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PROCEEDINGS OF INCOPED 5TH INTERNATIONAL SEMINAR ON COCOA PESTS AND DISEASES

Proceedings of

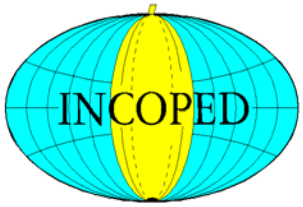
INCOPED 5TH

INTERNATIONAL SEMINAR

ON COCOA PESTS AND DISEASES

15TH -17TH OCTOBER, 2006, SAN JOSE, COSTA RICA.

EDITORS
AKROFI, A. Y. AND BAAH, F.



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Left: Cocoa pod infected by *Moniliophthora perniciosus*, courtesy Mirjam Bekker, CATIE, Costa Rica

Middle: *Sahlbergella sigularis*, courtesy J.E. Sarfo, CRIG, Ghana

Right: Mycelia and sporangia of *Phytophthora megakarya*, courtesy A. Y. Akrofi, CRIG, Ghana.

Proceedings

of

INCOPED 5th International Seminar

On Cocoa Pests and Diseases

15th -17th October, 2006,
San Jose, Costa Rica.

Theme: Developing Effective Sustainable Crop Protection Systems for Cocoa Production

Editors: Akrofi A.Y. and Baah F.

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Message from Hope Sona Ebai, Secretary General of Cocoa Producers' Alliance

The Chairman of INCOPED
The Chairman of INGENIC
The Chairman of INAFORESTA
Distinguished Scientists, Ladies and Gentlemen

I am very happy and honoured to be present at this joint opening ceremony. I am also happy to note that with the birth of INAFORESTA, our international working groups are growing in number and in output. Agroforestry brings us hope for additional income generation for farmers but I do believe that it is an opportunity to raise awareness of our collective environmental responsibilities.

As we fine-tune the various tools that are meant to enhance efficiency in cocoa growing, let us, as best as we can and where applicable give consideration to farmers' participation. In the same vein, I think it is important for the governments of the producer countries to be aware of on-going research and trials, because at a certain point in time, they would need to fit these results into their development programs and technology transfer schemes so the earlier they buy in the easier it would be eventually step up or step out or adopt the new technology. On our part, we will continue to play the role of the link to the policy makers of our member states and provide through these international cocoa research conferences an opportunity for your groups to meet and interact.

COPAL has set in motion an ambitious action plan to increase its membership so that we can have a larger exchange and debate on our respective efforts towards a sustainable world cocoa economy as well as provide a single address for the collection, documentation and dissemination of new technologies coming out of our research efforts.

I would like to salute industry for their efforts towards sustaining research through funding as well as their increased participation in COPAL activities especially our workshops and seminars. I would equally like to thank the governments of consuming countries for their continuous support to the cocoa sector. We will continue to promote dialogue so that the needs and concerns of the final consumer of cocoa products and those of farmers are taken on board as we set research priorities. As the need to properly manage scarce resources become more and more evident, we would need to encourage more collaborative efforts and a regional approach to problem solving.

Let us continue to work towards a sustainable cocoa economy. I wish you successful meetings and on this note declare open the 5th INGENIC Workshop on Cocoa Breeding for Farmers Needs, the 5th INCOPED International Seminar on 'Developing Effective Sustainable Crop Protection Systems for Cocoa Production and the 1st INAFORESTA group meeting.

Thank you for inviting me and thank you for your kind attention.

Mr. Hope Sona Ebai

Address by Chairman of the International Working Group on Cacao Trees, Forests and the Environment (INAFORESTA)

Distinguished guests, Dr. Har Adi Basri, from the Indonesian Cocoa Commission, members of the COPAL and CATIE organizing committees, scientists from around the cacao world, I am honored to address you this historic evening and to introduce you to **Inaforesta**, the International Working Group on Cacao Trees, Forests and the Environment. How did this vision come about? It is only 2 years since the 1st World Congress of Agroforestry where many of us gathered for the first time to discuss the formation of a group to be a partner with INGENIC and INCOPED in the future of cacao globally. Recognizing the urgency, we held a discussion at the World Agroforestry Centre (ICRAF) in Nairobi in 2005. This led to a second meeting at the Forest Research Institute of Ghana (FORIG) in mid 2006. From this meeting came the Kumasi Cocoa Quorum which formulated what it meant to be a partner in cacao science. There was universal agreement that a forum was needed within cacao agroforestry in its myriad of meanings and models to drive understanding of intensification of production, diversification of incomes and habit preservation and rehabilitation for biodiversity and environmental services. CATIE, The World Agroforestry Centre (ICRAF), IITA, CI, CIRAD and Mars working together have tonight launched **Inaforesta**. Over the next two days we will discuss how to lead the scientific endeavor in these disciplines. We need to learn from INGENIC and INCOPED how to promote this collaboration. We need to challenge our best thinking with all stakeholders from the cacao farmers to governments to donors to industry to policy makers on these critical issues and decisions.

There is no single model! There are many models to consider and understand. We must analyze, synthesize and disseminate the ideas through a series of filters or tests for the benefit of all stakeholders. What works in W Africa will likely be different in the Americas and E Asia. What works in the Americas will be probably different to W Africa and E Asia. What works in E Asia will probably be different in W. Africa and the Americas. And so on. But what is clear is a simple truth: We will all learn from each other and build models that will help the crop, the producers, their communities and landscapes to be economically viable, preserve and improve the production systems, maintain the habitat and forests outside the production systems, reduce the threat to fragile ecosystems and change the consumers perspective of what may be considered a maligned production system. Every option needs consideration without prejudice.

Tonight INGENIC and INCOPED begin their 5th respective meetings. We have to catch up fast. We cannot expect results tomorrow but we can and will tackle the issues. We as a group must consider the past, examine the present and suggest a series of models for the future to the myriad of questions that need answering. We have real knowledge but not all that is needed. Even with the best knowledge it still will need to be disseminated and put in practice with the cacao farmers' enthusiasm. The landscape of cacao production is under attack climatically, by soil depletion, boom and bust cycles, long term productivity and social non-sustainability.

We are here tonight to launch **Inaforesta**. The complications are many. The will to succeed is great. Join us in this endeavor in cacao agroforestry to improve the livelihood of cacao farmers globally through economic diversification, environmental

and biodiversity services to society. The first step has been taken. COPAL has a third sibling. Please allow us the chance to influence the future.

Thank you.

Howard-Yana Shapiro

Address by the Chairman of the International Permanent Working Group for Cocoa Pests and Diseases (INCOPED).

Mr. Chairman, Fellow Scientists, Ladies and Gentlemen, it is a pleasure to welcome you all on behalf of INCOPED

We select different seminar themes to help assemble our thoughts, directed at attending contemporary and future needs of the farmer. In this INCOPED 5th International Seminar we decided on '*Developing Effective Sustainable Crop Protection Systems for Cocoa Production'*. In this address, I normally list achievements made in our Group, committed to manage pests and diseases of cocoa. However, as our theme suggests, we do not operate on a single objective, but work towards improved production, productivity and sustainability of the cocoa farmer. Therefore, I opt to show that the three INs Specialized Groups (INGENIC, INCOPED and INAFORESTA) have to, and do, work jointly.

We have to understand that crop protection - the INCOPED mandate - in itself, does not increase production. In fact, the professionals in this area aim at protecting a potential crop, thus guarantee healthy development of flower buds to pods, subsequently provide a lucrative harvest, and/or ensure cocoa trees are in a state of health to produce. Therefore, to achieve sustainable cultivation of cocoa there has to be a strong link between INCOPED protective systems and other economic productive systems.

Implementing an entire crop protection schedule involves cost, which the farmer has to assume, and therefore, generating recommendations is not sufficient, if they cannot be applied in full in the field and thus be effective. However, in times as present, when sustainability is in question, this is difficult to accomplish. Cocoa prices are low, relative to crop protection inputs and plantations in greater part are decadent. As a result, farmers due to no fault of their own, seldom apply scientific based recommendations in full.

Ten years ago to the month, when invited to speak at one of the early INGENIC Workshops, that followed the 12th ICRC, I did say then (Dr. Eskers might recall), that the field applied components in strategies used for pest and disease management, by nature, are only stopgap measures while waiting for less cost-intensive recommendations. However, what we needed was to strengthen the genetic component in management strategies. Planting material, with inherent resistance to pests and diseases, not only maintains disease at lower levels, but also require less farmer-borne inputs; obvious, as we would then need to function on less intensive infections or infestations levels

Therefore, the good news is with members of the INGENIC Group it was possible to work jointly in intensive multi-disciplined projects undertaken in many countries, and as a result, demonstrated that by strengthening the genetic component in management, it is possible to have a more cost-effective IPM strategy.

Since then, we also have field use of a biological agent; the cost-effectiveness of which is demonstrated in that; farmer demand is greater then available supply. Consequently, the major benefits are: 1. A lower burden in recurring cost to be

sustained by the farmer; 2. In the process of introducing improved genetic material, decadent plantations can also be easily modernized at relatively little additional cost; 3. Also provide a greater probability of overcoming existing or new disease crises.

When dealing with a mono-crop one has to be aware of the obvious risk one takes. Cocoa cultivation in greater part is monoculture - an important and often sole money-earner in the humid belt of the tropics. But, we have seen disasters, for lack of alternative crops or ventures, to tide us over difficult times.

Therefore, to further add to the good news, we are now witnessing the 1st INAFORESTA meeting, showing a further link in our specialized groups towards sustainability. This, through diversification within the cocoa planted areas of farms, and/or surrounding land in farms, provides sources of additional income and thus ensures stability.

With this ladies and gentlemen, on behalf of INCOPED, I thank the Cocoa Producers' Alliance for continued assistance, which from the start has taken INCOPED under its umbrella; our host CATIE for their grateful local support; for vital grants towards staging the event, the British Mycological Society, Mars Inc., United States Department of Agriculture and the World Cocoa Foundation for its monetary pledge towards meeting the cost of preparing our Seminar Proceedings.

Also, I am sure you will join me in appreciating the dedication given by the National Organising Committee of INCOPED in this 5th International Seminar.

I thank you !

João Louis Pereira

Address by President of the International Group for Genetic Improvement of Cocoa (INGENIC)

Dear Mr. Andreas Ebert, Chairman of this joint opening ceremony
Dear Mr. Sona Ebai , Secretary General of COPAL
Dear organizers of the INCOPED and INAFORESTA events
Dear ladies and gentlemen

It is with pleasure that I welcome you here on behalf of the local organizing committee of the 5th INGENIC workshop, chaired by Wilbert Phillips, and on behalf of the INGENIC Board. INGENIC is very glad about the joint arrangements between the COPAL conference and the "IN" workshops. This has certainly helped to simplify and synergize the organization of these events. We are happy that the "IN" family is growing and wish INAFORESTA a very good start!

Since its creation in 1994, the mandate of INGENIC has not changed, i.e. firstly promotion of exchange of information and, secondly, enhancing collaborative approaches in the field of cocoa variety improvement and genetic research. INGENIC likes to consider itself as a platform linking cocoa geneticists as well as other interested persons. Currently, INGENIC's mailing list contains more than 300 addresses.

So what has INGENIC achieved in the 12 years that it has existed?" Shortly, we organized 4 workshops and published 10 newsletters. We can say that collaboration between cocoa breeders and other scientists has considerably increased; one example is the CFC/ICCO/Bioversity cocoa projects that were set up through discussions initiated at INGENIC workshops.

And what is new since the last workshop held in Accra in 2003? Firstly, a Molecular Biology Group was created in 2003 and this group has already held two meetings, the second one was earlier today. This group implemented two collaborative activities, an INGENIC micro-array study and agreed on a coordinated approach for the French Genoscope project. Secondly, INGENIC has set up a web site through the good services of Mark Guiltinan. At http://ingenic.cas.psu.edu/ you will find information on INGENIC, on its membership, on e-mail discussion lists including archives, the main conclusions from the workshops, scanned workshop proceedings, newsletters, lists of publications on cocoa genetics and breeding, and links to other related organizations and activities. Thirdly, at the Accra workshop a proposal was launched for increased regional collaboration in cocoa breeding (America, Africa and Asia). Coordinators were identified for each region and meetings were held during which activities were proposed. Unfortunately, financing to allow these activities to progress has not yet been obtained

Fourthly, INGENIC has participated actively in the discussions on the CacaoNet initiative to establish a Global Network for Cacao Genetic Resources and we are represented on the Steering Committee of this new network. Last Friday, INGENIC organized an open meeting to discuss "Cacao Germplasm Conservation, Evaluation, Utilisation and Exchange". It was agreed that INGENIC should collate opinions expressed on the development of the CacaoNet Conservation Strategy through its

internet discussion group. The outcome will be circulated to the discussion group and shared with the CacaoNet Steering Committee.

Can we feel satisfied with what was done? I think we should always ask ourselves the question: "What have we done that can have a real impact on the livelihoods of small cocoa farmers". This brings us to the central topic that we wish to address in the fifth INGENIC workshop, which has as title "Cocoa Breeding for Farmers' Needs". The topic of the workshop is in line with the increased involvement of farmers in the selection of new cocoa varieties. Examples are the selection for witches' broom resistant clonal varieties on farms in Bahia, Brazil and the participatory selection approach adopted in the CFC/ICCO/Bioversity project "Cocoa Germplasm Utilisation and Conservation: a Global Approach". The main topics of the workshop are:

- Farmers' perceptions and use of planting materials,
- Genetic diversity in farmers' fields, and
- Participatory selection of new cocoa varieties.

INGENIC is grateful for the financial and other types of support received from many institutions including BCCCA, CATIE, CIRAD, COPAL, CRIG, CRU, CTA, Bundesverband der Deutschen Susswarenindustrie (Germany), PennState University, Mars Inc., MCB, UESC, USDA and WCF.

I thank you for your attention and I am confident that the presence of so many of you will make the events very fruitful!

Dr. E. B. Eskes

1.1. Biological control of *Crinipellis perniciosa* by an isolate of *Trichoderma* sp.

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SUMMARY

Witches' broom caused by the basidiomycete fungus Crinipellis perniciosa (Stahel) Singer is the most important factor constraining cocoa production in the main cocoa growing regions of Brazil. Pod losses of up 90% are experienced in affected areas in Bahia and Amazon regions. Biological control by means of microrganisms is a powerful tool that could contribute substantially as an alternative or a part of an overall integrated management strategy of the witches' broom disease. The aim of this paper was studying in vitro and in vivo the effect of one isolate of Trichoderma sp. as a possible biocontrol agent for witches' broom disease. The antagonist was isolated from inner trunk tissues of cocoa, after surface desinfection with sodium hypochloride solution, rinsed once in 70% ethanol and twice in sterile distilled water. The isolate of Trichoderma sp. produced in liquid medium metabolites capable of inhibiting in 100% the mycelium growth and the germination of basidospores of C. perniciosa when culture filtrate was used in the concentrations of 2% and 10%, respectively. The production of basidiocarps on dead brooms was significantly reduced by one application of spore suspension (2 x 10^7 spores/ml) obtained from Trichoderma sp. grown on autoclaved rice grains. Experiments carried out in greenhouse showed that spore suspension (2 x 10^7 spores/ml) and culture filtrate in the concentration of 5% were able to reduce over 70.8% the incidence of witches' broom disease in cocoa seedlings, when applied six days before inoculation with the pathogen. These results suggest that this isolate of Trichoderma sp. is promising to be used as a biological control agent against C. perniciosa and therefore further studying is worthwhile.

INTRODUCTION

The basidiomycete fungus *Crinipellis perniciosa* (Stahel) Singer is the causal agent of witches' broom disease of cocoa (*Theobroma cacao* L.) which is the main factor limiting cocoa production in the Americas (Baker & Holliday, 1957). Breeding for resistance, phytosanitation and the application of fungicides are still the main tools for controlling this disease (Bastos, 1996).

Intensive wordwide research during the past several years has established biological control as a feasible alternative to the use of chemical for the reduction of plant diseases caused by pathogenic fungi (Lewis & Papavizas, 1993). Among the most studied antagonists are species of the genus *Trichoderma*, even for plant pathogens of phylloplane, when applied as sprays. This fungus has been studied in the control of several fungi (Tronsmo & Dennis, 1977; Moretto *et al*., 2001; Howell, 2002) including *C. perniciosa* (Bastos, 1988; Bastos, 2000). Key strategies for managing witches' broom disease include suppression of basidiocarp production by the pathogen inoculum sources (dead brooms) and protection of flushes and fruits against infection by basidiospores of the fungus. An antagonist fungus *Trichoderma*

stromaticum was shown to have potential to control witches' broom by reducing basidiocarp formation by 99% in brooms in contact with soil and 56% in brooms on trees (Costa *et al*., 1998).

With the increasing interest in developing alternatives to chemical control fungicides or integrated control of plant diseases, mass production of *Trichoderma* for use as a bioprotectant has become a focus of industrial research and development. Besides this, *Trichoderma* species are considered as standard in biological studies because they are easily isolated, grow fast in several substrates, affect several pathogens, act as mycoparasites, produce antibiotics and have an enzymatic system able to attack a range of plant pathogens (Well, 1986).

Epiphytic and endophytic fungi are being pursued as potential biological agents of the fungal diseases of cacao (Samuels *et al*., 2006). Endophytic fungi, in particular those that have coevolved with cacao or other *Theobroma* species, are being investigated for use as biocontrol agents within the framework of classical biocontrol (Evans *et al*., 2003)

The purposes of the research reported in this paper were to evaluate one isolate of *Trichoderma* sp. for the ability to control *C. pernciosa in vitro* and *in planta* under greenhouse conditions and on dead brooms.

MATERIALS AND METHODS

Trichoderma sp. isolate

The isolate of *Trichoderma* sp., potential biological control agent (BCA) was obtained from inner trunk tissues of cocoa tree, collected in the CEPLAC center for *Theobroma* Germplasm Collection from the José Haroldo Genetic Resources Experiment Station, Marituba, and Pará, Brazil, where the witches ' broom disease is endemic. For isolation of fungus, plant tissues were surface disinfected with hypochlorite solutions (3%) for 3 minutes, rinsed once in 70% ethanol and twice in sterile distilled water. Fragments were placed onto PDA plates and incubated at 25±1°C. The hyphal tips of mycelium that emerged from the fragments were transferred to PDA slants.

Trichoderma sp. and *Crinipellis perniciosa* growth *in vitro*

Examination of *Trichoderma* sp. and *C. perniciosa* growing in dual culture was made by placing mycelial disks of 5-mm diam. 4 cm apart on malt extract agar (MEA) plates. Controls were also set up using the antagonist or the pathogen alone, so that growth and interaction could be precisely measured. Plates were incubated at 25±1°C in the dark and after 12 days assesments of interaction and growth of *C. perniciosa* was made by measuring colony radii at right angles. Experiments were repeated at least twice each with five replicates.

Trichoderma sp. metabolite production

Three disks of medium with *Trichoderma* mycelium were placed in 250 ml-Erlenmeyer flasks containg 50 ml of potato dextrose medium (PD) and incubated for eight days at lab conditions without shaking. The fermented broth was filtered on paper filter (Whatman no. 4) and then, on a Millipore membrane (0.22μm).

Activity of *Trichoderma* sp. metabolite on germination of *C. perniciosa* basidiospores

Required concentrations of culture filtrates autoclaved and non-autoclaved (0.0, 0.1%, 0.3%, 0.5%, 1.0%%, 2.0% and 3.0%) were made by serial dilution with sterile distilled water. Drops (100 µl) of different concentrations of filtrates or sterile distilled water were pipetted separately into microscope cavity slides contained within Petri dishes lined with moist filter paper. An agar block (c 3 mm diam) containing freshly-deposited basidiospores was placed in each drop. Four replicate slides were prepared per treatment for each experiment. Plates were incubated at 25°C and after 24h a drop of a 1% cotton blue in lactophenol was placed into each cavity to stain spores and germ tubes and to arrest further growth. Spores were considered to have germinated when germ tube length was longer than the maximum spore diameter. Percentage germination was calculated based on germination of 100 spores at random in four microscope fields from each cavity slide.

In addition, culture filtrate was incorporated to MEA at concentrations ranged from 1% to 10% and poured into Petri plates (50 mm diam) which were centrally inoculated with agar discs (5 mm diam) of the pathogen. Plates with no filtrate served as control. Four replicates were prepared per treatment. Plates were incubated at 25±1°C in the dark for seven days and then the radial growth of the mycelium was recorded.

Fungicidal action of filtrate of *Trichoderma* sp. to basidiospores of *C. perniciosa*

Basidiocarps were taken from the brooms and stuck inside the lids of Petri dishes with vaseline to deposit basidiospores on 1.5% water agar. The basidiocarps in triplicate dishes were used. The spores "prints" were flooded with 5ml of a series of dilutions of culture filtrate. At various intervals, one plate from each treatment was harvested, the solution removed and the spore "prints" washed three times with sterile distilled water. These were then incubated at 25°C and after 24 h basidiospore germination was observed under the microscope.

Effect of *Trichoderma* sp. and its metabolite on the protection of cocoa seedlings against infection by *C. perniciosa*

The trials were done to test the effect of spore suspension of *Trichoderma* and its metabolites on the infection of cocoa seedlings by *C. perniciosa.* Culture filtrates of the antagonist were obtained as described above and fermented broths autoclaved and non- autoclaved were used. For production of inoculum the antagonist was grown on autoclaved rice grains in 250 Erlenmeyer flasks.

Seeds of cocoa cultivar PA 195, susceptible to witches' broom disease were peeled and placed on wet blotting paper in a seed germination tray. The germinated seeds were then planted singly in 9 x 25 cm conical tubetes containig soil and kept in a greenhouse. When the seedlings were 40 days old they were sprayd with culture filtrate autoclaved and non-autoclaved in the concentration of 5% and spore suspension (2 x 10^7 spores/ml) or water as control. The seedlings were sprayed individually to run off with the treatments six days before inoculation with basidiospore suspension (1 x 10^5 spores/ml) of the pathogen. Ten cocoa seedlings were used as replicates for each treatment. The basidiospore suspension was obtained by diluting the inoculum stock solution stored in liquid nitrogen with 0.25% agar-water. Aliquots of 30 µl of spore suspension were placed on the apical buds of plants.

Inoculations were carried out in a room fitted with a humidifier to maintain the temperature at approximately 25°C at 100% RH. After incubation for 24 h the plants were moved to a greenhouse. The presence or absence of vegetative brooms was recorded.

Effects of *Trichoderma* sp. on basidiocarps production

The experiment was conducted in "vassoureiro" conditions. Dead broom hung were sprayed to run off with a spore suspension (2 x 10^7 spores/ml) and the numbers of mature basidiocarps produced on each broom were subsequently recorded. Fifty brooms for each treatment were used. The antagonist was applied once only. The brooms were then subjected to a daily regime of 16 h wet and 8 h dry, when there was no rain. The numbers of mature basidiocarp produced were recorded twice a week for three months, starting from the week that they were first found. Once mature basidiocarps had been counted, they were removed from the brooms to avoid counting them twice.

RESULTS AND DISCUSSION

***In vitro* interactions and metabolite production**

In paired cultures, no visible inhibition zone was observed. *Trichoderma* sp. had growth velocity significantly higher than *C. perniciosa.* The antagonist overgrew the pathogen colonies, which often grew (28.7 mm diam) while the control continued to grow (68.25 mm diam) The isolate of *Trichoderma* sp. produced in liquid medium metabolic products with inhibitory activity against the witches' broom pathogen.

The results (Table 1) demonstrate that culture filtrates of *Trichoderma* sp. are highly toxic to spore of *C. perniciosa*. No germination was recorded in the broth at concentrations over 1% after 24 h due to spore plasmolyses compared with 100% germination in control. The ability of *Trichoderma* metabolite to inhibit basidiospore germination is an essential requirement for protecting young tissues *in vivo*

Similar culture filtrate when incorporated into MEA plates caused complete inhibition of the mycelium growth of *C. perniciosa* compared with the controls, MEA with no filtrate, 12 days after incubation (Figure 1). In addittion the metabolite produced by the *Trichoderma* isolate did not loose its activity after autoclaving (data not shown).

Table 1. Percentage basidiospore germination of *Crinipellis perniciosa* after incubating with different concentrations of culture filtrate of *Trichoderma* sp. for 24 h.

Concentration of culture filtrate (%)	Basidiospore germination (%)*
Control	100.0
0.1	100.0
0.3	100.0
0.5	83.3
1.0	33.3
2.0	0.0
3.0	0.0

* Values are means for 3 replicates; 100 basidiospores per replicate

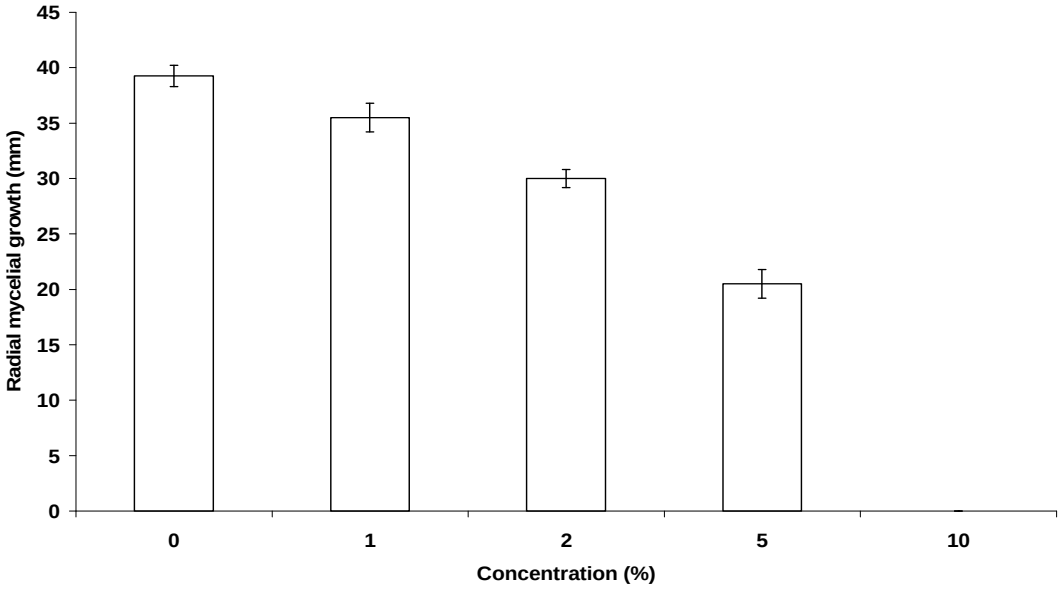

Figure 1. Effect of culture filtrate of *Trichoderma* sp. on mycelial growth of *Crinipellis perniciosa* after incubation on MEA plates for 7 days. Bars = S.E

Fungicidal action of the culture filtrate

The effect of exposing basidiospores of *C. perniciosa* to various concentrations of culture filtrate solution is shown in Table 2. Incubation at 5% and a 60 minute exposure to the antifungal agent was fungicidal to all the spores. Incubation in increasing concentration (10%) was increasingly fungicidal, and no germination took place.even from spores that had been in contact with the metabolites for only 10 minutes. Thus, under the conditions used, the metabolites of *Trichoderma* sp. were primarily fungicidal.

Table 2.Fungicidal action on germination of basidiospores of *Crinipellis perniciosa* suspended in various concentrations of *Trichoderma* sp.culture filtrate for different periods of time

Concentration (%) Germination after exposure to metabolites

	10 min	20 min	30 min	60 min
Control	++	++	++	++
1.0	++	++	++	++
2.5	++	+	+	+
5.0	+	+	+	-
10.0	-	-	-	-

* Values are means of 3 replicates.

** ++ = germination normally; + = partial germination; - = total inhibition.

Infection protection on cocoa seedlings

The results (Table 3) show that culture filtrates autoclaved, non-autoclaved and active conidia of *Trichoderma* sp. applied on cocoa seedling were able to reduce significantly the infection caused by *C. perniciosa*, compared with the untreated

plants, inoculated controls. The fungus successfully introduced into healthy tissues suppressed witches' broom symptoms under greenhouse conditions.

Table 3: Effect of *Trichoderma* sp.on basidocarp production of *Crinipellis perniciosa* on dead brooms

Treatment	Number of basidiocarps*
Control	689
*Trichoderm*a sp.	232

**Values are the total of basidiocarps produced in 50 dead brooms/treatment.*

The reduction on the infection percentage of cocoa seedlings produced by *Trichoderma* sp. when applied six days before inoculation, can be due either to the liberation of metabolites or parasitism of the antagonist against the pathogen. Although it was not able demonstrate mycoparasitism of *Trichoderma* sp., *in vitro* trials demonstrate that the isolate have an antibiotic effect against *C. perniciosa*. Thus, it suggests that the metabolite could be produced in planta and it could contribute to induce resistance to witches´broom disease.

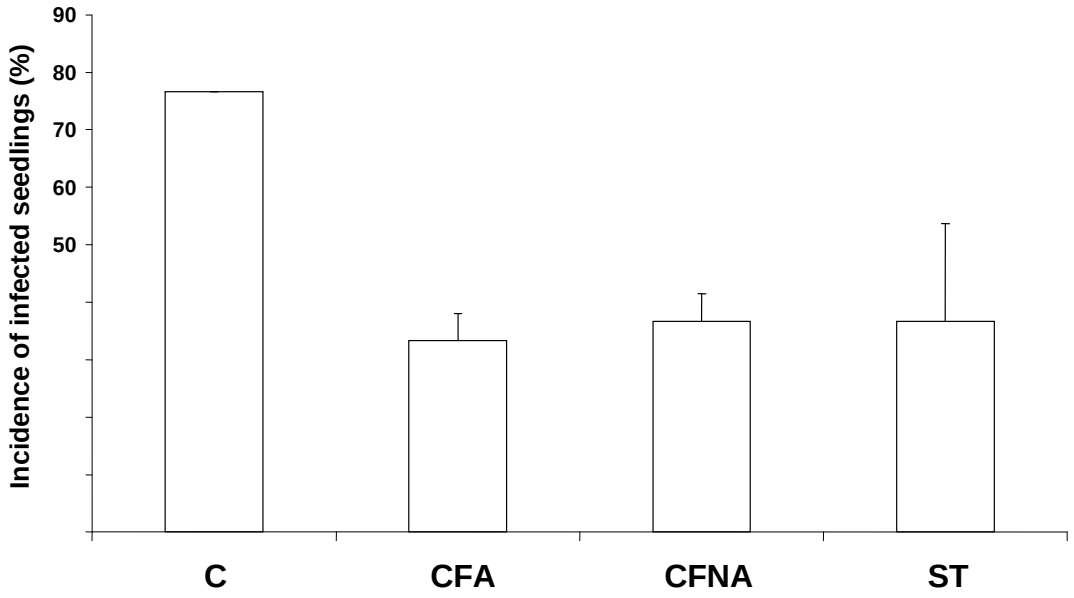

Figure 2 Effect of *Trichoderma* sp. on cocoa seedlings 6 days before inoculation with *Crinipellis perniciosa* . C= control; CFA = culture filtrate (5%) autoclaved; CFNA=culture filtrate(5%); not autoclaved; ST= spores of *Trichoderma* . Bars = S.E.

Trichoderma sp. on basidiocarps formation

The formation of basidiocarps on dead brooms was significantly suppressed by the Trichoderma sp.when compared with the control (Table 3). There was not effect of culture filtrates when applied on dead brooms (data not showed).

CONCLUSION

In conclusion, the isolate of Trichoderma sp. demonstrated potential to prevent infection on plant tissues and also to reduce pathogen inoculum on dead brooms. Thus, two alternative methods of control of the witches' broom pathogen through the use of the isolate of *Trichoderma* sp. are feasible: direct biological control, treatment of brooms and healthy cocoa tissues and chemical control, by treatment of healthy cocoa tissues with mycotoxin from culture filtrates. Finally, biological control of cocoa witches' broom is not regarded as a potential panacea, but rather an additional tool that can be applied in combination with partial disease resistance in the cultivar, sanitation measures and other practices.

REFERENCES

Baker, R. E. D. and Holliday, P. (1957). Witches' broom disease of cocoa (*Marasmius perniciosus* Stahel). Phytopathological Papers n. 2, 42p.

Bastos, C. N. (1988). Resultados preliminares sobre a eficácia de *Trichoderma viride* no controle da vassoura-de-bruxa (*Crinipellis perniciosa*) do cacaueiro. Fitopatologia brasileira, Brasília 13(4): 340-342.

Bastos, C. N. (1996). Mycoparasite nature of the antagonism between *Trichoderma viride* and *Crinipellis perniciosa*. Fitopatologia brasileira, Brasília 21(1): 50-54.

Bastos, C. N. (2000). *Trichoderma stromaticum* sp. nov. na produção de basidiomas e infecção de ramos e almofadas florais do cacaueiro por *Crinipellis perniciosa*. Agrotrópica, Ilhéus 12:59-62.

Costa, J. C. B., Bezerra, J. L.and Bastos, C. N. (2000). Ação antagonista de *Trichoderma stromaticum* sobre a produção de basidiomas de *Crinipellis perniciosa* no Estado da Bahia. Fitopatologia brasileira, Brasília 25 (Suplemento): 366.

Evans, H. C., Holmes, K. A. and Thomas, S. E .(2003). Endophytes and mycoparasites associated with an indigenous forest tree, *Theobroma gilleri*, in Ecuador and a preliminary assessment of their potential as biocontrol agents diseases. Mycological Progress 2: 149-160.

Howell, C. R. (2002). Cotton seedling premergence damping-off incited by *Rhizopus oryzae* and *Pythium* spp. and its biological control with *Trichoderma* spp. Phytopathology 92: 177-180.

Lewis, J. A. and Papavizas, G. C. (1993) *Stilbella aciculosa*: A potential biocontrol fungus against *Rhizoctonia solani*. Biol. Sc. and Tech. 3: 3-11.

Moretto, K. C. K., Gimenes-Fernandes, N. and Santos, J. M. (2001). Influence of *Trichoderma* spp. on *Colletotrichum acutatum* mycelial growth and morphology and infection of "Tahiti" lime detached flowers. Summa Phytopathologica 27(4): 357-364.

Samuels, G. J., Suarez, C., Solis, K., Holmes, K. A., Thomas, S. E., Ismaiel, A and Evans, H. C. (2006). *Trichoderma theobromicola* and *T. paucisporum*: two new species isolated from cacao in South America. Mycologocial Research 110: 381-392.

Tronsmo, A.and Dennis, C. (1977). The use of *Trichoderma* species to control strawberry fruits rots. Journal Plant Pathology, Netherlands, 85: 449-455.

Well, H. D. (1986). *Trichoderma* a biocntrol agent. *In:* Mukerjl, K. & Garg, K. L. (Ed.) Biocontrol of plant disease. Boca Raton CRC Press, p.71-82.

1.2 Early detection of frosty pod rot as key to cost-effective control

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SUMMARY

Moniliophthora roreri causes frosty pod rot (FPR) and losses of around 70-80% in most areas where it is well established. FPR can lead to complete crop failure. Cultural control has proven to be the central pillar of any integrated FPR control approach. Diseased pods have to be removed from cocoa trees before the pathogen sporulates on infected pods. The pathogen has a latent phase of approximately seven weeks. By the time external symptoms appear, the marketable product, the cocoa beans, are largely destroyed. Currently, growers in FPR-affected countries are able to recognize the disease one week before sporulation, necessitating costly phytosanitation in weekly intervals, if sporulation is to be prevented effectively. Although this is the only sound approach from an epidemiological point of view, this frequency is cost-effective in a few low-wage regions only. However, less frequent phytosanitation results in sporulating pods on the trees and thus exacerbates FPR and increases subsequent control costs. This paper presents a possible solution to this dilemma.If growers and their field workers could be trained to recognize early, subtle symptoms of FPR one to two weeks earlier than they can at present, this would reduce the necessary removal frequency to once in every 15-20 days and thereby the cost of disease management. This paper discusses possible training approaches and tools as well as likely challenges in achieving this goal.

PROBLEM DEFINITION

Moniliophthora roreri, the causal agent of frosty pod rot (FPR), is an invasive pathogen that has expanded from South America (Peru, Ecuador, Venezuela, Colombia) into Central America (Panama, Costa Rica, Nicaragua, Honduras) in the late 19-hundreds and, in recent years, established itself in Guatemala (2002), Belize (2004) and Chiapas, Mexico (2005) (Phillips-Mora *et al.*, 2006 a, b). Few pathologists doubt that the spread of the disease to countries in the Americas still free from it, is no more than a question of time. Such imminently threatened countries are the Dominican Republic, the world's largest producer of organic cocoa, and Brazil, the largest cocoa producer on the American continent. There is also concern that FPR might be introduced to West Africa in the longer term, where most of the global cocoa production is currently grown.

FPR causes losses of around 70-80% in most areas where it is well-established and can lead to complete crop failure. Cultural control has proven the central pillar on any integrated FPR control approach (Bateman *et al.*, 2004). Diseased pods have to be removed from cocoa trees before the pathogen sporulates on these pods. The pathogen has a latent phase of approximately seven weeks. By the time external

symptoms appear, the marketable product, the cocoa beans, are largely destroyed. Currently, growers in FPR-affected countries are able to recognize the disease one week before sporulation, necessitating a very costly weekly phytosanitation if sporulation is to be prevented. Although epidemiologically speaking, the only sound approach is weekly phytosanitation (Soberanis *et al*., 1999), this frequency is cost-effective only in a few low-wage regions (Leach *et al.*, 2002; Krauss *et al*., 2003). The paper suggests a practical solution to this dilemma.

Symptom	**Healthy Pods**	**Frosty Pods Rot-infected Pods**
Deformation		
Discolouration		
Internal rot		

Figure 1: Healthy fruit can show deformation and external discolouration similar to FRP-infected fruit.

POTENTIAL SOLUTION: EARLY RECOGNITION

If growers and their field workers could be trained to recognize early, subtle symptoms of FPR one to two weeks earlier than they can at present, this would reduce

the necessary removal frequency to once in every 15-20 days and thereby the cost of disease management. This paper discusses possible training approaches and tools as well as likely challenges in achieving this goal.

Figure 1 illustrates how easily the early symptoms of FPR can be mistaken with slight abnormalities on healthy pods, e.g. deformations and irregular colourations during ripening. No farmer wants to remove a healthy pod on the suspicion that it might be infected; therefore, growers need to learn to distinguish healthy from diseased pods at this early stage. Infected pods are heavier and, when apparently mature, the seeds do not move freely inside the pod hollow, a movement associated with a characteristic sound in healthy pods. Practical training is required, as well as familiarity (of the trainer and grower) with the germplasm presents in the field, as some odd shapes and colours are particular to certain cultivars. Certainty can be provided by a shallow cut under the pod skin: a brown striation indicates FPR, while a healthy pod would expose white tissue. This method should only be applied to mature pods, as secondary infections entering the wound could kill younger pods.

Pods showing only small external lesions can already be largely destroyed internally (Figure 1). Furthermore, other diseases produce symptoms which may be mistaken for FPR (Figure 2). For example black pod, caused by *Phytophthora* spp., produces brown lesions, which, in contrast to FPR, starts from the outside of the pod. Generally, black pod lesions exhibit clearer demarcation lines and little or no discolouration of the healthy tissue, but borderline cases are known to occur in certain cultivars (Figure 2).

Pod disorder	**Mistakable symptom**	**Frosty Pods Rot-infected Pods**
Black Pod: Clearer demarcation line around lesion Rot starts on pods shell and progresses inwards	 	 

Witches' Broom: Distinguishable only by absence of cream-coloured sporulation on pods in field and laboratory	 	   

Figure 2: Similarities and differences between FPR and other cocoa diseases.

Witches' broom, caused by *Moniliophthora perniciosa*, is indistinguishable from FPR at the early stages (Figure 2). This does not matter, as pods with either infection should be removed. Nevertheless, if confirmation of the presence of the pathogen *M. roreri* is desired, slicing the pods and incubating them in humid chambers will give clarity: *M. roreri* produces its characteristic tan spores within a week (Figure 2).

TRAINING TOOLS NEEDED

Apart from competent trainers, graphic training tools for the early recognition of FPR are needed. Figure 3 depicts a prototype chart developed by our team. With little additional effort, this can be adapted for particular regions and/or purposes, i.e. for FPR control in countries with or without witches' broom presence, or for phytosanitary quarantine officers at potential points of entry in FPR-free producer countries.

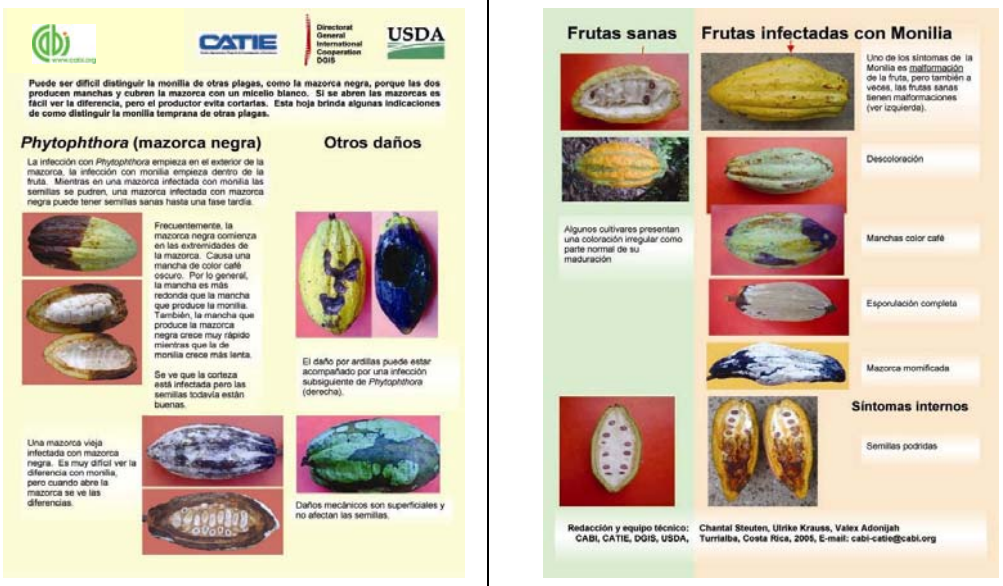

Figure 3: Training tools for growers and extensionists.

Our team also possesses decades of experience in participatory approaches to technology transfer in cocoa (e.g. Vos and Krauss, 2004).

CONCLUSION

Successful training will reduce the necessary removal frequency to once every 15-20 days and, thus, cost of disease management in FPR-affected countries. For countries still free of the disease, these tools will provide the necessary support to quarantine and extension services to detect any introduction in a timely manner, while eradication is still possible and economical.

ACKNOWLEDGEMENTS

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REFERENCES

Bateman, R.P., Holmes, K.A., Krauss, U., Padi, B. 2004. Future tactics and tools for pest management. *In*: Flood, J. and Murphy, R. eds. Cocoa Futures; A source Book of Some Important issues facing the Cocoa Industry., 1st ed. CABI-Federacafé USDA, Chinchiná, Colombia, p. 77-93.

Krauss U, Ten Hoopen M, Hidalgo E, Martínez A, Arroyo C, García J, Portugués A, Sánchez V (2003) Manejo Integrado de la moniliasis (*Moniliophthora roreri*) del cacao (*Theobroma cacao*) en Talamanca, Costa Rica. *Agroforestería en las Américas* **10**, 52-58.

Leach A.W, Mumford J.D, Krauss U (2002) Modelling *Moniliophthora roreri* in Costa Rica. *Crop Protection* **21**, 317-326.

Phillips-Mora, W., Cawich, J., Garnett, W., Aime, M.C. 2006a. First report of frosty pod rot (moniliasis disease) caused by *Moniliophthora roreri* on cacao in Belize. Plant Pathology 55: 584.

Phillips-Mora, W., Coutiño, A., Ortiz, C.F., López, A.P., Hernández, J., Aime, M.C. 2006b. First report of *Moniliophthora roreri* causing frosty pod rot (moniliasis disease) of cocoa in Mexico. Plant Pathology 55: 584.

Soberanis W, Ríos R, Arévalo E, Zúñiga L, Cabezas O, Krauss U (1999) Increased frequency of phytosanitary pod removal in cacao (*Theobroma cacao*) increases yield economically in eastern Peru. *Crop Protection* **18**, 677-685.

Vos J.G.M., Krauss U (2004).Working with farmers. In *Cocoa Futures*, J Flood, R Murphy (Eds.), CABI Commodities Press, Cali, Colombia, 141-149.

1.3. Biocontrol of cocoa mirid *Sahlbergella singularis* hagl. (Hemiptera: Miridae) with *Beauveria bassiana* Vuillemin: First results of activities carried out at IRAD, Cameroon*

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SUMMMARY

Mirids are the main detrimental pests of cocoa in Africa. In Cameroon, Sahlbergella singularis Hagl. is the most common species. The laboratories of entomology and biological control of the Agricultural Research Institute for the Development (IRAD) at Yaoundé, develop research activities aiming to control the populations of this pest by the use of entomopathogenic fungi. 5 strains of Beauveria bassiana Vuillemin were sampled and isolated on Water Agar medium from infected mirids, obtained in a laboratory rearing and in an experimental plot of Nkolbisson research station. Each isolate was grown on potatoes dextrose agar (PDA). The growth rate and the sporulation ability of each strain were studied for conidia suspension of 10^5, 10^6 and 10^7 conidia/ml. These two characters allowed the differentiation of the strains, and then, three among them were selected for the pathogenicity tests towards S. singularis. For these tests, cocoa cherelles were immersed in conidia suspension of the 3 selected strains at the concentration of 10^6 conidia/ml. Then, they were introduced in plastic boxes measuring 10x7x2cm. S. singularis nymphs of 4^{th} and 5^{th} stages, coming from a laboratory rearing, were introduced in the boxes, where they fed on the cherelle. The pathogenicity of the strains was assessed by observing the daily mortality of the nymphs. All the isolate tested were pathogenic to S. singularis, but to varying degrees. The mortality of the nymphs appeared since the third day and it was total on the sixth day, for two of the three strains.

