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Evaluation of Banana Bunchy Top Disease (BBTD) Epidemiological Parameters after Inoculation of Different Doses of Banana Bunchy Top Virus (BBTV) on Four Banana Varieties in A Controlled Environment

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Abstract

Bunchy Top Disease is the most devastating viral disease affecting banana plants. This study evaluates the epidemiological parameters (severity, prevalence and incidence) of Banana Bunchy Top Disease (BBTD) after inoculation of different doses of *Banana bunchy top virus* (BBTV) on four banana varieties in a controlled environment. The varieties tested include two dessert bananas, Fougamou (ABB) and Gros Michel (AAA), and two plantains, Elate (AAB) and Ebang (AAB). The plants, propagated using the PIF (Plants from Stem Fragments) method, were inoculated with a pure (100%) inoculum and a 50% dilution. The parameters of severity, prevalence, incidence and weekly leaf growth were monitored for 15 weeks. The results show a dose-response relationship in the expression of symptoms. At 100% inoculum, the Ebang, Elate and Gros Michel varieties showed increased severity, with a predominance of high symptom levels (levels 2 and 3). Fougamou stood out for its marked resistance, developing only mild symptoms (level 1) at high doses and maintaining significantly higher leaf growth than the other varieties. Total prevalence increases rapidly between weeks S10 and S14 (from 12.5% to 75%) and then stabilises. No symptoms are observed during the first nine weeks, indicating a latent period, followed by a sharp increase in incidence, reaching 75% (50% dose) and 79.17% (100% dose) at W15.The study confirms the high susceptibility of AAA (Gros Michel) varieties and reveals an unexpected susceptibility of AAB (Elate and Ebang) plantains in the context of this study. Fougamou (ABB) emerges as a tolerant variety with potential for varietal selection and sustainable management of BBTD. These results provide essential quantitative data on the infectious dynamics of BBTV and highlight the importance of viral dose and genetic background in disease expression.

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Keywords

Banana, BBTD, BBTV, dose, inoculum, epidemiological parameters, varietal resistance.

Introduction

The banana tree (*Musa spp*) is a crop that plays an important 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 these phytopathological threats to banana trees is *Banana Bunchy Top Disease* (BBTD). This viral disease, caused by the *Banana bunchy top virus* (BBTV), is one of the most devastating threats to banana trees (Kumar *et al.,* 2015). The BBTV, 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 seems to have been detected for the first time in Gabon in 1978, at the FAO centre in N'toum. A preliminary research conducted by the FAO in 2018 indicates that 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 Grand Libreville agroecological zone, to date, no work has been done to show the negative effect of BBTD through the determination of epidemiological parameters in a controlled environment.

In this context, this study aims to investigate the severity, prevalence and incidence of this disease on banana plants inoculated with different doses of the virus in a plant nursery. The results will provide a better understanding of the impact of this disease on banana varieties with a view to varietal selection and will also 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 Estuaire province, 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 technical high school of Bikélé, whose GPS coordinates are 00°25'00.22" N and 009°32'59.18" E. It was carried out from 25 January to 20 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 for plant propagation and to determine severity, prevalence and incidence as epidemiological parameters. This included:

- A germinator necessary for propagating healthy banana plants using the PIF (Seedling from Stem Fragment) method;
- Polyethylene sheeting and elastic bands to seal the germinator;
- Sawdust used as substrate;
- A kitchen knife;
- Level 5 diseased banana plants for preparing the inoculum;
- A spray bottle for infecting healthy plants with the inoculum.
- A table showing the different levels of severity of BBTD symptoms (Ngama, 2010).

Methods

Propagation of plants using the PIF (seedling from Stem Fragment) method

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. twelve (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 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 doses of 25 mL and 50 mL per 15 L of water, respectively. They are thenleft 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 pseudo stem left after peeling.
- **Making incisions: This** involves locating the apical meristem and making 2 to 3 cross incisions in the centre of the explant.
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- **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 trees 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 liters 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%).

