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Organic and phosphate fertilization on the aggressiveness of *Phytophthora megakarya* Brasier & M. J. Griffin in *Theobroma cacao* L.

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The cocoa tree (*Theobroma cacao* L.) is primarily cultivated for its beans, which are used to produce chocolate. However, cocoa cultivation is frequently hindered by parasitic attacks, including those caused by *Phytophthora megakarya*, which result in significant yield losses. To enhance cocoa's tolerance to this pathogen, a study was conducted using organic fertilizers (laying hen droppings (LHD) and broiler hen droppings (BHD)) and triple super phosphate (TSP), as well as their combinations at varying doses. In a greenhouse setting, the development of necrosis on whole leaves was evaluated on 5-month-old cocoa plants over four infection cycles following fertilizer application. The fertilizers were applied in two phases: initially, all fertilizers were applied, and subsequently, only organic fertilizers were applied, followed by TSP application at the 8th week. The results showed that TSP improved tolerance to *P. megakarya*, although its effects were rapid and transient. Correlation and principal component analyses revealed that fertilizer treatments were negatively correlated with the control treatment, except for BHD and TSP, which were positively correlated. The most effective fertilizers for improving tolerance to *P. megakarya* were found to be the combinations of TSP + LHD and BHD, TSP + LHD, and TSP + BHD. These fertilizers have potential for use in integrated management strategies to combat brown rot in cocoa fields.

Key words: broiler hen droppings, fertilization, laying hen droppings, necrosis, *Phytophtora megakarya*, *Theobroma cacao*, triple super phosphorus.

INTRODUCTION

The cocoa tree is an important cash crop for cocoa farmers in Africa, particularly in Central and West Africa. Cocoa production by cocoa farmers in this region represents approximately 70% of global cocoa production (ICCO, 2014; Ndoungue et al., 2021). This plant is cultivated for its beans, which regulate the income of

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many cocoa farmers and producing countries. However, species of the genus *Phytophthora* cause brown pod rot, which is one of the diseases responsible for major losses in cocoa production worldwide (Rego et al., 2022). In Cameroon, the species *Phytophthora megakarya* affects cocoa cultivation in all cocoa plantations in the country. This pathogen infects every stage of development and every part of the cocoa tree in humid conditions, leading to significant yield losses (Ndounbe-Nkeng et al., 2004; Andrews et al., 2015). According to some authors (Gregory et al., 1984; Iwaro, 1997; Ndumbe-Nking, 2003; Nyadanu et al., 2012), losses caused by the pathogen can reach 90 to 100% depending on the growing region, the variety cultivated, and environmental conditions in the absence of phytosanitary treatments. Faced with this problem that has lasted for decades, researchers have developed several methods to deal with the pathogen. Chemical control using contact fungicides, although the most widely used means of control, is very costly, causes diseases among farmers, pollutes the environment, particularly surface and groundwater, acidifies soils and therefore does not constitute a crop management process for sustainable development (Nyadanu et al., 2012). Cultural control through regular sanitary harvests in order to rid trees of all sources of contamination and make the conditions for the development of the pathogen less favorable unfortunately proves to be incomplete, unsatisfactory and the success of its measures is conditioned by environmental factors (Ndumbe-Nking, 2003). Biological control using microorganisms antagonistic to *P. megakarya* as well as the use of biofungicides comes up against the ability of the pathogen to develop resistance mechanisms. Therefore, improving host tolerance by strengthening its defense mechanism is an important way to reduce the effects of the pathogen on cocoa trees. Beyond these parasitic attacks, the context of cocoa cultivation in Côte d'Ivoire, Cameroon and Ghana is currently and naturally marked by deforestation or the disappearance of the forest used by producers as the ideal crop precedent for cocoa cultivation (Gockowski and Sonwa, 2010; Ouattara et al., 2017). Faced with this situation, producers are looking for new cultivable areas that do not necessarily respect the forest condition as a previous crop. These are mainly old cocoa or coffee plantations and natural fallows. This results in a difficult installation of cocoa plantations (Assiri et al., 2015), as well as a rapid decline in productivity. In the soils of these previous crops, there is a decrease in the main nutrients in the soil, particularly phosphorus (Assiri et al., 2015). This decline in fertility is accentuated by the use of new high-yielding hybrids. Subsequently, the soils become more acidic, causing interference with the availability and assimilation of major elements such as phosphorus (Stroia et al., 2011).

Indeed, in these soils, the problem of phosphorus adsorption and the inefficiency of phosphate fertilizers has been proven because 85 to 90% of inorganic phosphate fertilizers that, applied in acidic soils, become unavailable to plants during the year of their application. This observation results from the adsorption and precipitation of phosphorus with iron, aluminium and calcium hydroxides in the soil (Messiga et al., 2013; Ouattara et al., 2017). The availability of phosphorus for plants can be improved by the application of organic or inorganic amendments such as manures, composts, natural phosphates or the various commercialized inorganic phosphate fertilizers. Several studies have shown that the application of organic amendments of plant or animal origin in acidic soils can help increase the pH and improve the growth and development of young cocoa trees (Adejobi et al., 2014; Ouattara et al., 2017). In addition, the production and valorization of organic fertilizers are constantly expanding and positive effects on improving plant resistance to parasitic attacks have been demonstrated by several authors (Dordas, 2008). It is with this in mind that the application of organic and phosphate fertilization, adapted and reasoned, was initiated in order to improve the resistance of the cocoa tree to Phytophthora megakarya. The aim of this work was to find an economic and ecological solution in the context of sustainable development.

MATERIALS AND METHODS

Plant material

In this study, the plant material consisted of hybrid cocoa plants resulting from the cross between two parental genotypes of pure lines, one tolerant and the other sensitive. After manual pollination, some mature pods of this hybrid were harvested at the IRAD station in Baronbi-Kang and transported to the Biology and Physiology of Plant Biology (BPOV) laboratory of the Faculty of Science of the University of Douala.

Fertilizers and substrates used

The fertilizers used in this study were subdivided into two groups: Fertilizers consisting of triple super phosphate (TSP) with 45% P2O5 obtained in the Sandaga market in Douala. Organic fertilizers consisting of broiler hen droppings (BHD) obtained from a farm in the city of Douala and laying hen droppings (LPD) obtained from a farm in the city of Nkongsamba. The substrate used consisted of a mixture of humus soil from a plot of the Faculty of Science and sawdust from a sawmill in Maképé in Douala. The fertilizers thus collected were stored in the greenhouse of the Douala laboratory until their use.

Fungal material

The fungal material consisted of a pure strain of *P. megakarya* isolated from a pod infected with this fungus from a cocoa plantation in the city of Nkongsamba.

Experimental design

The creation of the nursery was carried out according to the method of Djocgoue et al. (2019). The plastic pots were filled with humus soil obtained from a mixture of surface soil from a field plot of the Faculty of Science of the University of Douala and fine sawdust in the proportions 3:1 (v / v). This soil was dried in the shade, and then sieved to remove stones and plant debris. The filled pots were placed in the greenhouse of the Laboratory. The beans were taken from inside the cocoa pods and washed in tap water made up of fine sand to remove the mucilage. On the eve of sowing, the pots were watered abundantly and the next day the cocoa seeds were sown at a rate of one bean per pot. Each seed was placed in the middle of each pot at a depth of 1 cm with the large end of the seed downwards to keep the tap straight. After sowing, the pots were watered daily at 6 am for the first 15 days after sowing, then every 2 days in order to maintain soil moisture.

Storage and preparation of fertilizers

The various fertilizers collected were stored in the greenhouse of the University of Douala until the day of fertilization, at which time the different doses of organic fertilizers were measured using a Pearson kitchen scale.

