

In vivo Expression and Evolution of *Phytophthora katsurae* (Pythiaceae) Symptoms on two Coconut Varieties EGD and PB 121+ after Treatment with Phosphorous Acid in Southern Côte d'Ivoire

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Abstract: This study was initiated to optimize chemical control against *Phytophthora katsurae* on coconut trees with different concentrations of the fungicide phosphorous acid. *In vivo*, different concentrations of phosphorous acid [2.8 g (TA), 5.6 g (TB), and 11.2 g (TC) of active ingredient (a.i.)] dissolved in water, were injected into the stipes of the coconut varieties Equatorial Green Dwarf (EGD) and the hybrid PB 121⁺ cultivars in comparison with the same untreated cultivars. Soft and brutal inoculation, a strain of *Phytophthora katsurae* from naturally infected nuts was used to follow its behavior on the nuts of these cultivars treated with phosphorous acid or not. As a result, external and internal symptoms on nuts evolved according to the concentrations of acid received. 11.2 g (TC) of a.i. of phosphorous acid provided protection beyond 12 months with good inhibition of pathogen growth, as opposed to 2.8 g (TA) and 5.6 g (TB) of a.i. fluctuated between 3 and 9 months depending on the varieties. This study showed a persistence of phosphorous acid in coconut tissues at high concentration in the control of *Phytophthora katsurae*.

Keywords: Coconut trees, Chemical control, *Phytophthora katsurae*, Phosphorous acid, Côte d'Ivoire

Introduction

The coconut tree, *Cocos nucifera* L. (Arecaceae), is a tropical perennial plant whose cultivation covers a global area of 11,800,000 Ha with a production of 10,500,000 T of coprah (Van der Vossen and Chipungahelo, 2007). Because of its diversity of usage (food, handicrafts, cosmetics, pharmacopoeia), it has been called "tree of hundred uses" (Fremon et al., 1966). In Côte d'Ivoire, it is one of the main cash crops for the coastal populations because it is the crop that best adapts to this type of soil and ensures the subsistence of nearly 12,500 families through direct and indirect income (Assa et al., 2006).

Moreover, it covers an area of 50,000 Ha, a part (93%) of which is located on the coast and 7% on average in Côte d'Ivoire (Ochs, 1977; Zakra, 1997). In terms of yield, average copra production in Côte d'Ivoire is estimated at nearly 1.3 T/Ha/year, compared to 0.5 T/Ha/year at world level (Amrizal, 2003).

Nowadays, in addition to traditional uses, new opportunities are opening up for the nucicultural sector, particularly the production of drinks based on coconut sap and coconut sugar (Konan et al., 2014;

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Konan et al., 2017; Somawiharja, 2018), which could revive this sector.

But the global economic stakes represented by its cultivation is compromised by several enemies and diseases (Mariau et al., 1981; Mariau, 1999; Konan et al., 2013; Arocha-Rosete et al., 2016), among them *Phytophthora* disease. This disease, caused by *Phytophthora katsurae*, origins either by the rot of nuts, early fall of immature fruits or the rot of the heart (Renard and Quillec, 1984) and leading to the death of the tree. Coconut loss from an infected tree can be up to 75% of its annual production and, in planting, a crop loss of 50% is possible (Bennet et al., 1988).

Different ways have been used to effectively combat this pathogen, including mechanical, genetic and chemical controls (Allou et al., 2002; N'Goran et al., 2010). Given the limits of mechanical and genetic controls, chemical control is still the best way of disease control. For example, phosphorous acid, an inorganic chemical used as a fungicide and injected into the trunk, causes immediate action on the parasite (Horner and Hough, 2013). Apart from its direct action on the parasite, this fungicide initiates an indirect action notably the modification of the metabolism by induction and stimulation of the activity of certain enzymes such as polyphenoloxidases (PPO) and phenolic compounds which have an antimicrobial role for the plant (Benhamou, 2009; N'Goran et al., 2015). Such an action leads the tree to develop its own defense system against the pathogen.

In Côte d'Ivoire, most of the work carried out on coconut with phosphorous acid has consisted in comparing its efficacy with other synthetic products (Renard and Quillec, 1984; Allou and de Franqueville, 2001). However, very few data exist on the duration of action of this fungicide when used alone in treatment. This study was initiated in order to re-evaluate its efficacy and optimize its use in the field.

2. Materials and methods

2.1. Study area

The study was carried out at Marc Delorme Research Station (Abidjan). It is subject to four-seasons *Attiéén* climate, including the long rainy season (April to mid-July), the short dry season (mid-July to mid-September), the short rainy season (mid-September to November) and the long dry season (December to

March). The average annual temperature is 26.1 °C. The average annual rainfall is about 2000 mm. The ferruginous, hydromorphic, predominantly sandy soil is suitable for coconut cultivation.

2.2. Materials

2.2.1. Plant and fungal material

Two coconut varieties, the cultivar Equatorial Green Dwarf (EGD) and the hybrid PB 121⁺, were used for this study. The choice of these two varieties was justified by their susceptibility to the disease caused by *Phytophthora katsurae*. The fungal material was composed of strains of *Phytophthora katsurae* isolated from naturally infected nuts.

2.2.2. Culture medium and fungicide

The modified V8-agar medium was used for the study. Phosphorous acid (H₃PO₃) in 98,5 % crystals (Panreac, Spain) was used for the tests. With a molecular weight of 82 g.mol⁻¹ and a density of 1.65 g.cm⁻³, this colorless product is highly soluble in water (Anonymous, 2015).

2.3 Methods

2.3.1. Isolation, cultivation and conservation of *Phytophthora katsurae* strain

The sick and fallen nuts were collected at the feet of the trees. The *Phytophthora* strain was isolated by the classical method of isolation from diseased organs (Bujung, 1990). Mesocarp fragments were taken from the symptom-expression front of diseased nuts. They were then inoculated on modified V8-agar solid medium (955 ml water, 45 ml V8 and 21 g of agar-agar medium) previously poured into Petri dishes (Meng and Wang, 2010). This strain isolation and inoculation operation was carried out under a laminar flow hood and in the presence of a flame. Four days after inoculation, the contents of the plates were observed under light microscopy and two purifications were performed to isolate the strain (Bujung, 1990). The method of purification consisted of transplanting the strains twice onto new culture media under sterile conditions so that they could be isolated only without contamination. The isolated *Phytophthora katsurae* strain was transferred to modified V8-agar medium and kept in the incubator.