Determination of the epidemiological parameters of BBTD

In order to study the impact of the disease on banana plants, three epidemiological parameters were selected for this study: severity, prevalence and incidence. These parameters were recorded as soon as the first symptoms of the disease appeared on the banana plants that had been inoculated with the virus.

→ **BBTD severity**: this was determined by visual observation of the symptoms on the leaves. This observation was made possible by the BBTD rating scale (Table 1) describing the different symptom levels (Ngama, 2010).

Level 0	**No symptoms (A)**
Level 1	Green streaks on the leaves (B)
Level 2	Dark striations up to the pseudo-trunk (C)
Level 3	Discoloration of the leaves with normal size
Level 4	Reduced size of discolored leaves (E)
Level 5	Bunchy top (F)

→ **Prevalence of the disease:** According to Benaldjia (2021), prevalence is defined as the ratio between the total number of cases (old and new) of a disease and the total population exposed at a given time or over a given period. In this study, the prevalence rate was determined using the following formula:

$$prevalence = \frac{Number\ of\ cases\ observed\ (new + old)\ at\ a\ T\ time}{\text{Population at risk at this moment}} X\ 100$$

→ **Incidence:** According to Allioui (2020), inplant pathology incidence is often used to measure the importance of a disease in a field or region. The incidence rate (IR) of BBTV, in the context of this study, is the ratio of the number of infected plants to the total number of plants in the batch. It is calculated using the following formula:

$$T\ I = \frac{\textit{number of new cases at a T time}}{\textbf{Total population at this moment}} X\ 100$$

Determination of leaf area

The leaf area is determined by measuring the length and width of the leaf and calculating according to the method proposed by Kumar *et al.,* (2002). In this study, its calculation was a prerequisite for determining the average weekly growth.

Average weekly growth

Average weekly growth was determined by calculating the difference between the leaf area measurement for the following week (LA2) and the leaf area measurement for the previous week (LA1). This formula is written as follows:

Average weekly area = LA2 - LA1

Results and Discussion

Severity

BBTD severity at Inoculum 50%

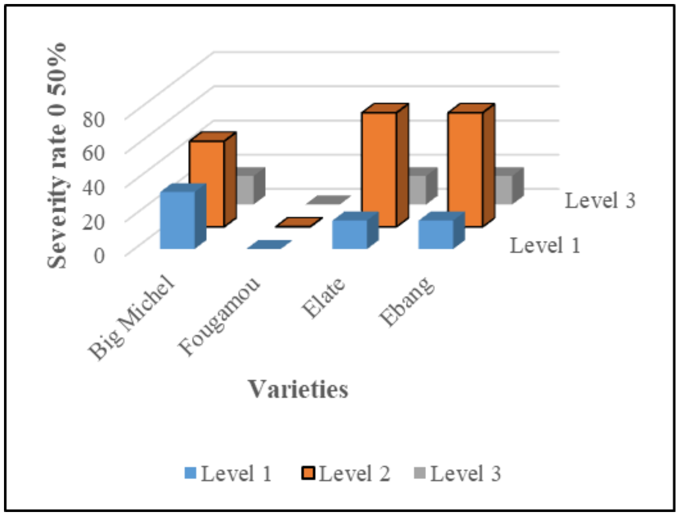
This study reveals different levels of BBTD severity in plants inoculated at 50% (Figure 1).

Observations of the Elate and Ebang varieties (plantains) show levels 1, 2 and 3 with severity rates of 16.67%, 66.67% and 16.67% respectively.

For these two varieties of plantains, level 2 is the most representative with a rate of 66.67%. Gros Michel, on the other hand, shows all three levels of symptomatic severity, with level 2 being the most prevalent (50%) and level 3 being the least prevalent (16.67%).

No symptoms were found on Fougamou, as the three levels of symptomatic severity (level 1, level 2 and level 3) all had a rate of zero.