Preparation of pea agar (PA) and potato dextrose agar (PDA) media for mushrooms

Pea agar (PA) culture medium

This culture medium is selective for the trapping and purification of the pathogen. On a scale, 70 g of peas were weighed and crushed using a mortar until a leg was obtained. Then, a small amount of distilled water was added to make it liquid. After filtration, 15 g of Agar and 250 mg of chloramphenicol were added to the solution and the volume was made up to 1000 mL with distilled water. After homogenization, the medium was autoclaved at 115ºC for 30 min and poured into petri dishes ready to be used for trapping and purification of the mycelial of the microscopic fungus *P. megakarya*.

Potato-Dextrose-agar (PDA) culture medium

This medium is used for subculturing and collecting mushrooms (Benhanou and Chet, 1996). Its preparation is carried out as follows: two hundred grams of previously washed and cut potatoes were weighed on a scale, and boiled for 45 min with distilled water on a hot plate. Then the boiled potato solution was collected and introduced into a graduated Erlenmeyer flask. Fifteen grams of agar and 20 g of dextrose were added to the solution. The volume was completed to 1000 mL with distilled water. After homogenization, the medium was autoclaved at 115°C for 30 min and poured into Petri dishes ready to be used for the subculturing of the mycelial fungus *P. megakarya*.

Isolation, pathogenicity test and maintenance of the *P. megakarya* strain

The isolation and culture of *P. megakarya* were carried out according to the method of Nyassé et al. (1995). The pathogen strain was isolated from a pod naturally affected by brown rot, whose necrosis was developing. This pod was used to infect a healthy pod previously washed with distilled water by placing them side by side in a plastic container soaked in distilled water and incubated at 26°C in the dark for two weeks. This new infected pod was washed with tap water and then serially disinfected with 95% ethanol for 30 s in 10% sodium hypochlorite for 2 min, then in 70% ethanol for 2 min; so as to eliminate microorganisms present on the epidermis of the cortex. The capsule was then rinsed three times with sterile distilled water to remove traces of disinfectant. The sampling area was selected and the superficial tissues were stripped with a sterile scalpel. Several cubic-shaped fragments of approximately 5 mm in size were removed from the subcortical tissues with a sterile punch at the growth front of the necrosis. Each fragment was placed on PDA and PPA media in 90 mm diameter Petri dishes. Incubation was carried out in the dark at 26 °C for 5 days. After colony formation, the isolates obtained were purified by successive subcultures on PDA media. The pathogenicity test was carried out to verify whether it was indeed an oomycete *P. megakarya*. The apparently healthy pods, harvested early in the morning, were used for the pathogenicity test. They were washed with distilled water and disinfected with 70° alcohol for 3 min. Then, a 0.5 cm diameter punch was used to make a 1 cm deep hole in the center of each pod. Then a fungal inoculum in the form of a disc taken from a 7-day-old mycelial colony of culture was introduced into the opening made on the pods (Assiri et al., 2017). The maintenance and conservation of the pathogen strain were done by successive subcultures in petri dishes containing the same culture medium every two weeks.

Fertilizer supply, foliar infection and necrosis assessment

The droppings of broiler hens and laying hens were inoculated on the first day of the experiment around the 5-month-old cocoa plants, so as to form a circle around the stem according to the doses found in Table 1. Each treatment was repeated three times. The control treatment (T0) that did not receive any fertilizer was considered as the control treatment.

Leaf infection was performed according to the method of Manga et al. (2016). Three healthy leaves were selected per treatment, then each leaf was scarified along the midrib using a sterile blade and then the scarified leaf was sterilized with a 70° ethanol solution. A 6 mm diameter disk of agar containing the *P. megakarya* mycelial was cut out and then placed on the scarified vein of the leaf blade and then covered with sterile hydrophobic cotton and the whole was held in place with adhesive tape. The cotton was constantly moistened with sterile distilled water in order to maintain the humidity essential for the growth of the pathogen (Figure 1).

The diameter of the necrosis was measured on the back of the infected leaf on days 3, 5 and 7 using a 30 cm graduated ruler and the area of the necrosis was calculated according to the formula of Blaha and Lotode (1976).

Infection cycles

Foliar infections were carried out four times during this work:

First infection cycle

One week after the application of organic fertilizers, infection of the entire leaves in the greenhouse was carried out and then the diameter of the necrosis was measured on the back of the infected leaf on days 3, 5 and 7. After taking the growth parameters of the necrosis on the 7th day, the infected leaves were eliminated and then the plants were watered with distilled water for two weeks to allow the plants to return to their normal physiological state and to end the first infection cycle of the plants.

Second, third and fourth infection cycle

During each of these cycles, whole leaf infection was carried out in the greenhouse on the first day of the cycle, followed by taking the

Table 1. Doses of different organic fertilizers applied.

Fertilizer input	Treatment	Doses (g)
Without input	T0	0
Broiler hen droppings	T1	150
	T2	300
Triple super phosphate	T3	150
	T4	300

Figure 1. a) Nursery setup, b) growth phase and c) leaf areas of cocoa seedlings infected by *Phytophthora megakarya*.

diameter of the necrosis on days 3, 5 and 7. The end of each infection cycle was marked by the removal of infected leaves after the 7th day and then followed by watering the plants with distilled water for a period of two weeks.

Evaluation of the effect of phosphate fertilizers on the tolerance of cocoa trees infected by *P. megakarya*

Storage and preparation of phosphate fertilizers

The triple super phosphate (TSP) was stored in the Laboratory of Biology and Physiology of Plant Biology Department of the University of Douala until the day of fertilization, when the different doses of chemical fertilizers were measured using a KERN precision balance.

Fertilizer application, foliar infection and necrosis assessment

TSP was inoculated on the first day of the experiment around 5-month-old cocoa plants, forming a circle around the foot. Each treatment was repeated three times on three different plants. The different doses of TSP are shown in Table 2.

Infection cycles and necrosis assessment

Foliar infections and necrosis assessment were carried out four times using the same method as in the case of organic fertilizers.

Assessment of the effect of the combination of phosphate and organic fertilizers on the tolerance of cocoa trees infected by *P. megakarya*

Fertilizer application, infection and necrosis assessment

The fertilizer application was carried out in two different ways.

First aspect of fertilization

This first aspect of fertilization consists of treatments from T8 to

Table 2. Doses of triple super phosphaté used.

Fertilizer input	Treatments	Doses (g)
Control	T0	0
Triple super phosphate	T5	1.9
	T6	3.8
	T7	7.6

T21. During which, triple super phosphate, broiler hen droppings and laying hen droppings were inoculated only on the first day of the experiment around 5-month-old cocoa plants (first aspect). The different combinations of fertilizers and the fertilizer doses are given in Table 3.

Second aspect of fertilization

This aspect of fertilization consists of treatment T22 to treatment T35, the fertilizer composition and doses of the different types of fertilizers of which are given in Table 3. The droppings of broiler and laying hens were only applied on the first day of the experiment on five-month-old seedlings (second aspect). Triple super phosphate was only applied in the eighth week of the experiment, more precisely at the beginning of the third infection cycle.

Infection cycles and necrosis assessment

First infection cycle

After the application of organic fertilizers, infection of the whole leaves in the greenhouse was carried out after one week, and then the diameter of the necrosis was measured on the back of the infected leaf on days 3, 5 and 7. After taking the growth parameters of the necrosis on the 7th day, the infected leaves being eliminated, the plants were watered with distilled water for two weeks in order to allow the plants to return to their normal physiological state and to end the first infection cycle of the plants.

Second, third and fourth infection cycle

During the third infection cycle, phosphate fertilizers were supplemented in the second treatment group (T22 to T35) according to the doses mentioned in Table 3. During each of these cycles, whole leaf infection was carried out in the greenhouse on the first day of the cycle, followed by taking the diameter of the necrosis on days 3, 5 and 7. The end of each infection cycle was marked by the removal of infected leaves after the 7th day and then followed by watering the plants with distilled water for a period of two weeks.

Statistical analyses

The results of the development of necrosis are represented as mean ± standard deviation. Statistical analyses were conducted using SPSS software version 22.0 to perform one-way analysis of variance (ANOVA). The Student-Newman-Keuls test was used to compare differences between means at the significance level $p<0.05$. Histograms were performed using Microsoft Excel 2016 software. Correlation circle and Principal Component Analysis were performed using R version 4.0 software.

