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Mycoparasitism by *Clonostachys byssicola* and *Clonostachys rosea* on *Trichoderma* spp. from cocoa (*Theobroma cacao*) and implication for the design of mixed biocontrol agents

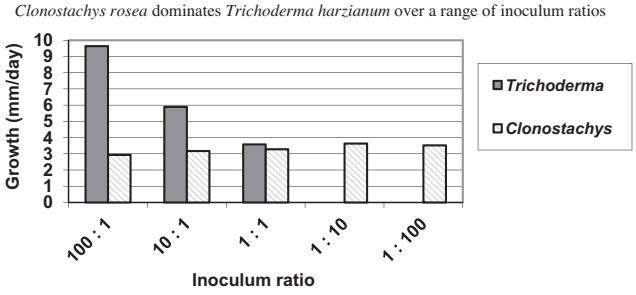
Ulrike Krauss [a,*], Martijn ten Hoopen [b], Robert Rees [c,d], Tim Stirrup [d], Tim Argyle [d], André George [a,e], Claudio Arroyo [a], Eduardo Corrales [f], Fernando Casanoves [f]

[a] CABI-CATIE-USDA, c/o Centro Agronómico Tropical de Investigación y Enseñanza (CATIE), 7170 Turrialba, Costa Rica
[b] CABI-CATIE-DGIS, c/o CATIE, 7170 Turrialba, Costa Rica
[c] Institute for Cell and Molecular Biology (ICMB), Rutherford Building, Mayfield Road, Edinburgh EH9 3JH, UK
[d] Department of Natural Sciences, Bath University, Bath BA2 7AY, UK
[e] Universidad de Costa Rica (UCR), Facultad de Agronomía, Sede Atlántica, Turrialba, Costa Rica
[f] Unidad de Bioestadística, CATIE, 7170 Turrialba, Costa Rica

HIGHLIGHTS

- In the laboratory, *Clonostachys* spp. *parasitized Trichoderma* spp.
- In the field, *C. rosea* dominated over *Trichoderma* sp.
- Native *Trichoderma* spp. were poor pioneer colonizers of surface-sterilized pods.

GRAPHICAL ABSTRACT



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ABSTRACT

Cocoa (*Theobroma cacao*) pod diseases cause tremendous losses, frequently eliminating over 80% of potential yield. Biological control has previously shown promise to supplement cultural control methods practiced by the majority of smallholders. Mixed *Clonostachys* inocula were most effective against multiple pathogens in Peru, but not in Costa Rica, where *Clonostachys* spp. seemed to antagonize *Trichoderma* spp. The objective of our study was to systematically investigate the mycoparasitic interactions between *Clonostachys* and *Trichoderma* species, in order to enable the design of mixed inocula in a rational manner. Specifically, we aimed to quantify dominance of one mycoparasite genus over the other using *in vitro* tests as well as field experiments. The compatibility of Peruvian and Costa Rican isolates of known efficacy was tested by pair-wise confrontations on water agar as well as two host-range assays, one of which mycoparasites were offered another mycoparasite as only host and one where conidia of a *Clonostachys* and a *Trichoderma* isolate were mixed at ratios ranging from 1:100 to 100:1 in order to determine dominance on the susceptible host, *Phytophthora palmivora*. A field study quantified survival and establishment of selected mixtures on surface-sterilized cocoa pods. Hyphal interactions and host-range studies coincided in identifying *Clonostachys rosea* as the most aggressive mycoparasite, closely followed by *C. byssicola*. *Trichoderma* spp. were least aggressive; instead, they were highly susceptible to *Clonostachys* spp. The outcome of interactions depended on the species used and occasionally even on the isolates confronting each other. The country of origin, however, was irrelevant. Parasitic growth of *C. rosea* was unaffected by *Trichoderma harzianum*, even at an inoculum ratio of 1:100. Parasitic growth of *Trichoderma* spp.

* Corresponding author. Present address: P.O. Box GM1109, Gablewoods, St. Lucia. Fax: +1 758 451 8162.
E-mail address: ulrike.krauss@gmail.com (U. Krauss).

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progressively slowed as *C. rosea* concentrations rose, but the effect varied in strength with different isolates. When *C. rosea* and *Trichoderma* sp. were applied to cocoa pods in the field either separately or in combination at a ratio of 5:1, *C. rosea* dominated at the expense of *Trichoderma* sp., which was suppressed. At equal or ten times higher concentrations of *T. harzianum*, *C. rosea* was temporarily suppressed, but from two weeks after application, remained the dominant and persistent pod colonizer. Native *Trichoderma* spp. were poor pioneer colonizers of surface-sterilized pods and applied populations vanished within less than four weeks. Our results suggest that, although *Clonostachys* spp. antagonize *Trichoderma* spp., the incompatibility can be partly overcome by adjusting the inoculum ratio of mixed inocula in favor of *Trichoderma* spp. Therefore, mycoparasitism among several promising biocontrol candidates do not necessarily rule out their combination into a mixed inoculum, but can be addressed by formulating susceptible isolates at higher inoculum ratios. This, however, will not sustainably improve surface coverage by poorly colonizing *Trichoderma* spp. Therefore, optimal strains for the habitat and task are needed first.

1. Introduction

Cocoa (*Theobroma cacao* L) is a neotropical understory tree that provides cocoa beans, the raw material for chocolate. Globally, cocoa diseases account for losses of more than 30% of the potential yield and have caused a steady decline in productivity and a reduction in bean quality in almost all the cocoa-producing areas in the world, especially on small-holder farms in Latin America and West Africa (Hebbar, 2007). The most destructive disease worldwide is black pod, caused by several *Phytophthora* spp., some of which are of pantropical distribution. However, possibly the greatest threat to sustainable cocoa production arises from frosty pod rot, caused by the invasive pathogen *Moniliophthora roreri*. Although global losses are still moderate, this is due to its absence from major bulk-producing countries, particularly in West Africa. Within a few years of disease outbreak in Central America, frosty pod rot reduced yields by over 80%. Witche's broom, caused by *Moniliophthora perniciosa* (previously *Crinipellis perniciosa*), may pose the second greatest threat, with losses ranging from 30% to 90% (Ploetz, 2007). In Brazil, the production of cocoa beans has dropped from 400,000 to 100,000 metric tons in just 10 years (Bowers et al., 2001). Numerous strategies for control of both diseases have been investigated and some are recommended. These, invariably, center on cultural control with various supplementary control options, such as chemical and biological control (Bateman et al., 2005a), at different stages of crop development. Biocontrol of cocoa pod diseases has shown field success in Peru, with mixed inocula achieving simultaneous control of three predominant pathogens: *M. perniciosa*, *M. roreri* and *Phytophthora palmivora*. In fact, the number of isolates in the mixture was negatively correlated with frosty pod rot and witche's broom (Krauss and Soberanis, 2001, 2002). However, the mixed inocula approach was less successful in Costa Rica (Bateman et al., 2005b; Krauss et al., 2003).

The number of biological control agents (BCAs) on the market is gradually growing. However, there remains a huge discrepancy between academic research and practical use. Many BCAs hailed as "promising" in scientific publications never reach the field stage, and if they do, very often perform poorly in comparison with conventional, usually chemical, control. Frequently, trial data of 1 year are good, followed by a disappointing second season. Krauss et al. (1998) attributed this inconsistency to the unrealistic expectation that a single BCA could control all races of a pathogen species in all seasons. They suggested to mix BCAs in order to achieve both a wider host-range, and increased environmental adaptability.

