

Full Length Research Paper

Influence of clam shells and *Tithonia diversifolia* powder on growth of plantain PIF seedlings (var. French) and their sensitivity to *Mycosphaerella fijiensis*

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Plantain prices in sub-Saharan markets are very high due to the fact that the supply does not cover the large demand. The main constraint of plantain cultivation is the seedlings unavailability in quantity and quality, which is essential to boost the creation of new plantations. The PIF technique could solve this problem if its substrate of production is amended with natural products for quality enhancement. This study aims to assess clam shells and *Tithonia diversifolia* effects on the growth of PIF plantain seedlings and their sensitivity to *Mycosphaerella fijiensis*. Plantain PIF seedlings were grown in an amended substrate. The treatment influences the seedlings germination rate, number of shoots, height, diameter, area of leaves and favours a less sensitivity to *M. fijiensis* compared to the controls. The presence of clam shells and *T. diversifolia* in the treatment especially enhanced the (1) vegetative growth and (2) less sensitivity as well as accumulation of proteins and polyphenols respectively. This combination shows a synergic action with dual role both as a biofertilizer and as a biopesticide. This work valorises the use of by-fishing products and bad herbs that are environmentally benign and affordable to poor smallholders' farmers, leading to a sustainable and responsible agriculture, as well as poor peasants' empowerment.

Key words: Plantain (*Musa* spp.), PIF seedlings, *Tithonia diversifolia*, clam shells, biofertilizer, biopesticide, *Mycosphaerella fijiensis*.

INTRODUCTION

Banana in the *Musaceae* family is a perennial monocotyledonous plant that originates from South East Asia and grows in tropical and subtropical regions. The *Musa* spp. is composed of many cultivars, notably need to be cooked before consumption as compared to dessert

bananas. The contribution of plantain (*Musa* spp., genome AAB) cultivation for income generation is significant and vital for food security of the population in tropical and sub-tropical zones, especially in Central and West Africa.

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In Central Africa, Cameroon is the first in term of plantain production and ranks 9th in the world (4.94 million tons per year) (FAO, 2017). Plantain production is very low and inadequately covers the large demand, leading to very high prices for this commodity in local markets. Based on this, it is necessary to create new plantations to improve the performance of this crop and meet up with the large demand despite the unavailability of seedlings in quantity and quality (Ewané et al., 2019).

Traditionally, a banana plant is obtained from suckers of another banana plant that is one plantain sucker for one plantain seedling, and is usually disseminated with farmlands soil which often contains pathogenic microorganisms. The vitroplants are ideal for new plantations creation since they are safe from any contamination, but are very expensive and not affordable to poor peasants. An innovative technique called PIF set up by the African Centre for Research on Banana-plantain (CARBAP) that is, plants issued from stem fragments is an alternative for smallholders' farmers owing to its many advantages (Kwa, 2002). When this technique is applied to one sucker, we can obtain 20 to 100 seedlings depending on the variety and the farmer's experience. This technique is essential for massive production of seedlings in quantity within a very short period of time (2 to 3 months) and at low cost. This innovative technique can help increase the number of plantain plantations in the subregion through an easy and cheap set up of plantain plantations, leading to an increase in the production as well as the purchasing power of the poor peasants. However, PIF seedlings are facing many problems during acclimatization like contamination of the seedlings on farmlands that could lead to plants mortality of about 60% during the establishment of new plantations and are now rejected by some farmers (Ewané et al., 2019).

Banana tree is permanently under the threat of many pathogens amongst a virulent, invasive and predominant pathogen called *M. fijiensis*, that causes severe reduction of the leaf area in all banana-growing countries. Moreover, *M. fijiensis* is responsible for black Sigatoka disease (BSD), the most economically destructive disease of bananas, that causes loses of about 50% of production (Onautshu, 2013). The use of synthetic products such as weed-killers, fertilizers, fungicides, pesticides in PIF seedlings production and on farmlands can be harmful to human and the environment, be responsible for the appearance of resistance in plant pathogens strains (Ewané et al., 2013) and is not affordable to the smallholder farmers.

It was recently demonstrated in Cameroon that clam shells powder has a strong influence on PIF plantain seedlings growth and susceptibility to BSD in nurseries by its dual role as a biofertilizer and as a biofungicide (Ewané et al., 2019). Therefore, regarding their properties, clam shells are a good candidate to improve the production in quality and quantity of PIF plantain

seedlings. Another good candidate is *Tithonia diversifolia*, a woody herb of 2-3 m tall in the family *Asteraceae*. It is highly rich in nutrients, averaging about 3.5% nitrogen (N), 0.37% phosphorus (P) and 4.1% potassium (K) and decomposes rapidly after its application to the soil thereby enriching the soil with N, P and K for the growth of crops. With its antifungal properties, it plays an important role in diseases control and induces the crude synthesis of defense metabolites (flavonoids, tannins, alkaloids, pathogenesis related-proteins) for plants defense (Chagas-Paula et al., 2012). Phytochemicals such as sesquiterpenoids, diterpenoids, alkaloids, flavonoids, chlorogenic acid derivatives, phenols, saponins, tannins, and terpenoids are present in the leaves, stems, and roots of *T. diversifolia* (Umar et al., 2015; Kerebba et al., 2019).

Utilization of these two natural products (clam shells and *T. diversifolia*) could be a new approach to improve the quality and the quantity of plantain. Based on their cost benefit ratio, the association of the PIF technique and the powders of clam shells and *T. diversifolia* in the production of plantain seedlings could lead to the enhancement of the number of plantation and the productivity in the subregion, the less utilization of synthetic inputs in agriculture, the less production cost leading to the poor small holder farmer poverty alleviation. The aim of this study is to examine the effect of clam shells and *T. diversifolia* powder on the growth promotion of plantain PIF seedlings in nursery and on their protection against *M. fijiensis*.

MATERIALS AND METHODS

Plantain suckers of French variety were obtained from Lékié division (Obala) of Centre Region of Cameroon. The short cycle of production and the good productivity capacity were the selected criteria for the choice of this variety.

The clam shells (organic matter) came from the municipality of Mouanko, located in the Littoral region and more specifically in the Sanaga Maritime division, precisely on the North bank of the Sanaga River about twenty kilometers east of its mouth in the Gulf of Guinea. To obtain the organic matter powder, the fresh clams were washed, dried in the sun, broken into large pieces, then reduced to powder and finally sifted.

T. diversifolia tissues were obtained from farm lands around the Biotechnology Centre of University of Yaoundé 1 located at Nkolbisson (Yaoundé-Cameroon).

The strain of the causal agent of black Sigatoka disease (*Mycosphaerella fijiensis*) was provided by the African Centre for Research on Bananas and Plantains (CARBAP) of Njombé in the Littoral region of Cameroon.

The sawdust, sand and black soil were used as substrates and sterilized in an oven at different temperatures and time intervals as described by Ewané et al. (2019). The sawdust was used for growth of plantain PIF seedlings in the greenhouse while a mixture at a ratio of 2/3 of black soil and 1/3 of sand was used in the shade.

Experimental design

This research was conducted in Yaoundé (Centre Region,

Table 1. Experimental design for the study of the influence of clam shells and *Tithonia diversifolia* powder on vegetative growth of plantain PIF seedlings and their sensitivity to *Mycosphaerella fijiensis*.

Location	Completely Randomized Block Device	
	Greenhouse	Shade
Phase	Germination	Acclimatization
Purpose	Production of the PIF seedlings	Survey of the seedling's growth
Experimental Unit (EU)	Each treatment	Each treatment
Substrate to amend	Sawdust	Black soil and sand
Number of plants/EU	Three (03) Explants	At least three (3) plants
Container	Propagator	Plastic planter bags
Block	A sterilized substrate block (B1)	A non-sterilized substrate block (B2)
Condition	Controlled Condition Use of Sterile Substrate (SS)	Uncontrolled (Farmer) Condition Use of non-Sterile Substrate (nSS)
Number of Treatment	Four (04)	Four (04)
Treatment	1. Sterile Substrate + Clam shells (SS+CS) 2. Sterile Substrate + <i>T. diversifolia</i> (SS+Td) 3. Sterile Substrate + Clam shells + <i>T. diversifolia</i> (SS+CS+Td) 4. Sterile Substrate only as Control (SS)	1. Non-Sterile Substrate + Clam shells (nSS+CS) 2. Non-Sterile Substrate + <i>T. diversifolia</i> (nSS+Td) 3. Non-Sterile Substrate + Clam shells + <i>T. diversifolia</i> (nSS+CS+Td) 4. Non-Sterile Substrate only as Control (nSS)

Cameroon), from September 2015 to March 2016 under controlled conditions in the laboratory and in the greenhouse (Table 1). The PIF technique was done in two steps the germination of the explants in the greenhouse and 2) an acclimatization phase of the seedlings under shade. During this second step (November 2015 to January 2016), the average temperature and the mean monthly rainfall of the locality were respectively 28 °C and 53 mm. The suckers were prepared through trimming, shelling and the trauma of the shoot apical meristem following the method used by Ewané et al. (2019). The different experimental units were classified by block on the shelves in a greenhouse and covered with a white and transparent plastic. Explants tracking (watering) in the greenhouse allowed them to germinate and produce seedlings.

