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Impact of Banana Bunchy Top Disease (BBTD) by Different Doses of Banana Bunchy Top Virus (BBTV) on Banana Tree Growth Parameters

Jean Alban Ondh-Obame[1]*, Bénédicte Ndeboko[2], Jean Aubin Ondo[1], Yvette Ogandaga[1] and Corine Oye Anda[3]

[1]Multidisciplinary Laboratory of Sciences (LAPLUS) of the Ecole Normale Supérieure de Libreville, BP 17009 Libreville (Gabon).
[2]Laboratoire du Centre Hospitalier Universitaire Mère-Enfant Fondation Jeanne Ebori, Libreville, Gabon, B.P. 20000 Libreville (Gabon).
[3]Centre National de la Recherche Scientifique et Technologique, Institut de Recherches Agronomiques et Forestières B.P. 2246 Libreville (Gabon).

**Corresponding author*

Abstract

Banana Bunchy Top Disease (BBTD) is the most devastating viral disease affecting banana plants. To study the impact of this disease on banana plant growth using different inoculum doses, an experiment was conducted at the Lycée Technique de Bikélé (Ntoum municipality). Two dessert banana varieties (Fougamou and Gros Michel) and two plantain varieties (Elate and Ebang) were multiplied using the PIF (Plants from Stem Fragments) method in a germinator for six weeks. The resulting plantlets were then transplanted into a nursery and contaminated by foliar spraying with inoculum (at 50% and 100% concentrations). Growth parameters (leaf area, collar diameter, and stem length) were monitored over a period of fourteen weeks. For each variety, three treatments were applied: control (T0), 50% inoculation, and 100% inoculation. The results showed that in the germinator, the Elate (59 plantlets) and Ebang (46 plantlets) varieties produced the highest number of plantlets. Regarding the impact of BBTD on growth parameters, only the Gros Michel, Elate, and Ebang varieties were affected, showing a slowdown in leaf area expansion, collar diameter increase, and stem elongation from the eleventh week (S11) onwards. Fougamou was the only variety whose growth parameters remained largely unaffected despite the different virus inoculations. The results of this study suggest Fougamou as a variety of interest for sustainable management programmes of this viral disease.

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Keywords

Banana, variety, BBTD, BBTV, inoculum, inoculation dose, growth.

Introduction

Banana cultivation (*Musa spp*) plays a crucial role in food security and the economy of many countries, particularly in tropical and subtropical regions (FAO, 2023). According to Kilimo (2014) and Ngatat *et al.,* (2024), more than 100 million people in sub-Saharan Africa depend on this crop for their livelihoods. Despite its importance, banana cultivation faces numerous phytopathological threats that affect farm productivity and profitability (Kwa and Temple, 2019). Among the phytopathological constraints recorded on banana trees, *Banana Bunchy Top Disease* (BBTD), caused by the *Banana bunchy top virus* (BBTV), is one of the most devastating threats (Kumar *et al.,* 2015). This virus, transmitted by the *Pentalonia nigronervosa* aphid, causes a significant reduction in plant growth, inhibition of flowering and a decrease in yield (Mandal *et al.,* 2021). According to Fouré and Manser (1982), the disease appears to have been detected for the first time in Gabon in 1978, at the FAO centre in N'toum. According to a preliminary study conducted by the FAO in 2018, the disease is present in 8 out of 9 provinces, covering 90% of the national territory (Ondh-Obame, 2021). Although the work of Ondh-Obame (2021) has determined the prevalence and severity in the Greater Libreville agroecological zone, to date no work has been done to show the negative effect of BBTD on banana growth parameters.

In this context, this study aims to assess the impact of this viral disease on banana growth parameters, including collar diameter, plant height and leaf area in nurseries. The results will provide a better understanding of the impact of this disease and contribute to the development of sustainable management strategies.

Materials and Methods

Study setting and period

This study was conducted in the commune of Ntoum, located in the province of L'Estuaire, more precisely in the department of Komo-Mondah. The climate of the commune of Ntoum is tropical. Average annual rainfall is 2716 mm.

The experiment was conducted at the Lycée Technique de Bikélé, whose GPS coordinates are 00°25'00.22" N and 009°32'59.18" E. It was carried out from 25 January to 18 June 2025.

Materials

The plant material used in this study consisted of two dessert banana varieties, Fougamou (ABB) and Gros Michel (AAA), and two plantain varieties, Elate (AAB) and Ebang (AAB).