RESULTS

Evaluation of the effect of organic on the tolerance of cocoa trees to *P. megakarya*

The effect of organic fertilizers on the tolerance of cocoa trees was evaluated during each infection cycle.

First infection cycle

The development of leaf necrosis was evaluated in cocoa trees under different treatments from the 3rd day after infection. Table 4 shows the average surface area of necrosis (cm^2) on leaves during the first infection cycle. Analysis of variance (ANOVA) indicated that the development of necrosis had a significant effect of day and treatment during the first infection cycle. These results show the presence of necrosis in all treatments from day 3. The control treatment (T0) had the highest necrotic surface area (0.735±0.08) compared to the laying hen droppings (LHD) treatment (T4 and T2) and broiler hen droppings (BHD) which had the smallest surface areas (0.031±0 and 0.17±0.04 respectively). These results show from this day a negative influence of organic fertilizers on the development of necrosis. The broiler hen droppings treatments showed low necrotic surfaces compared to the laying hen droppings treatments. An effect of organic fertilizer doses on the development of necrosis is also observed with the lowest surface areas in the T2 and T4 treatments (300 g). On day 5, the development of necrosis continues in all treatments but this development is quite rapid in the control (3.467±0.330). LHD treatments were found to be more effective in inhibiting the development of necrosis compared to BHD. These observations continued on day 7, where despite the development of necrosis in all treatments, organic fertilizer treatments generally showed the lowest surface areas.

Fourth infection cycle

Table 5 shows the average necrosis surface area (cm^2) on the leaves during the fourth infection cycle. On day 3

Table 3. Doses of different fertilizers applied.

Fertilizer input	Treatments	Fertilization aspect	Doses of different fertilizers (g)
Without input	T0	First aspect	0
Triple super phosphate + broiler hen droppings	T8	First aspect	0.95 + T1
	T9	First aspect	1.9 + T1
	T10	First aspect	3.8 + T1
Triple super phosphate + broiler hen droppings	T11	First aspect	0.95 + T2
	T12	First aspect	1.9 + T2
	T13	First aspect	3.8 + T2
Triple super phosphate + laying hen droppings	T14	First aspect	0.95 + T3
	T15	First aspect	1.9 + T3
	T16	First aspect	3.8 + T3
Triple super phosphate + laying hen droppings	T17	First aspect	0.95 + T4
	T18	First aspect	1.9 + T4
	T19	First aspect	3.8 + T4
Triple super phosphate + broiler hen droppings + laying hen droppings	T20	First aspect	0.5 + 0.75 + 0.75
	T21	First aspect	0.95 + 150 + 150
Triple super phosphate + broiler hen droppings	T22	First day and eighth week	0.95 + T1
	T23	Second aspect	1.9 + T1
	T24	Second aspect	3.8 + T1
Triple super phosphate + broiler hen droppings	T25	Second aspect	0.95 + T2
	T26	Second aspect	1.9 + T2
	T27	Second aspect	3.8 + T2
Triple super phosphate + laying hen droppings	T28	Second aspect	0.95 + T3
	T29	Second aspect	1.9 + T3
	T30	Second aspect	3.8 + T3
Triple super phosphate + laying hen droppings	T31	Second aspect	0.95 + T4
	T32	Second aspect	1.9 + T4
	T33	Second aspect	3.8 + T4
Triple super phosphate + broiler hen droppings + laying hen droppings	T34	Second aspect	0.5 + 0.75 + 0.75
	T35	Second aspect	0.95 + 150 + 150

after infection of the leaves in the greenhouse, with the exception of the control which showed fairly rapid development of necrosis, the organic treatments were free of this necrosis. On day 5, the LHD treatments were free of any contamination. The development of necrosis was observed in the control and the BHD treatment with fairly rapid development in the control. On day 7, the development of necrosis was observed in all treatments. On the other hand, this development is quite reduced in the BHD treatments with the T4 treatment which showed the smallest surface area T4 (0.423±0.067a).

Evaluation of the evolution of the necrotic surfaces of days 7 during the four infection cycles under organic treatment

The analysis of the evolution of the necrotic surface of days 7 of the different organic treatments over the cycles shows that the necrotic surfaces are very high in the first

Table 4. Development of necrosis (cm²) during the first infection cycle on organic fertilization.

Treatment	Day 3	Day 5	Day 7
T0	0.735 ± 0.08^{e}	3.467 ± 0.330^{e}	9.276 ± 0.240^{e}
T1	0.225 ± 0.04^{d}	3.145 ± 0.314^{cd}	3.145 ± 0.314^{d}
T2	0.17 ± 0.04^{c}	3.037 ± 0.177^{c}	3.037 ± 0.177^{cd}
T3	0.070 ± 0^{b}	1.195 ± 0.113^{b}	1.195 ± 0.113^{b}
T4	0.031 ± 0^{a}	1.020 ± 0.278^{a}	0.994 ± 1.108^{a}

Values with the same letter in the same column are not significantly different at $p < 0.05$ according to the Student-Newman-Keuls test.

Table 5. Development of necrosis (cm²) during the fourth infection cycle on organic fertilization.

Treatment	Day 3	Day 5	Day 7
T0	0.463 ± 0.068^{b}	2.640 ± 0.167^{i}	6.91 ± 0.268^{d}
T1	0 ± 0^{a}	0.225 ± 0.05^{b}	1.261 ± 0.113^{c}
T2	0 ± 0^{a}	0.389 ± 0.109^{c}	0.735 ± 0.086^{b}
T3	0 ± 0^{a}	0 ± 0^{a}	0.686 ± 0.086^{b}
T4	0 ± 0^{a}	0 ± 0^{a}	0.423 ± 0.067^{a}

Values with the same letter in the same column are not significantly different at $p < 0.05$ according to the Student-Newman-Keuls test.

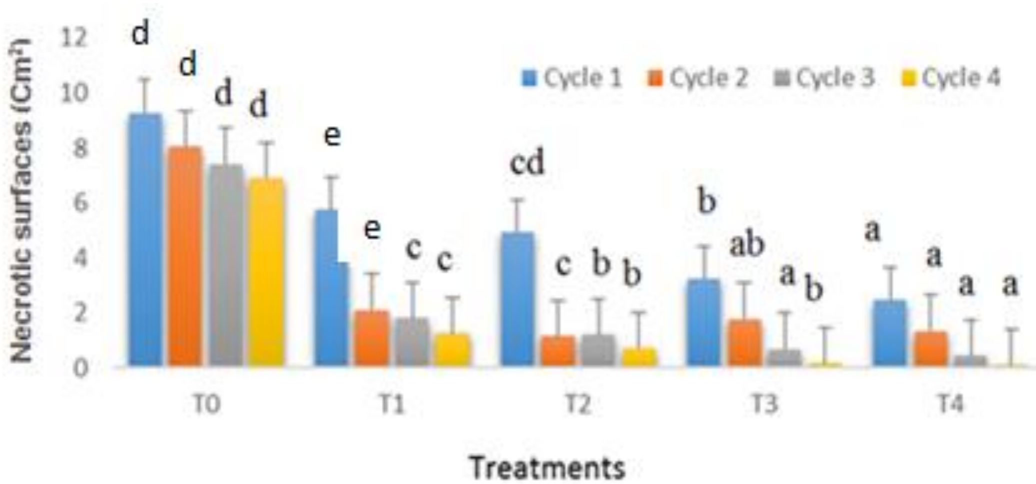

Figure 2. Evolution of necrotic surfaces on days 7 of the four infection cycles under organic treatment.

cycle for all treatments, low from the second cycle and gradually decrease until the fourth cycle (Figure 2).

Evaluation of the effect of phosphate fertilizers on the tolerance of cocoa trees to *P. megakarya*

The effect of phosphate fertilizers on the tolerance of cocoa trees was evaluated during each infection cycle.