* Full text of paper not available at press time

1.4. New sources of resistance to *Moniliophthora roreri*

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SUMMARY

Studies carried out within an old cocoa hybrid population at the Pichilingue Tropical Research Station showed twelve trees with a year incidence of frosty pod rot below 15%, while the population average was around 60%. In order to establish if this apparent resistance was genetic in origin, the present study was performed, challenging every tree with the disease under controlled conditions. A minimum of thirty pods per tree were inoculated with a spore suspension (5 X 10^4) of Moniliophthora roreri. Three highly susceptible trees from the same population (>50% infection) and the clones SCA12, EET387 (resistant), EET 19, EET 48 and EET 103 (susceptibles) were used as control. Fruits were hand pollinated using IMC 67 pollen, inoculated when 85 days old and were covered with plastic bags all throughout the study to avoid natural infection. Weekly monitoring of the selected trees was conducted to record incubation period. At maturity, fruits were harvested and the degree of infection or external and internal severity was recorded following formerly established scales. One tree performed as immune with no infection while EET387 and another two presented less than 30% infected fruits. SCA 12 presented 70 and 75% disease incidence, and the rest presented the expected >50% of infection. Components of resistance acting on these trees are discussed.

RESUMEN

Evaluando el comportamiento individual de una antigua población de híbridos de cacao en Pichilingue, se encontraron once árboles con una incidencia anual de Moniliasis < 15%, mientras el promedio de la población fue del 60%, para determinar si esta resistencia es genética se realizó un estudio inoculando un mínimo de treinta frutos por árbol, con una suspensión de esporas de 5 x 10^4. Como control se inocularon tres árboles susceptibles (> 50% de infección) y los clones SCA 12 y EET 387 (resistentes); EET 19, EET 48 y EET 103 (susceptibles). Los frutos se obtuvieron por polinización manual con polen del IMC 67; antes y después de la inoculación, los frutos se mantuvieron cubiertos para evitar la infección natural. Después de la inoculación se hicieron observaciones semanales para registrar el periodo de incubación y cuando los frutos alcanzaron la madurez se los cosecharon y evaluaron según escalas establecidas de severidad externa e interna. Un árbol no ha presentado reacción a las inoculaciones y dos se mantienen por debajo del 30% de infección. El SCA 12 presentó 77% de infección y los demás árboles se mantienen alrededor de la media esperada (> 50%). Se discuten algunos de los componentes de la resistencia que pudieran estar presentes en los cultivares.

INTRODUCTION

Cocoa has been an important economic crop for Ecuador since colonial times, when it depends almost exclusively on this crop; all expenses required by independence wars in America, the maintenance of troops and army supplies were covered by wealth generated with cocoa trade to Europe. Alter independence, initial trades, banks, factories were financed by cocoa. This continued until the first World War and the appearance of complex diseases that started affecting the crop around 1914-1920.

"Quevedo disease", "watery pod rot" or "frosty pod rot" were the most conspicuous at the time. This disease eventually was identified as caused by a fungus that Rorer (1916) named *Monilia roreri*; then, another common name starting to spread between farmers and stake holders: "Monilla" or "Moniliasis". As time and science progressed, we now know it is a basidiomycete and its actual accepted name is "*Moniliophthora roreri*".

Moniliasis or frosty pod rot is a disease that affects only pods, and its damage to harvest may easily reach eighty to a hundred per cent losses with an average around 40 to 60% annually. (Suárez, 1983). Losses due to *M. roreri* vary according to age of fruits, environment, the genetic of the crop and management. From Rorer´s times to date it has been a quest for control measures, and since cultural and chemical ones have proven elusive, to find resistance genes is considered the most sustainable possibility. For this reason, INIAP works on the development of new varieties looking for genotypes with traits for flavor, yield and disease resistance.

Studies on an old population of hybrids in Pichilingue ("2A" Plot) carried out by Agama (2004) who registered yield and diseases of each tree of this population and found that disease incidence was not similar to all of them, with two year of records, eleven trees were found highly productive and with a low (< 15%) incidence of pod infection. It was suspected of genetically resistant trees but it was argued as well that they may have escaped from the disease by some reason (climate during fruit development, location of plants within the plot etc) (Agrios, 2002). Sources of pod resistance, especially against *M. roreri* are very scarce and therefore any new finding in this area would be a significant contribution for cocoa breeding. These observations gave place to the present research to determine if the apparent resistance seen on this material was genetic in origin or it was some form of escape.

OBJECTIVES

Overall Objective

To find new sources of resistance to *M. roreri* in cacao as a contribution to a sustainable control of this disease.

Specific Objectives

1. To know the reaction of a group of cocoa trees to artificial inoculation with *Moniliophtora roreri.*

2. - Determine if there is a significant level of resistance to the disease and establish if that is genetic in origin or a consequence of phenology of the crop and weather circumstances.

3. - To relate natural infection of the selected trees with its reaction to the artificial inoculation.

METHODOLOGY

This research was developed in the Tropical Experimental Station at Pichilingue belonging to the National Institute of Agricultural Research of Ecuador. It is located 5Kms SouthWest of Quevedo, at 79º 28` 24`` South, 75 m above sea level, with 2400 mm of rain, 25°C of average temperatura, 85 % of Relative Humidity and with an average of 900 sunshine hours per annum; there are two well define weather seasons:

the rainy season (hot and humid) from December to May and the dry one (fresher and dry) from June to December.

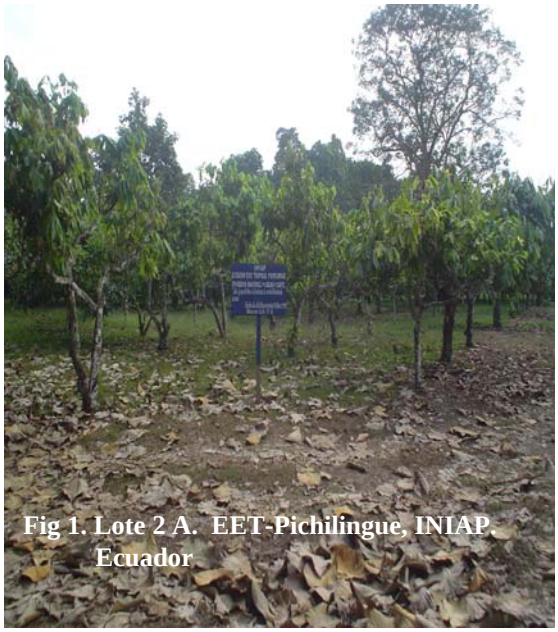


Fig 1. Lote 2 A. EET-Pichilingue, INIAP. Ecuador

Field work was conducted on a plot known as "2A". The whole orchard has 620 trees planted in 1954; they were rehabilitated on 1990 and since then this orchard is yielding more than 1500 kg of dry cocoa per year. Within this plot, 11 trees that had good productivity and presented, during two consecutive years, less than 15% pod infection were selected for this study. The plot (Figure 1) is maintained with recommended sanitary and agronomic practices; during the last three years only organic fertilizer has been added. The selected "resistant" trees were compared with two of the same population but with high percentage of pod infection, with SCA12 and EET387 as resistant, and with the commercial clones EET 19, EET48 & EET103, susceptibles (Tables 1 and 2)

Table 1.Identification and Genetic origin of the trees used for *M. roreri* resistant study

Code	**Resistant trees (Origin)**
2396	EET 161 x EET 166 (N. * V.A.) * (N. * V.A.)
2250	EET 156 x EET 166 (N. * V.M.9) * (N. * V.A.)
2228	EET 166 x EET 84 (N. * V.A.) * (V.M.)
2217	EET 238 x EET 106
2197	EET 238 x EET 106 (N. * V.M.) * (N.)
2195	EET48 x EET 195 (N.) * (N.)
2126	EET 48 x EET 195 (N.) * (N.)
2078	EET192 x EET 237 (N.) * (V.M.)
2076	EET 192 x EET237 (N.) * (V.M.)
2707	EET 1 x SCA 6 (N*VA) * FOR. AMARILLO
2748	LOST
SCA 12	FORASTERO AMAZONICO CONTROL RES.
EET 387	Selec hibrido cruce 2122 CONTROL RES.
	Susceptible trees
2327	EET6 x SCA 12 (V.M.) * (For.Amaz.)
2570	EET 250 x SCA 12 (V.A.) * (For.)

Table 2. Commercial clones used as susceptible control

Code	**Synonimia/Origin**
EET 19	Tenguel 15/Nac. Complex
EET 103	Tenguel 25/ Nac. Complex
EET 48	Santa rosa 34/ Nac. Complex

Trees and clones selected were irrigated and sprayed with B (22%) to increase flowering. All fruits present naturally at the beggining of this trial were eliminated.


Fig 2 Polinización artificial

Hand pollination was carried out covering buttons with plastic tubes (Eppendorf de 1.5 mL), to avoid natural fertilization with unknown pollen; the following day opened flowers were fertilized with pollen from IMC 67 (Figure 2). After 8 days, tubes were removed and small fruits covered with plastic bags that were kept until harvest. Enough number of flowers was pollinated considering only 40% success. Sometimes this estimate was even lower, and successive pollinations were done until complete at least 30 fruits/tree.

When fruits reached 85 days (had ± 8 cm in length) they were inoculated with *M. roreri* spore suspension of 50000 spores/ml; inoculum was prepared in the Plant Pathology lab. of the Crop Protection Department at Pichilingue. Cultures were freshly isolated from infected pods on Agar-Potato-Dextrose (Difco), and incubated for 30 days. Tween 80 was added to the spore suspension.

From inoculation, fruits were monitored twice weekly to register incubation period. When controls reached the mature stage, all fruits were harvested and evaluated according to external and internal symptoms, using scales developed by Sanchez (1982.

The experimental design considered was completely randomized in time; with each tree was an experimental unit and each pod/tree an evaluation unit within the population.

Scale used to measure External Severity.

Value	Symptom
0	No symptoms
1	Hydrosis or “oily” points
2	Early yellowing, irregular spots, pod deformation and small lumps
3	Bown patches and presence of mycelium
4	Necrosis + spores up to ¼ of the pod
5	Necrosis + spores covering more than ½ of the pod

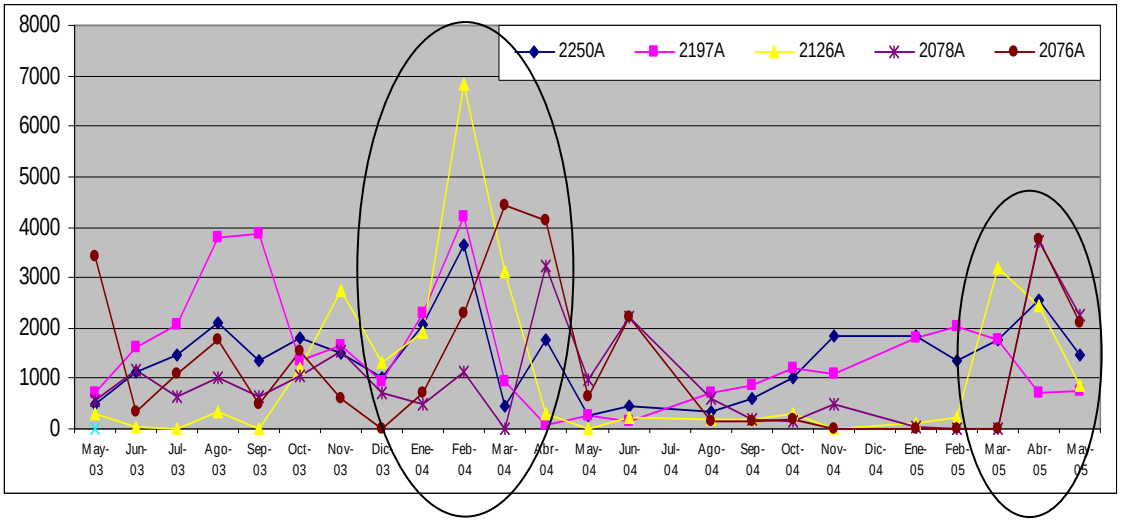
Scale used to measure External Severity

Value	Percentage of necrotic area
0	0
1	1 – 20
2	24 – 40
3	41 – 60
4	61 – 80
5	>80

RESULTS AND DISCUSSION

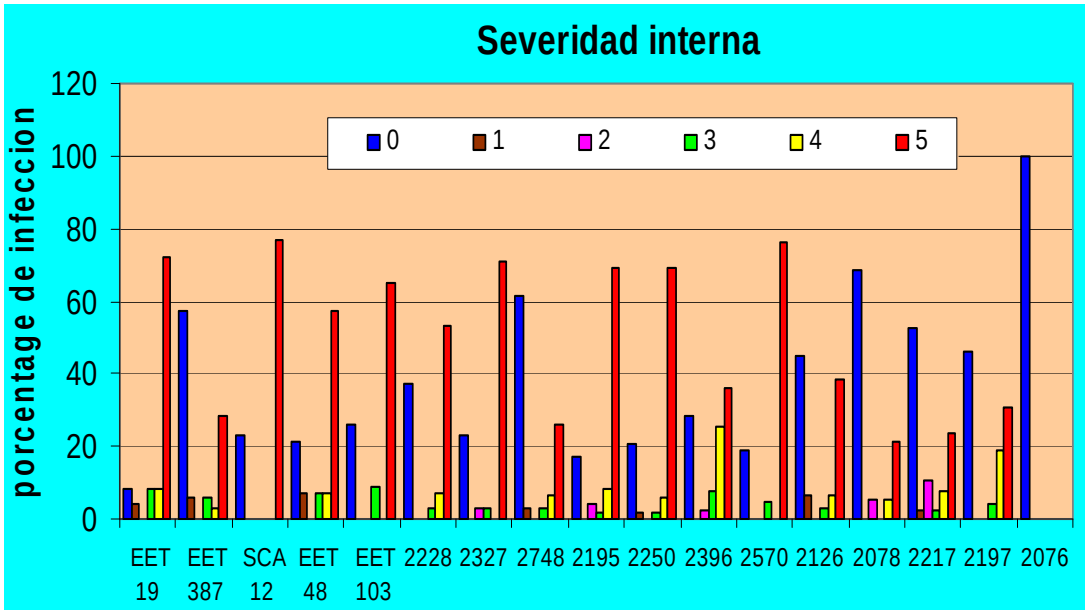
To obtain fruits for this kind of study presented a series of difficulties and it is necessary to work progresivally until the specified number of pods/tree is completed. This paper presents the results obtained so far although in most trees only 80% of pods have been inoculated and a few only 30%. Figure 3 shows pod harvest of a group of the selected trees where it is possible to see that in two epidemic seasons (2004 & 2005) the pick of fruit harvest for some trees coincide with January to March/April, which means they were set and pass the most susceptible stage when there was not conditions for the disease. Besides, the cultural maintenance of this plot may account for lower inoculum pressure that allowed trees to show its natural resistance/tolerance to the disease.

Figure 3. Pod development of some of the selected trees, showing its peak of harvest during January to March, Pichilingue, 2003-2005



Figures 4 & 5 shows cumulative Percentages of internal and external infection obtained on the different series of pods obtained. Three of the trees (2078, 2748, 2217) presented values similar to the resistant control EET387, with less than 25% infection; and there is another group of three (2396, 2126 & 2197) that maintained low levels of infection with less than 35%. On the other hand, two of the selected trees (2195, 2250) showed high susceptibility, comparable to the susceptible from the same population (2327 and 2570) and to the EET19 with more than 70% of infection. The tree selected as SCA12 was as well in this group of high susceptibility.

Figure 4 Percentage of internal severity on 16 cocoa trees inoculated with *M. roreri*. EET-Pichilingue, 2006



From the tree 2076 only six pods have been evaluated so far and none of them were infected, however we should wait until a larger group of pod is inoculated. The external severity of the infection was somewhat lower in all the trees; however the relative performance of each one followed the same pattern than for the internal severity and in any case, it is consider the latter as a better criteria to evaluate resistance, figures 6 and 7 illustrate this point.

Figure 5 Percentage of external infection presented on 16 cocoa trees inoculated with *M. roreri*. EET-Pichilingue, 2006

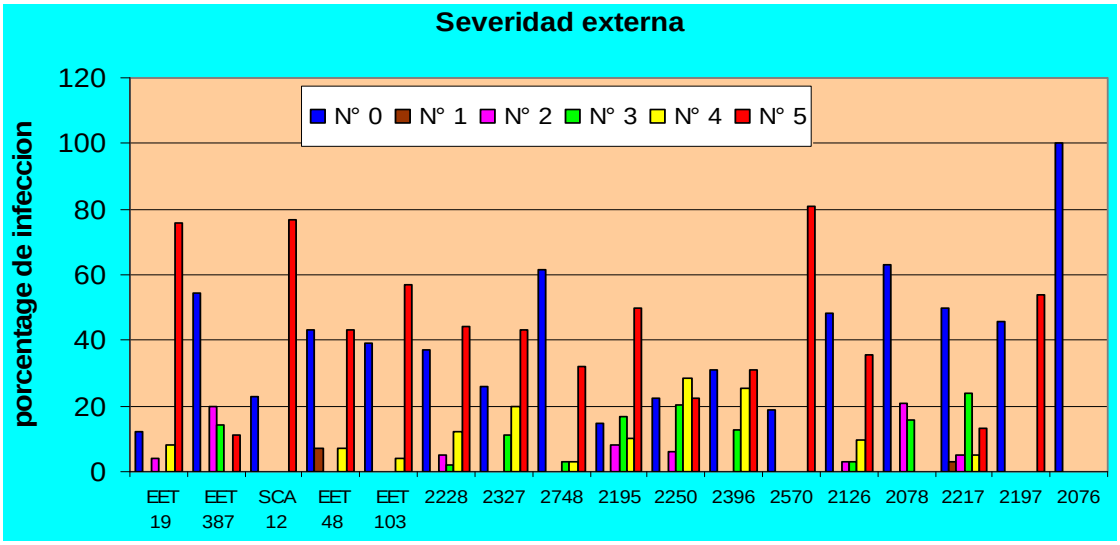


Fig. 6. Aspect of infection (Internal and External on pods from the tree 2078 with low incidence of *M. roreri*

Fig. 7. Aspect of infection (Internal and External on pods from the tree 2327 with high incidence of *M. roreri*.

CONCLUSIONS

- Once the trees were challengde with the disease most presented different levels of incidence and severity, so at field level may be working some escape mechanisms.

- Five out of the 11 trees presented infections similar or lower than the resistant control EET387 with less than 25% infection. The tree 2076 is not considered in this group yet as only 6 pods have been inoculated though none of them presented infection.

- The SCA12 do not showed any resistance, with 77% of infección and high severity. Since this clone is reported resistant in other countries its identity will be reviewed.

- It seems that there are two mechanisms present on this group of trees, escape at field level, with most of its pod formation taken place during the dryer months of the year, and some genetic resistance as in those trees that showed similar or lower infection than the control.

- It seems there is both mechanisms present in this population: scape from infection which is visible under natural conditions and some genetic resistance as shown by low levels of infection when challenge pods with the pathogen.

- We will continue with the observations, but we have recommended the multiplying of the five best trees already observed.

REFERENCES

Agama, P. J. E. (2005). Selección de Progênies y Plantas Elites de Cação (Theobroma cação L.) Mediante la Evaluación de Características Agronômicas y de Resistência a Enfermedades. Tesis. Ing Agr. Quito. Ecuador. Universidad Central del Ecuador. 112 pp

Agrios, G. N. (2002). Fitopatologia. UTHEA. Noriega Editorial. Segunda edicion. pp. 125-139.

Cruz. S. (1993). Determinación de Fuentes de Resistencia de Cacao de origen Nacional al Ataque De *Monilia roreri* (Cif & Par). Tesis. Ing Agr. Guayaquil. Ecuador. Universidad de Guayaquil. 102 pp

Delgado. J and Suarez. C (1993). Moniliasis del cacao. Quito. Ecuador. FUNDAGRO-INIAP. N°.10 Documento técnico. 8 pp

Sanchez. J. (1987). Metodología para la inoculación de mazorcas con el hongo *Moniliopthora roreri*. In 10° Conferencia Internacional de Investigación en Cacao, p. 467- 471.

SENASA. (2003). La Moniliasis del cacao. Disponible en http://www.senasa.gob.pe/sanidad_vegetal/programas_fitosanitarios/ci_moniliasis_cacao/moniliasis_cacao.htm. Consultado el noviembre 2005.

Suarez, C. C. 1971. Estudio de mecanismo de penetración y del proceso de infección de *Monilia roreri* (Cif & Par) en frutos de cacao (*Theobroma cacao*). Tesis Ing. Agr., Guayaquil, Facultad de Agronomía y Veterinaria, Universidad de Guayaquil. Ecuador. 59p.

Suarez, C 1993. Enfermedades de cacao y su control. In Suárez. C. ed. Manual del Cultivo de Cacao. 2da. Ed. Quevedo, Ecuador, EET-Pichilingue. Manual Técnico No.25.p. 90-116.

1.3.

Comparison of methods for early evaluation of resistance to witches broom (*Crinipellis perniciosa* (Stahel) singer*)* in cocoa (*Theobroma cacao L.*).

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SUMMARY

Three sets of trial were carried out in order to test early evaluation of resistance to Crinipellis perniciosa. In all experiments clons SCA 6, SCA 12, CCAT 4675, BE 10 and EET 95 cocoa clones were inoculated with a spore suspension of 1 x 10^4 basidiospores/ml. Two trials dealt with Basidiospores germination on leaf disks and plant extract. Clones showed statistically significance and highly significant for spore germination on leaf disks and water extract respectively. With methods dealing with seedling inoculation disease incidence was 100% and quite severe for all methods: Holliday test, a modified Holliday and the belt spray method. In theses trials, no differences between clones were observe for the incubation period or disease incidence; only the belt spray method gave statistically significant differences for cotyledon persistence on seedlings. In the field inoculation method, the clones showed significant differences for the infection incidence in branches and infection incidence per plant. Basidiospores germination percentage in the leaf disk method and watery extracts method showed a positive correlation with branch infection percentage (0.70 y 0.90) and plant infection percentage (0.90 y 0.70). Cotyledon permanence in the automatic inoculation method was positively correlated with the infection percentage in branches (0.70) and plants (0.60).

BACKGROUND

Different methods have been developed and used for early evaluation of witches' ´ broom disease in cacao. Some of these as spore germination on leaf disks (Ducamp & Thevenin, 1999), or in aquous extract of young shoots (Evans & Bastos, 1980), the Holliday test (Holliday, 1954), Semiautomatic system of inoculation-infection or "Belt Spray Method" (Frias and Purdy, 1995) and the agar drop method .have produced reliable results under different conditions and have been used on WB selection for breeding programs; however differences persists making difficult its extensive use by pathologists and breeders.

This study was designed to compare all these methods under similar conditions and with the same cultivars in order to determine which one gives more consistent results under Ecuadorian conditions.

METHODOLOGY

The study was conducted in the Laboratory and experimental fields of the Tropical Experimental Station "Pichilingue" from the Nacional Institute of Agricultural Research (INIAP). The Station is located in the central area of the Coastal plain of Ecuador, 5.5 km South of Quevedo. The methods to be evaluated were grouped in three sets of trials, according to the parameter used to evaluate results:

Group 1: spore germination on cocoa leaf disks and on aqueous extract of young shoots.

Group 2: Reaction of young seedlings to inoculation: Holliday test; a modified Holliday test and the Belt spray method.

Group 3: Reaction of plants shoots. Agar drop inoculation of young shoots on the field

Management of the trials.

All methods were tested using open pollinated seedlings and vegetative material of clones: SCA 6, SCA 12, (Resistant); CCAT 4675 (unknown reaction); BE 10 y EET 95 (Susceptible)

Inoculum preparation

Basidiospores were collected from dry broom collected in Pichilingue and placed in broom cabinets to produce sporophores, collected, stored and utilized as recommended by Suárez (1977) and Frias *et al* (1987).

Description of methods

Group 1. Evaluation parameter: spore germination

Trial No. 1 Spore germination on leaf disks (Ducamp & Thevenin, 1999)
Fully developed leaves were collected from the middle of healthy tree branches; they were placed on glass containers with 500 ml of sterile distilled water (SDW) kept on thermal boxes (RUBBERMAID) at approximately 9^{o}C to keep them fresh until taken them to the laboratory. Once there, leaves were washed with SDW and antibacterial soap (PROTEX) and tap dry on paper towels. Disks, 13 mm in diameter were cut with a sacabocado, between the veins of the leaf, they were washed again in SDW and eight disks were placed on Petri dishes lined with sterile filter paper. The paper was wetted with 1ml of SDW. One drop (0,1ml) of spore suspension on water agar (0.3%) was placed on the abaxial surface of each disk. Incubation took place during three hours on lab benches at an average temperature of 23^{o}C.

Trial No. 2. Spore germination on aqueous extract of young cocoa shoots (Evans & Bastos, 1980)
Cocoa shoots were collected early morning and placed on glass flasks with 20 ml of SDW and then maintain in thermal boxes at 9oC to keep them fresh. In the laboratory, shoots were washed with SDW and cut on pieces of about 1 cm. Those pieces were placed in a glass container and then boiling water was pour over them to prepare an infusion (250 ml of water over 2g of leaves pieces). The preparation was cool down in a water bath at 23^{o}C for five minutes. Ten µl of this infusion were placed on each well of a Elisa plate (CORNING 25802). On top of them 100 µl of the spore suspension in water agar (0,3%). Plates were incubated in a humid chamber for three hours on lab benches at an average temperature of 23^{o}C.

In both experiments, once the incubation period was over, germination was stopped applying one drop of Trypan blue on each leaf disk or Elisa well, and then proceed to measure the percentage of spore germination.

Group 2. Symptom evaluation on seedlings

Trial No. 3. Holliday test (Holliday, 1954)
Ripe, open pollinated pods from the selected cocoa clones were harvested & selected for uniformity. The testa was peeled from each seed and then they were placed on plastic basquets with towel paper wetted with SDW. Seeds were germinated in darkness for 4 to 6 days until the radicule was around 2cm in length. Every 48 hours SDW was sprayed over the seeds to keep them wet and favor germination. At this stage, germinated seedlings were rinse with SDW, tap dry with towel paper and submerge during 2 minutes in the spore suspension. Immediately after inoculation, germinated seeds were planted on plastic cones containing 135g of a mixture of soil + peat (BIOLAN[1]). The cones with the plants were kept in plastic racks inside a shade house at ambient temperature (±27 ^{o}C).

Trial No 4. Holliday test modified (Suárez, 2004[†])
In this case the procedure was exactly as describe for the Holliday test above but instead of submerging the germinated seeds, they were sprayed with a manual sprayer applying 1ml of spore suspension for each seedling.

Trial No 5. Belt Spray Method (Frías, 1987)
Peeled seeds were placed on the cones containing the soil and peat mixture as indicated before, taking care of leaving one fourth of the seed above soil level. Racks with cones were maintained in the shade house for 15 to 21 days until they presented first shoot with primary leaves no longer than 1.5 cm when they were sprayed with a spore suspension in a moving belt as describe by Frias (1987). Inoculated seedlings were places in an incubation chamber for 24 hours. Temperature in the chamber was kept at 27-28 ^{o}C, with 95 -100% relative humidity. Then seedlings in cones were transfered to a shade house until evaluation.

In trials 4 and 5, evaluation parameters were incidence by symptoms (%), incubation period (days) and the time of cotyledons remain attached to the plants.

Trial No 6. Field inoculation
Clonal plants of the selected materials were used. Young branches without lignifications were selected, the apical shoot, pruned so axilar buds become active. These branches were covered with plastic bags to avoid natural infection. Every active bud was inoculated with 0,1 ml of agar spore suspension as the one used in experiments 1 and 2. Following inoculation, branches were again covered with plastic bags (12 x 15cm) but this time a piece of wet paper towel was included to produce a humid chamber to ensure infection. The plastic bag was removed after 7 days.

In this trial parameters measured were percentage of infection per branch and per plant and the incubation period.

[1] *Material orgánico en descomposición o apenas ligeramente alterado, acumulado en medio anaerobio. Representa el primer estado en la formación de los carbones. Producto distribuido por Agripac S.A*
[2] *Comunicación personal*

RESULTS AND DISCUSSION

Group 1. Trials 1 and 2. Spore germination on two different substrates

Results obtained on the two trials can be seen on Table 1. Spore germination on leaves was very low, ranging from 5 to 7%. However the Chi^2 test showed significant difference between clones, with the resistant SCA 6 having the lowest value (4.83) and the CCAT 4675 having the largest percentage (7.91). It is well known that *M. perniciosa* prefer very young, meristematic tissue, therefore although the leaves used in this trial were still tender, they were fully grown and may have some inhibitor that prevented the fungus to germinate. It is not discarded as well the possibility stated by Brownlee *et al* (1993) about the presence of tannins on leaves that may act against the fungus.

Table 1. - *C. perniciosa* spore germination on two different substrates from five cocoa clones (*Theobroma cacao* L.). INIAP. Pichilingue. Quevedo-Los Ríos, 2005.

Clone	Subtrates (mean values)	
	Leaves disks *	Aqueous extracts
	Chi^2: 10.67 *	Chi^2: 18.43 **
SCA 6	4.83	88.64
SCA 12	4.87	89.89
CCAT 4675	7.91	92.58
BE 10	5.58	95.05
EET 95	5.19	95.39
Control +	-	95.86

*Chi2, Kruskal-Wallis Test, *p=0,01 and **p=0,001*
+ Control: Water + spores), Value not considered in the anaysis.

On the other hand, as may be expected on aqueous extract of young shoots, percentage of germination was comparable to that in pure water (Evans and Bastos, 1980). Again, although numerical differences are very short, germination on extracts from SCA6 and SCA12 presented some factor that reduced germination significantly (88.64 %, and 89.89% respectively). Various factors may have influenced the percentage of germination; one could be a high dilution of the content of the extract that prevented larger differences between clones; the use of Tween-80 as dispersant agent as well may have prevented tannins which, as in the leaves, could have prevented the development of the germ tubes. In the two experiments, the SCA reaction was as expected in a resistant material. The reaction of the other three clones is variable, especially for CCAT 4675 and EET 95 that presented opposite ranking in both trials; however differences are so short that it is not possible to reach clear conclusions. The clone BE10 kept same ranking in both trials, being on the susceptible side.

Group 2. Symptom evaluation on seedlings

This group of trials included those methods based on the reaction of seedlings to *M. perniciosa***:** Holliday test, H. test modified and the belt spray method**.**

Seedlings presented a range of symptoms as describe elsewhere for Witches broom infection (Table 2). Controls were symptomless, stem roughness (RT) and cotiledonary brooms (EC) were the most frequent symptoms, followed by hypersensitive reaction (RH) that kill inoculated seedlings.

Table 2. Percentage of incidence and Number of plants with different types of symptoms with three methods to evaluate, INIAP. Pichilingue. Quevedo-Los Ríos.2005.

Methods	Clones	No. of inoc. seedlings	Number of plants with symptoms								Infection %
			HH	HNC	HE	AH	RT	EC	ET	RH	
Holliday Test	SCA 6	103	21	19	13	7	73	23	0	35	100
	SCA 12	139	22	8	28	18	68	35	1	8	100
	CCAT 4675	116	29	29	29	0	66	51	3	10	100
	BE 10	91	23	14	13	6	52	29	0	24	100
	EET 95	172	38	26	66	4	73	83	2	12	100
	Testigo	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Holliday test modified	SCA 6	77	11	8	1	13	58	28	0	43	100
	SCA 12	123	8	8	7	16	69	29	0	8	100
	CCAT 4675	124	27	22	5	2	64	69	0	15	100
	BE 10	82	20	17	2	2	59	27	0	26	100
	EET 95	177	45	35	31	5	108	89	1	29	100
	Testigo	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Belt spray system (SAI)	SCA 6	73	0	0	10	0	61	29	7	21	100
	SCA 12	153	1	4	17	0	117	61	22	33	100
	CCAT 4675	82	0	1	6	0	62	27	5	16	100
	BE 10	57	0	0	16	0	51	8	15	6	100
	EET 95	148	0	12	12	0	102	73	12	55	100
	Testigo:	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

HH: *Hypocotile swollennes,* ***HNC:*** *Coteledonal node swollen,* ***HE:*** *Swollen Epicotile,* ***AH:*** *Ahilamiento of stem,* ***RT:*** *Stem roughgenness,* ***EC:*** *Cotiledonal broom,* ***ET:*** *Terminal broom,* ***RH:*** *Hypersensitive reaction*

The total numbers of symptoms override the number of plants because many seedlings presented more than one symptom. The relatively high incidence of the hypersensitive reaction on these trials indicates high inoculums pressure. This same factor plus the virulence of the Ecuadorian strain, may account for the high disease incidence (100%) on all treatments that did not allow discriminating levels of susceptibility (Frias *et al* 1995)

The incubation period was as well very similar (Table 3) in all treatments and differences found in Brazil by Andebrhan *et al* (1998) and others on this parameter was not observed under the conditions of this experiments. Concentration of the spore suspension and the age of the seedlings may require adjustments to the aggressiveness of the Ecuadorian strain of *M. perniciosa*.

In these studies, special attention was put on measuring for the first time the permanence of cotyledons on inoculated plants as compared with non inoculated. Although Holliday (1955) mentioned the non-abscission of cotyledons in artificially inoculated seedlings, it has not been considered a discriminator system and is not describe as such on the detailed symptoms descriptions published (Baker and Holliday, 1957 and Rudgard, 1989).

Table 3: Incubation period of M. perniciosa in days for three methods of early evaluation of resistance for five cocoa clones. INIAP., Pichilingue, 2005.

CLONE	Inoculation Methods		
	Holliday	Holliday Modified	Belt Spray
	Chi^2: 2.70 ns	Chi^2: 2.30 ns	Chi^2: 2.27 ns
SCA 6	17.86	17.71	18.39
SCA 12	16.60	17.75	19.43
CCAT 4675	17.25	18.34	18.55
BE 10	18.80	17.52	19.79
EET 95	16.34	17.14	17.72

***** *Mean values. ns=no significance for Chi^2 Test from Kruskal-Wallis*

Non-inoculated plants allowed abscission of cotyledons between 34 and 54 days, so we can speculate that it is a factor that depends on the genetic constitution of the clones; however cotyledons on the inoculated seedlings remain on the plants up to 84 days (Table 4). Both Holliday tests showed no differences between clones for this parameter, but the belt spray method gave statistically significant differences between clones, in agreement to the statement of Motilal *et al* (2003); with the SCA12 and 6 in one end with the lower value (0, 20 and 8 days difference respectively with their controls); and the susceptible EET95, the highest (22 days of difference from the control).

Table 4: Permanence of cotyledons in days after infection for three early evaluation methods for resistance against Witches Broom in cacao seedlings.

CLONE	Inoculation methods					
	Holliday	Control	Holliday Modificado	Control	SAI	Control
	Chi^2: 1.73 ns		Chi^2: 2.88 ns		Chi^2: 18.97 **	
SCA 6	84.40	45.74	68.38	45.74	42.67	33.79
SCA 12	81.31	47.60	78.14	47.60	41.36	41.16
CCAT 4675	81.84	39.45	79.34	39.45	54.69	42.24
BE 10	69.92	48.57	84.20	48.57	49.36	37.30
EET 95	80.74	54.94	74.94	54.94	70.48	47.88

***** *Valores Medios. Abreviatura ns muestra la no significancia en la prueba del Chi^2 del test de Kruskal-Wallis.*

Group 3. *Field inoculations*

Despite the very low values obtained in this test, differences were highly significant between clones (Table 5), both for disease incidence and for incubation period. Low infection may be a consequence of predominant weather conditions during this trial, with high light intensity and low relative humidity (low rain) which could have affected the fungus and the branches as well. However it may well be interesting to

take these factors into account and consider these variables for early tests as has been proposed by Almeida *et at* (1991).

Table 5: Percentage of infection per branch and per plant and incubation period on young shoots for five cocoa clones (*Theobroma cacao* L.) inoculated in the field by the agar drop method.

	Disease incidence %		**Incubation period Days**
CLONE	**Per branch**	**Per plant**	**Young shoots**
	Chi^2 : 10.64*	**Chi^2:** 5.33**	**Chi^2 :** 9.95 **
SCA 6	0.45	0.16	71.00
SCA 12	3.16	2.07	42.39
CCAT 4675	3.17	4.11	45.38
BE 10	4.91	5.09	49.04
EET 95	3.40	3.59	44.63

*Mean values: * p: 0.01; ** p: 0.001 for Chi^{2,} Kruskal-Wallis Test.*

Correlation between the evaluation methods

Attempts were done to correlate variables of the different methods evaluated. The variable percentage of germination in both methods used had high correlation with percentages of branch and plant infection (0.90 and 0.70 respectively) in the field. However, it is not recommended to use methods that require counting of spores to evaluate large number of materials, because it is a very tedious and time consuming. In the same line, it was very difficult to find adequate material in the field to collect shoots to prepare the extract, and this is a handicap in the use of this type of method on a large scale.

In the case of the Belt spray method, the variable permanence of cotyledons was as well highly correlated with branch (0.7) and plant (0.6) infection in the field. This method as stated by Frias (1987, 1995), and Purdy *et at* (1998) is showing the most promising possibilities because once the facilities are set up in place, the procedure allow handling of large amounts of plants, and reproduce infection consistently. It is necessary however to adjust the concentration of the inoculum and the stage of the shoot to inoculate, factors that are so far hampering this method.

The inoculation of shoots at the field presented as well interesting possibilities, but it is too dependent on selecting adequate material on the field, and we can estimate that there would always be weather factors altering the reaction of cocoa plants to artificial inoculation at this level.

CONCLUSIONS

- The reaction of the plants in the five methods compared were of a magnitude that did not allow proper discrimination between the clones;
- Variables to measure vary with the method;
- The permanence of cotyledons in the belt spray method; spore germination on both methods used, and field infection were the variables and methods that allowed to appreciate differences.

- Although differences found with the different methods were very short, clones SCA, 6 y 12 did showed its resistant conditions and the EET 95 maintained as well its susceptible condition.
- Efforts should be addressed to overcome factors and conditions that affect the efficiency of the methods;
- A "calibration" of the stage of the substrate to use and the spore concentration seems to be a condition to fulfill wherever and whenever one of these methods will be used.
- The modified Holliday test, the belt spray and the agar drop methods can be the tool breeders and pathologist require for WB resistant material selection, once the adjustments sugested in this comparative work be completed.

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REFERENCES

Almeida, L ; Andebrhan, T. (1991). Avaliacao de genótipos de cacau em relacao a producto de basidiocarpos de *C. perniciosa* nas vassouras. Bélem, Brasil, CEPLAC/CEPEC. 73-75 (Informe de Pesquisas 1991).

Andebrhan, T; De Almeida ; Nakayama, L. (1998). Resistencia de *Theobroma cacao* L. a *C. perniciosa (Stahel) Singer*: A Experiencia da amazonia brasileira. Agrotropica 10(2): 49 – 60.

Bartley, B. (1958). Observations on the reactions of young cocoa seedling to artificial inoculation by *Marasmius pernicious* Stahel. *In* Inter American Cocoa Conference. (7, 1958, Bogotá). Actas. Bogotá, Colombia. p. 104 – 116.

Brownlee, H; M. C Euen; Hedger, J; Scott, I. (1993). Antifungal effects of cocoa tannin on *Crinipellis perniciosa*. *In* Conferencia Internacional de Investigaciones de Cacao. (11, 1993, Yamoussoukro). Actas. Yamoussoukro, Costa de Marfil. p. 69 - 73.

Evans, H; Bastos, K. (1980). Um novo método para testar resistencia do cacau a Vassoura-de-bruxa *(Crinipellis perniciosa)*. Theobroma 10(4): 174-185.

Frias, G. (1987). An inoculation method to evaluate resistance to Witches broom Disease of Cocoa. Thesis PhD. Gainswille; Florida; United State. University of Florida. 111 p.

--------; Purdy, L; Schmidt, R. (1995). An inoculation Method for evaluating resistance of Cacao to *Crinipellis perniciosa*. Plant disease 79(8): 787 – 791.

Motilal, L; Sirju-Charran, G; Sreenivasan, T. (2003). Effect of *Crinipellis perniciosa* Infection on abscission of cacao cotyledons reserves mobilization and dry matter partitioning. Journal of Phytopathology 151: 546 – 552.

Suarez, C. (1977). Growth of *Crinipellis perniciosa*.(Stahel) Singer in vivo and in vitro. Thesis PhD. London, England. University of London. 120 p.

Surujdeo-Maharaj, S. 2002. Development of an optimised inoculation method to identify resistance to Witches broom disease in Cocoa. *In* Annual report 2002 .ST AGUSTINE, Trinidad and Tobago. Cocoa Research Unit. The University of the West Indies. p. 44 - 52.

1.6. A new morpho-type of *Phytophthora palmivora* on cacao in Central America

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SUMMARY

Several species of Phytophthora have been implicated as the cause of black pod disease, one of the most serious constraints to cocoa production worldwide. These include P. arecae, P. capsici, P. citrophthora, P. megakarya, P. megasperma, P. nicotianae var. parasitica and P. palmivora. Only P. palmivora has a global distribution and can cause up to 100% of crop losses in the Caribbean and Central America during periods of particularly high rainfall or in wet years. In the Americas, black pod disease is also caused, to a lesser extent; by P. capsici and P. citrophthora has been isolated from diseased cacao pods in Brazil. The primary infective agents are zoospores for all known isolates of P. palmivora, which are released singly and require free water for their liberation and subsequent host infection.

In contrast, recently two Phytophthora isolates (Cr10 and Ni5) from infected cacao pods from the Atlantic coasts of Costa Rica and Nicaragua, respectively, were observed to release the majority of their zoospores 'en masse'. These zoosporic balls germinate to form the infective agent causing the characteristic necrotic circular lesion on the pod surface. Isolates are papillate and perform as well as known P. palmivora isolates in aggressiveness tests although they release a thousand-fold fewer single zoospores. Sporangia characteristics for Cr10 (L=47.2 µm, B=29.9 µm, L/B ratio=1.6:1, pedicel length=2.6 µm) and Ni5 (L=49.5 µm, B=29.5 µm, L/B ratio=1.7:1, pedicel length=2.3 µm) are well within the range reported for P. palmivora. Both Cr10 and Ni5 were also determined to be of Mating Type A2, typical for the P. palmivora cacao pathogen in Central America.

Previous studies have shown sequence variation of five genes to be good indicators of species-level diversity in Phytophthora: these include three nuclear-encoded genes, the ITS (internal transcribed spacer regions 1 and 2 and intervening 5.8S rDNA gene), translation elongation factor EF-1α, and β-tubulin; and two mitochondrial-encoded genes, cytochrome c oxidase subunit I, and the NADH dehydrogenase subunit. Sequence data for these five genes were generated to compare Ni5 with six geographically diverse isolates of P. palmivora from Colombia (Co4), Costa Rica (Cr15), Ecuador (Ec1), Panama (Pa1), Trinidad (P-161) and Tobago (P-217). Sequence homology for all seven isolates were confirmed by BLAST analyses against the GenBank databases (http://www.ncbi.nlm.nih.gov/) and found to share 100% identity to each other and with other known strains of P. palmivora. Results indicate that these isolates are a new morpho-type of P. palmivora, which releases zoospores 'en masse'. Implications to cocoa production will be discussed.

INTRODUCTION

Black pod disease occurs in most cocoa (*Theobroma cacao*) producing regions worldwide. The disease is manifested as characteristic brown necrotic lesions on the pod's surface and rotting of the beans. Worldwide, an estimated 30% of the cacao crop is lost to black pod disease. At 2005 cocoa bean prices (ICCO, 2006) this is an estimated US$1.5 billion in lost revenue. In the Caribbean and Central America losses of up to 100% in susceptible genotypes can occur in years of high rainfall. Seven species of *Phytophthora* (*P. arecae*, *P. capsici*, *P. citrophthora*, *P. megakarya*, *P. megasperma*, *P. nicotianae* var. *parasitica* and *P. palmivora*) have been implicated in black pod disease of cacao. The latter occurs worldwide in all cacao producing regions and is the major pathogenic species in the Caribbean and Central America where black pod disease incidence is highest at crop harvest.

Black pod disease caused by *P. palmivora* is typified by a necrotic, lesion that spreads evenly in near circular fashion on the pod surface. Infection of the beans within the pods results in crop loss as infected beans produce off flavors during fermentation. The infectious agents for *P. palmivora* are free swimming, biflagellate, single zoospores produced in large numbers in sporangia. This is the major distinguishing character of the genus *Phytophthora* from *Pythium* in the peronosporalean line (Oomycota, Peronosporales), i.e., *Phytophthora* species differentiate zoospores in the cytoplasm before discharge from the sporangium, whereas *Pythium* species first extrude a vesicle (Brasier and Hansen, 1992).

Classical methods of *Phytophthora* spp. identification are based upon morphological characters of the sporangia and colony form or growth characteristics (Brasier and Griffin, 1979; Erwin and Ribeiro, 1996). However, variability of morphological and cultural characters does not always permit clear differentiation of *Phytophthora* at the species level (Erwin, 1995). Al-Hedaithy and Tsao (1979) showed pedicel length to be a highly stable diagnostic character among *Phytophthora* species.