A variety of technical equipment was used, both for plant propagation and for measuring leaf area, collar diameter and stem length which was used as a growth parameter. This included:

- ✓ A germinator necessary for propagating healthy banana plants using the PIF (Plant from Stem Fragment) method;
- ✓ Polyethylene sheeting and elastic bands to seal the germinator;
- ✓ Sawdust used as a substrate;
- ✓ A kitchen knife;
- ✓ A caliper for determining the collar diameter;
- ✓ A tape measure for taking measurements (length and width) of banana plants.
- ✓ Banana plants with level 5 disease severity for preparing the inoculum;
- ✓ A spray bottle for infecting healthy plants with the inoculum.

Methods

Propagation of plants using the PIF method (Plant Issu du Fragment de Tige)

This is a vegetative propagation method known for producing healthy plants quickly. It was carried out according to the steps described by Ngoula Meguassu (2016) and Bezard *et al.,* (2024).

Construction and preparation of the germinator: this was built at the Bikélé Technical High School. Subsequently, five bags of sawdust were obtained.

Selection and collection of suckers: the suckers selected were from the dessert banana and plantain varieties mentioned above (Fougamou, Gros Michel, Elate and Ebang), which were collected in the Bikélé and Kinguélé districts. A total of 48 suckers were needed, i.e. 12 suckers for each variety.

Cleaning and trimming of suckers: this involves removing all soil from the plant and removing all weevil tunnels and other pests present in the plant.

Shelling of the sheaths: this involves meticulously removing 3 to 5 leaf sheaths from the pseudostem.

Treatment and drying of explants: the explants are soaked in a solution containing an insecticide (Pyriga) and a fungicide (Azox) at respective doses of 25 mL and 50 mL per 15 L of water. They are then left to dry for 48 hours.

Rejuvenation of explants: This takes place 48 hours after the explants have dried. It consists of removing the tip of the pseudostem left after peeling.

Making incisions: This involves locating the apical meristem and making 2 to 3 cross incisions in the centre of the explant.

Placing the explants in the germinator: the explants are placed in the germinator and covered with a thin layer of sawdust. The germinator is then sealed with polythene and elastic bands to maintain an adequate heating temperature.

Weaning and transfer to the nursery: this was carried out 46 days after placing the explants in the germination chamber. This step consists of carefully removing the young plants from the chamber. The weaned plants are then transplanted into polyethylene bags (measuring 26 cm x 26 cm) containing black soil that has been watered beforehand. After transplanting, the seedlings are placed in a location sheltered from the sun and bad weather.

Counting the plants in the germinator

In order to study the behaviour of the four (4) banana varieties in the germinator, the number of seedlings was counted once a week for six (6) weeks.

Preparation of the inoculum

The preparation of the inoculum involved extracting sap from diseased banana trees, as this sap contains BBTV. The plants selected for this preparation were diseased banana trees with a severity level of 5. To do this, the diseased banana trees were first crushed using a mortar and pestle. The crushed banana trees were then pressed manually and the sap extracted from them was filtered to remove debris and other impurities. In total, approximately 1.1 litres of inoculum were collected at the end of this stage.

Infection of plants

Infection was carried out by spraying the inoculum (one week after transplanting the seedlings) onto the entire plant (leaves and stem) using a sprayer (Silué *et al.,* 2018). To do this, each variety, consisting of eighteen (18) plants, was divided into three treatments:

→ Six (6) uncontaminated plants served as controls;
→ Six (6) plants were contaminated with pure inoculum (I100%);
→ Six (6) plants contaminated with inoculum diluted to 50% (I50%).

Growth parameter measurement

In order to observe the impact of the virus on the growth of banana plants, three parameters were selected for this study: collar diameter, stem height and leaf area. These parameters were measured once a week for 14 weeks.

→ **The diameter at the collar** of the stems was measured using a calliper (Staley brand).
→ **The height of the plant** was measured from the collar to the top of the stem using a graduated ruler or a tape measure for plants over 30 cm tall on the two selected plants.
→ **The leaf area** was determined on the third leaf from the top of the plants. The leaf area was determined by measuring the length and width of the leaf and calculating according to the method proposed by Kumar *et al.,* (2002):

$$SFT = L \times La \times 0.80 \times N \times 0.662$$

Where:
SFT = total leaf area;
L = length of the leaf in question;
l = width of the leaf in question;
N = number of leaves.

