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Effects of climatic parameters on the expression of the black pod disease on *Theobroma cacao* in Côte d'Ivoire

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ABSTRACT

Objectives: To determine variation in black pod disease that is explainable by the climate and evaluate the changes in sensitivity of *Phytophthora palmivora* to *Trichoderma* species as influenced by variations in climatic parameters.

Methodology and results: The effects of 6 climatic parameters were evaluated. Four different zoospores' suspensions as bio fungicides constituting the treatments T1 as *Trichoderma spirale*, T2 as *Trichoderma harzianum*, T3 as *Trichoderma virens* and T4 as *Trichoderma asperellum* were assessed. Untreated (T5) was included as control. These 4 treatments were applied each on 100 cocoa trees according to Despreaux's random targets method. The rainfall, maximal and minimum temperature, relative humidity, mean temperature and temperature gaps as climatic parameters were recorded daily from June to February in the course of the years 2006-2007 and 2007-2008. The separation of means and Principal Component Analysis were used to determine the best climatic parameters disease occurrence. Non-linear polynomial multiple regression was used to quantify the link between intensity of the black pod disease and the climatic parameters through R^2-value. Due to their weak annual variations, maximal temperature, temperature gaps and relative humidity were eliminated. From the first to the second year, rainfall, minimal and mean temperatures explained 55.90 and 61.70%, respectively, of the black pod disease fluctuations in the untreated control. However, in the presence of 4 *Trichoderm* species, the fluctuations of black pod disease caused by *P. palmivora* varied from 12.10 to 39.60%, respectively, in the first and second year.

Conclusion and application of findings: Climate significantly influences black pod disease development. *T. asperellum* which proved to be the best will be used in large fields trials.

Key words: disease variation, sensitivity to climatic variations, biofungicides, random targets method.

INTRODUCTION

The cocoa tree is a perennial, allogamous and diploid plant of the Malvaceae family (Whitlock *et al*., 2001). It provides substantial incomes to farmers of producing countries (Wood & Lass, 1985; Mossu, 1991). Its average yields in farmers' fields are in the order of 250-500 Kg/ha, which is low compared to 2.5 t/ha attained in research stations (Mossu, 1991; Clément *et al*., 1996; Kéli *et al*., 2005). One of the ways to improve these yields is through control of pests and diseases that affect tree growth and development.

The black pod disease caused by *Phytophthora palmivora* is one of the most serious phytosanitary threats that reduce the durability of cocoa tree plantations. Indeed, global losses of production caused by *P. palmivora* range between 30 to 44% (Van der Vossen, 1997; Renard, 1999). The Côte d'Ivoire is the top cacao producing country in the world and records some production losses of 20 to 30% due to the disease. These losses further increase from 30 to 45% with the appearance of *P. megakarya* (Kébé *et al*., 1996). To reduce the impact of diseases, biocontrol is one of the proposed methods as part of integrated control (Krauss & Hebbar, 1999).

However, biocontrol trials both in laboratory and on farm are done under control of some physical parameters such as temperature, ambient humidity, and photoperiod, among others. To date most of the implemented studies on the antagonistic activity of *Trichoderma* species to control phytopathogenic *Fusarium*, *Pythium*, *Rhizoctonia*, *Crinipellis* and *Phytophthora* species took place under laboratory conditions where physical parameters are easily controllable (Bastos, 1996; Sanogo *et al*., 2002; Kredics *et al*., 2003; Kredics *et al*., 2004; Begoude *et al*., 2006). In the field, there are numerous climatic parameters that influence black pod disease occurrence (Wood, 1974; Blaha & Lotodé, 1976; Ndoumbé-Nkeng *et al*., 2004). However, such influences could be reduced by the antagonist activity of some fungi, e.g. *Trichoderma* species.

To date no study has reported the quantification of black pod disease variations caused by climatic parameters. Likewise, no information is available concerning the reduction of the fluctuations of black pod disease due to climatic parameters in the presence of *Trichoderma* species applied as biofungicides. . The quantification of black pod disease fluctuations would allow the appreciation of the real effect of climatic parameters. In the same way, the knowledge of sensitivity of *P. palmivora* to climatic variations in the presence of antagonistic *Trichoderma* species could enhance strategies for its application under field conditions.

