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Study of the Influence of Previous Vegetation and the Induction of Immunization of Oil Palm (*Elaeis guineensis*) against *Fusarium* Wilt

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Authors' contributions

This work was carried out in collaboration by all authors. Author GLR conducted this study at the National Center for Agronomic Research (CNRA) under the technical direction of author DS and Scientific Direction of author DAE. Author YW gave us all necessary means to carry out this work. All authors read and approved the final manuscript.

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ABSTRACT

Study Design: One of the major challenges in the control of oil palm *Fusarium* wilt at prenursery stage for seedlings protection by pre-inoculation with a saprophytic strain of *Fusarium oxysporum* lies in the persistence of the defence induced under a high parasitic strain's pressure characterizing old oil palm plantations areas at replanting.
Place and Duration of Study: Prenursery area of the National Center for Agronomic Research (CNRA), research station Robert Michaux of Dabou and oil Palm Physiology and Pathology Laboratory, between October 2009 and May 2010.
Methodology: The assessment of the influence of the *Fusarium* wilt history of an oil palm plantation on the acquisition of protection by the next generation of oil palms was performed on 2-months-old oil palm seedlings, sensitive to *Fusarium* wilt and growing on three types of soils

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characterizing the different *Fusarium* wilt history.
Results: The protection inducing treatment, characterized by the pre-inoculation of the non-pathogenic strain of *Fusarium oxysporum* (*Fot*) in the root system before the inoculation of the pathogenic strain *F. oxysporum* f. sp. *elaeidis* (*Foe*), has reduced the number of diseased seedlings at a rate of 24% against 35% when only the pathogenic strain Foe was inoculated in the seedlings, independently of the type of soil. Nevertheless, the expression of *Fusarium* wilt was delayed for a few weeks, only on the extension soil, and only in the case of pre-inoculation with *Fot*.
Conclusion: The biological control of oil palm *Fusarium* wilt at prenursery stage can only be, for the moment and under the conditions specified herein, rationally envisaged in new areas and not in replanting of old oil palms' areas.

Keywords: Oil palm; previous vegetation of soil; Fusarium wilt history; biological control.

1. INTRODUCTION

Oil palm is one of the most important worldwide oils, Côte d'Ivoire is the 7th world largest producer and the 3rd African one [1]. Despite the high potential of this crop, its expansion is limited by several constraints among which *Fusarium* wilt that causes the greatest damage on the African continent [2]. This disease, which pathogen is *Fusarium oxysporum* f. sp. *elaeidis* is favoured by a number of factors, including the previous vegetation of the oil palm plantation [3]. The incidence of *Fusarium* wilt in the first generation or planting of oil palms plays an important role in the expression of the disease in the second one [4]. Indeed, in extension over old-growth forests or savannahs, *Fusarium* wilt, an adulthood disease, has an early onset in replanting [5].

However, the control of this disease has greatly improved since its introduction in the 1960s [3]. It is based on the selection of tolerant material from the selection test at prenursery stage [6]. Individuals are resistant to the disease thanks to their genetic potentialities which favour the synthesis of biochemical compounds, but also the good agricultural practices associated [7]. However, this method, like most of those developed in order to control the whole vascular parasitic diseases, is limited so as to ensure total efficiency [8]. Indeed, the epidemic nature of the disease, added to long survival of the pathogen in the soil, as well as the time and material constraints of the selection technique (time-to-results and the large number of plants to be inoculated for selection), require the consideration of alternative methods.

This study aims at assessing, by simulating at prenursery in sensitive oil palm seedlings, the influence of the previous vegetation of an oil palm plantation on the induction of immune responses to *Fusarium* wilt, by pre-inoculation of a non-pathogenic strain of *Fusarium oxysporum*.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Fungal material

The antagonistic strains of *Fusarium oxysporum* f.sp. *elaeidis* (*Foe*) responsible for oil palm *Fusarium* wilt and *F. oxysporum* sp. (*Fot*) non-pathogenic, were the fungal material. *Foe* was isolated from tissues of diseased oil palms and *Fot* from safe oil palms. They were kept in single spore cultures until preparation of the inoculum.