Statistical analysis

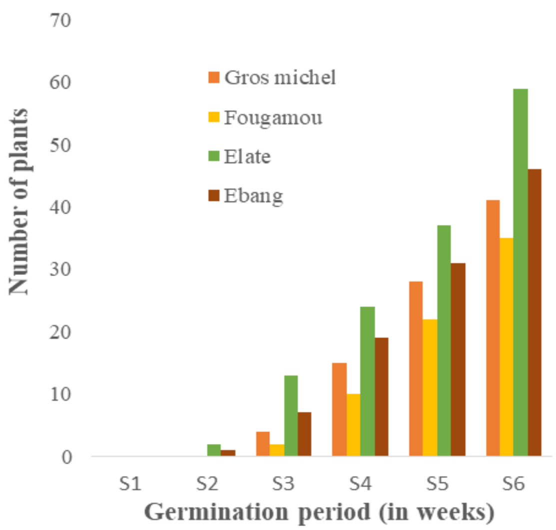
Statistical data processing, including data entry and graphical representations, was performed using Excel 19 and GraphPad Prism version 8 software. The test used to study the effect of the inoculum was the one-factor ANOVA test. To determine the significance of the test, a comparison was made between the probability associated with the test statistic (P) and the theoretical value a=0.05. If $P \leq 0.05$, it is concluded that there is a significant difference between the treatments; otherwise, the result is not significant.

Results and Discussion

Varietal evolution in the germinator

The study shows that no seedlings were observed for any of the varieties. The first seedlings were recorded at S2, notably with Elate (2 plants) and Ebang (1 plant). The first Gros-Michel and Fougamou plants were observed only from the third week onwards, with 4 and 2 plants respectively. Between S3 and S6, heterogeneous emergence was observed for all varieties. Elate stood out with the highest emergence, increasing from 13 to 59 seedlings during this period. For the Ebang variety, the number of seedlings increased from 46. The number of Gros-Michel plants increased from 4 to 41. The lowest number of seedlings was recorded for Fougamou, which increased from 2 to 35 (Figure 1).

Figure.1 Varietal evolution in germination



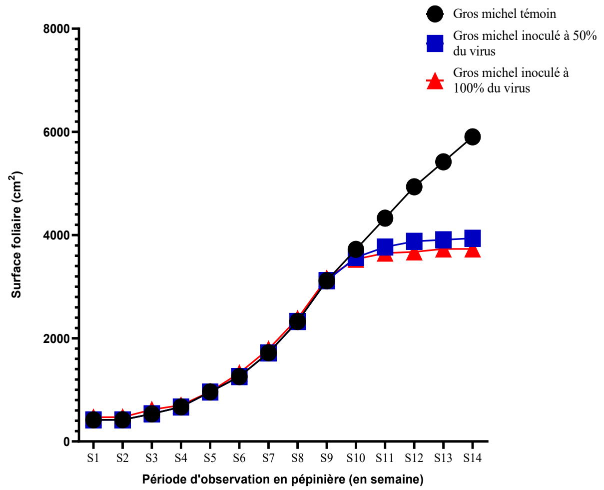
Impact of BBTV infection on dessert growth

Impact of BBTV infection on Gros Michel growth

Impact of BBTV on the leaf area of Gros Michel

For the three treatments: control Gros Michel (Gros Michel T0), Gros Michel inoculated at 50% (Gros Michel I 50%) and Gros Michel inoculated at 100% (Gros Michel I 100%), significant growth in leaf area was recorded from the first to the tenth week (Figure 2). In fact, it ranged from 418.8 cm^2 to 3722.2 cm^2 for the Gros Michel control; from 417.0 cm^2 to 3565.8 cm^2 for the Gros Michel inoculated at 50% and from 470.3 cm² to 3532.3 cm² for the Gros Michel inoculated at 100%. The leaf area continued to increase in the Gros Michel control plants (T0) until it reached exponential growth (5,903.9 cm²) in the fourteenth week. However, in the Gros Michel I 50% and Gros Michel I 100% plants, a slowdown in this growth was observed from S11 onwards. In fact, from S11 to S14, a stagnation in leaf growth was observed, ranging from 3769.6 to 3937.9 $^{cm^2}$for 50% inoculated banana trees and 3651.5 $^{cm^2}$to 3742.1 $^{cm^2}$for 100% inoculated banana trees. Although no significant difference was recorded between the two types of inoculation, the shape of the curves shows that among the inoculated plants, the slowest growth was recorded in the banana trees inoculated at 100%. Significant differences were recorded between the inoculated plants and the controls.