Molecular methods have proven to be very useful in the identification of fungal species. The internal transcribed spacer (ITS) region of nuclear ribosomal DNA (rDNA) is particularly useful for fungal identification at the species level. The rate of accumulation of mutations in ITS regions corresponds well with the rate of speciation in *Phytophthora* (Lee and Taylor, 1992). These mutations can be visualized by restriction analysis of amplified ITS regions (Cooke and Duncan 1997) or by direct sequencing of the ITS regions (Lee and Taylor, 1992). Direct sequencing is more accurate at detecting mutations for species identification in *Phytophthora* as fragments could have the same length but different sequences (Lee and Taylor, 1992). Sequence analyzes of mitochondrial (cytochrome c oxidase *CoxI* and *CoxII*; NADH dehydrogenase subunit I) and nuclear gene regions (translation elongation factor (EF1α) and β-tubulin) were useful in species differentiation and phylogenetic inferences in *Phytophthora* (Kroon et al., 2004; Martin and Tooley, 2003).

Two *Phytophthora* isolates (Cr10 and Ni5) from the Atlantic coasts of Costa Rica and Nicaragua, respectively, were purified from infected cacao pods. These isolates release the majority of their zoospores 'en masse' or a ball, which germinate to form the infective agent causing the characteristic necrotic, circular black pod lesion in healthy pods. The objective of this study was to characterize Cr10 and Ni5 to the species level.

MATERIALS AND METHODS

The isolates

The Latin American isolates were collected via opportunistic sampling by Wilbert Phillips in 1999. The isolates from Trinidad and Tobago were collected in 2001 in a randomized sampling design to determine the genetic diversity of the cacao black pod population on the islands. Details of the isolates used in this study are presented in Table 1.

Table1: The 41 isolates used in the characterization of Cr10 and Ni5. Unless an alternate 'Host' is stated, all isolates were purified from infected *Theobroma cacao* pods. The six isolates with numeric designations are from the CABI BioScience Genetic Resource Collection (GRC) and are used as standards for comparison.

Isolate Label	Origin	Region/Location	Species determined	Sporangia Length µm	Sporangia Breath µm	Pedicel Length µm
P-115	Trinidad	HA9, Sangre Grande	*P. palmivora*	51	31	5
P-119	Trinidad	HA1, Grande Riviere	*P. palmivora*	45	25	5
P-121	Trinidad	HA1, Matelot	*P. palmivora*	No Data	ND	ND
P-124	Trinidad	HA3, Manzanilla	*P. palmivora*	46	26	5
P-150	Trinidad	HA8, Tortuga	*P. palmivora*	54	30	5
P-161	Trinidad	HA3, Cushe Village	*P. palmivora*	52	25	5
P-164	Trinidad	HA2, Cumaca	*P. capsicii*	48	20	82
P-168	Trinidad	HA2, Cumaca	*P. palmivora*	50	33	5
P-181	Trinidad	HA6, Cedros	*P. palmivora*	49	26	5
P-183	Trinidad	HA6, Cedros	*P. palmivora*	51	27	5
P-185	Trinidad	HA9, Maracas St. Joseph	*P. palmivora*	40	29	5
P-188	Trinidad	HA1, Paria Brasso Seco	*P. palmivora*	51	28	5
P-201	Trinidad	HA5, La Lune Moruga	*P. palmivora*	46	34	5
P-206	Trinidad	HA9, Santa Cruz	*P. palmivora*	42	24	5
P-217	Tobago	HA11, Golden Lane	*P. palmivora*	49	31	5
P-220	Tobago	HA14, Mason Hall	*P. palmivora*	48	27	5
Co2	Colombia	Caldas	*P. palmivora*	48	28	3
Co3	Colombia	Caldas	*P. palmivora*	58	32	2
Co4	Colombia	Santander	*P. palmivora*	48	26	3
Co5	Colombia	Antioquia	*P. palmivora*	51	29	3
Cr10	Costa Rica	Limon	*P. palmivora*	47	29	3
Cr11	Costa Rica	Limon	*P. palmivora*	45	30	3

Cr13	Costa Rica	Turrialba	*P. palmivora*	Dead	Dead	Dead
Cr14	Costa Rica	Cabiria, Turrialba	*P. palmivora*	48	31	2
Cr15	Costa Rica	Cabiria, Turrialba	*P. palmivora*	49	31	2
Cr16	Costa Rica	Cabiria, Turrialba	*P. palmivora*	51	29	2
Cr17	Costa Rica	Cabiria, Turrialba	*P. palmivora*	51	31	3
Cr3	Costa Rica	Cartago	*P. palmivora*	Dead	Dead	Dead
Cr4	Costa Rica	Cartago	*P. palmivora*	48	27	3
Cr7	Costa Rica	Alajuela	*P. palmivora*	49	29	2
Cr8	Costa Rica	Heredia	*P. palmivora*	Dead	Dead	Dead
Ec1	Ecuador	Hacienda Secadal	*P. palmivora*	48	28	2
Ni4	Nicaragua	Tulimbina, (RAAN)	*P. palmivora*	48	28	3
Ni5	Nicaragua	Piwipilia (RAAN)	*P. palmivora*	50	31	2
Pa1	Panama	Colon	*P. palmivora*	50	28	2
348342	Indonesia	Host: *Cocos nucifera*	*P. arecae*			
386258	Ghana		*P. palmivora*			
386325	Tobago		*P. palmivora*			
325900	Brazil	Host: *Piper nigrum*	*P. capsici*			
352321	India	Host:*Capsicumannuum*	*P. capsici*			
40502	New Mexico	Host: *Piper nigrum*	*P. capsici*			

Morphological Characterization

A modified Al-Hedaithy and Tsao (1979) method was used for sporangia production and measurement of isolates used in this study. All isolates, except the six from the CABI Bioscience GRC, were cultured on V8-$CaCO_3$ agar (20% v/v Campbell V-8 juice, 0.3% w/v $CaCO_3$, 1.8% w/v agar) in 100ml Erlenmeyer flasks for 4 days in the dark immediately followed by 3 days under continuous fluorescent light at 25°C. All isolates produced abundant sporangia under this regime which were harvested by addition of 10 ml distilled water to the culture, a swirl to ensure complete coverage of the mycelial mat before incubating at 4°C for 30 min followed by another 30 min incubation at 25°C to release zoospores. The flask was subsequently swirled vigorously to dislodge sporangia and the resulting solution (inoculum) of zoospores and sporangia decanted. A 1:10 dilution of the solution was made for estimating zoospore release by count on a hemocytometer and the dimensions (length, breath and pedicel length) of 10 sporangia measured on a Nikon MicroPhot–FX light microscope fitted with a calibrated Filar micrometer in Costa Rica and a calibrated reticule in Trinidad. The entire procedure was repeated three times for each isolate.

The inoculum of isolates Co5, Cr7, Cr11, Ni4, Ni5 and Pa1 were used to compare the aggressiveness of Ni5, the isolate releasing its zoospores in a ball to other isolates

from different geographic origins releasing single zoospores. For these aggressiveness tests, 3 to 5 month old pods of Pound 7 (black pod resistant) and IMC 67 (black pod susceptible) were inoculated with a 1cm circle of Whatman #2 filter paper soaked in a 100,000 spores/ml dilution of the inoculum and a distilled water control. Pods were incubated individually in large plastic bags containing two folded sheets of moistened paper towels to maintain humidity at 25°C under diurnal conditions. Lesion diameter was measured daily for 5 days after which mycelia emerged on the pod surface.

Molecular Characterization by Fingerprinting with ITS4 and ITS6

A modified Duncan and Cooke (1997) method was used to generate restriction profiles of the amplified ribosomal gene 5.8 S, ITS1 and ITS2 regions for all 41 isolates. The six isolates from the CABI GRC were grown on V8 medium and DNA was extracted using the UltraClean Plant DNA Isolation Kit (MoBio, Cambridge, UK) according to the manufacturer's protocols. DNA was extracted by a CTAB method from the aerial hyphae produced by five 100mm petri-plate cultures of each of the other 35 isolates grown on 5% Oatmeal agar (5% w/v ground whole oats, 0.8% w/v agar) and incubated in the dark for 14 days. The 5.8S gene, ITSI and ITS2 regions were amplified using primer pair ITS6 (Forward primer) (5'-GAAGGTGAAGTCGTAACAAGG-3') ITS4 (Reverse primer) (5'-TCCTCCGCTTATTGATATGC-3') (Figure 1) in a 50µl PCR reaction mixture. Each reaction contained 2.5 units of Super Tth enzyme (HT Biotechnology Ltd, Cambridge, UK) with 5 µl 10 X manufacturer's buffer (containing 1.5 mM of $MgCl_2$); 0.2 mM dNTP's (Promega, Southampton, UK); 50 pmol of each ITS primer, ITS4 and ITS6 (Sigma Genosys, UK); 5-50 ng of DNA and PCR grade water was added to give a total reaction volume of 50 µl. The PCR reaction was performed in a Hybaid Express Thermal Cycler (Hybaid Ltd., Middlesex, UK) using the parameters a 2 min denaturation step at 95°C followed by 30 cycles at 95°C for 20 s, 55°C for 1 min and 72°C for 50 s, before the final step of 72°C for 10 min. Success of the amplification was determined by running 5 µl of the reaction products and a 1 Kb size marker (Invitrogen, Paisley, UK) on a 1.5% LE agarose gel (Flowgen, Nottingham, UK) which was subsequently stained with ethidium bromide and visualized under UV light.

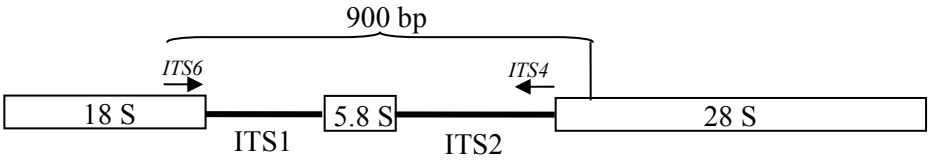

Figure 1: Schematic of the ribosomal gene 5.8S, ITS1 and ITS2 regions analyzed in this study. Arrows indicate the relative position and direction of amplification for the primers ITS4 and ITS6, which generate a 900 bp product.

The products of amplification were digested using the restriction enzymes *Alu* I, *Msp* I and *Taq* I (Promega, Southampton, UK) in accordance with the manufacturer's protocols. A 12.5 µl aliquot of products of digestion was separated by electropherisis on a 2.5% MetaPhor agarose gel for 3 h (Cambrex, Wokingham, UK) with 100bp size marker (Invitrogen, Paisley, UK) as a standard. Gels were stained with ethidium bromide and visualised under UV light..

The profiles for each of the 35 *Phytophthora* isolates were compared to that of the six standard cultures from the CABI Genetic Resource Collection (GRC), some of which are used on the web-based *Phytophthora* identification database http://www.phytid.org/

Molecular Characterization by Gene Sequencing

A subset of seven isolates (P-161, P-217, Co4, Cr14, Ec1, Ni5 and Pa1) (Table 1) representing the range of geographic diversity for the isolates in this study and including the different morpho-variant Ni5 was chosen for DNA sequencing. Five genes previously shown to be reliable at resolving species in the genus *Phytophthora* were sequenced for each sample: cytochrome c oxidase subunit I (Cox I), rDNA internal transcribed spacer (ITS), translation elongation factor 1-alpha (EF1α), NADH dehydrogenase subunit 1 (NADH1), and beta-tubulin (β-tub) (Martin & Tooley 2003, Kroon et al. 2004). Primer sequences and amplification parameters for Cox I followed Martin & Tooley (2003); primer sequences and amplification parameters for ITS, EF1α, NADH1, and β-tub followed Kroon *et al*. (2004). The PCR products were cleaned with Montage PCR Centrifugal Filter Devices (Millipore Corp., Billerica, MA) according to the manufacturer's protocol.

Cleaned PCR products were sequenced with BigDye Terminator sequencing enzyme v.3.1 (Applied Biosystems, Foster City, CA). Each reaction contained 2 μL of diluted BigDye in a 1:3 dilution of BigDye: dilution buffer (400 mM Tris pH8.0, 10 mM MgCl2); 0.3 μL of 10 μM primer (same used for amplification); 10–20 ng of cleaned PCR template; and H_2O to 5 μL total reaction volume. Cycle sequencing parameters consisted of a 2 min denaturation step at 94 C, then 35 cycles of 94 C for 39 s, 50 C for 15 s, and 60 C for 4 min. Sequencing reactions were cleaned by ethanol precipitation and sequenced on an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequencing reactions were edited and contiguous sequences were assembled in Sequencher v.4.1.4 (Gene Codes Corp., Ann Arbor, MI). Sequences obtained were compared using BLAST (**B**asic **L**ocal **A**lignment **S**earch **T**ool) in GenBank http://www.ncbi.nlm.nih.gov/ to confirm identity of each isolate for each locus. All isolates were further compared to each other for all loci in Sequencher to identify any potential polymorphisms.

RESULTS AND DISCUSSION

Morphological Characterization

Sporangia dimensions for all the Latin Amercian isolates were well within those published for *P. palmivora* (Sporangia length 35-70 μm, Breath 20-40 μm, L/B ratio 1.2:1 – 1.8:1 and pedicel length <5 μm) (Brasier and Griffin, 1979). For the Trinidad and Tobago isolates, the sporangia of isolate P-164 with a pedicel length of 82 □m were clearly different from the rest, exhibiting similar morphology to those from Latin America. Isolate P164 was also different from all other isolates in lesion characteristics on the pod, colony morphology and Mating Type of A1. All the *P. palmivora* isolates from the Caribbean and Latin America were determined to be of Mating Type A2. As such isolate P164 was postulated to be *P. capsicii*.

Although the Latin American isolates Cr10 and Ni5 demonstrated the morphological characteristics of other *P. palmivora* isolates, they produced 1,000 fold fewer single zoospores the majority being released in a ball, which germinates directly (Figure 2). In aggressiveness tests with other Latin American isolates (Co5, Cr7, Cr11, Ni4, and

Pa1) Ni5 was just as competitive as other *P. palmivora* isolates and produced the typical necrotic lesions on the black pod susceptible cacao clone IMC 67 (Figure 2).

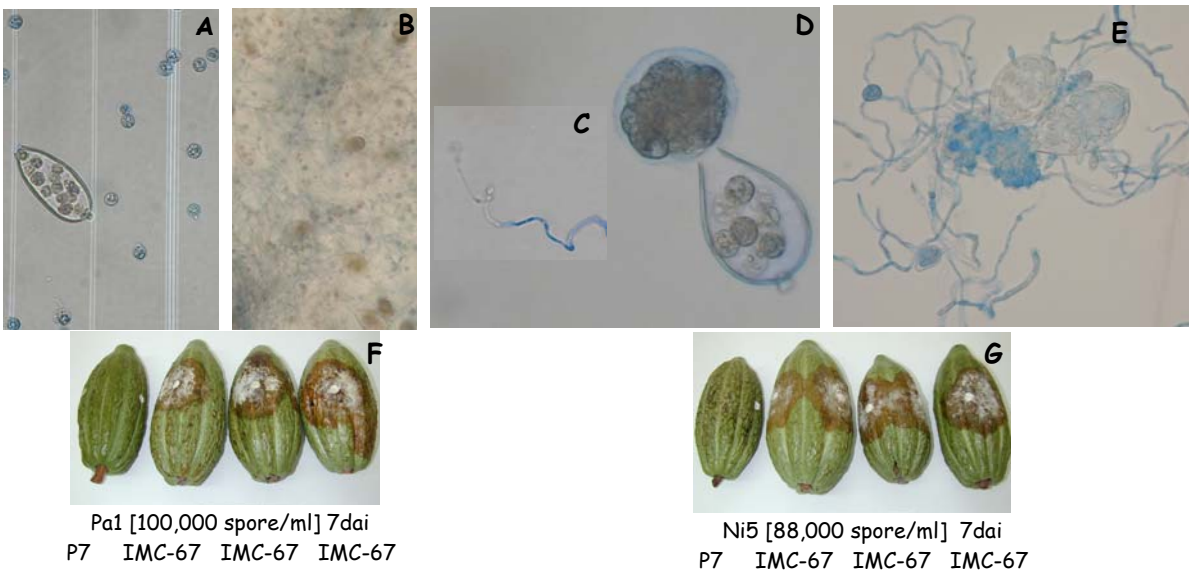

Figure 2: Morphological characteristics of *Phytophthora* isolate Ni5 from Nicaragua and Pa1 from Panama. **Panel A**: Sporangium of Pa1 releasing zoospores singly each of which could germinate to form a mycelial mat as in **Panel B** after 16 hrs in water. **Panel C** is a single zoospore of Ni5 after 16 hrs in water. **Panel D** is the more frequently observed condition for Ni5 in which zoospores are released in 'en masse' which directly germinates as in **Panel E**. **Panels F and G** show the results of the aggressiveness tests and the typical *P. palmivora* lesions formed on the black pod susceptible IMC-67.

Molecular Characterization by Fingerprinting with ITS4 and ITS6

Primers ITS4 and ITS6 produced the expected 900 bp product in all 41 (35 test and 6 standard) isolates used in this study. The number and size of the bands generated by restricting the 900 bp product with the enzymes *Alu* I, *Msp* I and *Taq* I is a fingerprint enabling species identification when compared to the banding pattern obtained for a standard or known isolate. Thirty-four of the 35 isolates from Latin America and Trinidad and Tobago were observed to have identical banding patterns or fingerprints to each other and the 3 *P. palmivora* standards 348342, 386258 and 386825 from the CABI Bioscience GRC collection. Isolate Phy-164 from Trinidad showed a completely different banding pattern to the other *P. palmivora* isolates and seems to share a more similar banding pattern to the three standard isolates 325900, 352321, 040502 for *P. capsicii* (Figure 3).

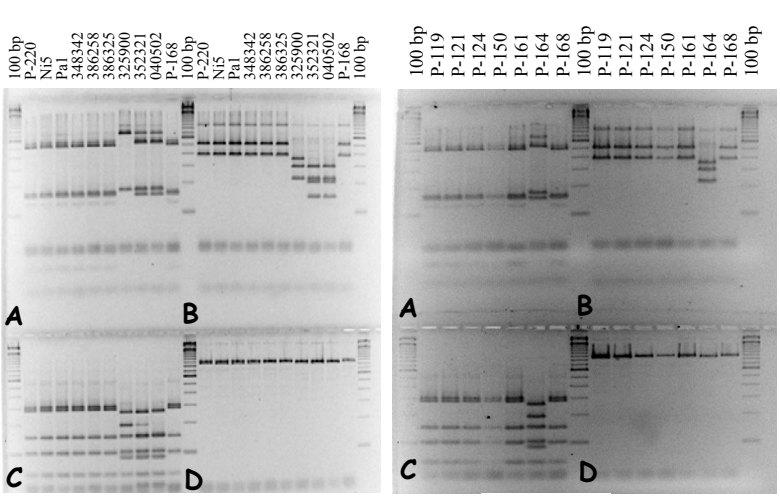

Figure 3: Digestion products of *Alu* I **Panel A**; *Msp* I **Panel B** and *Taq* I **Panel C** for the 900 bp product **Panel D** of ITS4 and ITS6**.** Isolate P-164 from Trinidad is similar to *P. capsicii*. Ni5 from Nicaragua is *P. palmivora*