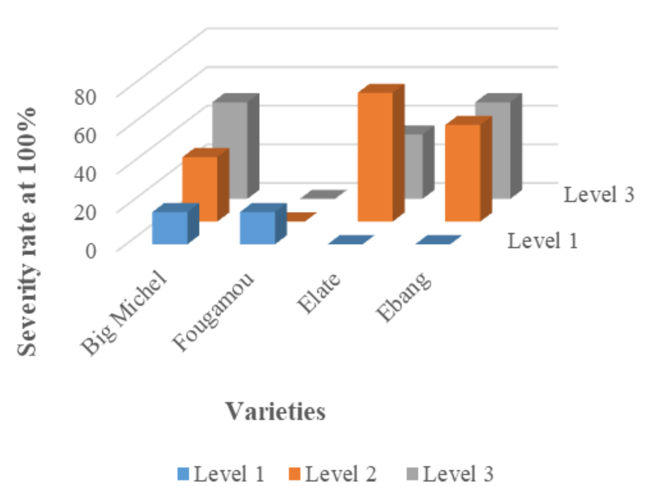
Figure.1 BBTD severity at Inoculum 50%



BBTD severity at Inoculum 100%

This study reveals that at a dose of 100%, the Elate and Ebang varieties show only two levels of BBTD severity, namely level 2 (66.67% for Elate and 50% for Ebang) and level N3 (33.33% for Elate and 50% for Ebang), with level 1 being absent (Figure 2). The Gros Michel variety, on the other hand, shows the first three levels of symptomatic severity of the disease, with level 3 representing the highest severity rate (50%), followed by level 2 (33.33%) and level 1 being the lowest level (16.67%). Fougamou, on the other hand, only shows level 1 with a severity rate of 16.67%, with levels 2 and 3 absent.

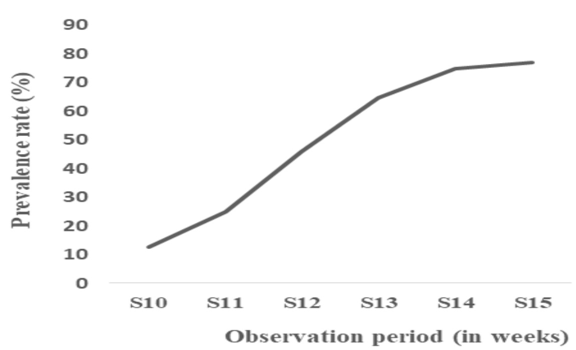
Figure.2 BBTD severity at Inoculum 100%



Prevalence

The results obtained from week S10 to week S15 show a change in the prevalence of foot rot (Figure 3). This study reveals an increase over time in the total prevalence rate of BBTD, with a slowdown in the last two weeks. From week 10 to week 14, there was a significant increase in prevalence, from 12.5% to 75%. From week 14 to week 15, this prevalence rate slowed, rising from 75% to 77.08%.

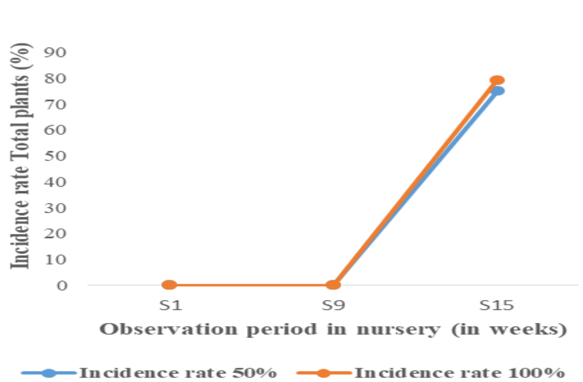
Figure.3 Prevalence of foot rot



Total incidence

Observations show an incidence of BBTD on plants inoculated at 50% and 100% between weeks W1, W9 and W15 (Figure 4). This study shows that no symptoms of BBTD were observed during the first nine weeks, regardless of the inoculation rate, as the incidence was 0% for both types of inoculation. However, after W9 to W15, there was a sharp increase in the incidence rate in both types of inoculation, with a 75% incidence rate for plants with 50% inoculation. The highest incidence was recorded in plants inoculated with 100% BBTV (79.17%).