First infection cycle

The development of leaf necrosis was evaluated in cocoa trees under different TSP treatments. Analysis of variance (ANOVA) indicated that the development of necrosis showed a significant effect of day and treatment during the first infection cycle.

Table 6 shows the mean necrosis areas (cm²) on leaves during the first infection cycle. On day 3, the

Table 6. Development of necrosis (cm²) during the first infection cycle on phosphate fertilization.

Treatment	Day 3	Day 5	Day 7
T0	0.735±0.08^{d}	3.467±0.330^{c}	9.276±0.240^{c}
T5	0.070±0^{bc}	2.640±0.168^{b}	5.738±0.720^{b}
T6	0.071±0^{bc}	2.451±0.159^{a}	4.942±1.011^{b}
T7	0.008±0^{a}	2.462±0.418^{a}	3.247±0.185^{a}

Values with the same letter in the same column are not significantly different at $p < 0.05$ according to the Student-Newman-Keuls test.

Table 7. Development of necrosis (cm²) during the fourth infection cycle on phosphate fertilization.

Treatment	Day 3	Day 5	Day 7
T0	0.463±0.068^{b}	2.640±0.167^{d}	6.91±0.268^{c}
T5	0.008±0^{a}	0.502±0^{c}	0.949±0^{b}
T6	0.008±0^{a}	0.351±0.059^{b}	1.08±0.274^{b}
T7	0.008±0^{a}	0.172±0.040^{a}	0.463±0.067a

Values with the same letter in the same column are not significantly different at $p < 0.05$ according to the Student-Newman-Keuls test.

development of necrosis was observed in all treatments. This development was much faster in the control, which recorded the largest area (0.735±0.08) compared to the phosphate treatments. To date, it is observed that among the different phosphate treatments, treatment T7 (7.6 g) had the lowest necrotic surface (0.008±0). These results show from this day a negative influence of TSP on the development of necrosis. On the fifth day after infection, necrosis had developed in all treatments and more quickly in the control. Treatments T6 and T7, on the other hand, had the smallest surfaces. This evolution of the necrotic surface is also observed on day 7. To date, the control treatment has always had the largest necrotic surface (9.276±0.240) compared to the phosphate treatments. Among the different phosphate treatments, treatment T7 is always the one with the lowest necrotic surface (3.247±0.185).

Fourth infection cycle

Table 7 shows the average necrosis surface area (cm^2) on the leaves during the fourth infection cycle. Day 3 shows the development of necrosis in all treatments. This development is much faster in the control (0.735±0.08) which records the largest surface area to date; on the other hand the TSP treatments presented the smallest surfaces (0.008±0). On day 5 after infection, an increasing evolution of the necrotic surface was observed with T0 which always shows the highest necrotic surface area (2.640±0.167) while the TSP treatment T7 has the smallest surface area (0.172±0.040). This growth was also observed on day 7. To date the T7 treatment (3.247±0.185) presented the best inhibition of the development of necrosis.

Evaluation of the evolution of necrotic surfaces on days 7 during the four infection cycles under phosphate treatments

The analysis of the evolution of the necrotic surface of the different phosphate treatments over the cycles also shows that the necrotic surfaces are very high in the first cycle and then gradually decrease from one cycle to the next (Figure 3).

Assessment of the combination of organic and phosphate fertilizers on the tolerance of cocoa trees to *P. megakarya*

The effect of the combination of organic and phosphate fertilizers on the tolerance of cocoa trees was assessed on two aspects of fertilization during four infection cycles.

First aspect of the combination of fertilizers

The development of leaf necrosis was assessed on cocoa plants after amendment of organic and phosphate fertilizers. Analysis of variance (ANOVA) indicated that the development of necrosis had a significant effect of day and treatment during each of the four infection

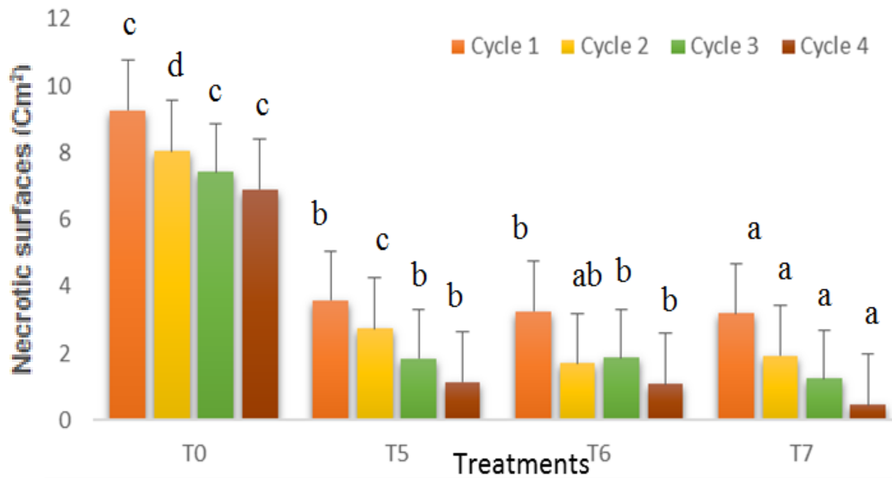

Figure 3. Evolution of necrotic surfaces from day 7 during the four infection cycles under phosphate treatment.

Table 8. Development of necrosis (cm²) during the first infection cycle of the first aspect of the combination of organic and phosphate fertilizers.

Treatment	Day 3	Day 5	Day 7
T0	0.735±0.08i	3.467±0.330f	9.276±0.240^{h}
T8	0.197±0^{gh}	1.771±0.236^{d}	2.849±0.616^{def}
T9	0.10±0.03^{cd}	1.201±0.218^{c}	2.744±0.51^{de}
T10	0.009±0^{fg}	0.424±0.068^{a}	2.289±0.493^{de}
T11	0.197±0^{gh}	2.559±0.503^{ef}	3.271±0.942^{fg}
T12	0.149±0.04^{ef}	2.381±0.551de	3.14±0.503^{fg}
T13	0.127±0de	2.193±0.341^{de}	2.368±0.442^{cde}
T14	0.031±0^{a}	0.656±0.231ab	2.469±0.534^{cde}
T15	0.031±0^{a}	0.641±0.141^{ab}	2.374±0.267^{cde}
T16	0.031±0^{a}	0.508±0.12^{a}	2.195±0.251^{cd}
T17	0.07±0^{bc}	0.591±0.077^{ab}	2.195±0.370^{cd}
T18	0.031±0^{a}	0.596±0.163^{ab}	1.857±0.418^{bc}
T19	0.031±0^{a}	0.468±0.145^{a}	1.543±0.421^{a}
T20	0.031±0a	0.463±0.068^{a}	1.6928±0.394^{ab}
T21	0.031±0a	0.384±0^{a}	1.543±0.394^{a}

Values with the same letter in the same column are not significantly different at $p < 0.05$ according to the Student-Newman-Keuls test.

cycles.

First infection cycle

Table 8 presents the average surface area of necrosis (cm^2) on leaves during the first infection cycle. On day 3 after infection of the leaves by the pathogen, necrosis was observed in all treatments. In the control treatment T0, a fairly rapid development of necrosis (0.735±0.08) was observed compared to the other treatments where a reduced development of necrotic surfaces was observed. The combinations of TSP + LHD + BHD and TSP + LHD are those having shown the smallest surfaces independently of the doses used. These results show from this day, an influence of the combination of fertilizers on the development of necrosis with the smallest necrotic surfaces observed in treatments T10 (0.009±0), T14, T15, T16, T18, T19, T20 and T21 (0.031±0). These observations continue on day 5 in all treatments with an evolution of the necrotic surface in all treatments. However, some treatments had more significant effects over time. Thus, the smallest necrotic surfaces were observed for the most part in the treatments that received

Table 9. Development of necrosis (cm²) during the fourth infection cycle of the first aspect of the combination of organic and phosphate fertilizers.