2.1.2 Plant material

Two-months-old oil palm seedlings were used during our different trials. They were developed within the experimental prenursery of the National Centre for Agronomic Research (CNRA) station Robert Michaud of Dabou (Fig. 1). Seedlings originated from *Foe* sensitive cross L4156D x L2277P belonging to the hybrid category C1901 (D5D AF x L2T AF).

2.1.3 Experimental nursery location

The study medium was located within the CNRA research station Robert Michaux of Dabou. This site is located in the sub-prefecture of the said town at 70km west of Abidjan. Included in the low forest area of Côte d'Ivoire with the characteristics of a tropical forest having an equatorial climate and an average annual rainfall of about 1600mm, this place has had anyway a vegetation of savannah for many years and due to a strong anthropogenic activity [9]. This site which is favourable to the cultivation of oil palm is endemic to *Fusarium* wilt of this plant and the relatively clay-loam soil has a high proportion of coarse elements [10].

2.2 Methods

2.2.1 Inoculum production

In order to perform all manipulations, the laboratory equipment used as well as the different media prepared was sterilized at 120°C under a pressure of 1 bar during 30 minutes.

Thus, the medium for solid mould (MM) was first used for the cultivation of the single spores of both antagonistic *Fusarium* of our study. Then, after 7 days of cultivation, samples of mycelium of fungi were transplanted into erlenmeyer flasks containing 75ml of liquid Armstrong medium and incubated for 5 days. A volume of 2ml of this suspension of conidia obtained was used to seed 100ml of Armstrong medium contained in Roux flasks. After 8 days of incubation, the contents of the Roux flasks were crushed and diluted. Finally, 20ml of ground material of each fungal species present, containing an average of 2.5 x 10^6 conidia determined using a cell of Malassez were selected as inocula [3].

2.2.2 Creation of prenursery and maintenance of plant material

The germinated seeds provided by the selection department of the CNRA were checked in and planted in 1 litre-white polybags filled to 2/3 with sterile soil. These bags were then spread over nursery beds. Among the seedlings obtained after a few days, the most viable were selected and placed on trial beds within the prenursery premises according to an experimental system of 8 randomized Fisher blocks set up by the FOXPRO software [5].

A regular weeding of the premises was made, and each plant received once per month, in addition to daily watering, 0.2 l of a mixture of kieserite (Mg), urea (N), tricalcium phosphate and potassium chloride in order to meet its mineral needs [1].

For each experimental treatment, five repetitions of 20 seedlings were used, and each experiment was repeated three times.

2.2.3 Inoculation

20 ml of inoculum Foe and/Fot depending on the treatment, containing 2.5 x 10^6 spores were poured on the roots previously extracted and injured l, around the collar of the oil palm seedling. [7].

2.2.4 Methods of study

Two techniques enabled us to highlight the influence of *Fusarium* wilt history of the soil and the protective treatment on the expression of *Fusarium* wilt and the protection of sensitive oil palm seedlings faced with this disease.

Fig. 1. Two-months-old oil palm seedlings in the experimental prenursery

Oil palm can be planted on plots that have been used for several types of previous vegetation. The most common examples are the extension over savannah or forest or replanting on old oil palm plantations. In the latter case, the index of *Fusarium* wilt in first generation plays an important role in the expression of the disease.

It seemed useful for us to try to simulate the different histories made up of sensitive or tolerant oil palms at the prenursery level (Table 1).

Three types of soil from oil palm planted plots or otherwise above mentioned were used (Table 2).

2.2.4.1 Study of the influence of the nature of the soil on the expression of Fusarium wilt

In the first experiment, seedlings growing on different soils were inoculated with the pathogenic strain *Foe* only in order to study the influence of the nature of the soil on the expression of *Fusarium* wilt.