Figure.4 Incidence of BBTD on plants inoculated at 50% and 100%

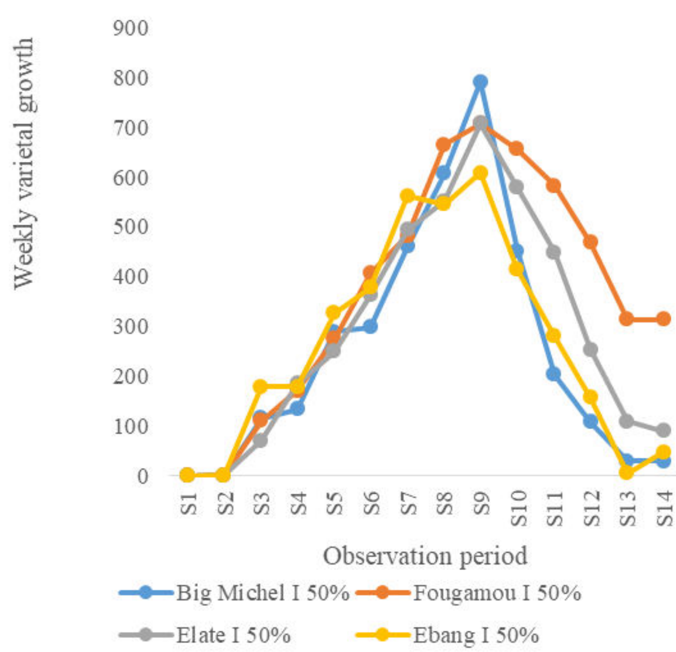


Varietal susceptibility

Varietal susceptibility of plants inoculated at 50%.

The results show that plants inoculated at 50% exhibit different varietal sensitivities (Figure 5). All varieties show progressive weekly growth in leaf area until a peak growth around S9 for all varieties. At S9, the highest average growth was recorded for Gros Michel (790.51 cm^2), followed by Fougamou (706.97 cm^2), Elate (706.92 cm^2) and finally Ebang (608.28 cm^2). This was followed by a decline between S10 and S14. During this period, all varieties saw a significant decrease in their weekly leaf area growth. At S14, they will be 29.87 cm^{2}for Gros Michel, 90.83 cm^{2}for Elate and 48.35 cm^{2}for Ebang. The highest weekly growth recorded was for Fougamou (313.40 cm^2), despite the observed decrease.

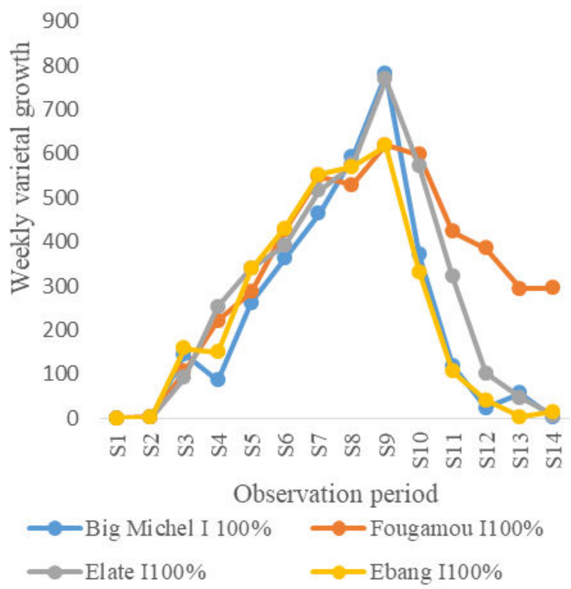
Figure.5 Varietal susceptibility of plants inoculated at 50%.



Varietal sensitivity of 100% inoculated plants.