Treatment	Day 3	Day 5	Day 7
T0	0.463±0.068[b]	2.640±0.167[i]	6.91±0.268[c]
T8	0±0[a]	0.070±0[bc]	0.125±0[ab]
T9	0±0[a]	0.031±0[ab]	0.07065±0[ab]
T10	0±0[a]	0.008±0[ab]	0.031±0[ab]
T11	0±0[a]	0.008±0[ab]	0.149±0.040[ab]
T12	0±0[a]	0.008±0[ab]	0.070±0[ab]
T13	0±0[a]	0.031±0[ab]	0.125±0ab
T14	0±0[a]	0.031±0[ab]	0.075±0.048[ab]
T15	0±0[a]	0.031±0[ab]	0.031±0[ab]
T16	0±0[a]	0.008±0[f]	0.008±0[a]
T17	0±0[a]	0.031±0[ab]	0.031±0[ab]
T18	0±0[a]	0.008±0[ab]	0.031±0[ab]
T19	0±0[a]	0.008±0[ab]	0.008±0[a]
T20	0±0[a]	0.008±0[ab]	0.172±0.040[b]
T21	0±0[a]	0±0[a]	0±0[a]

Values with the same letter in the same column are not significantly different at $p < 0.05$ according to the Student-Newman-Keuls test.

the highest doses of fertilizers T21 (0.384±0), T10 (0.424±0.068), T20 (0.463±0.068) and T19 (0.468±0.145). On day 7, the results observed on day 5 were confirmed. As time progressed, some treatments had more negative effects on the development of necrosis. To date, more negative effects of the combination of fertilizers on the development of necrosis have been observed in treatments T19 (1.543±0.42), T20 (1.6928±0.394) and T21 (1.543±0.394) (Table 8). Generally, treatments with the combination of the three types of fertilizers regardless of the dose showed the lowest necrotic surfaces.

Fourth infection cycle

Table 9 shows the average necrosis surface area (cm^2) on the leaves during the fourth infection cycle. On day 3 after infection of the entire leaves in the greenhouse by the pathogen, all treatments of the fertilizer combination showed no signs of necrosis development. On the other hand, necrosis was recorded only in the control treatment T0 (0.463±0.068). These results support the negative influence of fertilizers on the establishment of pathogen infection on the cocoa tree. Then on day 5 after infection, it was also observed that the control treatment T0 (2.640±0.167) had the largest necrotic surface area while T21 (0±0) showed no signs of necrosis development. Thus, all phosphate treatments recorded necrotic surfaces less than 1 cm². In this context, some combinations were revealed by even more reduced developments of necrotic surfaces. This is how the most reduced necrotic surfaces are recorded in treatments T10 (0.008±0); T11 (0.008±0); T12 (0.008±0); T16 (0.008±0); T18 (0.008±0); T19 (0.008±0) and T20 (0.008±0), which allows to testify the negative action of phosphorus on the development of necrosis. It was also observed on day 7, that the control treatment T0 (6.91±0.268), still presents the largest necrotic surface, on the other hand the phosphate treatment T21 did not record any symptoms of the pathogen. At this date, all treatments of the combination of organic and phosphate fertilizers presented necrotic surfaces less than 1 cm². This is how the smallest necrotic surfaces are recorded in treatments T16 (0.008±0); T19 (0.008±0); T10 (0.031±0); T15 (0.031±0); T17 (0.031±0) and T18 (0.031±0). Some new treatments T10 (0.008±0), T11 (0.008±0) and T12 (0.008±0) for day 5, T10 (0.031±0), T15 (0.031±0) and T16 (0.008±0) on day 7 were revealed at the end of this cycle with a more marked negative action on the development of necrosis.

Evaluation of the evolution of the necrotic surfaces of days 7 during the four infection cycles of the first aspect of the combination of fertilizers

Figure 4 presents the evolution of the surface of the necrosis of days 7 of the four infection cycles. The analysis of this figure also showed that the necrotic surfaces of the different phosphate treatments are very high in the first cycle and then decrease progressively over time.

Second aspect of fertilization

The development of necrosis under different treatments

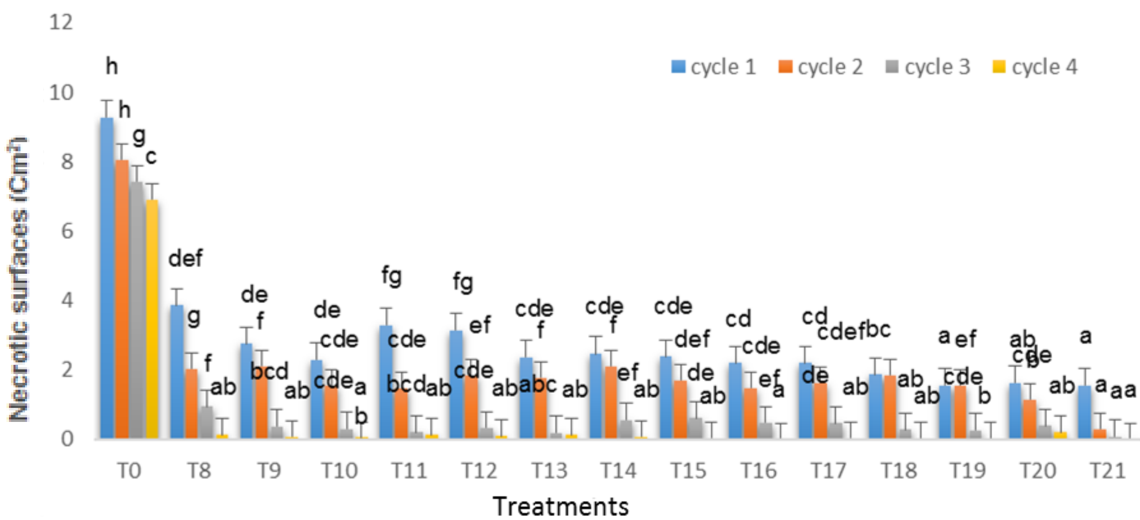

Figure 4. Evolution of necrotic surfaces from day 7 during the four infection cycles of the first aspect of the combination of organic fertilizers and phosphates.

Table 10. Development of necrosis (cm^2) during the first infection cycle of the second aspect of the combination of organic and phosphate fertilizers.

Treatment	Day3	Day 5	Day 7
T0	0.735±0.086^{h}	3.467±0.330^{f}	9.276±1.107^{e}
T22	0.508±0.125^{fg}	2.640±0.167^{e}	5.311±0.408^{d}
T23	0.283±0^{cd}	2.451±0.158^{e}	5.173±0.231^{d}
T24	0.196±0^{bc}	2.360±0.159^{e}	5.178±0.471^{d}
T25	0.468±0.145^{ef}	1.847±0.14^{d}	4.649±0.222^{cd}
T26	0.385±0de	1.614±0.131^{cd}	4.157±0.361^{cd}
T27	0.351±0.058^{d}	1.468±0.122^{bcd}	4.034±0.209^{abc}
T28	0.196±0^{bc}	1.363±0.513^{abc}	4.649±0.222^{cd}
T29	0.597±0.163^{bc}	1.467±0.122^{bcd}	4.649±0.222^{cd}
T30	0.071±0^{a}	1.261±0.113^{abc}	4.521±0^{bcd}
T31	0.126±0^{ab}	1.201±0.218^{ab}	3.804±0.345^{ab}
T32	0.126±0^{ab}	1.201±0.218^{ab}	3.359±0.380^{a}
T33	0.126±0^{ab}	1.201±0.218^{ab}	3.804±0.345^{ab}
T34	0.196±0^{bc}	1.075±0.217^{a}	4.286±0.565^{bc}
T35	0.126±0^{ab}	1.08±0.274^{a}	4.066±0.886^{abc}

Values with the same letter in the same column are not significantly different at $p < 0.05$ according to the Student-Newman-Keuls test.

of organic and phosphate fertilizer combinations was evaluated in the nursery on cocoa leaves from the 3rd day during the second aspect of fertilization. Analysis of variance (ANOVA) revealed that the development of necrosis reveals a significant effect of day under different fertilizer treatments.