2.3.2. Treatment of trees of coconut varieties EGD and PB 121⁺

The variety Equatorial Green Dwarf (EGD) and the hybrid PB 121⁺ were used in this study. Fifteen coconut trees were used per variety and per treatment. For the choice of trees to be treated, those presenting

good nut production with very few attacks of the pest *Pseudaeraptus devastans* (Heteroptera: Coreidae) were selected. Four treatments were applied: the first one was characterized by perforated trees which received distilled water (control T0); the second, by trees having received 2.8 g of active ingredient of phosphorous acid in 5 ml of water (TA); the third by those trees that received 5.6 g of phosphorous acid active ingredient in 5 ml of distilled water (TB). The fourth treatment was constituted by coconut trees having received 11.2 g of active ingredient of phosphorous acid in 7.5 ml of distilled water (TC), to allow the fungicide to dissolve easily. An oblique hole was made by a self-propelled drill for the TA and TB treatments to a depth of about 12 cm at 1 m from the ground. For these two treatments, the total volume of product obtained could be absorbed through a single hole. For the TC treatment, two holes located on the same side were made, as the total volume obtained could not be absorbed by a single hole. These holes were plugged with a wooden dowel, coated with motor oil and grease, to prevent insect attack.

2.3.3. Collection of nuts after treatment

To facilitate the harvesting of the nuts, four different colors (black, yellow, green and blue) were chosen and assigned to each treatment. Useful trees were girdled with these colors at human height. On the different dates 15, 45, 60, 90, 180, 270 and 366 days after treatment, 3 nuts aged 7 to 8 months were harvested per tree and per treatment. A total of 45 nuts were obtained per treatment and per variety, i.e. 180 harvested for each variety and 160 used for inoculations. Phosphorous acid monitoring was carried out on detached nuts from coconut trees treated according to two inoculation modalities. The first one is closed to the natural way (soft inoculation) and the second one requires a prior opening on the nuts (brutal inoculation). The tests were carried out in a shed.

2.3.4. Sporocyst production and inoculum preparation

In order to allow the strains to retain their pathogenicity, healthy nuts were picked and contaminated with the strain. Once the symptoms were expressed, different strains were isolated and purified after successive transplantations on V8 medium. Each strain of *Phytophthora katsuræ* obtained after purification was transplanted onto solid V8-agar medium with added β -sitosterol. It was incubated at 25 °C in the dark. β -Sitosterol is a

hormone that promotes early and abundant sporulation of *P. katsuræ* (Hendrix, 1970; Bunjung, 1990; Allou, 1992; Yao, 2005). Its contribution to the V8 medium is indispensable, as this hormone, which is not synthesized by the pathogen, plays an important role in asexual reproduction (Leroux, 1985). After 8 days, the Petri dishes containing the strains were first removed from the incubator. Then, it flooded with 15 ml of distilled water and placed in cold storage at 4 °C for 30 min (Allou, 1992). This refrigeration step allowed the release of zoospores by dehiscence of sporocysts (Allou and de Franqueville, 2001; Yao et al., 2009). Subsequently, the boxes are removed and brought back to room temperature for 10 to 15 min. This temperature contrast favors the release of zoospores by sporocysts. A few drops of each inoculum were collected and observed under a light microscope to ensure the existence of zoospores prior to inoculation. Strain inoculums, composed of zoospores, were used to inoculate the nuts.

2.3.5. Inoculation of nuts

The nuts collected after treatment were inoculated using two inoculation techniques: soft inoculation and brutal inoculation.

For the soft inoculation, pieces of rubber tubing, 0.5 cm long and 0.8 cm in diameter, were first cut. The nuts in the shed were cleaned with distilled water and a soft cloth. Then, a selected piece of hose was glued with Neoprene contact glue (GEB, France) from one side, on the equatorial part of the nut to provide a receptacle for the suspended inoculum. 20 μ l of inoculum were deposited in each receptacle using a pasteur pipette, without injuring the nut, and the receptacle was kept closed with a piece of adhesive tape to avoid any external contamination. In order to evaluate the importance of the evolution of the symptoms according to the different treatments, a circle of 20 cm in diameter was made around the inoculation point. Finally, each nut was bagged to maintain relative humidity (Allou, 1992). The nuts were left to incubate for 14 days at the ambient temperature of the shed (due to the variability and irregularity of symptoms in mild inoculation, only the data from the brutal inoculation were taken into account in this work).

For the brutal inoculation, a wound was made, using a sterilized punch, on the equatorial part of the nut. Thus, 20 μ l of inoculum were introduced into the wound using a Pasteur pipette. After inoculation, this wound was closed with adhesive tape (Allou, 1992). To assess the extent of symptom progression with different treatments, a circle 20 cm in diameter was

made around the inoculation point. Similarly, the nuts were bagged and, as with gentle inoculation, left to incubate for 14 days at shed temperature.

2.3.6. Experimental design

After treatment with the different concentrations of phosphorous acid, the collected nuts of the EGD and PB 121⁺ varieties underwent the same inoculation tests at 15, 45, 60, 90, 180, 270 and 366 days after treatment. Thus, on each harvest date, 80 nuts were subjected to soft inoculation and 80 nuts to brutal inoculation, at a rate of 20 nuts per treatment and per variety. For all these trials, a block system was adopted. Thus, for each type of inoculation, 4 blocks were formed. In each block, 20 nuts, i.e. 5 nuts from each treatment, were inoculated with *Phytophthora katsurae* strain. Nuts with different phosphorous acid concentrations constituted the different treatments:

- nuts treated with 2.8 g of phosphorous acid in 5 ml of distilled water (TA);
- nuts treated with 5.6 g of phosphorous acid in 5 ml of distilled water (TB);
- nuts treated with 11.2 g of phosphorous acid in 7.5 ml of distilled water (TC);
- control nuts not having received phosphorous acid (T0).

The nuts were harvested one day before inoculation and placed under shelter. After inoculations, the nuts were kept in plastic bags to maintain sufficient humidity, favorable to the development of the fungus.

2.3.7. Data collection

At all times, the observations started from the first day of inoculation, called day 0 (D-0), until the 14th day. Using a tape measure, a direct measurement of the lesion on the nut was made. It consisted of measuring the height (H) and diameter (D) of the spot caused by each strain of *P. katsurae* on each nut. These measurements were made on a daily basis until day 14. The mean diameter of the lesions was determined as follows:

$$D \text{ avg} = (H+D)/2$$

D avg = average diameter of lesions

H= lesion limit along the vertical axis through the inoculation point

D= lesion limit along the horizontal axis passing through the inoculation point

These data were obtained from the total number of inoculated nuts.

