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Trichoderma asperellum PR11 soil treatments for *Phytophthora megakarya* control

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Many *Phytophthora* species have a soil borne phase in their natural life cycles even though disease expression often occurs on aerial plant parts. In Cameroon, black pod rot of cacao (*Theobroma cacao* L.), is caused by *P. megakarya*. Primary inoculum of *P. megakarya* is located in the soil and with the onset of the rainy season, through rain splash, cacao pods become infected. From there the repeated cycles of pod infection and sporulation are at the origin of losses that can reach up to 80% when no control measures are in place. Thus, if this soil borne inoculum could be eliminated or prevented from reaching cacao pods, losses from black pod could be reduced considerably. In Cameroon, successful biological control of black pod rot has been obtained with the use of *Trichoderma asperellum* PR11 applications directed at cacao pods. The objective of this project therefore, was to investigate the possibility of using *T. asperellum* PR11 in soil applications in order to reduce black pod disease incidence due to *P. megakarya*.

The study was undertaken in a farmer's field near Nkolbisson, Centre region of Cameroon. *Trichoderma asperellum* PR11 was applied on a three weeks basis over two consecutive production seasons. A water only and a fungicide, Ridomil Gold 66 WP (6 % metalaxyl-M & 60 % CuO) treatment were used as controls. Weekly data collection consisted of counting all healthy and diseased pods. For each treatment, six replicate plots each containing 16 cocoa trees were used.

The absolute number of rotten pods and percentage pod rot was lower for *T. asperellum* treated plots when compared with the water control yet higher than the fungicide control, albeit these differences were not significant. There was however, a significant difference between treatments with regard to disease progression over time. Given that *T. asperellum* applications directed at cacao pods have shown efficiency in controlling black pod rot, the use of soil applications of *T. asperellum,* which slows disease progression, should therefore lead to additive effects when both control strategies are employed simultaneously.

Introduction

Many *Phytophthora* species have a soil borne phase in their natural life cycles (Ristaino and Gumpertz, 2000) even though disease expression often occurs on aerial plant parts. The importance of this soil borne phase differs according to species. In many cases, sporangia or zoospores released from direct germination of sporangia are the primary infective units that are dispersed from inoculum that has survived unfavorable conditions (e.g. the winter or a dry season) and are responsible for primary infections. Subsequently, repeated asexual cycles of sporangium production and dispersal are involved in secondary spread for many species of *Phytophthora* (Ristaino and Gumpertz 2000).

Black pod rot of cacao (*Theobroma cacao* L.), caused by several *Phytopthora* spp., is present in all regions were cacao is grown (Guest, 2007). Yet in Cameroon, black pod rot of cacao is principally due to *Phytophthora megakarya*. Control of *P. megakarya* is obtained primarily through cultural control together with chemical control. Yet, cultural control is time consuming and may provide insufficient control (e.g. Ndoumbe Nkeng et al., 2004), whereas chemical control comes with many negative externalities. Several authors have therefore advocated alternative control methods such as biological control (Deberdt et al., 2008; Tondje et al., 2007). In Cameroon, biological control of *P. megakarya* has been obtained with the use of *Trichoderma asperellum* PR11 (Deberdt et al, 2008, Mbarga et al., 2014; Tondje et al., 2007). This biocontrol agent has also been used to control soil borne inoculum of *Phytophthora ramorum* and *Phytium myriotylum* (Mbarga et al., 2012; Widmer, 2014)

Since primary inoculum of *P. megakarya* is located in the soil (Mfegue, 2012; Ristaino and Gumpertz, 2000), if soil borne inoculum could be eliminated or prevented from reaching cacao pods, losses from black pod should possibly be reduced considerably. Efforts to confine this pathogen in the soil by adding mulch or using soil applications with fungicides have given variable results (Gorenz, 1974 and references therein). One of the problems with soil oriented fungicide applications is how to assure that the fungicide arrives at the biological target. The use of a biological control agent, a living organism, in this case a *Trichoderma* isolated from the soil environment, has the benefit that this organism is adapted to the environment it's applied to and could actively grow in this environment.

Therefore, the objective of this project was to investigate the possibility of using *T. asperellum* PR11 as a soil application to reduce black pod disease incidence due to *P. megakarya*.