The present work aimed to quantify black pod disease variations explainable by the climatic parameters and evaluate changes in the sensitivity of *P. palmivora* to *Trichoderma* species as affected by climatic parameters.

MATERIALS AND METHODS

Plant and fungal materials: Two different types of materials were used: 1) fungus isolates T4, T40, T7, T54 identified as *Trichoderma spirale*, *Trichoderma harzianum*, *Trichoderma virens* and *Trichoderma asperellum*, respectively; 2) plant material constituting of cacao hybrid progenies.

The four isolates of *Trichoderma* used were isolated from the cocoa rhizosphere soil in cocoa production areas of Côte d'Ivoire. They were shown to be effective antagonists of *Phytophthora palmivora*, causing black pod disease (Mpika *et al*., 2009). Species identification was done on the basis of sequence analysis of internal transcribed spacers ITS1 and ITS2 of ribosomal DNA using TrichoBLAST database (Druzhinina *et al*., 2005). The *Trichoderma* species were then conserved in the ACBR culture collection and representative samples deposited in the Gene Bank at CPK of Institute of Chemical Engineering, Research Area Gene Technology and Applied Biochemistry, Vienna, Austria. Cultures of the isolates were also retained in Côte d'Ivoire.

Plants of the hybrid cocoa progenies used were in a factorial trial design between four female parents (PA13, P19A, PA121 and ICS89) and two male parents (IMC67 and PA150). Except for ICS89 which is a Trinitario, all other parents belong to Upper Amazon group. This trial, installed in cocoa plot (C2/1) at the Bingerville station in 1988, consisted of 8 hybrid progenies. The latest were planted according to a

completely randomized design in three blocks with one-tree plot, at a density of 1333 plants/ha.

Experimental site for field trials: Experiments were conducted in experimental plots of the Centre National de Recherche Agronomique (CNRA) situated about 25 Km North West of Abidjan. Conditions at the site were average maximal temperature of 31.6 °C, minimal temperature of 21.9°C, mean relative humidity of 83.45% and an annual rainfall of 1583 mm.

Production of biomass by *Trichoderma* species: Biomass of the four *Trichoderma* species isolates was obtained by liquid fermentation according to the Hebbar and Lumsden (1999) method. After sterilisation, four starter flasks (250 mL) containing 100 ml of molasse-brewer's yeast medium (in g/l: 15 molasses and 2.5 grams of brewer's yeast) were inoculated with conidial suspensions obtained by delicately scraping on the surface of 7-day old fungal cultures. These starter culture flasks were incubated on a rotary shaker (150rpm, 3-5d, 26 - 28°C) and tested for bacterial contamination 1 day before they were inoculated into the laboratory fermenter. The fermenter (20 L capacity) contained the autoclaved molasse yeast medium (450 g molasses/75 g brewer's yeast) and was filled to 15 litres and dabbled with filtered air for 10 days at 26 ± 2°C. The carboy was shaken to encourage formation of chlamydospores. The liquid biomass obtained after 10 days of cultivation was used directly by mixing with water for large scale field trials.

Experimental design, treatments and data collection: The experiment was a random-targets design considering a pod on a tree as a random target (Despreaux *et al*., 1984). The cocoa trees in the field experiment were divided into five groups including each 100 cocoa trees randomly chosen. These five cocoa tree groups corresponded to the five treatments that were applied. The fifth treatment was represented by an untreated control. The cocoa trees of the same group were marked by numbered placards bearing the same colour. Identity of colour allowed the reconnaissance of cocoa trees belonging to the same group, thus facilitating the application of treatments. One row out of two was chosen for treatment. On each chosen row, one tree out of two was treated. Thus, one tree treated was encircled by eight trees as border.

Five treatments were applied as follows: the first corresponded to *Trichoderma spirale* T4, second represented *Trichoderma harzianum* T40, third was *Trichiderma virens* T7, fourth was *Trichoderma asperellum* T54 and fifth represented the untreated control. The *Trichoderma* spp. biomass was applied using knapsack sprayers (Solo, 15l) at a concentration of about 10^7 cfu ml^{-1}. Sprays were directed at the entire cocoa tree. In the first year, six applications of biocontrol candidates were carried out at 21-day intervals from June to November 2006, while in the second year applications started from July to November 2007. Conidia suspensions of *Trichoderma* strains were prepared and sprayed on each of 100 cocoa trees from the month of June. After one month, the observations were performed until December.