Evaluation of the vegetative growth in the greenhouse and in the shade

The germination rate and the number of PIF seedlings per experimental unit were evaluated after every seven days starting from the second week of introduction of explants in the greenhouse for a period of four successive weeks. This evaluation was done according to the method reported by Ewané et al., (2019). The seedlings with two to three small open leaves and three to four radicles were transferred after eight weeks in plastic planter bags in the shade for acclimatization.

The height and the diameter of the seedlings' pseudo-stems, and the total leaf area of the seedlings' leaves were evaluated for three plants selected per experimental unit in the shade. The total leaf surface (TLS) of each plantain seedling was determined using the method reported by Ewané et al., (2019). Every seven days starting from the day the seedlings entered the shade, the measurements were taken for each experimental unit for three successive weeks.

Evaluation of the sensitivity to black Sigatoka disease

M. fijiensis's strain was used for artificial inoculations of the leaves

of plantains seedlings and was obtained according to the protocol of Ewané et al., (2019).

The leaves of the same age i.e. about 12 weeks from three plants per experimental unit were selected the day of the experimentation, detached and transported to the laboratory for inoculation. Before inoculation, a leaf of each plant was conserved at - 45°C in a plastic sachet for biochemical analysis of the before inoculation stage, while the ones to be inoculated were cleaned and kept for two hours at air temperature. A 100 µL droplet of *M. fijiensis* suspension (10^6 zoospores/mL) was then deposited on the middle of leaf surface. The infected leaves were kept under controlled condition of relative humidity in the laboratory in a basin and covered with transparent film. The evaluation of necrosis's progression was done by measuring the length (L) and the width (W) of the necrotic surface after every two days for 12 days in order to visualize the rot spreading on the leaf's surface. The 'necrotic surface area' (NSA) in mm² was calculated for each measurement by assuming a rectangular shape to the necrosis as in the formula of Ewané et al. (2019): $NSA = L \times W$.

Biochemical analyses

The determination of the content of total native protein and total phenolic compounds were carried out in two stages (before and after inoculation) on the whole leaves. The leaves samples involved were cut at 1 cm beyond the necrotic point or beyond the marked scar (sections with no symptoms). For these analyses, each treatment was repeated thrice.

Extraction samples were carried out according to the method reported by Pivorani et al., (2008) with modification and by El Hadrami et Baaziz (1997) respectively for total native protein and phenolic compounds. 1 g of fresh leaf was used for each extraction followed by quantification as described by Ewané et al. (2019). The protein concentration was expressed in mg equivalent (Eq) of bovine serum albumin (BSA) per g of fresh weight (FW) while that of phenolic compounds was measured in mg equivalent of gallic

Table 2. Variance analysis of clam shells and *Tithonia diversifolia* powder effects on the percentage of germination and number of cumulative shoots of plantain seedlings in the greenhouse.

Source	Percentage of germination ($R^2 = 100\%$)			Number of shoots ($R^2 = 99\%$)		
	DF	F	P	DF	F	P
Condition	1	2190	< 0.0001	1	6	0
Treatment	3	1653	< 0.0001	3	590	< 0.0001
Day	3	9881	< 0.0001	3	769	< 0.0001
Condition×Treatment	3	3	0	3	1	0
Condition×Day	3	451	< 0.0001	3	2	0
Treatment×Day	9	277	< 0.0001	9	17	< 0.0001
Condition×Treatment×Day	9	78	< 0.0001	9	1	1

DF is the degree of freedom; F is the value of F test and P is the probability.

acid per g of fresh weight.

Statistical analyses

The effects of *T. diversifolia* and clam shells powders on plantain seedlings vegetative growth, sensitivity to BSD, and total proteins and polyphenols were analysed by subjection of the value (percentage of germination, number of shoots, height and diameter of seedlings, leaves surface area, necrotic surface area, total proteins and total polyphenolic) to mixed three-way ANOVA performed with XLSTAT software. Each plant being taken as experimental unit and condition, treatment and day as factors. Multiple comparisons of the means were done by applying Tukey's test at 5% probability level. Pearson correlation analysis between the different variables was also performed with XLSTAT software.

RESULTS

Effect of clam shells and *T. diversifolia* on the PIF seedlings vegetative growth

The germination rate and the number of shoots were found to be significantly influenced ($P < 0.0001$) by the three variables (condition, the treatment and the day) with respective R^2 values of 1 and 0.99 (Table 2). The most influential variable of the three was the day. The percentage of germination was consistently higher in the treated PIF substrates compared with the controls.

The number of shoots was consistently higher in the amended PIF substrates compared with the controls. The germination occurs fast in the controlled condition (SS) compared to the non-controlled condition (nSS), and the significant difference was very low between both conditions for the number of shoots (Figures 1 and 2).

Treatment effect was almost the same for all the amended substrate in the greenhouse with 100% of germination obtained 28 days after seeding (DAS) regardless of the condition. A significant interaction ($P < 0.0001$) between the condition and the day, the treatment and the day, and the condition, the treatment and the day was observed (Table 2 and Figure 1). However, the total germination (100%) was obtained after 35 DAS in the control experimental unit. Showing thus,

two statistically different groups between the amended and the control PIF seedlings regardless of the condition in term of germination percentage.

Treatment effect was especially marked for CS + Td amendment that generated more shoots in PIF substrate 35 DAS (average value: 17), followed by Td amendment (average value: 13) and CS amendment (average value: 11) compared to the control (average value: 8) as confirmed by the significant interaction ($P < 0.0001$) between the treatment and the day, although no significant interaction was observed between the condition and the day; the condition, the treatment and the day (Table 2 and Figure 2). Showing thus, four statistically different groups were distinguished between the amended and the control PIF seedlings regardless of the condition in terms of the number of shoots.

The PIF seedlings height and diameter of shoots, and the area of leaves were found to be significantly influenced ($P < 0.0001$) by the condition, the treatment and the day with respective R^2 values of 0.97, 0.96 and 0.96 (Table 3). Between these three variables, the most influential variable was the treatment for the height of shoots, and the condition for the diameter of shoots and area of leaves. The height, the diameter and the leaves surface area were consistently higher in the amended PIF seedlings compared with the controls. The difference between the controlled condition (SS) and non-controlled condition (nSS) was significant for the height and the diameter of pseudo stems, and for the leaves area surface (Figures 3 to 6).

Treatment effect was especially marked in non-controlled condition (nSS) for the CS + Td amendment that had 21 days after weaning (daw), seedlings with the higher height (average value: 13.93 cm), the bigger diameter of pseudo-stems (average value: 2.25 mm), and the larger leaves area surface (average value: 73.52 cm^2), followed by the *T. diversifolia* amendment (average value: 12.01 cm; 1.81 mm and 68.68 cm^2 respectively) and the CS amendment (average value: 10.35 cm; 1.58 mm and 60.61 cm^2 respectively) compared to the control (average value: 8 cm; 1.22 mm and 48.7 cm^2 respectively). A significant interaction ($P < 0.0001$) was