On that respect, the seedlings growing on different soils received at the time T_0, an inoculation of the pathogenic strain *Foe*.

2.2.4.2 Study of the influence of the nature of the soil on the expression of protection against Fusarium wilt

In the second trial, the plants arranged in lots on the different predetermined soils, underwent two types of treatments (Table 1):

- -In the first case, a *Foe* inoculation was applied at the time T_0+3 days after prior scraping of seedling roots and water rinsing at the time T_0 (FOE treatment);
- -In the second case, the pre-inoculation of *Fot* (time T_0) was followed three days later by that of *Foe* (FOT treatment).

2.2.5 Parameters measured

2.2.5.1 Rate of Fusarium wilted seedlings

In the inoculation tests of the oil palm *Fusarium* wilt agent at prenursery stage, the first symptoms appeared 4 to 5 weeks after inoculation. On this basis, monthly observations were made by first considering the external symptoms (yellowing and perforation of leaves, stunting of the seedling). The expression of the disease was subsequently confirmed in the last month of each experiment carried out, by cutting the pseudobulb of young diseased seedlings (Fig. 2) in order to look for the internal symptom (browning of the pseudobulb fibres). Finally, a counting of *Fusarium* wilt oil palms was made and the rate of diseased seedlings was determined according to the following formula [2]:

$$RDS_{\%} = \frac{NDS}{TNS} \times 100$$

RDS = Rate of Diseased Seedlings; NDS = Number of Diseased Seedlings; TNS: Total Number of Seedlings.

Table 1. Different soil types used for the experiences

Soil type	Origin	Objective
Extension soil, virgin from oil palm cultivation (TE)	It has previously been used for the setting up of selection tests or various trials.	It has simulated here the conditions of extension;
Replanting soil tolerant cross (TT)	Soil recovered from an inoculation test and that have been habitat for a tolerant cross (5.6% *Fusarium* wilt 5 months after inoculation).	This treatment has simulated replanting on tolerant cross (TT);
Replanting soil sensitive cross (TS)	Soil recovered from an inoculation test and that has been habitat for a sensitive cross (40.5% *Fusarium* wilt 5 months after inoculation),	This has simulated replanting on sensitive cross (TS).

Table 2. Trials conducted for the study of the influence of soil type and associated treatments on the expression of protection against *Fusarium* wilt

Soil	Treatments				Method
	Foe		Fot		
	T_0	T_0+3days	T_0	T_0+3days	
Extension (TE)	Water	Foe	Fot	Foe	
Tolerant cross (TT)	Water	Foe	Fot	Foe	Gbongué, 2012
Sensitive cross (TS)	Water	Foe	Fot	Foe	

Fig. 2. Young oil palm plant *Fusarium*-wilted seedlings dissected and showed some external and internal symptoms of *Fusarium* wilt

2.2.5.2 Evolution of the disease

The external symptoms of *Fusarium* wilt were observed and assessed every 2 weeks in order to determine the progression of the disease during the trials from the rate of diseased seedlings [1].

2.2.5.3 Reisolation of fungi

Strands of browned fibers were removed from the pseudobulb of *Fusarium* wilted seedlings and cultured for 7 days on mould medium (MM). Samples of conidia of the fungi that developed were stained Carmino green. The optical microscope observations enabled to identify the presence of *Fusarium oxysporum* f. sp. *elaeidis*.

2.2.5 Statistical analysis

Statistica 7.1 software was used to perform analyses of variance necessary for the interpretation of results. In order to ensure the normality and homogeneity of variance of these results, the data collected were subject to an arcsin transformation

$$\sqrt{\text{Rate of Diseased Seedlings (TPM)/100}}$$

prior to a two-factor variance analysis carried out at the threshold of 5%. The results then noted: "index of the rate of *Fusarium* wilted seedlings" helped to form homogeneous groups from the Newman-Keuls test.