2.3.8. Data analysis

All the data obtained were entered in Excel, calculated and analyzed with the Statistica 7.1 software. An analysis of variance on lesion diameters was performed with the Newman-Keuls test at 5 % of probability

3. Results

3.1 Expression of external and internal symptoms of *P. katsurae* on treated nuts according to treatments after soft inoculation

The symptoms on the nuts presented in this work were obtained after soft inoculation which is the closest to the natural route of infection (Figure 1). Thus, the external symptoms caused by the pathogen according to the different treatments show that at the level of control nuts (T0), in both cultivars, the evolution of the parasite on the epidermis was significant and regular. In addition, the evolution of the parasite in the internal parts was important, leading to mesocarp rot and reaching the shell. For hulls with cracks, the rot reached the almond, which has a foul-smelling odor. For nuts that received the treatment (TA), in general, the symptoms on both varieties were quite similar to those observed on the control nuts with rot observed at the mesocarp level and progressing towards the shell. On EGD and PB 121⁺ nuts having received the treatment (TB), the symptoms observed were less compared to those of the first two treatments but progressive in the mesocarp. For nuts having received the treatment (TC), the evolution of symptoms on both varieties was relatively less important and localized around the inoculation points with a low extension in the wadding.



Figure 1: External and internal symptoms after inoculation on nuts treated or not with phosphorous acid

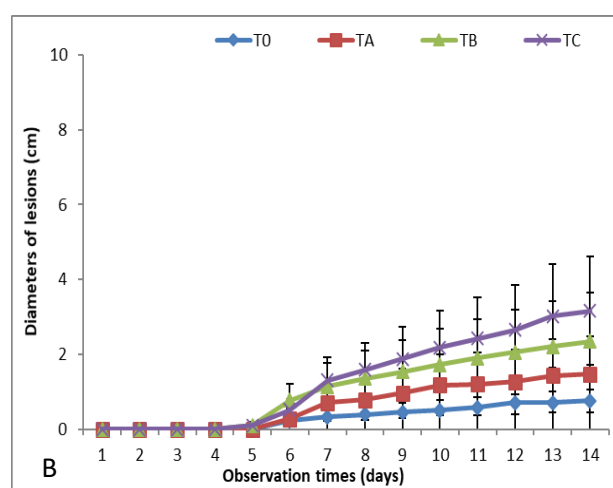
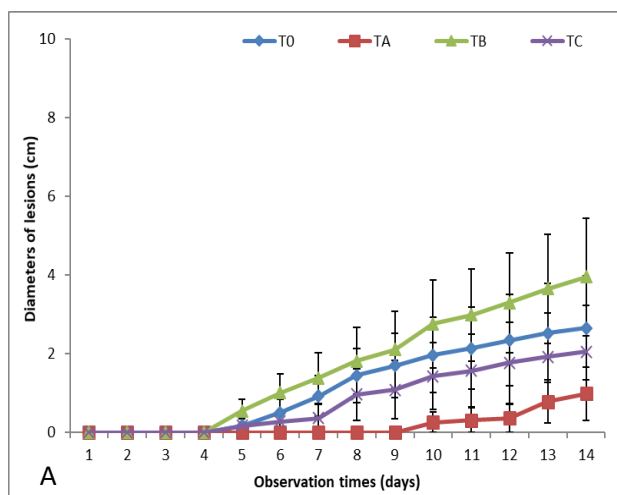
3.2 Effects of treatment on the evolution of symptoms according to the type of inoculation

3.2.1. Daily evolution of lesion diameters after soft inoculation

- Cultivar Equatorial Green Dwarf (EGD)

The incubation period was 3 to 9 days for all treatments. Thus, after the onset of symptoms, they evolve. After 14 days of observation on the nuts of the cultivar EGD, the diameters of lesions caused by the parasite were different on the nuts, depending on the treatments received and the inoculation periods. Thus, at T+15 d, the changes in diameter on the nuts from the different T0 to TC treatments ranged from 0.9 to 3.9 cm (Figure 2 A). Also, for those at T+45 d,

symptoms evolved between 0.7 and 3.16 cm (Fig. 2 B). Symptoms at T+60 d ranged from 5.17 to 8.03 cm (Figure 2 C). After T+90, T+180 and T+270 days, all inoculated nuts showed no symptoms (Figure 2 D, E, F). Lesion diameters ranging from 4.92 to 8.69 cm were observed after T+366 days (Figure 2 G). Statistical analyses of the effect of treatments on the evolution of lesion diameters were different ($p < 0.001$) at T+15 days, T+45 days, T+60 days, T+366 days. For the periods T+90 days, T+180 days and T+270 days, they could not be carried out because the symptoms were not expressed on the nuts.



In vivo Expression and Evolution of *Phytophthora katsurae* (Pythiaceae) Symptoms on two Coconut Varieties EGD and PB 121+ after Treatment with Phosphorous Acid in Southern Côte d'Ivoire

