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# Relative susceptibility of *Musa* genotypes to banana bunchy top disease in Cameroon and implication for disease management

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#### ABSTRACT

Banana bunchy top disease (BBTD) is a serious threat to banana and plantain (Musa spp.) production. BBTD is caused by the Banana bunchy top virus (BBTV, genus Babuvirus) which is spread through infected plant propagules and banana aphid, Pentalonia nigronervosa. A high level of resistance to BBTD in Musa spp. has not been yet discovered, but there is a wide range of susceptibility among Musa genotypes. In this study, performance of 16 Musa genotypes to BBTD infection was studied during 37 months in two replicated field experiments planted in a BBTD-endemic location in the South region of Cameroon. BBTD expression varied among genotypes without any specific patterns related to their genomic composition. In Abang Minko'o, highest BBTD incidence was observed in Cavendish Williams (100%) and in the plantain hybrid variety PITA 23 (91.6%). A larger group of 11 genotypes including 4 plantain landraces, 3 hybrid plantains, 3 hybrid bananas and a Cavendish Grande Nain were susceptible with incidence between 40 and 80%. The disease incidence was least in Gros Michel and Fougamou which after 37 months had less than 20% symptomatic plants. In Kou'ou-si, 60% BBTD incidence was observed on PITA 23 while two plantain landraces (Asung Mbele, Big Essong) and Gros Michel remained uninfected at 37 months after planting. The banana aphid was present on all genotypes; but excepting PITA 23 and Williams in Abang Minko'o and PITA 14 and PITA 23 in Kou'ou-si, the occurrence of infection was not correlated to aphid abundance. BBTD expression on the same genotype planted in different areas reveals that BBTD expression is not only related to the varietal characteristic but also to the epidemiological situation of the area.

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#### 1. Introduction

Bananas and plantains (*Musa* spp.) contribute to food security for people in the intertropical area of the world. They are important food crops in sub-Saharan Africa, providing more than 25% of the carbohydrate and 10% of the calorie for approximately 70 million people in the region (Swennen, 1990). Banana bunchy top disease (BBTD) is the most economical important viral disease of bananas and plantains in several countries in Asia, Africa and South Pacific, including Australia (Dale, 1987; Kumar et al., 2015). Plants infected early do not produce fruits thus, losses attributed to BBTD are 100% in case of early infection. Fruits from late infection are dwarf and unmarketable (Hooks et al., 2009). BBTD is an emerging threat to banana and plantain production in Africa. Actually, the disease has been reported in 14 countries in Africa (Adegbola et al., 2013; Blomme et al., 2013; Kumar et al., 2011, 2015). BBTD is caused by the *Banana bunchy top virus* (BBTV), which is a single-stranded DNA virus belonging to the genus *Babuvirus*, family Nanoviridae. BBTV is specific to *Musa* species (Magee, 1940; Hu et al., 1996; Geering and Thomas, 1997; Thomas et al., 1994). BBTV can only spread through infected plant propagules (suckers, corms, tissue culture plants) and banana aphid, *Pentalonia nigronervosa* Coquerel (Hemipterae:







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Aphididae) (Anhalt and Almeida, 2008). The aphid transmits BBTV persistently (Hu et al., 1996). The banana aphid is widespread in tropical and subtropical parts of the world (Blackman and Eastop, 2000). When a field is started with clean planting materials, the banana aphid is responsible for primary inoculum and for secondary plant-to-plant spread of the disease within banana field. Banana aphid plays an important role in disease epidemiology (Fouré and Manser, 1982). Hu et al. (1996) established that BBTV can be efficiently transmitted by a single aphid but the transmission efficiency increases when the number of adult increases to five per plant. Aphid abundance varies with Musa genotype (Ngatat, 2010). Plantains are more suitable for aphid growth than bananas. There are no reports of immunity or high levels of resistance to BBTV in any Musa species or cultivar. Host plant resistance is recognized as the most effective, and in many cases the only, strategy for the control of plant virus diseases. Several studies reported differences in susceptibility among Musa genotypes (Espino et al., 1993; Ariyaratne and Liyanage, 2002; Gondwe et al., 2007; Niyongere et al., 2011). Magee (1948) reported the susceptibility of Cavendish cultivars and the tolerance of Gros Michel. Espino et al. (1993) mentioned greater susceptibility and higher percentage of infection of AA and AAA cultivars and some degree of resistance and low percentage of infection among AAB, ABB, ABBB and BB/BBBB sub group. Hooks et al. (2009) highlighted the potential use of less susceptible cultivars for BBTD management and the importance of screening banana varieties under field conditions for their response to disease and associated vector.