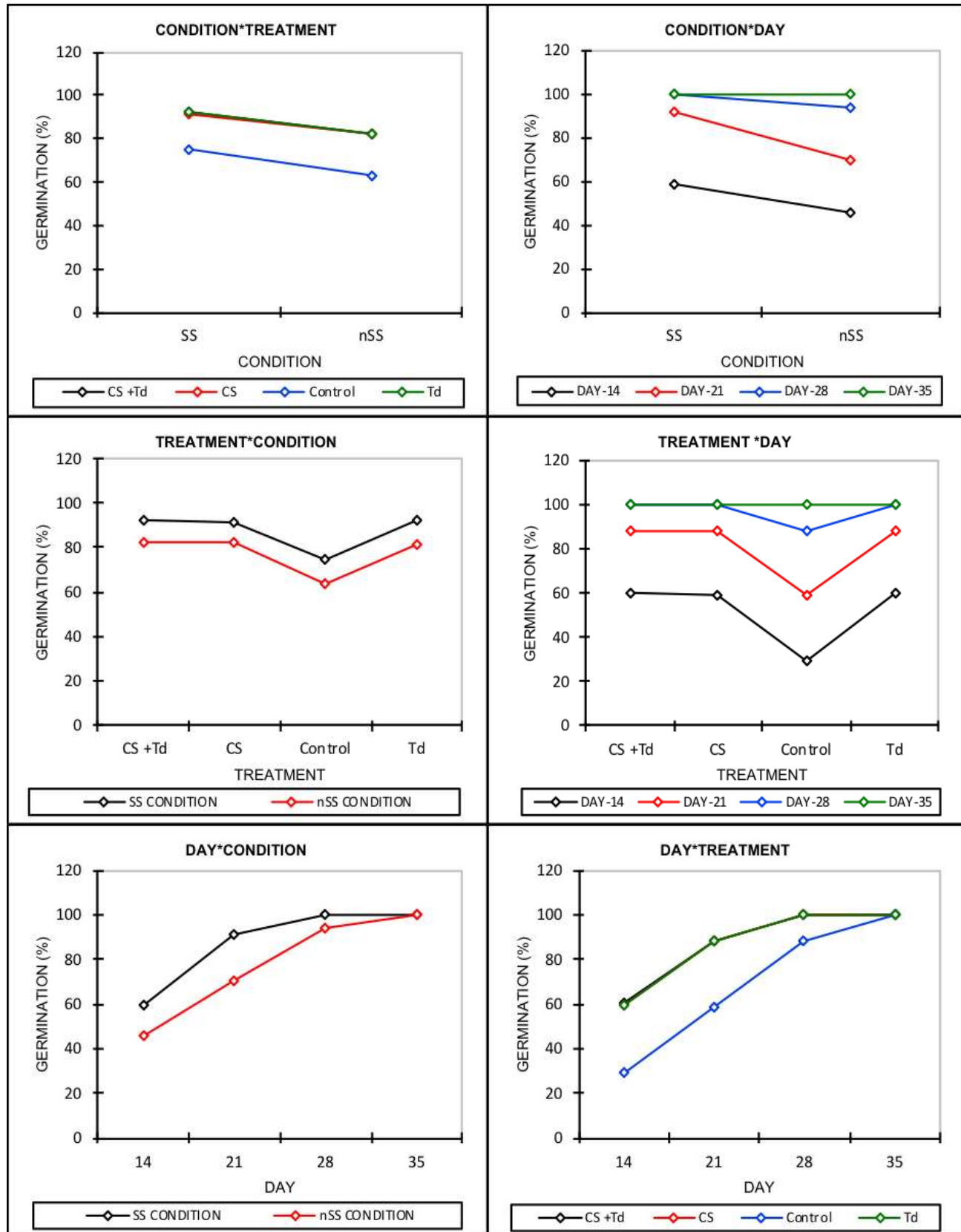


Figure 1. Interaction plots (condition, treatment and day) of the clam shells and *T. diversifolia* powder effects on the percentage of germination of PIF plantain seedlings in course of time. Each point represents the average mean of three replicates for each treatment.

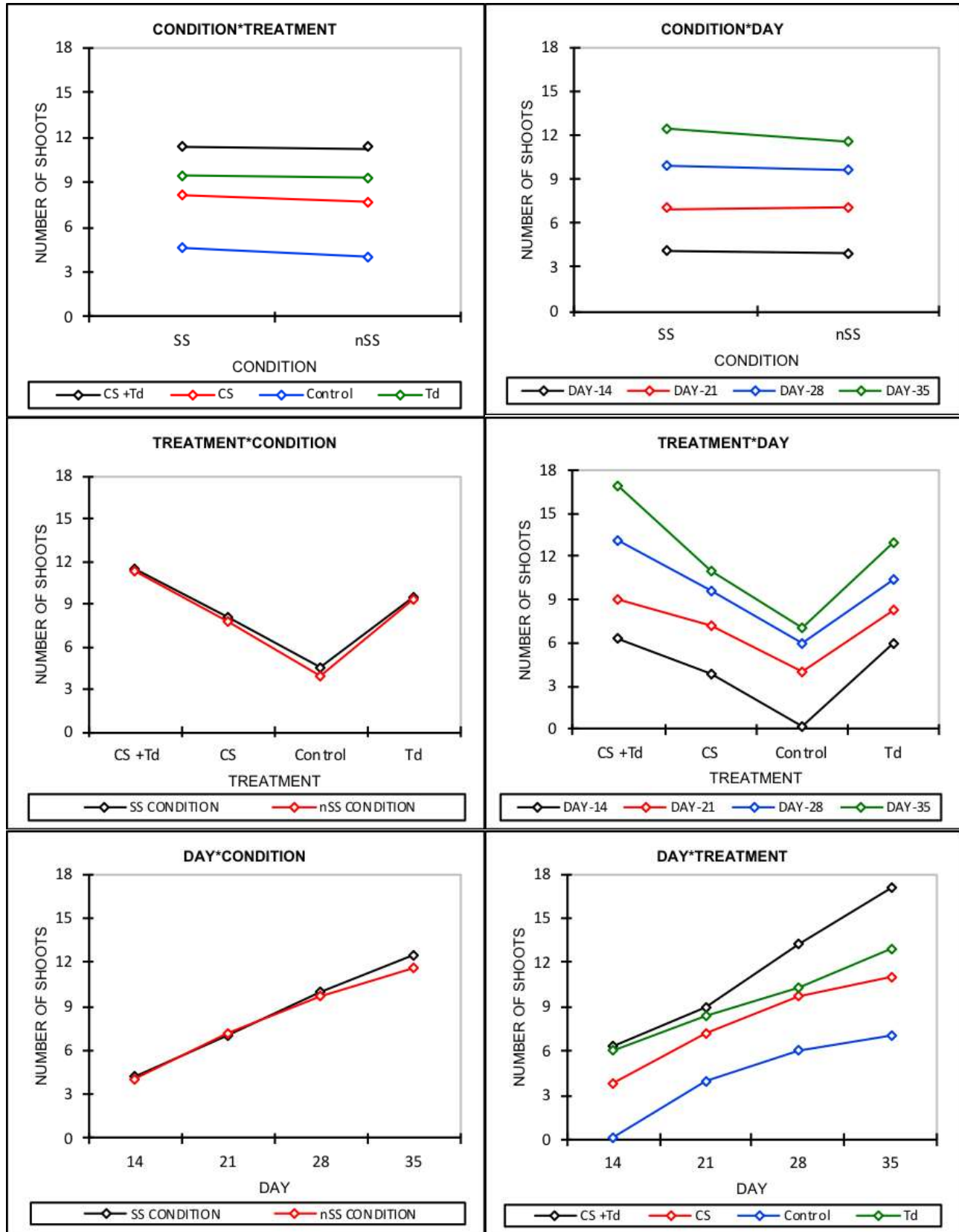


Figure 2. Interaction plots (condition, treatment and day) of the clam shells and *T. diversifolia* powder effects on the number of cumulative shoots of PIF plantain seedlings in course of time. Each point represents the average mean of three replicates for each treatment.

Table 3. Variance analysis of clam shells and *Tithonia diversifolia* powder effects on the height of shoots, the diameter of shoots, the foliar surface area of leaves of plantain seedlings in the shade.

Source	Height of shoots (cm) [R ² = 97%]			Diameter of shoots (cm) [R ² = 96%]			Area of leaves (mm ²) [R ² = 96%]		
	DF	F	P	DF	F	P	DF	F	P
Condition	1	377	< 0.0001	1	865	< 0.0001	1	959	< 0.0001
Treatment	3	488	< 0.0001	3	93	< 0.0001	3	121	< 0.0001
Day	3	22	< 0.0001	3	57	< 0.0001	3	5	0
Condition×Treatment	3	63	< 0.0001	3	8	< 0.0001	3	11	< 0.0001
Condition×Day	3	0	1	3	6	0	3	0	1
Treatment×Day	9	1	1	9	3	0	9	0	1
Condition×Treatment×Day	9	1	1	1	1	0	9	0	1

DF is the degree of freedom; F is the value of F test and P is the probability.

found between the condition and the treatment, although no significant interaction was observed between the condition and the day, the treatment and the day for the height of pseudo-stems and the leaves surface area, and between the condition, the treatment and the day for all the three variables (Table 3, Figures 3 to 6). Showing thus, four statistically different groups between the amended and control PIF seedlings in terms of the height and diameter of shoots, and the area of leaves.

Effect of clam shells and *T. diversifolia* on the PIF seedlings sensitivity to BSD

The PIF seedlings sensitivity to black Sigatoka disease was found to be very significantly influenced ($P < 0.0001$) by the condition, the treatment and the day with R² value of 0.97 (Table 4) and the most influential variable was the day. The black Sigatoka disease severity was consistently lower in the treated PIF substrates compared to the controls. The difference between the level of severity in the controlled condition (SS) and the non-controlled condition (nSS) was significant but very low (Figure 7).

Treatment effect was especially marked for the amendment containing clam shells (CS + Td and CS) that had seedlings with consistently lower necrotic surface area (average value: 1.46 cm² and 1.23 cm²) 12 daw, followed by *T. diversifolia* amendment (average value: 3.39 cm²) compared to the control (average value: 4.84 cm²). A significant interaction ($P < 0.0001$) was found between the condition and the treatment, the condition and the day, the treatment, and the day and the condition, the treatment and the day (Table 3 and Figure 7). Showing thus, three statistically different groups between the treated and the control PIF seedlings in terms of sensitivity to *M. fijiensis*.

Effect of clam shells and *T. diversifolia* on proteins and polyphenols accumulation

The proteins accumulation (R² = 0.96) in PIF seedlings

was found to be very significantly influenced ($P < 0.0001$) by the treatment and the stage while the variables influencing very significantly ($P < 0.0001$) the polyphenols accumulation (R² = 0.98) were the condition, the treatment and the stage (Table 5). The difference between the amount of proteins and polyphenols accumulated in the controlled condition (SS) and non-controlled condition (nSS) was significant only for polyphenol accumulation but very low for both variable (Figures 8 and 9). The most influential variable in the accumulation of proteins and polyphenols was respectively the treatment and the stage. The proteins and polyphenols amount were high in the amendment PIF substrates compared with the controls regardless of the condition.