Observations on individual trees consisted of weekly counting of the healthy mature pods, and also the pods infected with black pod disease and other rotted pods on the treated and untreated cocoa trees. Observations started from June or July and ended in February. After counting, sanitary harvest was practiced in all treatments including the untreated control. For this purpose, diseased pods were removed every week. Simultaneously, the counted healthy pods were harvested on the treated and untreated cocoa trees.

Measured variables: Two groups of variables were used. The first group comprised of variables measured upon each tree and was represented by the percentage of diseased black pods. The second group included 6 climatic variables. These were: 1) the rainfall, 2) minimal temperature, 3) maximal temperature, 4) temperature gaps, 5) mean temperature and 6) relative humidity. Among these variables, the percentage of diseased black pods and the rainfall underwent arcsin$\sqrt{}$ and logarithm transformations, respectively, so as to normalize and equalize the variances of populations.

Data analysis: The most variable climatic parameters were identified by means separation and Principal Component Analysis using SPSS 12.0.1 and XIstat 2007.6. From year to year, the comparison of means was used to search for the least variable climatic parameters and this was performed according to Student-Newman and Keuls' test at 95% confidence probability. Pearson's Principal Component Analysis with varimax rotation was used. From this, analysis of conformity of the best climatic parameters from the first to the second year allowed the exclusion of those of which the variation is not stable. The identified best climatic parameters were then submitted to linear and non-linear multiple regressions, to test the link between intensity of the black pod disease and the climatic parameters. Link intensity is appreciated through the correlation coefficient of fitted curve called R^2, which contains the essential of information concerning the regression (Baradat, 1982). The best identified

regression model was used to quantify the percentage variation of black pod disease that is explainable by the identified climatic parameters compared to the untreated control (T5). For each of the 4 treatments, the reducing rate of the sensitivity of *P. palmivora* to *Trichoderma* species caused by climatic variations was calculated from the difference between R^2 of untreated control (T5) and that of each treatment. The climatic parameters most linked to the expression of black pod disease were identified from the highest partial regression coefficient of the regression equation designated ″b″. For the regression, only the months between September and December were chosen.

RESULTS

Most variable climatic parameters: From comparison of averages, the 6 climatic parameters as a whole varied significantly, from year to year (Table 1). Therefore, none was excluded from the study. Nevertheless, due to existence of such annual variations, the analyses were done year by year. The amplitude of gaps between the means of each climatic parameter and performed individual observations oscillated from 0.02 to 0.73%.

Taking into account the quality of representation of climatic parameters on the factorial axes as well as the sign of correlation coefficients among these parameters for each of years of study, the maximal temperature and the relative humidity were eliminated. In contrast, the minimal temperature, the temperature gaps, the rainfall and the mean temperature were chosen as the most influential climatic parameters on which the study continued.

Table 1: Classification of averages of six measured climatic parameters as a function of year.

Dependent variable *	Year	Partially transformed average*	RC(%)	Untransformed average
Rain	Year2	0.825 a	0.73	7.25 mm
	Year1	0.942 b	0.53	8.42 mm
Tmax	Year1	30.875 a	0.04	-
	Year2	31.111 b	0.05	-
Tmin	Year2	19.375 a	0.1	-
	Year1	23.927 b	0.08	-
Hrela	Year1	83.792 a	0.03	-
	Year2	87.232 b	0.03	-
Tmoy	Year2	25.243 a	0.02	-
	Year1	27.401 b	0.02	-
Etm	Year1	6.948 a	0.4	-
	Year2	11.736 b	0.26	-

Legend : Rain : Average of weekly average pluviometric total. **Tmax :** Average of weekly average temperature. **Tmin :** Average of weekly minimal temperature. **Hrela :** Average of weekly relative humidity. **Tmoy :** Average of average maximal temperature. **Etm :** Average of gaps between the weekly maximal and minimal temperatures. **Partially transformed average* :** Values followed by different letters are statistically different according to the Student-Newman and Keuls' test at the 95% confidence probability level. Here, only the rain underwent the log(x+1) transformation. **RC(%) :** Reliability coefficient in percentage. **Untransformed average :** Values were obtained from inverse function of the one used for their transformation.