Molecular Characterization by Gene Sequencing

For the seven isolates (P-161, P-217, Co4, Cr14, Ec1, Ni5 and Pa1) products averaged 1035 bp in length for CoxI, 775 bp for ITS, 890 bp for EF1α, 780 bp for NADH I, and 905 bp for β-tub. Products for all seven isolates and all five loci shared 100% sequence similarity with other isolates of *P. palmivora* in GenBank, and also shared 100% sequence similarity with each other at each locus (Figure 4).

```
ni5.coxr4   1     ATAATATGAG AGCTCCTGGA TTAAGTTTTC ATAGATTACC
p217.coxr4 1      ATAATATGAG AGCTCCTGGA TTAAGTTTTC ATAGATTACC
ec1.coxr4   1     ATAATATGAG AGCTCCTGGA TTAAGTTTTC ATAGATTACC
pa1.coxr4   1     ATAATATGAG AGCTCCTGGA TTAAGTTTTC ATAGATTACC
p161.coxr4 1      ATAATATGAG AGCTCCTGGA TTAAGTTTTC ATAGATTACC
cr15.coxf4 1      ATAATATGAG AGCTCCTGGA TTAAGTTTTC ATAGATTACC
co4.coxr4   1     ATAATATGAG AGCTCCTGGA TTAAGTTTTC ATAGATTACC
palmivora   1     ATAATATGAG AGCTCCTGGA TTAAGTTTTC ATAGATTACC
ni5.coxr4   41.    TTTATTTGTG TGGTCTATTT TAATTACTGC TTTTCTTTTA
p217.coxr4 41     TTTATTTGTG TGGTCTATTT TAATTACTGC TTTTCTTTTA
ec1.coxr4   41    TTTATTTGTG TGGTCTATTT TAATTACTGC TTTTCTTTTA
pa1.coxr4   41    TTTATTTGTG TGGTCTATTT TAATTACTGC TTTTCTTTTA
p161.coxr4 41     TTTATTTGTG TGGTCTATTT TAATTACTGC TTTTCTTTTA
cr15.coxf4 41     TTTATTTGTG TGGTCTATTT TAATTACTGC TTTTCTTTTA
co4.coxr4   41    TTTATTTGTG TGGTCTATTT TAATTACTGC TTTTCTTTTA
palmivora   41    TTTATTTGTG TGGTCTATTT  TAATTACTGC TTTTCTTTTA
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Figure 4: Alignments for a segment of the Cox I gene for the seven isolates P-161, P-217, Co4, Cr14, Ec1, Ni5 and Pa1 representing the geographic range of the 35 isolates from Latin America and Trinidad and Tobago**.** A dot (.) indicates identity with the *P. palmivora* sequence in GenBank.

The results of morphological and molecular characterization showed isolates Ni5 and Cr10, from the Atlantic coasts of Nicaragua and Costa Rica respectively, to be a new morpho-type of *Phytophthora palmivora*. The coastal regions of these two countries tend to be hotter and drier than other parts of these countries. Currently black pod disease cause severe cacao crop losses during the rainy season in producing countries. If the new morpho-type is an adaptation to drier conditions, the resulting year round inoculum levels for black pod disease could far exceed the 30% crop losses currently reported. Research into the epidemiology of and pathology to find host resistance to these new *P. palmivora* morpho-types are required.

ACKNOWLEDGEMENTS

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REFERENCES

Al-Hedaithy, S.S.A. and Tsao, P.H. (1979). Sporangium pedicel length in *Phytophthora* species and the consideration of its uniformity in determining sporangium caducity. Trans. Br. Mycol. Soc. 72:1-13.

Brasier, C.M. and Griffin, M.J. (1979). Taxonomy of the *Phytophthora* on cocoa. Trans British Mycol. Soc. 72,111-143.

Brasier, C.M. and Hansen, E.M. (1992). Evolutionary biology of *Phytophthora*. Annu. Rev. Phytopathol. 30:173-200.

Cooke, D.E.L and Duncan, J.M. (1997). Phylogenic analysis of *Phytophthora* species based on ITS1 and ITS 2 sequences of the ribosomal RNA gene repeat. Mycol. Res. 101:667 – 677.

Erwin, D.C. and Ribeiro, O.K. (1996). *Phytophthora* Diseases Worldwide. St. Paul: APS Press, Am. Phytopathol. Soc.

Erwin, D.C. (1995). Variability within and among species of *Phytophthora*. In: *Phytophthora* Its Biology, Taxonomy, Ecology and Pathology. Eds. D.C. Erwin, S. Bartnicki-Garcia and P.H. Tsao. St. Paul: APS Press, Am. Phytopathol. Soc.

ICCO (International Cocoa Organization) (2006). ICCO Monthly and Annual Averages of Daily Prices of Cocoa Beans, 1960 – 2006. http://www.icco.org/prices/pricesave

Kroon, L.P.N.M., Bakker, F.T., van den Bosch, G.B.M., Bonants, P.J.M. and Flier, W.G. (2004). Phylogenetic analysis of *Phytophthora* species based on mitochondrial and nuclear DNA sequences. Fungal Genet. Biol. 41: 766-782.

Lee, S.B. and Taylor, J.W. (1992). Phylogeny of five fungus-like protoctistan Phytophthora species, inferred from the internal transcribed spacers of ribosomal DNA. Mol. Biol. Evol. 9:636-653.

Martin, F.N. and Tooley, P.W. (2003). Phylogenetic relationships among *Phytophthora* species inferred from sequence analysis of mitochondrially encoded cytochrome oxidase I and II genes. Mycologia 95:269-284.

1.7 Highlights of an international collaborative research initiative for the biological control of the black pod disease in Cameroon.‡

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SUMMARY

Phytophthora pod rot, commonly called "black pod", is the most serious disease affecting cocoa production in Cameroon, where losses can reach up to 100% of annual crop in the absence of control measures. Typically, the control of black pod disease in Africa focuses on the uses of metalaxyl and copper fungicides, phytosanitation, and the availability of less susceptible planting material. Additionally, sources of sustainable genetic resistance are difficult to obtain and high dose fungicide applications remain costly and polluting. The last decade has witnessed meaningful changes in the method of controlling cocoa diseases worldwide, in particular with the development of biological control approaches on cocoa. The development of highly effective, self-sustaining classical biological control agents remains the "holy grail"; but, before reaching this point, it should be considered as experimental and hence requires further research and development.

International coordination and cooperation are essential to get a more comprehensive knowledge of the black pod disease issue as needed to underpin research efforts in biological control in any cocoa producing country. Since 2003, EBCL and SBML laboratories within USDA-ARS, CIRAD, Masterfoods-US and Imperial College London have been collaborating with IRAD and IITA of Cameroon to develop the first pilot biological control programme in this African region. The main objective of this programme is mainly to provide baseline data for the evaluation of the potential of biological control agents originating in Cameroon – Trichoderma asperellum –, under different ecologies and disease pressures. In its 3-year scope, the project has given emphasis to the following tasks:

- *Conduct epidemiological survey in smallholder plots treated with biological control agents in comparison with fungicides.*
- *Assess the diversity, dynamics and aggressiveness of the pathogens in these smallholder plots to provide insights into the adaptive capacity of these pathogens.*
- *Monitor the persistence and the colonization of the biological control agents.*
- *Strengthen IRAD's capacity to improve local production, formulation and other delivery aspects for microbial control agents*
- *Support development of scientific capabilities including microbial systematics.*

‡ Full text of paper not available at press time

After the first year, the major outcomes of this project were the development of collaborative relationships between researchers in USA, France, UK and Cameroon and the setting-up of the first biological control laboratory in this African region. Initial trends from the two-years of treatments indicated that biological control agents performed differently under different ecological conditions. We will emphasize the necessity of strong collaborative networks that enable different approaches to be taken for problem solving. In addition, specific results obtained in the various disciplines will also be presented by each scientist involved in the project. This international collaborative research programme could provide conceptual bases for the development of any future biological control programme in Africa.

[*] Full text of paper not available at press time

1.8. Evidence of polygenic inheritance of resistance to witches' broom disease caused by *Moniliophthora perniciosa* in *Theobroma cacao* L[§].

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SUMMARY

Witches' broom disease caused by Crinipellis perniciosa severely restricts cocoa production in the Americas. Breeding for resistance has however been unsuccessful due to lack of an effective screening method and a poor understanding of the genetics of resistance. The genetic basis of resistance to the disease was investigated using the new agar-droplet screening method. Twelve genotypes were intercrossed in 7 M x N mating designs, while another 12 parents were used in biparental crosses. Resistance was assessed based on incubation period and broom diameter and data analyzed by North Carolina Design II and Parent-Offspring Regression analysis. Significant differences were observed among parents and among F_1 progenies for the measures of resistance. Frequency distributions of symptom severity for the progeny families were continuous with evidence of transgressive segregation. Additive genetic effects were predominant for both measures of resistance and was 8-9 fold larger than non-additive effects. Estimates of narrow sense heritability were 0.79 and 0.78 for broom base diameter and incubation period respectively. There was a strong linear relationship (r^2 = 0.85) between mid parental values and progeny means for broom base diameter. These results indicate that resistance to the Witches' Broom disease is polygenic and biparental with a predominance of additive genetic effects. The utilization of this information in effective breeding programmes for resistance to witches' broom disease is discussed.

[§] Full text of paper not available at press time

1.9. Towards integrated control of frosty pod rot (*Moniliophthora roreri*) of cacao: a model programme for pest and disease control[**]

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SUMMARY

Frosty pod rot (Moniliophthora roreri) of cocoa (Theobroma cacao) is a major biological constraint to cocoa production in Latin America. The pathogen is still in an invasive phase and poses a continuing threat to other cocoa growing areas of Latin America (Brazil and Bolivia), having recently invaded Mexico. The consequences of failing to manage frosty pod rot may be devastating should the pathogen spread further afield to West Africa. It has already impacted on the livelihoods of many smallholder farmers and their communities, who had traditionally relied on cocoa for their income. The potential for economic and environmental impact is very real. With this in mind, in 1998 USDA-ARS looked to develop a collaborative research programme, to find a means to control this devastating disease. Conventional control measures, including phytosanitation, have failed to halt the progress of frosty pod rot through Latin America. Alternative strategies were pursued to reduce the impact of frosty pod rot. Plant breeding, crop sanitation, rational pesticide use and biological control were, and are, still being investigated as a means to develop an integrated crop management (ICM) strategy, tailored to meet the needs of the smallholder farmers. Individual pathways for control of the frosty pod pathogen were investigated with an eventual view to combine all components in a definitive integrated control. Here we report on the development of this research programme and how the components have been combined in the field to produce the beginning of a control strategy for frosty pod rot and the identification of resistance genes as a long-term means of identifying resistant germplasm. This foresighted control programme can be a model for facing the challenges of current, or future, pest and disease in cacao.

[**] Full text of paper not available at press time

Discussion on Integrated Pest Management: Advances in Conventional Methods

Chairpersons: J. L. Periera, Ceplac, Brazil and Brian Bailey, ARS, USDA, USA.

Q: Roy Bateman, IPARC, Imperial College, UK

How much importance would you give to searches for isolates from Heteroptera in other crops and environments?.

A: Regis Babin, CIRAD/IRAD, Cameroon

The fungi strain available now at Nkolbison come exclusively from cocoa mirid bodies, but it would be interesting to screen different species of Heteroptera, on other crops to collect new strains. Cocoa mirids are not the only Heteroptera attacking cocoa and it would be good to collect strains efficient for all the Heteroptera pests of cocoa.

Q: Roy Bateman, IPARC, Imperial College, UK

Are we trying to (eventually) develop Beauvaria bassiana product that can can be marketed in the whole region?

A: Regis Babin, CIRAD/IRAD, Cameroon

I would like that this activity could be conducted at IPARC, using the mycoharvester and we are waiting for your proposition.

Comment: Carmen Suarez Carpello, INIAP, Equador

This is a comment to reinforce the statement by Ulrike Krauss, especially, in favour of African countries. It is crucial to bring down canopies of cocoa trees since there is no way to manage Moniliophthora roreri and even Phytophthora; no cultural or chemical will be effective.

In relation to Prakash's comment as well as we have hopes that the use of biocontrol (which apparently are finding their way in cocoa orchards) will restore source equilibrium between pathogen and biocontrol so other control measures may become more efficient.

Q: Gary Samuels, USDA-ARS, Beltsville, USA

Beauvaria bassiana has a wide host range. Is it necessary to consider environmental impact of selected strains on other insects?

A: Regis Babin, CIRAD/IRAD, Cameroon

Yes, I agree with you. B. bassiana could be dangerous for pollinating insects and natural enemies of mirids. So it is very important to test and identify strains more specific to Heteroptera species that are globally harmful to cocoa tree.

Q. Prakash Hebbar, Mars Inc., USA

Can phytosanitation be done after spraying with a mycoparasite with anti-sporurulant properties? This could avoid spore dispersal during phytosanitation eg Peru field work with SENESA reduction of sporulation offFrosty pod rot by *T. stromaticum*

A: Ulrike Krauss/K. Holmes, CABI Bioscience, Trinidad and Tobago

Yes, this should work. Our results show that, especially Chlonostachys formed a cup over the pod as soon as *M. roreri* mycelium started emerging. This prevented sporulation.

We have not quantified this but in field studiesand in surveys; we have observed colonization and sporulation on the pseudostroma by Trichoderma and Bionectria species with the associated apparent reduction in sporulation.

Q: Pierre Tondje, IRAD, Cameroon

Could INCOPED recommend more awareness to quarantine workers on the diseases that are reported in South and Central America to prevent their spread to the main cocoa producing countries of West Africa?

A: Julie Flood, CABI Bioscience, UK

In response to Pierre Tondje's comment about raising awareness of alien species in West Africa, there are plans to have a workshop in the region in mid-2007 to report on the Witches broom CFC project and to plan a future CFC project to strengthen quarantine awareness with farmers, researchers, extensionists and conducting PRAs.

Q. Prakash Hebbar, Mars Inc., USA

There is the neeed to raise awareness of the FPR programme in the Carribean regions

A: Julie Flood, CABI Bioscience, UK

Yes, we are planning one. A concept paper has been developed by Ulrike Krauss and Keith Holmes.

Q: Julie Flood, CABI Bioscience, UK

For pathogenicity testing of Beauveria against mirids, are mirids collected from the field or are they raised artificially?. How are they reared and what artificial diet do you use?.

A: Regis Babin, CIRAD/IRAD, Cameroon

Mirids used for pathogenicity were reared on cocoa pods and twigs from Nkolbisson station in Cameroon. More information on the rearing method can be obtained from a paper devoted to this subject in the proceedings of the 15th ICRC.

Q: A. Y. Akrofi, CRIG, Ghana

Is it possible to have this early detection manual for incorporation in our training material in West Africa so that extension personnel and farmers in the region can become aware of these diseases?

A: Ulrike Krauss, CABI Bioscience, Trinidad and Tobago

Definitely. This is a prototype tool we developed for adaptation to our clients' needs and use, which can happen immediately. The colour chart and manual done, however, can be misinterpreted by the inexperienced. Therefore, this process has to be initialed as "accompanied leor" ie. We have to facilitate and train our future trainers to disseminate this knowledge further. This is why we don't hand out hundreds of colour charts or upload them on the webpage. We want to ensure that people get high quality production (colour quality and laminated) and the necessary training support.

Q: I.Y. Opoku, CRIG, Ghana

I wonder why you appear to encourage farmers to leave black pod infected pods on trees a little longer whilst frosty pods are removed at the earliest detection. Why is this so?

A: Ulrike Krauss, CABI Bioscience, Trinidad and Tobago

I do not mean to encourage pods infected by any pathogen to be left in the field. They should of. Be removed. But if a black pod infected pod is missing only one was o ripen and harvest. The farmer will very reasonably leave this pod for the remaining week and reap these benefits. This is alright. The point I tried to make is that this "extra week" approach is never alright for frosty pod rot as the farmer would not be able to harvest healthy beans.

Q: I.Y. Opoku, CRIG, Ghana

What do farmers do when frosty pods are removed from the trees?. Do they carry them away from the farms?

A: Ulrike Krauss, CABI Bioscience, Trinidad and Tobago

Our recommendation based on field observation and some solid research done in Columbia is MINIMAL AGITATION, because agitation of sporulating pods is the best way to inoculate the field. Farmers are to leave the pods at the foot of the tree. It is best to cover them with litter for faster decomposition. You can spray decomposition enhancing inoculum including *Trichoderma.* Urea is also an anti-sporulant, but the main thing is to encourage decomposition with minimal agiatation of sporulated pods.

Q. Prakash Hebbar, Mars Inc., USA

Can we better prepare for frosty pod rot spread with more modern techniques for spore estimation using ELISA techniques or spore trapping?. If it is so, work in Brazil's experience on spore trapping should be continued.

A: Ulrike Krauss, CABI Bioscience, Trinidad and Tobago

It is very difficult to quantitatively collect *Moniliophthora roreri* spores from pods in the field or the air, and if you get them, they are tiny, transparent and are easily mistaken with many other species. Your suggestion is good and we should work on it, but implementation is tricky.

Q: F. Kouame N'guessan, CNRA, Cote d'voire

You said that early recognition of symptoms caused by *Moniliophthora roreri* could help reduce infection through phytosanitation. Practically, how do you detect symptoms in the canopy?

A: Ulrike Krauss, CABI Bioscience, Trinidad and Tobago

Pruning your trees to a manageable height is crucial to this approach. There is no short cut to that, but if farmers invest in this pruning and also learn early recognition, they will reduce labour cost in the medium- to long-term.

2.1. Alteration in fungal/plant gene expression following colonization of *Theobroma cacao* by endophytic *Trichoderma* species: a role in defense or stress tolerance?

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SUMMARY

Endophytic Trichoderma species are being studied for their potential for moderating the responses of cacao to biotic and abiotic stresses. Using three different methods of inoculation, Trichoderma harzianum isolate DIS 219f and Trichoderma ovalisporum isolate DIS 70a actively and endophyticly colonized cacao stems, roots, leaves, plumules, and cotyledons based on tissue plating results. Gene expression was quantified using RNA markers and QPCR. The Trichoderma isolates induced, in various combinations, genes putatively encoding an ornithine decarboxylase, a GST, a zinc finger protein, a carbohydrate oxidase, a wound response gene, and an unknown protein. Fungal gene expression was also altered during cacao colonization. Our ability to detect altered fungal gene expression depended on the isolate studied. The fungal genes characterized included genes encoding/putatively enzymes involved in breakdown of plant cell components including carbohydrates (cellulases and alcohol oxidase), proteins (serine protease) and lipids (Enoyl-CoA hydratase/isomerase). Q-PCR using genomic DNA indicated DIS 219f was more aggressive in colonizing cacao hypocotyls than DIS 70a. Isolates DIS 219f and DIS 70a were also endophytic on the leguminous crop pigeon pea with isolate DIS 219f again being more aggressive. The endophytic relationship between some isolates of Trichoderma involves a complex system of genetic cross-talk involving both partners. Trichoderma isolates colonize plant tissues with different efficiencies, a result that indicates it may be possible to use molecular techniques to select for strong colonizers of cacao tissues among Trichoderma isolates with significant biocontrol capabilities.

INTRODUCTION

Biocontrol of soil-borne plant diseases with *Trichoderma* species has been extensively studied (Kubicek and Harman, 1998; Harman and Kubicek, 1998). *Trichoderma* species can function in commercial systems to control disease of aerial plant parts (Elad, 2000; Samuels et al., 2000; Holmes et al., 2004). The primary aerial targets for control of cacao diseases are meristematic tissues, flower cushions, and the cacao pod (Woods and Lass 2001). The concept of endophytic/epiphytic colonization of plant tissues by *Trichoderma* isolates holds the potential for limited application events and long-term disease control (Harman *et al*., 2004).

We have designed methods for characterizing cacao colonization by *Trichoderma* isolates in order to: 1. understand the nature of the interaction between cacao and *Trichoderma*, 2. determine the *Trichoderma* carrying capacity of various cacao tissues, and 3. identify factors that influence the ability of *Trichoderma* to colonize cacao tissues. Our goal is to identify *Trichoderma* isolates with the greatest potential for colonizing cacao tissues among those that have significant biocontrol potential.

MATERIALS AND METHODS

Trichoderma isolates and their culture.

The collection and characterization of *Trichoderma* isolates was described by Evans *et al*. (2003). Two isolates were chosen for molecular analysis (Holmes *et al*., 2004) of their interactions with cacao seedlings: DIS 70a-*T. ovalisporum,* (Holmes *et al*., 2004) and DIS 219f -*T. harzianum* (Evans *et al*., 2003). Mycelia of each isolate were produced by growth at 23°C in stationary cultures, frozen in liquid nitrogen, and stored at -80ºC before use in RNA extractions.

Isolates were screened for mycoparasitic ability on *M. roreri* as previously described (Holmes et al., 2004; Bailey *et al*., 2006). Inoculum of the *Trichoderma* sp. to be evaluated was placed at one edge of a 9 cm diam PDA plate pre-colonized by *M. roreri* and maintained at 25^{o}C in the dark. Agar plugs (5 mm) were collected at weekly intervals and plated out onto 20% PDA and incubated at 25 ^{o}C under black light (near UV) and observed over 14 d for the growth of the *Trichoderma* sp. or *M. roreri*.

Cacao seedling production.

Seeds of *T. cacao* variety Comun (Lower Amazon Amelonado type) were collected by Alan Pomella from established plantings at the Almirante Cacau, Inc. farm (Itabuna, Bahia, Brazil). The seed coat was removed and the seed surface sterilized by incubation in 14% sodium hypochlorite for 3 min followed by three washes in sterile distilled water. Three sterile seeds were placed on 1.5% water agar in 10-cm-diam Petri dishes and sealed with parafilm. Seeds were pre-germinated under fluorescent lights at 23°C.

Cacao seedling inoculation and colonization methods.

For the studies of gene expression, after being pregerminated 3 d, two 0.6-cm agar plugs of one of the four *Trichoderma* isolates were placed on the water agar surface below the emerging roots. The *Trichoderma* was allowed to grow out of the agar plug through the water agar and onto the cacao seedlings. After 6 days of colonization, the cotyledons were removed and the seedlings were frozen in liquid nitrogen and stored at -80ºC until used for RNA isolation.

Alternatively, cacao seedling colonization (Method 1) was determined after rating the seedlings for discoloration using a scale from 0 (no discoloration) to 4 (severe browning). Colonization was also studied using two additional methods. With Method 2, after colonization on plates for 6 days, the germinating seed were planted in 3 cm of sterile soil-less mix (80 g of 2:2:1, sand: perlite: promix) in double magenta boxes (20 cm high and approx. 6.5 cm square)**.** Twenty mL of sterile distilled water was added to the dry soil-less mix after planting and seedlings were grown on fluorescent light benches at 23°C for 2 weeks. With Method 3, a colonized agar plug was transferred to the soil surface of magenta boxes and 20 mL of sterile

distilled water was added. The magenta boxes were incubated at 23°C for 10 days before a sterile pregerminated seed (3 days on water agar) was planted in each box. The seedlings were grown 3 weeks after inoculation before being dissected.

For each inoculation method, cacao seedlings were dissected and 1 cm tissue sections were plated on cornmeal dextrose agar (CDA). For Method 1, sections of roots, stems, cotyledons, and plumules were surface sterilized as previously described (Bailey et al., 2006) for the cacao seed and plated on CDA. For Method 2 and 3 leaves were also sampled. All the plated sections were incubated on the lab bench (23°C) for 5 to 7 days and were then counted as positive or negative for colonization.

RNA Isolation and Real-time quantitative PCR (Q-PCR).
Total RNA was isolated from cacao seedlings and treated with DNase I as previously described (Bailey et al., 2006). Total RNA of fungal mycelia was extracted using RNeasy mini kit (Qiagen, Valencia, CA) according to the manufacturer's recommendation, with an extra DNase I treatment (Bailey *et al*., 2006). Four micrograms of each RNA sample isolated as described above were used to generate first strand cDNA (Bailey *et al*., 2006). Primers (Bailey *et al*., 2006), 23 to 27 oligomers, for selected genes were designed to generate a product of 200 to 250 bp, and to have a T_m (melting temperature) of 60 ± 3°C. Q-PCR was carried out and altered expression was determined by calculating the expression of plant genes in colonized tissues verses control tissues (Bailey *et al*., 2006). The constitutively expressed cacao *ACTIN* gene (P55) and *Trichoderma ACTIN* gene**,** GJS 01-07 (AY376676) were used as expression references. The data for fungal EST expression were calculated as Log_{10} of expression levels normalized against *Trichoderma ACTIN* (Bailey *et al*., 2006). Altered expression of fungal ESTs was calculated as expression in cacao tissue versus expression in mycelia (Bailey *et al*., 2006).

Colonization of cacao and pigeon pea by *Trichoderma.*
Cacao seedlings grown in magenta boxes as described above were inoculated with *Trichoderma* isolates DIS 70a and DIS 219f. A colonized agar plug was placed either between the cotyledons or on the soil surface at the seedlings base using sterile technique. The magenta boxes were closed and the seedlings maintained as described above. Hypocotyls were harvested 14 days after inoculation and frozen until DNA could be extracted. Genomic DNA was isolated using a modified DNeasy® Plant Mini Kit Protocol.

Seed of pigeon pea (*Cajanus cajan*) were surface sterilized as described above and planted into sterile soil using the magenta box system. Immediately after planting, an agar plug of isolate DIS 70a or DIS 219f was placed on the soil surface using sterile technique. The magenta boxes were closed and the seedlings maintained as described above. The seedlings were harvested 3 weeks after planting and sectioned into roots stems and leaves. Individual sections were surface sterilized and plated on CDA with the remainder of each tissue being frozen for DNA extraction using the modified DNeasy® Plant Mini Kit Protocol.

Real Time QPCR was carried out using Genomic DNA following procedures similar to those described for cDNA. Genomic DNA samples were analyzed with cacao specific primers for *ACTIN* (P55) and TEF (P57), pigeon pea specific primers for *ACTIN*, or *Trichoderma* specific primers (TEF and *ACTIN* for DIS 70a and DIS

219f). The data is expressed as picograms of fungal DNA per nanograms of plant DNA. Means and standard errors are provided.

RESULTS

Mycoparasitism, cacao seedling colonization, and seedling reaction.

Isolates DIS 70a and DIS 219f are mycoparasites of *M. roreri* (Table 1), each isolate completely colonizing *M. roreri* colonies in plate culture. DIS 219f caused a strong reaction in cacao roots resulting in a discoloration rating of 3.0 versus 1.7 for DIS 70a (Table 1). The *Trichoderma* isolates were observed growing out of surface sterilized cacao tissue isolated from magenta box grown seedlings when plated on CDA (Table 1). Both isolates were strong colonizers of cacao stems regardless of method of inoculation.

Table 1.Mycoparasitism of *M. roreri* and cacao root discoloration, and cacao tissue colonization by *Trichoderma* isolates DIS 219f and DIS 70a.

				Colonization (method)		
Isolate	Mycoparasitism	Discoloration	Tissue	1	2	3
	%	Rating 1-4		----------%----------		
DIS 219f	100	3.0	Root	100	100	100
			Stem	87	100	100
			Leaf	nd	100	100
			Cotyledon	100	100	100
			Plumule	50	100	70
DIS 70a	100	1.7	Root	75	100	100
			Stem	100	100	100
			Leaf	nd	60	60
			Cotyledon	87	100	100
			Plumule	50	80	40

QPCR analysis of gene expression in response to colonization.

Genes showing altered expression in response to colonization of cacao by DIS 70a and DIS 219f are presented in Tables 2 (Bailey et al., 2006). Based on real time Q-PCR analysis of colonization of cacao seedlings, isolate DIS 70a induced accumulation of transcripts for 5 plant ESTs (P1, P4, P13, P59, and U4). Transcripts for plant ESTs P12, and P31 were repressed in DIS 70a-colonized tissues more than 50%. Expression of 9 fungal ESTs (F2, F3, F5, F7, F9, F11, F12, F14, and F19) was enhanced in seedlings colonized by DIS 70a as compared to expression in liquid culture grown mycelia (Table 2).

Plant ESTs P1, P4, P13, P26, P59, and U4 were induced in DIS 219f-colonized seedlings compared to non-colonized seedlings. Transcript levels for EST P12 decreased in response to seedling colonization by DIS 219f. Of the fungal ESTs presented in Table 3, only Transcripts F9 and F19 were detected in cacao seedlings colonized by DIS 219f. ESTs F9 and F19 accumulated in cacao seedlings colonized by DIS 219f to levels 99.5 and more than 1,000,000-fold higher levels in DIS 219f-

colonized seedlings as compared to expression in liquid culture grown mycelia (Table 2), respectively.

Table 2.Summary of Cacao and *Trichoderma* ESTs showing altered expression in cacao seedlings colonized by *Trichoderma* species using Q-PCR analysis.

Clone #	Source[1]	Clone ID	Putative ID
P1	DIS 219b[2]	AF029349	*Lycopersiconesculentum*/ornithine decarboxylase
P4	DIS 219b	AB087837	*Pisum sativum* /glutathione S-transferase
P12	DIS 70a	AM117766	*Theobroma cacao*/Unknown Protein/ extensin-like protein
P13	DIS 219b	CA992708	*Gossypium hirsutum* /EST-Zinc-Finger protein, putative Cys2-His2 type
P26	DIS 219b	CA798633	*Theobroma cacao*/Putative wound protein
P29	DIS 70a	AF531362	*Gossypium barbadense*/EF-hand, calcium binding motif
P31	DIS 219f	BT012976	*Lycopersicon esculentum*/tonoplast intrinsic protein
P59	DIS 219b	AF503442	*Nicotiana* spp./Nectrin 5-glucose oxidase activity
U4	DIS 219b		No homology
F2	DIS 219b	XP_369811	*Magnaporthe grisea*/Vacuolar ATP synthase subunit
F3	DIS 219b	XM_654907	*Aspergillus nidulans* /Glycosyl hydrolases family 2
F5	DIS 219b	XM_385607	*Gibberella zeae*/Phenylalanyl-tRNA synthetase
F7	DIS 219b	XM_745951	*Aspergillus fumigatus* /Glycosyl hydrolase family 7
F9	DIS 219b	XM_327884	*Neurospora crassa*/Uracil phosphoribosyltransferase
F11	DIS 219b	AY258899	*Trichoderma hamatum*/alkaline proteinase
F12	DIS 219b	XM_322385	*Neurospora crassa*/Enoyl-CoA hydratase/isomerase
F14	DIS 219b	CF872154	*Trichoderma reesei*/Nuclear pore membrane glycoprotein
F19	DIS 219f	AF232903	*Cochliobolus victoriae*/alcohol oxidase

[1]Source for P#, U#, and F# ESTs represents the cacao/*Trichoderma* isolate combination from which the EST was isolated.
[2]DIS 219b is a *Trichoderma hamatum* isolated at the same time and from the same tree as was 219f (Bailey *et al*., 2006).

Colonization of cacao and pigeon pea by *Trichoderma.*

When compared on a genomic DNA basis, DIS 219f was detected in cacao hypocotyls at higher levels than was observed for DIS 70a (Table 3). When seedlings were inoculated at the soil surface, DIS 219f was detected at 38 times the level of isolate DIS 70a. When inoculated at the cotyledons, DIS 219f was detected at 20.6 times the level of isolate DIS 70a.

Both DIS 219f and DIS 70a were able to colonize pigeon pea leaves, stems , and roots. Colonization rates were similar for both isolates based on results from tissue plating (Table 4). As was the case for cacao colonization, isolate DIS 219f tended to colonize pigeon pea tissues to higher levels than isolates DIS 70a although the variation in colonization was large (Table 4), especially for leaves. Colonization for both isolates was higher in the roots.

Table 3.Colonization of cacao hypocotyls by endophytic *Trichoderma* isolates based on Q- PCR using genomic DNA.

Treatment	Fungal DNA	Std. Error pg/ng
Control/DIS 70a	0.0019	0.0008
DIS 70a/Control	0.0072	0.0060
Control/DIS 219f	0.0739	0.0380
DIS 219f/Control	0.1475	0.0590

Seedlings were inoculated between the cotyledons (Control / Isolate) or at the soil surface (Isolate / Control).

Table 4. Colonization of pigeon pea seedlings by endophytic *Trichoderma* isolates based on tissue plating and Q-PCR using genomic DNA.

Tissue	DIS 219f	DIS 70a
Tissue plating	# colonized/12 sections	
Leaf	9	9
Stem	9	9
Root	10	7
Q-PCR	pg fungal DNA/ ng plant DNA (S.E.)	
Leaf	3.17 (1.57)	0.25(0.08)
Stem	0.31(0.29	0.23(0.13)
Root	4.49(1.43)	1.31(0.67)

DISCUSSION

The primary tissues targeted for control of aerial cacao diseases are meristems, young stem and leaf tissues, flowers, brooms, and of course pods (Wood and Lass, 2001). *Trichoderma* can be isolated from most of these tissues on occasion but apparently does not dominate these tissues under field conditions (Samuels *et al*., 2000; Arnold

et al., 2003; Holmes *et al.,* 2004; Rubini *et al*., 2005). *Trichoderma* isolates associated with cacao showed a differential ability to colonize hypocotyls of cacao seedlings (Table 3). Isolate 219f, *T. harzianum*, was most aggressive in colonizing cacao regardless of the inoculation site (soil surface or cotyledons). Similar results were observed when pigeon pea seedlings were inoculated with DIS 219f and DIS 70a. Isolate DIS 219f established the largest populations in both cacao and pigeon pea. Populations were greatest in the roots of pigeon pea. Although the *Trichoderm*a isolates were collected from cacao environments in Ecuador, DIS 219f from *Theobroma gileri* in Esmeraldas Province (Evans *et al*., 2003) and DIS 70a from a witches' broom on a liana (*Banisteriopsis caapi*, Malpighiaceae) in Sucumbios Province (Holmes *et al*., 2004), they actively formed endophytic associations with pigeon pea, an old world crop. They both also heavily colonized pigeon pea roots despite being originally isolated from above ground tissues.

The level of colonization varied greatly even under the highly controlled environment within the magenta box. DIS 219f was detected in cacao hypocotyls at levels as high as 0.367 pg fungal DNA per ng plant DNA while the fungus could not be detected in some replications of the same treatment. This suggests that the application techniques and formulations used in applying *Trichoderma* to foliar tissues are likely to be critical to achieving as uniform colonization as possible. If *Trichoderma* is to colonize a tissue it must have nutritional support and a proper environment (Kubicek and Harman, 1998; Harman and Kubicek, 1998). Both isolates studied here heavily colonized the roots of pigeon pea. On aerial plants surface nutrition can come from several sources including rainfall, other organisms living on the plant surface, formulation additives, or breakdown of the plants surface. The surface of the cacao stem is constantly changing, a fact that is obvious when it changes from a green tissue covered with trichomes to a brown tissue with a corky bark (Wood and Lass, 2001). Once inside the plant, *Trichoderma* may feed on intercellular material or intracellular material by causing local cell lysis (Harman *et al*., 2004). As observed here (Table 2) and elsewhere (Kubicek and Harman, 1998; Harman and Kubicek, 1998), many *Trichoderma* species posses the required enzymes to break down plant cells.

Endophytic/epiphytic *Trichoderma* populations are unlikely to be static in nature. Competition, available nutrition, and environment change over time and the endophyte population will change over time also to a greater or lesser degree. Also, it should not be assumed that the presence of an organism means activity by that organism. In the case of mycoparasites, the endophyte should be in a state primed for rapid response if not in a state of rapid growth. *Trichodermas* are good at occupying niches (Kubicek and Harman, 1998; Harman and Kubicek, 1998), consuming available resources and then sporulating in search of new resources. What remains behind must be in equilibrium with available resources and environment. After isolate selection, the best way to increase *Trichoderma* populations in the canopy may be to increase resource availability. This may be done by planting genotypes that provide more nutritional resources, altering the endophyte in a manner so it is primed to exploit available nutritional resources (for example applying *Trichoderma* in formulations that carry inducers of enzymes required for endophytic colonization), applying the *Trichoderma* to specific tissues rich in nutritional resources, or applying more nutritional resources with the *Trichoderma*. Knowing when resources are at their optimum for use by *Trichoderma* may also influence establishment of endophyte populations. For example, the senescing flower may serve as a resource for

Trichoderma proliferation. Points of rapid tissue expansion may have similar potential. By understanding the potential of *Trichoderma* to colonize cacao tissues, including the factors that impact colonization, it may be possible to optimize its use for biocontrol. The combination of *Trichoderma* isolates that are active and persistent colonizers having strong biocontrol capabilities with optimum formulations applied at the appropriate time to appropriate tissues continues to hold the promise of long term disease control with limited applications.

REFERENCES

Arnold, A., Mejia, L., Kyllo, D., Rojas, E., Maynard, Z., Robbins, N., and Herre, E. (2003) Fungal endophytes limit pathogen damage in a tropical tree. PNAS 100. 15649-15654.

Bailey, B., Bae, H., Strem, M., Roberts, D., Thomas, S., Crozier, J., Samuels, G.J., Choi, I, and Holmes, K. (2006) Fungal and plant gene expression during the colonization of cacao seedlings by endophytic isolates of four *Trichoderma* species. Planta DOI 10.1007/s00425-006-0314-0.

Elad, Y. (2003) Biocontrol of foliar pathogens: mechanisms and application. Commun Agric. Appl. Biol. Sci. 68. 17-24.

Evans, H., Holmes, K., and Thomas, S. (2003) Endophytes and mycoparasites associated with an indigenous forest tree, *Theobroma gileri*, in Ecuador and a preliminary assessment of their potential as biocontrol agents of cocoa diseases. Mycological Progress 2. 149-160.

Harman, G. and Kubicek, C. (1998) Trichoderma & Gliocladium, Vol. 2. Bristol: Taylor and Francis Inc.

Harman G., Howell, C., Viterbo, A., and Chet, I. (2004) *Trichoderma* spp. - Opportunistic avirulent plant symbionts. Nature Reviews 2:43-56

Holmes, K., Schroers, H., Thomas, S., Evans, H., and Samuels, G.J. (2004) Taxonomy and biocontrol potential of a new species of *Trichoderma* from the Amazon basin in South America. Mycological Progress 3.199-210.

Kubicek, C. and Harman, G. (1998) Trichoderma & Gliocladium, Vol. 1. Bristol: Taylor and Francis Inc.

Rubini, M., Silva-Ribeiro, R., Pomella, A., Maki, C., Araujo, W., dos Santos, D., and Azevedo, J. (2005) Diversity of endophytic fungal community of cacao (*Theobroma cacao* L.) and biological control of *Crinipellis perniciosa*, causal agent of Witches' Broom Disease. International Journal of Biological Sciences 1:24-33.

Samuels, G. J, .Pardo-Schultheiss, R., Hebbar, K., Lumsden, R., Bastos, C., Costa, J., and Bezerra, J. (2000) *Trichoderma stromaticum* sp. nov., a parasite of the cacao witches broom pathogen. Mycol. Res. 104. 760-764

Wood, G. and Lass, R. (2001) Cacao. 4th ed. Oxford: Blackwell Science Ltd.

2.2. Endophytic biocontrol agents; most effective isolate, at the right time?

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SUMMARY

Co-evolved endophytic fungi, in particular Trichoderma spp., have been identified as prospective novel Classical biocontrol agents for the fungal diseases of cacao (Theobroma cacao) in Latin America; witches' broom and frosty pod rot. Trichoderma ovalisporum, a recently identified new species, has shown potential in in-vitro and in-vivo studies as a biocontrol agent of Moniliophthora roreri, the causal agent of frosty pod rot of cacao. A number of isolates from different hosts have been collected from the Amazon basin. A field study was carried out in CATIE's La Lola Field Station in Costa Rica to assess the ability of different strains of T.ovalisporum, and selected other Trichoderma species, to colonise and persist on cacao pods. Approximately 6 week old, hand pollinated and protected pods were sprayed with spores of the Trichoderma isolates. The pods were subsequently harvested and assessed for the presence of the Trichoderma species after 12 weeks. All Trichoderma isolates were re-isolated but to varying degrees.

A study was also undertaken in Ecuador at EET – INIAP to compare the ability of a selected Trichoderma spp., T. ovalisporum, T. stromaticum and T. koningiopsis (another newly identified endophytic Trichoderma species) to colonise flower cushions and subsequently colonise flowers and persist to mature pods. Flower cushions were initially sampled to determine the presence of indigenous Trichoderma spp. in the tissues. The flower cushions were subsequently inoculated with either T.ovalisporum or T.koningiopsis. Isolations were made from flower cushions, emerging flowers to determine the presence of the applied Trichoderma spp.

Here we report on the results of these studies and discuss the implications for control of frosty pod rot, and cacao pests and diseases in general.

INTRODUCTION

Fungal endophytes have been identified as a potential novel source of biocontrol agents for the basidiomycetous pathogens of cacao, in particular *Moniliophthora roreri*, the causal agent of frosty pod rot and *Moniliophthora perniciosa*, the causal agent of witches' broom (Evans, Holmes and Thomas, 2003).

Trichoderma endophytes have been collected associated with *Theobroma* species and the witches' broom and frosty pod rot pathogens, in Ecuador and Brazil (Evans *et al*., 2003; Holmes, Schroers, Thomas, Evans and Samuels, 2004; Samuels, Suarez, Solis, K, Holmes, Thomas, Ismaiel, Evans, 2006). A few species are currently being field tested. In Costa Rica a new species, *Trichoderma ovalisporum* is being field tested at

the CATIE La Lola field station, for biocontrol activity (Holmes**,** Krauss, Samuels, Bateman, Thomas, Crozier, Hidalgo, García, Arroyo, Bekker, and Evans, 2006). Initial results have indicated that its application can increase yield to similar levels to those of chemical control agents such as copper hydroxide (Holmes *et al*., 2006). However, the isolate currently being used may not be the best within the *T.ovalisporum* isolates, or *Trichoderma* isolates. A number of other *T. ovalisporum* and *Trichoderma* isolates will be tested to determine the most effective coloniser of cacao pods.

In addition, application of the endophytes is currently being targeted at the growing cocoa pods, with applications on a monthly basis (Holmes *et al*., 2006). This may not be the most efficient or cost effective approach to application of the endophyte. As endophytes the *Trichoderma* species, such as *T.ovalisporum*, may be able to persist and colonise cocoa pods after one application. It is possible that it may need only one application to the flower cushions to allow season long colonisation of emerging flowers and pods. To determine the ability of *Trichoderma* endophytes to colonise flower cushions and persist to pods a study was initiated in Ecuador, in collaboration with EET-INIAP.

This paper reports on studies to select the best strain/isolate for colonisation and determine the ability of the *Trichoderma* endophytes to colonise flower cushions and subsequent emerging flowers.

METHOD

Strain/isolate selection for colonisation

This study was carried out at CATIE's La Lola Field station and at CATIE, Turrialba.

Trichoderma isolates were selected for screening; these included 3 *T. ovalisporum* isolates, as well as additional *Trichoderma* isolates of interest as potential biocontrol agents;

1. *T.ovalisporum* TK1 = Dis 70a, collected from a witches' broom on liana (*Banisteriopsis caapi, Malpighiaceae*) in tropical forest along the Panacocha-Río Yanayacu, Napo River, Sucumbios Province, Ecuador by H.C.Evans & S.E. Thomas, 26.03.99
2. *T. ovalisporum* TK20 = Dis172h, collected from stem of 50-60 year old *Theobroma grandiflorum* (cupuaçu), Embrapa, Belem, Para, Brazil, by H. C. Evans & K. A. Holmes, 29.2.00
3. *T. ovalisporum* TK21 = Dis 203c, collected from stem of 20 m tall *Theobroma speciosum*, BellaVista Farm, Rio Xingu, Brazil, by H. C. Evans & K. A. Holmes, 5.3.00
4. *Trichoderma harzianum*. TK3 = Dis 219f, collected from pod of *Theobroma gileri*, Guadual, Lita, Esmeraldas Province, Ecuador, by H. C. Evans & K. A. Holmes, 05.05.00.
5. *T. endophyticum* TK23 = Dis 320c, collected from stem of *Theobroma gileri*, Arasha Resort forest, Km 120, Rio Caoni, Vicente Maldonado, Pichincha Province, Ecuador, by H. C. Evans, 03.11.01
6. *Trichoderma theobromicola* TK24 = Dis 85f: collected from *Theobroma cacao* at forest edge, Betsaida, Marañon, Peru, by H. C. Evans & D. H. Djeddour, 1.5.99

Field screening for colonisation was carried out as described previously (Holmes *et al.*, 2004). Two-month-old, hand-pollinated and protected (covered with polythene bag) pods were sprayed with the treatments; 1 – 6. A spore suspension (10^6 spores ml^{-1} in 0.01% NP-7) of each individual *Trichoderma* isolate (listed above)

7. Kocide control (0.015g a.i/ml)
8. Water control

This was carried out for 6 replicate plots containing 4 trees, 10 pods treated per tree, for each treatment and plots distributed randomly.

After ten weeks, 9 pods were harvested from each replicate tree and assessed for the presence of the isolate, both on the surface and internally (surface sterilised).

Twelve, 5 mm plugs were removed from the surface of the pods (six surface sterilised and six direct) along a transect following the length of the pod, using a flame-sterilised cork borer. The plugs were transferred to Petri dishes containing selective medium (20% PDA, 50 μg ml^{-1} Penicillin, 50 μg ml^{-1} Streptomycin and 50 μg ml^{-1} Rose Bengal), and five plugs were arranged equidistantly around the periphery of each plate. The plates were incubated at 25^o C and emerging fungi observed for three weeks.

Statistical analysis

Angular transformed data (percentage recovery) were analysed by analysis of variance (ANOVA).

Colonisation of flower cushions and transfer to flowers

This study was carried out at the commercial CCN51 cacao farm Rio Lindo (Agrotropical S.A.) and EET-INIAP, Pichilingue, Ecuador.

Experimental design was a fully randomised field study. 10 trees were identified for each of the 4 treatments, each tree representing a single plot. Each of the trees was labelled and areas containing 20 flower cushions were marked. The treatments to be used were;

1. *Trichoderma ovalisporum* (Dis 70a) (10^6 spores ml^{-1})
2. *Trichoderma stromaticum* (10^6 spores ml^{-1})
3. *Trichoderma koningiopsis* (10^6 spores ml^{-1})
4. Water

Application of the treatments was carried out once, at the beginning of the study using hand-held aerosol sprayers.

Sampling of the flower cushions and flowers was carried out prior and post treatment application, to determine the presence of indigenous *Trichoderma* spp. in the plant material. Following initial assessment sampling was carried out every 4 weeks.

Sampling of flower cushions was carried out using a modification of Evans, Thomas and Holmes (2003). Small portions of the flower cushion were removed from the surface and placed in plastic bags to return to the laboratory. In the laboratory these were surface sterilised for 2 minutes in 5% chlorine and subsequently washed twice in sterile water. The surface sterilised tissue was then placed on 20% PDA containing 50

μg ml^{-1} Penicillin and 50 μg ml^{-1} Streptomycin. These were incubated and observed for emerging fungi.

In addition, internal cushion material was removed using a flame sterilised scalpel and plated out directly onto 20% PDA (antibiotics as above). These were incubated and observed for emerging fungi.

10 flowers per tree were also collected. These were surface sterilised 2 minutes in 5% chlorine and subsequently washed twice in sterile water. The surface sterilised tissue was then place on 20% PDA containing 50 μg ml^{-1} Penicillin and 50 μg ml^{-1} Streptomycin. These were incubated and observed for emerging fungi.

Statistical analysis

Angular transformed data (percentage recovery) were analysed by analysis of variance (ANOVA).

RESULTS

Strain/isolate selection for colonisation

It was observed (Table 1) that there was a significant difference ($p>0.001$) between recovery of *Trichoderma* isolates from the surface and internal tissues of the cacao pod. Significantly greater recovery of *Trichoderma* was observed from the unsterilised plugs than the surface sterilised material. It was also observed that chemical and water control had a low level of recovery of *Trichoderma* species.

All applied *Trichoderma* isolates were recovered from the cacao pods. *Trichoderma ovalisporum* (TK1- Dis 70a) and *Trichoderma harzianum* (TK3- Dis 219f) having the highest percentage recovery.

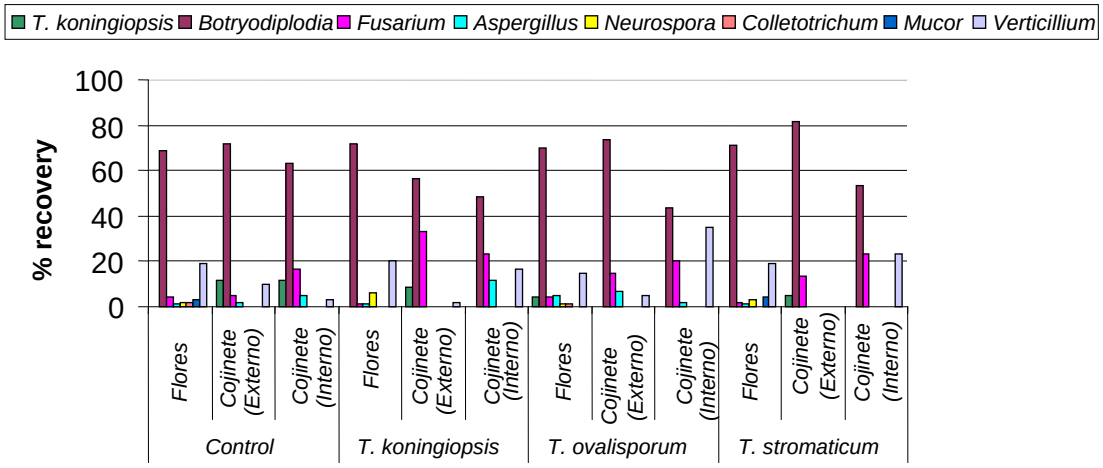
Table 1. Percentage recovery (colonisation) of *Trichoderma* isolates from cacao pods after 12 weeks

Treatments	CABI Code (DIS)	Field Code	% Recovery *Trichoderma* (Arcsin)	
			Unsterilised	Surface sterilized
Trichoderma ovalisporum	70a	TK1	94.44 (84.6)	61.57 (54.11)
Trichoderma ovalisporum	172h	TK20	75.46 (66.53)	45.83 (41.77)
Trichoderma harzianum	219f	TK3	93.06 (83.75)	58.80 (51.53)
Trichoderma ovalisporum	203c	TK21	82.41 (72.90)	40.28 (36.77)
Trichoderma endophyticum	320c	TK23	31.94 (28.35)	7.41 (7.32)
Trichoderma theobromicola	85f	TK24	55.09 (48.93)	17.59 (16.74)
Kocide			9.72 (10.7)	1.85 (1.52)
Water			6.48 (7.14)	0.93 (1.34)
		LSD=10.832		

Colonisation of flower cushions and transfer to flowers

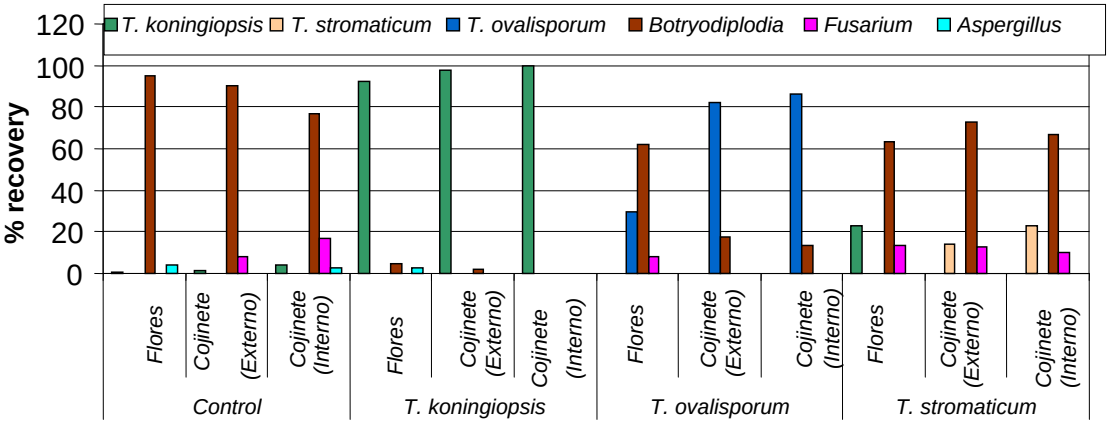
Initial observation (figure 1) of pre-treated material identified a number of opportunistic colonisers of cacao, such as *Botryodiplodia, Colletotrichum* and *Fusarium* species. In addition low levels of *T .koningiopsis* were recovered from all assigned plots.

Figure 1. Isolation of fungi from flower cushions and flowers prior to *Trichoderma* application..