The stage effect was especially marked for amended PIF seedlings that had consistent amount of proteins and polyphenols after inoculation compared to the amount before inoculation as confirmed by the significant interaction ($P < 0.0001$) of stage (Table 5, Figures 8 and 9). The treatment effect was especially marked for amended PIF seedlings before inoculation (BI) and after inoculation (AI) which had respective consistent average values of proteins and polyphenols especially for treatment CS + Td (BI 0.255 mg and 0.041 mg; AI: 0.526 mg and 0.089 mg), followed by treatment CS (BI: 0.247 mg and 0.038 mg; AI: 0.461 mg and 0.079 mg) and treatment Td (BI: 0.113 mg and 0.028 mg; AI: 0.257 mg and 0.050 mg) compared to the controls (BI: 0.082 mg and 0.020 mg; AI: 0.150 mg and 0.037 mg) as confirmed by the significant interaction ($P < 0.0001$) of treatments (Table 5, Figures 8 and 9). The amounts of total proteins and total polyphenols were expressed in mg equivalent of BSA per g of fresh weight and mg equivalent of gallic acid per g of fresh weight. Moreover, a significant interaction was found ($P < 0.0001$) between the treatment and the stage, although no significant interaction was observed for the condition; between the condition and the treatment for total proteins, between the condition and the stage; the condition, the treatment and the stage for both variables (Table 5, Figures 8 and 9). Showing thus, four statistically different groups were distinguished

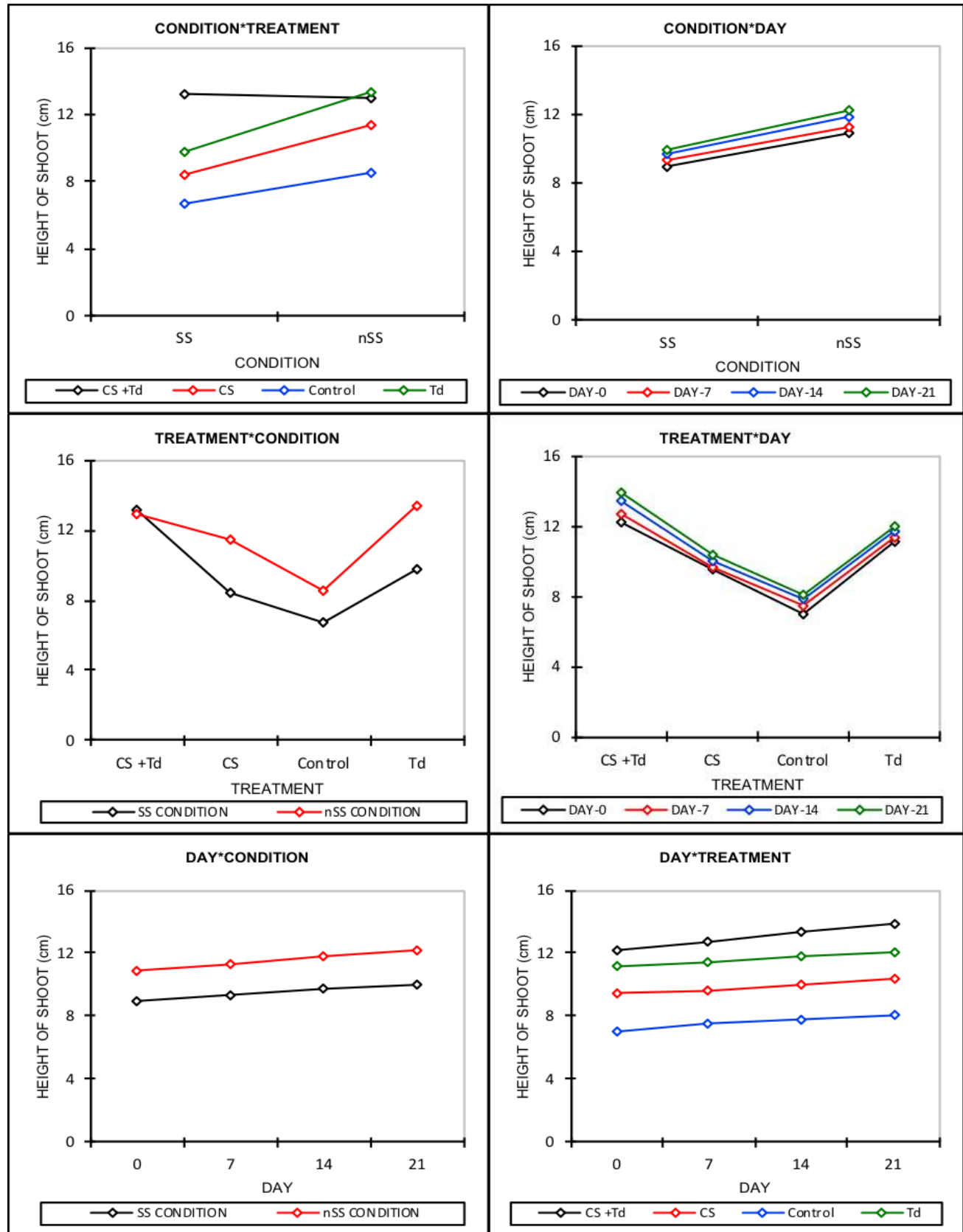


Figure 3. Interaction plots (condition, treatment and day) of the clam shells and *T. diversifolia* powder effects on the height of PIF plantain seedlings in course of time. Each point represents the average mean of three replicates for each treatment.

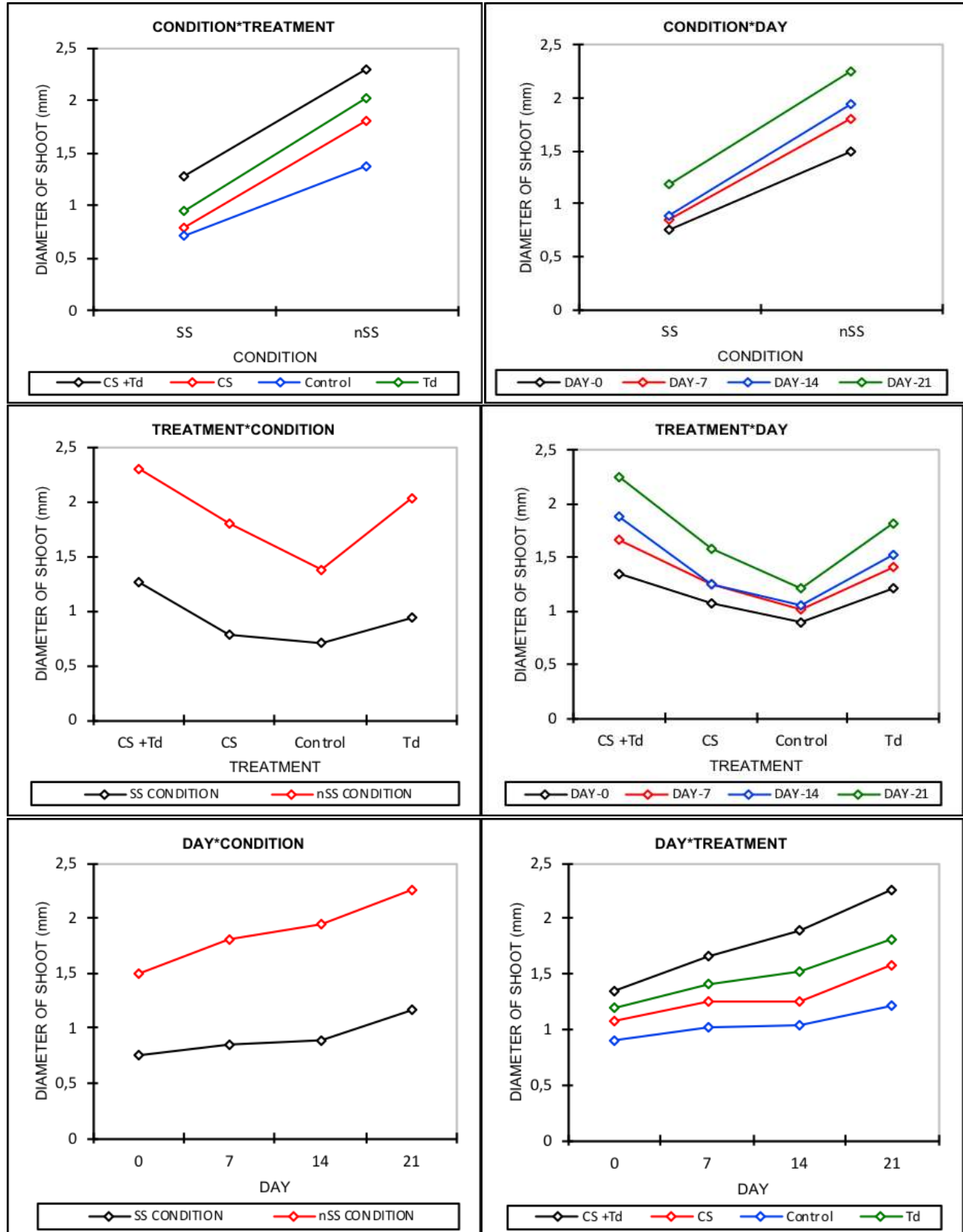


Figure 4. Interaction plots (condition, treatment and day) of the clam shells and *T. diversifolia* powder effects on the diameter of PIF plantain seedlings pseudo stem in course of time. Each point represents the average mean of three replicates for each treatment.