3. RESULTS

3.1 Influence of Nature of Soil on the Expression of Protection against *Fusarium* Wilt

Results relating to the effect of the nature of the soil on the expression of *Fusarium* wilt by oil palm seedlings are shown in Fig. 3. The analysis of variance revealed no significant difference between the different soils studied (p = 0,14).

However, differences in behaviour were observed in the evolution of the disease (Fig. 4) in plants growing on the three different soils (extension soil, sensitive and tolerant). Apart from the extension soil on which the appearance of the disease in the plants was early (as early as the 2nd week after treatment), *Fusarium* wilt was reported from the 4th week in plants growing on tolerant and sensitive soil.

3.2 Effect of Treatments on the Induction of Protection According to Soils

The indexes of average rate of *Fusarium*-wilted oil palm seedlings were assessed according to the different treatments FOE and FOT. These values were significantly different ($F_{(1 ; 8)}$ = 9,12 ;

p = 0,0006). Fig. 5 shows that the highest value was obtained with the treatment FOE (0.35) and the lowest was recorded with FOT (0.24).

Moreover, if the first signs of the disease occurred in the same period on the extension soil (Fig. 6) for all treatments applied, they appeared differently on sensitive and tolerant soils.

On the sensitive soil (Fig. 7), the treatment which consisted in creating root injuries by scraping them with a piece of wood and then rinsing them with water before inoculation of Foe (FOE treatment) presented the first diseased seedlings as from the 2nd week of the test. *Fusarium* wilt was delayed by two weeks on seedlings treated with *Fot* and *Foe* (FOT treatment).

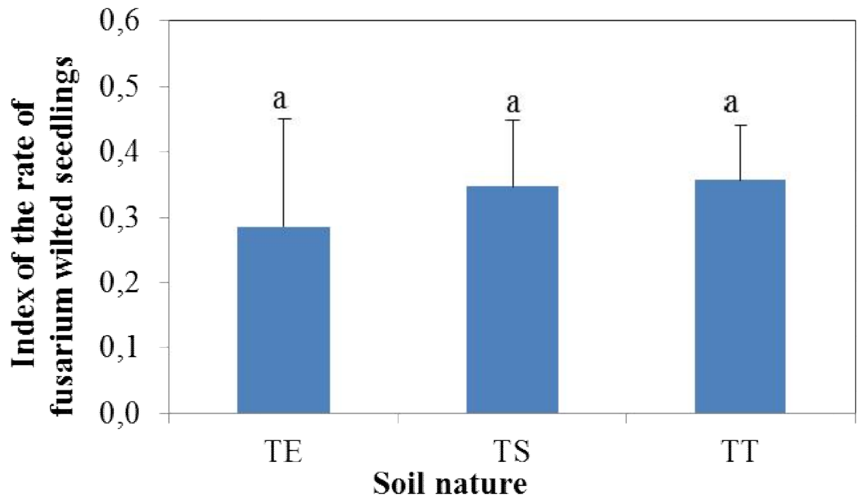

Fig. 3. Impact of soils on the expression of *Fusarium* wilt of oil palm seedlings
TE: Extension soil; TS: Sensitive soil; TT: Tolerant soil.The averages assigned with the sale letter on the histograms are not significantly different at the threshold of 5% according to the Newman-keuls test