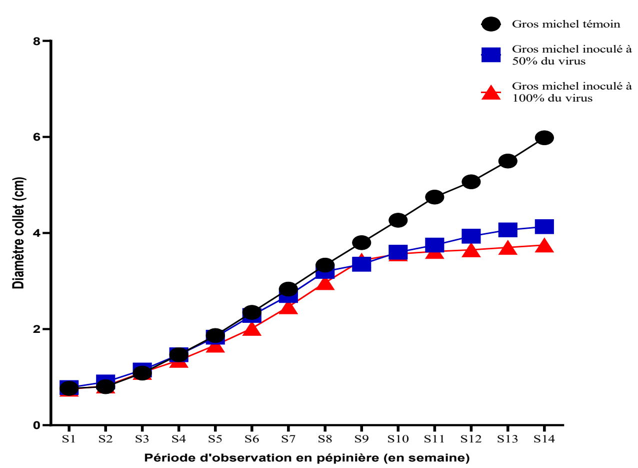
Figure.2 Effect of BBTV on the leaf area of Gros Michel



Impact of BBTV on the collar diameter of Gros Michel

This study reveals significant growth in the diameter of Gros Michel for all treatments during the first nine weeks (Figure 3). From week S10 onwards, a divergence appears: the control plants continue to grow steadily, reaching the largest diameter (5.98 cm) at S14, while the inoculated plants show a marked slowdown. Those infected at 50% stagnated at around 4.13 cm, and those inoculated at 100% had the smallest diameter (3.75 cm).

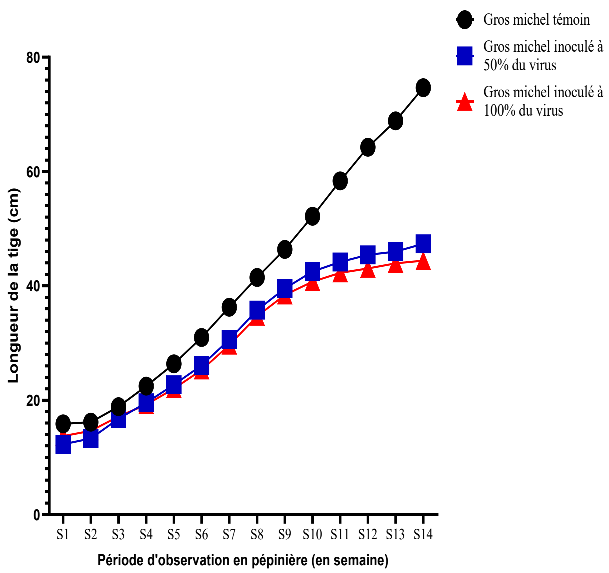
Figure.3 Effect of BBTV on the collar diameter of Gros Michel



Impact of BBTV on the stem length of Gros Michel

During the first ten weeks (S1 to S10), growth was relatively similar for all treatments (Figure 4). However, from S11 onwards, a clear difference emerged: the control plants showed the greatest length, reaching approximately 74.65 cm at S14, while the inoculated plants showed slower growth in length. Plants inoculated with a 50% inoculum concentration reach approximately 47.35 cm, and those inoculated with 100% show the shortest length at 44.42 cm.

Figure.4 Effect of BBTV on the stem length of Gros Michel

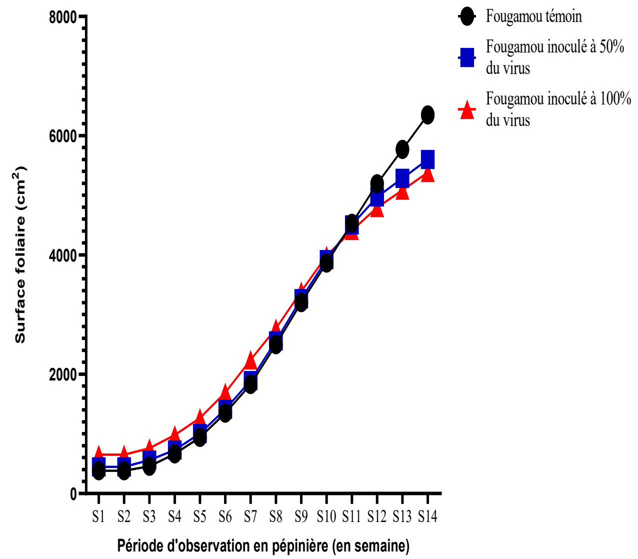


Impact of BBTV on Fougamou growth

Impact of BBTV on the leaf area of Fougamou

This study shows that from S1 to S10, all plants exhibit significant growth in leaf area (Figure 5). During this period, it ranged from 382.2 $^{cm^2}$to 3866.$^{1\ cm^2}$for Fougamou T0; from 447.0 $^{cm^2}$to 3922.8 $^{cm^2}$for Fougamou I 50%; and from 650.5 $^{cm^2}$to 398.7 cm^2 for Fougamou I 100%. Between S11 and S14, the leaf area continues to increase for all three treatments. Although leaf growth continues to increase for inoculated plants (Fougamou 50% and Fougamou 100%), it differs significantly from that of the control, which has the highest leaf area. The lowest growth rates are recorded in plants inoculated at 100%. However, statistically, there is no significant difference between all treatments.