The results show different varietal sensitivities to BBTD in plants inoculated at 100% (Figure 6). The study reveals that all varieties have a progressive average weekly growth in leaf area until a peak growth around S9 for all varieties. In fact, at S9, the highest average growth is recorded with Gros Michel (781.^{01} cm^2), followed by Elate (769.63 cm^2), Fougamou (619.43 cm^2) and Ebang (617.57 cm^2). This was followed by a decline between S10 and S14. During this period, all varieties saw their weekly leaf area growth decrease considerably to almost zero, except for Fougamou. At S14, they were 1.22 cm^2 for Gros Michel, 5.69 cm^2 for Elate and 14.42 cm^2 for Ebang. Although Fougamou's average weekly growth also decreased (295.17 cm^2), it remained the highest compared to the others.

Figure.6 Varietal susceptibility of plants inoculated at 100%.



When studying the severity of BBTD in this study, at the 50% dose (Figure 1), the two plantain varieties (Elate and Ebang) showed a similar distribution of BBTD symptoms, with level 2 being dominant (66.67%) and levels 1 and 3 being the least frequent (16.67% each). This suggests that at a moderate dose, these varieties mainly develop moderate symptoms (level 2), with a small proportion of plants showing mild (level 1) or severe (level 3) symptoms. At the highest concentration, which is 100% (Figure 2), for these two varieties, level 1 disappears, tending towards the highest levels (levels 2 and 3). Thus, the absence of level 1 and the increase in level 3 indicate that the severity of symptoms intensifies with the inoculation dose, especially for Ebang, which shows the most severe symptoms.

As regards dessert bananas, for Gros Michel at a dose of 50% (Figure 1), the most severe level is represented by level 2 (50%), with level 3 being the least severe (16.67%). At a dose of 100%, again for Gros Michel, level 3 becomes dominant (50%), while level 1 has the lowest severity (16.67%). This shows that plants subjected to a moderate dose of inoculum exhibit moderate levels of symptoms (level 2), and that the higher the inoculation dose (100%), the more severe the symptoms exhibited by the plants (level 3). This observation is consistent with the principle of "dose dependence" in plant pathology, where higher inoculums exacerbate symptoms (Hull, 2002). For Fougamou at a dose of 50%, no symptoms were recorded. However, only at a dose of 100% was level 1 (16.67%) recorded. Fougamou appears to show resistance to BBTD, even at high doses. The very limited occurrence of N1 suggests tolerance or resistance to BBTD.

Comparing the proportions of plants by symptom level, it can be said that at 50% inoculation, the most dominant level is N2 (66.67%) recorded in Elate and Ebang, followed by Gros Michel (50%). With the highest inoculation (100%), the most dominant level (N2 = 66.67%) is recorded in Elate, followed by level N3 (50%) recorded in Ebang and Gros Michel.

Analysis of the results in Figure 3 shows a rapid increase in the total prevalence of BBTD on banana plants, from 12.5% to 75% between weeks S10 and S14, followed by a slowdown in progression between S14 and S15 (from 75% to 77.08%). This dynamic reflects a very rapid initial spread of the virus, followed by a saturation phase where most plants are already infected. The marked increase in prevalence at the beginning of the observation period can be explained by the presence of many plants that were initially healthy but began to show symptoms of BBTD. This reflects active spread of the virus within the plant population. Studies such as those by Hooks *et al.,* (2009) have shown that under favourable conditions, BBTV spreads efficiently once vectors acquire and disseminate the virus, reaching a prevalence of up to 80%. The slowdown observed from S14 onwards reflects the exhaustion of the plants. In fact, the majority of plants show all the symptoms of BBTD, thus limiting the possibility of new infections.

With regard to the total incidence of BBTD on inoculated plants, analysis of Figure 4 shows that no symptoms are observed during the first nine weeks (W1 to W9), regardless of the inoculation dose. This suggests a latency or incubation period for the virus before symptoms appear (the plant is not yet infectious), which is consistent with the characteristics of the BBTV virus, known for its prolonged incubation period. These results corroborate the work of Hooks *et al.,* (2008), whose results reported a latency period of between 25 and 85 days (approximately 3 to 12 weeks) before the onset of

BBTV symptoms, confirming that the virus requires an incubation period before manifesting itself. This asymptomatic phase is crucial for the undetected spread of the virus.