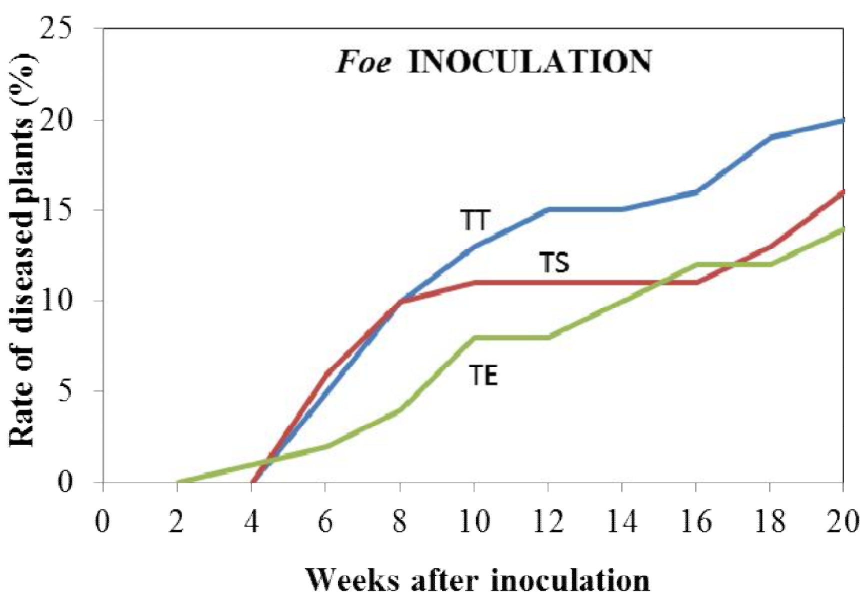

Fig. 4. Impact of soils on the evolution of *Fusarium* wilt of oil palm seedlings
TE: Extension soil; TS: Sensitive soil; TT: Tolerant soil. FOE: Inoculation of Foe after previous scraping of seedlings roots and water rinsing, FOT: preinoculation of Fot followed by inoculation of Foe three days later, Foe: Fusarium oxysporum f.sp. elaeidis (pathogenic); Fot: Fusarium oxysporum sp. (non-pathogenic)

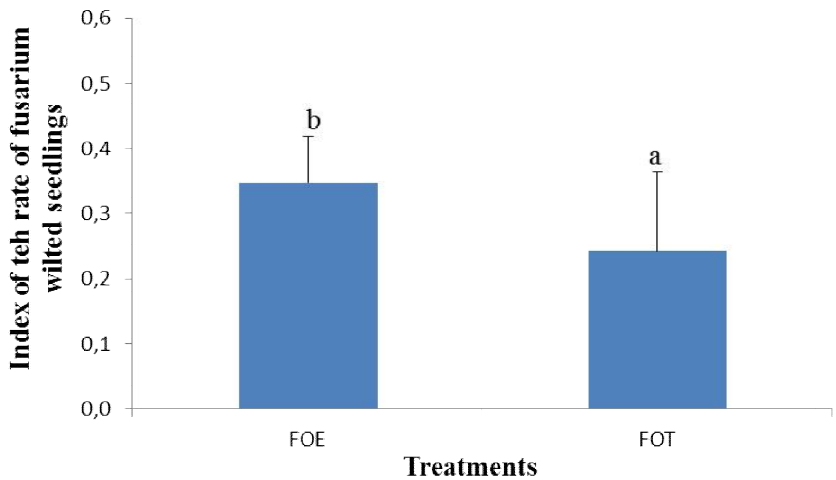

Fig. 5. Impact of the treatments applied to the different soils on the expression of *Fusarium*-wilt of oil palm seedlings

FOE: Inoculation of foe after previous scraping of seedlings roots and water rinsing, FOT: Preinoculation of fot followed by inoculation of foe three days later; the averages assigned with the sale letter on the histograms are not significantly different at the threshold of 5% according to the Newman-keuls test

