Development of Biological Control Agents for Use Against Pathogens of Cocoa

Introduction

The three main pathogens of cocoa in South and Central America are *Phytophthora palmivora*, *Crinipellis perniciosa* and *Moniliophthora roreri*. These pathogens cause the diseases black pod, witches broom and frosty pod respectively and result in enormous crop losses every year. This has led to the abandonment of farms in many cases. Chemical treatment of these diseases is expensive and often relatively ineffective necessitating development of alternative methods of disease control. At the Fitoprotecion (plant pathology) lab at CATIE, Costa Rica, Dr Ulrike Krauss and team are at the forefront of research towards development of a successful biological to combat these pathogens. It is hoped that by using mycoparasites with ability to degrade these pathogens, a universal biocontrol innoculant can be developed with the ability to reduce disease.

Within this research I was involved in two main areas:

- Compatibility analysis of various strains of mycoparasites that show promise for use in biocontrol innoculants.
- Implementation of a field survival trial of different mixtures of mycoparasites with the aim of determining how well these mixtures will survive in natural conditions over a period of two to three months.

Methods

Compatibility experiments

Host range experiments involved pre-colonising ½ potato dextrose agar (PDA) petri dishes with the various mycoparasites until the plate was completely colonised. Once fully colonised a 2.5 x 0.5cm strip of agar colonised by a different mycoparasite was placed hyphal surface down onto the colonised plate and was incubated at 25°C for a further 7 days. The growth rate of the challenger (mm/day) was then noted. The challenger had no access to agar on the new plate and therefore any growth was deemed the result of outcompetition or parasitism of the host fungi.

Hyphal interaction experiments involved placing small discs of agar colonised by different mycoparasites at opposite ends of a glass slide coated in a thin layer of agar. The hyphae then grew towards each other and any interactions were noted.

Field Survival Trial

The field survival trial was carried out in CATIE's experimental research station at La Lola and was designed to assess the ability of mycoparasites to remain as a viable population over a period of two months after application to healthy, unripe cocoa-pods. Pods were first surface sterilised with alcohol to remove any surface dwelling native mycoparasites before being promptly inoculated with various mixtures of mycoparasites. Differing conformations of mixtures were made up using strains of *Clonostachys rosea* and a single strain of *Trichoderma longibrachiatum*. A negative control was used to monitor the recolonisation of pods by native mycoparasites. Discs were cut from the

pods on days 0, 7, 14 and so on for a period no less than two months and were placed on plates fully pre-colonised with *Phytophthora palmivora* for incubation at 25°C for approximately six days.

Results

Compatibility Experiments

Host range experiments showed that all strains of *Clonostachys* tested parasitised *T. longibrachiatum*T4, but that *T. longibrachiatum*T4 was unable to parasitise *C. rosea* strains.

Hyphal interaction experiments also showed evidence of antagonism of *T. longibrachiatum*T4 by the various strains of *C. rosea*. Destruction of *T. longibrachiatum* T4 hyphae on contact with *C. rosea* hyphae was often observed whilst the opposite was almost never true.

Field Survival Trial

The results of this experiment have now been collected and are being processed for statistical analysis. Since the statistical analysis has not yet been completed a completely accurate summary of the results cannot be given unequivocally. However, on preliminary observation of the data it was seen that *Clonostachys* displayed high levels of survival throughout the experiment, showing a gradual decline in samples. *T. longibrachiatum* did not display the 100% level of presence that was expected on first sampling and did not show high levels of survival throughout the experiment. However, later in the trial *T. longibrachiatum* appeared in larger quantities than would have been expected at this time.

Discussion

This work displayed the ability of *C. rosea* to degrade the hyphae of a promising biocontrol strain of *T. longibrachiatum*. It also displayed that *C. rosea* tended to persist in natural environmental conditions better than *T. longibrachiatum*. However, when *T. longibrachiatum* is added to biocontrol innoculants a better biological control capability is displayed. An explanation for this may be the ability of *Trichoderma* to thrive in different environmental conditions than *C. rosea*, thus improving suppression of pathogens throughout the fruiting season. The increased incidence of *T. longibrachiatum* late in the field survival trial may be an example of this increased fitness under different environmental conditions.

The work I undertook in the summer is currently being assembled and combined with other work undertaken in the lab for publication in the near future. I would like to thank Dr Ulrike Krauss for providing me with this fantastic opportunity, to her team for providing me with great help and support and also to the Barnson Bequest and James Rennie Bequest for making this trip possible through generous financial contribution.

Robert Rees