Following application of the *Trichoderma* isolates to the flower cushions and flowers sampling was made after one month. All the applied *Trichoderma* isolates were re-isolated from flower and cushions, both external and internal cushion tissues (figure 2).

Figure 2.Isolation of fungi from flower cushions and flowers post *Trichoderma* application



DISCUSSION

Local *Trichoderma* isolates were recovered at a low level in the chemical and water control. These were primarily epiphytic in nature and recoveries were significantly less than from those applied *Trichoderma* treatments. Current TK1 was the best of the *T.ovalisporum* isolates for colonisation with a similar performance to that of TK3, others such as TK23/24 were not as efficient colonises endophytically, although were still present as epiphytes. This would suggest that from current isolates of still present

as epiphytes. This would suggest that from current isolates of *T.ovalisporum,* TK1 is the most efficient coloniser and that there are potentially other isolates still to pursue within other species of *Trichoderma.* Although, all ideally need to be screened in formal field trials to assess their ability to control disease and increase yield, the cost and time involved in this process is such that a means to screen these is required. This is one of a series of screens to determine the 'best bet' isolates for field assessment.

The study of the ability of the *Trichoderma* isolates to colonise the flower cushions and subsequent developing flowers has initially demonstrated that the *Trichoderma* isolates are able to colonise and persist in the flower cushions and pass to the flowers. This result supports the idea that one mechanism by which the applied *Trichoderma* isolates, in the field trials to date, have led to increase yield is through increased pod set. The applied *Trichoderma* sanitises the flower cushions and flowers. The next few field samplings will hopefully demonstrate the ability of the applied endophytes to colonise pods to maturity.

From this small selection it can be seen that although the *T. ovalisporum* currently being used in the field is the best of the current collection for colonisation, the others are similarly effective colonisers. However, to distinguish further between them would require full scale field trials which would be prohibitively expensive, not to mention problems in identifying appropriate sites. It is also evident that within the current collection there are additional *Trichoderma* isolates which would be worth pursuing if the aforementioned constraints could be overcome.

The observation that the *Trichoderma* isolates are able to colonise and persist to flower stage, and hopefully beyond, does open the possibility that a single application each season may provide season long protection. These endophytes may then provide protection against pathogens and/or pests. Ideally, one application of an endophytic biocontrol agent would endow the host plant with life-long protection against pest and diseases, once established.

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REFERENCES

Evans, H.C., Holmes, K.A. and Thomas, S.E. (2003). Endophytes and mycoparasites associated with an indigenous forest tree, *Theobroma gileri*, in Ecuador and a preliminary assessment of their potential as biocontrol agents of cocoa diseases. *Mycological Progress 2 (2)*, 149-160.

Holmes, K.A., Schroers, H-J, Thomas, S.E., Evans, H.C. and Samuels, G. J. (2004.) Taxonomy and biocontrol potential of a new species of *Trichoderma* from the Amazon basin of South America*. Mycological Progress,* 3(2): 199-210.

Holmes, K.A., Krauss, U., Samuels, G., Bateman, R.P., Thomas, S.E., Crozier, J., Hidalgo, E., García, J., Arroyo, C., Bekker, M and Evans, H.C. (2006) *Trichoderma ovalisporum*: a potential biocontrol agent of frosty pod rot (*Moniliophthora roreri)*. 15th Cocoa Producers Alliance International Cocoa Research Conference, San Jose, Costa Rica, 8th – 13th October 2006 (in press)

Samuels, G.J., Suarez, C., Solis, K., Holmes, K.A., Thomas, S.E., Ismaiel, A., Evans, H.C. (2006) *Trichoderma theobromicola* and *T. paucisporum:* two new species from South America. Mycological Research, Mycological Research, vol. 110. 381-392.

2.3. *Bacillus* spp. as biological control agents of cacao disease

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SUMMARY

The ability of endospore-forming Bacillus spp. from vegetable crops to colonize cacao leaves and to reduce disease severity of black pod rot in a detached leaf assay was evaluated. Bacillus cereus isolates BT8 and BP24 colonized cacao leaves for the duration of the experiment (68 days) when applied to foliage with the polysilicon adjuvant Silwet L-77. Colonization with BT8 was characterized by fluctuating ratios of epiphytes to endophytes and ratios of vegetative cells to endospores. Colonization with BP24 consisted primarily of epiphytes and vegetative cells during the experiment. Bacterial colonists did not travel systemically to colonize leaves that emerged after initial colonization. Colonization of cacao leaves with BT8 resulted in significantly reduced Phytophthora capsici disease severity in detached leaf assays from 32 to 68 days following initial colonization. Newly emerged leaves of colonized cacao plants exhibiting reduced disease despite lacking bacterial colonist support a concept of systemically induced resistance. The signal for induced resistance probably originates from colonized leaves, causing a defence response in both colonized and non-colonized leaves. These results indicate that Bacillus spp. could potentially be developed into biological control agents for cacao diseases.

INTRODUCTION

Endospore-forming bacteria as biological control agents

Endophytes are microbes that reside in the internal tissues of a plant without causing disease. Endophytes offer a wide range of benefits to plants, which have allowed them become an active area of biological control research utilized in annual, biannual, and perennial crops (Bargabus *et al.*, 2004; Kloepper *et al.*, 2004). Several commercial biological control agents act under the principal that endophytes isolated from one specific plant may have the ability to colonize a range of plants and reduce disease through activation of plant defense mechanisms. *Pseudomonas* sp. strain PsJN, a nonpathogenic onion endophyte, inhibited the growth of *Botrytis cinerea* in grapevine as well as promoted vine growth (Barka *et al.,* 2002). This phenomenon has been seen with a range of endophytes and plants, such as *Pseudomonas putida* 89B-27 and *Serratia marcescens* 90-166, which suppressed Cucumber Mosaic Virus in tomatoes and cucumbers (Raupach *et al.*, 1996) in addition to foliar anthracnose and fusarium wilt in cucumber (Liu *et al.*, 1995). The ability of endophytes to inhabit a range of hosts makes them excellent potential biological control agents since they can be commercially labeled for use on multiple crops.

Many biological control products consist of endospore-forming bacteria. Thick walled endospores are formed by some Gram-positive bacteria in response to stress. Endospores provide bacteria with resistance to environmental stresses and are long-term resting structures (10-9,000 years) (Nilsson and Resenber, 1990). Endospore-

forming bacteria, such as *Bacillus* spp., are excellent candidates for biological control agents of cacao diseases, as formulations consisting of endospores could readily survive the heat and humidity of cacao growing regions. Additionally, expensive mycoharvesters are not needed to ferment the bacterial suspension; therefore, farming unions could produce the biological control product.

Biological control of cacao diseases

The increasing severities of disease problems in South America and the large numbers of consumers and farmers who are interested in ecologically based pest management have lead to an interest in biocontrol options for the management of cacao diseases. Arnold *et al.* (2003) evaluated cacao fungal endophytes for their ability to colonize leaves and reduce disease severity of *Phytophthora palmivora* on challenge plants. *Colletotrichum* spp. was the most successful leaf colonist as well as reduced lesion size on leaves challenged with mycelium of *Phytophthora* sp. Although *Colletotrichum* spp. had some success in reducing black rod rot, *Trichoderma* spp. has been the focus of more biocontrol research projects. In 2006, de Souza *et al*. investigated the genetic distribution of naturally occurring *T. stromaticum*, a mycoparasite of *Moniliophthora perniciosa*, in Bahia, Brazil. *T. stromaticum* strain TVC is produced as the biological control agent Trichovab by CEPLAC in Brazil. Naturally occurring strains of *T. stromaticum* were found to be genetically diverse and some strains were effective in suppressing witches' broom. The success of fungal endophytes has lead to investigations of bacterial endophytes, which have been effective in vegetables for their abilities to suppress diseases of cacao.

MATERIAL AND METHODS

Plant material and growth conditions

Theobroma cacao var. *commun* seeds were obtained from open pollinated trees on the Masterfoods plantation in Bahia, Brazil and shipped to the USDA-ARS Sustainable Perennial Crops Lab in Beltsville, MD. Seedlings were maintained in a greenhouse in a soil mix consisting of one part potting soil, two parts sand, and two parts perlite. During colonization studies, plants were maintained in an environmental room (Conviron, Winnipeg, Canada). The growth room was maintained at 28^{o}C with 12-hours light at 55% RH and 12-hours dark at 75% RH. Daytime light was provided by high intensity discharge lamps at 300 µmol/m^{2}s PAR. Plants were irrigated with 1/10 strength Hoagland's solution to maintain adequate levels of soil moisture and nutrition.

***Theobroma cacao* var. *commun* leaf developmental stages**

Theobroma cacao var. *commun* leaf development was separated into four stages based upon morphological differences, as described in Bailey *et al*. 2005 (Table 1).

Table 1. Morphological differences in the different leaf stages

Leaf stage	Abbrev.	Description
Unexpanded leaves	UE	Leaves < 1 cm long with limited pigment
Young red leaves	YR	Leaves 5-10 cm long, flexuous, and semi-translucent
Immature green leaves	IG	Leaves 10-20 cm long, flexuous, and light green
Mature green leaves	MG	Leaves 10-20 cm long, rigid, and dark green

Bacterial isolates

Bacterial endophytes were routinely cultured on yeast-extract-dextrose-calcium-carbonate (YDC) Agar. *Bacillus cereus* isolate BP24 from potato and *Bacillus cereus* isolate BT8 from tomato plants were collected from plants sprayed with a colloidal chitin suspension in 1991 (D. Ploper and P. Backman, Auburn University, unpublished data). All cultures are stored in Tryptic Soy Broth (TSB) (Difco, Becton Dickinson and Company, Franklin Lakes, NJ) with 20% glycerol in liquid nitrogen.

Bacterial inoculum preparation and colonization

Bacillus spp. were grown in 500 ml of sterile TSB in 2.8 l Fernbach flasks inoculated with a loop of bacteria. Flasks were incubated on a rotary shaker (New Brunswick Scientific, Edison, NJ) at 28^{o}C and 150 rpm for 7 days. The concentration of the bacterial solution was adjusted to $1x10^{8}$ CFU ml^{-1}. Silwet L-77 (GE Silicons, Tarrytown, NY) was added to the bacterial solutions so that it was 0.24% the total volume. The solution was sprayed onto appropriate plants using hand-held aerosol sprayer (Crown power pack, Aerovoe Pacific, Gardenerville, NV) until leaves were wetted. Control leaves were sprayed with a solution of 0.24% Silwet L-77 in sterile 0.1M phosphate buffer. Immediately following spraying, leaves were evaluated on a light box to verify infiltration of solution into substomatal cavities, which were visible as translucent areas of internal leaf tissues. Trees were kept with the same treatment until leaves dried; then placed into a randomized block design in the environmental growth room.

Pathogen isolates and inoculum preparation

Phytophthora spp. were collected from infected trees by H. Purdy (Univ. of Florida), shipped to The Pennsylvania State University, and stored in 20% glycerol in liquid nitrogen. Cultures were removed from storage and routinely cultured on unclarified V8 agar. Strains were tested for their ability to incite infection on detached leaves and aggressiveness. *P. capsici* isolate 73-73 from Ecuador was used for the detached leaf assay, and periodically isolated from infected cacao leaves to maintain virulence. Inoculum for the detached leaf bioassay was prepared with one-week-old isolates grown on unclarified V8 agar. Zoospores were induced and released following the Lawrence (1978) protocol. Zoospore concentration was adjusted to $5x10^{3}$ zoospores ml^{-1} (50 zoospores per 10 µl drop).

Determination of bacterial colonization

Levels of bacterial colonization were determined at weekly intervals for three trees per treatment. Two MG leaves were removed from the top of each tree. One leaf was washed for four hours under running water to remove epiphytic colonization, while the other remained unwashed. Five 1.73 cm diameter leaf discs (2.35 cm^{2}) were removed from the unwashed leaf in a "W" pattern using a size 10 cork borer. Leaf discs were placed with into a stomacher bag with 3 ml of sterile 0.1 M phosphate buffer. Leaf tissue was agitated by placing the bag into a stomacher blender at 100 oscillations min^{-1} for 30 seconds, with the paddles pushed back to achieve agitation while preventing tissue disruption. Fifty µl of the supernatant was plated in triplicate in the exponential mode onto YED agar using a spiral plater (Interscience, Markham, Ontario). The remaining supernatant was heated at 80^{o}C for 15 minutes in a water bath to destroy all vegetative cells. Once cooled, 50 µl of the supernatant was plated as previously described. The leaf discs were removed from the stomacher bag, placed into filter bags with 3 ml of fresh 0.1 M phosphate buffer. Leaf tissue was triturated

at 100 oscillations min^{-1} for 30 seconds in a stomacher blender with the paddles pushed forward followed by spiral plating the supernatant as previously stated. The supernatant was heated and plated as previously mentioned. The procedure was repeated for leaves that were washed for four hours to remove epiphytic growth, and counts were thus expected to reflect endophytic populations. Bacterial plates were incubated and enumerated. Two leaves were removed from the middle of the same trees to ensure uniform distribution bacterial colonists across the tree and the aforementioned procedure was repeated.

Pathogen challenge to determine disease suppression

Plants were sampled for disease suppression by challenging 100 mm leaf discs with *P. capsici* zoospores. Immature green (IG) leaves were used for the detached leaf assay, as they are the susceptible stage. IG leaves were detached from plants and washed as in the colonization experiment. The lid of a 100 mm Petri dish was used to cut leaf discs out of leaves with the midrib in the center of the disc. Discs were placed adaxial side up on a moist 10 cm Whatman #2 filter paper in the lid of a Petri dish. Four 10 µl drops of the zoospore suspension were placed to the left of the midrib, while four 10 µl drops of sterile distilled water were placed to the right of the midrib to serve as a control. Petri dishes were sealed with parafilm and incubated at 28°C with a 12-hour light-dark cycle. Leaves were rated every 6-12 hours for approximately 52 hours after inoculation by evaluating the lesion diameter, percent necrosis of the lesion, and disease severity ratings. Initial infections began as small specks of necrosis, which eventually coalesced with neighboring spots to form confluent necrosis on the leaf. Disease progress curves were created using disease severity measurements and the area under the disease progress curve (AUDPC) was determined (Shanner & Finney 1977). Data was statistically analyzed for significance using ANOVA analysis followed by a Tukey test using the SAS program (SAS Institute Inc., Raleigh, NC). A 95% confidence level (P=0.05) was used for all analyses.

RESULTS

Colonization of cacao leaf tissue with *Bacillus* spp.

Total colonization (Fig. 1) lasted the duration of the experiment (68 days). Little variation in population levels occurred between replicates. Although total colonization dropped less than a log during the course the 9-week experiment, there were fluctuating ratios of epiphytes to endophytes and vegetative cells to endospores.

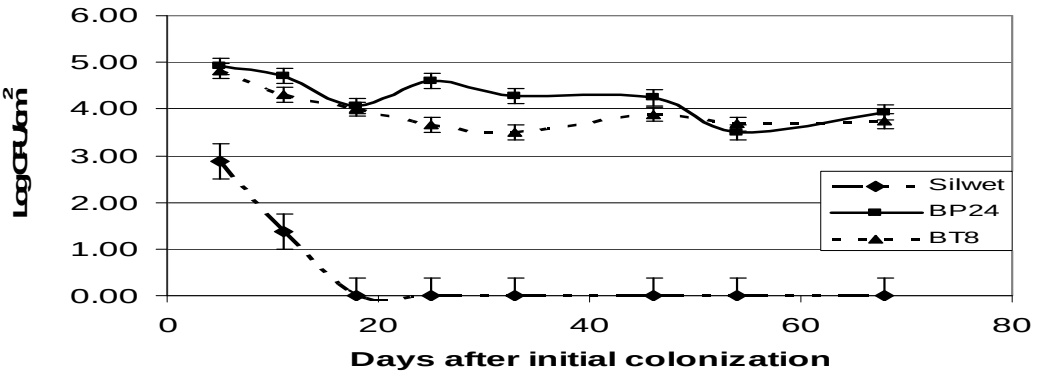

Figure 1 – Mean total colonization of *T. cacao* var. commun leaves sprayed with *B. cereus* isolates BT8 and BP24. Silwet represents leaves sprayed with the Silwet L-77 formulation. Leaves on Silwet control plants had low levels of bacterial colonist due to cross

contamination from treated plants while in the randomized block design. Initial colonization of cacao occurred at day 0. Bars extending around the means represent the standard error of the mean.

BT8 showed a slow decline in epiphytic populations, with endophytes usually representing less than 10% of total population. BP24 had a similar slow decline in epiphytes, with no measurable endophytic population at 25 or 54 days after colonization. With the exception of these two dates, endophytes appeared to hold near levels observed for BT8. The number of vegetative cells and endospores of BT8 colonized MG leaves remained stable at approximately 3.6 log CFU/cm^2 over the 68 days of the experiment. There was no detectable level of BP24 endospores 18 days after initial colonization until the termination of the experiment.

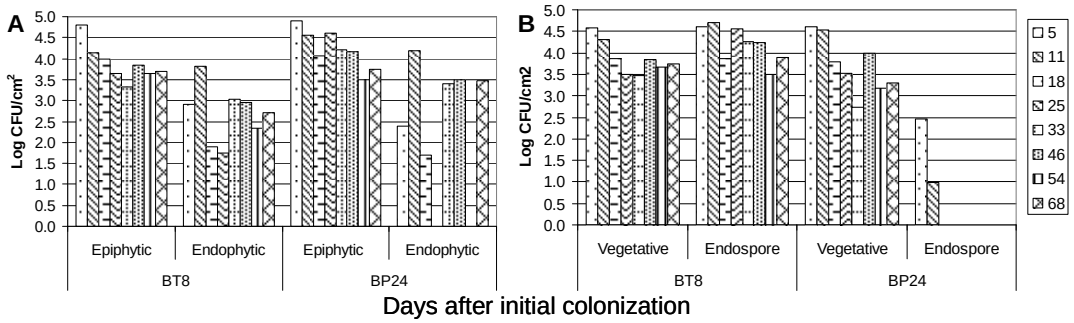

Figure 2: (a) Mean distribution of *B. cereus* isolate BT8 and *B. cereus* isolate BP24 colonizing mature green cacao leaves. (b) Cell stages of *B. cereus* isolate BT8 and *B. cereus* isolate BP24 colonizing mature green cacao leaves. Initial colonization of cacao leaves occurred at day 0 with colonization measured at approximately 5-7 day intervals until 70 days after initial colonization.

Disease suppression due to bacterial colonization

Colonization of cacao leaves with BT8 suppressed black pod rot in detached leaf assays. No significant differences were seen in disease severity until 32 days after initial colonization. Washed and unwashed leaves from BT8 treated plants had significantly lowered disease severity ($P = 0.0151$) at 32 days after initial colonization. Disease suppression occurring in washed as well as unwashed leaves indicated that epiphytic colonists were not essential to disease suppression. Endophytic colonists are most likely required for disease suppression. Similar disease suppression results were obtained at 46, 52, and 68 days following initial colonization, demonstrating that BT8 suppressed disease in the detached leaf assay for extended periods of time. IG leaves challenged 32 to 68 days after initial colonization were formed after colonization, therefore lacked bacterial colonists, but were produced on plants with colonized mature green leaves, indicating a probable plant defense response.

DISCUSSION

Colonization of cacao leaf tissue with *Bacillus* spp.

Research was conducted to provide a detailed look at the distribution of bacteria (epiphyte vs. endophyte) in colonized cacao leaves and the stage of the bacterial colonists (vegetative cells vs. endospores) present. The short-term colonization of control plants was likely due to bacterial transfer from sprayed leaves after plants were placed into a random block design, as plants were contained in plastic tents

during spraying with the bacterial solution. Redistribution of bacteria probably occurred when epiphytic colonists from treated plants dripped onto the leaves of control plants due to the high humidity of the environmental chamber.

The fluctuation between epiphytic and endophytic populations and vegetative cells and endospores is possibly a response to stress in the form of inducible plant defense products. Although Silwet L-77 provided substomatal infiltration, colonization was not strictly endophytic in nature. Colonization consisted of both epiphytes and endophytes. Colonization with BT8 consisted of endospores present throughout colonization, while BP24 colonized leaves lacked endospores 18 – 68 days after initial colonization. BP24 may have endophytically colonized leaf tissue without adequately activating plant defense mechanisms; therefore, no plant stresses were present in BP24 colonized plants to incite the production of endospores. The endospores seen at 6 and 11 days after colonization were possibly due to Silwet L-77 activating plant defense mechanisms, as Silwet is know to have short-term activation of plant defenses (Jetiyanon 1994). The stable total colonization of BT8 and BP24 either resulted from germinating endospores maintaining the long-term stable populations in the leaves or the epiphytic populations serve as sources for endophytic populations. These results raise the possibility that plant defense products may not only induce disease suppression, but may also affect the nature of the endophytic colonization by biological control agents.

Disease suppression due to bacterial colonization

Long-term colonization of cacao leaves with BT8 had a long-term effect on disease suppression, while colonization with BP24 did not affect disease development. Even though BP24 was capable of long-term colonization of cacao leaves, it probably did so without activating plant defense mechanisms. The disease suppression incited by BT8 was a unique response. Disease suppression occurred in both washed and unwashed leaves indicating that the epiphytic colonist did not affect disease suppression. BT8 and BP24 did not have antagonistic effects on *P. capsici* mycelium (unpublished data); therefore, the bacteria themselves did not likely parasitize the pathogen. The aforementioned results indicate that the endophytic population likely affected disease suppression. As endophytes were the only colonist to impact disease, disease suppression was likely a response to plant defense mechanisms. Endophytes are known to activate plant defense mechanisms and bring about positive disease suppression in a range of pathosystems (Kloepper *et al*. 2004). Even though a disease response occurred, it likely took time before BT8 activated plant defense mechanisms to suppressive levels.

BT8 colonized cacao leaves for 4 weeks before disease suppression was evident. Once disease suppression occurred, it was a durable response as BT8 colonization reduced disease from 32 days after initial colonization until the termination of the experiment (68 days). Although colonization was durable, the onset of disease suppression took 7-10 days longer than in previous cacao colonization experiments (unpublished). The increased time for disease suppression may not have been a failure of the bacteria to activate plant defense mechanisms, but may reflect unknown environmental differences in the experimental methods. The time from detachment until pathogen challenge increased, as leaves where challenged with zoospores approximately 45 minutes after removal from the plant in previous experiments. The increased time before challenge with *P. capsici* may have allowed for the activation of

plant wounding pathways, which would have uniformly increased the levels of plant defense products. The sampling procedure could also have masked the benefits of bacterial colonization.

CONCLUSIONS ON *BACILLUS* SPP. AS BIOLOGICAL CONTROL AGENTS FOR CACAO DISEASES AND FUTURE RESEARCH

Bacillus cereus isolate BT8 (from tomato) and *Bacillus cereus* isolate BP24 (from potato) were effective long-term colonists of cacao leaf tissue, indicating that *Bacillus* spp. from vegetable crops could act as biological control agents against cacao diseases. Endospore-forming bacteria isolated from cacao trees may provide better colonization and disease suppression, as they are naturally adapted to the environment present within the cacao plant tissues. Natural colonists of cacao may be better suited for long-term colonization and may activate plant defense mechanisms more rapidly than BT8. Natural cacao endophytes from pods may be more likely to colonize pods than non-native bacteria. Since one of the main economic impacts of cacao diseases is damage to the pod, protection of the pod is a key element in obtaining economic returns. An additional benefit of finding natural cacao endophytes is that they are native to the region where they would be applied, reducing regulatory and environmental problems associated with the introduction of non-native microbes.

An additional area that requires more attention is the verification that disease suppression occurs in response to plant defense mechanisms, or alternatively due to antagonistic products produced by the added bacteria Molecular studies should be conducted to determine which defense pathways are turned on by the presence of the bacteria as well as when peak activation occurs. An understanding of defense mechanisms may provide further support that bacterial endophytes induce broad-spectrum disease suppression, therefore may be effective against several cacao diseases. Overall, colonization of cacao leaves with BT8 activates long-term disease suppression in cacao when applied to foliage with the polysilicon adjuvant Silwet L-77. In conclusion, this research demonstrates that *Bacillus* spp. have potential as biological control agents of cacao diseases.

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REFERENCES

Arnold, A.E., Mejia, L.C., Kyllo, D., Rojas, E.I., Maynard, Z., Robbins, N., and Herre, E.A. (2003). Fungal endophytes limit pathogen damage in a tropical tree. Proceeding of the National Academy of Sciences 100 (26):15649-15654.

Bailey, B.A., Bae, H., Strem, M.D., Antúnez de Mayolo, G., Guiltinan, M.J., Verica, J.A., Maximova, S.N., Bowers, J.H. (2005). Developmental expression of stress response genes in *Theobroma cacao* leaves and their response to Nep1 treatment and a compatible infection by *Phytophthora megakarya*. Plant Physiology and Biochemistry 43:611-622.

Bargabus, R.L., Zidack, N.J., Sherwood, J.E., and Jacobsen, B.J. (2004). Host Response-Based Screening for the Identification of Potential Biological Control Agents. Biological Control. 30: 342-350.

Barka, E.A., Gognies, S., Nowak, J., Audran, J.-C., and Belarbi, A. (2002). Inhibitory effect of endophytic bacteria on *Botrytis cinerea* and its influence to promote the grapevine growth. Biological Control 24:135-142.

de Souza, J.T., Pomella, A.W.V., Bowers, J.H., Pirovani, C.P., Loguercio, L.L., and Hebbar, K.P. (2006). Genetic and biological diversity of *Trichoderma stromaticum*, a mycoparasite of the cacao witches'-broom pathogen. Phytopathology 96:61-67.

Jetiyanon, K. (1994). Immunization of cabbage for long-term resistance to black rot. M.S., Plant Pathology, Auburn University, Auburn, Alabama.

Kloepper, J.W., Ryu, C.M., and Zhang, S. (2004). Induced systemic resistance and promotion of plant growth by Bacillus spp. Phytopathology 94:1259-1266.

Lawrence, J.S. (1978). Evaluation of methods for assessing resistance of cocoa (*Theobroma cacao* L.) cultivars and hybrids to *Phytophthora palmivora* (Butler) Butler. Bolletin tecnico 62 (Com. Exec do Piano da Lavoura Cacaucira, Brazil):47 pp.

Liu, L., Kloepper, W., and Tuzun, S. (1995). Induction of systemic resistance in cucumber against Fusarium wilt by plant growth-promoting Rhizobacteria. Phytopathology 85:695-698.

Nilsson, M., and Renberg, I. (1990). Viable endospores of Thermoactinomyces vulgaris in lake sediments as indicators of agricultural history. Applied Environmental Microbiology 56 (7):2025-2028.

Raupach, G.S., Liu, L., Murphy, J.F., Tuzun, S., and Kloepper, J.W. (1996). Induced systemic resistance in cucumber and tomato against cucumber mosaic cucumovirus using plant growth-promoting rhizobacteria (PGPR). Plant Disease 80 (8):891-894.

Shanner, G., and Finney, R.E. (1977). The effect of nitrogen fertilization on the expression of slow mildewing resistance in Knox wheat. Phytopathology 67:1051-1056.

2.4. Genetic population structure of *Clonostachys byssicola* and genetic disease resistance in cocoa (*Theobroma cacao* L.).

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SUMMARY

A previous study has shown that the genetic disease reaction of cocoa (Theobroma cacao) towards Phytophthora palmivora and Moniliophthora roreri, the two most important cocoa pod diseases in Central America, and mycoparasite abundance on cocoa flowers and pods, are independent phenomena. The most commonly isolated native mycoparasites encountered in that study belonged to the genus Clonostachys. The purpose of this study was to look in more detail at the relationship between Clonostachys and cocoa genotypes and to determine whether resistance to M. roreri and P. palmivora is mediated by genetically distinct populations of C. byssicola. A total of 62 isolates of Clonostachys were used in this study: 58 isolates of C. byssicola, originating from cocoa hybrids with differential genetic disease reactions towards M. roreri and P. palmivora, two C. rosea isolates, one C. cf. byssicola isolate from the rhizosphere of cocoa and one C. byssicola isolate from cocoa from a different agro-ecological production area. Molecular analysis using ISSR primers was undertaken to study the genetic variation between the C. byssicola isolates. Results show that the 58 isolates could be divided into five groups. No relationship was found between the genetic composition of C. byssicola isolates/groups and the cocoa genotypes with differential genetic disease reactions they originated from. These results strengthen previous findings that mycoparasite composition and genetic disease reaction on cocoa are independent and control strategies such as biological and genetic control should lead to additive effects.

INTRODUCTION

A previous study by Ten Hoopen *et al*. (2003) has shown that the abundance and composition of native, epiphytic mycoparasites did not differ between the three main cocoa cultivars, 'Criollo', 'Forastero' and 'Trinitario', nor between clones within this group. Different susceptibility classes of segregating F1 populations of hybrids with resistance against moniliasis, caused by *Moniliophthora roreri*, and black pod, caused by *Phytophthora* spp., also supported similar population levels and taxonomic assemblages of mycoparasites. *Clonostachys byssicola* was the most commonly isolated mycoparasite. On the basis of their results the authors concluded that mycoparasite abundance and genetic disease resistance to black pod and moniliasis are independent phenomena.

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However, it has been hypothesized that certain plant species and in particular perennial plant species may selectively enrich microbial communities with organisms that provide a certain benefit, such as disease protection. Most studies on this subject have taken place in the rhizosphere (Garbeva *et al*., 2004). Briones *et al*. (2002) compared the diversity and activity of ammonia-oxidizing bacteria in the rhizosphere of different cultivars of rice and found that gross nitrification rates were significantly higher in a modern rice variety than in two traditional varieties. Population levels of these ammonia-oxidizing bacteria also varied between the modern and the two traditional varieties. Berg *et al*. (2001) showed that the abundance, taxonomic composition and diversity of bacteria antagonistic to plant pathogens, isolates originating from the rhizosphere of three host plants of Verticillium dahliae, strawberry, potato and oilseed rape, were plant species dependent. The proportion of antagonistic bacteria was highest for the strawberry rhizosphere (9.5%), followed by oilseed rape (6.3%), potato (3.7%), and bulk soil (3.3%). Thus, plants can affect the microbial communities present in their rhizosphere in a functional way. Davelos et al. (2002) found that the rhizosphere of different prairie plant species differed with respect to the density of antibiotic-producing Streptomycetes. In addition they found that Streptomycete communities from the rhizosphere of different plant species varied significantly in their inhibitory activity. Streptomycetes from different plant species also varied in their inhibitory activity against different soil-borne pathogens (Verticillium, Fusarium, Streptomyces, and Rhizoctonia). Work on aerial plant parts to support or refute this theory is greatly lacking.

The objective of this study was to determine whether a relationship exists between the genetic make-up of *Clonostachys byssicola* isolates found on flowers and pods, and the genetic resistance of segregating F1 populations of hybrids with resistance to *M. roreri* and *P. palmivora*.

MATERIALS AND METHODS

A total of 10 *Clonostachys* isolates were collected from a segregating population (F1-generation) of the hybrid 'Catongo x Pound 12' in La Montaña Turrialba. Individual trees were identified as susceptible, intermediate or resistant to *Phytophthora palmivora* (Lainez Mejia, 1991). Another 47 Clonostachys isolates were isolated from a segregating population (F1-generation) of the following hybrids: UF-712 x Catie 1000; CC137 x UF273; UF712 x CCN 51; UF 712 x CC137; CCN 51 x CC137 and ICS 95 x CCN 51, in La Montaña, Turrialba. Individual trees were classified as susceptible, moderately susceptible, moderately resistant or resistant to *Moniliophthora roreri* (W. Phillips-Mora, unpublished results). Based on morphology, all 57 *Clonostachys* isolates were identified as *C. byssicola.*

In addition to the 57 *C. byssicola* isolates from cocoa, five other strains of *Clonostachys* were included for molecular analysis; two strains of *C. byssicola* from different geographic locations, one *Clonostachys* cf. *byssicola* isolated from the rhizosphere of a cocoa plant in Cabiria, Turrialba, and two strains of *Clonostachys rosea*. The fungi were grown in universal tubes with 10 ml of GYM liquid media for four days at 25 °C, at 150 rpm in an orbital incubator. The cultures were collected by filtration. The mycelium was subsequently freeze-dried for 24 hours. DNA was extracted by grinding the freeze-dried mycelium to a fine powder, under liquid nitrogen, using pestle and mortar. Around 20 mg of ground mycelium was used for

DNA extraction using the Qiagen DNeasy® Plant Mini kit following the manufacturer's instructions.

Inter simple sequence repeat polymerase chain reaction (ISSR-PCR) was performed using five primers; ISSR-ACA, ISSR-CCA, ISSR-TGT (Sigma) and ISSR-UBC888 and ISSR-UBC890 (University of British Colombia). PCR reactions were carried out in 20 µl volumes containing 0.5 µl primer, 200 µm of each dNTP, 1 unit of *Tth* DNA polymerase and 5 ng of template DNA. Amplification products were separated on a 1.5% agarose gel in 0.5x TBE buffer and stained with ethidium bromide.

Gel images were examined and analysed using GEL COMPAR II. A binary matrix of the presence or absence of bands was created. This matrix was subsequently analysed using principal co-ordinate analysis based on standardized Manhattan distance using Infostat (Infostat, 2004).

RESULTS

A total of five primers gave rise to a total of 202 polymorphic loci which were used in a principal coordinate analysis (PCA). The results of the principal coordinate analysis of the ISSR study are shown in Fig. 1. Genetic distances between isolates ranged from 0.1 to 1.23 (Manhattan, Infostat). The first three axes (principal coordinates=PCs) from the principal coordinate analysis accounted for 38.3% of the variation (PC1=15.9%, PC2=12%, PC3=10.4%, respectively). The first axis (PC1) divides the isolates into two groups: Group A: Isolates belonging to genetic clusters I, III and IV and group B: Isolates belonging to genetic cluster II, while genetic cluster V has affinity with both groups. The second axis divides the isolates into another two groups: Group C: Isolates belonging to genetic clusters II and V and Group D: Isolates belonging to genetic clusters III and IV, while genetic cluster II has affinity with both groups. When looking at the graphs of principal coordinates 2 and 3 and principal coordinates 1 and 3 (results not shown), it is clear that genetic clusters I, II, III and IV have great affinity and that the only genetic cluster that is distinctly different is cluster V, which contains the two isolates of *C. rosea,* three closely related isolates from three different trees (two trees susceptible to *M. roreri* and one tree susceptible to *Phytophthora*) and the *C.* cf. *byssicola* from the rhizosphere of cocoa. Cluster I, the largest, contains *C. byssicola* isolates from 15 trees off all four classes of susceptibility towards *M. roreri* as well as from all three of the *P. palmivora* susceptibility classes. Cluster II consists of 18 isolates from seven different trees: two susceptible trees to *M. roreri*, one moderately susceptible tree, one resistant tree and three isolates from the hybrid 'Catongo x Pound 12' (one intermediately susceptible and two trees resistant to *Phytophthora*). Cluster III contains five isolates from three different trees, all with differential genetic reaction to *M. roreri* (resistant, moderately resistant and susceptible). Cluster IV contains seven isolates, all from the same tree (resistant to *M. roreri*). The only isolate with no clear affinity was isolate number 40 which has a different geographic origin (isolate from Ecuador).

DISCUSSION

Using only five primers, a total of 202 polymorphic loci were obtained which indicates a high level of genetic diversity within *C. byssicola,* especially when considering the small geographic origin of most isolates (La Montaña, Turrialba, Costa Rica). However, the high number can partially be explained by the relatively low annealing temperature used in the PCRs. As no previous knowledge on genetic

variability was available, low annealing temperatures were used to ensure sufficient bands for analysis. In retrospect this may not have been necessary.

Clonostachys byssicola AMR56 (nr 75 in the ISSR study, Fig. 1) is closely related to the ex-epitype strain of *Bionectria byssicola*. However, previous investigations have shown the existence of at least two distinct clades (based on partial sequence data from the beta-tubulin gene) within *C. byssicola* isolates from Costa Rica (H.-J. Schroers, pers. comm.). The existence of at least two clades could explain why we find two large genetic clusters (I and II). The *Clonostachys* cf. *byssicola* isolate ARB37 (nr 20 in the ISSR study, Fig. 1) is morphologically indistinguishable from *C. byssicola,* although sequence data (not presented) claims the isolate as unrelated to *C. byssicola*.

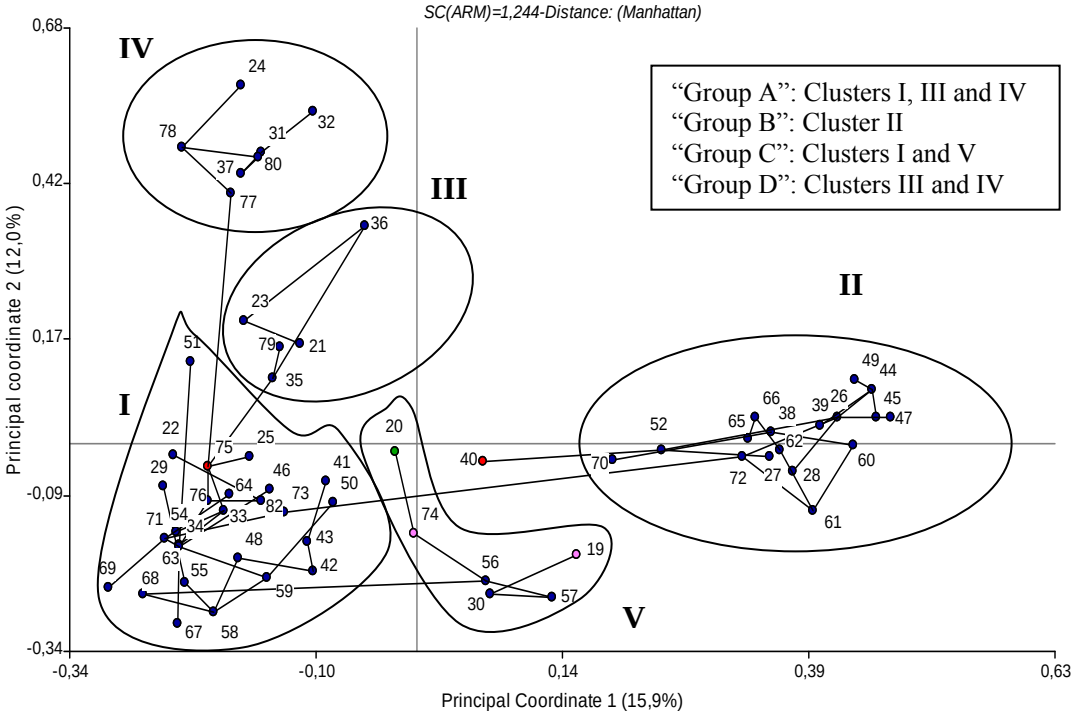

Fig. 1: Principle coordinates analysis (PCA) and minimum spanning tree of the ISSR study using 202 bands of 62 isolates of *Clonostachys.* Numbers 19 and 74 (purple) belong to the two *C. rosea* isolates, number 20 (green) is the *C.* cf. *byssicola* isolate from the rhizosphere, numbers 40 and 75 (red) are the two *C. byssicola* isolates with different geographical origin

Based on the sequence data, this isolate would belong to an undescribed species (H-J Schroers, pers. comm.). Isolate ABR37 seems closely related to the two isolates used in this study that were identified as *C. rosea* (CTR71-18 and GJS95-7, ISSRs nrs. 74 and 19 respectively, Fig. 1). It seems clear that the correct identification of *Clonostachys* spp. solely on morphological characteristics is difficult at best. If more than one species of *Clonostachys* was present in this study (next to the two *C. rosea's* and the *C.* cf. *byssicola*) this could provide an alternative explanation for the high level of genetic variation within this population.

Due to self-incompatibility, cocoa is usually grown as a mixture of different clones and/or hybrids (Mossu, 1990). For practical purposes, it is desirable that a single biocontrol inoculum is suitable for all genotypes grown in one field. Based on the results by Ten Hoopen *et al*. (2003) this seems to be a realistic goal. However, Davelos *et al*. (2002) found that *Streptomycetes* communities from the rhizosphere of different plant species varied significantly in their inhibitory activity as measured against a collection of ten standard *Streptomycete* test isolates. These test isolates were genetically distinct (Davelos *et al.,* 2004). Saratchandra *et al*. (1993) isolated fluorescent *Pseudomonas* spp. from 62 root, leaf and fruit samples covering 24 horticultural crops and 8 pastoral soil samples. These were tested in vitro for their ability to inhibit the growth of 5 pathogenic fungi (*Botrytis cinerea*, *Fusarium oxysporum* f.sp. *lycopersici*, *Phytophthora nicotianae* var. *nicotianae*, *Pythium debaryanum* and *Pythium ultimum*). They found that the isolates from the rhizosphere of beetroot, lettuce, loquat, pasture, strawberry and the fructoplane of lemon had the greatest percentage of antifungal bacteria and that there were differences between plant species in the percentage of antifungal *Pseudomonas* isolates showing antifungal activity against *P. ultimum*, *P. debaryanum*, *P. nicotianae* and *B. cinerea*. It is therefore, conceivable that resistant varieties do not support higher numbers of mycoparasites but maintain genetically distinct populations of mycoparasites through which resistance is mediated. However, results presented here indicate that genetic disease reaction in cocoa and genetic make-up of the *C. byssicola* populations present on cocoa pods and flowers are independent phenomena, strengthening our previous believe that biocontrol and genetic disease resistance are independent phenomena and should lead to additive effects.

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REFERENCES

Berg, G., Roskot, N., Steidle, A., Eberl, L., Zock, A., and Smalla, K. 2001. Plant-dependent genotypic and phenotypic diversity of antagonistic rhizobacteria isolated from *Verticillium* host plant. Appl. and Environ. Microbiol. 68: 3328-3338.

Briones, A.M., Okabe, S., Umemiya, Y., Ramsing, N.B., Reichardt, W., and Okuyama, H. 2002. Influence of different cultivars on populations of ammonia-oxidizing bacteria in the root environment of rice. Appl. Environ. Microbiol. 68: 3067-3075

Davelos, A., Kinkel, L.L., Xiao, K., and Rodgers, M. 2002. Do some prairie plants enhance soil microbial antagonists to protect against disease? Poster presented at the 87th meeting of the Ecological Society of America, Tucson, Arizona, (abstract only).

Davelos, A.L., Xiao, K., Samac, D.A., Martin, A.P., and Kinkel, L.L. 2004. Spatial pattern in *Streptomyces* genetic composition and diversity in a prairie soil. Microbial Ecol. 48: 601-612.

Garbeva, P., Van Veen, J.A. and Elsas, J.D. 2004. Microbial diversity in soil: selection of microbial populations by plant and soil type and implications for disease suppressiveness. Ann. Rev. Phytopathol. 42: 243-270.

Infostat, 2004. Infostat, version 2004. Grupo Infostat, FCA, Universidad Nacional de Córdoba, Argentina.

Lainez Mejia, J.R. 1991. Estudio de la descendencia del cruce interclonal de cacao "Catongo × Pound 12" bajo condiciones de Turrialba, Costa Rica. M.Sc. Thesis, CATIE, Costa Rica, pp. 125.

Mossu, G. 1990. *Le Cacaoyer*. Maisonneuve et Larose, Paris, pp. 160.

Saratchandra, U., Duganzich, D., and Burch, G. 1993. Occurrence of antifungal fluorescent *Pseudomonas* spp. on some horticultural and pastoral plants. New Zealand J. Crop Horticultural Sci. 21: 267-272.

Ten Hoopen, G.M., Rees, R., Aisa, P., Stirrup, T., and Krauss, U. 2003. Population dynamics of epiphytic mycoparasites of the genera *Clonostachys* and *Fusarium* for the biocontrol of black pod (*Phytopthora palmivora*) and moniliasis (*Moniliophthora roreri*) on cocoa (*Theobroma cacao*). Mycological Research 107: 587-596.

2.5 Use of endophytes to protect cocoa seedlings against cocoa diseases[§§]

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SUMMARY

A large scale field trial has been planned in Ecuador with some of the most promising endophytes previously isolated from cocoa stems. While the selected hybrid seed is being prepared, some assays were performed in order to define best inoculation methods for seedlings. Pregerminated open pollinated seedlings of EET 19 (susceptible to both diseases) and SCA 12 (Tolerant to FPR and resistant to WB) were inoculated with Trichoderma koningiopsis, T. ovalisporum and four non identified Trichoderma species (214, 233g, 219b and 219f-CABI). Methods used were 20 min immersion; seedling spray and direct exposure of seeds to the fungus, leaving them in contact with each fungus on Petri plates for two days. A suspension of 1 x 10^6 spores or infection units for mL were used for each. Seedlings were then kept in cones with sterile substrate and irrigated weekly with sterile water. First harvests of seedlings were carried out five weeks after infection; each seedling was fragmented in 10 sections from collar to apex and placed on weak PDA. T. ovalisporum and T. koningiopsis were recovered from all stem pieces; 233g, 219b and 219f were recovered on 50-80% of the samples; the strain 214 now identified as T. paucisporum that has shown strong antagonistic effect to Moniliophthora roreri, was not recovered. Implications for a large scale experiment are discussed.

[§§] Full text of paper not available at press time

Discussion on IPM: Novel Methods

Chairperson: I.Y. Opoku, CRIG, Ghana

Q: Gary J. Samuels, USDA, USA

Do *Bacillus* endophytes present the possibility of self sustaining biological control?

A: Rachel Menlick, Penn State University, USA

Endophytic *Bacillus* sp. may be self-sustaining biological control agents. Endospore germinating can maintain the population levels especially after a period of plant stress, such as drought. Additionally, environmental dispersal such as rain dispersal can allow spread throughout. Further research will test the ability of self-sustainability of *Bacillus* sp.

Q: Gary J. Samuels, USDA, USA

How long does induced resistance that is observed at a distance from a point of inoculation last?

A: Rachel Menlick, Penn State University, USA

Bacillus sp are known to induce resistance in a wide range of plants. The fact that I have seen disease suppression in immature leaves with or without bacteria colonist that produced on peanuts with mature colonized leaves indicates that a signal is unraveling from mature leaves to protect unevenly emerged non-colonized leaves. In my studies, induced resistance was seen two months after colonization

Q: Martijn Ten Hoopen, CABI-CATIE, Costa Rica

Why did the Bacillus sp. that run-off from your treated plants onto your control plants do not colonize your control plants for a longer period of time as with your treated plants?

A: Rachel Menlick, Penn State University, USA

The Bacillus sp. colonization of the control plants was strictly epiphytic in nature. The low population levels coupled with the epiphytic nature of colonization prevented long term colonization of control plants.

Q: J. L. Pereira, CEPEC/CEPLAC, Brazil

What distinguishes an endophyte from an epiphyte?

A: Bryan Bailey, USDA, USA

Trichoderma endophytes are only similar in their epiphytic phase. I have not seen spores inside tissue. A single Trichoderma does not normally dominate a tree. I believe and the data supports that cacao endophytes ability covers a broad range from isolates with endophytic ability to very weak endophytes.

Q: H. Dzahini Obiatey, CRIG, Ghana

I did not quite follow the link between resistance biocontrol that you said were additive. Can you explain this further?

A: Martijn Ten Hoopen, CABI-CATIE, Costa Rica

Trees with differential genetic disease reactions towards *M. roreri* and *Phytophthora palmivora* maintain similar assemblages of potential control agents with respect to species composition, relative abundance and in the case of C. byssicola, their genetic composition. Trees thus can support a wide array of generalist biocontrol agents or their pods, irrespective of their resistance to diseases. This should lead to additive effects when applying biocontrol agents on disease resistant trees to control diseases.

Q: J. L. Pereira, CEPEC/CEPLAC, Brazil

What is the difference between endophytes and epiphytes other than their selective nitches?.

A: Bryan Bailey, USDA, USA

We do not currently know of any difference.

Q: J. L. Pereira, CEPEC/CEPLAC, Brazil

Is there any difference at all between an endophyte and epiphyte of the same species? Are endophytes better protected within the plant tissue as opposed to epiphytes?

A: Bryan Bailey, USDA, USA

Endophytes as biocontrol agents should be less susceptible to environmental fluctuation once internalized, as opposed to epiphytes which are impeded by environmental conditions.

Q: J. L. Pereira, CEPEC/CEPLAC, Brazil

Are endophytes more resistant to fungicides as opposed to epiphytes?

A: Bryan Bailey, USDA, USA

Studies in-vitro shows no difference between epiphytes and endophytes in the presence of copper and the systemic fungicide, flutolanil.

Q: Prakash Hebbar, Mars Inc. USA

Can we at some stage recommend the use of endophytes for improving cacao seedling health. It could either be Bacillus sp. mycorrhyzae, Trichoderma. We have no data on the effects of root rotting fungi, nematodes etc. on root health.

A: Keith Holmes, CABI Bioscience, Trinidad and Tobago.

We have some isolation that can be commercialized. It is possible that there are isolates in the rhizosphere component not endophytes which could protect seedlings.

Trichoderma are commercialized for promoting seedling growth and controlling seedling disease in other crop systems. It is possible that our isolates have similar potential. It might help in marketing a product.

Q: Pierre Tondje, IRAD, Cameroon

Are you aware of some effects of endophytes on pods and on some pathways that are involved in plant resistance to diseases in cacao?

A: Bryan Bailey, USDA, USA

Trichoderma alter gene expression in cacao. We do not have solid evidence that they penetrate the pod husk and alter seed development. It is possible that Trichoderma can cause system effects that influence seedling development.

3.1. Meeting Farmers' Demand: Production and Supply of Safe Planting Materials from *Phytophthora megakarya* Infected Cocoa Seed Gardens in Ghana.

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SUMMARY

Cocoa is the main commodity crop of Ghana and it is financially important for the livelihood of many rural communities. The recommendation is for cocoa farmers to establish new farms and rehabilitate old ones with improved hybrid seed materials from approved polyclonal seed gardens. The movement of infected planting materials in the form of cocoa pods and seedlings, plantain suckers, cocoyam corms and other materials associated with soil to uninfected areas has, however, been identified as means by which P. megakarya spreads. To reduce the spread, several measures including a complete ban on the use of pods from Phytophthora megakarya infected seed gardens at Bechem and Akomadan as planting material were put into place in the early 1990's. Presently, P. megakarya has been found in six other seed gardens (Jamasi, Goaso, Sankore, Akaa, Ampeyo, Buako). To meet the increasing farmers' demand for seed pods in the country, the policy on the complete ban was reviewed and precautionary measures to facilitate the distribution of hybrid planting materials without spreading the pathogen was instituted. The measures included production of hybrid pods outside the black pod season (October to March), the transportation of fungicide treated seeds and pods from seed gardens, and thorough washing of vehicles and soil associated materials before leaving the premises of the seed gardens. These practical measures have resulted in the production and supply of safe planting materials for farmers in the P. megakarya endemic seed pod producing gardens in Ghana. The impact of the measures on seed pod production and distribution for sustainable cocoa production in the country are also discussed.