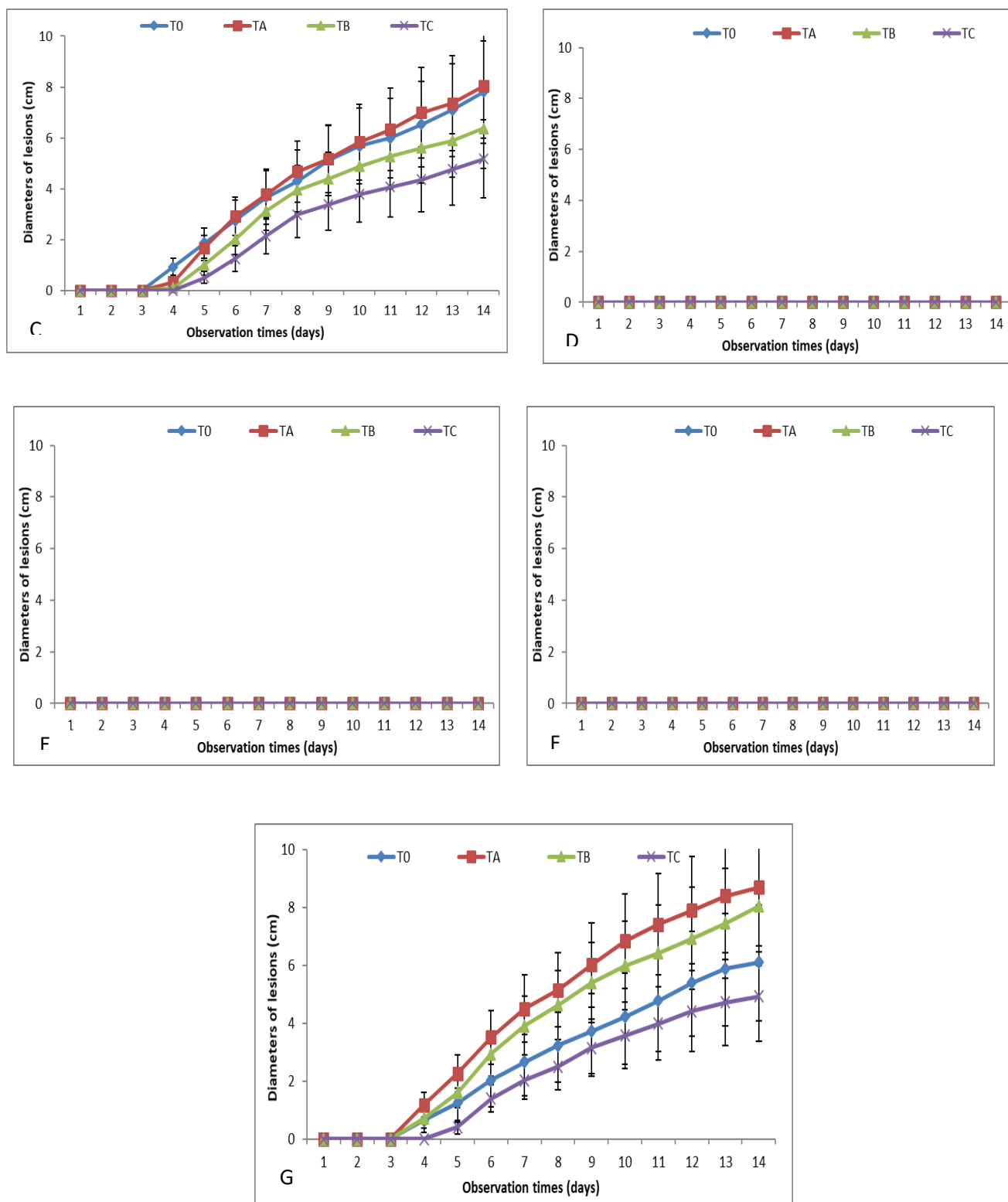
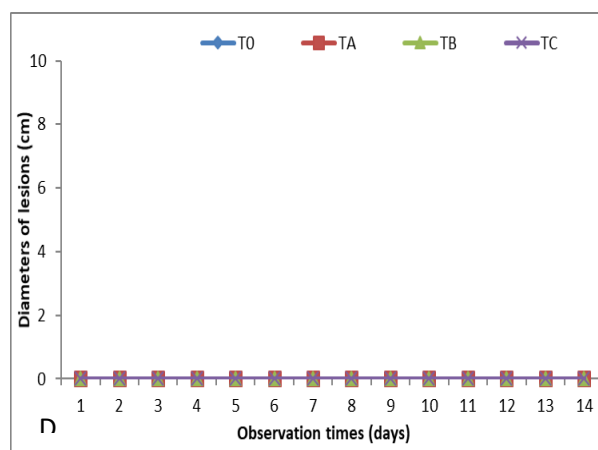
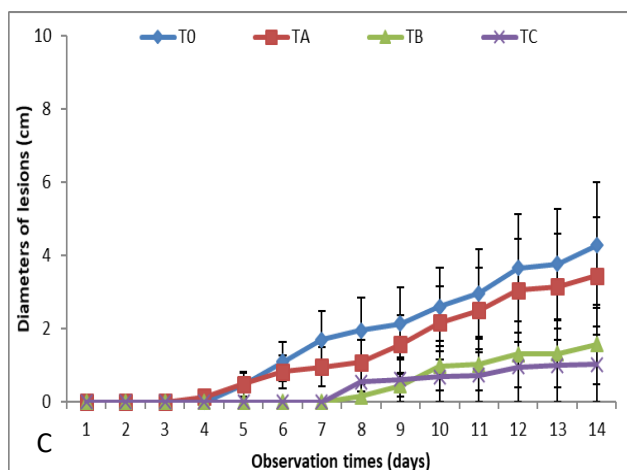
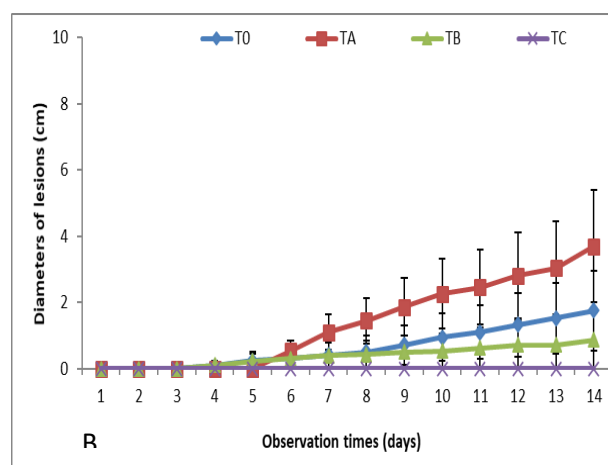
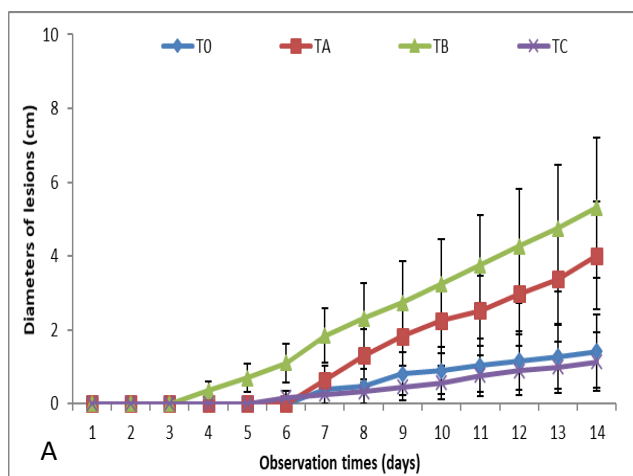


Figure 2: Evolution of *P. katsurae* lesion diameters after soft inoculation on cultivar Equatorial Green Dwarf (EGD) nuts treated or not at different phosphorous acid concentrations after T+15, T+45, T+60, T+90, T+180, T+270 and T+366 days of treatment

- Cultivar Hybrid PB 121⁺

The incubation period was 3 to 7 days. Over the same observation period, at T+15 days, the diameters of lesions caused by the fungus on individual treated nuts ranged from 1.13 cm to 5.3 cm (Fig. 3 A). At T+45 days, from T0 to TC, symptom diameters ranged from 0 to 3.7 cm (Figure 3 B). At T+60 days, the evolution of symptoms ranged from 1.02 to 4.28 cm, for T0 to TC treatments (Figure 3 C). For the periods between T+90 days and T+270 days, the inoculations carried out did not result in the

expression of symptoms on the different nuts treated (Figure 3 D, E, F). At T+366 days, symptoms varied between 1.24 and 4.58 cm in diameter for T0 to TC (Figure 3 G). A highly significant difference ($p < 0.001$) was also observed between the changes in lesion diameters at T+15 days, T+45 days, T+60 days, T+366 days according to the different treatments applied. For the periods T+90 days, T+180 days and T+270 days, they could not be carried out because the symptoms were not expressed.