Figure.5 Effect of BBTV on the leaf area of Fougamou

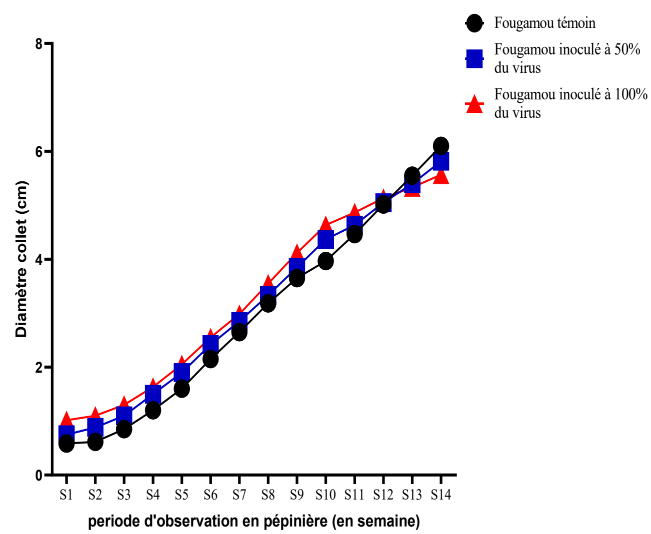


Impact of BBTV on the collar diameter of Fougamou

This study reveals a gradual increase in the diameter of Fougamou plants for all treatments from S1 to S14 (Figure 6).

Although the control plants recorded the highest diameter (6.1 cm) at S14, there was no significant difference compared to the Fougamou plants inoculated with 50% (5.82 cm) and 100% of the virus (5.57 cm).

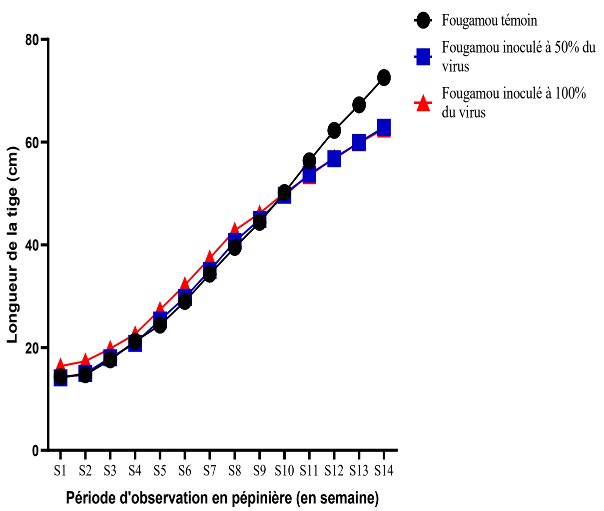
Figure.6 Effect of BBTV on the collar diameter of Fougamou



Impact of BBTV on the stem length of Fougamou

This study reveals that, overall, all plants show progressive stem growth over time. However, a clear difference between treatments appears from S11 onwards (Figure 7). The control shows continuous growth and reaches a maximum length of approximately 72.56 cm at S14. Compared to the control, the Fougamou plants inoculated at 50% and 100%, although their stem length continued to grow, showed a slowdown from S11 to S14, where they reached 62.87 cm and 62.85 cm respectively for the plants inoculated at 50% and 100%.

Figure.7 Effect of BBTV on the stem length of Fougamou



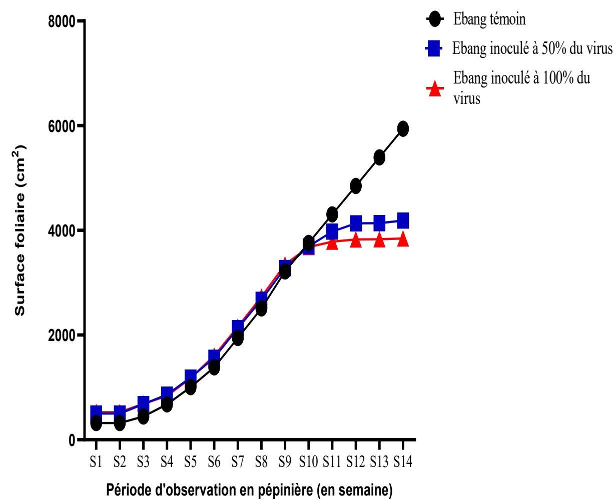
Impact of BBTV infection on plantain growth

