, the only location from which these data are available.

Different mixtures of antagonists were evaluated in comparison with a fungicide treatment and an absolute control in the presence or absence of fertilization. The results are shown in Table 3. Moniliasis incidence was consistently lower with fertilization than without fertilization ($P = 0.009$). The mixture of all four mycoparasites was the most effective control measure, closely followed by the mixture of *T. longibrachiatum* and *T. virens*. Whereas all biocontrol treatments reduced moniliasis significantly compared with the absolute control, the fungicide treatment was again ineffective (Table 3). There was no significant interaction between fertilization and control measure (deviance ratio 0.88). The incidence of witches' broom was not affected by fertilization ($P = 0.67$). Only the mixture *T. longibrachiatum* + *T. virens* significantly reduced the incidence of witches' broom as compared with the control (Table 3). The incidence of black pod was below 1% with no significant differences between treatments; therefore, this disease was not tabulated. For both percentage of healthy pods and the number of healthy pods

Table 2
Effect of fertilization on soil chemical characteristics

Location	Bella (sandy loam)		Castillo (silt loam)		Pendencia (silt loam)		Mean		*P* (pair-*t* test)
Fertilization	No	Yes	No	Yes	No	Yes	No	Yes	
pH (in water)	4.2	4.6	4.7	5.0	6.8	6.8	5.2	5.5	0.096
CO_2Ca (%)	0	0	0	0	1.61	1.30	nd[a]	nd	nd
Organic matter content (%)	0.84	4.66	1.42	3.29	3.80	3.87	2.02	3.94	0.109
Nitrogen (%)	0.03	0.20	0.06	0.14	0.17	0.17	0.09	0.17	0.116
Phosphorus (ppm)	6.6	10.2	6.8	9.6	7.1	9.8	6.8	9.9	0.004
K_2O (kg ha^{-1})	121	146	118	108	161	186	133	147	0.186
K (me per 100 g)	nd	nd	nd	nd	1.1	10.6	NA[b]	NA	NA
Ca (me per 100 g)	nd	nd	nd	nd	12.4	13.6	NA	NA	NA
Mg (me per 100 g)	nd	nd	nd	nd	2.1	2.7	NA	NA	NA
Ca + Mg (me per 100 g)	3.8	4.2	3.9	4.3	14.5	16.3	7.4	8.3	0.102
Al + acidity (me per 100 g)	2.0	2.1	2.0	2.2	nd	nd	NA	NA	NA
Effective cation exchange capacity (me per 100 g)[c]	5.0	6.3	5.9	5.3	nd	nd	NA	NA	NA
Cation exchange capacity (me per 100g)[c]	nd	nd	nd	nd	15.9	17.7	NA	NA	NA

[a] nd, not determined.
[b] NA, not applicable.
[c] For soils of pH $\leqslant 5.0$, the effective cation exchange capacity was determined, comprising the four bases Ca, Mg, K, Na, and exchangeable acidity (Al + acidity); whereas for soils of pH > 5.0, the cation exchange capacity is based on all cations, many of which can be measured individually.

Table 3
Effect of fertilization on biocontrol efficiency

Fertilization	No	Yes
Moniliasis incidence (%)		
Control	19.2[a]	16.7[abc]
Fungicide	19.5[a]	17.1[ab]
T. longibrachiatum + *T. stromaticum*	15.6[bcde]	13.5[de]
T. longibrachiatum + *T. virens*	14.8[bcde]	12.8[e]
T. longibrachiatum, *T. stromaticum* + *T. virens*	15.8[bcd]	13.7[de]
C. rosea, *T. longibrachiatum*, *T. stromaticum* + *T. virens*	14.7[cde]	12.7[e]
Witches' Broom incidence (%)		
Control	4.5[ab]	3.7[abcd]
Fungicide	5.5[a]	4.6[ab]
T. longibrachiatum + *T. stromaticum*	4.2[abc]	3.4[bcd]
T. longibrachiatum + *T. virens*	2.8[de]	2.2[e]
T. longibrachiatum, *T. stromaticum* + *T. virens*	4.0[abcd]	3.3[cde]
C. rosea, *T. longibrachiatum*, *T. stromaticum* + *T. virens*	4.0[abcd]	3.3[cde]
Percentage healthy pods (%)		
Control	76.2[ab]	79.4[c]
Fungicide	75.0[a]	78.3[bc]
T. longibrachiatum + *T. stromaticum*	79.8[c]	82.7[def]
T. longibrachiatum + *T. virens*	82.0[cde]	84.7[f]
T. longibrachiatum, *T. stromaticum* + *T. virens*	80.2[cd]	83.0[ef]
C. rosea, *T. longibrachiatum*, *T. stromaticum* + *T. virens*	81.2[cd]	83.9[ef]
Number of pods (per tree)		
Control	23.7[e]	26.3[cd]
Fungicide	24.6[de]	27.3[bc]
T. longibrachiatum + *T. stromaticum*	27.7[bc]	30.7[a]
T. longibrachiatum + *T. virens*	26.3[cd]	29.1[ab]
T. longibrachiatum, *T. stromaticum* + *T. virens*	27.4[bc]	30.4[a]
C. rosea, *T. longibrachiatum*, *T. stromaticum* + *T. virens*	26.1[cd]	29.0[ab]