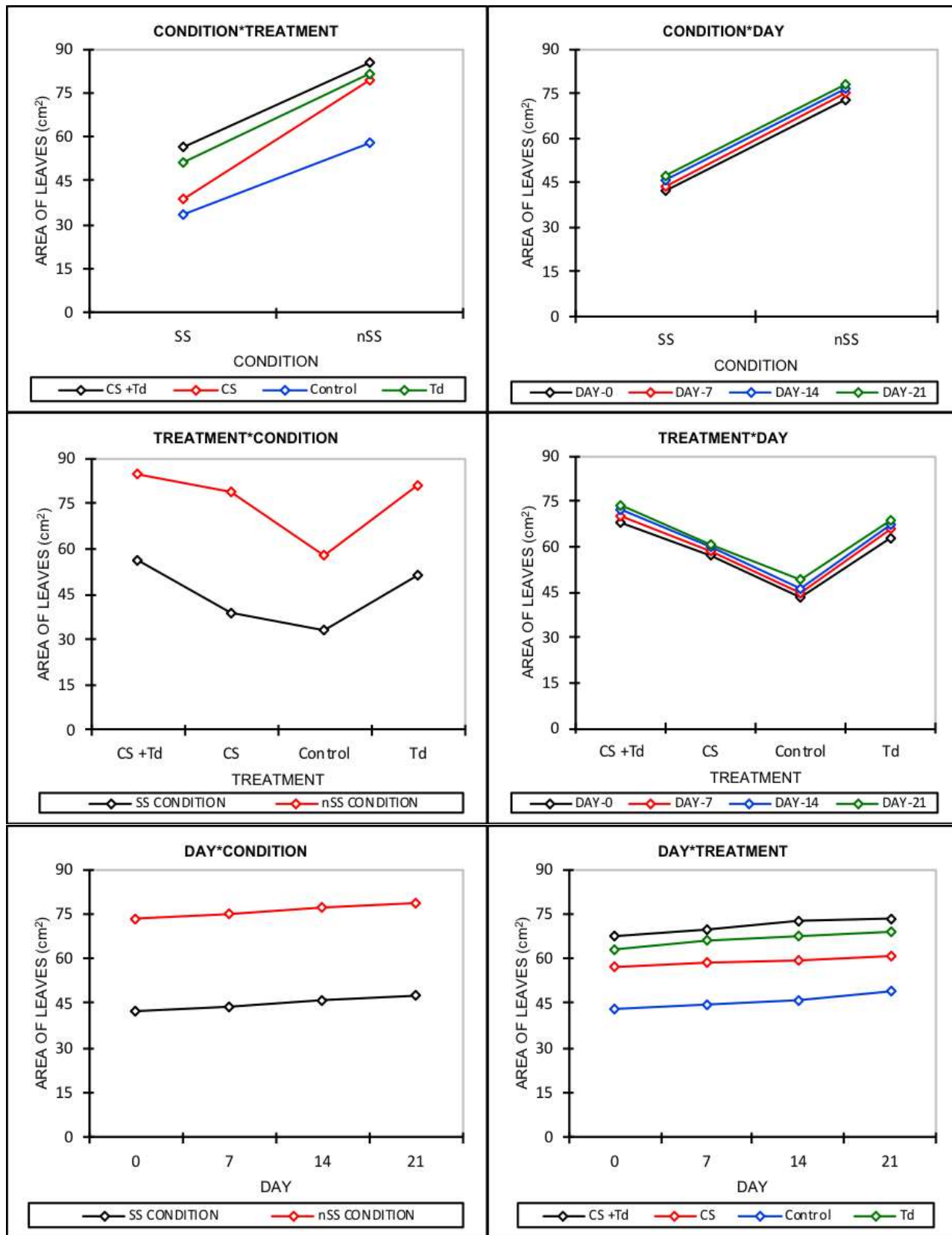


Figure 5. Interaction plots (condition, treatment and day) of the clam shells and *T. diversifolia* powder effects on the PIF plantain seedlings leaves surface area in course of time. Each point represents the average mean of three replicates for each treatment.

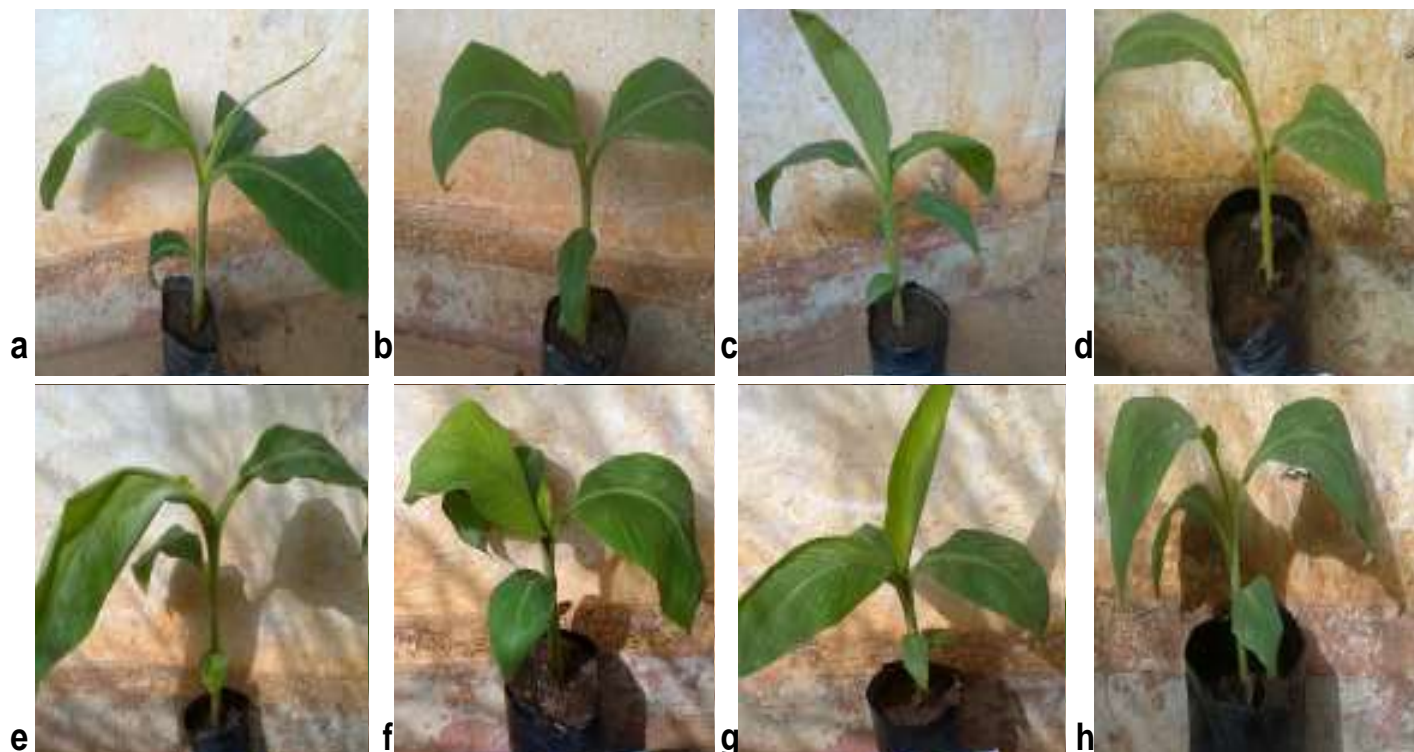


Figure 6. PIF seedlings 40 days old after weaning, grown on (a) sterile substrate, clam shells and *T. diversifolia* (SS+CS+Td), (b) sterile substrate and *T. diversifolia* (SS+Td), (c) sterile substrate and clam shells (SS+CS), (d) sterile substrate only as control (SS), (e) non-sterile substrate, clam shells and *T. diversifolia* (nSS+CS+Td), (f) non-sterile substrate and *T. diversifolia* (nSS+Td), (g) non-sterile substrate and clam shells (nSS+CS), (h) non-sterile substrate only as control (SS).

Table 4. Variance analysis of clam shells and *Tithonia diversifolia* powder effects on the plantain seedlings sensitivity to black Sigatoka disease.

Source	BSD sensitivity (cm ²) [R ² = 97%]		
	DF	F	P
Condition	1	46	< 0.0001
Treatment	3	15857	< 0.0001
Day	6	17723	< 0.0001
Condition×Treatment	3	441	< 0.0001
Condition×Day	6	45	< 0.0001
Treatment×Day	18	1451	< 0.0001
Condition×Treatment×Day	18	50	< 0.0001

DF is the degree of freedom; F is the value of F test and P is the probability.

between the amended and the control PIF seedlings in terms of total proteins and polyphenols accumulation.

Pearson correlation analysis between the different variables

The amount of total proteins and total polyphenols were negatively correlated with the BSD severity in the French variety as confirmed by the scatter plots (Figure 10).

Between most vegetative growth variables (germination percentage, height of shoots, diameter of pseudo stems and area of leaves), a strong positive correlation was found. It was evidenced that germination percentage, height of shoots, diameter of pseudo stems and leaves surface area were positively and strongly correlated to BSD severity, as well as being poorly linked to total proteins and total polyphenols content of PIF seedlings in nursery.