Impact of BBTV infection on Ebang growth

Impact of BBTV on the leaf area of Ebang

The findings show an increase in leaf growth for the three treatments during the period from S1 to S10 (Figure 8). During this period, it increased from 318.5 to 3760.5 $cm^2$for Ebang T0, from 502.0 to 3696.^{2} $cm^2$for Ebang I 50%, and from 526.8 to 3679.0 $cm^2$for Ebang I 100%. However, during the 11th and 14th weeks, a difference in leaf growth was observed between the three treatments. A continuous increase in leaf growth was observed for the non-inoculated Ebangs, reaching the highest leaf area (5939.4 cm^2) in the 14th week. According to statistical analyses, no significant difference was recorded between the inoculated plants, but differences were recorded between the control and the inoculated plants (P≤0.05). According to the curves, the plants inoculated at 50% and 100% showed a slowdown in the growth of their leaf area. The lowest leaf growth was recorded with Ebangs inoculated at 100%.

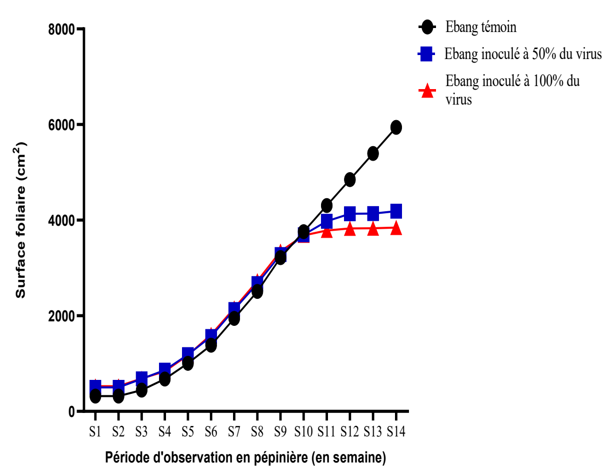
Figure.8 Effect of BBTV on the leaf area of Ebang



Impact of BBTV on the collar diameter of Ebang

The results show that the control plants continued to grow in diameter until S14, reaching their maximum growth. The inoculated plants (at 50% and 100%) showed an increase in growth between S1 and S10. However, slower growth in Ebang diameter was observed for these two treatments between S11 and S14 (Figure 9).

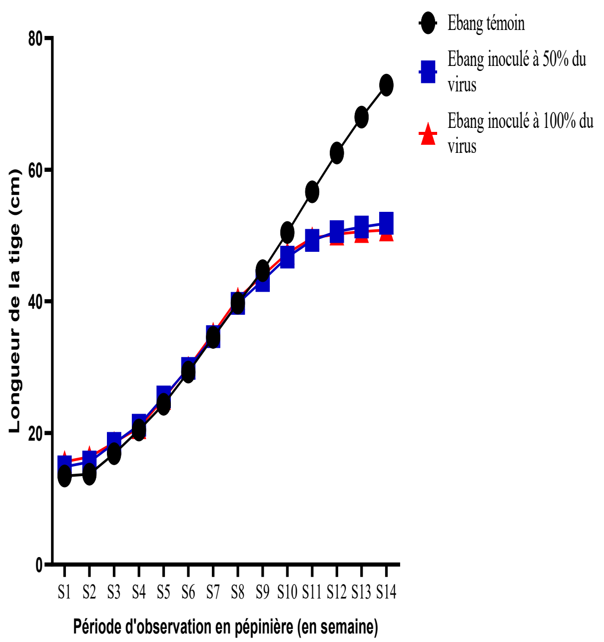
Figure.9 Effect of BBTV on the collar diameter of Ebang



Impact of BBTV on Ebang stem length

The results of this study reveal that the most significant stem growth was recorded in the control Ebang (black curve), increasing from 13.45 cm to 72.85 cm from S to S14 (Figure 10). In contrast, plants inoculated with the virus showed significant stem growth during the first ten weeks (from S1 to S10). Between S11 and S14, the plants inoculated at 50% and 100% experienced a slowdown in stem growth, with the lowest growth recorded in the plants inoculated at 100% (50.85 cm at S14).