Complementary information relating to Principal Component Analysis: In the first year 4 factorial axes synthesized the information as a whole contained in 6 initial parameters, as against 3 in the second year (Tables 2 and 3; Figures 1 and 2). In the first year, 4 chosen factorial axes explained 100% of total variability, while 3 of the second year explained 95.62%. In the first year, F1 axis expressed 52.21% of variability, whereas in the second year, it showed 69.68%. In the first year, this axis described the thermic amplitude, while in the second year it represented the low temperatures. In the first year, the temperature gaps and the maximal temperature were well represented and both described similar phenomenon

(Table 2). Because of the positive sign of their correlation coefficient, the maximal temperature which is the least well represented was eliminated from the study (Table 3). However, in the second year, the minimal temperature and the temperature gaps were salient and since both parameters did not express similar behaviour (Table 2) they were retained in the study (Table 3).

In the first year, F2 axis revealed 18.75% of variability unexplained by F1 axis, as against 15.81% in the second year. In the first year, it represented the thermic mean, while the second year it described the rainfall. In the first year, the mean temperature and relative humidity were prominent, whereas in the second year the rainfall and the maximal temperature were prominent and negatively correlated (Table 2). Therefore, both were retained in the study.

Table 2: Relationship among 6 climatic parameters through Pearson's linear correlations.

Year	Variable	Rain	Tmax	Tmin	Hrela	Tmoy	Etm
Year 1	**Rain**	**1**	-0.320**	0.284**	0.063**	-0.173**	-0.331**
	Tmax	-0.320**	**1**	-0.688**	-0.117**	0.729**	0.947**
	Tmin	0.284**	-0.688**	**1**	0.171**	-0.005	-0.884**
	Hrela	0.263**	-0.117**	0.171**	**1**	0.001	-0.151**
	Tmoy	-0.173**	0.729**	-0.005	0.001	**1**	0.472**
	Etm	-0.331**	0.947**	-0.884**	-0.151**	0.472**	**1**
Year 2	**Rain**	**1**	-0.151**	0.306**	0.385**	0.364**	-0.262**
	Tmax	-0.151**	**1**	-0.844**	-0.715**	-0.505**	0.931**
	Tmin	0.306**	-0,844**	**1**	0.685**	0.889**	-0.981**
	Hrela	0.385**	-0.715**	0.685**	**1**	0.492**	-0.721**
	Tmoy	0.364**	-0.505**	0.889**	0.492**	**1**	-0.784**
	Etm	-0.262**	0.931**	-0.981**	-0.721**	-0.784**	**1**

Legend : as indicated under table 1. Value followed by ** indicates that both climatic parameters for which it was measured are highly and significantly correlated according to Pearson's test at 1% probability level.

In the first year, F3 axis showed 15.05% of variability untaken into account by F2 axis, as against 10.13% in the second year. During the first year this axis described the low temperatures while in the second year, it described the thermic means. In the first year, only the minimal temperature was salient, while in the second year the mean temperature and the relative humidity were well represented. In the second year, these 2 later parameters varied in the same manner (Table 2). Thus, the relative humidity which was the least well represented parameter was eliminated from the study (Table 3). In the first year, F4 axis explained 13.99% of variability unexplained by F3 axis. It was suited for the rainfall, because this one was well represented there (Table 3).

Identification of the best model expressing the effects of some identified climatic parameters upon the black pod disease: After testing of linear and non-linear models of the multiple regression, the non-linear polynomial model of third degree (equation 1) was identified as the best, from correlation coefficient of fitted curve R^2. It is valid for both the first and second years of study. The preliminary tests of simulation of modelling revealed a weak contribution of temperature gaps to black pod disease variations, and it was thus excluded from the study. In this equation, the dependent variable Y represents the percentage of diseased pods. The constant b_1 expresses the ordinate at origin, and it indicates the percentage of black pod when there is no variation of 3 climatic parameters. Constants b_1, $b_2 \ldots b_{10}$ are some partial regression coefficients. Variables X_1, $X_2 \ldots X_3$ represent the 3 most variable climatic parameters.

$$Y = b1 + b_2 X_1 + b_3X_2 + b_4X_3 + b_5 X_1^2 + b_6X_2^2 + b_7X_3^2 + b_8X_1^3 + b_9X_2^3 + b_{10}X_3^3 \quad \ldots\ldots(1)$$

Table 3: Relationship between climatic parameters and factorial axes.