[a,b,c,d,e,f] Values followed by the same letter do not differ at $P = 0.05$ (comparison of 12 values for the same parameter only).

per tree (Table 3), parallel trends were observed. Fertilization increased relative ($P = 0.002$) and absolute yield ($P < 0.001$) without interaction with the disease control measure (deviance ratios 2.63 and 0.05, respectively). All biocontrol treatments increased yields to a similar extent, whereas the fungicide had no significant effect.

For an economic comparison (Table 4) of cultural, chemical, and biological disease controls, the different yields from biocontrol treatments were averaged because they did not differ significantly (Table 3). Chemical control was the least economical. Biocontrol not only led to the highest yield increase but was also most profitable in terms of net returns. The benefit-cost ratio was highest for cultural control alone, followed by biocontrol, and lowest for fungicidal control. Fertilization increased production costs, but this was more than compensated for by increased gross returns independent of the disease control measure. Although net returns were consistently improved by fertilization, the benefit-cost ratio was decreased. Overall, net returns were highest for biocontrol combined with fertilization (9.1% above cultural control without fertilization), followed by biocontrol without fertilization (3.6% increase) (Table 4).

3.3. Biocontrol application frequency

A third field trial had the objective to optimize the application frequency of biocontrol agents. A mixed inoculum containing all strains used in 1998 and 1999, except the inefficient *C. rosea* G-4, was employed (Fig. 1). All biocontrol application frequencies greater than one significantly reduced the incidence of moniliasis compared with the control (zero applications). There was no significant difference between three, five, and ten regular applications. Three adjusted applications fell into the same range of efficacy. However, a clear trend of lower moniliasis incidence with higher regular application frequencies was observed ($r = -0.793$, df $= 13$, $P < 0.01$, $y = -0.70x + 27.38$). Only the highest application frequency (ten applications at two-week intervals) significantly reduced witches' broom incidence. Again, there was a significant decrease in disease incidence with increasing frequency of regular applications ($r = -0.870$, df $= 13$, $P < 0.001$, $y = -0.43x + 13.61$). The percentage of healthy pods was positively correlated

Table 4
Economic extrapolation of fertilization and disease control

Disease management option	Control		Fungicide		Biocontrol	
Fertilization	No	Yes	No	Yes	No	Yes
Extrapolated yield (kg ha^{-1} yr^{-1})	1244	1381	1292	1433	1411	1565
Gross returns ($ ha^{-1} yr^{-1})[a]	1033	1146	1072	1190	1171	1299
Production cost ($ ha^{-1} yr^{-1})[b]	293	381	576	664	404	492
Net returns ($ ha^{-1} yr^{-1})	740	765	496	526	767	807
Benefit-cost ratio	3.53	3.01	1.86	1.79	2.90	2.64

[a] Based on a farm-gate price of US$ 0.83 kg^{-1}.

[b] Composed of fixed production costs of US$ 293 ha^{-1} yr^{-1} including weekly removal of diseased pods, biocontrol costs of US$ 111 ha^{-1} including materials and labor, fungicidal control of US$ 283 ha^{-1} yr^{-1} (both for ten annual applications), and fertilization costs of US$ 88 ha^{-1} yr^{-1}.