INTRODUCTION

Cocoa is the main commodity crop of Ghana and it is financially important for the nation's economy, in terms of foreign exchange generation, domestic incomes for many rural communities and a major source of revenue for the provision of socio-economic infrastructure. It is currently estimated that 1,212,000 ha of land is cultivated to the crop with about 800,000 cocoa farm families i.e. 60% of the national agricultural labour force involved in its production (Appiah, 2004). The hub of production of the crop has moved from the Eastern region, where the crop was first introduced in 1879, through the Ashanti and Brong Ahafo Regions to the Western Region. The Western region currently has an estimated area of 502,161 ha, 42% of the total land under cocoa, and currently accounts for over 30% of the national output (Table 1). The region also has the largest proportion of new plantings (COCOBOD, 2005).. The relatively recent arrival of the more aggressive black pod pathogen, *Phytophthora megakarya,* coupled with the declining fertility status of soils after decades of cultivation and the endemic nature of the cocoa swollen shoot disease in

the other regions partly account for the movement of cocoa farmers from the Eastern, Ashanti and Brong Ahafo to the virgin forests of the Western Region.

Table 1. Regional distribution in ha of cocoa in Ghana.

Region	*Area (ha)*	*% of total*
Western	502,161	41.5
Ashanti	295,938	24.5
Eastern	174,832	14.5
Brong Ahafo	130,773	10.8
Central	81,029	6.7
Volta	24,630	2.0
Total	1,209,403	100

Source: Research Dept, Ghana COCOBOD

For sustainable cocoa production in Ghana, the use of improved planting materials has become the focus of research through several stages of varietal development (Table 2) (Edwin and Masters, 2005). This aims at providing farmers with improved materials with less gestation period and better resistance to diseases and pests. Earlier cocoa types introduced into Ghana in 1879 by Tetteh Quarshie mainly consisted of the Amelonado variety which needed a period of 6 to 8 years to come into pod bearing. The current Series II hybrids (the hybridisation between the Upper Amazons and selected Amelonado/Trinitario clones) set pods within 2 to 3 years and it is better resistant to some notable cocoa diseases and pests (Legg and Lockwood, 1981).

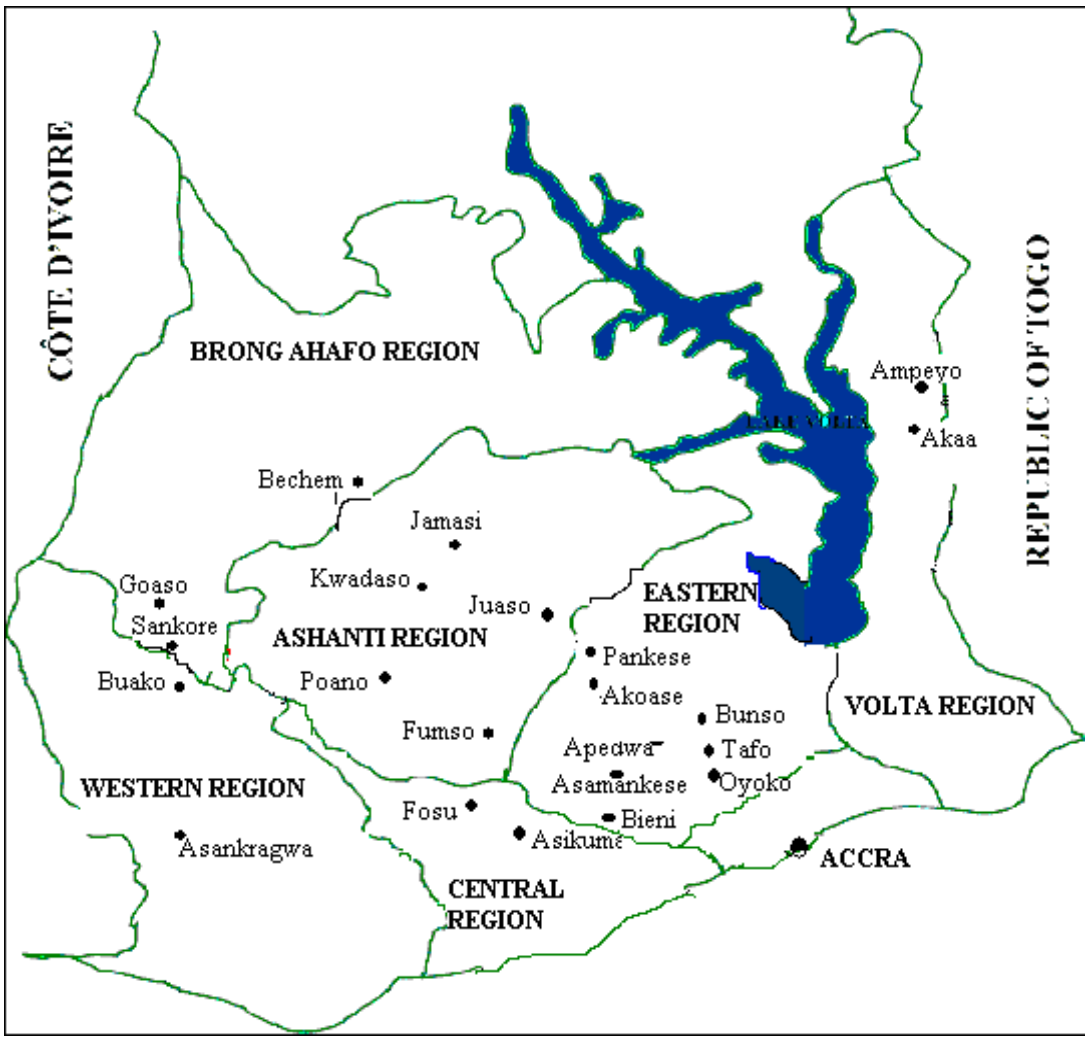
Table 2. Stages in cocoa varietal development programme in Ghana

Collections/Introductions	Source	Period of release	Years to bearing
"Traditional Varieties"			
Amelonado	Equatorial Guinea	before 1887	6-8
Trinitario	Trinidad, Jamaica, and Venezuela	1900-1909	6-8
Mixed Amazon	Peru via Trinidad	1950s	5-6
Upper Amazon x Amelonado and local Trinitario (originally series II Hybrids)	Peru and WACRI	1966-1970	4-6
Upper Amazon x Amelonado hybrids (Modified Series II hybrids)	WACRI	1971-1985	2-3
"New varieties"			
Inter-Amazon (BRT collection)	British Research Team (BRT)	Mid-1980s	2-3
Mutant hybrids (MV5)	Current CRIG collections	1990s	4

Source: Edwin and Masters (2005).

Following the production of these hybrids, bi-clonal seed gardens were established in the cocoa growing regions of the country (Fig.1) to mass produce improved seed planting materials relying on natural pollination in the 1960s. In 1969, a manual pollination technique was developed that allowed large scale production of high quality seed pods with a measure of control over seasonal periodicity of production (Edwards, 1973). Consequently, seed production has increased dramatically, and ccurrently, over 55% of planting materials in farmers' farms are Series II and Amazon Hybrids (COPAL, 2005).

Fig. 1. Map of Ghana showing locations of cocoa seed gardens



However, the production of planting materials in Ghana is hampered by diseases such as black pod, which is caused mainly by *Phytophthora palmivora* and *P. megakarya.* The soil is known to be the main site of survival as well as source of primary infection of the more virulent *P. megakarya* (Griffin *et al*., 1981). Therefore, the movement of soil or materials associated with soil, such as cocoa seedlings, plantain suckers, cocoyam corms, yam tubers (particularly those harvested from cocoa plantations) carry the danger of spreading the disease to new areas or farms. It is also suspected that the movement of vehicles from one cocoa farm to the other within and between districts helps to disseminate the pathogen. Movement of apparently healthy but infected cocoa pods is also considered as a source of infection to previously

uninfected areas. The possible spread of *P. megakarya* in or on planting materials led to a ban in 1991 on the supply of hybrid cocoa pods to farmers from seed gardens of the then Cocoa Services Division (CSD) in *P. megakarya* infected areas at Akomadan (Ashanti Region) and Bechem (Brong Ahafo Region). Presently, *P. megakarya* has been found in six other seed gardens at Jamasi (Ashanti), Goaso and Sankore (Brong Ahafo), Akaa, and Ampeyo (Volta) and Buako (Western) (Opoku *et al*., 1999), and several non-contiguous cocoa districts confirming that human factors are involved in the spread. As the pathogen continues to spread, it is only a matter of time that all the seed gardens in the country will be affected. There is, therefore, the need to develop strategies to produce and distribute healthy planting materials for farmers in the midst of *P. megakarya* to sustain cocoa production in the country. This paper presents a review and highlights of the precautionary measures put in place in a *P. megakarya* endemic seed pod garden in Ghana to facilitate production of disease-free cocoa seed pods for farmers.

POD PRODUCTION OUTSIDE THE BLACK POD SEASON

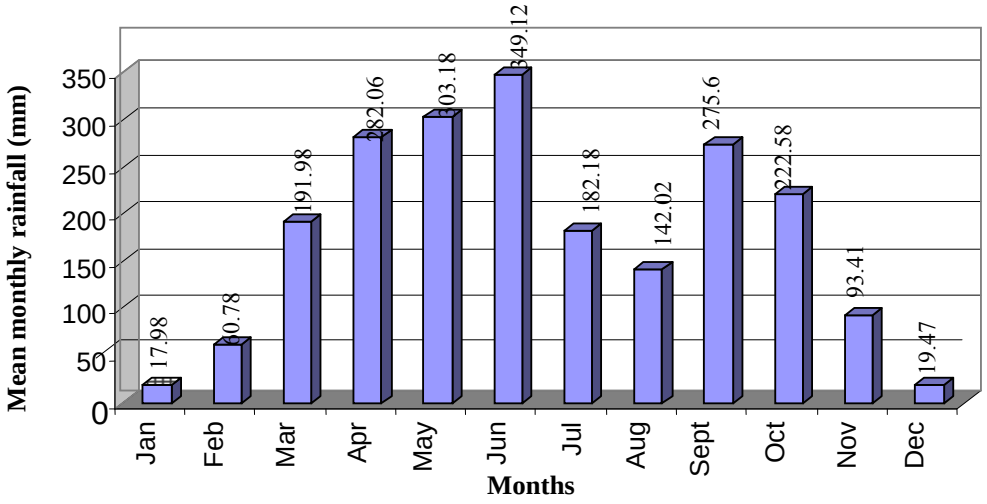
Cocoa pod development takes an average of five months from flowering to pod ripening. During this period, the pod is exposed to the vagaries of the weather and the biotic environment. Environmental factors including frequent rainfall, high humidity and low temperature favour black pod disease development (Maddison and Griffin, 1979). Recent observations from cocoa farms in Ghana also suggest that in *P. megakarya* areas, September to October is the most critical period for black pod infection and confirms earlier report that August to October is the peak period for *P. palmivora* infection (Dakwa, 1974). These months are associated with frequent rainfalls and coincide with abundant pods on trees, which serve as substrate for *Phytophthora* infection. Thereafter, both rainfall and the incidence of black pod disease declines until March. The 13-year mean rainfall at Bechem (Fig. 2) showed a typical bi-modal distribution with the peak of the major season in June, the minor in September and a relatively dry period between the months of November and March, with less than 100mm. This suggests that cocoa trees that produce the bulk of their pods between November and March would escape the effects of black pod infections.

At Bechem, following the review on the ban of production of seed pods for farmers in 2001, the cropping patterns of the established clones and the rainfall regimes were critically examined. Consequently, a programme to produce seed pods at a time when environmental conditions were less suitable for disease development was instituted. This involved two-weekly manual pollination schedules from November to February and rigorous farm sanitation, including regular weeding and pruning, judicious shade adjustment, frequent harvesting (2-weekly intervals) and removal of diseased pods at 2-3 weekly intervals to protect the developing pods from infection. With the five months period from flowering through pod set to ripening, apparently healthy seed pods were available from April to June for planting by farmers. The advantage with this policy is that farmers get planting materials at the onset of the main rain season which is suitable for seedling establishment.

PRECAUTIONS AT SEED GARDENS

In order to minimize pod loss from black pod at the peak of infection, harvesting is done frequently (at most at two weekly intervals) .All infected pods are removed to reduce sporulation and hence inoculium for subsequent infections.

Fig. 2 Thirteen-year (1990-2002) monthly mean rainfall (mm) at the Bechem Seed Garden, Bechem.



Management of the Seed Gardens ensures that harvested seed pods are gathered at least 500 metres away from the farm to prevent farmers from contaminating themselves with infected soil. All vehicles leaving the seed gardens are thoroughly washed to remove mud before entering non-*P. megakarya* areas. Root crops such as yams, cocoyams and cassava are also not permitted to be transported out of the seed gardens and *P. megakarya* farms. Farmers are periodically educated on the consequences of *P. megakarya* infections and how to prevent its spread.

PRECAUTIONS AT DISTRIBUTION CENTRES

At each harvest, pods are sorted and apparently healthy ripe pods are conveyed to a collection point outside the seed garden on a clean dry cemented floor previously sprayed with fungicides. After a quarantine period of 3 to 5 days, pods without black pod symptoms are then sold to farmers. The farmers split open these healthy pods, remove and clean the beans with saw dust to reduce the flow of mucilage. The beans are then dusted with any of the recommended fungicides and transported in perforated polythene bags. Such beans are planted immediately on arrival as the beans tend to lose their viability with time.

IMPACT OF THE PRECAUTIONARY MEASURES ON SEED POD PRODUCTION AND BLACK POD DISEASE SPREAD

Table 3 shows the categories of seed pods produced in the period 2003/04 to 2005/06 at the Bechem Seed Garden. Over the three year period, the mean percentage useable pods (%UP) and pods rejected due to black pod disease (%R) were 85.55%, 73.99, 60.27% and 7.93%, 6.03%, 4.38% respectively. Relatively low numbers of rejected pods were harvested between the months of December and March, whereas, pods apparently free from black pod infection and sold to farmers as planting materials were relatively high in all the months compared to the other two categories. The category of fermentable pods (%FP) was higher in April and May in 2004/05 and 2005/06. Such pods were infected during the ripening stage of development and the beans could therefore be used, though not recommended as planting material. This suggests that apparently healthy pods can be produced when pollination is well timed and rigorous husbandry practices are undertaken.

Table 3.Categories of cocoa pods harvested at the Bechem Seed Garden, 2003/04 – 2005/06

Month	2003/2004			2004/2005			2005/2006		
	%UP	%FP	%R	%UP	%FP	%R	%UP	%FP	%R
OCT	63.09	0.00	36.91	68.42	13.90	17.68	73.82	17.51	8.67
NOV	75.45	2.60	21.91	81.92	16.97	1.11	62.30	30.97	6.73
DEC	89.20	1.96	8.85	81.04	17.65	1.32	63.30	35.39	1.31
JAN	96.50	3.50	0.00	86.94	12.14	0.92	57.48	40.86	1.66
FEB	84.80	15.2	0.00	87.04	10.97	1.99	66.18	31.53	2.29
MAR	94.80	5.20	0.00	76.24	20.9o	2.86	65.76	31.35	2.89
APR	92.12	5.14	2.74	54.61	42.54	2.85	50.21	48.52	1.27
MAY	92.49	6.58	0.93	68.59	29.80	21.61	44.45	52.77	2.78
JUN	90.54	9.46	0.00	61.12	34.99	3.89	58.91	29.27	11.82
Mean	85.55	5.52	7.93	73.99	22.21	6.03	60.27	35.35	4.38

Source: Seed Production Unit, Ghana COCOBOD

The precautions taken since the introduction of the ban has also prevented the spread of the pathogen to the Eastern Region of the country in spite of frequent travels to the seed garden and other *P. megakarya* areas by research scientists.

CONCLUSION

The production of safe seeds for farmers in *P. megakarya* infected seed gardens is feasible if the production programme is appropriately timed and the necessary husbandry practices are undertaken. It is noted that the introduced precautionary measures on movement of planting materials from *P. megakarya* endemic areas have prevented the spread of the *P. megakarya* pathogen to the Eastern Region of the country. However, since there is no legislation on the movement of materials across regions, it is only a matter of time that *P. megakary*a will spread to all the cocoa growing regions in the country.

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REFERENCES

Appiah, M.R. (2004). Impact of Cocoa Research innovations on poverty alleviation in Ghana. Inaugural lecture of the Ghana Academy of Arts and Sciences, August 2004, 32pp.

COCOBOD,(2005). Raising awareness on the importance of cocoa in Ghana. www.ghanascience.org (consulted at 10/08/2006).

COPAL (2005) Report on the Meeting of the Scientific Research Committee on Relevant Technologies Available Towards the Sustainability of the Cocoa Economy in Cocoa Producing Countries, Ilheus, Bahia – Brazil, 9-13th August,2004, 43pp.

Dakwa, J.T. 1974. The development of blackpod disease (*Phytophthora palmivora*) in Ghana. Turrialba 24: 367-372.

Edwards, D. F. (1973). Pollination studies on Upper Amazon cocoa clones in Ghana in relation to the production of hybrid seed. *Journal of Horticultural Science* 48:247–259.

Edwin, J and Masters, W.A.(2005) Genetic improvement and cocoa yields in Ghana. *Expl Agric.* 41, 491–503

Griffin, M.J., Idowu, O.L., Maddison, A.C., Taylor, B and Ward, M.R. (1981). Sources of infection. In: Epidemiology of *Phytophthora* on cocoa in Nigeria, pp75- 95.

Legg, J.T. and Lockwood, G. (1981). Resistance of cocoa to swollen-shoot virus in Ghana. I. Field trials. *Annals of Applied Biology* 97:75-89.

Maddison, A. C. and Griffin, M.J. (1981). Detection and movement of inoculum. In: Gregory, P.H. and Maddison, A.C. (Eds). Epidemiology of *Phytophthora megakarya* on cocoa in Nigeria. Commonwealth Mycological Institute, Kew, pp31-49.

Opoku, I.Y., Akrofi, A.Y. and Asante, A.A. (1999). The spread of *P. megakarya* in Ghana. *Journal of the Ghana Science Association*, Vol. 2, 3, 110-116.

3.2. Assessing the options for spray interventions to control the *Moniliophthora* cocoa disease complex of cocoa in Ecuador[***]

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SUMMARY

We describe a series of field tests that examine the relative efficacy of control agents for the management of frosty pod rot (Moniliophthora roreri) and the witches' broom pathogens (Moniliophthora perniciosa). Research and development to date has focused on the optimisation of delivery systems for chemical fungicides and microbial control agents (MCAs) through improved MCA formulation, field testing and spore monitoring techniques. Only copper hydroxide sprays significantly increased yield in the Rio Lindo fungicide trial during 2005: which was a dry year with relatively low FPR incidence. Taken together with other trials, copper fungicides remain the most robust recommendation for the protection of pods against the Moniliophthora diseases. In order to be cost-effective, the number of spray applications should be kept to a minimum, and we report that an economic benefit can be achieved with only 5 sprays per season. Trichoderma spp. have a potential role in lowering levels of disease inoculum and reducing the number of chemical sprays. Results to date indicate they may best be deployed as part of a "mixed regime" that includes copper hydroxide for pod protection: and which appears to have little deleterious impact on Trichoderma in the field. The use of such interventions is discussed in the context of safety with pesticide use and other IPM measures, such as the need for good crop canopy management. This research programme serves to back-stop farmer participatory training co-ordinated by the Andean Countries Cocoa Export Support Opportunities (ACCESO) initiative.

[***] Full text of paper not available at press time

3.3. Development and use of 'mycoharvesters' for formulating conidia of *Trichoderma* spp. and *Beauveria bassiana* for insect pest and disease management in cocoa†††

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SUMMARY

The development of a promising microbial control agent into a successful biopesticide product depends on a number of factors, but efficient mass production systems (using cost-effective substrates) and reliable formulations are especially crucial. Current mass production techniques for anamorphic beneficial fungi, including Trichoderma spp. and Beauveria bassiana, usually involve 2-stage production techniques culminating in aerial conidiation on solid substrates such as broken rice. For most application systems, the fungal product must be formulated in a way that is easy for operators to handle and does not cause blockages in filters, restrictors and other spray nozzle parts. High quality spore separation enables the development of other stable, suspension formulations, and by concentrating conidia into a pure product, facilitates drying to low moisture contents (in order to maintain shelf life). In addition, the process reduces operator contamination hazards - especially inhalation of dust. This paper describes recent development of 'MycoHarvesters': at a laboratory-scale in Ecuador and Cameroon and production-scale in Brazil. Depending on the scale of the equipment, initial separation of the conidia from the rice is achieved either by tumbling (in large scale) or by creating a fluid-bed effect on the inoculated substrate (laboratory scale). Spore products can now meet the size specification required for reliable spraying of cocoa and other crops, and methods of particle size analysis and subsequent formulation are discussed.

††† Full text of paper not available at press time

3.4 What causes *Moniliophthora roreri* to germinate?

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SUMMARY

An integrated approach for the control of Moniliophthora roreri requires understanding of the epidemiology of the pathogen and in particular of its infection process; without germination there is no infection. Surprisingly, very little is known about the factors that regulate germination of M. roreri. In laboratory experiments the effect of different media on germination of M. roreri was tested (V8-juice agar, half-strength potato dextrose agar, water agar and yeast extract agar). For the latter different pH-values, ranging from pH = 3 to pH = 12, were tested. Subsequent germination tests were done using water agar to which amino acids, present in yeast extract agar (YEA), were added individually. The effects of prolonged laboratory cultivation of M. roreri on germination, spore volume and pathogenicity were also investigated. YEA supported the highest germination rate for M. roreri (up to 80%). Water agar enriched with the amino acids tyrosine, leucine and or asparagine showed significantly higher germination levels than water agar; however, germination levels did not exceed those on YEA. Optimum pH value for germination on YEA was pH = 7. For germination experiments the range of pH = 5 to pH = 9 is acceptable. Neither germination, nor spore volume was altered by prolonged laboratory cultivation. Results of field pathogenicity tests are reported. The implications of these results in obtaining a better understanding of the epidemiology of M. roreri and in particular the processes that may regulate germination, and thus the beginning of the infection process, are discussed.

INTRODUCTION

Cocoa farming, particularly in Latin America, has become increasingly difficult due to several economic and biological factors (Holmes *et al.,* 2004). One of the main biological constraints is frosty pod rot (FPR), caused by *Moniliophthora roreri*. This disease is still in an invasive phase (Evans, 2002), having recently been confirmed in Belize (Phillips-Mora *et al.,* 2006a) and Mexico (Phillips-Mora *et al.,* 2006b). In areas where *M. roreri* is present the pathogen can result in total crop loss, leading to immense economical losses (Bateman *et al.,* 2004). After introduction of the disease in Costa Rica in 1978, pod losses of 60 – 90 % have been reported (Evans *et al.,* 2003; Enríquez *et al.,* 1982). Control of this disease in cocoa-growing areas is essential to protect farmers' livelihoods but also to avoid further spread to yet unaffected neighbouring countries such as Brazil, Bolivia and the Caribbean.

For a suitable control system to be implemented and effective, a greater understanding of the pathogen's life cycle is needed. Here we investigated different factors that influence the germination of *M. roreri* under laboratory conditions. Field trials

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investigating on the pathogenicity of *M. roreri* have also been initiated.

MATERIALS AND METHODS

Source of inoculum

All isolations used in this study have their origin in Costa Rica and thus belong to the Co-West group according to Phillips-Mora (2003). The inoculum of *M. roreri* used in this study was collected by three different methods: from the laboratory culture collection, from pods using the sliced pod method (SPM) or direct isolation (DI). For the laboratory inoculum, strains maintained under laboratory conditions for at least five years were used. For field inoculum, pods infected, but not yet sporulating, were collected at two locations, the experimental farm 'La Lola' (Siquirres, Costa Rica) and Cabiria (Turrialba, Costa Rica). Pods were cleaned using tap water, dried with tissue paper, sprayed with alcohol and flamed. The outer layer of the pod was removed and again the pod was sprayed with alcohol and flamed to surface sterilise. For the SPM, the pod was then cut into horizontal slices and stored in humid chambers at room temperature. This was referred to as harvest day 0. For DI, discoloured internal tissue was placed on potato dextrose agar (PDA). After colonisation, mycelium was transferred to a new plate of PDA: this sub-culture was used for spore collection. Inoculum obtained via either of these methods is referred to as field inoculum.

Effect of different media on germination

Here, the SPM was used on pods collected in La Lola. At harvest days 4, 5, 7, 13, 20, 27 and 41, three replicate spore suspensions were collected from the sporulating slices (10^6 – 10^7 spores ml^{-1}). Of each suspension 100µl was spread out uniformly on the following media: yeast extract agar (YEA: 20g granulated agar (Difco), 10g yeast extract (Difco) in 1l distilled water), V8-juice agar (V8 agar: 25g granulated agar (Difco), 250ml V8-juice (Campbell soup company), 3.75g $CaCO_3$, 1.25g asparagine (Sigma) in 1l distilled water) and half strength potato dextrose agar (½ PDA: 20 g PDA (Difco), 10g granulated agar (Difco) in 1l distilled water). Antibiotics (penicillin-G procaine with dihydro-streptomycin, Phenix) were added to final concentrations of 50ppm and 30ppm respectively. The plates were incubated at room temperature (±27ºC) and normal daylight.

Effect of different amino acids on germination

Not all amino acids present in yeast extract agar were available for testing. Asparagine is not present in yeast extract, but was included in this experiment as asparagine is widely used in many standard growth media as a nitrogen source. Amino acids were added separately (Table 1) to water agar (20g bacto-agar (Difco) in 1l distilled water). Antibiotics were added as above.

Inoculum was obtained by the SPM with pods from La Lola. Spores were collected after one week. On each agar plate, 100µl of the suspension ($5.3x10^6$ spores ml^{-1}) was spread out uniformly and plates were incubated at 24 ± 1 ºC with 12/12 hours dark/light.

When amino acids were added singly to water agar, tyrosine, asparagine and leucine showed a stimulatory effect on germination in comparison with water agar. Therefore tyrosine, asparagines and leucine were also added in mixture to water agar following the same procedure as described for the single amino acid experiment.

Table 1. Overview of amino acids and the amount added to 50ml water agar. In the column on the right, a complete list of all amino acids as analysed in yeast extract (Difco) is given.

Amino acid	Mg	% in yeast extract	Amino acid	Mg	% in yeast extract
Alanine	26.80	5.36	Lysine	25.75	5.15
Arginine	15.10	3.02	Methionine	5.25	1.05
Asparagine	50.00	0.00	Phenylanaline	n.a.	2.53
Aspartic acid	33.45	6.69	Proline	13.00	2.60
Cysteine	3.70	0.74	Serine	14.20	2.84
Glutamic acid	71.00	14.20	Threonine	14.75	2.95
Glycine	16.25	3.25	Tryptophan	6.80	1.36
Histidine	n.a.	1.20	Tyrosine	6.00	1.20
Isoleucine	n.a.	3.23	Valine	n.a.	3.79
Leucine	23.45	4.69			

Source:http://www.voigtglobal.com/Anonymous/DIFCO_Yeast_Agar_Formulations.pdf, consulted at 10/01/2006).
Asparagine is not present in yeast extract but was included in this experiment.
n.a.= not available for this experiment.

Effect of pH and origin on germination

Field inoculum was obtained by DI from three replicate pods collected in La Lola and Cabiria. Also strains from the laboratory culture collection with origin La Lola and Cabiria were used for this 2 x 2 factorial experiment. The experiment was carried out three times.

YEA was used in this experiment for the assessment of germination. For each repetition of the experiment a new batch of YEA plates in a pH range of three to 12 was poured; normal YEA was prepared, after autoclaving the pH was adjusted (Corning pH meter 215) by adding sodium hydroxide (Sigma) (stock solution: 1M) or hydrochloric acid (1M, Fisher). On each plate 100µl of the spore suspension (10^6 – 10^7 spores ml^{-1}) was spread out uniformly and plates were incubated at 24 ±1 ºC in complete darkness.

Spore morphology

Field inoculum was obtained in the same way as described for the pH experiment. Three pods from La Lola and three pods from Cabiria were used. Two strains from the collection were used for laboratory inoculum, one with origin La Lola, one with origin Cabiria. This 2 x 2 factorial experiment was repeated twice, each repetition with new field isolates. For the laboratory strains, inoculum was obtained from new plates for each repetition.

Ten spores from each suspension were measured with a graticule along two axes. Spores with a length-width ratio ≤ 1.3 were classified as globose, spores with a ratio > 1.3 were classified as ellipsoidal (Hawksworth *et al.,* 1983). Spore volume (μm^3) was calculated using the formula $^4/_3\ \pi\ r^3$ if the spore was globose. If the spore was ellipsoidal the formula $^4/_3\ \pi\ r^2\ h$ was used (where r is the radius of the circular face and h is half the length of the spore) (Verkerk *et al.,* 1986).

Aliquots (100µl) of the suspension were spread out uniformly on YEA. Plates were incubated at 24 ±1 ºC in 8/16 hours light/dark.

Assessment and statistical analysis

Germination in all experiments was counted after two days for 200 spores per plate. Germination percentages were arcsine-transformed to normalize the error distribution and analysed by ANOVA on InfoStat (InfoStat, 2004) using the appropriate model. Length-width ratios and volumes of spores were analysed directly by ANOVA; their relation to germination was assessed using covariance.

RESULTS

Effect of different media on germination

Yeast extract agar showed significantly higher germination rates than V8-agar and ½ PDA (P<0.0001) (Figure 1).

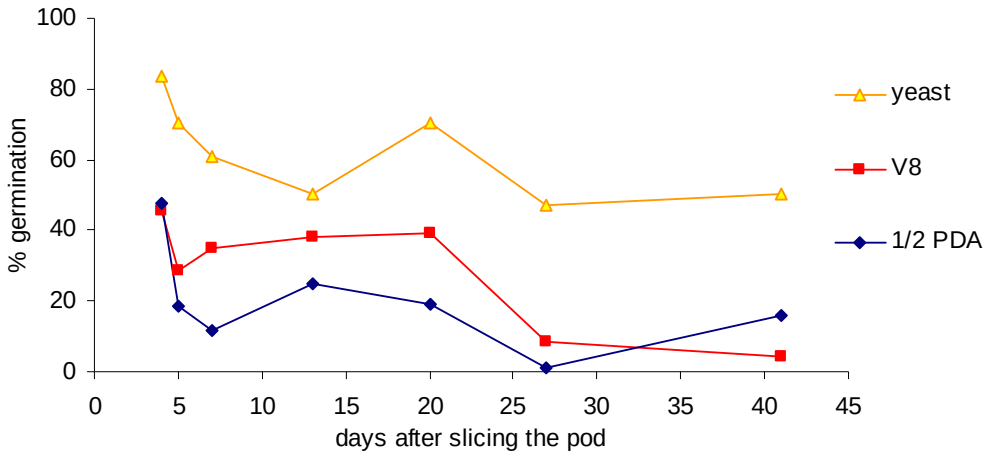

Figure 1. Germination percentages on different harvest days for different media.

Effect of different amino acids on germination

Germination percentages of the agar media with different amino acids differed significantly (P<0.0001). Yeast extract and tyrosine had the highest germination percentages: 63% and 52%, respectively. The control medium had a significantly lower germination percentage (11%) than yeast extract (63%), tyrosine (52%), asparagine (42%) and leucine (39%) (Figure 2).

Different media showed significantly different germination percentages (P<0.0001). Yeast extract agar and the mixture of asparagine with leucine resulted in the highest germination percentages: 85% and 78% respectively. Germination on the control medium (35%) did not differ significantly from tyrosine (30%) Other amino acid(s) (mixtures) were intermediate (Figure 3).

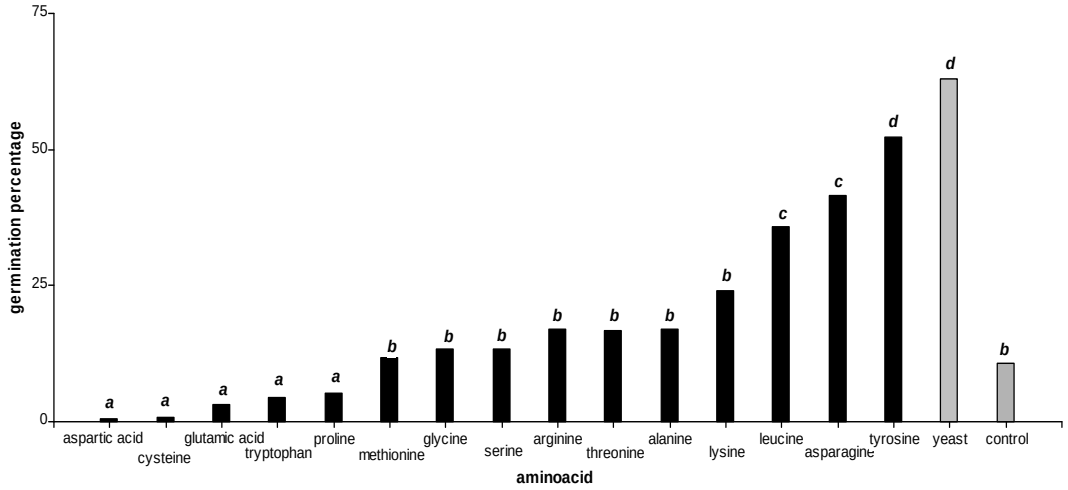

Figure 2. Germination percentages for the different media, containing one single amino acid, or yeast extract agar, or no amino acid (controls).
[a b c d] bars with the same letter indication do not differ at $p < 0.05$.

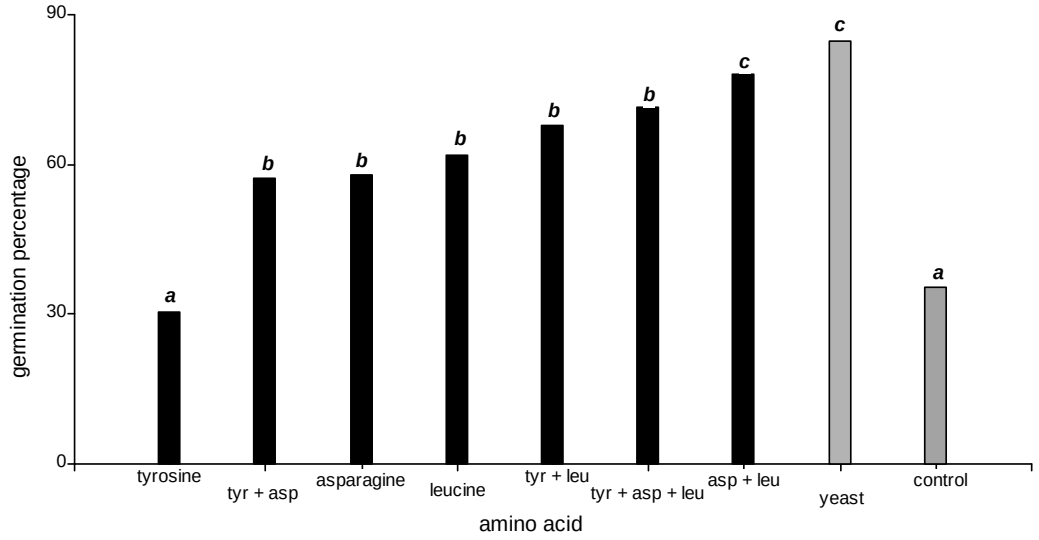

Figure 3. Germination percentages for the different media, containing one or more amino acids or yeast extract agar or no amino acid (controls).
Tyr = tyrosine, asp = asparagine, leu = leucine.
[a b c] values followed by the same letter do not differ at $p<0.05$.

Effect of pH and origin on germination

Germination percentages of the laboratory strains were significantly higher than the field strains ($P<0.0001$). The highest germination percentages, for both the laboratory and field strains, were found at pH = 6, 7 and 8 (Figure 4). No interaction between historic origin and pH was found ($P = 0.8233$). This means that germination percentages between laboratory and field strains differ, but optimum pH values are the same.

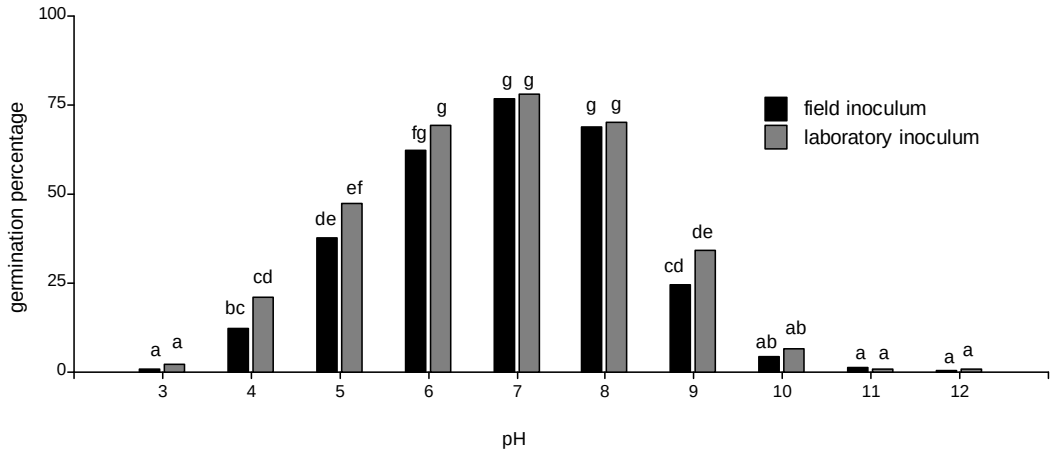

Figure 4. Germination percentages per pH value for laboratory and field strains. [a, b, c, d , e, f, g] bars with the same letter do not differ at p<0.05.

Spore morphology

Spores from La Lola isolates had significantly higher volumes (487µm^3) than spores from Cabiria isolates (344µm^3) (*P* = 0.0001) (Table 2). Spores from laboratory isolates had just significantly higher volumes (473µm^3) than field isolates (404µm^3) (*P* = 0.0492).

Table 2. Spore volume (µm^3) per geographic and historic origin on different harvest days.

		Geographic origin						
Day	Historic origin	La Lola	Cabiria	Mean	Mean per harvest day		Mean per historic origin	
1	Laboratory	522	225	347 [a]			473	α
1	Field	464	354	409 [a]	391	A	404	β
8	Laboratory	490	407	449 [ab]				
8	Field	464	313	389 [a]	419	AB		
15	Laboratory	475	449	597 [b]				
15	Field	509	318	413 [a]	438	B		
Mean per geographic origin		487	II	344	I			

[a b] Spore volumes averaged for historic origins followed by the same small letter do not differ at *P*<0.05.

AB Mean spore volumes per harvest day followed by the same capital letter do not differ at *P*<0.05.

I II Mean spore volumes per geographic origin followed by the same Roman number do not differ at *P*<0.05.

α, β Overall means on historic origins followed by the same Greek letter do not differ at *P*<0.05

The length-width ratio of spores from La Lola isolates was significant smaller (1.66) than the length-width ratio of Cabiria isolates (2.10) (P = 0.0009). No difference in length-width ratio was found between spores from laboratory and field strains (P = 0.1458).

Isolates from the geographic origin La Lola had significantly higher germination percentages than Cabiria isolates (P = 0.0164) (Table 3). No effect was found of historic origin (P = 0.5259). Harvest day 1 had the highest germination percentages, followed by day 8 and then by day 15 (P = 0.0008). No interaction was found between geographic origin and historic origin (P = 0.0992).

La Lola isolates on harvest day 1 (both historic origins) showed the highest germination percentages. These differed significantly from Cabiria isolates on harvest day 15, of which the field isolate had significantly lower germination than any other sample (Table 3). No covariance of germination percentages was found with spore volume (P = 0.8514), nor with the length-width ratio of the spore (P = 0.8109).

Table 3. Germination percentages per historic and geographic origin on different days.

		Geographic origin				
Day	Historic Origin	La Lola		Cabiria		Mean per harvest day
1	Laboratory	84.67	c	71.00	bc	
1	Field	87.33	c	78.33	bc	80.25^{C}
8	Laboratory	89.17	c	59.17	bc	
8	Field	65.17	bc	54.00	bc	66.75^{B}
15	Laboratory	53.33	bc	47.50	b	
15	Field	78.67	bc	8.83	a	47.25^{A}
Mean per geographic origin		76.33	II	53.17	II	

$^{a, b, c, d}$ Germination percentages followed by the same letter do not differ at $p < 0.05$.

$^{I, II}$ Mean per geographic origin followed by the same number does not differ at $p < 0.05$.

$^{A, B, C}$ Mean per harvest day followed by the same capital letter does not differ at $p < 0.05$.

DISCUSSION

These experiments showed that YEA resulted in highest germination percentages. Main ingredients of yeast extract are amino acids. When amino acids were added single or in mixture to water agar, leucine, asparagine and tyrosine had a positive effect on germination, with the mixture of asparagine and leucine being as stimulatory as YEA. This is interesting, as asparagine is not present in YEA. In the field, potential natural nutrient sources are found in mixtures. Griffin (1994) stated that for fungi a mixture of amino acids, generally, allows greater and more rapid growth than any single amino acid. Several fungi cannot use any form of inorganic nitrogen. These fungi have no specific amino acid requirements: the biochemical basis for this has not been investigated (Griffin, 1994).

Surprisingly laboratory strains exhibited higher germination percentages than field isolates. Several authors have observed a deterioration of fungal vigour during continuous sub-culturing. Smith and Waller (1992) stated that continuous sub-culturing of fungi over a prolonged period can cause reduced viability. However in this experiment, repeated sub-culturing over several years of *M. roreri* did not influence germination ability, or length-width ratio.

Although germination percentages of laboratory and field strains differed from each other, their optimum pH values do not differ. For experiments on germination a pH

range of pH = 5 until pH = 9 is acceptable. For optimal germination, pH values between pH = 6 until pH = 8 are recommended. Findings of Donnan *et al*. (2006) showed that immature pods have an average pH value of pH = 9.5, whereas mature pods were found to have an average value of pH = 8. This is in accordance to the findings described in this study which shows that *M. roreri* can and will germinate at these pH values, although, based on pH values found on the exterior of cocoa pods, lower infection of immature pods would be expected due to their higher pH compared to that of mature pods. However, experiments of Suárez (1971) showed that immature pods show earlier disease symptoms than mature pods. Information about the process of disease infection of mature and immature pods is greatly lacking and many other factors may be involved.

Spores from La Lola isolates (from field and laboratory) showed higher germination, a larger spore volume and a lower length-width ratio than spores from Cabiria isolates. This is in agreement with Steiner and Lockwood (1969) who stated that spores with a higher volume, in general and across several genera, germinated faster than spores with a lower volume. Griffin (1994) found that spores of fungi at the onset of germination swell because of the uptake of water from the environment.

It would be interesting to see if amino acid presences on the pod surface and the pH of the pod have a relationship with genetic resistance. Further investigations are required to explain why strains with origin La Lola have higher germination rates than Cabiria isolates, given the fact that they are assumed to be clonal or near clonal populations (Phillips-Mora, 2003).

Results from this study suggest that, for future experiments on germination of *M. roreri*, strains from the laboratory or sub-cultures directly from the field can be used, depending on experimental ease. YEA with a pH of around 7 is the preferred medium.

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REFERENCES

Bateman, R.P., Holmes, K.A., Krauss, U., Padi, B. 2004. Future tactics and tools for pest management. *In*: Flood, J. and Murphy, R. eds. Cocoa Futures; A source Book of Some Important issues facing the Cocoa Industry., 1st ed. CABI-Federacafé USDA, Chinchiná, Colombia, p. 77-93.

Donnan, Y., Arroyo, C., Gamboa, A., ten Hoopen, M., Krauss, U. 2006. Cocoa pod pH and implications for the screening of biological control agents of cocoa disease. Proceedings of the 5th INCOPED Seminar, San José, Costa Rica 16-18 Oktober 2006 (in press).

Enríquez, G.A., Brenes, O., Delgado, J.C. 1982. Desarrollo e impacto de la Moniliasis del cacao en Costa Rica. Proceedings, 8th International Cocoa Research Conference, Cartagena, Colombia, p. 375-380.

Evans, H.C. 2002. Invasive Neotropical Pathogens of Tree Crops. *In*: Watling, R., Frankland, J.C., Ainsworth, A.M., Isaac, S., Robinson, C.H. eds. Tropical Mycology, Vol. 2. CABI Publishing, Wallingford, UK, p. 83-112.

Evans, H.C., Holmes, K.A., Thomas, S.E. 2003. Endophytes and mycoparasites associated with an indigenous forest tree, *Theobroma gileri*, in Ecuador and a preliminary assessment of their potential as biological control agents of cocoa diseases. Mycological Progress 2: 149-160.

Griffin, D.H. 1994. Fungal Physiology. John Wiley & Sons Ltd, Chichester, USA.

InfoStat 2004. www.InfoStat.com.ar Release 2004. Grupo InfoStat, Universidad Nacional de Córdoba, Argentina.

Hawksworth, D.L., Sutton, B.C., Ainsworth, G.C. 1983. Ainsworth and Bisby's Dictionary of the fungi. Seventh Edition.

Holmes, K.A., Schroers, H.J., Thomas, S.E., Evans, H.C., Samuels, G.J. 2004. Taxonomy and biocontrol potential of a new species of *Trichoderma* from the Amazon basin of South America. Mycological Progress 3: 199-210.

Phillips-Mora, W. 2003. Origin, Biogeography, Genetic Diversity and Taxonomic Affinities of the Cacao (*Theobroma Cacao* L.) Fungus *Moniliophthora Roreri* (Cif.) Evans *et al.* as Determined using Molecular, Phytopathological and Morpho-Physiological Evidence. Ph.D. Thesis, University of Reading, Reading, UK.

Phillips-Mora, W., Cawich, J., Garnett, W., Aime, M.C. 2006a. First report of frosty pod rot (moniliasis disease) caused by *Moniliophthora roreri* on cacao in Belize. Plant Pathology 55: 584.

Phillips-Mora, W., Coutiño, A., Ortiz, C.F., López, A.P., Hernández, J., Aime, M.C. 2006b. First report of *Moniliophthora roreri* causing frosty pod rot (moniliasis disease) of cocoa in Mexico. Plant Pathology 55: 584.

Smith, D., Waller, J.M. 1992. Culture collections of microorganisms: their importance in tropical plant pathology. Fitopatologia Brasileira 17: 5-12.

Steiner, G.W., Lockwood, J.L. 1969. Soil fungistasis: sensitivity of spores in relation to germination time and size. Phytopathology 59: 1084-1091.

Suárez, C.C. 1971. Estudio del Mecanismo de Penetración y del Proceso de Infección de *Monilia roreri* (Cif en Par) en Frutos de Cacao (*Theobroma Cacao* L.), Universidad de Guayaquil, Pichilingue, Ecuador.

Verkerk, G., Broens, J.B., Kranendonk, W., Puijl van de, F.J., Sikkema, J.L., Stam, C.W. 1986. Binas Informatieboek VWO-Havo voor het Onderwijs in de Natuurwetenschappen. 2nd ed. Wolters-Noordhoff, Groningen, NL.

3.5. Cocoa pod pH and implications for the screening of biological control agents of cocoa diseases

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SUMMARY

Effectiveness of biological control agents (BCAs) in field trials is often disappointing compared with results from tests carried out in controlled environments. The BCA identification procedures and the lack of ecological considerations on target disease before application have been blamed for these failings. Cocoa pod pH is a physiological property with potential to directly influence BCAs of cocoa disease and their effectiveness. Little is known, however, about the pH of the flowers and pods of cocoa (Theobroma cacao). The objective of this study was to investigate the pH of cocoa flowers and pods. This will provide insight into the potential of pH to influence the effectiveness of BCAs and its importance in the elaboration of biocontrol strategies. A series of pH measurements were taken in different positions on the pod as well as the pH of pods of different colour, developmental stage, shading conditions and health. Pod pH was measured using pH indicator paper applied to the area of interest with distilled water. The average cocoa pod pH was basic (average 8.5). Significant differences were found between pods of different colour (8.33 for green compared to 8.69 for red, P= 0.0089), along the surface of the pod (increase from 9.11 to 9.86 from peduncle to apex, P=0.0001), throughout the developmental stages (7.37 for flowers, 9.56 and 9.53 for cherelle and immature pods, respectively and 8.32 for mature pods, P<0.0001) and between shading conditions (9.23 in shaded and 8.26 in non-shaded conditions, P<0.0001). Although the exact biological significance of these variations remains to be determined, the range of pH values encountered has clear potential to influence BCAs and their effectiveness. Successful BCAs of cocoa disease need to be effective under these pH conditions. Furthermore, it would be of interest to preferably select for BCAs able to withstand similar changes in pH without loosing effectiveness. Therefore, we suggest that screening procedures for successful BCAs not only focus on desirable characteristics for biocontrol, but also on how they perform under the different pH conditions encountered at the target site.

INTRODUCTION

Every year, it is estimated that 40% of all cocoa production is lost to just five diseases (Flood *et al.*, 2004). This high disease incidence clearly indicates the lack of effective control methods. Biocontrol is a control option with much promise and the development of an effective biocontrol strategy for these diseases could dramatically improve cocoa production. Effectiveness of biological control agents (BCAs) in field trials is often disappointing compared with results from tests carried out in controlled

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environments. The BCA identification procedures and the lack of ecological considerations on the target disease before application have been blamed for these failings (Deacon, 1991). A more in depth knowledge of the factors affecting BCAs performance may lead to improved selection procedures and the development of more efficient biocontrol strategies.

Biocontrol agents are required to be functional throughout the development of the fruit they are applied to and thus must be compatible with the varying physiological factors that they will encounter on its surface. Incompatibility of the BCA and the conditions present on the pod would defeat the effectiveness of the agent and lead to the failure or limited effectiveness of the BCA. One potential factor that could influence the survival and effectiveness of the biocontrol agent is pH. We found no literature on the pH of the cocoa pod, and therefore its potential to influence biocontrol strategies is unknown. The purpose of this work was to document this aspect of cocoa pod physiology and attempt to predict its impact on biocontrol. A series of field trials were carried out comparing the pH of cocoa pods of different colour, on different areas of the pod, during development, in different conditions of shading, and of different health status.

MATERIALS AND METHODS

Field trials

Randomly selected pods from clones in the Cabiria germplasm collection at CATIE, Turrialba, Costa Rica were used to investigate pod pH. The pH was measured using Micro Essentials Laboratory Hydrion Insta-Chek pH papers with a total range of pH 0-13. Prior to the measurement, distilled water was applied to the area of interest with a spray bottle. This dispensed a fine mist on the pod without creating running water on the surface. The paper pH indicator was then applied to the humidified area and the pH read by comparing the change in colour to the scale provided with each roll of paper.

A total of five experiments were carried out. In each trial, bar the third, a total of 15 pods were used for each factor, with three pods being selected from five individual trees. For the third trial, the pods of four clones were used, two with red pods and two with green pods, and five flowers or pods of each developmental stage (flower, cherelle, immature, mature, and ripe) were measured per clone. Each trial was repeated three times on different days.

The first trial compared red versus green pods and cherelle versus mature pods, with measurements taken at the apex of the pod. The second trial compared red versus green pods, cherelle versus mature pods, while measurements were taken at three different locations on the pod: apex, middle and top. The third trial compared red versus green and different developmental stages: All measurements were taken at the apex. The fourth trial compared red versus green pods in sun and shade conditions. All measurements were taken at the apex. The final trial compared the pH of pods affected by Frosty Pod Rot (FPR) and Black Pod Rot (BPR). Areas exhibiting symptoms were compared to areas not exhibiting symptoms.

Statistical analysis

All five trials were completely factorial with either two or three factors. Data was analyzed by ANOVA using Infostat (InfoStat, 2004). Differences between means

were assigned using Tukey. In the third trial differences between pod colour were compared using orthogonal contrasts.

RESULTS

The pH at apex of the pod

There was a significant difference between green and red pods (P=0.0089), with green pods having a lower pH than red ones (8.33 and 8.69, respectively). There was also a significant difference between cherelles and mature pods (P=0.0001), with cherelles having a higher pH than mature pods (10.02 compared to 6.99).

Different positions on pods

pH-values differed significantly according to the measurement location on the pod surface (P<0.0001). The pH was highest at the top and decreased towards the bottom (apex) (9.12, 9.42, and 9.86, respectively).

There were significant differences (both P<0.0001) between green (pH=9.10) and red pods (pH=9.86) and between cherelles (pH=10.46) and matures pods (pH=8.47).

There was an interaction between colour and size (P<0.0001), with a significant difference being reached between green and red mature pods (7.81 and 9.13, respectively) but not red and green cherelles (10.39 and 10.53). This was a trend that was already noticed in the previous trial, but failed to reach significance.

Developmental stages

Developmental stages showed significant differences in pH (P<0.0001). Flowers had a pH of 7.37, cherelles and immature pods of 9.56 and 9.53, respectively, and mature pods of 8.32.

Growth in sun or shade

Sunny and shaded conditions had a significant influence on pod surface pH, with shaded pods having a higher pH (9.23) than unshaded pods (8.67). Consistent differences in the pH in relation to colour were found (P<0.0001) with both green cherelles and green mature pods having lower pH values than the red equivalents (6.49 for green mature and 10.23 for green cherelles pods compared to 8.12 and 10.97 for the red equivalents). The interaction of colour with shading was significant (P<0.0001): Only green pods showed significant differences in pH (P<0.0001) between shaded and unshaded conditions (8.99 in shade and 7.73 in sun). Red pods failed to reach significance between shading regimes. Both cherelles and mature pods, however, showed significant differences (P<0.0001) in shade and sun (7.14 in sun and 7.47 in shade for big pods and 10.20 and 11 respectively for cherelles). No 3rd order interaction between the factors colour, size and shading was found (P=0.5035).

The pH of diseased and healthy pods

The pH of healthy pods was significantly higher than the pH of areas presenting symptoms for either FPR or BPR (P=0.0003, pH=9.49 compared to pH=9.11 and pH=9.14 respectively). No differences were found between non-affected parts of diseased pods and healthy pods. Only pods affected with BPR showed differences between affected and non affected parts, with the diseased part having a significantly lower pH (P=0.0003, 9.11 compared to 9.39).

DISCUSSION

Exterior pod pH appears to be highly variable. Factors that are most likely to determine exterior pod pH are pod secretions, environmental deposits, microbial populations and microclimatic factors. Secretions are known to differ during the development of leaves and fruits with older tissue excreting more nutrients than young tissue (Tukey, 1970; Fourie & Holz, 1998). Environmental factors may also influence pod secretion which will, in turn, affect microbial population structure (Fourie & Holz, 1998). Microbial population structure is, however, also governed by external processes such as exposure to rain (Breeze and Dix, 1981) and UV radiation, for example (Hirano and Upper, 2000; Rotem *et al.*, 1985). The importance and effect of each factor with regards to pH is unclear. It is most likely that all factors interact to determine pH. Here we found development stage, of cocoa pods to be of paramount importance, with 2.2-3.0 pH units variation. Pod colour (average variation 0.77 pH units), location of measurement on pod (average variation 0.74 pH units), shading regime (average variation 0.56 pH units), and health status (average variation 0.37 pH units) also had an effect. The biological significance of these changes remains to be fully determined. Although variations in pH of <0.4 units are unlikely to yield significant differences, shifts of >2.2 log units are likely to be of importance.

A successful biocontrol agent needs to be effective at the eco-physiological conditions that it meets on the pod, one of which is pod pH. Variations of pH on the pod surface and during the development from flower to ripe pod mean that the BCA needs to be effective over a range of pH values. Many fungi support a broad range of pH, between 2 to 9, in which they are comfortable, but exhibit preferred optima generally in the range of 4 to 7 (Griffin, 1994). In contrast, *Moniliophthora roreri*, the causal agent of FPR, exhibited an optimum of pH=7.0 for germination (Bekker *et al.*, 2006). The pH of diseased areas of pods showed significant differences to healthy pods, however, the difference was only of 0.4 log units which may only be of limited eco-physiological importance.

The pH values found on pods, however, were considerably basic in that regard. The lowest average being of pH=7.4 and the highest pH=11.0. These values could be near or beyond the upper limit of the optimum for fungal, especially antagonist, growth. Experiments investigating *Trichoderma* spp. showed that maximum biomass production occurred at values between pH=4.6 and pH=6.8 (Jackson *et al*., 1991). *Trichoderma viride* strains stopped growing at values higher than pH=8 on yeast medium, and at values higher than pH=7 in soil extract medium (Kredicks *et al.*, 2004). Variations in one pH unit from the growth optima of *T. pseudokoningii* were shown to decrease average biomass by 4.55% and 26% for a two unit change. Decreases of 11% and 4.5% were observed for a one unit change in two different *Trichoderma viride* strains and a decrease of 22.5% and 15.5% for a two pH shift (Jackson *et al*., 1991). Average linear mycelium growths of two additional *T. viride* strains decreased by 20% and 6.2% for one unit changes and 46.2% and 45.1% for two unit changes (Kredicks *et al.*, 2004). Similar trends were also found for *T. aureoviride* and *T. harzianum* strains (Kredicks *et al.*, 2004).

These results show that the pH conditions of the cocoa pod surface have clear potential to restrict the growth of BCAs, such as *Trichoderma* spp., and may be an explanation for the erratic performances of some BCAs in the field.