		Factorials axes *			
Year	**Climatic Parameters**	**F1**	**F2**	**F3**	**F4**
Year 1	Rain	-0.457	0.085	-0.305	**0.831**
	Tmax	**0.972**	0.195	-0.076	0.102
	Tmin	-0.798	0.465	**-0.291**	-0.251
	Hrela	-0.200	**0.597**	0.756	0.182
	Tmoy	0.588	**0.708**	-0.380	-0.096
	Etm	**0.978**	-0.079	0.079	0.177
Year 2	Rain	-0.408	**0.892**	-0.095	-0.167
	Tmax	0.877	**0.308**	0.264	0.256
	Tmin	**-0.978**	-0.090	0.186	-0.028
	Hrela	-0.807	0.079	**-0.466**	0.355
	Tmoy	-0.825	0.118	**0.525**	0.173
	Etm	**0.978**	0.171	-0.032	0.111

Legend : Factorial axes* : Principal components obtained by linear combination of initial variables represented by 6 climatic parameters. **F1* :** First principal component expressing the maximal variability. **F2* :** Second principal component non-linked with F3 axis and showing the variability unexplained by F1 axis. **F3* :** Third principal component non-correlated with F4 axis presenting the variability untaken into account by F2 axis and so on. **In bold type :** Values of the most strong correlations between the principal component and the best represented climatic parameters.

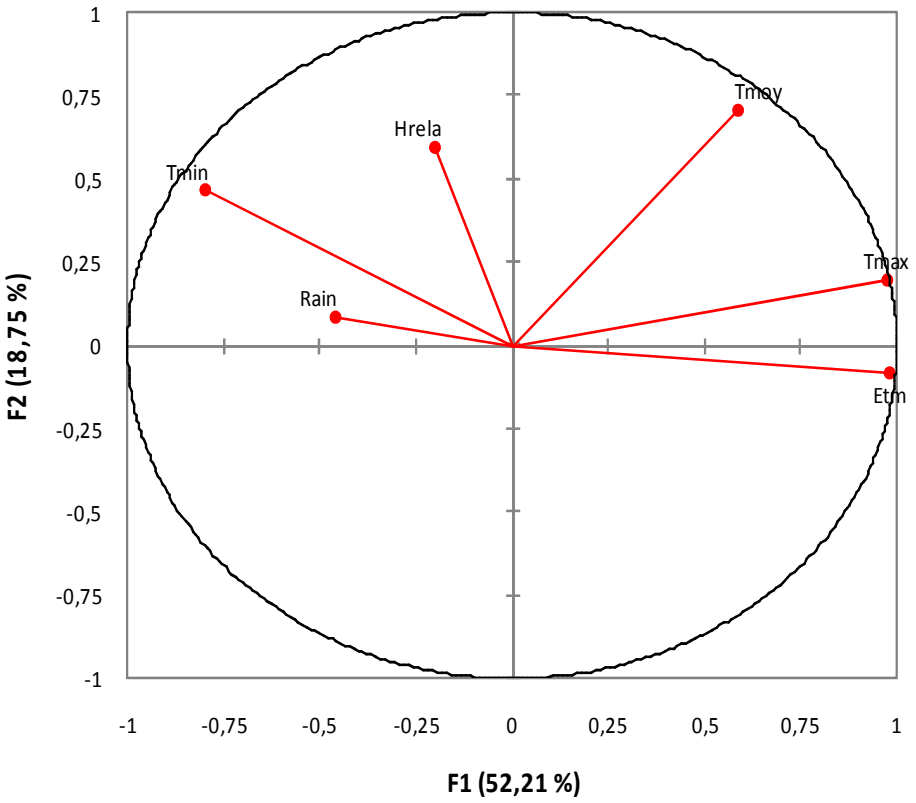

Figure 1: Quality of representation of 6 climatic parameters on the plane 1-2 of correlation circle of Principal Component Analysis for the first year of study. Legend is as indicated under table 1.