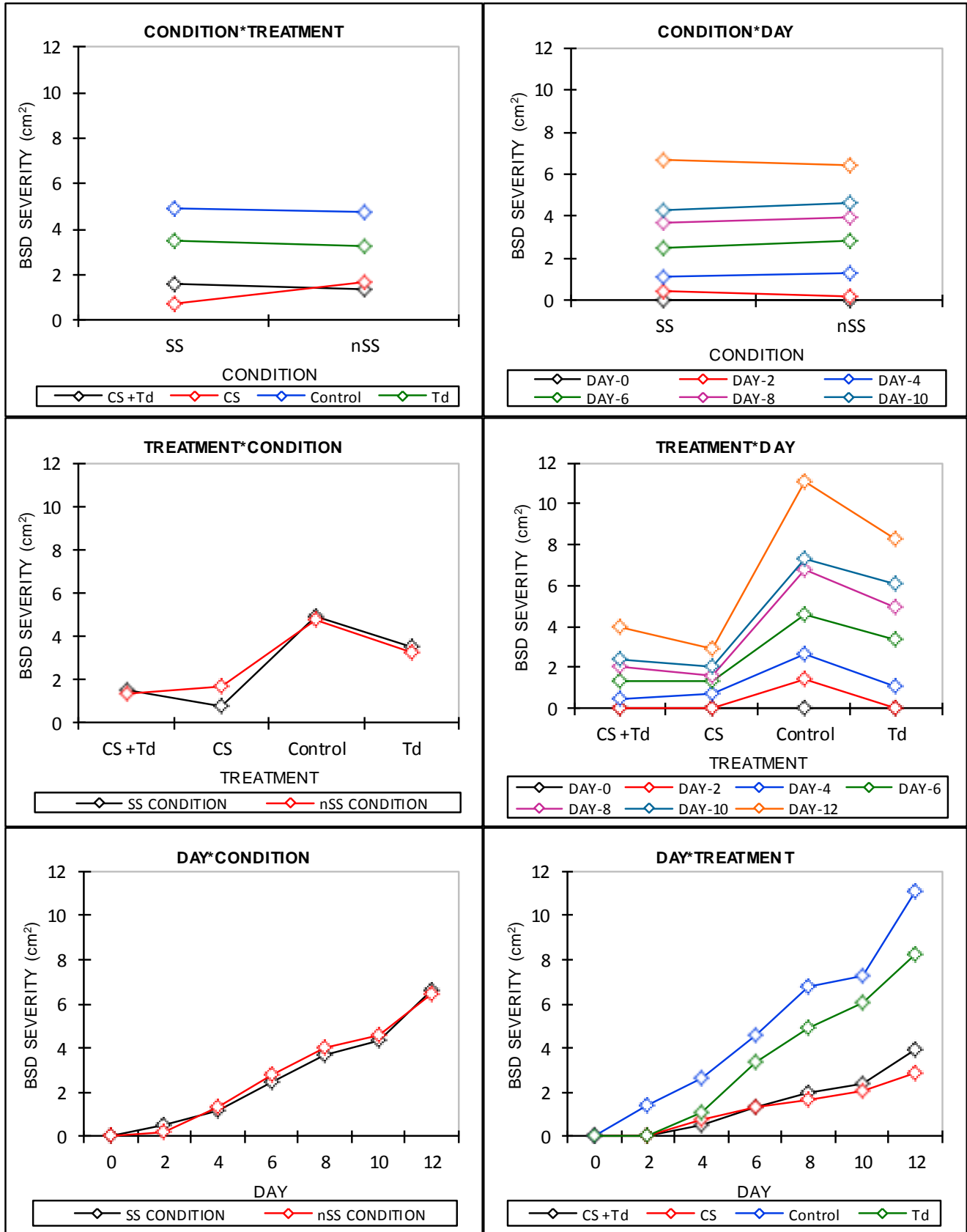


Figure 7. Interaction plots (condition, treatment and day) of the clam shells and *T. diversifolia* powder effects on the PIF plantain seedlings sensitivity to BSD in course of time. Each point represents the average mean of three replicates for each treatment.

Table 5. Variance analysis of clam shells and *Tithonia diversifolia* powder effects on the accumulation of proteins and polyphenols in plantain seedlings for both stages (before inoculation and after inoculation).

Source	Total proteins (mg Eq BSA/g FW) [R ² = 96%]			Total polyphenols (mg Eq Cat/g FW) [R ² = 98%]		
	DF	F	P	DF	F	P
Condition	1	0	1	1	5	0
Treatment	3	170	< 0.0001	3	267	< 0.0001
stage	1	297	< 0.0001	1	972	< 0.0001
Condition×Treatment	3	0	1	3	3	0
Condition×Stage	1	1	0	1	0	1
Treatment×Stage	3	19	< 0.0001	3	52	< 0.0001
Condition×Treatment×Stage	3	1	0	3	2	0

DF is the degree of freedom; F is the value of F test and P is the probability.

DISCUSSION

The aim of this work was to assess the effects of clam shells and *T. diversifolia* powders amendment on the growth promotion of plantain PIF seedlings and their sensitivity to *M. fijiensis*. The results of this study have provided evidence for wide variations in the germination rate, number of shoots, height of shoots, diameter of pseudo stems, area of leaves, the number and length of roots (data not shown), in the sensitivity to *M. fijiensis* of amended plantain PIF seedling and in the accumulation of total proteins and polyphenols before and after inoculation as recently shown on banana treated with shells in nursery (Ewané et al., 2019), as well as on cocoa (Téné et al., 2017, 2019). The clam shells and *T. diversifolia* powder treatment affects positively the generation of shoots, sensitivity to BSD and accumulation of proteins and polyphenols regardless of the condition as proven by less difference between both conditions. However, for the vegetative growth characters, the treatment effect is more important in the non-sterile condition (nSS) compare to the sterile condition (SS). The efficiency of the treatment in sterile condition as well as in the non-sterile condition, which is suitable for the poor peasant, seems to be proven through this result.

The clam shells treatments (Td+CS and CS) especially stimulated the PIF seedlings defense response with respective percentage of protection of 74.59 and 69.84% compared to the controls. These results are in accordance with previous study that have shown an increase in pre-existing (before inoculation) and *de novo* synthesized (after inoculation) proteins, polyphenols as well as some enzymes involved in plant tissues defense on plantain PIF seedlings (Ewané et al., 2019), and on cocoa (Téné et al., 2017, 2019). Indeed, plant antifungal metabolites are preformed inhibitors that are pre-existing in healthy plants (phytoanticipins), or they may be synthesized *de novo* in response to pathogen attack or various non-biological stress factors (Pusztahelyi, 2018; Pusztahelyi et al., 2015). These compounds are considered as chemical or physical barriers, playing key

roles in the defense against pathogens infection. This protective effect relies on (1) the improvement of the soil microbial communities in both the abundances and structures (Malerba and Cerana, 2019), (2) the interaction between the substrate, the plant and the plant microbiome leading to the recognition by specific receptors present on the plant cell plasma membrane, the triggering of biochemical pathways associated with defense responses and activated immunity through the systemic acquired defense (Pusztahelyi, 2018). It would be interesting to assess the defense enzymes involved in the protection of plantain PIF seedlings against diseases.

The *T. diversifolia* treatments (Td+CS and Td) especially enhance respectively the germination rate (25.97 and 25.84%), the number of shoots (58.82 and 46.15%), the height of shoots (74.13 and 50.13%), diameter of pseudo stems (84.43 and 48.36%) and leaves area (50.96 and 41.40%) of plantain PIF seedlings compared to the controls. The results of this study are in accordance with one from a recent study that has shown the increase of the growth and yield after the use of *T. diversifolia* green biomass alone in the culture of cassava (Bilong et al., 2017). *T. diversifolia* seems to act as an organic fertilizer that probably improve the quality of the soil physicochemical and biological properties through increases of the seedling's growth and sensitivity to pathogens. Moreover, the combined effect of *T. diversifolia* leaves with inorganic fertilizers on the yield of maize, tomato and cassava has also been demonstrated (Kaho et al., 2011; Ngosong et al., 2016; Bilong et al., 2017). *T. diversifolia* tissues decompose rapidly and are richer in excellent physicochemical properties which probably provide the PIF substrates with elements such as nitrogen, magnesium, potassium (Oyerinde et al., 2009), coupled to the action of key enzymes of nitrogen metabolism (nitrate reductase, glutamine synthetase and protease), as well as the amelioration of nitrogen transport in functional leaves (Kaho et al., 2011), for the acceleration of germination and plant growth promotion. However, the inhibitory effects of *T. diversifolia* during this research (data not shown) was noticed for some