Recently much focus has been put on endophytic BCAs. Endophytic BCAs, unlike epiphytic BCAs, are only subject to pod surface pH conditions during short germination and establishment periods. Although we have not yet looked at the interior pH of cocoa pods, which is relevant for continued growth, in any detail, preliminary studies indicate that interior pod pH may be closer to the lower pH optimum for fungal growth. Provided that establishment and germination can occur successfully in the exterior pH conditions, this may be further encouragement to the use of endophyte BCAs, but also requires rather pH-tolerant isolates.

In light of these findings, effectiveness of BCAs in the field may be improved by introducing pH tolerance into BCA screening procedures at early stages, covering a range of 7.0 to at least 11.0. Mixed inocula could also be designed to cover the range of pH-values encountered on a pod if antagonists effective in that range are found. Further research should look at the performance of known or potential BCAs under different pH conditions and test in the field the efficacy of those that are the most promising.

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REFERENCES

Bekker, M., Arroyo, C., Donnan, Y., Krauss, U. (2006) What causes *Moniliophthora roreri* to germinate? Proceedings of the 5th INCOPED Seminar, San José, Costa Rica, 17-18 October, 2006 (in press).

Breeze, EM; Dix, NJ (1981) Seasonal Analysis of the Fungal Community on Acer platanoides Leaves. *Trans. Br. Mycol. Soc.* ***77***: 321-328.

Deacon, J.W. (1991) Significance of ecology in the development of biocontrol agents against soil-borne plant pathogens. *Biocontrol Sci. Technol*. **1**: 5-20.

Flood, J., Guest, D., Holmes, K.A., Keane, P., Padi, B., Sulistyowati, E. (2004) Cocoa under attack. **I**n: Flood, J. and Murphy R. eds. *Cocoa Future*s, pp. 33-53. Colombia: The Commodities Press.

Fourie, J.F., Holz, G. (1998) Effects of fruit and pollen exudates on growth of *Botrytis cinerea* and infection on plum and nectarine fruit. *Plant Disease* **82**: 165-170

Griffin D.H. (1994) Fungal Physiology. 2nd edition. New York: Wiley-Liss, Inc.

Hirano, S.S., Upper, C.D. (2000) Bacteria in the Leaf Ecosystem with Emphasis on Pseudomonas syringae-a Pathogen, Ice Nucleus, and Epiphyte, Microbiology and Molecular Biology Review 64: 624-653.

InfoStat 2004. www.InfoStat.com.ar. Release 2004. Grupo InfoStat, Universidad Nacional de Córdoba, Argentina.

Jackson, A.M., Whipps, J.M., Lynch, J.M. (1991) Effects of temperature, pH and water potential on growth of four fungi with disease biocontrol potential. *World Journal of Microbiology and Biotechnology* **7**: 494-501.

Kredics, L., Manczinger, L., Antal, Z., Pénzes, Z., Szekeres, A., Kevei, F., Nagy, E. (2004) In vitro water activity and pH dependence of mycelial growth and extracellular enzyme activities of Trichoderma strains with biocontrol potential. *Journal of applied Microbiology* **96**: 491-498.

Rotem, J., Wooding, B., Ayler, D.E. (1985) The role of solar radiation, especially ultraviolet, in the mortality of fungal spores. *Phytopathology* **75**: 510-515.

Tukey, H.B.Jr. (1970) The leaching of substances from plants. *Annu. Rev. Plant Physiol.* **77**: 1341-1345.

Discussion on Integrated Pests Management: Basic Scientific and Technological Support

Chairperson: Carmen Suárez-Capello, INIAP, Ecuador

Q: Roger Dehnel, Mars Inc., USA

Has the nature of the part of the tree on which we spray biocontrol agents been overlooked? Is it a limiting factor or an opportunity?

A: Ulrike Krauss : CABI Bioscience, Trinidad and Tobago

No, I think it is on the list of our priorities. But we were all surprised to see that the development stage was the overriding factor.

A: Keith Holmes, CABI Bioscience, Trinidad and Tobago

Trichoderma has been isolated from cocoa pods and other *Theobroma* species pods and have been shown to be able to colonise pods in the field. As these are often adapted to this niche application to the pods should not be a barrier to development as a biocontrol.

Q: Piere Tondje, IRAD, Cameroon

Did you apply this model on the fungus now being used as a biocontrol agent in your trials to explain the differences that we observed.

A: Ulrike Krauss : CABI Bioscience, Trinidad and Tobago

We will from now onwards – and infact has started. But please bear in mind that the last of the results presented here were from September this year.

Q: Prakash Hebbar, Mars Inc.: USA

Can we improve formulations by modifying pH?

A: Ulrike Krauss : CABI Bioscience, Trinidad and Tobago

We don't know yet, but it is one thing we could look at.

Q: Prakash Hebbar, Mars Inc.: USA

Can we add substrates to improve efficacy? But we should be careful not to aid the pathogen.

A: Ulrike Krauss : CABI Bioscience, Trinidad and Tobago

We have just published an article on that topic in Biological Control. Molasses did not enhance biocontrol nor did it aid the pathogen. But the molasses solution only increased yield over the absolute control.

Comment: Q: Prakash Hebbar, Mars Inc.: USA

Flower cushion colonisation may be due to readily available nutrients, but could be pathogens themselves.

Q: Hugo Hermelink, Organic Cocoa Farmer, Costa Rica

Did you also try copper oxide, coppersulfate (Bordeaux mixture)?

A:Roy Bateman,Imperial College, -IPARC,UK

The common copper formulations are copper sulfate (often turned into Bordeaux mixture –WHO toxicity class I -).
Copper oxide – WHO toxicity class II -
Copper hydroxide – WHO toxicity class III –
Copper oxychloride (formulated) – WHO toxicity class IV –
(for toxicity classes see: Tomlin "The Pesticide Manual").
We prefer class III - IV compounds for unprotected small holder farmers, and copper hydroxide is widely available in places where we work.

Q: Hugo Hermelink, Organic Cocoa Farmer, Costa Rica

Aren't you afraid of disseminating *Monilia* spores by using a motorized mistblower?

A: Roy Bateman, Imperial College, -IPARC,UK

A number of people have expressed concerns about this. I would like to see prove in replicated, controlled trials. It may be a real phenomenon but I would like to have thought that over the course of a month natural wind events would be more important than a single spraying.

Q: J. L. Pereira, Ceplac, Brazil

I am confused with the common use of the word "formulation" for *Trichoderma* bio-agent, with or without the original substrate rice. It does not follow it is a "formulation" or "formulated" when it only is an active ingredient, or at the most with some wetting agent.

A: Roy Bateman, Imperial College, -IPARC,UK

According to the Crop life International classification "technical material (TC)" is a legitimate formulation, but I agree that further formulation of *Trichoderma* will indeed be required. One option to consider would be the "twin pack" method used for *Ampelomyces quisqualis* products against grape powdery mildew. In this case, the dry spore powder is premixed by the farmer with emulsifiable oil, before mixing with water for the spray tank.

Q: Bryan Bailey, ARS-USDA, USA

Comment: take note of buffering capacity when screening.

How does pH optimum for *M. roreri* (pH 7) germination interpret with the pod pH (9) observed by Dr. Krauss?

A: Mirjam Bekker, CABI Bioscience, Costa Rica

The optimum values of pH for germination were found to be between pH 6-8. Observations made by U. Krauss showed average pH values of 9.5 for cherelles, for mature pods this was 8. Only based on pH one would expect more infection of mature pods. This is not the case; infection of pods depends on more factors than pH of the exterior of the pod alone

4.1. Farmer field schools and the management of cocoa pests and diseases in Ghana

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SUMMARY

A major bottleneck in the management of the major pests and diseases of cocoa in Ghana is farmers' lack of knowledge of the dynamics of these biological agents. This is often a consequence of the top-down extension approach used by agents in their interactions with farmers. Farmers are often told what to do with little attempt to explain the underlying principles involve in the control and management practices. Farmer field schools (FFS), an experiential farmer training and learning model based on adult education principles have been used successfully in South-Asia in particular and elsewhere to educate farmers in the control of major pests and diseases. FFS was introduced in Ghanaian cocoa extension recently in pilot projects in the Central and Ashanti regions. This paper examines how the FFS approach could enhanced farmers' knowledge of the management of the major cocoa pests and diseases in Ghana.

INTRODUCTION

Agricultural extension as an activity has been used as an instrument to induce change in agricultural communities over the years. The transfer of technology (ToT) model of agricultural extension by which technological innovations are transferred from research to farmers via extension agents has been the dominant mode of information transfer until quite recently. It is now largely acknowledged that farmers are not merely passive receivers of the ideas of scientists; they are active researchers and experimenters themselves (Röling, 1994). This perception has led to the desire to involve farmers more in agricultural research and extension supported by the growing realization that the socio-economic and agro-ecological conditions of farmers are complex, diverse and risk-prone, and that conventional approaches such as the ToT model are unlikely to be fruitful (Farrington, 1998).

In response to these concerns, a number of participatory approaches have been adopted in the past in national research and extension systems to involve farmers in the research and technology dissemination processes, including, farming systems research and extension (Simmonds, 1986; Wiggins, 1995; Jiggins, 1981; Chambers, 1980, 1986; Tripp, 1989; Biggs, 1984), farmer participatory research (Okali et al., 1994; Tripp, 1989; Sumberg and Okali, 1988; Richards, 1986) and the farmer first and last (FFL) model (Chambers and Ghildyal, 1985). Each approach has had its fair share of criticisms (Baah, 2001a). The consensus appears that these approaches have not integrated and empowered farmers enough for them to reap the benefits of the technology development and dissemination processes. A popular model at the moment is the farmer field schools (FFS) approach which appears to have won the hearts of farmers and development workers alike. It is an experiential learning approach originally developed in Southeast Asia for the integrated management of rice pests but adaptable to other crops and even to livestock and fisheries. FFSs are

intensive, season-long programmes where farmers meet regularly to learn and experiment on a given topic.

FFSs have shown remarkable impacts in terms of reducing farmers' use of pesticides for environmental and health benefits, increasing their on-farm productivity, improving knowledge gain among farmers, and empowering rural communities (Davis, 2006; van de Fliert, Pontius and Röling, 1995). However, the model has come onto the spotlight for criticisms in recent times because of its perceived limited effect beyond the local level, FFS graduates' inability to share their knowledge and skills with non-participants (Davis, 2006) and its high cost of implementation which raises questions about sustainability (Feder, Murgai and Quizon, 2003). In other words, questions are being asked whether the FFS is value for money.

FFS in Ghanaian Cocoa

Cocoa remains an important sector in the Ghanaian economy contributing 4.35 to agricultural GDP in 2004 (ISSER, 2005). Low yields on farmers' farms relative to those obtainable in neighbouring Cote d'Ivoire and Malaysia have been a source of concern. Efforts to increase yields on farmers' farms have included the institution of a national control of cocoa pests and diseases and increases in the producer price paid to farmers. Extension remains the key instrument of transfer of research results to farmers. Cocoa research institute of Ghana (CRIG) collaborated with Conservation International, an international non-governmental organization, the national Integrated Crop and Pest Management (ICPM) unit of the Ministry of Food and Agriculture (MOFA) to introduce the FFS model in cocoa production.

An important objective of this initiative was to use the FFS as a platform for information exchange and interactive communication between farmers, researchers, extensionists and other stakeholders in the cocoa sector. Communities around the Kakum National Park in the Central region were chosen for the pilot phase. The project commenced in March 2001 and ended late 2003 and reviews are provided in Baah et al. (2003) and Osei-Bonsu et al. (2003). Following the perceived success of the Kakum study, USAID[‡‡‡] provided funds through the sustainable tree crops programme (STCP) for its extension in the Atwima and Amansie West districts of Ashanti region. This programme commenced in 2003 and is on-going and is the focus of this study which had the objectives of identifying constraints to higher productivity, analyzing farmers' information sources and linkages with other stakeholders and evaluating the impact of the FFS programme on farmers' knowledge and management of cocoa pests and diseases.

Major cocoa pests and diseases in Ghana

Capsids, also known as mirids (Heteroptera: Miridae) are the most economically important insect pests of cocoa in Ghana with crop losses estimated at 25-30% per annum (Wills, 1962). The two main species are *Sahlbergella singularis* Hagl and *Distantiella theobroma* (Dist.) and occur in low overall densities but often aggregate in 'pockets' created by a break in the cocoa shade canopy followed by growth of vegetative chupons and fan tissue. Feeding by cocoa mirids is characterised by dark markings referred to as 'lesions' on pods and shoots and result from the collapse of

[‡‡‡] United States Agency for International Development

plant tissue caused by the toxic saliva (Entwistle, 1965). Although integrated control strategies are being adopted to control capsids, control with insecticides predominates.

Black pod disease is caused by the fungus *Phythopthora* and until recently, the main species was *palmivora* which cause losses estimated at 4.9-19% (Dakwa, 1987). Damage caused by the black pod disease has assumed alarming proportions following the emergence of the more virulent *P. megakarya* which is advancing steadily to the all-important southwest of the Ghana cocoa belt (Opoku et al. 1997).

There are other pests and diseases of cocoa in Ghana of major economic importance (for instance, cocoa swollen shoot virus disease) but from the perspective of the farmers encountered in this study, capsids and black pod are the major pest and disease respectively.

METHODS

A mixed method approach (Neuman, 2003) involving the use of focus group discussions, questionnaire survey and in-depth interviews was used to elicit information from farmers. Twenty-five focus group discussions were followed by the administration of questionnaires on 278 randomly selected cocoa farmers using a two-stage stratified sampling procedure, the sample size being determined by procedures suggested by Casley and Kumar (1989). After the questionnaire survey, 10 farmers were purposively selected and interviewed for further clarification and insight. The study was carried out in ten villages in Atwima and Amansie West districts of Ashanti region, Ghana between November 2004 and May 2005. The questionnaire data were analyzed using the statistical software for the social sciences (SPSS version 11.5).

RESULTS

Sample Characteristics

Studies have shown that cocoa farmer characteristics such as age, marital status, number of children and level of education have a bearing on farm management behaviour and decision making processes (such as adoption), and hence agricultural output (Donkor, Henderson and Jones, 1991; Boahene, 1995). The sample was 'gender sensitive' in that both sexes were fairly represented (Table 1). Majority of the farmers were married (79%) with a mean of 9 (the median and mode were 6) children.

Table 1: Personal characteristics of sample

VARIABLE	CHARACTERISTIC
Gender: Male Female	59.7% 40.3%
Age groups (years)	2.9%: 20-29 years 13.1%: 30-39 years 28.0%: 40-49 years 27.6%: 50-59 years 28.4%: 60 and above years
Marital Status	79.5%: Married 3.6%: Single 8.3%: Divorced 8.3%: Widowed
Number of Children (mean)	9.5

Number of Children with own farms (mean) Number of children helping respondent on the farm (mean)	4.4 4.9
Level of education (categories)	33.1%: None 23.7%: Primary 35.3%: Junior secondary 4.7%: Senior secondary 2.2%: College/University

Source: Survey data. Percentages may not add up to 100 because of non-response.

Over half the sample (56%) was aged 50 years and above, reflecting the ageing nature of Ghanaian cocoa farming population (Table 1). In terms of education, majority (66%) had some form of education ranging from the basic primary education to college or university. Gender correlated positively and significantly (P<0.01) with education (Cramer's V = 0.316, P=0.000) and the differences between men and women were significant (χ^2 = 55.653, df = 10, P <0.01). Age also showed a positive correlation with level of education (Cramer's V = 0.358, P<0.001).

Socio-economic Characteristics

Majority of the farmers (64%) had been in cocoa cultivation for 10 years or more (Table 2) with 15% belonging to a farmer association. Farming experience showed a positive correlation with membership of farmer association (Cramer's V = 0.195, P<0.001). Cocoa farmers could be classified into 3 production classes: *low class farmers, medium class* and *high class* on the basis of their level of management and ultimately, yield (see FAO/World Bank, 1986). In this sample, 62.7% were low class, 31.0% medium and only 6.3% in the high class category (Table 2). Class of farmer correlated positively with level of education (Cramer's V = 0.298, P <0.001) and farming experience (Cramer's V = 0.224, P<0.001).

The mean yield of dry cocoa beans was 111.1 kg/ha (Table 2). This was quite lower than the 250-350kg/ha often reported (e.g., FAO/World Bank, 1986; MASDAR, 1998; Donkor et al, 1991; Appiah, 2004). Highly significant differences were found between gender and yield (t = 3.138, df = 271, P = 0.002) and between class of farmer and yield (kg/ha) (F=11.315, P = 0.000). Farmers do obtain substantial income from non-cocoa sources (Table 2).

Table 2: Socio-economic characteristics of sample

VARIABLE	CHARACTERISTIC
Total land size (ha). Mean	14.7 (S.D. 88.83) (median = 4.0)
Total land under cocoa (ha)	8.4 (S.D. 66.23) (median = 2.0)
Yield (kg/ha)	111.4 (S.D. 150.1) (median = 63.2, mode = 62.5)
Proportion of annual income from cocoa (mean)	69.31 (S.D. 99.455) (median = 60)
Proportion of annual income from other farming (mean)	40.29 (S.D. 102.079) (median = 30)
Proportion of annual income from non-farming activities (mean)	21.7 (S.D. 103.743)

Source: Survey data

Constraints analysis

The study sought to identify factors militating against farmers in their quest to raise farm-level productivity. The results are as shown in Table 3.

Table 3: Constraints mentioned by cocoa farmers, Ashanti, Ghana

CONSTRAINT	% REPORTING
Weeds Infestation	1.4
Capsid infestation	6.0
Mistletoes infestation	5.1
Lack of credit	20.3
Inadequate labour	7.8
Inadequate extension support	9.9
Low yield	6.8
Black pod infestation	9.9
Termite Infestation	0.6
Inadequate supply of improved seeds	7.4
Inadequate knowledge of cocoa practices	8.8
Inadequate spraying machines	1.0
Inadequate pruners	1.0
Bush fires	0.4
Surface mining activities	13.6
TOTAL	100.0

Source: Survey data

Lack of access to institutional credit is the main stumbling block to farmers' attempts to improve the maintenance of their farms. Farmers reported that the lack of credit compel them to use the services of Sherlock money lenders with often disastrous consequences including the forfeiture of their farms when there are drown by mounting interests on the loans. In addition, farmers were concerned by the widespread incidence of pests and diseases (see Table 3) especially black pod.[§§§]

FFS and non-FFS farmers compared

Because the FFS was meant to raised farmers awareness and knowledge of practices associated with cocoa cultivation, attempts were made to find out if FFS graduates fared better in these matters compared to non-participating farmers. In terms of awareness, respondents were asked a number of questions related to cocoa research recommendations made by CRIG[****]. Their responses were then summed into an 'awareness index'. Using the t-test for independent samples, no significant differences ($P < 0.05$) were found between the two groups ($t = 0.873$, df (degree of freedom) = 274, P (asymptotic significance, 2-sided) = 0.383). Using similar procedure, the study found no significant differences ($P<0.05$) between the groups in respect of yield of dry cocoa beans per ha ($t = 0.73$, df = 274, df = 272, $P = 0.383$.

Table 4: FFS versus non-FFS farmers

[§§§] Farmers suggested that since the inception of the national programme to control cocoa pests and diseases (CODAPEC)

[****] Cocoa Research Institute of Ghana

CRITERIA	FFS FARMERS* (%)	NON-FFS FARMERS** (%)
Frequency of weeding		
Once	1.4	12.6
Twice	51.1	56.3
Thrice	43.3	29.6
Four times	4.3	1.5
Spraying against capsids?		
Yes	88.7	87.4
No	11.3	11.9
Yield (kg/ha)	192.08 (288.10)	218.92 (215.71)

Source: Survey data.
* N =141; ** N = 135. Figures in parentheses are standard deviations.

However, highly significant differences were found between the groups in relation to their carrying out of field activities on their farms. For instance, FFS farmers carried out more frequent weeding than non-FFS farmers (χ^2 = 21.31, df = 4, P<0.001). Differences in terms of spraying the cocoa against capsids (mirids) were however not significant (P<0.05) (χ^2 = 1.351, df = 4, P = 0.853) (see Table 4). Also FFS graduates generally exhibited higher knowledge of the cocoa ecosystem especially knowledge of desirable cocoa shade trees species and the relationship between shade regimes and black pod incidence on the farms. They also showed better understanding of the reasons behind some research recommendations. For instance, they could explain why research recommends spraying of farms to control black pod and capsids at certain months of the year.

DISCUSSIONS

This study found no significant difference between FFS and non-FFS farmers in terms of awareness and yield. This contradicts findings from the Kakum studies (Baah et al, 2003) where in these aspects, FFS farmers showed significant advances over the others. Perhaps the Ashanti FFS is still quite young and need time for whatever skills and knowledge that the farmers have learnt to be translated into yield and other indicators. Nevertheless, the findings are in consonance with those of Tripp, Wijeratne and Piyadasa (2005) in their evaluation of an FFS project in Sri Lanka.

Whilst FFS participants did not exhibit significantly higher levels of awareness of certain issues in the Ghanaian cocoa sector, they showed greater knowledge of the working of the cocoa ecosystem including the relationship between shade and black pod disease management, the role of natural enemies in the control of cocoa pests and their farms appeared better management in terms of pest and disease control compared non-participants. A key indicator of success of the FFS model is the facilitation of farmer-to-farmer information exchange and learning (Pontius et al., 2002; Simpson and Owens, 2002). This study found very little evidence of horizontal farmer - to- farmer dissemination of information, knowledge and skills acquired from the FFS programme within the cocoa communities. This is quite worrying because FFS groups are expected to survive beyond project phases into viable community development groups (Pretty and Ward, 2001), a process which hinges on how well the FFS groups integrate into the larger farming community.

Experiences elsewhere (Tripp et al., 2005; Röling and van de Fliert, 1994) indicate that in many instances it has been difficult to scale up FFS beyond the project phase because 'FFS requirements of well-trained facilitators, season-long courses, and first-hand participation make the economics of scaling up problematic' (Tripp et al., 2005:1717). Nevertheless, given the potential benefits of enhanced farmer understanding and knowledge of the various management practices associated with cocoa, especially the management of pests and diseases which training regimes such as the FFS provides, it is imperative that the necessary enabling environment be provided to enhance the scaling up of this approach to farmer training and education. In this regard, the major challenge appears to be finance because the initial cost of an FFS programme appears to be high relative to other extension methods (Bruin and Meerman, 2001).

CONCLUSIONS

The search for approaches to meet the information needs of different farmer categories is likely to continue as new challenges emerge. There is no doubt however that no single approach is likely to suit the different farmers and their environments. Approaches that seek to empower farmers to become better managers of their farms and the environment are more likely to be useful than those which merely 'dish out' information to farmers. The FFS has been widely adopted because it has worked in a wide range of institutional and environmental contexts. It offers a platform for creative learning, enhanced awareness and group interaction (Bruin and Meerman, 2001). Farmer Field Schools may not be the ultimate solution in the search for 'ideal' extension strategy or approach but what perhaps sets the FFS apart from other extension approaches is that it has tended to empower farmers to make more informed decisions about their farm enterprise. As Bruin and Meerman (2001) indicated, the FFS should be used to complement other approaches that have the capacity to cater for the needs of individuals and farmer groups by responding to their concerns and needs. The FFS could be used countrywide in the Ghanaian cocoa sector to enhance the effective management of cocoa pests and diseases on farmers' farms.

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REFERENCES

Appiah, M.R (2004). Impact of Cocoa Research Innovations On Poverty Alleviation in Ghana. Fellowship Inaugural Lectures, Ghana Academy of Arts and Sciences, Accra.

Baah, F (2001a). Farmer participatory approaches towards cocoa IPM implementation. In: J. Vos and P. Neuenschwander (Eds). Proceedings of the West Africa Regional Cocoa IPM Workshop, Cotonou, Benin, Nov. 13-15, 2001, pp 30-38.

Baah, F., Osei-Bonsu, K., Afrifa, A.A., Afreh-Nuamah, K and Amoako-Gyedu, G (2003). Farmer Field School as Extension Strategy in the Ghana Cocoa Industry: Prospects and Challenges. In: Proceedings, 14th International Cocoa Research Conference, Accra, pp. 1177-1183.

Biggs, S.D (1984). Agricultural Research: A review of social science analysis. Discussion paper 115, School of Development Studies, University of East Anglia, Norwich.

Boahene, K (1995*). Innovation Adoption as a Socio-economic Process: The Case of the Ghanaian Cocoa Industry*. Amsterdam: Thesis Publishers.

Bruin, G.C.A and Meerman, F (2001). New ways of developing agricultural technologies: the Zanzibar experience with participatory integrated pest management. Wageningen University / CTA, 167 pp.

Chambers, R (1980). The small farmer is professional. *Ceres* **13** (2): 20-23.

Chambers, R (1986). Normal professionalism, new paradigms and development. IDS Discussion paper No. 227, IDS, University of Sussex, Brighton.

Chambers, R and Ghildyal, B.P (1985). Agricultural research for resource-poor farmers. The Farmer First and Last model. *Agricultural Administration*, **20**:1-30.

Casley, D.J and Kumar, K (1988). *The Collection, Analysis, and Use of Monitoring and Evaluation Data*. Washington D.C: The World Bank / John Hopkins University Press.

COCOBOD (1995). Report on causes of decline in cocoa production and measures to revamp the industry. Accra: Ghana Cocoa Board (COCOBOD), 103 pp.

Dakwa, J.T (1987). A serious outbreak of black pod disease in marginal area of Ghana. *Proc. 10th Int. Cocoa Res. Conf.,* Santo Domingo, 447-451.

Davis, K (2006). Farmer Field Schools: A Boon or Bust for Extension in Africa? *Journal of International Agricultural and Extension Education*, **13** (1): 91-98.

Donkor, M.A., Jones, A.P and Henderson, C.P (1991). Survey to Quantify Adoption of Cocoa Production Technologies in Ghana, FSU Research Report 3, Tafo: Cocoa Research Institute of Ghana.

Entwistle, P.F (1965). Entomology. *Rep. Cocoa Res. Inst., Nigeria,* 1963-64: 66-72

FAO and World Bank (2000). Agricultural Knowledge and Information Systems for Rural Development (AKIS/RD): Strategic vision and guiding principles. Rome: Food and Agriculture Organisation / The World Bank. Online: www.fao.org/sd/Ex direct/Ex re 0027.htm

Farrington, J (1998). Organisational Roles in Farmer Participatory Research and Extension: Lessons from the last decade. *Natural Resource Perspectives* **27**, London: Overseas Development Institute, 4 pp.

Feder, G., Murgai, R and Quizon, J.B (2003). Sending Farmers Back to School: The Impact of Farmer Field Schools in Indonesia. *Policy Research Working Paper* **3022**, Washington D.C.: The World Bank.

ISSER (2005). State of the Ghanaian Economy in 2004. Legon: Institute of Statistical, Social and Economic Research (ISSER), University of Ghana.

Jiggins, J (1981). Farming Systems Research: New name for old habits or the key to increasing farm productivity? An exploration with reference to sub-Saharan Africa. Nairobi: The Ford Foundation, 16 pp.

MASDAR (1998). *Socio-Economic Study of Cocoa Farming in Ghana*. Consultancy Report, Accra: Ghana Cocoa Board / MASDAR International Consultants.

Neuman, W. L (2003). *Social Research Methods: Qualitative and Quantitative Approaches* (5th Edition). Boston and New York: Allyn and Bacon

Okali, C., Sumberg, J and Farrington, J (1994). Farmer Participatory Research. London: Intermediate Publications.

Opoku, I.Y., Akrofi, A.Y. and Appiah, A.A (1997). The Spread of *Phytophthora*

megakarya on cocoa in Ghana. In: Proceedings, 1st Int. Cocoa Pests and Diseases Seminar, 6-10 Nov, 1995, Accra, Ghana, pp. 98-107.

Osei-Bonsu, K., Baah**,** F., Afrifa, A.A., Amoako-Gyampah, G and Osei-wusu Y.B (2003). The Status of Cocoa in the Agrarian System of Some Rural Communities Surrounding the Kakum Forest Reserve, Ghana. Proceedings of the 14th International Cocoa Research Conference, Accra, pp. 1017-1022.

Pontius, J., Dilts, R and Bartlett, A (Eds) (2002). *Ten Years of IPM Training in Asia – from Farmer Field School to Community IPM.* Bangkok: FAO.

Pretty, J and Ward, H (2001). Social capital and the environment. *World Development,* **29**: 209-227.

Richards, P (1986). What's wrong with farming systems research? Paper for the conference of the development studies association, University of East Anglia, 15-16 September, 1986

Röling, N (1994). The Changing Role of Agricultural Extension. In: Agricultural Extension in Africa, Proceedings of an international workshop, Yaounde, Cameroon, pp 7-20.

Röling, N and van de Fliert, E (1994). Transforming extension for sustainable agriculture: the case of integrated pest management pest management in rice in Indonesia. *Agriculture and Human Values*, **11** (2/3): 96-108.

Simmonds, N. W (1986). A short Review of Farming Systems Research in the Tropics. *Experimental Agriculture*, **22**:1-13.

Simpson, B.M and Owens, M (2002). Farmer Field Schools and the future of Agricultural Extension in Africa. In: Proceedings, 18th Annual Conference of AIAEE, Durban, South Africa, pp. 405-412.

Sumberg, J and Okali, C (1988). Farmers, on-farm research and the development of new technology. *Experimental Agriculture,* **24** (3): 333 – 342.

Tripp, R., Wijeratne, M and Piyadasa, V.H (2005). What should we expect from Farmer Field Schools? A Sri Lanka Case Study. *World Development*, **33** (10): 1705-1720.

Tripp, R (1989). Farmer participation in Agricultural research: new directions or old problem? Discussion paper 256, IDS, university of Sussex, Brighton.

Van de Fliert, E., Pontius, J and Röling, N (1995). Searching for strategies to replicate a successful extension approach: Training of IPM trainers in Indonesia. *European Journal of Agricultural Education and Extension* **1**:41-63.

Wills, J.B (1962). Agriculture and Land use in Ghana. London: Oxford University Press.

Yech, P (2003). Farmer Life Schools in Cambodia. *Leisa*, **19** (1): 11-12.

4.2. Report on an unusual attack on cocoa by a defoliator, *Anomis leona* Schaus (Coleoptera: Noctuidae) in Ghana.

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SUMMARY

Anomis leona Schaus (Lepidoptera: Noctuidae), is considered a minor pest of cocoa in Ghana, causing insignificant damage to leaf flushes at the onset of rains. In February 2006, there were reports of an unusual severe attack of the insect in five of the 16 cocoa growing areas, in the Bibiani district of the Western Region of Ghana. From March to April, 2006 a survey was carried out on farms from each affected area to assess the situation. Two farms were selected randomly in each of the five areas and 100 trees selected from 0.5ha plot demarcated from the farm. A census of infested and uninfested pods was taken, and the infested pods were categorized as severe if the endocarp was eaten to expose the beans, and less severe if the damage was only superficial (restricted to the pericarp). Larvae of the insect within hand height of the trees were recorded. The outbreak covered about 500 hectares and affected about 100 farmers. Larval stages of the insect were implicated and they initially attacked leaves on which the eggs had probably been laid. The attack was initially restricted to young and succulent leaves but it extended unto pods as the leaves aged and hardened. Green pods of all stages were attacked, even though cherelles and younger pods were more susceptible. The pods were damaged as the larvae ate the pericarp and gradually bore through the endocarp to reach the beans, resulting in premature pod ripening and eventual wilting. Severe pod damage ranged from 30% to 50% and larval numbers per tree were between six and nine. Three pathogens, Colletotrichum gloesporium, Lasiodiplodia theobromae and Trachysphaera fructigena were associated with the insect damage. To control the menace, the farmers were advised to spray with any of the recommended insecticides for mirid control in Ghana. Confidor (Imidacloprid) was therefore, applied at 150ml in 55 litres of water per hectare and a 24hr post-treatment assessment showed that the insect had been successfully controlled. For prompt control of future outbreaks, it was recommended that cocoa farmers and extension personnel should be educated on the early detection and reporting of such outbreaks.

INTRODUCTION

Cocoa (*Theobroma cacao*) was introduced into the Eastern region of present day Ghana in the nineteenth century. Since then its cultivation has spread to cover six regions such that by 1998 about 1.2 million hectares of land was under cocoa cultivation in Ghana (Anon, 1998). Presently it accounts for about half the country's foreign exchange earnings with over six million people making livelihoods from it. The cocoa plant is attacked by insects at every stage of development causing considerable reduction in yield especially by mirid pests (Wills, 1962; Stapley and Hammond, 1959). The leaves provide food for larvae of defoliators such as *Anomis Leona* Shaus (Lepidoptera: Noctuidae) (Entwistle, 1972).

Anomis Leona is considered a minor pest of cocoa in Ghana causing insignificant damage to leaf flushes at the onset of rains. The life history of the insect in the laboratory shows that the adult lays eggs three days after emergence which incubates

for seven days and takes 20-28 and 7-8 days for larval and pupal developments respectively (Owusu Manu, 1985; Smith, 1965). According to Smith (1965), it probably causes more defoliation than any other cocoa defoliator. Occasionally, however, there may be some limited feeding on green stems and the pericarps of unripe pods (Entwistle, 1972). The insect is known to cause sporadic attacks on cocoa during which it causes considerable damage to the leaves only leaving the pods, and the plants always invariably recover completely by profuse production of flushes. In February 2006, there were reports of unusual attacks of the insect in five of the 16 cocoa growing areas in the Bibiani district of the Western Region of Ghana. Additional districts in the region were also later found to have suffered the attacks. Although sporadic attacks by this insect have been reported in this region earlier, this seems to be the worst outbreak in recent history. Contrary to previous experiences, leaves were rarely attacked; instead it was pods that were seriously damaged by the larvae.

Due to the severity of the damage, surveys were conducted in the infested areas to assess the damage and any secondary opportunistic infections of the pods. This paper reports the results of the surveys, discusses the control measures taken and recommends measures to be adopted in response to future outbreaks.

METHODOLOGY

Survey

From March to April, 2006 a survey was carried out on farms from each of the following affected operational areas in Bibiani district of Western Region: Lineso, Nzema Nkwanta, Dominebo, Bibiani Old Farm and Wenchi to assess the situation. Two farms were selected randomly in each of the five areas and 100 trees selected from 0.5ha plot demarcated from the farm. A census of infested and uninfested pods was taken, and the infested pods were categorized as severe if the endocarp was eaten to expose the beans, and less severe if the damage was only superficial (restricted to the pericarp). Larvae of the insect within hand height of the trees were recorded. The outbreak covered about 500 hectares and affected about 100 farmers. Damaged pods were incubated in the laboratory at the Cocoa Research Institute of Ghana (CRIG) for pathogens. Impact of the outbreak on yield could not be assessed before the submission of this paper.

Chemical control

After assessing the situation in the plots it was decided to apply CRIG recommended insecticide for cocoa pests to knock off the larvae which were vulnerable because they were exposed. Confidor (Imidacloprid) was therefore, applied at 150ml in 55 litres of water per hectare using motorized knapsack. Farms were assessed 24 hours after insecticide application for the insects.

RESULTS AND DISCUSSION

Infestation levels

The outbreak covered about 500 hectares and affected about 100 farmers. All farms visited were infested by the insect pest to various degrees. . Severe pod damage ranged from 30% to 50% and larval numbers per tree were between six and nine. Post treatment assessment showed that the insects were absent and there was no fresh damage.

Damage

Both leaves and pods were attacked. Larval stages (caterpillars) of the insect were implicated and they initially attacked leaves on which the eggs had probably been laid. The attack was initially restricted to young and succulent leaves (Fig.1A) but it extended unto pods as the leaves aged and hardened. Green pods of all stages were attacked, even though cherelles and younger pods were more susceptible (1B). The pods were damaged as the larvae ate the pericarp and gradually bore through the endocarp to reach the beans (1C & 1D), resulting in premature pod ripening and eventual wilting (1E).Three pathogens, *Colletotrichum gleosporium, Lasiodiplodia theobromae* and *Trachysphara fructigena* were associated with the insect damage.

Fig.1 Effect of caterpillar of *Anomis leona* on young succulent cocoa leaves (A), on pods at different stages of development (B), pericarp of pod (C), boring into pod (D) and on young pods with some showing premature ripening and wilting (E).

A. leona is known to prefer flushes (Smith, 1962) but observation during the survey seems to suggest that softer tissues of the plant especially young pods may be at risk during outbreaks. Outbreaks are usually associated with rise in population numbers; therefore, knowledge of the population cycle of the pest would be useful for predictive purposes. Studies by Owusu Manu (1991) show that the population of the insect changes frequently with peaks differing from month to month in the respective years. Incidentally, the outbreak at Bibiani fell within one such recorded peak periods

(Owusu Manu, 1985 and 1986). While this may suggest interplay between flushes, population peaks and factors such as abundance of fresh tissues as possible precursors to *A. leona* outbreak, the varying peak months suggests significant influence of climatic and edaphic factors in the outbreaks.

Considering the success of the control measure applied, it is expected that farmers would justifiably readily resort to the application of insecticides in future outbreaks. It may also be argued that the exclusion of the area from insecticide application in the government's current pest control programme might have led to the population build up of the insect. However, caution must be exercised to avoid liberal application of chemical insecticides to control the insects, so that the parasitoid natural enemies (Padi, 1994) which generally have been keeping *A. leona* populations low, are not eliminated, to trigger off more outbreaks.

CONCLUSION

Prompt and early application of Confidor (Imidacloprid) at 150ml in 55 litres of water per hectare using motorized knapsack is essential for effective control of *A. Leona* caterpillar outbreak, therefore, cocoa farmers and relevant stakeholders must be educated on early detection and prompt reporting of outbreaks. In as much as chemical application may be necessary as a short term measure, in the long term its use should be minimized and more environmentally friendly and sustainable methods found. Researchers in Ghana should, therefore, carry out detailed studies on the insect for relevant information especially on pest forecasting to help in its control.-

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REFERENCES

Anon (1998). Final report on Cocoa Tree Stock Project. Project GCP/INT/632/COA. Report submitted to the Ghana Cocoa Board and ICCO: 107pp.

Entwistle, P. F. (1972). Pests of Cocoa, Longman Group Ltd. 779p.

Owusu Manu, E. (1991). Miscellaneous Pests. *Ann. Rep. Cocoa Res. Inst. Ghana:* 56-59

Owusu Manu, E. (1986). Miscellaneous; Minor Pests. *Ann. Rep. Cocoa Res. Inst. Ghana.* 70 - 79

Owusu Manu, E. (1985). Miscellaneous; Minor Pests. *Ann. Rep. Cocoa Res. Inst. Ghana:*29-30

Padi, B and Kumah, N. K.(1994). Miscellaneous insects on cocoa: Parasitism of *Anomis leona* Schaus. *Ann. Rep. Cocoa Res. Inst. Ghana, 1991/92*: 181-182.

Smith, M. R. (1962). Minor Pests; Lepodoptera. *Ann. Rep. W. Afr. Cocoa Res. Inst. Ghana,1960/61:* 49

Stapley, J.H. and Hammond, P.S. (1959): Large scale trials with insecticides against capsids on cocoa in Ghana. *Emp. J. Exp. Agric*. **27**: 343 – 353.

Wills, J. B.(1962). Agriculture and land use in Ghana. Oxford University Press, London.

4.3. Delivering integrated pest management technology to cocoa farmers in Ecuador††††

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SUMMARY

It is acknowledged that the integrated pest management strategy is a knowledge intensive activity, it demands from users' full understanding of the plant as a crop, and of the growing conditions. However IPM usually gets to producers as a large instruction list stating what has to be done on his crop. It has not been taking into account that great majority of farmers do not have a good perception of the microscopic nature of disease agents, of changing processes of many insects, neither from the most simple epidemiological processes that may allow them to relate a disease like Witches´ broom or Frosty pod rot with events that happened two, three or more months back. This study intends to share with the cocoa community, attempts made in Ecuador to overcome this bottleneck between generation and use of IPM research results. Some teaching tools about IPM principles, cause-effect relationships of factors that favor diseases, and life cycles of Moniliophthora roreri and M. perniciosa are presented along with testimony collected from farmers that participated on the preparation of these tools.

†††† Full text of paper not available at press time

4.4. Towards effective management of cocoa pests and diseases in Ghana: the role of the radio

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SUMMARY

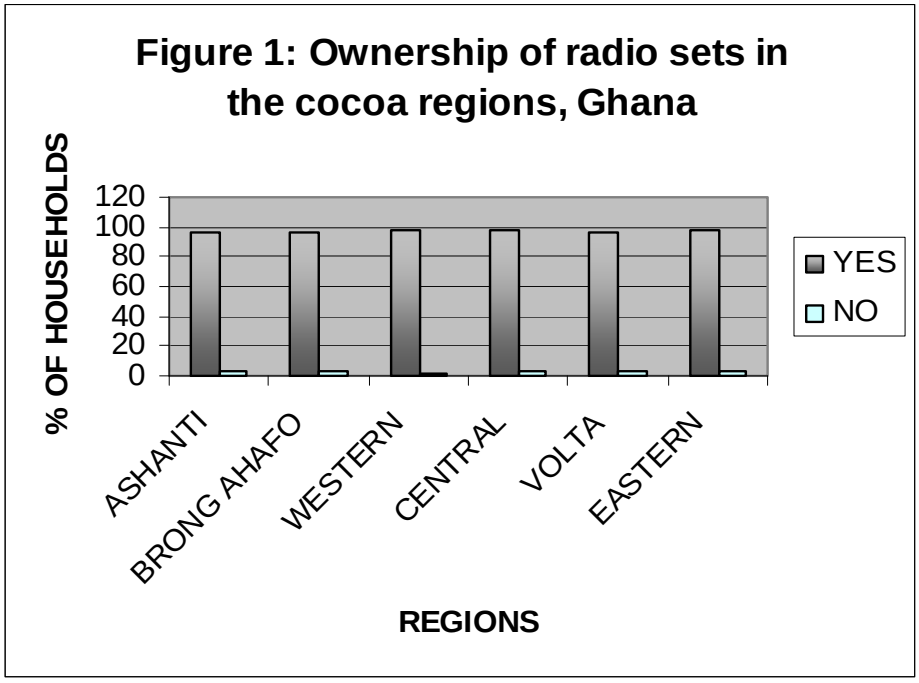
The effective management of cocoa pests and diseases in Ghana remain a key challenge in the effort to raise farm-level productivity. Farmers consider the prevalence of pests and diseases on their farms as a major constraint. Studies have shown that often there is dearth of knowledge among farmers on the nature and control of these biological agents. This situation has not been helped by difficulties in the cocoa extension machinery in recent times following the unification of cocoa extension with mainstream Ministry of Agriculture extension services. Recent developments in the telecommunications sector in Ghana including the liberalisation of the airwaves have resulted in the springing up of several frequency modulation (FM) stations all over the country. These radio stations have shown the potential of providing platforms for the education of cocoa farmers in the management of the major pests and diseases. This paper discusses how this potential could be harnessed by extension and research institutions to enhance interactive communication between them and farmers.

INTRODUCTION

Cocoa continues to play a dominant role in the Ghanaian economy. Production has averaged 370,000 metric tones in the last five years, though the country has the potential to produce more. Constraints to higher productivity include low producer prices, high costs of inputs, inadequate extension support and poor infrastructural facilities among others (COCOBOD, 1995). Information and knowledge are regarded as essential for farmers to respond positively to the opportunities and challenges of the physical, social and policy environments in which they operate (McQuail, 2005). Indeed, it has been said that empowering the poor is about providing them with information (World Bank, 2004), and the demand for agricultural information is stronger than ever (LEISA, 2002). Knowledge gaps and information problems are key constraints to the efficient functioning of markets and equitable growth and development (Garforth, Khatiwada and Campbell, 2003). They also influence the adoption of innovations. Boahene (1995) for example, found that the adoption of hybrid cocoa in Ghana is influenced by lack of information concerning the existence and availability of the hybrid cocoa. Available communication strategies being used in farming communities to promote farmer learning include interpersonal exchanges, group processes (such as farmer field schools), mass media (largely the radio), mixed-media campaigns and in more sophisticated environments, Internet delivery from community telecentres (Coldevin, 2003).

Extension workers have utilized the mass media to provide general and current information on agriculture, nutrition, health and rural development to rural households. Extension through the mass media will only be effective if farmers have access to the medium (Garforth, 1994). The radio remains the most important medium

for communicating with the rural populations of developing countries (Odame and Kassam, 2002). Not all farmers have equal access to all mass media, but many will have access to radio at home. This is very true of most Ghanaian cocoa farmers who regard the radio as a useful companion. In a review of the costs and benefits of information and communication technologies for direct poverty alleviation worldwide, Kenny (2002) suggested that the radio is by far the cheapest electronic communication technology (he quoted an average cost of receivers as $10 or between $70-$100 for the wind-up models; at the time of this study, one could purchase a radio for $5 in Ghana). The radio is a particularly useful mass medium for extension because it is readily available. A recent audience research report by the Ghana Broadcasting Corporation (GBC) indicates that 97.3% of respondents in a nationwide survey own radio sets; 98.7% listen to the radio regularly and 75.0% said they listen to the radio everyday (GBC, 2001). The results for the rural households in the cocoa producing regions are shown in Figure 1 (YES = household owns a radio set; NO = household do not own a radio set).



Source: GBC (2001)

In addition, the radio is relatively affordable, and information can reach households directly and instantly throughout the country. Consequently, the radio has penetrated deep into otherwise inaccessible rural areas (Osborn and Landorthe, 1995). Another advantage of radio communication is that despite its mass audience, a good presenter can make programmes seem very informal and personal, giving the impression that an individual listener is being spoken to directly (Garforth, 1994). The radio however has its limitations. Batteries may be expensive or unavailable, especially in rural areas. There may be no repair facilities when there is breakdown. People often listen to the radio rather casually whilst engaged in other activities (Garforth, 1994). This latter problem may be overcome if prior notice is given of an intended broadcast. The perception that the radio is a one-way flow of information may have been overcome in many communities by improvements in telecommunication allowing call-in programmes (Bennett, 2002).

The objectives of the study were to assess from farmers' perspectives the state of cocoa extension in the context of recent institutional changes (merger of cocoa extension, formerly carried out by the Ghana cocoa Board, with mainstream Ministry of Food and Agriculture extension service with its attendant retrenchment and or retraining of extension staff) in the cocoa sector, identify farmers information sources and provide a general overview of the state of the cocoa-based agricultural information and knowledge system as it affects farmers' ability to effectively manage the major pests and diseases affecting their cocoa.

METHODS

A mixed method approach involving the use of focus group discussions, questionnaire survey and in-depth interviews was used to elicit information from farmers. Twenty-five focus group discussions were followed by the administration of questionnaires on 278 randomly selected cocoa farmers using a two-stage stratified sampling procedure, the sample size being determined by procedures suggested by Casley and Kumar (1989). After the questionnaire survey, 10 farmers were purposively selected and interviewed for further clarification and insight. The study was carried out in ten villages in Atwima and Amansie West districts of Ashanti region, Ghana between November 2004 and May 2005.

RESULTS

Sample Characteristics

Cocoa farmer characteristics such as age, marital status, number of children and level of education have a bearing on farm management behaviour and decision making processes (Boahene, 1995). Fifty nine percent of the sample was male and majority (79%) of the farmers were married with a mean of 9 children (the median and mode were 6). Fifty six percent of the farmers were in the 50 years and over age bracket, reflecting an ageing farming population. In terms of education, majority (66%) had some form of education ranging from the basic primary education to college or university. Gender correlated positively and significantly ($P<0.01$) with education (Cramer's $V = 0.316$, $P=0.000$) and the differences between men and women were significant ($\chi^2 = 55.653$, $df = 10$, $P <0.01$). Age also showed a positive correlation with level of education (Cramer's $V = 0.358$, $P<0.001$).