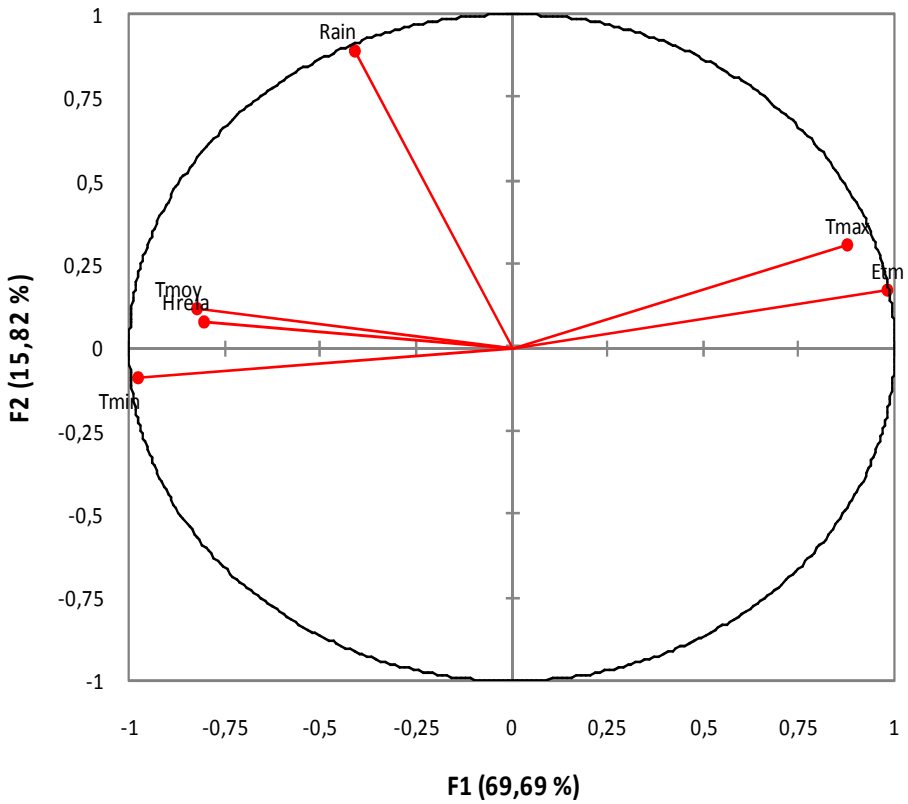

Figure 2 : Quality of representation of 6 climatic parameters on the plane 1-2 of correlation circle of Principal Component Analysis for the second year of study. Legend is as shown under table 1.

Effects of the 3 most variable climatic parameters on black pod disease for each of 5 treatments: As regards the untreated control (T5), the first year of study 55.90% of variations of black pod were due to rainfall, the minimal temperature and the mean temperature, as against 61.70% in the second year. In the first year, the polynomial of third degree which links the variations of black pod to those of climatic parameters was as shown (equation 2) and for the second year, it was as shown in equation 3. In the first year the rainfall was found to be the most linked climatic parameter to black pod (Eq. 4). On the other hand, in the second year the minimal temperature was proved to be the most associated with the black pod (Eq. 5).

Blapod = 5.92045 + 0.48567 Rain + 0.09983 Tmin – 0.29782 Tmoy – 0.074842 $Rain^2$.....(2)

Blapod = -41.94798 – 0.60574 Rain + 3.71108 Tmin – 0.41654 Tmoy + 0.77050 $Rain^2$ – 0.21790 $Rain^3$ – 0.00263$Tmin^3$.....(3)

(b/Rain-Blapod/year1 = +0.48567).....(4)

(b/Tmin-Blapod /year2 = +3.71108).....(5)

With respect to the actual treatments, with treatment T1 (*Trichoderma spirale)* in the first year 17.90% of fluctuations of the black pod were triggered by the 3 climatic parameters, as against 44.10% in the second year, i.e. a reduction occurred of the influence of the 3 climatic parameters upon *Phytophthora palmivora* because of antagonist action of *Trichoderma spirale* of 38% in the first year, and 17% in the second year relative to the untreated control (T5). In the first year, Eq. 6 shows the polynomial model of third degree while Eq. 7 shows for the second year. From the first to the

second year, the rainfall was the most associated climatic parameter with the black pod (Eq. 8).