First infection cycle

Table 10 shows the average of the necrosis surfaces (cm^2) on the leaves during the first infection cycle of the second aspect of fertilization. On day 3 after infection of the leaves by the pathogen, necrosis was observed in all treatments. The control treatment T0 (0.735±0.08) showed a fairly rapid development compared to the other treatments where a limited development of necrotic surfaces was observed. At this date, the most restricted areas were observed in treatments T30 (0.071±0) and T31, T32, T33 and T35 (0.126±0). These observations continued on day 5 in all treatments with an influence of organic fertilizers on the development of necrosis. In addition, some treatments presented more significant negative effects over time. Thus, the most restricted

Table 11. Development of necrosis (cm²) during the fourth infection cycle of the second aspect of the combination of organic and phosphate fertilizers.

Treatment	Day 3	Day 5	Day 7
T0	0.463±0.068[c]	2.64±0.168[f]	7.065±0[i]
T22	0.031±0[b]	0.895±0.095[e]	0.686±0.086[f]
T23	0±0[a]	0.351±0.059[c]	0.547±0.077[e]
T24	0±0[a]	0.071±0[a]	0.149±0.04[bc]
T25	0±0[a]	0.008±0[a]	0.008±0[a]
T26	0±0[a]	0.008±0[a]	0.196±0[c]
T27	0±0[a]	0.008±0[a]	0.149±0.04[bc]
T28	0.008±0[ab]	0.641±0.141[e]	0.463±0.068[d]
T29	0±0[a]	0.071±0[a]	0.316±0.059[cd]
T30	0±0[a]	0.031±0[a]	0.031±0[a]
T31	0.031±0[b]	0.641±0.141[d]	1.261±0.113[h]
T32	0.008±0[ab]	0.196±0[b]	0.39±0.11[cd]
T33	0±0[a]	0.071±0[a]	0.071±0[a]
T34	0±0[a]	0±0[a]	0.05±0[a]
T35	0±0[a]	0±0[a]	0±0[a]

Values with the same letter in the same column are not significantly different at $p < 0.05$ according to the Student-Newman-Keuls test.

development of necrotic areas was observed in treatments T34 (1.075±0.217) and T35 (1.08±0.274). On day 7, the treatments that inhibited necrosis the most were treatments T32 (3.359±0.380), T31 (3.804±0.345) and T33 (3.804±0.345). These results show from this day, an action of treatments based on the combination of fertilizers on the development of necrosis.

Fourth infection cycle

Table 11 shows the average necrosis surface area (cm²) on leaves during the fourth infection cycle of the second aspect of fertilization. On day 3 after infection of the leaves by the pathogen, necrosis was observed in all treatments. A fairly rapid development of the necrotic surface was observed in the control treatment T0 (0.463±0.068) and apart from treatments T22, T28, T31 and T32 (0.031±0) which recorded very small necrotic surfaces, most treatments remained free of any necrosis.

On day 5 after infection, the control treatment T0 (2.640±0.167) still stood out from the others by a fairly rapid development of its necrotic surface. While to date, treatments T34 and T35 (0±0) have been shown to have no necrosis on the leaves. In addition, the other treatments recorded areas of less than 1 cm². The largest necrotic area was observed on day 7 in the control treatment T0 (6.91±0.268), while treatment T21 did not record any symptoms of the pathogen. This last day of the infection cycle confirms the negative action of the combination of fertilizers on the pathogen, which is reinforced over time. At this date, treatment T35 did not show any symptoms of necrosis. Throughout this second aspect of fertilization, treatments T30, T31, T32, T33, T34 and T35 have been shown to have more negative actions on the development of the pathogen.

Evaluation of the evolution of necrosis during the four infection cycles of the second aspect of fertilization

The evaluation of the evolution of the effect of organic and phosphate fertilizers on the tolerance of *Theobroma cacao* after infection of the leaves by the pathogen was made during days 7 of the 4 cycles, also for the 2nd aspect of fertilization (Figure 5). Very high necrotic surfaces were generally noted during the first cycle with a progressive decrease in this necrotic surface from the 2nd to the 4th cycle. Thus, the first cycle is the one that presented the largest surface of necrosis on the leaves (greater than 3.4 cm²). The same observation is made during the 2nd cycle where the necrotic surfaces always remain high; but with all the same a decrease in the necrotic surfaces observed compared to the first cycle. A very significant decrease in the necrotic surface (all surfaces are less than 1.5 cm²) is observed during the third cycle of infection, a period which corresponds to the addition of TSP. Cycle 4 is characterized by an even more significant decrease in the necrotic surface (less than 0.7 cm²).

Study of the correlations between the different treatments studied

The correlation circle of the means of the necrotic

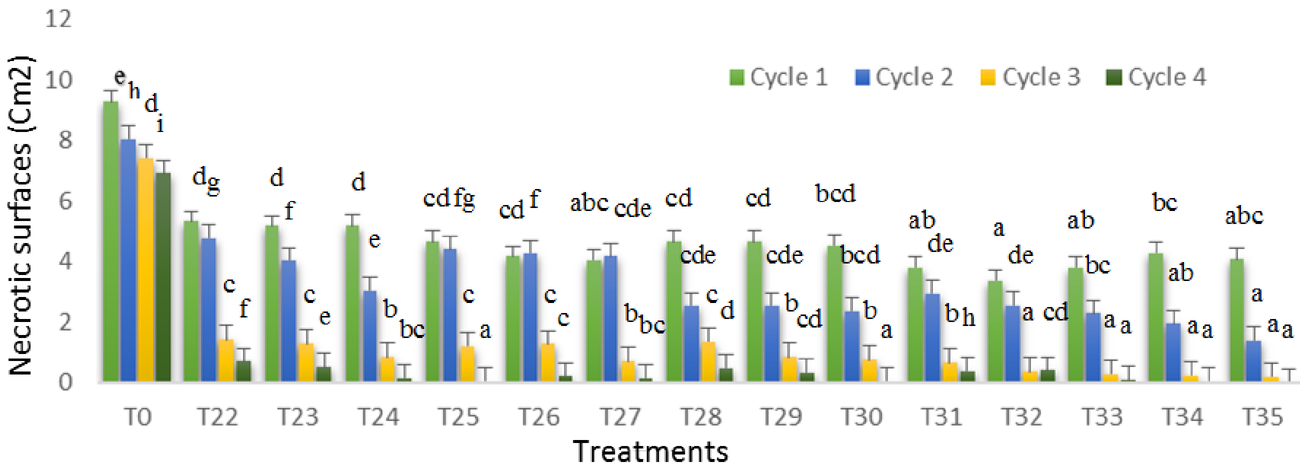

Figure 5. Evolution of necrotic surfaces from day 7 during the four infection cycles of the second aspect of the combination of organic fertilizers and phosphates.