While some inoculum mixtures yield clear advantages, not all BCAs are compatible. Synergism, antagonism as well as neutral interactions have been reported between fungal antagonists (Elad et al., 1998; Etebarian et al., 2000; Krauss et al., 1998; Krauss and Soberanis, 2001; Mendoza et al., 2003; Ruano Rosa and López Herrera, 2009; Sangeetha et al., 2009; Sundram et al., 2008; ten Hoopen et al., 2010; Thrane et al., 2000; XiangMing et al., 2010). The cited authors quantified disease and/or yield for single versus mixed BCAs, but only a few studies investigated fungal population dynamics in such systems. Elad et al. (1998) found no significant reduction in *Trichoderma harzianum* when co-applied with *Ampelomyces quisqualis*. Similarly, mycoparasitism of mildew by *A. quisqualis* was unaffected by the co-applied *T. harzianum*. Co-application of *Talaromyces flavus* with one other BCA: *Bacillus subtilis*, non-pathogenic *Fusarium oxysporum* or *Clonostachys rosea* (formerly known as *Gliocladium roseum*) in eggplant did not significantly reduce colonization of stems by the pathogen, *Verticillium dahliae*. In the mixed inoculum, the dose of each antagonist was halved; surprisingly, their population levels in soil soon doubled to the level of the single-strain inoculant (Nagtzaam et al., 1998). Naár and Kecskés (1998), in a detailed study, found that the interaction of various *Trichoderma* spp. with competing fungi depended mostly on the *Trichoderma* spp. in question, and to a lesser extent on the test fungus it was confronted with. In contrast, interactions of *Trichoderma* spp. with bacteria were less complex and were determined predominantly by the test bacterium, i.e. the antibiotic(s) it produced. Of the fungal species tested, *C. rosea* had the greatest influence on *Trichoderma* spp. Tolerance to *C. rosea* was accompanied by overall increased competitiveness of those *Trichoderma* spp.

In order to design better, i.e. compatible, synergistic and robust mixtures of BCAs, we need to expand our knowledge of their interactions. The research presented here focuses on *Clonostachys* and *Trichoderma* species used as BCAs in Latin American cocoa. Both genera have previously been shown to possess a wide host-range (Krauss and Soberanis, 2001). This study forms part of a larger project on mycoparasite compatibility (Krauss et al., 2004; ten Hoopen et al., 2010). Our objective was to systematically investigate mycoparasitic interactions of selected isolates of *Clonostachys* and *Trichoderma* species from Costa Rica with Peruvian strains of known effectiveness against multiple cocoa diseases (Krauss and Soberanis, 2001, 2002), i.e. *Clonostachys byssicola*, *C. rosea*, *T. harzianum* and *Trichoderma* sp. T4, with the ultimate goal of designing compatible, mixed inocula for application as BCA in Central American cocoa in a rational manner. Specifically, we aimed to quantify dominance of one mycoparasite genus over the other using *in vitro* tests as well as field experiments.

2. Materials and methods

2.1. Fungal isolates

Fungal isolates used in experiments are shown in Table 1. They were deposited at CABI, Egham, United Kingdom and at the *Centro Agronómico Tropical de Investigación y Enseñanza* (CATIE), Turrialba, Costa Rica. Mycoparasites were isolated using the precolonized plate method (Krauss et al., 1998). Fungi were maintained on

Table 1
Mycoparasites and their origin used in this study.

Mycoparasite species	Strain number	Antagonist source	Country
Clonostachys byssicola	AMR0006	Young cocoa pod	Costa Rica
"	AMR0055	Cocoa pod	Costa Rica
"	AMR0057	Cocoa pod	Costa Rica
Clonostachys rosea	APP0023	Cocoa petals	Costa Rica
"	APP0043	Cocoa pod	Costa Rica
"	AMR0038	Young cocoa pod	Costa Rica
"	G1	Basidiocarp of *Moniliophthora perniciosa*	Peru
"	G2 (=IMI 382477)	Basidiocarp of *Moniliophthora perniciosa*	Peru
"	G3 (=IMI 382478)	Healthy shoot tip	Peru
"	G4	Healthy cocoa pod	Peru
"	G7 (=IMI 382480)	Healthy cocoa pod	Peru
Trichoderma sp.[a]	T4 (=IMI 382482)	Frosty pod (sporulating)	Peru
Trichoderma harzianum	APP0129	Cocoa pod	Costa Rica
"	APP0156	Mature, healthy cocoa pod	Costa Rica
"	APP0160	Mature, healthy cocoa pod	Costa Rica

[a] The International Mycological Institute originally identified this isolate as *Trichoderma longibrachiatum*. Subsequently, however, Gary Samuels (USDA) assigned this isolate to *Trichoderma asperellum* (pers. comm., 2003). Until an identification is confirmed, it will be referred to as *Trichoderma* sp. T4.

half-strength potato-dextrose agar (PDA) in liquid nitrogen and as up to 20 agar blocks (5 mm × 5 mm) stored at 4 °C in 30 ml vials filled with 20 ml sterile distilled water. Antagonist inoculum for field trials was produced by a two-step liquid/solid fermentation as described by Krauss et al. (2002).

2.2. In vitro interactions

Host-range studies and hyphal interactions on water agar were carried out as described by Krauss et al. (1998), only that mycoparasites instead of plant pathogens were used as experimental hosts. The former measures overgrowth of a presumptive host by a mycoparasite, the latter the percentage hyphae destroyed upon contact of a fungus with another, so that either can act as host in addition to as attacker.

In order to be able to look at individual hyphal interaction, sterile glass coverslips (22 mm × 64 mm) were dipped in molten 2% Bacto agar (Difco Laboratories, Detroit, MI) to coat them with a thin film of agar. Coated coverslips are places on solidified water agar (Oxoid) plates and inoculated according to the interaction to be observed as follows. Small agar disks with mycoparasite inoculum (4 mm diam, <1 mm high) of each fungus were placed on opposite ends of the coverslip and incubated until the colonies just made contact. The fine mycelia formed on nutrient-free agar allowed inspection of interaction between individual hyphae by light microscopy. For each contact made, hyphal damage (coagulation of cytoplasm and/or lysis) of either partner was scored. All possible combinations were tested, including self-self interactions for hyphal interactions. Due to the large number of measurements in this experiment, it was set up in three stages: first only Costa Rican isolates were paired, then only Peruvian ones, and lastly a selection of Costa Rican isolates was confronted with a selection of Peruvian isolates. Three replicate plates were used for each host-range combination; three to five replicates were scored for hyphal confrontations, depending on the frequency of contact, which was affected by antibiosis for certain isolates, as developed by ten Hoopen et al. (2010).

Selected fungi were subsequently used for a double host-range experiment, as described in detail by ten Hoopen et al. (2010): *Trichoderma* sp. T4, *T. harzianum* APP0129, *Clonostachys rosea* G2, G7 and APP0023, and *C. byssicola* AMR0057 were the mycoparasites. These were tested as paired mixtures at different ratios on the highly susceptible host, *Phytophthora palmivora* strain C13 Phy, as follows. Mycoparasite spore suspensions were prepared in sterile distilled water (SDW), filtered through Whatman N° 1 to remove mycelial aggregates and adjusted to the desired concentration (10^6 spores ml^{-1}, except for AMR0057, which had an initial spore concentration of 5.4 (10^5 spores ml^{-1}). Subsequently, ten-fold dilutions were prepared and combined into pair-wise mixtures with rations of 100:1, 10:1, 1:1, 1:10, and 1:100. Sterile strips (5 mm × 35 mm) of filter paper (Whatman N° 3) were dipped into the mixed spore suspensions. The inoculated paper strips were then placed on top of the mycelium of each pre-colonized plate of *P. palmivora*. Plates were sampled, following the procedure of Krauss et al. (1998), in two-day intervals until day 10; thereafter cuts were made every 5 days until fungal growth had reached the end of each plate. Throughout this procedure, filter papers were kept in their original positions. Three replicate plates were prepared for each treatment. All plates were incubated at 26 °C on an alternating basis of 12 h of darkness and 12 h of light.

2.3. Cocoa field study

This field survival trial measures the establishment and persistence of inoculated mycoparasites on cocoa pods as well as the recolonization of the surface-sterilized pod surface by naturally present mycoparasites. The experiment was carried out in the so-called Clonal Garden of CATIE's La Lola Research Station, Matina, Limón. Trees were spaced 2 m × 4 m with heights of 3–4 m; the experimental area was well-shaded (⩾70%). Meteorological data were measured on site. Ten healthy replicate cocoa pods, 3–5 months of age, per treatment were chosen randomly. Pods were surface sterilized on the trees by wiping them with 95% alcohol and spray-inoculated to incipient run-off with the antagonist using a hand-held, pressurized sprayer (Volpi & Bottoli, Piadena, Italy). Fungal detection on the pod surface followed the methodology of Piper et al. (2000) as follows.