Blapod = 0.98341 + 0.34266 Rain + 0.14091Tmin – 0.15292 Tmoy – 0.07959 $Rain^2$…… (6)

Blapod = - 21.51412 – 1.32002 Rain + 1.28698 Tmin + 0.13422 Tmoy + 1.52035 $Rain^2$ – 0.48368 $Rain^3$ – 0.00090 $Tmin^3$…….. (7)

(b/Rain-Blapod/year1 = +0.34266; b/Rain -Blapod/year2 = -1.32002)……...(8)

With treatment T2 (*Trichoderma harzianum*), from year to year, the rainfall, the minimal temperature and the mean temperature, respectively, explained 31.3 and 45.9% of variations of black pod disease. This corresponds to decreased sensitivity of *P. palmivora* to the 3 climatic parameters caused by *Trichoderma harzianum* of 24.60% in the first year of study, as against 15.18% in the second year in relation to the untreated control (T5). In the first year, the association is shown by equation 9. In the second year, the polynomial of third degree is shown by Equation 10. The mean temperature parameter was most linked to climatic effects on black pod in the first year, while in the second year it was the minimal temperature (Eq. 11). The minimal temperature and the black pod occurrence had an inversely proportional variation relationship.

Blapod = 9.84661 – 0.01626 Rain – 0.01118 Tmin - 0.34088 Tmoy – 0.00960 $Rain^2$……(9)

Blapod = - 24.73088 + 0.241477 Rain + 1.99845 Tmin - 0.16623 Tmoy - 0.26596 $Rain^2$ + 0.10500 $Rain^3$ - 0.00133 $Tmin^3$……(10)

(b/Tmoy-Blapod /year1 = -0.34088; b/Tmin-Blapod /year2 = +1.99845)……(11)

With treatment T3 (*Trichoderma virens)*, in the first year 43.8% of the fluctuations of black pod incidence were attributed to 3 climatic parameters, while in the second year 22.1% were caused by the rainfall, the minimal temperature and the mean temperature. From the first to the second year, these variations corresponded with reduction of the sensitivity of *Phytophthora palmivora* to antagonist effect of *Trichoderma virens* of 12.1 and 39.6%, respectively, in comparison to the untreated control. Equation 12 and 13 model the variations of black pod disease in the first and second year, respectively. In the first year, based on the pattern of results of the untreated control (T5), rainfall was the most linked climatic parameter to the black pod (equation 14) while in the second year the minimal temperature was the most associated to the black pod (equation 15).

Blapod = 3.98527 + 0.46174 Rain + 0.20860 Tmin - 0.32221 Tmoy - 0.140895 $Rain^2$……..(12)

Blapod = - 33.21669 + 0.39148 Rain + 2.82844 Tmin - 0.25194 Tmoy - 0.63517 $Rain^2$ + 0.27265 $Rain^3$ - 0.00206 $Tmin^3$……..(13)

(b/Rain -Blapod /year1 = +0.46174) ……..(14)

(b/Tmin -Blapod /year2 = +2.82844)……...(15)

With treatment T4 (*Trichoderma asperellum*), in the first year the part of variations of black pod attributable to 3 climatic parameters was 30.4%, as against 30.3% in the second year. This, respectively, represents a decreasing rate of sensitivity of *P. palmivora* to 3 climatic parameters because of *T. asperellum*'s bio fungicide effect of 25.5 and 31.4%, compared to the untreated control (T5). Equation 16 and 17 show the model in the first and the second year, respectively. In the first year, rainfall was the climatic parameter that was most associated with black pod (equation 18) while

in the second year it was the minimal temperature (equation 19).