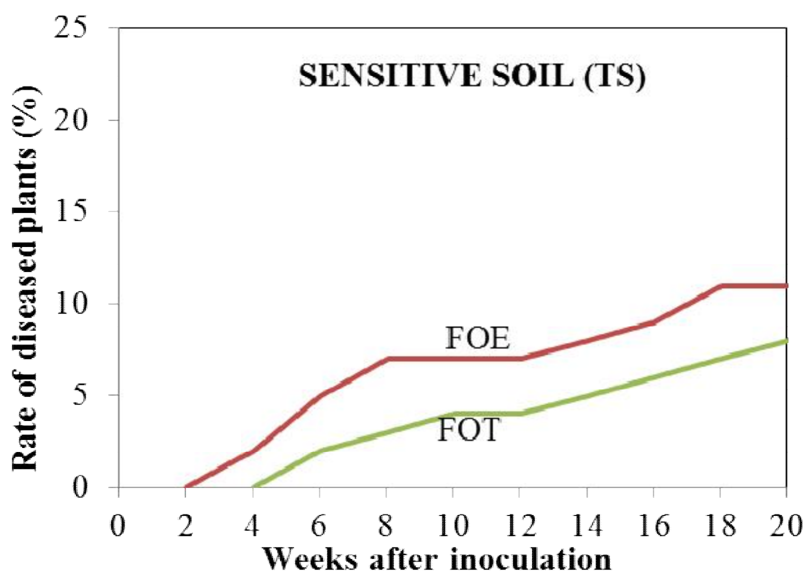

Fig. 6. Kinetics of treatments on the evolution of *Fusarium* wilt of oil palm seedlings on sensitive soil

TS: Sensitive soil, FOE: Inoculation of foe after previous scraping of seedlings roots and water rinsing, FOT: Preinoculation of fot followed by inoculation of foe three days later

Foe: Fusarium oxysporum f. sp. elaeidis (pathogenic), Fot: Fusarium oxysporum sp. (non-pathogenic)

On the tolerant soil, the FOT treatment caused, in seedlings, a delay of 2 weeks in the appearance of *Fusarium* wilt compared to FOE treatment in which the seedlings showed the disease as of the 2nd week (Fig. 8).

3.3 Reisolation of Fungi

Microscopic observations after 7 days of incubation of fungi isolated from fibres collected in the brown pseudobulb were compared to the strain of *F. oxysporum* f. sp. *elaeidis* used for inoculation of seedlings. The fungi cultured were similar to the Foe strain.

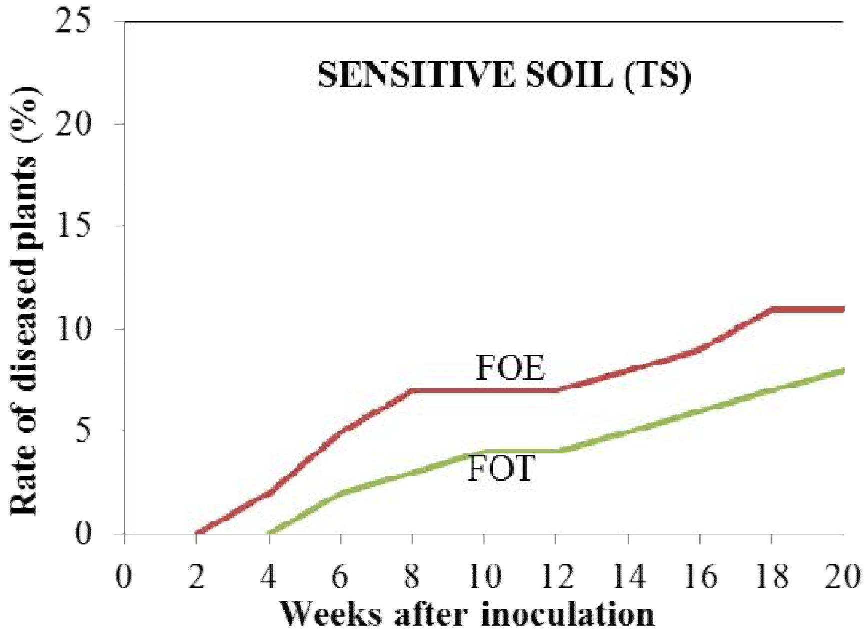

Fig. 6. Kinetics of treatments on the evolution of *Fusarium* wilt of oil palm seedlings on sensitive soil

TS: Sensitive soil, FOE: Inoculation of foe after previous scraping of seedlings roots and water rinsing, FOT: Preinoculation of fot followed by inoculation of foe three days later

Foe: Fusarium oxysporum f. sp. elaeidis (pathogenic), Fot: Fusarium oxysporum sp. (non-pathogenic)