Three peel discs were cored (12 mm cork borer) from each replicate cocoa pod per treatment and date. An excess of treated pods served as replacement for pods lost due to rots, especially after injury. The surface layer of the discs was removed with a scalpel and air-dried (ca. 3 days) in the laboratory. The dried discs were then placed, with their peel facing downwards, onto agar plates fully pre-colonized with *P. palmivora*, a highly susceptible bait host (ten Hoopen et al., 2003). The development of native and applied mycoparasites on the host was observed for approximately one week, scored as presence or absence.

The first field survival trial was conducted from 17 July to 17 September, 2001. Four treatments were: a water control, *Trichoderma* sp. T4, a mixture of five *C. rosea* isolates (G1, G2, G3, G4 and G7) and the mixture of the five *C. rosea* isolates plus *Trichoderma* sp. T4 (total concentration: 10^6 spores ml^{-1} with equal pro-

portion of all isolates). Eight samples were taken at approximately weekly intervals, starting with the day of inoculation.

The second field survival trial was conducted from 21 March to 23 May 2003. The Peruvian *C. rosea* G7 and the Costa Rican *T. harzianum* APP129 were co-inoculated onto eight replicate pods at ratios of 10:1, 1:1, and 1:10; water served as control. Ten samples were taken in approximately weekly intervals, starting with the day of inoculation.

2.4. Experimental design and statistical analysis

Laboratory experiments and the field survival study followed a completely randomized design. Fungal growth rates measured during the host-range and double host-range studies in addition to hyphal interactions were analyzed by nested analysis of variance (ANOVA) using InfoStat (Infostat, 2004) with fungal species constituting groups and isolates within species, where applicable, the subgroup. In the third hyphal interaction experiment, in which Costa Rican and Peruvian mycoparasites were set up against each other, genera were compared instead of species and the country of origin was added to the model as a factor, in an attempt to obtain clues on possible effects of co-evolution in susceptibility. Proportion of hyphal damage data were arcsine-transformed to normalize the error distribution (Zar, 1996) prior to ANOVA. When significant effects were detected, the Di Rienzo Guzmán and Casanoves (DGC) test was applied to separate conglomerates with similar attacker or host characteristics. The DGC-test is particularly useful when comparing large data sets, where differences between individual isolates are plentiful but are contributing little to understanding of significant differences (Di Rienzo et al., 2001). Percentage pod surface colonization during the field study was analyzed using generalized mixed models (GMM) in order to take into account lack of normality and correlations among experimental units measured at different dates. A binomial distribution with a logit link canonical function was used. Because the proportions were obtained from two or three independent runs, the variable number of experiments was used as offset variable. The best fitted model for each experiment was selected using the Akaike's Information Criterion (AIC) and the Bayesian information criterion (BIC). The model considers the effect of treatments, time and their interaction. Fisher's protected least significant difference (LSD) (alpha = 0.05) was used to compare the means. When there was a significant treatment effect, but no interaction, treatments means were compared across all dates. If the interaction was present, treatments were compared within each date. Data were analyzed with R (R Development Core Team, 2009) using the interface implemented in software InfoStat professional version 2013 (Di Rienzo et al., 2013).

3. Results

3.1. In vitro parasitism among mycoparasites

Host-range experiments with Costa Rican isolates are summarized in Table 2. The identity of some strains could not be ascertained when inoculated onto another member of the same species, which resulted in missing data for some treatments. Nevertheless, there were significant differences between host species as well as isolates within species (both $P < 0.001$). *T. harzianum* was the most susceptible host, followed by *C. byssicola* and then *C. rosea*. Within *T. harzianum*, APP156 was less susceptible than the other two hosts. Within *C. byssicola*, only AMR0006 was slightly susceptible. All *C. rosea* isolates were resistant to mycoparasitism.

Similarly, attacker species and isolates within species differed in their aggressiveness (both $P < 0.001$). *C. rosea* was the most aggressive mycoparasite, followed by *C. byssicola*. Within *C. byssicola*, AMR0055 was less aggressive than the other two strains; within *C. rosea* AMR0038 was the least aggressive. *T. harzianum* was unable to attack *Clonostaychys* with no differences between isolates (Table 2).

Host species interacted with attacker species as well as host isolate with attacker isolate (both $P < 0.001$; Table 2). *T. harzianum* was unable to parasitize any of the offered mycoparasites. At the same time, it was the most susceptible host for either *Clonostachys* sp. *C. byssicola* AMR0006 was as susceptible to *C. rosea* as *T. harzianum* APP129, whereas the other *C. byssicola* isolates were resistant to *C. rosea*. *C. byssicola* AMR0057 invaded the other two *C. byssicola* isolates measurably.

All Peruvian *C. rosea* isolates overgrew *Trichoderma* sp. T4, while T4 was unable to invade any *C. rosea* (data not tabulated). There was no visible evidence of parasitism among *C. rosea* isolates, but microscopic distinction of the strains was not possible.

3.2. Hyphal interactions among mycoparasites

Antibiosis by *C. byssicola*, particularly AMR0057, prevented some direct interactions which resulted in some missing data (Table 3). Overall, hyphal interactions of Costa Rican isolates on water agar paralleled the host-range observations in that *T. harzianum* was by far the most susceptible host, followed by *C. byssicola* and then *C. rosea* ($P < 0.001$). Again, within *C. byssicola* AMR0006 was more susceptible than the other two isolates ($P < 0.001$), and there were no differences between *C. rosea* isolates. In contrast to host-range studies measuring overgrowth, no differences in hyphal damage was detected between *T. harzianum* isolates.

As with overgrowth, *C. rosea* was also the most aggressive towards hyphae and was followed by *C. byssicola* and then *T. harzianum* ($P < 0.001$). However, against hyphae, *C. byssicola* AMR0006 and *C. rosea* APP0043 caused less hyphal damage than the other two isolates of their respective species ($P < 0.001$). Damage caused by *T. harzianum* was negligible, with no distinction between isolates (Table 3).

Host and attacker species interacted significantly ($P < 0.001$). *C. rosea* was resistant to attack by the other two species. *C. byssicola* succumbed to *C. rosea* only. *T. harzianum* was severely damaged by *C. rosea*, less than half that much by *C. byssicola* and caused a low, but measurable, percentage of lysis to its own hyphae (Table 3). Highly significant interactions between host and attacker strains ($P < 0.001$) indicate that individual behavior has to be taken into consideration despite overall tendencies at the species level. For example, while AMR0006 was the overall most susceptible isolate of *C. byssicola*, it was less susceptible to *T. harzianum* APP0160 than *C. byssicola* AMR0055. Conversely, while all *C. rosea* strains exhibited a high degree of hyphal resistance, both *C. byssicola* AMR0057 and *C. rosea* APP023 inflicted considerable damage to *C. rosea* APP0038. The largest variation was observed when *T. harzianum* isolates of overall similar susceptibility were attacked by different isolates of *C. byssicola*. The reactions ranged from resistant (when attacked by AMR0006) to highly susceptible (APP0129 attacked by AMR0055; Table 3).

Peruvian *C. rosea* also dominated over *Trichoderma* sp. Again antibiosis, particularly by G2, excluded certain contacts from occurring (Table 4). *Trichoderma* sp. T4 was far more prone to hyphal damage than *C. rosea*, while *C. rosea* was the more aggressive attacker species (both $P < 0.001$). The five *C. rosea* isolates did not differ in susceptibility ($P = 0.324$). While all *C. rosea* strains significantly damaged *Trichoderma* sp. T4, they differed in aggressiveness ($P < 0.001$): G2 caused more damage than the other *C. rosea* strains. Host and attacker isolates interacted significantly with each other ($P = 0.010$). The highest hyphal lysis was observed when *Trichoderma* sp. T4 was attacked by *C. rosea* G2 or G7 (Table 4).