Blapod = 1.08700 - 0.30734 Rain + 0.03140 Tmin – 0.06429 Tmoy + 0.21981 $Rain^2$……..(16)

Blapod = -15.76600 – 1.08396 Rain + 1.52093 Tmin – 0.25782 Tmoy + 1.90135 $Rain^2$ – 0.71315 $Rain^3$ – 0.00098 $Tmin^3$……(17)

T5 (b/Rain-Blapod/year1 = -0.30734)……..(18)

(b/Tmin -Blapod/year1 = +1.52093)……..(19)

DISCUSSION

The effects of 6 climatic parameters on the expression of black pod disease on *Theobroma cacao* were evaluated. All of the 6 climatic parameters evaluated varied significantly from the first to the second year. In spite of these annual variations, the maximal temperature, the relative humidity and the temperature gaps were the least variable within each year of study, and thus they were eliminated from the study.

Based on the results of the untreated control, results showed that the climate significantly influences the expression of black pod disease in *Theobroma cacao*. Indeed, more than 50% of black pod variations are triggered by the rainfall, the minimal temperature and the mean temperature. These parameters are necessary for the releasing, germination, penetration and colonization of pod tissues by the zoospores of *P. palmivora* (Wood, 1974). . In further studies concerning the natural effects of climate on the black pod disease, it would be desirable to investigate only the effect of rainfall, the minimal and mean temperatures.

In the presence of biofungicides, all of the 4 treatments significantly reduced the effects of climate on the expression of the black pod disease. Nevertheless, such a reduction varied from *Trichoderma* species to another and from the first to the second year. In the first year, it is treatment T1 (*T. spirale*) which diminished most the effects of 3 climatic parameters on the expression of the black pod disease (diminishing rate = 38%). It was followed by treatment T4 (*T. asperellum* with a reducing rate = 25.4%), T2 (*T. harzianum* with reducing rate = 24.6%), and T3 (*T. virens* with decreasing rate = 12.1%). In contrast, in the second year, treatment T3 (*T. virens*) had the strongest rate of reduction (diminishing rate = 39.60%), followed by *T. asperellum* (reducing rate = 31.40%), *T. spirale* (17.6%), and *T. harzianum* (15.80%). Furthermore, variation percentage of black pod disease attributable to 3 climatic parameters for treatment with *T. asperellum* was nearly similar from the first to the second year (year 1 = 30.4%; year 2 = 30.3%). Some similar results, where *T. asperellum* was the best, were obtained by ANOVA incorporating separation of means (Mpika *et al*. submitted for publication). For the field trials, it would be judicious to test the effect of *T. asperellum* to draw some definitive conclusions on its biofungicide activity.

Effects of *Trichoderma* species seemed to reduce the sensitivity of *P. palmivora* to variations induced by the rainfall, minimal temperature and mean temperature. Sure enough, although the zoospores of *P. palmivora* germinated earlier and their mycelium colonized the cocoa tree tissues, the *Trichoderma* conidia germinated also and their mycelium were mycoparasitic towards those of *P. palmivora*. In the course of this parasitism, *Trichoderma* hyphae coil around the *P. palmivora* hyphae and forms some appressoria on the host surface. It produces a range of hydrolytic enzymes and antibiotics which digest the cell wall of *P. palmivora* and penetrates it. Such a digestion triggers the destruction of *Phythophthora* sp. (Inbar & Chet, 1992; Kubicek *et al*., 2001; Rocha-Ramirez *et al*., 2002). The theory of mycoparasitism developed from laboratory trials seems to be in accordance with what takes place in the natural environment in field trials. Most of the reported studies analysed the influence of physical parameters such as temperature, photoperiod, luminous intensity among others upon the antagonist activity of *Trichoderma* species towards *P. palmivora*. Our results clearly establish that the *Trichoderma* mycoparasitism reduces the sensitivity of *P. palmivora* to climatic variations that ordinarily favour the growth and proliferation of *P.palmivora*.

Moreover, the climatic parameter that is most associated with the expression of black pod disease varied from one year to another. In the first year, it is the rainfall that was most linked to the expression of the black pod disease, whereas in the second year it was the minimal temperature. Thus, our results showed that

rainfall and the minimal temperature are the most dominant climatic parameters in the germination, growth, and colonization of floral cushions and pods by the mycelium of *P. palmivora*. Both climatic parameters must have some complementary effects in the expression of biological phenomena previously described. Some authors have suggested that the effect of rainfall is more important in the expression of the black pod disease (Babacauh, 1976; Deberdt *et al*., 2008), while others reported the preponderant influence of the minimal temperature. Our study demonstrated the effects of both, though it varies over time.

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