Materials and Methods

The study was undertaken in a farmer's field (3.900727 N, 11.446748 E), in the department of La Lékié, Centre Region of Cameroon, over two consecutive production seasons. A total of 18 plots, each containing 16 cocoa trees, were established. Every three weeks, using side lever knapsack sprayers, approximately 0.5 liter was applied to the soil surrounding the cocoa trees. Three treatments (with six replicate plots per treatment) were applied, a water only control treatment, a fungicide treatment (Ridomil Gold 66WP, containing 6% Metalaxyl-M & 60% CuO) at 5g l^{-1} and the biocontrol treatment containing *T. asperellum* PR11 at 1 x 10^6 spores ml^{-1}.

Weekly data collection consisted of counting all healthy and diseased pods on individual cocoa trees. Diseased pods were removed from the trees. Total number of healthy and rotten pods were log, and total pod rot percentage, arcsin transformed prior to analysis of variance (ANOVA). Area under the curve (AUC) was calculated for the number of rotten pods and pod rot rate over time. As observations periods differed between years (in length as well as period), AUC was divided over the number of days in the observation period to provide an AUC day^{-1} (Duveiller et al., 2005). This data was used in an ANOVA.

Results

There were marked differences in disease progression over time for the different years and treatments (Fig. 1) for both the number of rotten pods as well as pod rot rate. Yet, overall total pod loss due to black pod, as absolute number of rotten pods (P=0.642) and total percentage pod rot (P=0.164), was not different between years. Therefore data was pooled for further analysis. However, neither the total absolute number of rotten pods, nor total percentage pod rot, differed significantly between treatments (P=0.118 and P=0.096, respectively).

Nonetheless, when looking at the disease progression curves, differences were observed between treatments. When comparing the daily increase of area under the curve (AUC day^{-1}) for the number of rotten pods and pod rot rate (Fig. 2a and b) between soil application treatments, the daily increase was significantly higher (P=0.017 and P=0.02, respectively) for the water control treatment compared with the Ridomil treatment (Fig. 2a and b). In the case of the number of rotten pods the *Trichoderma* treatment was equal to the fungicide treatment and different from the water treatment (Fig 2a). In the case of pod rot rate (Fig. 2b), the *Trichoderma* treatment was not significantly different from either the fungicide or water treatment. For the number of ripe pods no differences (P=0.218) in daily increase in AUC was observed (data not shown).