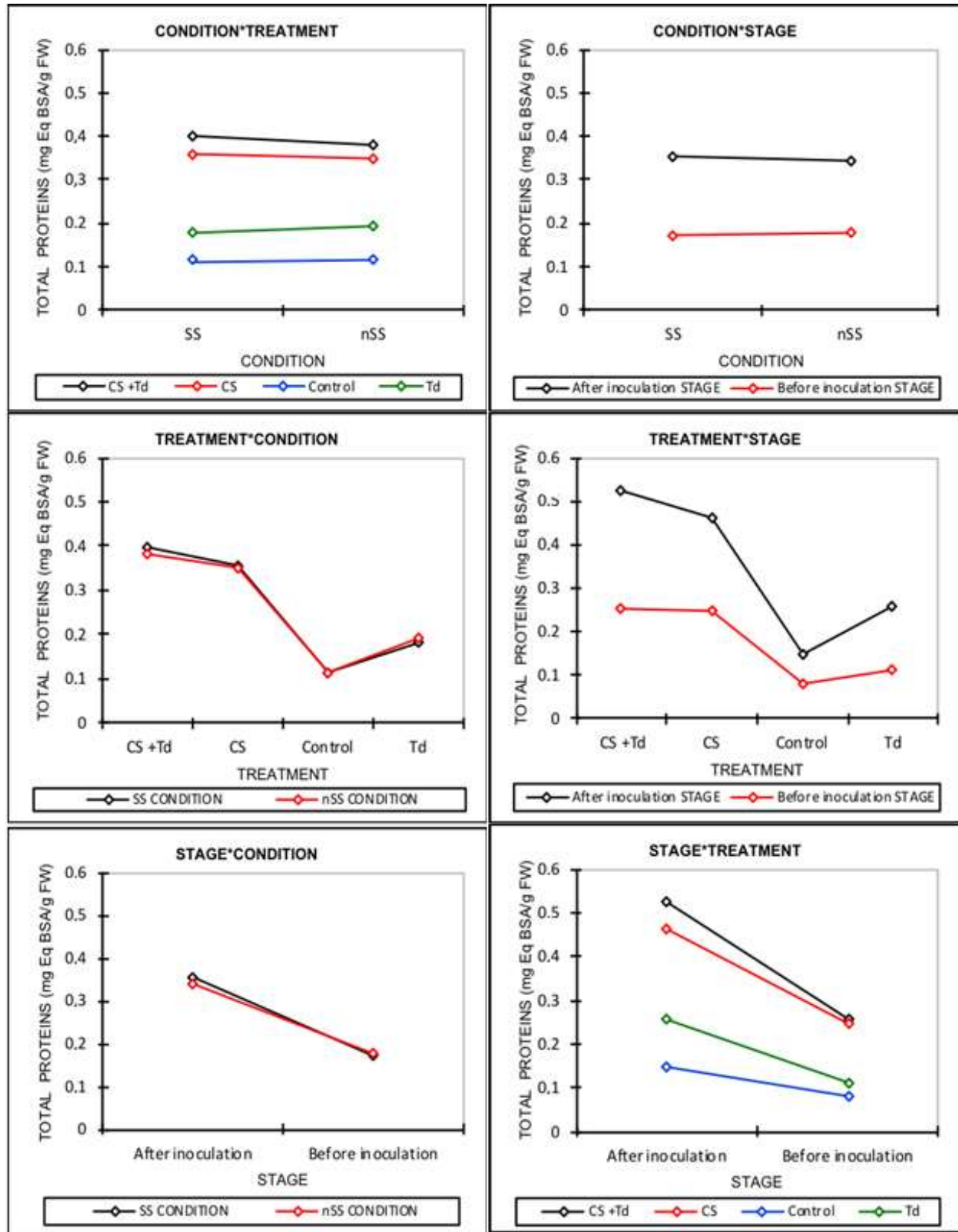


Figure 8. Interaction plots (condition, treatment and day) of the clam shells and *T. diversifolia* powder effects on the PIF plantain seedlings accumulation of total proteins at both stages (before inoculation and after inoculation). Each point represents the average mean of three replicates for each treatment.

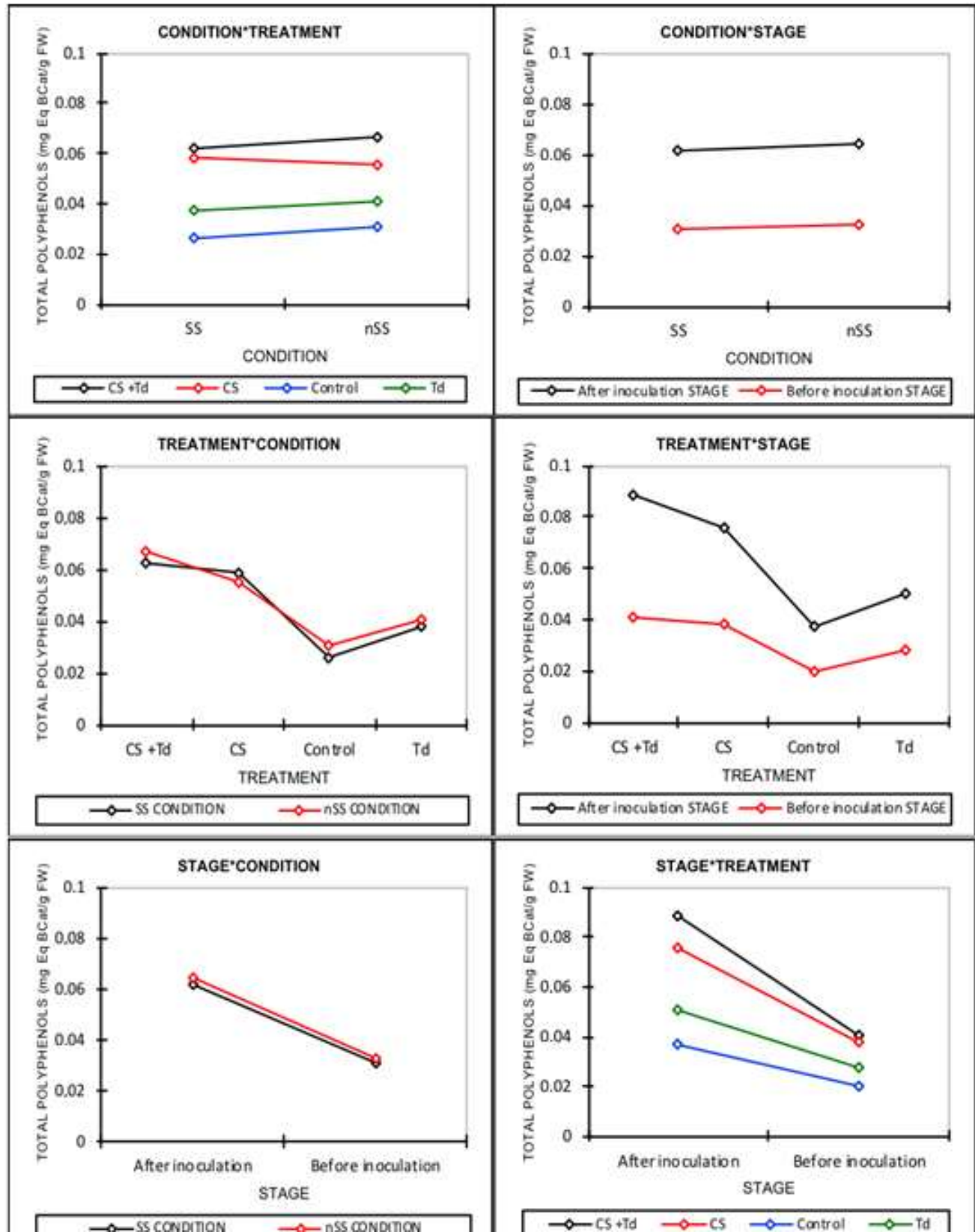
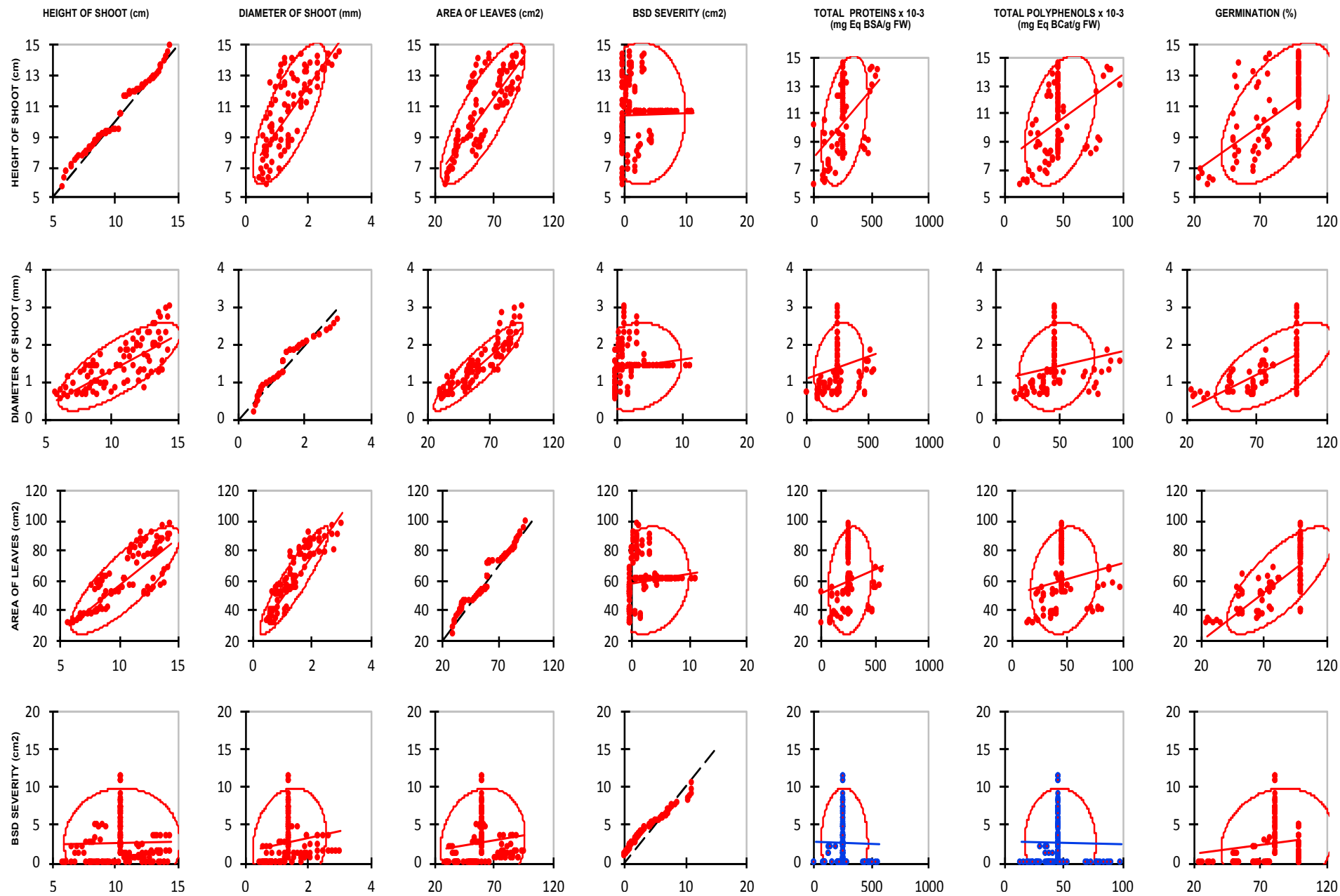


Figure 9. Interaction plots (condition, treatment and day) of the clam shells and *T. diversifolia* powder effects on the PIF plantain seedlings accumulation of total polyphenols at both stages (before inoculation and after inoculation). Each point represents the average mean of three replicates for each treatment.