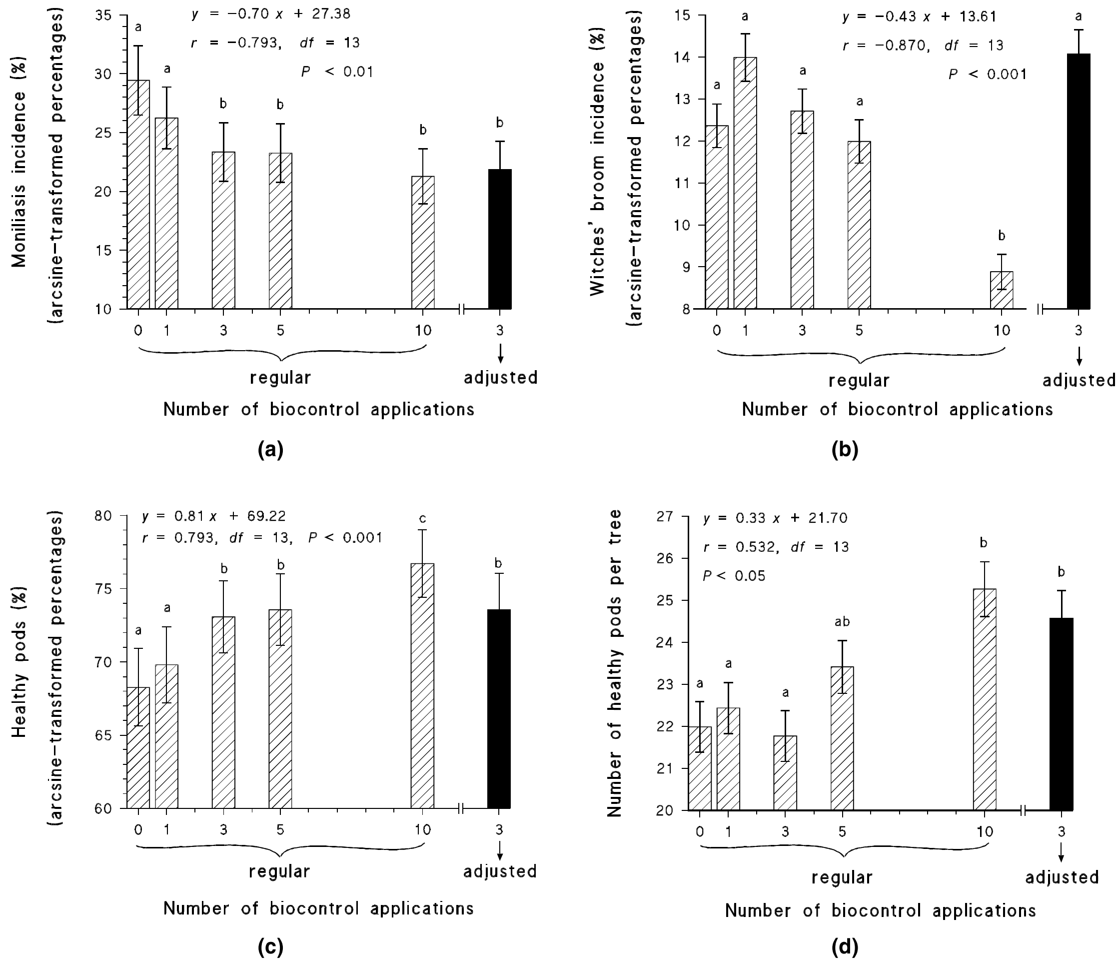

Fig. 1. Effect of frequency of application of mixed biocontrol inoculum on (a) moniliasis, (b) witches' broom, (c) percentage healthy pods, and (d) number of healthy pods per tree (mean ± SE). Bars with the same letter do not differ at $P = 0.05$ (comparison of all treatments). Regression equations refer to regular application intervals only (hatched bars). Adjusted application: first application was applied at the onset of flowering as for all the other treatments; the remaining applications were applied eight and ten weeks later, respectively (solid bars).

with regular application frequency ($r = 0.793$, df $= 13$, $P < 0.001$, $y = 0.81x + 69.22$). The percentage was highest with ten applications; a single application had no significant effect. Similarly, absolute yields increased with regular application frequency ($r = 0.532$, df $= 13$, $P < 0.05$, $y = 0.33x + 21.70$). They were highest for either ten regular applications (15% yield increase) or three adjusted applications (12% yield increase), followed by five regular applications (6.4% yield increase). One and three regular applications did not increase yield significantly (Fig. 1).