Table 2
Growth (mm day^{-1}) of Costa Rican mycoparasites (in columns as attackers) on plates precolonized with these mycoparasites acting as hosts (in rows).

Attacker	*Clonostachys byssicola*			*Clonostachys rosea*			*Trichoderma harzianum*			Weighted host mean
Strain number	AMR0006	AMR0055	AMR0057	APP0023	APP0043	AMR0038	APP0129	APP0156	APP0160	
Host										
Clonostachys byssicola										**0.26^{B}**
AMR0006	na[1]	0.06^{a}	0.65^{b}	1.64^{d}	1.64^{d}	1.69^{d}	0.00^{a}	0.00^{a}	0.00^{a}	**0.71^{B}**
AMR0055	0.00^{a}	na	0.48^{b}	0.00^{a}	0.00^{a}	0.00^{a}	0.03^{a}	0.00^{a}	0.00^{a}	**0.06^{A}**
AMR0057	0.00^{a}	0.00^{a}	na	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	**0.00^{A}**
Clonostachys rosea										**0.00^{A}**
APP0023	0.00^{a}	0.00^{a}	0.00^{a}	na	?[2]	0 ?	0.00^{a}	0.00^{a}	0.00^{a}	**0.00^{A}**
APP0043	0.00^{a}	0.00^{a}	0.00^{a}	?	na	0 ?	0.00^{a}	0.00^{a}	0.00^{a}	**0.00^{A}**
AMR0038	0.00^{a}	0.00^{a}	0.00^{a}	?	0 ?	na	0.00^{a}	0.00^{a}	0.00^{a}	**0.00^{A}**
Trichoderma harzianum										**1.27^{C}**
APP0129	2.05^{e}	1.82^{d}	1.76^{d}	1.77^{d}	1.63^{d}	1.60^{d}	na	?	?	**1.33^{D}**
APP0156	1.58^{d}	1.40^{c}	1.70^{d}	1.65^{d}	1.70^{d}	1.32^{c}	?	na	?	**1.17^{C}**
APP0160	1.99^{e}	1.34^{c}	1.28^{c}	2.17^{e}	2.34^{f}	1.23^{c}	?	?	na	**1.31^{D}**
Weighted attacker mean	**0.70$^{\gamma}$**	**0.58$^{\beta}$**	**0.73$^{\gamma}$**	**1.20$^{\varepsilon}$**	**1.23$^{\varepsilon}$**	**0.97$^{\delta}$**	**0.00$^{\alpha}$**	**0.00$^{\alpha}$**	**0.00$^{\alpha}$**	
		0.67$^{\beta}$			**1.14$^{\gamma}$**			**0.00$^{\alpha}$**		

^{a,b,c,d,e,f}Isolate means followed by the same common letter do not differ at $P = 0.05$ (DGC-test).
^{A,B,C,D}Host means followed by the same capital letter to not differ in susceptibility at $P = 0.05$ (DGC-test). Comparison within columns only.
$^{\alpha,\beta,\gamma,\delta,\varepsilon}$Attacker means followed by the same Greek letter to not differ in aggressiveness at $P = 0.05$ (DGC-test). Comparison within rows only.
[1] Na, not applicable; self-self interaction.
[2] ?, Strains not discernible with certainty; no data.

Table 3
Hyphal damage (re-transformed percentages) resulting from interaction of Costa Rican isolates of *Clonostachys* spp. and *Trichoderma harzianum* on water agar-coated coverslips. Isolates acted as attackers (in columns) as well as hosts (in rows).

	Attacker									Weighted host mean
Species	*Clonostachys byssicola*			*Clonostachys rosea*			*Trichoderma harzianum*			
Strain number	AMR0006	AMR0055	AMR0057	APP0023	APP0043	APP0038	APP0129	APP0156	APP0160	
Host										
Clonostachys byssicola										**3.7^{B}**
AMR0006	2.7^{a}	13.0^{b}	12.7^{b}	43.4^{c}	40.0^{c}	59.8^{c}	2.7^{a}	nd[1]	1.6^{a}	**14.2B**
AMR0055	1.8^{a}	0.0^{a}	1.3^{a}	0.0^{a}	0.0^{a}	0.7^{a}	0.3^{a}	2.4^{a}	5.0^{b}	**0.7^{A}**
AMR0057	0.2^{a}	0.0^{a}	0.2^{a}	nd	nd	2.0^{a}	1.0^{a}	1.1^{a}	1.8^{a}	**0.7^{A}**
Clonostachys rosea										**0.5^{A}**
APP0023	0.0^{a}	1.7^{a}	nd	0.1^{a}	1.2^{a}	2.7^{a}	0.0^{a}	0.0^{a}	0.8^{a}	**0.4^{A}**
APP0043	0.0^{a}	0.0^{a}	nd	1.0^{a}	0.7^{a}	0.2^{a}	0.0^{a}	0.1^{a}	0.3^{a}	**0.2^{A}**
APP0038	0.0^{a}	0.3^{a}	7.4^{b}	11.6^{b}	0.0^{a}	0.2^{a}	0.0^{a}	0.0^{a}	2.8^{a}	**1.0^{A}**
Trichoderma harzianum										**14.6^{C}**
APP0129	0.9^{a}	56.3^{c}	25.9^{b}	53.3^{c}	5.5^{b}	40.1^{c}	2.1^{a}	5.0^{b}	7.2^{b}	**17.3^{B}**
APP0156	nd	14.4^{b}	10.1^{b}	58.9^{c}	15.7^{b}	44.2^{c}	24.3^{b}	0.1^{a}	0.0^{a}	**15.8^{B}**
APP0160	0.5^{a}	20.8^{b}	17.4^{b}	39.2^{c}	15.4^{b}	25.0^{b}	2.6^{a}	0.3^{a}	7.4^{b}	**11.2^{B}**
Weighted attacker mean	**0.4$^{\alpha}$**	**6.2$^{\beta}$**	**8.7$^{\beta}$**	**18.5$^{\gamma}$**	**5.7$^{\beta}$**	**12.9$^{\gamma}$**	**1.6$^{\alpha}$**	**0.5$^{\alpha}$**	**2.2$^{\alpha}$**	
		4.1$^{\beta}$			**11.8$^{\gamma}$**			**1.3$^{\alpha}$**		

^{a,b,c}Isolate means followed by the same common letter do not differ at $P = 0.05$ (DGC-test).
^{A,B,C}Host means followed by the same capital letter to not differ in susceptibility at $P = 0.05$ (DGC-test). Comparison within columns only.
$^{\alpha,\beta,\gamma}$Attacker means followed by the same Greek letter to not differ in aggressiveness at $P = 0.05$ (DGC-test). Comparison within rows only.
[1] Nd, no data due to antibiosis.

When Costa Rican and Peruvian mycoparasites were confronted, the country of origin was not significant for either the host ($P = 0.595$) or the attacker ($P = 0.861$), and there was no interaction between the two factors ($P = 0.218$). *Trichoderma* was again by far more susceptible than *Clonostachys* ($P < 0.001$), with no difference between isolates within genera ($P = 0.627$; Table 5). *Clonostachys* was a more aggressive attacker than *Trichoderma* ($P < 0.001$), but differences between isolates within species were detected ($P = 0.026$): *C. byssicola* AMR0057 was more aggressive than the three *C. rosea* strains. The results of this experiment are summarized by the highly significant ($P < 0.001$) interaction between host genus and aggressor genus: damage occurred whenever and only when *Clonostachys* attacked *Trichoderma.* The only slight deviation from this rule, statistically expressed by a significant host strain with aggressor strain interaction ($P = 0.043$), was the mutual damage *C. rosea* G2 and AMR0057 inflicted on each other's hyphae on occasions when contact occurred despite strong antibiosis. Neither isolate in this pair was dominant, suggesting a balanced, moderate incompatibility (Table 5).