Majority of the farmers (64%) had been in cocoa cultivation for 10 years or more with 15% belonging to a farmer association. Farming experience showed a positive correlation with membership of farmer association (Cramer's $V = 0.195$, $P<0.001$). Cocoa farmers could be classified into 3 production classes: *low class farmers, medium class* and *high class* on the basis of their level of management and ultimately, yield (see FAO/World Bank, 1986). In this sample, 62.7% were low class, 31.0% medium and only 6.3% in the high class category (Table 2). Class of farmer correlated positively with level of education (Cramer's $V = 0.298$, $P <0.001$) and farming experience (Cramer's $V = 0.224$, $P<0.001$). The mean yield of dry cocoa beans was 111.1 kg/ha. This was quite lower than the 250-350kg/ha often reported (e.g., FAO/World Bank, 1986; MASDAR, 1998; Donkor *et al*, 1991; Appiah, 2004). Highly significant differences were found between gender and yield ($t = 3.138$, $df = 271$, $P = 0.002$) and between class of farmer and yield (kg/ha) ($F=11.315$, $P = 0.000$).

Analysis of constraints

During the focus group discussions, farmers were asked to mention and rank the main constraints militating against them. The results (see Table 1) indicate that limited availability and high cost of institutional credit and the prevalence of pests and diseases are farmers' main concern. Farmers did concede that the level of damage of their cocoa due to capsids and blackpod infestations have ceded since the inception of the national control of cocoa pests and diseases in 2000. In addition, farmers were vociferous about what they perceived to be poor extension support to them since the responsibility for cocoa extension was taken over by the ministry of food and agriculture from the Ghana cocoa board (COCOBOD). This perception perhaps is reflected in their response to the question about their main source of information and advice on cocoa (see Table 3) and their apparent lack of knowledge about basic dynamic relationships in the cocoa ecosystem (for instance, the relationships between shade regimes and blackpod and capsid incidence).

Table 1: Ranked problems of farmers in Ashanti, Ghana

RANK 1	**RANK 2**	**RANK 3**
o High cost of credit o Blackpod disease attack o Capsids infestation o Poor extension support o Termites infestation o Mistletoe infestation	o Surface mining o Ageing trees o High cost of farm inputs including cutlasses o Unavailability of land for new plantings	o Unavailability and high coat of labour o Declining soil fertility

Source: Survey data

Ownership of radio and television sets

Relative to the television, the radio is more affordable to farmers perhaps reflecting the low incomes of many farmers who cannot afford television sets. This study found highly significant differences ($P<0.01$) between men and women in the ownership of radios ($\chi^2 = 27.379$, df = 4, $P = 0.000$) and television sets ($\chi^2 = 14.534$, df = 2, $P = 0.001$) with men more likely to own these gadgets, the differences perhaps reflecting the observed differences in cocoa yields (kg/ha) ($t = 3.138$, df = 271, $P = 0.002$) and incomes ($t = 1.981$, df = 271, $P = 0.049$). Similarly, highly significant differences ($P<0.01$) were found between Atwima and Amansie West farmers and ownership of both radio ($\chi^2 = 48.297$, df = 4, $P = 0.00$, Phi (correlation coefficient) = 0.417, $P = 0.000$) and television sets ($\chi^2 = 7.889$, df = 2, $P = 0.019$). In both cases, Atwima farmers were found to be more likely to own radios and television sets (Table 2), again a reflection of highly significant differences ($P<0.01$) in cocoa yields (kg/ha) ($t = 3.000$, df = 273, $P = 0.003$) and incomes ($t = 2.680$, df = 273, $P = 0.008$).

However, though class of farmer correlated positively with ownership of radio (Cramer's V = 0.099, P = 0.489) and ownership of television sets (Cramer's V = 0.042, P = 0.921), no significant differences ($P<0.05$) were found between the three farmer classes and ownership of radio ($\chi^2 = 5.434$, df = 6, $P = 0.89$) or television sets ($\chi^2 = 0.492$, df = 3, $P = 0.921$).

Table 2: Ownership of radio and television sets by districts

OWNERSHIP OF ELECTRONIC GADGETS	DISTICTS		OVERALL
	ATWIMA	AMANSIE WEST	
	%	%	%
RADIO			
YES	93.6	52.5	66.2
NO	6.4	47.0	33.5
TOTAL	100	99.5	99.7
TELEVISION SETS			
YES	27.7	14.4	18.7
NO	72.3	85.6	81.3
TOTAL	100	100	100
N	94	181	275

Source: Survey data. Percentages may not add up to 100 because of non-response.

Information sources

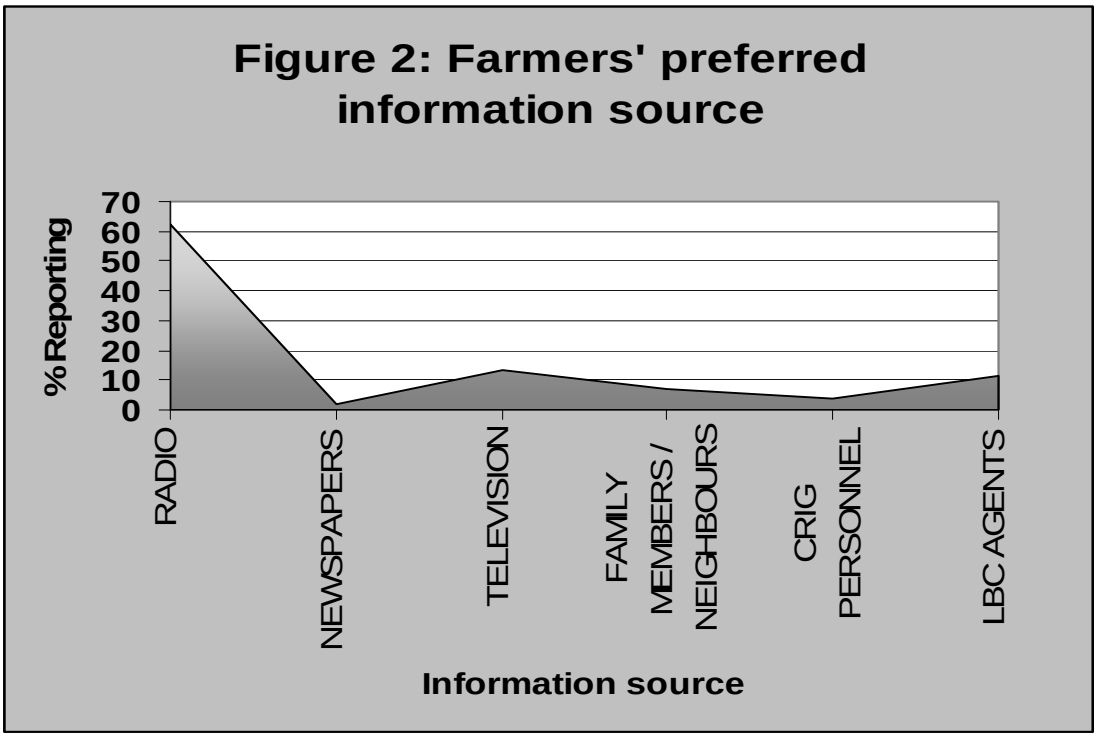
Majority of the farmers (70%) rely on their social networks – friends, neighbours, family members – for information and advice on cocoa. Only 13% regarded extension agents as their main source of information (Table 3). This may be indicative of the poor state of extension support for farmers as evident by the fact that a whopping 84% of farmers reported not having seen an extension agent in a year.

Table 3: Farmers' main source of information and advice

INFORMATION SOURCE	FREQUENCY	PERCENT
Other farmers/family	195	70.1
Extension officers	37	13.3
Licensed buying agent	20	7.2
Input dealers	4	1.4
Other	16	5.8
Not answered	6	2.2
Total	278	100.0

Source: survey data

When farmers were asked to indicate their preference for particular information sources, *the radio* was clearly the preferred information source, with television and agents of the licensed buying companies also featuring well (Figure 2). This study found no significant differences ($P<0.05$) between men and women and their main and preferred information sources ($\chi^2 = 8.025$, df = 10, P = 0.625, main information sources; $\chi^2 = 10.259$, df = 10, P = 0.418, preferred information sources).



Source: Survey data, 2004-2005

Information needs

Farmers revealed during the focus group discussions that their main information needs are:

- Sources of improved seeds, insecticides, fungicides and pruners. Farmers were particular about seeds as they mentioned that use of unproductive seeds in the past is a major cause of their low yields.
- Sources of credit available to cocoa farmers and its cost.
- Information about the effective management of capsids and blackpod disease which they considered their main pest and disease menace respectively.
- Uses to which they could put some of their cocoa processing by-products (such as sweating and pod husks (as they have learnt that these could be converted into useful products such as soaps (the husks), gin, brandy and wine (the sweating)).

DISCUSSION

Farmers consider the continued menace of pests and diseases on their farms as a major challenge. There was no evidence in this study to suggest that farmers possess the requisite knowledge of these biological agents and their effective management. This may be attributed to the poor state of extension support to farmers. The structural changes in extension service support to cocoa farmers which commenced in 2000 with the objective of introducing 'more cost effective extension' (MOF, 2005:1) has unfortunately resulted in many farmers being deprived of extension support. Though farmers make extensive use of their informal social networks of friends, neighbours, relatives and cocoa buying agents to seek for information and advice, the findings of this study suggest that the radio is the key in any effort to reach a mass of the people at the same time, and at a relatively cheaper cost. The potential of the radio to achieve this has been demonstrated elsewhere (Garforth *et al*, 2003; Coldevin, 2003; Odame and Kassam, 2002).

Modern information and communication technology (ICT) such as the Internet remains the technology for the future for many farming communities in the developing world (LEISA, 2002). The radio, an 'old' technology has penetrated deep into otherwise inaccessible rural areas. The radio is a dominant, effective and cost-efficient medium which caters for the information needs of both literate and illiterate populations (Lucas, 1999). The liberalization of the airwaves in Ghana in recent times has raised the status of the radio as a channel of information flow especially to rural communities. The radio is affordable and readily available. Over 90% of the respondents in the Ghana Broadcasting Corporation survey (GBC, 2001) own radios. The same survey showed that less than 10% had television. The radio is thus the channel to reach the poor and inaccessible (Kenny, 2002). Baah (2003) showed that there are large numbers of radio stations in the cocoa producing regions already broadcasting agricultural programmes, which could be used as a platform for tailor-made information to cocoa farmers to enhance the effectiveness of their management of the main pests and diseases.

There was little evidence from the perspective of farmers of interactive communication between them and researchers and other key stakeholders in the cocoa sector. It is suggested that the radio could be used as a platform to remedy this situation. Farmers' display of lack of in-depth knowledge about several aspects of cocoa pests and disease management could be addressed through programmes (on the radio) which promote interactive communication between them and especially researchers and extension personnel. The criticism of the radio as a one-way flow of information has been largely invalidated by improvements in telephone communication all over the country. Farmers can and do respond to broadcasts suggesting that they could potentially influence the contents of radio programmes. However, a lot of work needs to be done to make the radio more participatory and relevant to the needs of farmers. The mere existence of a number of radio stations does not equate to relevant, incisive and timely information provision.

At the moment, the radio airwaves are dominated by privately owned and controlled frequency modulation (FM) radio stations with a commercial agenda. Nevertheless, the FM stations in the cocoa producing regions have demonstrated (Baah, 2003) their willingness to accommodate the interests of farmers. In this regard, audience research needs to be undertaken to ascertain the kinds of information that farmers want, the format in which it is to be delivered, and the times and frequency of broadcasts that is desirable. Farmers must be involved in the development of programmes to enhance ownership and community identification with the communication products, an important pathway to the establishment of community-based radio broadcasting. Such radio broadcasts will be complementing other communication strategies including individual farm visits, open days, on-farm studies and use of leaflets and pamphlets.

CONCLUSIONS

The radio has broad appeal. It is relatively cheap and accessible to most rural households. It has helped to change rural communities worldwide by making them aware of developments around them. Improvements in telecommunications have removed a major drawback of the radio, namely the inability of listeners to respond to broadcasts. Now listeners can potentially influence the agenda for programmes. It is suggested that on the basis of the evidence adduced in this study, the radio should be the main channel through which cocoa farmers are provided with needed information

about the management of the main pests and diseases plaguing their farms. In addition, the use of the radio on a sustained basis through educative and farmer-centred programmes involving all the key stakeholders in the cocoa sector will enhance stakeholder interactions and communication.

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REFERENCES

Appiah, M.R (2004). Impact of Cocoa Research Innovations on Poverty Alleviation in Ghana. Fellowship Inaugural Lectures, Ghana Academy of Arts and Sciences, Accra.

Baah, F (2003). Dissemination Pathways for Cocoa Research Results in Ghana: The Potential Role of the Radio. Unpublished report, Tafo: Cocoa Research Institute of Ghana.

Bennet, N (2002). Connecting farmers world wide through radio. *LEISA*, **18** (2): 20-21

Boahene, K (1995). Innovation Adoption as a Socio-economic Process: The Case of the Ghanaian Cocoa Industry. Amsterdam: Thesis Publishers.

Casley, D.J and Kumar, K (1988). *The Collection, Analysis, and Use of Monitoring and Evaluation Data*. Washington D.C: The World Bank / John Hopkins University Press.

COCOBOD (1995). Report on causes of decline in cocoa production and measures to revamp the industry. Accra: Ghana Cocoa Board (COCOBOD), 103 pp.

Coldevin, G (2003). Participatory Communication: Key to Rural Learning Systems. Rome: FAO.

Donkor, M.A., Jones, A.P and Henderson, C.P (1991). Survey to Quantify Adoption of Cocoa Production Technologies in Ghana. FSU Research Report 3, Tafo: Cocoa Research Institute of Ghana.

FAO/World Bank (1986). Draft Report on Third Cocoa Project for Ghana. Ghana Agricultural Sector Review, World Bank Report No. 5366-GH, Washington D.C: The World Bank.

Garforth, C (1994). FAO Guide to Extension Training. Draft Chapter: Extension and communication, pp.1-37, Rome: FAO

Garforth, C., Khatiwada, Y., and Campbell, D (2003). Communication Research to Support Knowledge Interventions in Agricultural Development: Case Studies from Eritrea and Uganda. Paper presented at the Development Studies Association Conference, Glasgow, 10-12 September 2003.

GBC (2001). Audience Research Report on Nationwide Survey on Radio and Television. Accra: Ghana Broadcasting Corporation (GBC)

GoG (1995). Government of Ghana. Report on Causes of Decline in Cocoa Production and Measures to Revamp the Industry. Accra: Government of Ghana (GoG).

FAO/World Bank (1986). Draft Report on Third Cocoa Project for Ghana. Ghana Agricultural Sector Review, World Bank Report NO. 5366-GH, Washington D.C.: The World Bank.

FAO/World Bank (2000). Agricultural Knowledge and Information Systems for Rural Development (AKIS/RD). Rome: Food and Agriculture Organization. Online: www.fao.org/sd/Exdirect/Ex re 0027.htm [08/10/03].

Kenny, C (2002). Information and Communication Technologies for Direct Poverty Alleviation: Costs and Benefits. *Development Policy Review,* **20** (2): 141-157.

LEISA (2002). Changing Information Flows. Editorial Introduction, *LEISA* **18** (2): 4-5

Lucas, F.B (1999). Rural Radio in the Philippines. Online: http://www.fao.org/sd/Cddirect/Cdan0026.htm

MASDAR (1998). Socio-economic study of cocoa farming in Ghana. Consultancy Report, Accra: Ghana Cocoa Board / MASDAR International Consultants

McQuail, D (2005). Mass Communication Theory (5th Edition). London: Sage Publications.

MOF (2005). Report of the Review of Cocoa Extension under Ministry of Food and Agriculture (The Cocoa Extension Review Committee). Accra: Ministry of Finance (MOF), Ghana.

Odame, H.H and Kassam, A (2002). Listening to Stakeholders: Agricultural Research and Rural Radio Linkages. ISNAR *Briefing Paper* **48.** The Hague: ISNAR.

Osborn, P. and Landorthe, A. (1995). A Review of the Trends in the Supply of Information for Rural Development from Europe to ACP countries. In: Proceedings, International Seminar on The role of information for rural development in ACP countries: review and perspectives, Montpellier, France, 12-16 June 1995, pp. 139-155

World Bank (2004). Democratizing HIV Communication: *Development Outreach*. Washington D.C: The World Bank. Online: http://www1.worldbank.org/devoutreach/july04/extonly

Discussion on Other Pests and Technology Transfer

Chairperson: K. F. N'Guessan, Cote d'Ivoire

Q: Roy Bateman, IPARC, Imperial College, UK

Why did you choose a neonicotinoid such as imidacropid for your control campaign?. Wouldn't a pyrethroid have been cheaper?.

Do you have field trial evidence on your lower inpact on natural enemies?. If so, where have these trials been reported?.

A: J.E. Sarfo, CRIG, Ghana.

We chose imidacropid because it has been tested and recommended for use against mirids in Ghana. In fact, it is one of the insecticides being applied in the national cocoa pests control programme. In terms of cost, I am not sure a pyrethroid would have been cheaper than the neonicotinoid used.

In testing the insecticide, its impact on the environment especially, on natural enemies and insect pollinators was assessed and it was found not to be deleterious. There are a lot of publications on the field trials of imidacropid in the Annual reports of the Cocoa Research Institute of Ghana.

Comment: J.B. Ackonor, CRIG, Ghana

As a contribution to Sarfo's answer to Roy Bateman's question, I would want to indicate that the imidacropid (confidor) has been tested for 4 years and found effective against mirids and other cocoa pests including *Anomis leona*. The product was the one readily available in the outbreak area at the time of the outbreak. Since we needed to immediately control the situation we used what was readily available to remedy the situation.

Comment: Prakash Hebbar, Mars Inc., USA

There is the need for better forecasting methods to modernize cacao pests and diseases management. At present, very little effort is being made.

Comment: J.E. Sarfo, CRIG, Ghana.

That is true. In Ghana, we hope to develop methods to forecast mirid incidence from our pheromone work.

Comment: Prakash Hebbar, Mars Inc., USA

Secondly, chemical (pesticides/fertilizer) companies do a good job of selling and advertising their products. Those of us who advocate for IPM do a relatively poorer job of advertising.

Comment: J.E. Sarfo, CRIG, Ghana.

I agree with you perfectly well. We are poor at selling and advertising ourselves. We need to do better than we are doing now.

Q. Solomon Kofi Addo, Barry Callebaut, Ghana

Based upon my experience as a pod counter, the occurrence of this insect appeared well before February, 2006. My major concern based upon early detection is whether there are any future control measures?

A: J.E. Sarfo, CRIG, Ghana.

For early detection, we have plans to educate all stakeholders such as farmers, extension officers, and district agricultural and cocoa officers on the identification of the insect, its damage and the possible periods of occurrence.

For future control of outbreaks, we have recommended that Ghana COCOBOD make available at all the districts, reserve stocks of insecticides, fuel and knapsack mist blowers for any eventuality. Meanwhile, scientists at CRIG are engaged in detailed studies on the insect for information to enable

us come out with a monitoring scheme that would make it possible to forecast future outbreaks.

Q. Solomon Kofi Addo, Barry Callebaut, Ghana

Has any seasonality in occurrence been observed for the insect in Ghana?

A: J.E. Sarfo, CRIG, Ghana.

They are known to occur when there are new flushes at the beginning of the rainy season or when there is precipitation immediately after a period of dryness.

Q. J. L. Pereira, CEPLAC, Brazil

During the better days when growers received subsidies for purchase of agrochemicals and equipment, IPM was followed to the full. While I agree that we have deficiency in technology transfer and level of farmers' knowledge in the project you mentioned was there a funding component and due to which the farmers followed the recommendations to the full?.

A: Carmen Suarez, INIAP, Pichilingue, Equador

We have taken care of not giving farmers anything but the knowledge to avoid what is considered one of the reasons for projects relative failure. We are only developing with them and putting in their hands tools to promote awareness of the biological processes in the orchards.

Q. N'guessan Kouame Frances, CNRA, Cote d'Ivoire

All the cocoa producing countries in West and Central Africa (Cote d'Ivoire, Ghana, Nigeria and Cameroon) have been trying the FFS method through the STCP programme. I know Ghana is ahead and has more experience in this area. Can you comment on any foreseeable impact on the farmers and to touch on all cocoa producers?

A: Francis Baah, CRIG/Univ. of Reading, Ghana

The prospects of the FFS on cocoa are bright. In the Indonesian model, there was strong governmental support including the promulgation of a presidential decree which provided budgetary and other support to the incorporation of the FFS into government extension services delivery. Governments in West and Central Africa need to do this. Over-reliance on donor support will hamper progress.

Q: H. Dzahini Obiatey, CRIG/Univ. of Reading, Ghana

Have you had time to go back to the areas where the radio programme was done to assess the impact of the programme? If not, when and how do you intend to do this?.

A: Francis Baah, CRIG/Univ. of Reading, Ghana

The CRIG radio programme in which cocoa farmers were educated on selected radio stations was evaluated in 2003. We found out that many farmers wanted the programme to continue and that they rated the information they received and the exchange highly. I am happy to mention that this programme has been revitalized and it is on-going at the moment. Besides the radio, the development of the participatory video as mentioned by Mr. Akrofi at the cocoa conference and my presentation on the FFS are other approaches being developed to improve interactive communication between researchers and farmers.

Reports from INCOPED Regional Sub-groups

A. Report of the African Sub-group

Dr. I.Y. Opoku of the Cocoa Research Intitute of Ghana presented the report for the African sub-group. He indicated that the major fungal diseases of cocoa are *Phytophthora* pod rot, stem canker, thread blight and pink disease in that order of importance whilst the cocoa swollen shoot is the most important viral disease. Mirids and stem borers were reported as the important insect pests.

In the African sub-region, black pod disease, particularly, that caused by *Phytophthora megakarya* is the most important fungal disease. It is managed by combining farmer education, sanitation practices, fungicide application and the use of tolerant varieties. As a long term measure, efforts are being made to select and breed cocoa varieties for resistance to the disease. Currently, there is a sub-regional project in Ghana, Cote d'Iviore, Cameroon and Nigeria funded by the CFC, ICCO and IPGRI to screen various cocoa clones and progenies for resistance to the disease. A similar project sponsored by the European Union (EU) under EU/ STABEX project in Ghana focused on collecting clones apparently resistant to the black pod disease from farmers' farms in *P. megakarya* endemic districts and budded them unto Amelonado root stocks for further evaluation. On biocontrol of the disease, i*n-vitro* studies have shown that some potential biological agents against the black pod pathogen exist, but tests with the agents in the field have been on a very limited scale. A range of soil microorganisms that produce antibiotics against *Phytophthora in-vitro* have been reported including bacteria, actinomycetes and fungi.

Phytophthora megakarya and *P. palmivora* stem cankers have also become a major disease in the West African sub-region, particularly, in Ghana. Multiple cankers with large lesions are more common with *P. megakarya* infections than with *P. palmivora*. Stem cankers are managed by regular inspection of farms and removal of pods infected with the black pod disease; a measure that prevents the fungus from growing into the flower cushions through the peduncle to cause the canker. Infected barks are also treated by scrapping to expose the lesion to light. The exposed lesions could either be painted with slurry of fungicide or left to dry up; a method that has been found to be very effective, particularly at the early stages of infection. It is also recommended that trees and branches with severe and/or multiple infections are removed and burnt.

Pink disease caused by *Corticium salmonicolor* Berk. and Broome and thread blight disease caused by *Marasmius scandens* Mass which were hitherto considered as minor diseases have now become important diseases, particularly in Ghana The pink disease is controlled by the removal and burning of all infected branches. Affected branches are removed about 5-10 cm below the apparent point of infection and burnt immediately. This is followed by spraying at 3-weekly intervals with Ridomil 72 plus. In order for the fungicide to reach the canopies where the disease is prevalent, motorized spraying machines are used. Farmers are also being educated on the identification, prevention and control. An illustrated manual on the identification and control of major and minor diseases is in preparation to facilitate the education process.

Black and white thread diseases caused by *Marasmius scandens*, Mass and *Marasmius equicrinis* repectively are controlled by removal of infected parts and burning. The screening of fungicides for its control is underway.

Other diseases, such as root rot caused by *Phellinus noxius*, cushion gall believed to be caused by *Colonectra rigidiuscula*, charcoal pod rot caused by *Lasiodiplodia theobromae*, warty pod of unknown etiology, mealy pod caused by *Trachysphaera fructigena* are all minor diseases of cocoa in the West African sub-region. All these minor diseases are effectively controlled by good farm sanitation practices such as thinning, pruning, improving drainage and removal of infected trees or pods.

Cocoa swollen shoot virus disease (CSSVD) is, by far, the most serious and economically important and has been for many years a major problem for the cocoa industries of Ghana and Nigeria and more recently Togo. Serious problems have been encountered with the control by cutting out of infected trees and they largely relate to the expense and difficulty of operating routine survey and treatment operations on a massive scale and with the required efficiency. An additional limitation of the approach adopted has been the method of field detection. At present, the only practical method of detecting infection in the field is the inspection of cocoa trees for symptoms at regular intervals. Consequently, the success of the cutting-out programme depends largely on the efficiency of the inspectors. In addition, symptoms are not clearly expressed in the Upper Amazons or their hybrids. There has been a complete reappraisal of the cutting out campaign and a revision of previous practices. The current policy in Ghana is to survey and treat the cocoa farms annually in the *Cordon Sanitaire* and at 3 or 5 years interval in other areas, depending on the previous incidence of infection. Re-treatment is done as and when necessary at each site until no further infection has been found over a period of two years when the outbreak is deemed to have been controlled. To further decrease the early re-infection of newly established cocoa from adjacent old planting, the likely benefits of some form of barrier or mixed cropping systems have been evaluated in Ghana and Togo. Results of field investigations conducted at the Cocoa Research Institute of Ghana indicate citrus and oil palm are effective in preventing early CSSV re-infection of newly established farms from old adjacent cocoa and compensated adequately for the cocoa space they occupied. Farmers are therefore encouraged to plant these crops in the 10m cordon.

A range of mealybug species is known to vector CSSV in West Africa with *Planococcoides njalensis* being the most important. Past attempts at the control of the mealybug vectors of CSSV have been reviewed. These include the use of conventional insecticides for the control of mealybugs and their attendant ants and the use of biological control agents including parasitiods, predators and pathogenic fungi. However, both methods proved unsuccessful for various reasons including high cost and adverse side effects in the case of insecticides, and the influence of hyperparasitism, inefficient breeding and release methods and the introduction of ineffective exotic natural enemies in the case of biological control. Studies on the biological control of cocoa mealybugs have been re-visited recently in Ghana and several parasitoids and predators have been identified. Laboratory studies on the biology and effectiveness of the most abundant natural enemies have been initiated.

As a basis for the development of a sound integrated control strategy, studies aimed at accurately identifying the mealybug species within the *P. citri* complex using

conventional and biochemical methods as well as studies on parthenogenesis and the development of laboratory methods for rearing mealybugs are in progress.

A considerable effort has been made over several years to select resistant or tolerant genotypes from those already being grown in the sub-region or from subsequent introductions. Currently, the most tolerant hybrids available in Ghana are the Inter-Upper Amazon hybrids and these are supplied to farmers to plant after CSSV treatments. However, the resistance obtained in these hybrids may be insufficient to prevent virus spread under the prevailing conditions of high inoculum pressure.. Promising mutants have been obtained from mutation breeding using gamma irradiation at CRIG. The mutants are being assessed in the field.

Wide range of mistletoes is found on cocoa in West Africa. At least six species have been found on cocoa in Ghana and elsewhere in the subregion. *Tapinanthus bangwensis* (Engler and Krause) is the most widespread. There is evidence that mistletoe infestation of cocoa in Ghana and Nigeria is increasing. This may be accounted for by the general reduction of shade in these countries but it is possible that mistletoes have been slowly adapting to a new host. Preventive control of mistletoes aims at limiting the spread of infestation by maintenance of shade which prevents mistletoe germination in the cocoa farm. Early pruning is recommended for the control of mistletoes. Manual cutting-out is carried out as soon as parasite is noticed. In Ghana, the best time of year to carry out this work is during the August flowering peak, when the plants are easily seen in the canopy and farmers are not involved in harvesting.

Mirids (Heteroptera; Miridae) are the most economically important insect pests of cocoa in the region with two species, *Sahlbergella singularis* and *Distantiella theobroma*, the most widespread attacking cocoa from Sierra Leone in West Africa to the Congo and Central African Republics in the East. The insects' damage shoots and pods which may serve as entry points for fungal pathogens. Termites and stem borers, especially *Eulophonotus myrmeleon*, have in recent times been of economic importance in the region. Sporadic attacks by other insect pests including pod and feeders of minor importance also occur. Throughout the region, mirids have been controlled by the application of chemical insecticides. Two control programmes are being vigorously pursued: i) Integrated Pests Management (IPM) programme using female mirid pheromones and fungal pathogens such as *Beauvaria bassiana* and ii) breeding for cocoa varieties resistant to mirids. Trials on the pheromones have started in Ghana and Cameroon. Biological control of mirids is being done as collaboration between CRIG and CABI Bioscience, Kenya. It has led to the isolation of two promising isolates of *Beauvaria bassiana.*

The stem borer, *Eulophonotus myrmeleon* is widely distributed in West Africa from Sierra Leone to Cameroon and it is assuming economic importance in Ghana, Cote d'voire and Nigeria. Eight genera of termites, *Macrotermes, Cubitermes Nasusitermes, Microtermes, Coptotermes and Pseudacathotermes, Ancistrotermes* have been reported to damage cocoa in Ghana and young cocoa less than 3 years old and seedlings are particularly vulnerable. Sporadic attacks by other insect pests of minor importance also occur, causing varying degrees of damage. These insects include the pod feeders *Bathycoelia thalassina (H-s) (*Heteroptera.: Pentatomidae*)* and *Pseudotheraptus devastans* (Dist.) (Heteroptera; Coreidae) and the pod borer

Characoma stictograpta Hmps (Lepidooptera; Noctuidae) There are also the Lepidopteran foliage pests *Anomis leona* Schaus*., Earias biplaga* Wlk*., Orgyia basalis* Wlk *and Prodenia litura* F*.,* the psyllid *Tyora tessmanni* (Aulm*),* the aphid *Toxoptera aurantii* (Fonsc.) the thrips *Selenothrips* rubrocinctus (Giard.) and the stem tip feeder *Tragocephala spp* (Coleoptera; Cerambycidae). In addition to the insect pests, rodents, for example rats and squirrels, also attack cocoa in the region especially pods and seedlings particularly in Nigeria, Ghana and Sierra Leone.

In terms of technology transfer, there is emphasis and focus on cocoa farmers' knowledge and training. This is being facilitated through farmer field schools and the use of farmer videos and identification manuals. There is good collaboration between the Sustainable Tree Crop Programme, scientists and farmers in the various countries. Scientists are providing technical backstop and continuosly fine-tuning protocols with farmers to ensure that farmers' understand the principles underlying diseases and pests management within the whole context of sustainable cocoa production.

B. Report of the Americas Sub-group

Dr. J. L. Pereira presented the report for the American sub-group, initially generalising that diseases, endemic to the region, such as frosty pod rot and witches' broom continue to demand considerable attention and dedication from farmers, extension personnel and researchers. However, Phytophthora pod rot that continues, as an economically important disease, is often not controlled, although effective management recommendation exists. This is due to the need to weigh the low price of cocoa against required farm inputs; as a result growers often decide not to adhere fully or not at all, to application needed for control of this disease. Monitoring other diseases that occur sporadically, allows for appropriate management measures, when registered. Compared to cocoa diseases, insect pests are of lesser importance in the region.

Frosty pod rot (*Monilophthora roreri*) continued its rapid spread over all of Central America, and is now established in Mexico. The pathogen's adaptation to disseminate over wide geographic areas, in a relatively short period of time, emphasizes even more that Brazilian cocoa in the Amazon and Bahia States face an imminent risk of having to deal with this disease.

While, witches' broom caused by *Moniliophthora (=Crinipellis) perniciosa*, is present in all of South America, the measures taken to stop it's entry into Central America at Panama, in 1990, following implementation of eradication and exclusion management methods, were effective to the present day,. On the other hand, from 1989, following the introduction of witches' broom into the main cocoa growing region of Bahia, Brazil, the disease spread unchecked, and only after intensive investigations in other components of management (cultural, chemical, biological and genetics/breeding), a good level of success may be assumed, considered in the fact that to date, cocoa trees in 150,000 ha of existing farms have been grafted with genetically improved material. Further, an antagonistic, hyperparastic fungi (*Trichoderma stromaticum*), produced semi-commercially in the laboratories of Cocoa Research Centre, Brazil, is being routinely applied as a bio control agent to the witches' broom pathogen; with signs that once establish may self-perpetuate in the

field. Following increased research, cultural and chemical control methods also saw improvements of more effective management, involving timely removal of infected material and introduction of newer systemic fungicides, respectively.

Phytophthora pod rot that can be caused by as many as three species, *P. palmivora, P. capsici* and *P. citrophthora* in this Region, at least in Brazil, seems to have taken a second place in terms of economic importance and management. However, observations on farms have shown that this is a misconception, when crop loss caused by Phytophthora pod rot has been wrongly attributed to witches' broom.

Ceratocystis fimbriata that causes a wilt of cocoa is known to be present in South America since 1918. However, in Bahia, Brazil this disease only attain importance, when newer genetic material was released to growers selected for resistance to witches' broom. Some of this very material was highly susceptible to *Ceratocystis fimbriata* and was a cause for concern. To overcome the problem, material identified as susceptible to Ceratocystis wilt was reserved.

Pesticide application: During the Seminar reference was made to developments and continued use of motorised mist-blowers for application of pesticides in cocoa. Dr. Pereira informed that the motorised mist-blowers were researched on and developed about 50 years ago for spraying cocoa, and is efficient in generating and carrying droplets in an air stream to deposit on targets within and around the tree canopy. However, better a application hydraulic system based on hoses and lances, powered by a single 4-stroke petrol or diesel engine exist, and supersedes the motorised mist-blower. The first prototype assembled in 1983*, and compared to motorised mist-blowers, could effectively treat a maximum of eight cocoa trees simultaneously, less the half the cost in initial purchase, operation, maintenance and depreciation, for the same work output. At the same time afford comparable disease control and equally important, eliminate the discomfort/fatigue to the operators in carry weight, noise and vibration, associated to the mist-blowers. Since then, in Bahia, Brazil considerable improvements on the system have been undertaken, more so, with the advent of smaller and less expensive petrol or diesel engines coupled to high pressure generating diaphragm pumps. The use of motorised mist-blowers is not being promoted after development and evaluation of the hose and lance system shown to be superior.

C. Report of the South East Asia Sub-Group

The report of the South East Asia Sub-group was presented by Dr. Lee Choon Hui of Malaysian Cocoa Board. The South East Asia Sub-Group comprises Malaysia, Indonesia and the Philippines. At the 3rd INCOPED Meeting, October 2000, in Kota Kinabalu, it has been quite apparent that the main pests and diseases problems in all the three countries are the Cocoa Pod borer (CPB), Black Pod and Vascular Streak Dieback (VSD).

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* Pereira, J. L., Cezar, J. O., Andrade Filho, E. N. Concept, industrial design, field operation performance and the economics of a hose and lance system for phytosanitory treatments of cocoa. (1983), Technical Bulletin CEPLAC/CEPEC, Brazil. v. 113.

Occasionally, rodents pose a problem in the Phillipines and this group of pests can be managed and mitigated. It was stressed that collaboration of research in the region for CPB particularly biological control, with prospecting for natural enemies, ants and other potential entomopathogens. Other aspects such as host plant resistance should be in the long term perspective with resistance to Black Pod and VSD to be research into. There is the need to look into the taxonomy of CPB from different hosts in order to have a better understanding of the pest problem.

By the 4th INCOPED Meeting on October 2003 in ACCRA, CPB still remains the factor limiting cocoa production despite the main focus of Research and Development over the years. It was emphasised that chemical control was costly in the long-run, limited in effectiveness and economically may not be sustainable and definitely environmentally unfriendly. Approaches adopted then were strategically geared towards reduction in infestation and monitored such that an Integrated Pest Management concept is being applied. All measures of control including cultural, physical, biological and chemical were integrated with emphasis on reduced inputs costs with maximising benefits, economically sustainable and environmentally friendly and meets social needs.

Among the approaches and management strategies adopted that have been beneficial are:

(i) Sleeving of cocoa pods
This method is cultural and requires intensive labour requirements. In areas where it is practised, the cost is far less than 10% of the revenue that can be obtained; however, it would increase with the improving standard of living. The constraint on labour would eventually be the limiting factor, although the use of bio-degradable plastic offers scope for environmental friendly control measures. Although sleeving may be an option, the loss from high humidity leading to black pod would compound on the losses incurred.

(ii) Utilization of *Trichogrammatoidea bactrae fumata* (TBF)
Recent studies into the taxonomic status of TBF have led to a reclassification of it to be *Trichogramma toidea cojuangcoi* (TTC) and *not Trichogramma toidea bactrae fumata.* TTC, earlier known as TBF, is being used as an egg parasitiod seeks the host and destroys the eggs is being fine –tuned with the timing, frequency, duration and interval of release as well as on-farm application. Generally loss due to CPB can be reduced to a low threshold level but sudden occasional severe crop loss due to resurgence of CPB can occur and thus the need for investingation into other synergistic bioagents as well as the need for further fine-tuning.

(iii) Use of Cocoa Black Ants (CBA)
This has been shown to be possible to bring about a decline in CPB infestation over time and mitigate the crop loss encountered. However, population of CBA does fluctuate and vary over time and there are possible occasions where crop loss could increase due to a sudden surge of CPB infestation. Studies on CBA populations need to be continued to understand the mechanisms of the population and ensure stability and maintained for effective reduced CPB presence and loss.

(iv) Chemicals and pheromones

It has been shown that chemicals are more of a short-term control measure ensuring that crop loss at a sudden pest infestation can be minimised and management of the pest is more important than killing of the pests, as eradication has not been a possibility. The utilisation of chemical attractants (pheromones) to indicate initial infestations (in-coming infestations) of CPB so that strategies for management and control can be adopted to ward of the pest and thus mitigate losses to an acceptable level is being assessed. This would be within the norm of a pest within the ecosystem and yield of the crop is economically sustainable to the farm.

Technology for the management of CPB in Malaysia is available, is being utilised and is continuously fine-tuned. At times and infrequently, well managed cocoa fields have been inflicted by a sudden outbreak of the insect, leading to severe crop loss. Thus, the population pattern of CPB is being investigated continuously. In addition the possibility of monitoring in-coming and new infestation with pheromone traps is also being pursued. The mechanism of host-plant resistance and selection for resistant varieties is still in progress.

Although research impectus on CPB in Indonesia is yet to be seen, emphasis and focus has been on cocoa farmers' knowledge and training. This is an important point in Technology Transfer & Adoption within the scope of Extension Services; which is the basic essence of Cocoa Pest and Disease Management. These aspects are given priority and continuously being undertaken not only in Indonesia but also in Malaysia with focus and targeting on farmers and smallholdings. As progress in CPB management and control strategies are achieved, the information and technology is continuously being channelled to the target group, *i.e.* the cocoa farms. This shows the importance of maintaining a continuous information chain with no disconnectivity (no break in the chain of information).

The Cocoa Pod Borer (CPB)

CPB, *Conopomorpha cramerella* (Snellen), though, first discovered infesting cocoa in Java, Indonesia 1895, in Sulawesi towards the nineteenth century, in Sabah in 1980 and Peninsular Malaysia in 1986 (Azhar 2000), can be said to have been with the crop for more than two decades. It has been a serious pest of cocoa in the Philippines, where cocoa is planted as an intercrop in bananas, papayas and tapioca (Dumatol *pers. comm.*). Over the years of coping with the pest, the research and development of management and control strategies has led to the pest to be accepted as part of the Malaysian cocoa ecosystem. As outlined above, we have reached a phase where the technology for its control is available and utilised; and being fine-tuned for further improvements towards mitigating their losses with possible monitoring and management of threshold limits and the selection of resistant varieties as the long term solution. Such an approach and strategy has also been adopted in Indonesia with the potential of coping with the pest. Recently, the problem of CPB has been reported to have spread to Papua New Guinea. In some of the latter islands infestation and damage have been very severe; inflicting heavy crop losses. Mitigation of Crop losses and the CPB infestation has been undertaken and has yet to be fruitful. In cocoa areas where management and control strategies have yet to be adopted, severe infestations and losses are being experienced.

Diseases

Occasional severe crop loss due to Black Pod (BP) and Vascular Streak Dieback (VSD) in certain areas has been attributed to sudden weather changes and and periodic dry and wet spells that has its toll on the plants. A sudden wet season with prolong rain would lead to environmental conditions highly conducive to the spread and infestation of *Phytophthora palmivora*, thereby causing black pods. Management and control strategy such as reducing dense leaf canopy and leaf-cover is essential to reduce dampness within the cocoa tree canopy and thus avoid the problem of black pod. Screening and selection of resistant cocoa varieties is still being research into.

Occasional outbreak of VSD in well managed cocoa fields is also experienced in some areas where sudden weather changes bring about dry spells. With sub-optimum shade and prolong drought and water stress, VSD infestation can lead to severe withering of leaves and subsequent yield loss/reduction. Optimising shade requirements and ensuring proper and adequate nutrient and water management is highly important in the short term and the selection and screening for VSD resistant cocoa varieties would be the best approach.

ELECTIONS

Dr. J. L. Pereira informed the meeting that Mr. Andrews Y. Akrofi of the Cocoa Research Institute of Ghana who was elected at the 4th INCOPED meeting in Ghana in 2003 to understudy him for the Chairmanship of the group was ready to take up the position of INCOPED Chairman. The meeting unanimously voted to confirm Mr. Andrews Y. Akrofi as the new Chairman of the group

Closing address by Andrews Y. Akrofi, Elected Chairman of INCOPED.

Fellow participants of the 5th INCOPED Seminar, we must congratulate ourselves for a successful meeting to deliberate on issues related to cocoa crop protection. Considering the broad interest and importance of cocoa crop protection, especially during this period where pests and diseases account for more than 30% of crop losses, this seminar, I believe, has provided the opportunity for the international community associated with the cocoa crop to share experiences and research results.

Since the first INCOPED Seminar in Accra in 1995, we have witnessed the emergence of a broad yet simple scientific framework to reflect the experiences, practical achievements, theory and fundamental principles now identified with cocoa crop protection. The growth of the group is readily documented by the increase in the number of scientific papers at the International Cocoa Research Conferences and the Group's Seminars, and most recently, information on microbial control of cocoa pests and diseases.

The Seminar as the name implies is a meeting place where participants with common interests work towards common goals. The setting is informal and encourages exchange between and among speakers and discussants. Towards this end, the programme for the just ended seminar was not developed until after abstracts had been received. Our attention to structure has been mainly to organize the submitted abstracts into generally similar topics.

We cannot over emphasize the importance of the topics treated at this seminar to sustainable cocoa production. Likewise, we cannot overemphasize the importance of international commitments and cooperation within the scientific community if the science and practice of cocoa crop protection is to achieve its full potential. What remains to be done is to tease out the results from our work into forms applicable by the peasant farmers involved in cocoa production. These have been the challenge of INCOPED and let us charge ourselves to develop strategies and work to meet farmers' demands. It is only when we do this that we can be proud to be cocoa crop protectionists, working towards the betterment of the lives of the majority of peasant cocoa farmers.

Consumers demand for minimal pesticide use on cocoa puts pressure on us to work towards finding alternative sustainable and better management strategies. The possibility of spread of diseases and pests to areas, currently not infected or infested should be of concern to us and we need to put up the necessary strategies. Early detection and management, and means to this end should be pursued. I am happy that these areas have featured prominently in our discussions.

Fellow scientists, join me in saluting Dr. J.L. Pereira of CEPLAC, Brazil for his role in nurturing INCOPED after its birth to this age. His contribution to the group has been enormous, and we will continuously rely on his rich experience both in cocoa crop protection and administration to steer the affairs of the group. To this end, I wish to suggest for the approval of the group a position of INCOPED ADVISOR/CONSULTANT (voluntary position as the group is cash strapped).

We will also need the co-operation of all subregional co-ordinators (Africa, Asia and Americas) as well as all those involved in cocoa crop protection to steer the affairs of INCOPED and to let the group live up to its aims and objectives. We require suggestions and contributions from every one of you. I am also suggesting the establishment of National Co-ordinators who will feed Sub-regional Co-ordinators with pests and diseases status of various producing countries.

With the emerging role of biological control in the cocoa IPM, I will also want to suggest a position of Vice-chair for biocontrol group who will feed into the main group. With such a structure, we should be able to feed stakeholders with pests and diseases status and management strategies available.

Fellow participants, it is regrettable to note that in spite of the contribution of INCOPED to sustainable cocoa production, very little attention has been devoted to the group financially. We have to work to get the group on a better financial standing and will appreciate donation from various stakeholders. Suggestions to this end will be greatly appreciated.

We acknowledge with thanks the dedication to duty of the Local Organising Committee and the support of CATIE for a successful 5th INCOPED Seminar. The cooperation of COPAL and the financial assistance from the various organizations and groups is greatly appreciated.

On behalf of the Local Organizing Committee of the 5th INCOPED Seminar, I wish to thank you all for the successful seminar and look forward to seeing you at the 6th Seminar.

Long Live the International Working Group for Cocoa Pests and Diseases.

Andrews Y. Akrofi

Acknowledgement

The Chairman and organizers of INCOPED 5th International Seminar would like to express their appreciation and gratitude to all sponsors, contributors and participants for a successful meeting.

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