An economic extrapolation (Table 5) indicates that the highest net returns were achieved with three adjusted applications, followed by ten regular applications. Three regular applications were least profitable, even less than the cultural control alone (zero applications). Interestingly, the benefit-cost ratio for cultural control alone (3.26) was only second to the three adjusted applications (3.28). In contrast to net returns, the benefit-cost ratio (2.72) was lowest for ten regular applications.

4. Discussion

Whereas the earlier study (Krauss and Soberanis, 2001a) concentrated on previously abandoned cocoa fields, our work focused on farms under low-input management in accordance with the aim of gradually rehabilitating abandoned and neglected cocoa farms into productive ones (Servicio Nacional de Sanidad Agraria, 2000). Previously, the effectiveness of biocontrol agents plus cultural control was compared with cultural control alone (Krauss and Soberanis, 2001a). In the trials presented here, a copper fungicide (Akuprox Q) was also included. This treatment proved consistently ineffective (Tables 1 and 3).

All single-strain antagonists, except *C. rosea* G-4, effected a significant reduction of moniliasis, the main disease of the area. As single strains, only *T. longibrachiatum* and *T. stromaticum* reduced witches' broom (Table 1), and the combinations of *T. longibrachiatum* + *T. virens* and *C. rosea* + *T. longibrachiatum* +

Table 5
Economic extrapolation of biocontrol application frequency

Number of applications Interval	0 NA[a]	1 NA	3 45 days	5 30 days	10 15 days	3 Adjusted[b]
Extrapolated yield (kg ha^{-1} yr^{-1})	1150	1176	1139	1223	1323	1287
Gross returns ($ ha^{-1} yr^{-1})[c]	954	976	945	1015	1098	1068
Production cost ($ ha^{-1} yr^{-1})[d]	293	304	326	349	404	326
Net returns ($ ha^{-1} yr^{-1})	661	672	619	667	694	742
Benefit-cost ratio	3.26	3.21	2.90	2.91	2.72	3.28

[a] NA, not applicable.
[b] The first application was administered in early December, at the onset of flowering as for all the other treatments. The remaining applications were applied in February in 15-day intervals.
[c] Based on a farm-gate price of US$ 0.83 kg^{-1}.
[d] Composed of fixed production costs of US$ 293 ha^{-1} yr^{-1} including weekly removal of diseased pods and biocontrol costs of US$ 11.12 ha^{-1} per application including materials and labor.

T. stromaticum + *T. virens* controlled witches' broom at high application frequencies (Table 3 and Fig. 1). However, witches' broom control was erratic, and the latter treatment was only convincing in one of the two trials in the same season. *T. virens* controlled black pod in the first year, but disease incidence was too low to corroborate conclusions in the second year. Neither disease had been controlled successfully in the earlier study (Krauss and Soberanis, 2001a). *T. stromaticum* is a known mycoparasite of *C. perniciosa* (Bastos, 1996; Samuels et al., 2000), and *P. palmivora* is highly susceptible to *T. virens* strain GL-21 (Krauss and Soberanis, 2001a). In previously abandoned farms, black pod incidence was higher than in the present study, and *T. virens* failed to reduce this disease. Weekly removal of all diseased pods was practiced in both studies, but it is likely that higher disease pressure was present on abandoned farms with only recently resumed crop management than on neglected farms. This may have facilitated a more rapid re-infection by this polycyclic disease in the first study. Further research would be worthwhile to investigate high application frequencies against witches' broom and strain mixtures containing *T. virens* against black pod in areas where these diseases predominate.