3.3. Competitive mycoparasitism of combinations of Clonostachys and Trichoderma spp. on P. palmivora

In the double host-range experiment, two mycoparasites (a *Trichoderma* and a *Clonostachys* species) were mixed in different proportions and simultaneously inoculated onto *P. palmivora* as a highly susceptible primary host. None of the *C. rosea* isolates was affected in its parasitic growth by either *Trichoderma* species at any of the concentrations tested (Fig. 1, $0.102 \leqslant P \leqslant 0.505$). *T. harzianum* APP0129 was not significantly affected ($P = 0.397$) by *C. rosea* G2 but highly significantly ($P = 0.001$) affected by *C. rosea* G7. The higher the concentration of G7, the less the growth of APP0129. Nevertheless, as shown in Fig. 1, growth rates of APP0129 seem to decline in response to G2 between the ratios

Table 4
Hyphal damage (re-transformed percentages) resulting from interaction of Peruvian isolates of *Clonostachys rosea* and *Trichoderma* sp. on water agar-coated coverslips. Isolates acted as attackers (in columns) as well as hosts (in rows).

	Attacker						Weighted host mean	
Species	*Clonostachys rosea*					*Trichoderma* sp.		
Strain number	G1	G2	G3	G4	G7	T4		
Host								
Clonostachys rosea								**1.2^{A}**
G1	2.2^{a}	21.8^{a}	0.6^{a}	9.6^{a}	0.0^{a}	0.8^{a}	**3.4^{A}**	
G2	2.9^{a}	nd[1]	nd	nd	0.0a	0.0^{a}	**0.3^{A}**	
G3	2.0^{a}	nd	0.0^{a}	0.0^{a}	10.2^{a}	3.0^{a}	**1.6^{A}**	
G4	4.1^{a}	nd	7.5^{a}	0.3^{a}	0.0^{a}	0.0^{a}	**1.1^{A}**	
G7	1.3^{a}	0.0^{a}	1.4^{a}	0.0^{a}	0.2^{a}	0.0^{a}	**0.2^{A}**	
Trichoderma sp.								**28.5^{B}**
T4	27.0^{a}	81.1^{b}	21.9^{a}	12.4^{a}	53.7^{b}	0.2^{a}		
Weighted attacker mean	**4.8$^{\alpha}$**	**26.1$^{\beta}$**	**3.7$^{\alpha}$**	**2.1$^{\alpha}$**	**3.9$^{\alpha}$**			
			5.3$^{\beta}$			**0.3$^{\alpha}$**		

^{a,b}Isolate means followed by the same common letter do not differ at $P = 0.05$ (DGC-test).
^{A,B}Host means followed by the same capital letter to not differ in susceptibility at $P = 0.05$ (DGC-test). Comparison within columns only.
$^{\alpha,\beta}$Attacker means followed by the same Greek letter to not differ in aggressiveness at $P = 0.05$ (DGC-test). Comparison within rows only.
[1] Nd, no data due to antibiosis.

Table 5
Hyphal damage (re-transformed percentages) resulting from interaction of Costa Rican and Peruvian isolates of *Clonostachys* spp. and *Trichoderma* spp. on water agar-coated coverslips. Isolates acted as attackers (in columns) as well as hosts (in rows).

	Attacker						Weighted host mean	
	Clonostachys				*Trichoderma*			
	C. byssicola	*C. rosea*			*T. harzianum*	*Trichoderma* sp.		
Host	AMR0057	APP0023	G2	G7	APP0129	T4		
Country of Origin	Costa Rica	Costa Rica	Peru	Peru	Costa Rica	Peru		
Clonostachys spp.								**1.1^{A}**
C. byssicola AMR0057	0.0^{a}	0.0^{a}	16.8^{b}	nd[1]	0.0^{a}	6.5^{a}	**1.8^{A}**	
C. rosea APP0023	0.0^{a}	5.0^{a}	1.1^{a}	1.5^{a}	0.0^{a}	0.0^{a}	**0.6^{A}**	
C. rosea G2	16.8^{b}	2.1^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	**0.9^{A}**	
C. rosea G7	nd	0.0^{a}	4.8^{a}	2.1^{a}	1.0^{a}	2.9^{a}	**1.6^{A}**	
Trichoderma spp.								**48.1^{B}**
T. harzianum APP0129	93.4^{c}	85.9^{c}	89.1^{c}	63.1^{c}	0.0^{a}	0.0^{a}	**48.9^{B}**	
Trichoderma sp. T4	93.5^{c}	65.4^{c}	81.4^{c}	84.4^{c}	0.0^{a}	0.0^{a}	**47.2^{B}**	
Weighted attacker mean	**32.8$^{\gamma}$**	**16.4$^{\beta}$**	**24.5$^{\beta}$**	**20.5$^{\beta}$**	**0.0$^{\alpha}$**	**0.5$^{\alpha}$**		
	23.0$^{\beta}$				**0.2$^{\alpha}$**			

^{a,b,c}Isolate means followed by the same common letter do not differ at $P = 0.05$ (DGC-test).
^{A,B,C}Host means followed by the same capital letter to not differ in susceptibility at $P = 0.05$ (DGC-test). Comparison within columns only.
$^{\alpha,\beta,\gamma}$Attacker means followed by the same Greek letter to not differ in aggressiveness at $P = 0.05$ (DGC-test). Comparison within rows only.
[1] Nd, no data due to antibiosis.

10:1 and 1:100. *Trichoderma* sp. T4 was not significantly ($P = 0.749$) affected by *C. rosea* APP0023 but again, a trend of reduced growth in response to increased proportions of APP0023 is visible in Fig. 1. *Trichoderma* sp. T4 was significantly affected by *C. byssicola* AMR0057 ($P = 0.021$) and a clear tendency with concentrations between 10:1 and 1:100 was found (Fig. 1).

In many instances *Trichoderma* species were not observed in the first and second sampling cuts of the pre-colonized plate, but later on they suddenly appeared. It is possible that some hyphae managed to evade the attack of *Clonostachys* species unharmed due to the faster growth rate of *Trichoderma* spp., resulting in the establishment of the *Trichoderma* in areas still free from the slower growing *Clonostachys*.

3.4. Cocoa field study

Fig. 2 shows the population dynamics of main mycoparasite genera on the cocoa pod surface after application of a mixture of five *C. rosea* strains with or without added *Trichoderma* sp. T4, with *Trichoderma* sp. T4 alone and a water control (Fig. 2). Control pods show the re-colonization by mycoparasites naturally occurring in the experimental field. *C. rosea* showed high colonization levels throughout. Detection varied significantly between treatments and dates; both variables interacted significantly (all $P < 0.001$). *C. rosea* detection on control pods remained lower than any of the three biocontrol treatments for 15 DAI. The *Trichoderma* sp. T4 only treatment was intermediate on DAI 8 and 15. At 22 DAI, recolonization of control pods by *C. rosea* was still lower than pods treated with *Trichoderma* sp. T4, whether alone or in mixture with *C. rosea.* After 29 days, all treatments had equalized (Fig. 2i).

For *Trichoderma* recovery, both variables and their interaction were highly significant (all $P < 0.001$; Fig. 2ii). Initial colonization with *Trichoderma* exceeded 80% on pods to which *Trichoderma* sp. T4 had been applied on its own, but populations collapsed between 15 DAI and 22 DAI, with little difference between treatments from 22 DAI onwards. *Trichoderma* detection in the *C. rosea* + *Trichoderma* sp. T4 mix was negligible on DAI 0, but increased to intermediate levels for two weeks after application. A second, transient *Trichoderma* peak was observed after *ca* six weeks, with no difference between treatements (Fig. 2ii).