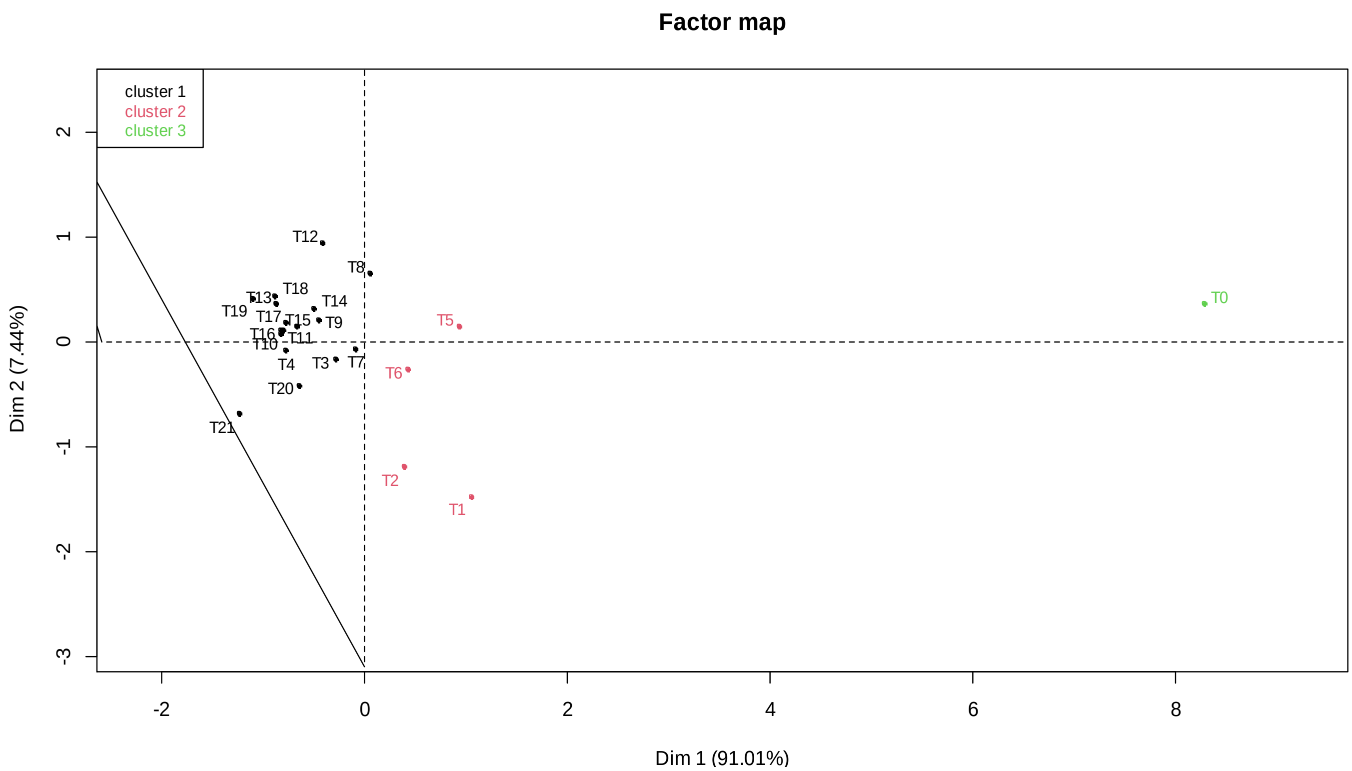

Figure 6. Correlation circle showing the relationship between the means of necrotic areas of days 7 between the different treatments of the first aspect of fertilization.

surfaces of the 7th day of the four infection cycles of the different treatments studied during the first aspect of fertilization (Figure 6) shows the correlation arrows of the four cycles very close to the correlation circle; which means that the data of the four infection cycles are well representative and correlated with each other at P = 0.005. The principal component analysis carried out between these different treatments also made it possible to visualize these correlations. Axes 1 and 2 represent 98.45% of the total variability.

Thus, it was observed that treatment T0 is significantly and positively associated with treatments T1, T2, T5 and T6. On the other hand, this treatment T0 is significantly and negatively associated with treatments T3, T4, T7 to

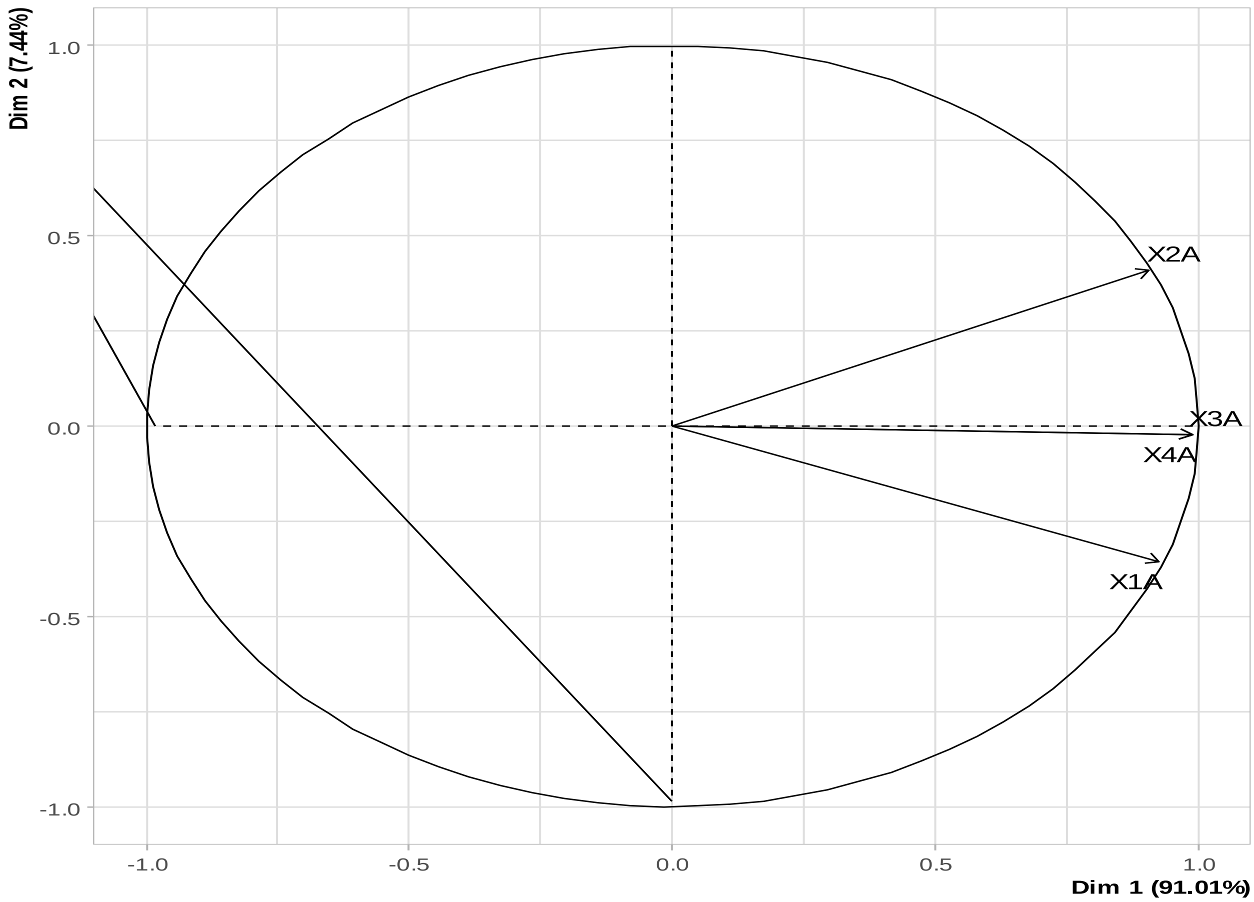

Figure 7. Principal Component Analysis showing the relationship between the means of necrotic areas of day 7, infected leaves, of the different treatments of the first aspect of fertilization.

T21 (Figure 7).

DISCUSSION

This study is an experiment conducted in a greenhouse to contribute to reducing the problems caused by *P. megakarya*, the causal agent of brown rot on cocoa trees (*T. cacao*) through the use of phosphate and organic fertilizers.

Evaluation of the effect of organic and phosphate fertilizers on the tolerance of *T. cacao* to *P. megakarya*

Evaluation of the effect of organic fertilizers

The observation of necrosis on the leaves from the 3rd day is due to the presence of *P. megakarya* mycelia. These results are in agreement with those obtained by Ondobo et al. (2014) and Manga et al. (2016). The development of necrosis under organic fertilization showed a significant effect of day and treatment during each of the infection cycles. These results obtained showed that the development of necrosis on leaves that received organic fertilization (LHD and BHD) showed low areas of necrosis significantly different from the control treatment; suggesting that hen droppings improved the tolerance of *T. cacao* via *P. megakarya*. These results corroborate with those obtained by Luong and Heong (2005) who showed that organic fertilizers minimized the pressure of insect pests and some plant diseases. Bruns et al. (1996) also showed that manures and organic fertilizers based on green waste are effective against the disease caused by *Pythium ultimum* in pea and by *Phytophthora parasitica* in tomato. While Hoitink et al. (1997) and Zhang et al. (1998) also showed that organic fertilizers reduce cucumber anthracnose attacks.