Krauss and Soberanis (2001a) found that a mixture of five *C. rosea* strains increased biocontrol efficiency compared with a single antagonist or various combinations of two. Earlier, *C. rosae* strains antagonized *T. virens* GL-21 in pre-screens, and this combination had not been field tested by the authors. However, the *C. rosea* strain G-7 used here was compatible with the three *Trichoderma* species. Given the frequently observed higher effectiveness and consistent performance of mixed biocontrol inocula (Krauss and Soberanis, 2001a; Krauss et al., 2001; Raupach and Kloepper, 1998), systematic studies on antagonist compatibility could enhance our capability to design promising mixtures.

Effective biocontrol in terms of reduced disease incidence does not necessarily translate into improved net yields. In a shaded, 34-year-old, formerly abandoned cocoa field, yields were more than doubled in the first year of rehabilitation by using cultural and biological control; whereas unshaded plots in the same age group failed to respond to effective biocontrol (Krauss and Soberanis, 2001). The contrary effect was observed in young vigorous cocoa in Panama (Krauss et al., unpublished data). Because the vigor of cocoa is relevant, we investigated the effect of fertilization. It is compatible with biocontrol and even reduces moniliasis. Fertilization increased the percentage of healthy pods as well as absolute yield (Table 3). No interaction with biocontrol was observed, and the two techniques can be implemented independently of each other.

Biological control was the most economical disease control measure and resulted in the highest net returns (Table 4). Biocontrol was followed by cultural control alone. Chemical control was least efficient and least economical. Fertilization was economical, independent of the disease control measure chosen, and increased net returns by nearly 5%. Thus, the combination of biocontrol and fertilization best improves farmers' incomes. However, growers are more likely to recognize only the benefit of biocontrol. Fertilization, despite increasing net returns, reduced the benefit-cost ratio. Fertilization is a more expensive investment and less likely to be adopted. Technology transfer programs should first introduce biocontrol and recommend fertilization from the second season onward, after the producer has already reaped some benefits from improved crop management and gained confidence in the recommendations of the extension service.

A highly effective biocontrol treatment, the mixture of the four antagonists *C. rosea*, *T. longibrachiatum*, *T. stromaticum*, and *T. virens*, was also used in the trial that investigated the frequency of application. Both moniliasis and witches' broom incidence decreased with increasing frequency of regular application, but moniliasis was more sensitive to changes. It responded to lower frequencies, whereas witches' broom was reduced significantly only by ten applications every two weeks, the treatment that led to the overall highest percentage of healthy pods (Fig. 1). In contrast, moniliasis was already reduced by three applications in 45-day intervals. Interestingly, the three adjusted applications (one at the onset of flowering and an additional two applications eight and ten weeks later) were intermediate between five monthly and ten biweekly applications, not differing either for moniliasis incidence or number of pods per tree (Fig. 1).

The improved yields and, thus, increase in gross returns with ten applications were not justified in economic terms because production costs rose at a higher rate (Table 5). Net returns were better with three adjusted biocontrol applications than with any other disease control strategy. Compared with cultural control alone, earnings rose by 12.3%. Cultural control alone was not the least economical strategy. In fact, its benefit-cost ratio was the second highest, and ten regular applications had the least favorable benefit-cost ratio, despite having the second best net return (Table 5). Extension personnel should thus encourage farmers to practice the weekly removal of diseased pods (Soberanis et al., 1999) and simultaneously introduce a biocontrol regime adapted to the production cycle of cocoa. Although not tested here, an economic extrapolation of both the fertilization and application frequency trials suggests that fertilization combined with three adjusted applications will improve net returns by almost 10% compared to cultural control without fertilization as practiced on these farms prior to the trials.

In areas like eastern Peru (Servicio Nacional de Sanidad Agraria, 1998) or Ecuador (Maddison et al., 1995), which possess a unimodal rainfall pattern and where moniliasis is the most important disease of cocoa, three adjusted applications of biocontrol can be recommended. Although circumstantial evidence suggests that the first application should be administered at flowering (Krauss and Soberanis, 2001), we do not yet know the optimum dates for the other two applications. Their optimization merits further study. The recommendations established for regions with unimodal rainfall and a single flowering peak are unlikely to be transferable to other cocoa-growing regions where seasons are less pronounced and flowering is more continuous. Future research should not only collect pathological data but also meteorological data and relate these to production patterns. This research should develop predictable disease progress curves. One outcome is modeling with the aim to develop a decision making tool with wider geographic application.

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