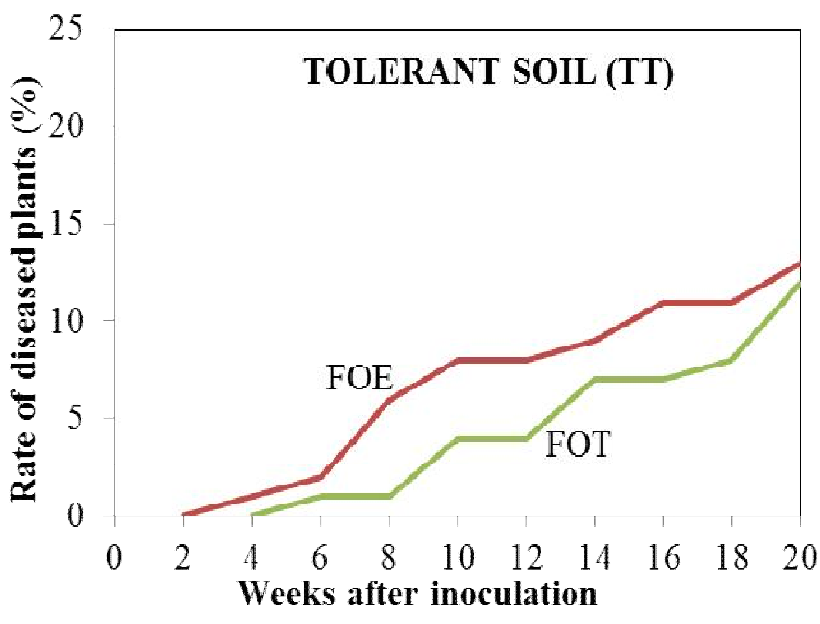

Fig. 7. Kinetics of the impact of treatments on the evolution of *Fusarium* wilt of oil palm seedlings on tolerant soil

TT: Tolerant soil, FOE: Inoculation of foe after previous scraping of seedlings roots and water rinsing, FOT: Preinoculation of fot followed by inoculation of Foe three days later

Foe: Fusarium oxysporum f. sp. elaeidis (pathogenic), Fot: Fusarium oxysporum sp. (non-pathogenic)

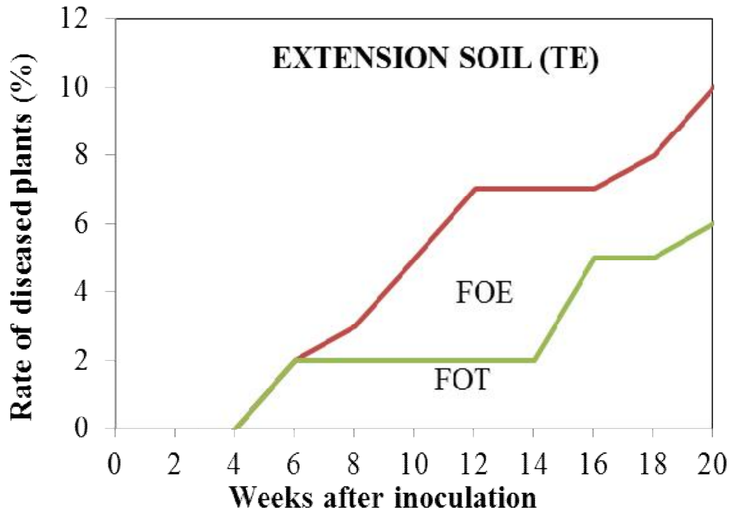

Fig. 8. Kinetics of the impact of treatments on the evolution of *Fusarium* wilt of oil palm seedlings on extension soil

TE: Extension soil, FOE: Inoculation of foe after previous scraping of seedlings roots and water rinsing, FOT: Preinoculation of fot followed by inoculation of Foe three days later