Analysis of this figure shows that a sharp increase in incidence was recorded for both doses between S9 and S15. Plants inoculated at 50% reached an incidence rate of 75%, while those inoculated at 100% reached an incidence of 79.17%. This highlights the virulence of BBTV and its ability to rapidly infect banana plants once the latency period has passed. Although the difference in incidence between the two doses is not very marked (75% vs 79.17%), it still suggests that higher concentrations of the virus could accelerate or intensify infection. However, the relatively small difference could also indicate that other factors, such as varietal susceptibility, play an important role in the progression of the disease (Allen., 1978; Hooks *et al.,* 2008).

The results obtained following controlled BBTV inoculations (Figures 5 and 6) highlight significant differences in varietal susceptibility among the four banana varieties tested: Gros Michel, Fougamou, Elate and Ebang. Analysis of weekly leaf growth, used as an indicator of susceptibility, allows several conclusions to be drawn. The Gros Michel, Elate and Ebang varieties show a sharp decline in leaf growth after the peak at S9, reaching very low values at S14 with both types of inoculation, but more particularly with 100% inoculation (Figure 6). This decline reflects the high susceptibility of these three varieties to BBTV, manifested by an inhibition of weekly leaf area growth, a typical symptom of viral infection. This varietal susceptibility can be explained by their genetic diversity. Indeed, genetic diversity influences varietal susceptibility or resistance. In the case of Gros Michel (genotype AAA), its susceptibility observed in this study is confirmed by the work of Ondh-Obame (2021) and Shadari Salumu *et al.,* (2024). These authors have shown that cultivars of the AAA genotype, including Gros Michel, are the most vulnerable to BBTV. However, the susceptibility observed in Elate and Ebang (both of the AAB genotype) is not consistent with the findings of these authors. According to the work of Ondh-Obame (2021) and Shadari Salumu *et al.,* (2024), cultivars of the AAB genotype are more resistant to BBTV. The resistance observed in these cultivars by these authors is thought to be due to the presence of the B genome. The B genome contains genes that confer a greater ability to limit viral multiplication or restrict the spread of the virus within the plant. This results in a reduction in viral load and less severe symptoms.

In the case of Fougamou (genotype ABB), it showed low sensitivity and the average weekly leaf growth remained high (313.40 cm² at S14) at 50% inoculation. At 100% inoculation, Fougamou again showed remarkably low sensitivity (295.17 cm² at S14), confirming its potential as a tolerant variety. These results are corroborated by Ondh-Obame (2021) and Shadari Salumu *et al.,* (2024), whose work has proven the resistance of this variety, also due to the presence of the B genome. The results of this study also showed the susceptibility of two plantain varieties (Elate and Ebang) to the virus, thus contradicting these same authors.

This study, conducted in a controlled environment, evaluated the effect of different doses of *Banana bunchy top virus* (BBTV) inoculums on four banana varieties, focusing on epidemiological parameters (severity, prevalence, and incidence) and varietal resistance to Banana Bunchy Top Disease (BBTD). The results of this study revealed a clear dose-dependent relationship in the expression of symptoms: higher inoculation doses (100%) resulted in increased severity, particularly in the Ebang (AAB), Elate (AAB) and Gros Michel (AAA) varieties. Conversely, the Fougamou (ABB) variety showed notable resistance, with minimal BBTD symptoms even at high doses and significant maintenance of weekly leaf growth. Epidemiological analysis revealed an asymptomatic latency period of approximately nine weeks, followed by a rapid increase in prevalence and incidence, reaching high plateaus (75-79%) at the end of the observation period. This confirms both the virulence of BBTV and its ability to spread once the incubation phase is over. The differences in varietal sensitivity observed, particularly the vulnerability of the Elate, and Ebang (AAB) plantain varieties in this study, highlight the complex influence of genetic background and indicate that resistance associated with the B genome is not absolute and may vary among cultivars. Overall, this work provides valuable quantitative data on the dynamics of BBTD and identifies Fougamou as a promising variety for varietal selection and sustainable management strategies for this devastating viral disease.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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