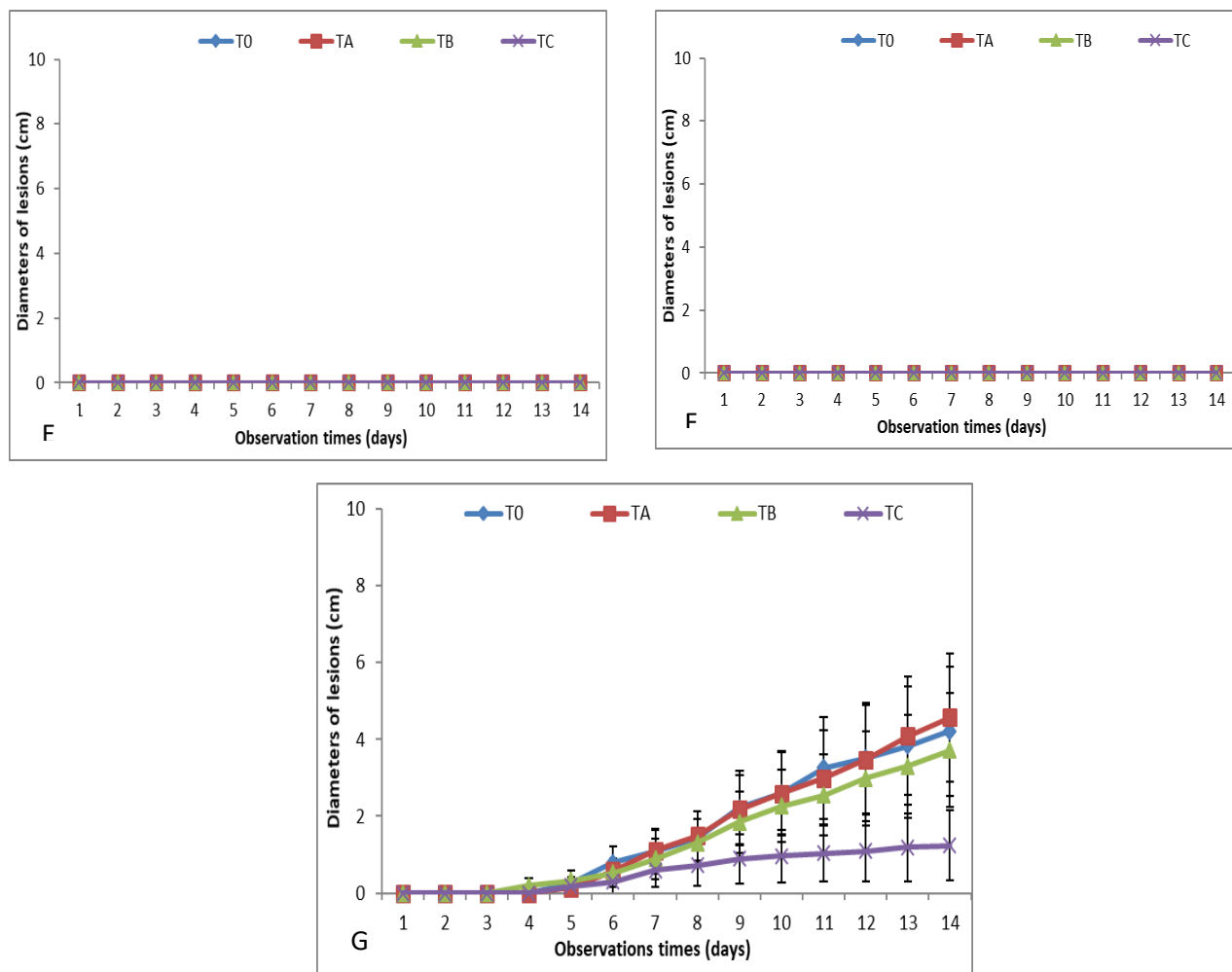


Figure 3 : Evolution of the diameters of lesions due to *P. katusrae* after soft inoculation on nuts of the Hybrid PB 121⁺ variety treated or not with different concentrations of phosphorous acid after T+15, T+45, T+60, T+90, T+180, T+270 and T+366 days of treatment