Figure.10 Effect of BBTV on the stem length of Ebang

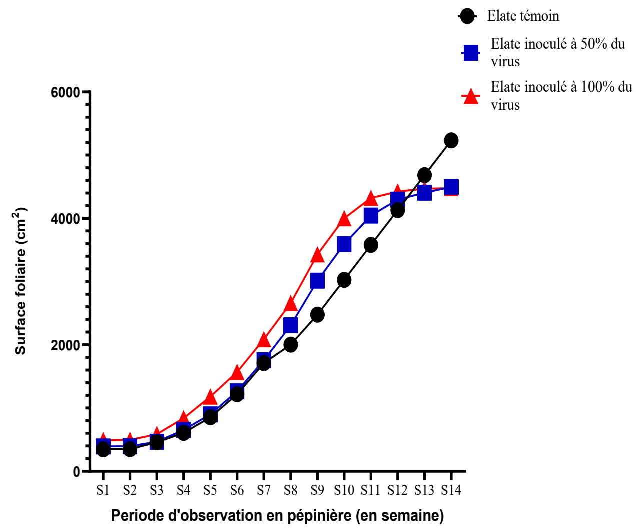


Impact of BBTV infection on Elate growth

Impact of BBTV on the leaf area of Elate

During the first ten weeks (Figure 11), leaf area growth was recorded for all three treatments, with the highest growth rates recorded for Elate I 100% (4001.5 $^{cm^2}$at S10). From S11 to S14, the trend reversed: healthy plants (T0) accelerated their growth to reach the largest leaf area at S14 (5234.655 $^{cm^2}$). The plants inoculated at 50% and 100% showed a similar slowdown in their leaf areas, reaching 4497.2 cm^2 (Elate I 50%) and 4477.0 cm^2 (Elate 100%). According to statistical analyses, there were no significant differences between the inoculated plants. Differences were observed only between the control and the inoculated plants (P≤0.05).

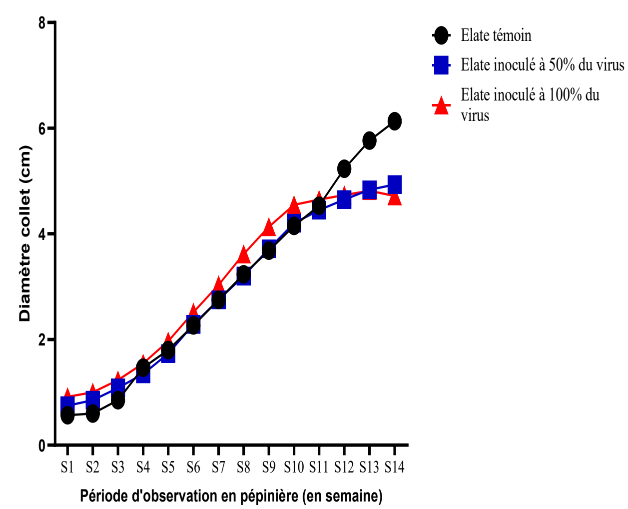
Figure.11 Effect of BBTV on the leaf area of Elate



Impact of BBTV on the collar diameter of Elate

In general, regardless of the treatment, all plants show progressive growth in collar diameter over time. The control treatment shows continuous growth, reaching the highest diameter t S14 (6.13 cm). Plants inoculated at 50% and 100% show significant diameter development during the first 10 weeks. From S11 onwards, a slowdown in diameter growth was observed. Plants inoculated at 100% showed comparable initial growth, with a slight advantage between S1 and S10, but a slowdown was observed between S11 and S14 (Figure 12).

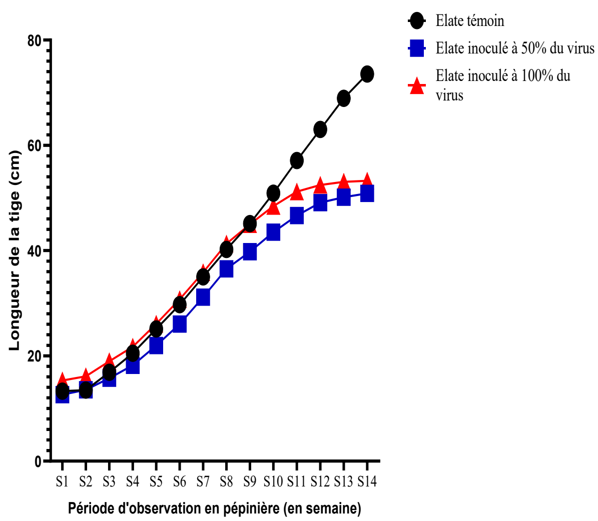
Figure.12 Effect of BBTV on the collar diameter of Elate



Impact of BBTV on Elate stem length

This study reveals that stem growth was greater in the control plants, with a length reaching approximately 73.53 cm at S14. In contrast, plants inoculated with 50% and 100% of the virus showed significantly slower growth (Figure 13). The results show that plants inoculated with 50% and 100% of the virus experienced a significant increase in stem length up to S11, reaching 46.65 cm and 51.2 cm respectively. Subsequently, for both treatments, a slowdown in stem growth was observed between S11 and S14.