2010 **2011**

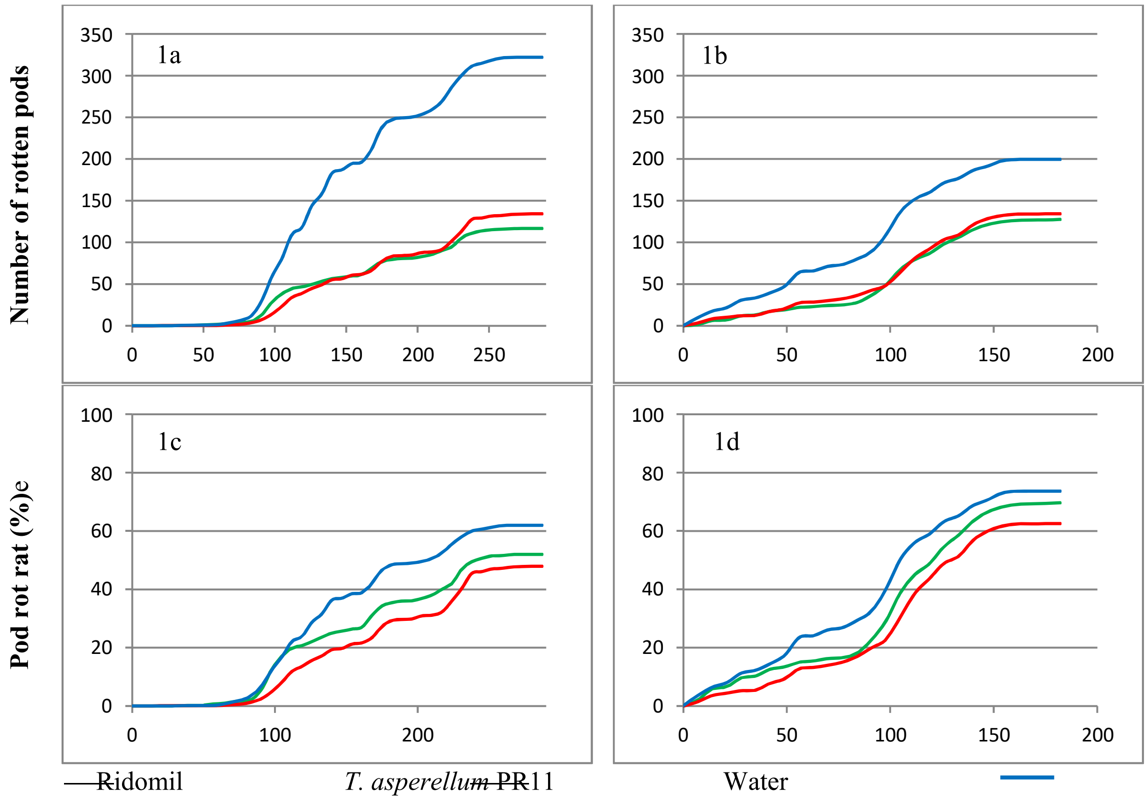

Figure 1 : The cumulative number of rotten pods per treatment in a) 2010 and b) 2011 and the cumulative pod rot rate in c) 2010 and d) 2011

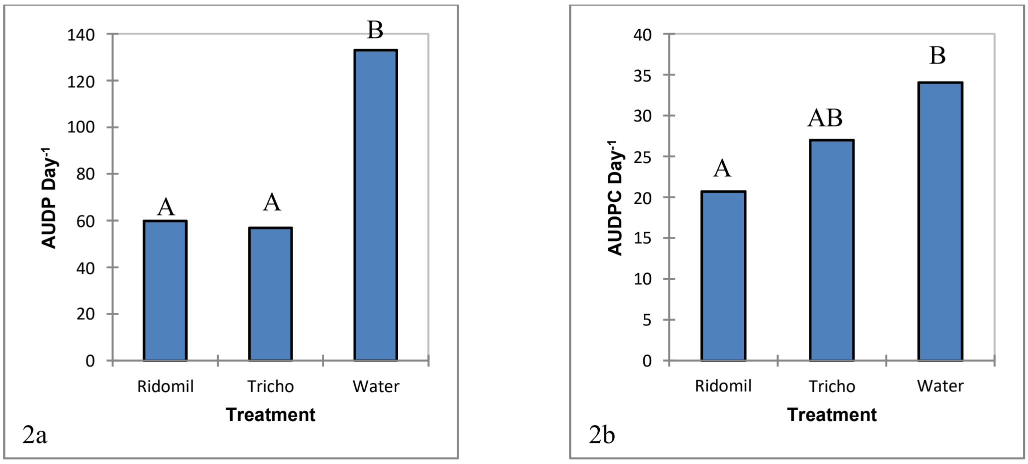

Figure 2: Differences between treatments in disease progression (as daily increase in area under the disease progression curve) for a) number of rotten pods and b) pod rot rate (data for both production seasons combined). Bars with the same letter do not differ significantly at $P<0.05$

Discussion

Although soil applications with *T. asperellum* PR11 and Ridomil Gold 66 WPdid not significantly reduce the total number of diseased pods, nor overall percentage pod rot in cacao trees, soil applications did change disease dynamics by reducing the speed in which the disease progressed (Figs. 1 and 2). That the total number of diseased pod and total percentage pod rot were not significantly different between treatments is not surprising given the fact that the only measure taken to reduce pod rot on the trees was weekly phytosanitation. Once an infection of a pod occurs on the tree, sporocysts will be produced and will give rise to secondary infections.

Although by itself soil applications will likely not be able to reduce black pod disease incidence sufficiently to be of interest to farmers, in combination with aerial sprays directed at the tree and its cocoa pods, which have proven to be effective (Deberdt et al, 2008, Mbarga et al., 2014; Tondje et al., 2007) these treatments should lead to additive effects and should result in reduced incidence, especially when applied over an extended period of time.

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