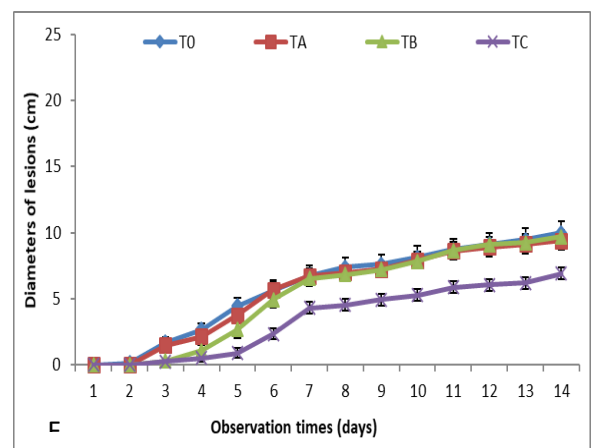
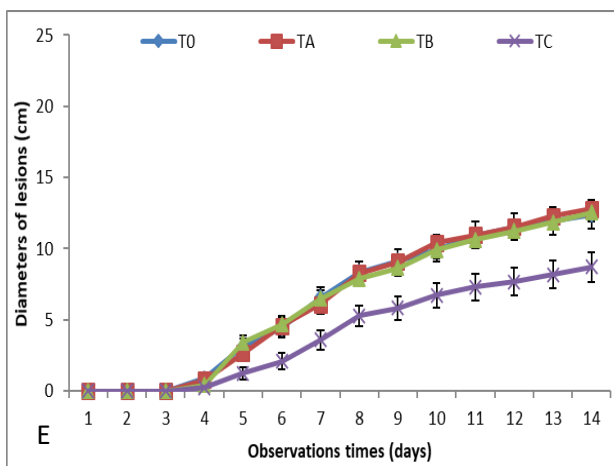
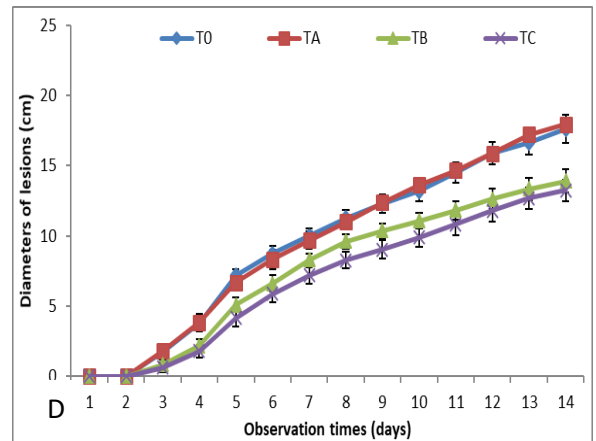
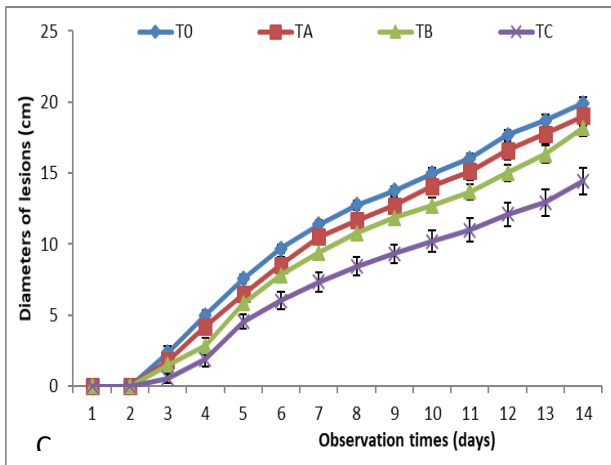
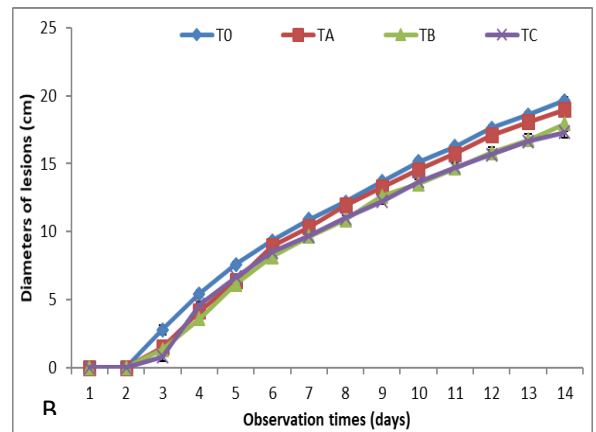
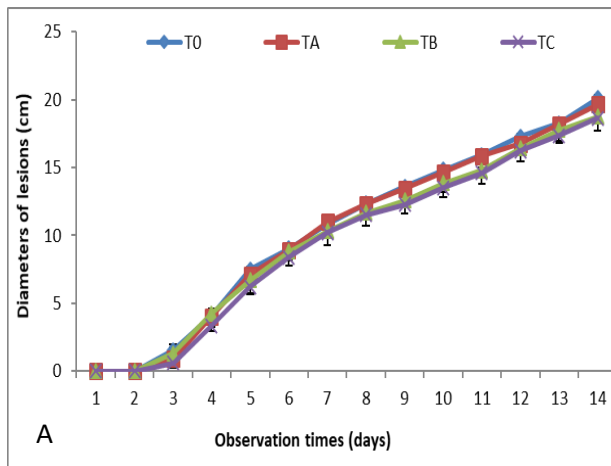
3.2.2. Daily evolution of lesion diameters after brutal inoculation

- Cultivar Equatorial Green Dwarf (EGD)

The incubation period was 2 to 3 days. After 14 days of observation, the course of symptoms with different treatments and different inoculation periods varied differently. Thus, at T+15 days, lesions on the nuts ranged from 18.63 to 20.09 cm for the T0 to TC treatments (Figure 4 A). At T+45 days, the observed symptoms ranged in size from 17.30 to 19.65 cm for T0 to TC (Figure 4 B). After T+60 days of treatment, recorded lesions ranged from 14.45 to 19.93 cm

(Figure 4 C). At T+90 days, the dimensions of the lesions noted varied between 13.24 and 17.95 cm (Figure 4 D). After T+180 days, lesions on the nuts with the different treatments ranged from 8.72 to 12.81 cm (Figure 4 E). For the periods T+270 days and T+366 days of treatment, the lesion trends were, respectively, between 6.9 and 9.9 cm and 17.46 and 20.97 cm (Figure 4 F and G). For all periods of inoculation, statistical analyses carried out showed significant differences in the evolution of symptom lesions on the nuts according to the treatments received by the nuts.

In vivo Expression and Evolution of *Phytophthora katusae* (Pythiaceae) Symptoms on two Coconut Varieties EGD and PB 121+ after Treatment with Phosphorous Acid in Southern Côte d'Ivoire



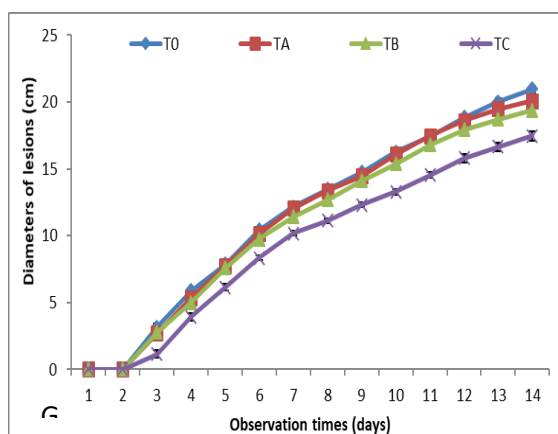
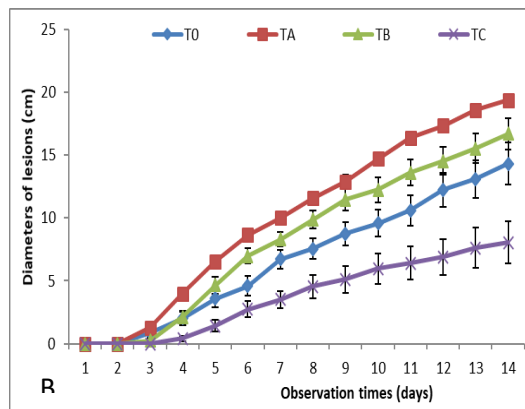
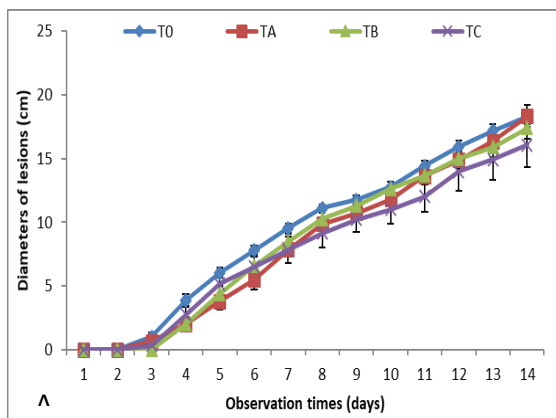