Naturally occurring *C. byssicola* and other verticilliate fungi colonized less than 20% of cocoa pod surfaces with no differences between treatments ($P = 0.194$; Fig. 2iii). Naturally occurring *Fusarium* spp. re-colonized control pods more rapidly than those treated with biocontrol agents ($P < 0.001$). There was no interaction with DAI ($P = 0.315$; Fig. 2iv). For re-colonization by *Aspergillus*

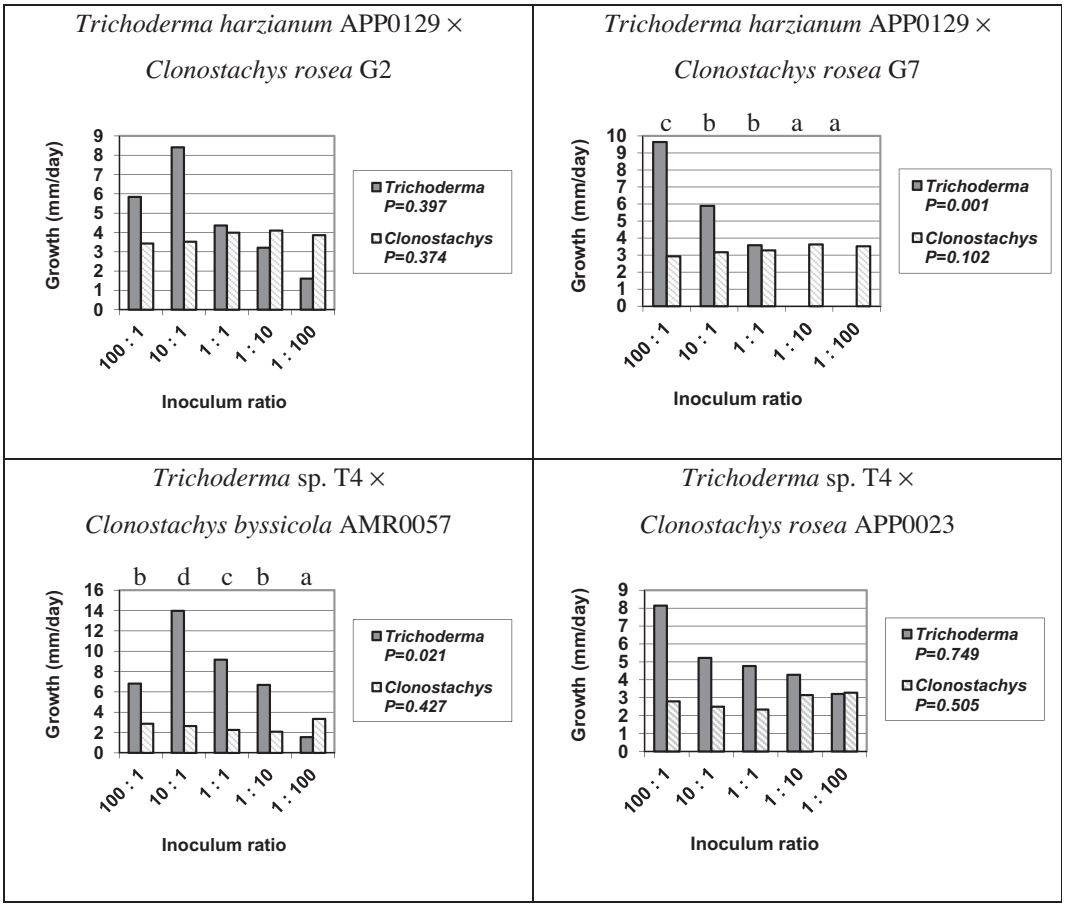

Fig. 1. Growth (mm day^{-1}) of *Trichoderma* and *Clonostachys* species on host *Phytophthora palmivora* when inoculated simultaneously at different inoculum ratios of the two mycoparasite genera. Bars of *Trichoderma* sp. labelled with the same letter do not differ at $P = 0.05$ (ANOVA; Comparison within each fungal pair only).

and *Penicillium* species, treatment, day and their interaction were significant (all $P < 0.001$). For the first 29 DAI, populations levels were erratic with no difference between treatments. Subsequently pod coverage by *Aspergillus* and *Penicillium* spp. was enhanced in treatments containing *Trichoderma* T4 and leveling off around 50%, whereas the control and *Clonostachys*-only treatments remained below 20% (Fig. 2v). Other, unidentified fungi did not exceed 30% colonization at any time with no significant differences between treatments ($P = 0.336$; Fig. 2vi).

Fig. 3 shows the daily and cumulative rainfall during the same time period and Table 6 summarizes other relevant meteorological data. Temperature, relative humidity and total precipitation in 2001 were within the typical range for the time of year. However, August was 50% wetter than normal; this was compensated for by below average rains in July and September (Table 6). August rainfalls peaked four weeks after inoculation (Fig. 3), which coincides with the collapse of *Trichoderma* populations (Fig. 2).

When *C. rosea* G7 was coapplied with *T. harzianum* APP0129 at different ratios, *C. rosea* was again the dominant mycoparasite (Fig. 4). Pods treated with *C. rosea* ratios equal or larger than *T. harzianum* APP0129 exhibited high *C. rosea* levels from day zero until the end of the trial. However, treatment, day, as well as their interaction, influenced *C. rosea* recovery (all $P < 0.001$). Half of the pods treated with 10 times more *T. harzianum* APP0129 than *C. rosea* G7 gave rise to *C. rosea* isolates on day zero. Within one week, *C. rosea* recovery from these pods did not differ from those with 100 times the *C. rosea* G7 dosage. Control pods were also quickly colonized by *C. rosea* and within 14 DAI all treatments were equally colonized by *C. rosea* (Fig. 4i). The recovery of *Trichoderma* spp. was also influenced by inoculum ratio, day and their interaction (all $P < 0.001$). Whereas almost complete pod colonization on day zero was observed when applied *T. harzianum* APP0129 levels were at least equal to *C. rosea* G7, no *Trichoderma* were recovered from the control or the treatment with 10 times higher *C. rosea* G7 than *T. harzianum* APP0129 in the inoculum (Fig. 4ii). The control was not significantly colonized by *Trichoderma* spp. throughout the 10 week duration of the trial. One week after inoculation, the treatment with the highest *T. harzianum* APP0129 ratio yielded 60% pod coverage, which was the highest on that day. Treatments with *T. harzianum* APP0129 inoculum levels lower than or equal to *C. rosea* G7 did not differ from each other, but were higher than the control for 7 DAI. Populations of applied *T. harzianum* APP0129 collapsed rapidly: from 14 DAI no treatment differed from the water control for recovery of *Trichoderma* spp. (Fig. 4ii). The re-colonization of surface-disinfected pods by *Fusarium* spp. was not influenced by the biocontrol treatments ($P = 0.189$; Fig. 4iii). Other, less common re-colonizers were influenced by treatment ($P = 0.008$) and date ($P < 0.001$) without interaction between the two parameters ($P = 0.119$). The control and *T. harzianum* APP0129-dominated treatment were re-colonized more quickly by other fungi than the treatment with equal applied concentrations of *T. harzianum* APP0129 and *C. rosea* G7. The *C. rosea* G7-dominated treatment was intermediate (Fig. 4iv).

4. Discussion

Piper et al. (2000) showed that *C. rosea* can parasitize *Trichoderma* sp. and hypothesized that *C. rosea* dominates over *Trichoderma*. Krauss and Soberanis (2001) used biocontrol efficacy