The results also showed that the dose of 300 g of hen droppings showed the lowest necrotic surfaces, which means that high doses of organic fertilizers are the most effective in stimulating the tolerance of cocoa trees. These results are similar to those obtained by Kabore (2013) who also concluded that high doses of organic manures can significantly reduce the incidence of

Macrophomina phaseolina on cowpea. Several authors explain these results through the suppressive ability of organic fertilizers to the pathogen. Indeed, the suppressive ability of organic fertilizers to plant diseases is due to the improvement of soil structure, the balanced supply of nutrients and even more by the action of the microorganisms they contain (Mouria et al., 2013; Biaou et al., 2017; Ouattara et al., 2017). The balanced supply of nutrients thus facilitates good water and mineral nutrition of the plant. The nutrients thus taken up constitute the first promoter of defense against diseases (Don and Haneklaus, 2007). In other words, nutrients act on the growth and development of the plant, on the ability to implement defense molecules and to limit the ability of the pathogen to infect and develop in the plant (Don and Haneklaus, 2007).

Over the cycles, the laying hen droppings showed the lowest necrotic surfaces and significantly different from those of the broiler hen droppings. They are therefore considered the best organic fertilizer. These results are consistent with the conclusions of authors such as Hoitink et al. (1997), Dissanayake and Hoy (1999), Fuchs and Bieri (2000), and Fuchs and Larbi (2004) who have shown that not all organic fertilizers have the same potential to protect plants against diseases.

Evaluation of the effect of phosphate fertilizers

The development of necrosis was observed on all the leaves of the cocoa tree tested in the greenhouse on days 3, 5 and 7 in all TSP treatments during the 4 infection cycles with a very significant difference compared to the control treatment. This suggests that TSP induced the tolerance of *T. cacao* to *P. megakarya*. These results are similar to those obtained by Pitan et al. (2000), Asiwe (2009) and Nitiema (2019) who showed that mineral fertilization containing phosphorus allows a considerable reduction in the number of *Maruca vitrata*, and *Clavigralla* species, on cowpea crops. In addition, Imrani et al. (2014) observed a reduction in the incidence and severity of *Helminthosporium spiciforme* after phosphorus supply. According to Larbi (2006), phosphate fertilization provides the plant with nutrients that are compounds controlling metabolic activities that are necessary for the synthesis of antimicrobial compounds with active response mechanisms or inhibitory phytoalexins such as phenols, flavonoids and other defense compounds that accumulate around plant infection sites. On the other hand, our results are contrary to those found by Bouet et al. (2012), who showed in their work that phosphorus has no significant effect on the development of yellow mottle of rice. This difference could be due to the plant-pathogen system studied. Fertilizers therefore do not have the same effects on plant defense systems (Larbi, 2006). These results also indicated that on day 7 of the 4 cycles, the T7 treatment with the highest dose of TSP (7 g) showed the lowest necrotic surface during the four infection cycles. It is therefore considered the best dose of phosphorus fertilization allowing good tolerance of *T. cacao*. Similar results have been observed by several authors such as Nyiransengiyumva (2007), Selvarajan et al. (2009), Moutassem et al. (2018) who have shown that the use of high doses of phosphorus decreases the production and viability of *Helmentosporium solani* conidia, helps to control *Banana Bract Mosaic virus* and significantly reduces chickpea vascular wilt disease.

Evaluation of the evolution of necrotic surfaces of organic and phosphate treatments from day 7 during the four infection cycles

Necrotic surfaces were generally very high in all control treatments of organic fertilization in the first infection cycle, and then gradually decreased until the fourth cycle. However, the most significant decrease was observed between the first and second infection cycles. This indicates that *T. cacao* developed a systemic immune response from the first infection, which nevertheless made it possible to set up defense mechanisms and thus respond quickly and more effectively during subsequent infections. In addition, this immune response is more noticeable in organic, phosphate treatments and their combination than in the control treatment. These results can be explained by the fact that improving the nutrition of *T. cacao* by organic fertilizers also improves the establishment of the systemic immune response. Rehman et al. (2020) also showed that PGPRs, by improving plant nutrition, indirectly promote the induction of systemic resistance.

Assessment of the effect of organo-phosphate fertilizers on the tolerance of the cocoa tree to *P. megakarya*

The development of necrosis on the leaves of *T. cacao* from the 3rd to the 7th day during the four infection cycles under organo-phosphate fertilization generally showed a very negative and significant effect on the development of necrosis.

These results confirm the negative action of fertilizers on the development of the pathogen. In addition, the organo-phosphate treatments T21 and 35 did not show any symptoms of the pathogen during the fourth infection cycle. This proves that the negative action of fertilizers on the tolerance of the cocoa tree is reinforced over time during many infections. In other words, the more the plant is infected, the more its defense system is strengthened.

The results also showed that the negative action of the combination of fertilizers depends on the dose and type of fertilizer used in the combination. After combining the three types of fertilizers (TSP, LHD and BHD), treatments T21 and T35, which consisted of the highest doses, proved to be the most effective. These results are in agreement with those found by Nitiema, 2019 who showed that the use of organic and mineral fertilizers best reduces thrips pressure on cowpea.

Comparative study

Comparative study between the different types of fertilizers used

The comparison of the necrotic surfaces induced by the different types of fertilizers as well as the different combinations showed that the combination of the three fertilizers (TSP + LHD + BHD) recorded the lowest surfaces throughout the four infection cycles; which is not significantly different from the combinations TSP + LHD, TSP + BHD and LHD. These results stipulate that these fertilizers, whether single or combined, induce better tolerance to *T. cacao* against the pathogen.

Comparative study between the first aspect and the second aspect of fertilization

The comparative study made between the first and second aspect of fertilization on the basis of the averages of the necrotic surfaces recorded on days 7 of the 4 infection cycles made it possible to note that the first aspect of fertilization of the combination of fertilizers significantly reduced the development of necrosis compared to the second aspect of fertilization. This aspect of fertilization is therefore considered the best method to adopt for the combination of fertilizers.

These results could be explained by the fact that the actions of the combination of organic and phosphate fertilizers are more effective in inducing tolerance to *T. cacao* when they are amended at the same time. This helps us understand why the combination of fertilizers stimulates the tolerance of the cocoa tree more than the application of simple fertilizers.

Study of the correlations between the different treatments studied

The correlation circle carried out on the different treatments of day 7 of the four infection cycles demonstrates the representativeness of the data. The principal component analysis showing the relationships between the different treatments of day 7 of these four infection cycles showed that axes 1 and 2 represent 98.45% of the total variability. Thus, on axis 1, treatment T0 is negatively and significantly correlated with treatments T3, T4, T7 to T21. These results mean that the development of necrosis is negatively correlated with fertilization with laying hen droppings at high doses of TSP and with the combination of organic and phosphate fertilizers. These results are similar to those reported by Moutassem et al. (2018) who indicated a negative correlation between the level of phosphate fertilization and a significant decrease in chickpea vascular wilt disease observed in the case of high phosphate availability.

Conclusion

At the end of this study, it appears that all the different types of fertilizers as well as their different combinations have improved the tolerance of *T. cacao* against the attack of *P. megakarya* and therefore attest that these fertilizers have an antifungal activity on leaf discs of *T. cacao* infected by *the pathogen*. However, the fertilizers that have shown the most significant effects on the tolerance of the cocoa tree are: the combination of triple superphosphate + laying hen droppings + broiler hen droppings, the combination of triple superphosphate + powdered hens droppings, the combination of triple super phosphate + broiler hen droppings and laying hen droppings. These fertilizers can therefore be used in fields to reduce brown rot caused by the pathogen on *T. cacao* while also reducing the exclusive dependence on chemical fungicides and fertilizers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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