Figure 4 : Evolution of the diameters of lesions due to *P. katsurae* after brutal inoculation on nuts of the cultivar Equatorial Green Dwarf (EGD) variety treated or not with different concentrations of phosphorous acid after T+15, T+45, T+60, T+90, T+180, T+270 and T+366 days of treatment.

- Cultivar Hybrid PB 121+

The incubation period was between 2 and 7 days. On treated nuts of this variety, at the different inoculation dates, the lesions obtained from T0 to TC were as follows: 16.06 to 18.32 cm for T+15 days (Figure 5 A); 8.02 to 19.35 cm for T+45 days (Figure 5 B); 5.03 to 19.48 for T+60 days (Figure 5 C); 5.52 to

18.08 for T+90 days (Figure 5 D); 6.25 to 14 cm for T+180 days (Figure 5 E); 3.7 to 6.1 cm for T+270 days (Figure 5 F); 16.26 to 20.81 cm for T+366 days (Figure 5 G). Analyses of the evolution of symptom lesions as a function of treatment at different periods differed between them (Figure 5 G).



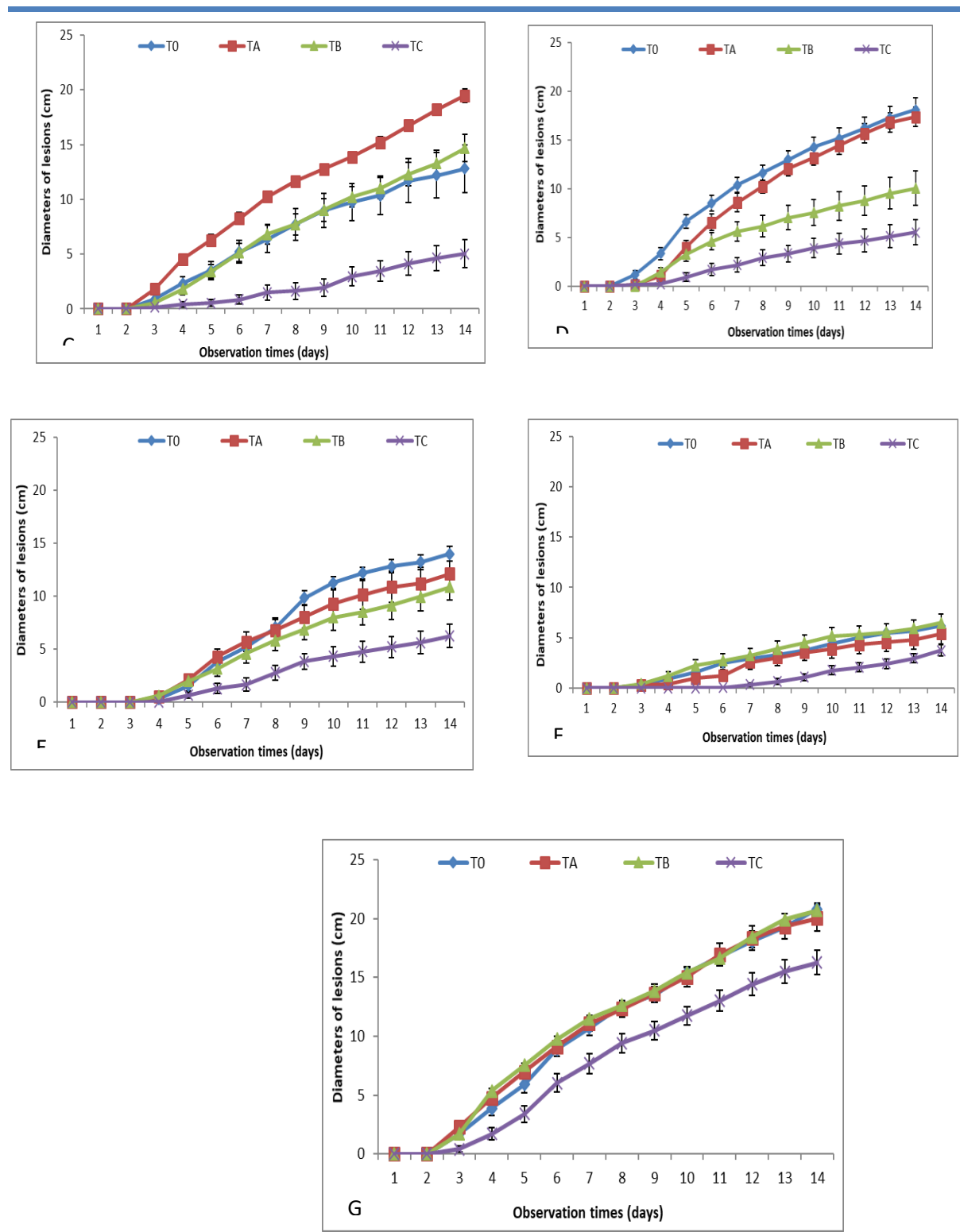


Figure 5 : Evolution of the diameters of lesions due to *P. katsurae* after brutal inoculation on nuts of the Hybrid PB 121⁺ variety treated or not with different concentrations of phosphorous acid after T+15, T+45, T+60, T+90, T+180, T+270 and T+366 days of treatment.

Discussion

The epidermis of the coconut represents the physical barrier limiting the diffusion of water and nutrients from the inside to the outside. It also provides protection against attacks by pathogens. It consists of a cuticle which has a chemical composition comprising cutin which is an insoluble polymer.