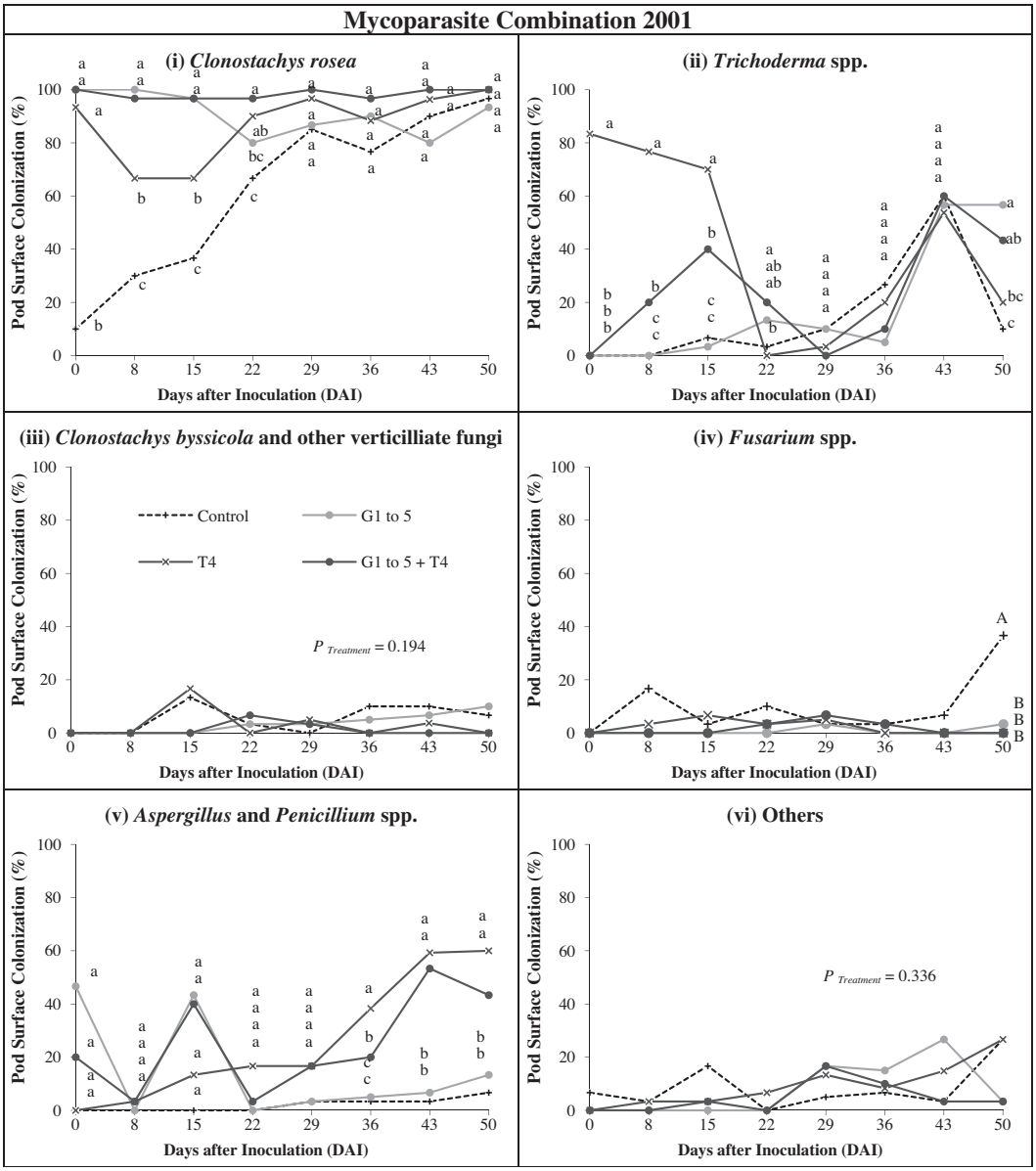

Fig. 2. Percentage surface colonization on cocoa pods by naturally occurring and inoculated mycoparasite genera following surface sterilization and application of the mycoparasites: *Clonostachys rosea* G1, G2, G3, G4, G7; *Trichoderma* sp. T4; and their combination. Treatments marked with the same common letter do not differ at $P = 0.05$ on that day; treatments marked with the same capital letter do not differ across DAI (GMM, followed by LSD).

scores in bioassays to eliminate incompatible combinations prior to costly field trial. They found *Trichoderma* spp. to be least compatible, independent of the other mycoparasites in the mixture. Both host-range studies and hyphal interactions in our present study confirm and further expanded their hypothesis. Both *Clonostachys* spp. tested were resistant to parasitism by *Trichoderma* spp. and, in turn, parasitized the latter consistently, independent of the geographic origin (Tables 2–5), thus ruling out mycoparasitic co-evolution as an important factor in this hierarchy. *C. rosea* was more aggressive than *C. byssicola.* Host-range studies and hyphal interactions exhibited remarkable parallels at the genus and species level; only at the isolate level did dominance ratings vary occasionally.

Clonostachys spp. were the most aggressive mycoparasites and were themselves the most resistant to parasitism. The reverse was true for *Trichoderma* spp. However, within *C. byssicola* the aggressiveness of different isolates was not always associated with resistance to parasitism. For example, AMR0006 and AMR0057 did not differ in aggressiveness; yet, AMR0006 was the more susceptible host of the two (Table 2). Ten Hoopen et al. (2010) also found the aggressiveness as and susceptibility to mycoparasites of 18 *Clonostachys* isolates to be independent phenomena. Instead, the level of aggressiveness and/or susceptibility of an isolate was largely dependent on the attacker isolate. *Clonostachys* and *Trichderma* spp. may possess fundamentally different mechanism of competitiveness. Antibiosis prevented most hyphal contact in *Clonostachys* here and the study by ten Hoopen et al. (2010), a phenomenon not observed for *Trichoderma* spp. A mycoparasite with prolific release of lytic enzymes would be resistant to the type and quantity of enzymes it produces. This may confer enhanced resistance to similar enzymes deployed by other mycoparasites. At this stage, it is too early to say under which circumstances aggressiveness of a mycoparasite may be accompanied by high levels of resistance to parasitism.

Another interesting outcome of both experimental approaches was the fact that host and attacker interacted significantly. This

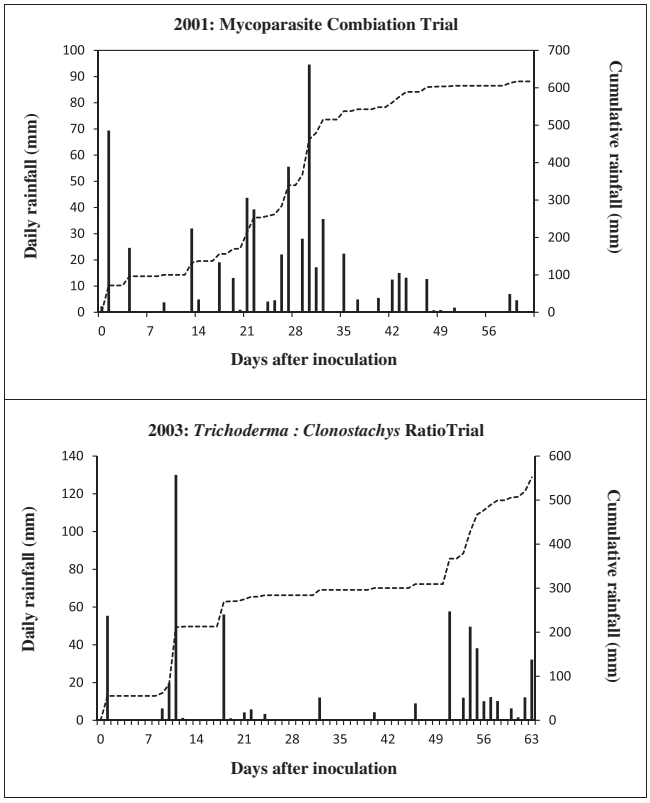

Fig. 3. Rainfall (in mm) during 2001 and 2003 field survival studies.

means that the susceptibility of a host depended on the organisms it was challenged by. This result agrees with the findings of ten Hoopen et al. (2010) for *Clonostachys* spp., but differs from the observation of Naár and Kecskés (1998) who, using a different experimental approach, found that the competitive saprophytic ability (CSA) of *Trichoderma* spp. was largely intrinsic to the *Trichoderma* spp. and independent of the test organism. In the present study, *T. harzianum* susceptibility could be differentiated not only between *C. rosea* and *C. byssicola*, but also between some isolates within these two species.

Naár and Kecskés (1998) found no correlation between *in vitro* growth and CSA of *Trichoderma* spp. The *Trichoderma* strains in our study grew markedly faster than *Clonostachys* spp.; yet, both *C. rosea* and *C. byssicola* consistently affected the growth rate of *Trichoderma* spp. at an inoculum ratio of 1:1 (Fig. 1), which agrees with the observations of Naár and Kecskés (1998). At the same time, the authors postulated that the growth rate of *Trichoderma* spp. interacting with other fungi was of greater importance than in competition with bacteria, since the fast growth rendered physical contact between hyphae more likely. Thus, the faster growth of *Trichoderma* spp. leads to more exposure to mycoparasitism and may, contrary to instinctive interpretation, contribute to their high susceptibility to hyphal damage. It may also allow occasional escape from a parasite, as seems to have happened in our double host-range experiment.