Figure.13 Effect of BBTV on the stem length of Elate



In this study, analysis of Figure 1 shows a clear difference in behaviour between the varieties: Elate and Ebang show early emergence, with seedlings observed (2 and 1 seedlings respectively) as early as the second week. At S6, the highest number of seedlings was observed in these two varieties (59 for Elate and 46 for Ebang). This rapid response to the PIF method indicates a genetic ability to quickly reactivate dormant buds after the incisions were made. According to Kengue *et al.,* (2021), certain banana varieties are sensitive to the mechanical stress induced by the PIF method, thus promoting earlier emergence. The high number of seedlings observed could be explained by the density and reactivity of latent buds (Nkengla *et al.,* 2022). According to these authors, the higher the density and reactivity of latent buds in a variety, the greater its capacity to produce numerous shoots when incisions are applied. Gros Michel and Fougamou only show their first shoots from the third week (W3) onwards. These two dessert varieties had the lowest number of seedlings at S6 (41 for Gros Michel and 35 for Fougamou). These results reflect longer dormancy or lower bud reactivity, as mentioned by Nkengla *et al.,* (2022).

Analysis of leaf area (Figures 2, 5, 8 and 11), collar diameter (Figures 3, 6, 9 and 12) and stem length (Figures 4, 7, 10 and 13) shows significant growth in the control plants for all four varieties throughout the 14 weeks (98 days) of observation. For the inoculated plants, growth in leaf area, diameter and stem length was recorded during the first ten weeks for all varieties. Subsequently, marked slowdowns in leaf area, collar diameter and stem length were observed from S11 to S14 in Gros Michel (Figures 2, 3 and 4), Ebang (Figures 8, 9 and 10) and Elate (Figures 11, 12 and 13). These slowdowns in leaf area, collar diameter and stem length show that BBTV affects banana growth parameters. This is consistent with Hooks *et al.,* (2008), whose work showed that BBTV has an impact on banana leaf area, collar diameter and stem length. Considering the two types of inoculation (I50% and I100%) for these three varieties, although no significant difference was observed, the behaviour of the curves shows that the most significant slowdowns are observed in plants inoculated at 100%. This confirms that BBTD has a negative impact on these three banana growth parameters and that the higher the inoculation dose, the more marked the slowdowns in leaf area, collar diameter and stem length, thus showing that the inoculation dose has an effect on their growth response. The slowdown in leaf area, collar diameter and stem length growth caused by BBTD can be explained by several physiological and viral mechanisms (Caruana, 2003; Simbare, 2017). According to these authors, BBTV infects phloem tissues, disrupting nutrient transport. Furthermore, according to Hooks *et al.,* (2008), the virus causes a significant decrease in chlorophyll production, which directly affects photosynthetic capacity and limits leaf development.

With regard to the effect of BBTV on the growth of Fougamou, analysis of Figures 5, 6 and 7 revealed that although the best growth (in leaf area, collar diameter and stem length) was recorded with the controls, the growth of the inoculated plants (Fougamou I 50% and Fougamou I 100%), although slower, remained stable compared to other varieties, particularly Gros Michel, which is a dessert banana. The maintenance of growth in these three parameters, despite the different inoculum doses, would indicate a certain resistance of Fougamou to BBTV. This resistance could be explained by genetic factors. Indeed, genetic factors influence BBTD resistance, which modifies the viral load dynamics and thus limits the speed of virus propagation in the tissues. This is in line with Dale and Harding (1998), whose work has shown that certain cultivars possess resistance genes or defence mechanisms (through the expression of antiviral proteins) that limit viral replication or its systemic spread.

This study aimed to evaluate the impact of *Banana bunchy top virus* (BBTV) at different inoculum doses on the growth parameters of four banana varieties (Gros Michel and Fougamou for dessert bananas; Elate and Ebang for plantains). By adopting a rigorous experimental design, including plant propagation using the PIF (Plants derived from stem fragments) method and controlled inoculation of the virus at two different concentrations (50% and 100%), this work made it possible to study the evolution of leaf area, collar diameter and stem length over a period of 14 to 15 weeks. This study clearly demonstrated the negative effect of BBTV on the growth of Gros Michel, Ebang and Elate banana varieties observed from the eleventh week (S11) onwards for all parameters measured (leaf area, collar diameter and stem length), with a more pronounced effect with the pure inoculum (100%). However, the Fougamou variety behaved differently. Although a slowdown in growth was noted in inoculated plants compared to controls, it remained less severe and statistically insignificant between inoculum doses. This behaviour of Fougamou suggests the existence of varietal resistance, potentially linked to genetic factors limiting the replication or systemic spread of the virus.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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