In vivo, the techniques used to inoculate the pathogen on coconuts have greatly influenced the onset and evolution of symptoms. With brutal inoculation, the physical barrier constituted by the epidermis of the fruit was destroyed. The expression of pathological symptoms on coconuts due to the absence of this barrier therefore required a short delay (3 to 4 days). With soft inoculation, the existence of a variable incubation period (4-9 d) seems to be a consequence of the presence of the nuts' pericarp, the first physical barrier to penetration of the strains followed by symptom expression (Bujung, 1990; Allou, 1992). The latter would therefore reduce the activity of hydrolytic enzymes involved in plant wall destruction (Yao et al., 2009) and host contamination. But the presence of phosphorous acid in the nut tissues would substitute the action of these enzymes by preventing the pathogen from spreading in the different tissues. Thus, symptoms in coconuts that received the high concentrations of phosphorous acid progressed more slowly from the epidermis towards the shell and kernel. Sometimes they remained confined around the inoculation points. On the other hand, for low and intermediate concentrations of phosphorous acid in coconuts, the evolution of the external and internal symptoms was progressive, and greater than in coconuts with the highest concentration of phosphorous acid. As for the controls, the symptoms evolved very rapidly on the epidermis and within the wadding, reaching the shell and the kernel. It appears that the external and internal symptoms observed on the nuts varied according to the type of treatment received.

- After treatment of coconut trees with phosphorous acid

In relation to the host-pathogen-environment factors and inoculation modes influencing successful infection, our results showed that the sensitivity of the plant material and the anatomy of the fruit would be factors in the success of tests in soft inoculation (Pohé, 1993; Allou et al., 2002). Thus, the cultivar EGD would present a high susceptibility to *Phytophthora katsurae* (Allou et de Franqueville, 2001) in contrast to the variety PB 121⁺, which would

inherit a tolerance from its male progenitor (Bourdeix et al., 2005). In addition, without taking into account the action of phosphorous acid, the expression of symptoms during the work could therefore be dependent on the combined action of different natural factors such as rain, minimum temperature and average temperature as observed by Wood (1974) and Mpika et al. (2009a). Considering the action of acid, our results are contrary to those obtained by de Franqueville and Allou (1992) who on EGD and PB 121 varieties, with the same method, showed that after two weeks of treatment, symptoms on treated nuts were weak. On the other hand, in brutal inoculation, whatever the treatment received and whatever the coconut variety, the majority of inoculated nuts showed symptoms. In fact, despite the presence of the fungicide, symptoms appeared as much on the majority of treated nuts as on the controls. Thus, the sudden inoculation, characterized by the need for prior wounding of the nut, does not make it possible to distinguish the level of aggressiveness of the strains (Yao, 2005). Destruction of the pericarp and mesocarp, which are the primary defense structures of coconuts, would facilitate penetration of the fungus into the tissues. In addition, the relative humidity brought to the pathogen by the presence of the plastic bag would reinforce its action. As a result, the lack of barrier constituted by the epidermis of the nut allowed the strain to have a short latency time to express symptoms on the nuts (Yao et al., 2009), hence the importance of symptomatic nuts.

- Effect of treatments over time

Visual evaluation of the action of phosphorous acid was made on the surface of the nuts through the diameter of the lesions. It thus made it possible to assess the evolution of symptoms on control and treated nuts. As a result, the action of the acid was much more perceptible from the 45th day after treatment because, at that date, the phosphorous acid would have been spread in the tissues of the coconuts and in all parts of the plant which delayed the development of the parasite. This is consistent with the work of Jiaming et al. (2015) who noted a rapid translocation of the acid into various tissues of the organs of the coconut tree. Also, the presence of the fungicide would contribute to the strengthening of the plant cell walls (pectins and celluloses). Indeed, the fungicide, through the phloem and cortex, would activate the production of lignin and suberin, at the edge of the infected area (Jiang, 1990). Thus, the low-concentration acid would participate in the repair of tissues slightly damaged by the parasite by

eliciting the host plants' defense mechanisms, through an indirect action as observed by Smilie et al. (1989), Sala et al. (2004) and N'goran et al. (2015).

Lesion diameters in soft inoculation, for both EGD and PB 121⁺ varieties, over the whole study period were variable. In contrast to this inoculation method, with brutal inoculation, the efficacy of the treatments was clear. Indeed, the control behaved differently from the other treatments. The latter, up to 3 months, gradually increased their effect. However, after that date, up to 366 days, the effectiveness of the treatments in nuts with medium and low acid concentrations decreased to approach that of the control. On the other hand, the treatment with the high dose of acid, over the whole period, was the one that gave better protection to the nuts. Thus, the acid injected into the trunk migrated rapidly into the plant, and systemically spread to act directly on the pathogen. Whatever the concentration applied, it protected the plant by preventing the development of parasites. This action on the fungus was characterized by a difference in the evolution of the diameters of the symptoms on the nuts. However, the results showed a progressive decrease in the efficacy of phosphorous acid over time. Such a decrease in efficacy could be related, on the one hand, to the progressive disappearance of the direct action of the acid and also to the resumption of the rainy season, which was a favorable period for the evolution and emergence of the disease. Our results corroborate the observations made by Reglinski et al. (2009) who noted a considerable variation in the longevity of phosphite action that would be influenced by environmental factors and plant physiology. On the other hand, with the loss of product related to the different nut crops for seed production, copra and, incidentally, to nut theft and coconut leaf drop. Moreover, as observed by Jiaming et al (2015), ageing and the appearance of new diets over time could explain these losses. Indeed, each month a new regime is produced on the tree, and the old ones are moved to a higher rank. Contrary to our work, Jiaming et al. (2015) used concentrations twice as high. Moreover, they did not observe any phytotoxicity in treated trees. This difference in concentration could be explained by the large number of treated trees and varieties used in our work.

Conclusion

In vivo, treatment of coconut varieties EGD and PB 121⁺ with different concentrations of phosphorous acid to assess their effect on the progression of the

pest on the surface and inside the coconuts resulted in a reduction of the parasite. Parasite growth was very low or confined around the inoculation point in coconuts receiving 11.2 g a.i. of phosphorous acid and only slowed down at half the dose (5.6 g and 2.8 g a.i.). Phosphorous acid therefore has a direct action on the pathogen. For the three doses of phosphorous acid, the highest dose of 11.2 g a.i. provided prolonged protection of the coconut trees and was not toxic to the trees. Thus, to compensate for product losses and to further prolong the effectiveness of the treatment, the use of phosphorous acid concentrations above 11.2 g a.i. could be recommended.

Conflict of interests

The authors have not declared any conflict of interests.

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