Whereas Naár and Kecskés (1998) found the CSA of *Trichoderma* spp. unaffected by inoculum density within a range of $10^{3.4}$ to $10^{7.2}$ conidia g^{-1} soil, Wardle et al. (1993) found the equilibrium between *T. harzianum* and *Mucor hiemalis* strongly dependent on the initial inoculum ratio of biomass. However, Naár and Kecskés (1998) explained the discrepancy between their own results and those of Wardle et al. (1993) and other researchers by the fact that these workers supplied significant amounts of nutrients together with their ill-defined biomass, whereas the conidial suspensions employed by Naár and Kecskés (1998) were washed free of external nutrients and, thus, provided the starting inoculum in a quantitative manner. Using comparable conidial suspensions, we found the *in vitro* competitive ability of *Trichoderma*, but not of *Clonostachys*, to depend on the initial inoculum ratio of the two genera (Fig. 1). The effect was only statistically significant in two out of four cases, although inoculum ratios stretched across a factor of 10,000. Under conditions of natural equilibrium, an effect of this order of magnitude may indeed be of minor importance. However, in the design of biocontrol inocula it is of relevance, because choosing very different proportions in a formulation may allow the combination of otherwise poorly compatible organisms.

Under field conditions *C. rosea* remained the dominant species (Figs. 2 and 4). It maintained applied populations throughout the

Table 6
Summary of meteorological data measured during the field survival study and long term (>50 years) averages for La Lola, Costa Rica, 2001 and 2003.

	2001			
	July (from 17th)	August	September (up to 17th)	Mean
Mean monthly temperature	24.2	24.7	24.6	24.5
Long term average (°C)[1]	25.1	25.2	25.5	25.3
Mean monthly relative humidity	93.3	94.0	93.3	93.5
Long term average (%)[1]	92.2	91.4	90.8	91.5
Mean minimum relative humidity	60.0	59.0	64.0	61.0
Long term average (%)[1]	67.7	64.2	61.2	64.4
				Total
Precipitation total	137.0	452.2	27.9	617.1
Long term average (mm)[2]	192.5	305.6	99.3	597.3
	2003			
	March (from 21st)	April	May (up to 23rd)	Mean
Mean monthly temperature	24.5	24.2	24.4	24.4
Long term average (°C)[1]	24.5	25.1	25.6	25.1
Mean monthly relative humidity	86.8	86.7	91.2	88.2
Long term average (%)[1]	88.7	88.9	90.8	89.5
Mean minimum relative humidity	50.0	46.0	56.0	50.7
Long term average (%)[1]	59.7	61.8	64.3	61.9
				Total
Precipitation total	81.4	219.0	251.4	551.8
Long term average (mm) [2]	114.9	224.2	242.5	581.6

[1] Long term averages are for entire months.
[2] Long term averages scales proportionate to trial days in month.

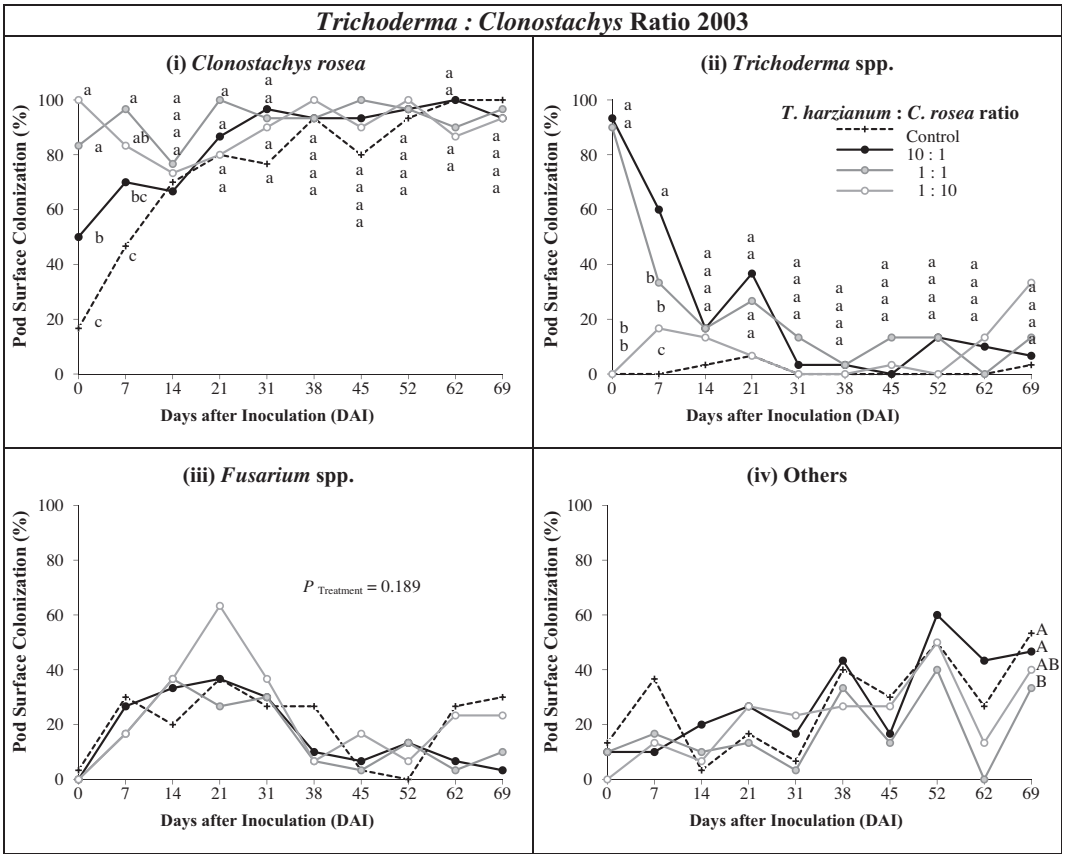

Fig. 4. Percentage surface colonization on cocoa pods by naturally occurring and inoculated mycoparasite genera following surface sterilization and application of different ratios of *Trichoderma harzianum* APP129 and *Clonostachys rosea* G7 to surface-sterilized pods. Treatments marked with the same common letter do not differ at $P = 0.05$ on that day; treatments marked with the same capital letter do not differ across DAI (GMM, followed by LSD).

trial duration and was the strongest colonizer of surface-sterilized control pods. In contrast to the laboratory, where *Clonostachys* was unaffected by *Trichoderma* even at 100 times higher concentrations, in the field a 10-fold higher *Trichoderma* concentration affected *C. rosea* for 7 DAI. At the same time, epiphytic *Trichoderma* pod coverage was generally too low and too ephemeral for a promising biocontrol candidate. *Trichoderma* detection even declined rapidly after application of *Trichoderma* sp. T4 on its own (Fig. 2). In the mixture of *C. rosea* with *Trichoderma* sp. T4, *Trichoderma* populations failed to surpass those of the *C. rosea*-only cocktail, confirming that *C. rosea* antagonized *Trichoderma* spp. in the field. Tropical rains (Fig. 3) may also have affected the persistence of *Trichoderma* spp. on the pod surface (Figs. 2ii and 4ii). Nevertheless, for 15 DAI more *Trichoderma* spp. were recovered from pods to which *C. rosea* + *Trichoderma* spp. had been applied than from control pods. Apparently some of the applied *Trichoderma* spp. did survive and/or escape suppression by *C. rosea* locally for about 2 weeks.

Our results confirm the hypothesized dominance of *Clonostachys* spp. over *Trichoderma* spp. Epiphytic trichodermas investigated here seem to be inadequate pod surface colonizers to bring about reliable biocontrol. Therefore, the focus of our research group has redirected to endophytic *Trichoderma* isolates. Nevertheless, unfavorable natural compatibility can be manipulated by adjusting the inoculum ratios in mixed inocula. Thus, the optimum inoculum ratio of fungi in a mycoparasite mixture has to be determined before formulation and application in the field. Differences at the species and even isolate level require further investigations on compatibility and establishment of mycoparasite mixtures on natural cocoa pods in the field in order to obtain the quantitative resolution necessary to optimize inoculum design.

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