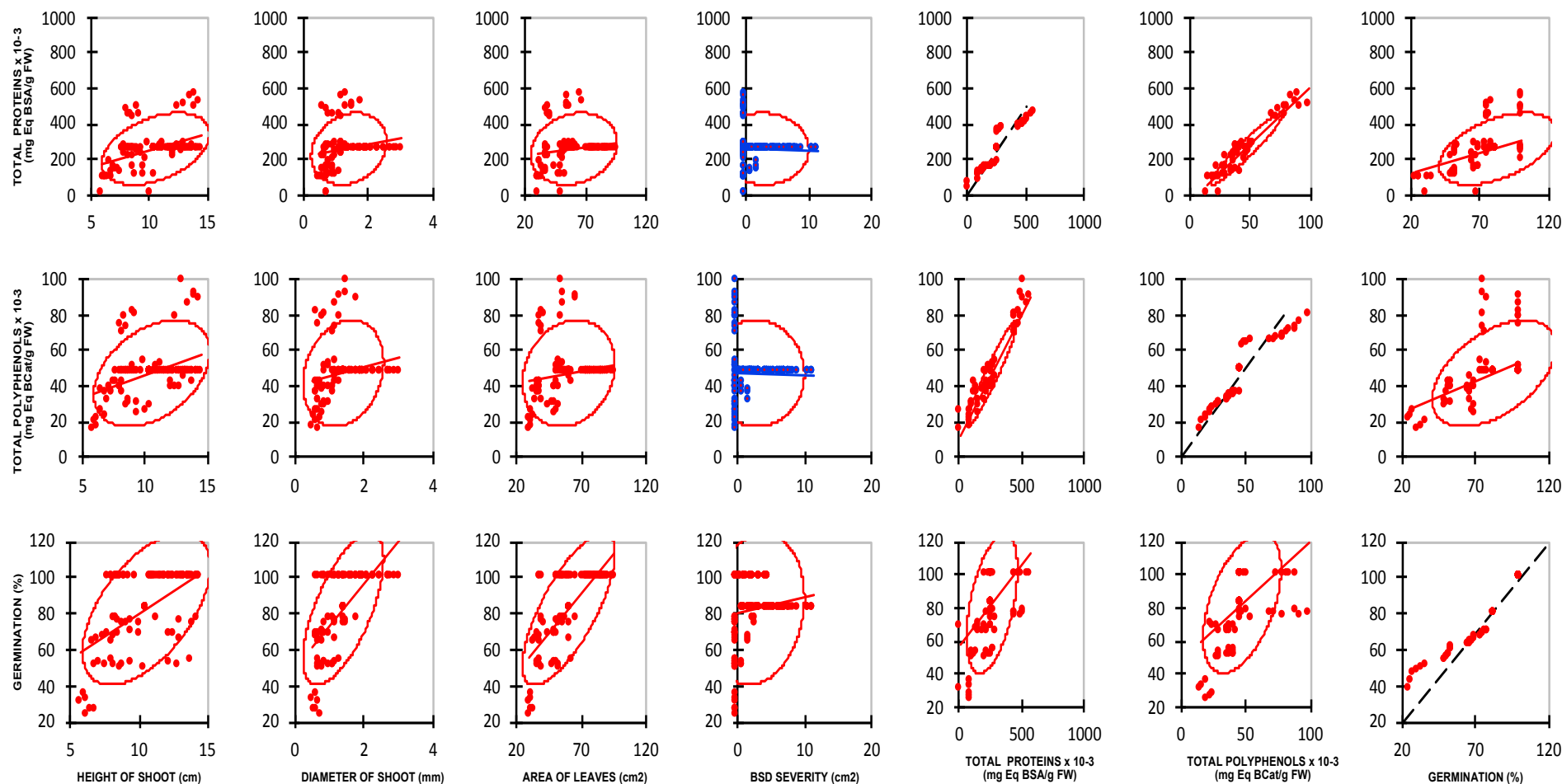


Figure 10. Relationship between the different variables of PIF plantain seedlings: germination, height of shoots, diameter of pseudo stems, area of leaves, BSD severity, total proteins and total polyphenols. The Scatter plots shows positive (red) or negative correlation (blue), but also the strength of the relationship.

concentrations. Indeed, the use of aqueous fresh shoot extract of *T. diversifolia* have shown *in vitro* both stimulatory and inhibitory effects on *Cleome gynandra* (spider plant) germination and growth (Hemsley and Gray, 2005).

The combination of clam shells and *T. diversifolia* powder show a synergic action with dual role both as a biofertilizer and a biopesticide since they affect the growth promotion of PIF plantain seedlings and their protection against *M. fijiensis*

in nursery and could probably enhance agricultural yield in the field. Indeed, the major compounds present in *T. diversifolia* are nitrogen, magnesium, potassium, flavonoids, sesquiterpene lactone and alkaloids... (Oyerinde et al., 2009),

while the ones in the clam shells are chitin, calcium and magnesium carbonate, proteins, (Khoushab and Yamabhai, 2010) and they all activated the growth promotion and natural defense systems through the increased synthesis of nutrients and defensive metabolites (Mondal et al., 2012; Akter et al., 2018; Malerba and Cerana, 2019). This association for plantain PIF seedlings treatment revealed significant increase of growth characters and less sensitivity to BSD compared to the controls, confirming a positive effect compared to the individual effects of *T. diversifolia* alone and clam shells alone. There is a need to assess this dual effect of the combined products at different ages and on other pathosystems.

The treatments containing clam shells (CS) especially improved significantly the total proteins and phenolics accumulation in plantain PIF seedlings. The amount of pre-existing proteins and polyphenols compounds is important in the treated seedlings and rises significantly after inoculation (*de novo* synthesized) compared to the controls. This amount double after infection for the treatment Td+CS compared to other treatments, especially that of *de novo* synthesis of Td treatment. Suggesting thus, different rates of accumulation depending probably on the level of sensitivity to diseases and the type of interaction (compatible or incompatible) establish between the plantain PIF seedlings and the *M. fijiensis* strain (Ewané et al., 2012). The treatment confers an important pool of pre-existing and *de novo* synthesized proteins and polyphenols that seem to be enough to participate in defense reactions and to overcome infection.

A positive correlation was found between the total amount of proteins and polyphenols before and after inoculation, and all the agromorphological vegetative growth variables which are involved in their growth promotion, while it was negative for the BSD severity. A lack in this study lies in the fact that 12 days after inoculation (DAI) seem to be almost too late for the assessment of the biochemical events occurring in plantain PIF seedlings in the first hours and days after inoculation and the establishment of infections as previously suggested by Ewané et al. (2019). Therefore, there is a need to access the physiological mechanisms involved in the combination of clam shells and *T. diversifolia* powder effect on growth promotion and protection against diseases in plantain PIF seedling.

Conclusion

The clam shells and *T. diversifolia* treatment enhance efficiently plantain PIF seedlings quality in nursery and therefore behave as a seedling's vaccine against mortality in the fields. These results have shown that clam shells and *T. diversifolia* alone, or in association are able to play a dual role (biofertilizer and biopesticide) in PIF plantain seedlings growth positive regulation and

improved defense responses against phytopathogens in terms of germination rate, number of shoots, length of shoots, diameter of pseudo stems, area of leaves, BSD severity, proteins accumulation and phenolic accumulation. However, the effect of the combination of both products was more efficient and has shown the best effects. There is a need to investigate the biochemical and molecular stimulation mechanisms involved in growth promotion and induced resistance against pathogens stimulation in the plantain PIF seedlings by clam shells and *T. diversifolia* powder treatments. Moreover, there is a need to continue this experimentation to the field in order to show the impact of this result compared to the conventional agriculture in terms of production costs, yield, productivity and the gains. Despite the fact that by-fishing products and bad herbs are environmentally benign compared to synthetic products, they are commonly neglected; hence, this study opens a way for their utilisation for an improved productivity, a sustainable and responsible agriculture, affordable for poor African small holders' farmers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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