Foe: Fusarium oxysporum f. sp. elaeidis (pathogenic), fot: Fusarium oxysporum sp. (non-pathogenic)

4. DISCUSSION

The ability of the nature of the soil, as well as that of the associated protective treatment, to induce protection against *Fusarium* wilt in sensitive young oil palm seedlings, was assessed. The nature of the soil has no effect on the expression of *Fusarium* wilt. The disease was expressed with the same intensity in extension (on soil having previously been habitat to no oil palm) as well as in replanting (on sensitive or tolerant soil). However, seedlings infected by *Fusarium* wilt showed the disease quicker on extension soil compared to replanting soil. Similarly, the soil in which the non-aggressive strain was brought had no significant importance. But we noted nevertheless that a soil artificially contaminated several months ago by the pathogen, slowed the expression of protection, whether tolerant or sensitive crosses were grown on the soil. This shows, as stated by Weller et al. (2002), that even in a soil which was habitat to a tolerant crossing, the present pathogen kept its potentiality to induce the disease. It turns out that tolerance does not determine the destruction of the pathogen, but it depends on the regulatory mechanisms of microbial populations in the soil. So that during replanting, the manifestation of the disease was not prevented but rather attenuated.

If one dares to extrapolate these results under conditions of plantation, this would mean that a biological control, efficient only thanks to the prior presence of the non-pathogenic strain in contact with the roots of oil palm, could be considered rationally only on extension soils rather than on replanting soils. Indeed, on the latter, the development of the pathogenic type is considerably favoured to the detriment of other microorganisms, by the cultivation of oil palm [12]. This corroborates the thesis of Weller *et al*. who argued that nutrient competition determines the level of tolerance or resistance of a soil to vascular *Fusarium*-wilt. Indeed, in the case of a monoculture of oil palm where nutrients of *Fusarium oxysporum* f. sp. *elaeidis* are predominant, it goes without saying that the latter will be favoured in its development and its abundance in the medium. It would be useful, as increasingly indicated that the combination of crops in plantation be recommended face to the idea of an intensive monoculture, for a more efficient integrated fight against the numerous health problems [1].

Thus, under extension condition where various plants existed previously before the establishment of the oil palm plantation, the presence of antagonists causes significant effects on the contamination and infection of seedlings. This has an impact on the microbial balance in the soil in favour of the plant, either by inhibition of the growth of the pathogen or by altering its phytopathological capacities [13].

On the other hand, in the case of a perennial crop, the permanence of protection seems difficult to maintain. This problem has been studied in the case of *Fusarium* wilt of banana tree, caused by *Fusarium oxysporum* f. sp. *cubense*, towards which the biological control can be considered only to the extent that the pathogen is not installed and by favouring the development and maintaining of an antagonistic flora that would inhibit its appearance [14]. Thus we agree with the assumption made by various authors, according to which the lack of development of *Fusarium* wilt in oil palm plantations of Southeast Asia would largely be due to the abundant presence of non-aggressive strains of *Fusarium* [15-21]. An answer to this is found in the nature of the modes of action developed by the antagonistic fungi. Overall, the profitable effects they achieve in biocontrol can be explained by three major mechanisms: competition for nutrients and infection sites, strong root colonization and induction of systemic resistance by the synthesis of phenolic compounds [22,23].

The different microscopic observations of conidia of the fungus responsible for the browning of pseudobulbs of 2-month-old oil palm seedlings, have called Foe into question. It is the strain of *F. oxysporum* f. sp. *elaeidis* indeed that is responsible for the appearance of these symptoms in oil palm seedlings at prenursery stage.

5. CONCLUSION

In the search for an alternative method of fight against *Fusarium* wilt of oil palm, the efficient protection technique, from saprophytic strains of *Fusarium oxysporum,* of oil palm seedlings sensitive to this disease, appears to be possible only in extension. In replanting, the prior existence of an oil palm plantation, even made up of tolerant seedlings, favours in no way the control of the pathogen or the induction of beneficial effects to plant protection.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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