This study was undertaken to evaluate prominent varieties for West and Central Africa for BBTD under natural environment, to understand variety performance in two fields (Abang Minko'o and Kou'ou-si) in a BBTD endemic area in southern Cameroon.

#### 2. Materials and methods

#### 2.1. Field trial

Field trials were set up in two sites in September 2010 in the Southern region of Cameroon where BBTD is present; Abang Minko'o (02°19.513'N/011°26.362'E, 563m a.s.l.) and Kou'ou-si (02°18.263'N/011°26.785'E, 555m a.s.l.). Climatic data were recorded using a hobo installed under a shelter at Kou'ou-si from September 2010 to October 2013; the average daily temperature was 24.14 °C (min 16.15 °C, max 37.48 °C), while the average relative humidity was 89.01% (min 34.5%, max 100%). Sixteen banana

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| List of genolypes planted in the held th | List | of | genotypes | planted | in | the | field | trial |
|--|------|----|-----------|---------|----|-----|-------|-------|
|--|------|----|-----------|---------|----|-----|-------|-------|

| Genotype    | Genomic group | Туре                  | Use                  |
|-------------|---------------|-----------------------|----------------------|
| Asung Mbele | AAB           | Local plantain        | Fries, chip, cooking |
| Essong      | AAB           | Local plantain        | Fries, chip, cooking |
| Big Essong  | AAB           | Local plantain        | Fries, chip, cooking |
| Batard      | AAB           | Local plantain        | Fries, chip, cooking |
| PITA 14     | AAAB          | Hybrid plantain       | Fries, chip, cooking |
| PITA 23     | AAB           | Hybrid plantain       | Fries, chip, cooking |
| PITA 24     | AAB           | Hybrid plantains      | Fries, chip, cooking |
| PITA 27     | AAB           | Hybrid plantain       | Fries, chip, cooking |
| FHIA 21     | AAAB          | Hybrid plantain       | Fries, chip, cooking |
| FHIA 25     | AAB           | Hybrid cooking banana | Flour, cooking       |
| BITA 3      | AAAB          | Hybrid cooking banana | Flour, cooking       |
| BITA 8      | AAB           | Hybrid cooking banana | Cooking, flour       |
| Fougamou    | ABB           | Cooking banana        | Cooking              |
| Grande Nain | AAA           | Dessert banana        | Dessert, Cooking     |
| Gros Michel | AAA           | Dessert banana        | Dessert, Cooking     |
| Williams    | AAA           | Dessert banana        | Dessert, Cooking     |

PITA 24 and Batard was overlapping; Batard was planted in Abang Minko'o while PITA 24 was planted in Kou'ou-si. and plantain genotypes were tested (Table 1). Planting materials were produced and provided by the laboratory of tissue culture of IITA-Cameroon. These planting materials generated in tissue culture were kept for about one year post flask in an area known to be free of BBTD. All the planting materials used were certified BBTV free. Plants were arranged as  $2 \text{ m} \times 2 \text{ m}$  row and line spacing for a density of 2500 plants/hectare. Each genotype was a treatment represented by a row of 5 plants. The experimental layout was a complete randomized block design with 3 replicates (15 plants per genotype tested in each location). At the edges of each row, spreader plants (BBTV infected suckers sourced from disease affected farms) were planted and were not replaced.

#### 2.2. Disease incidence and aphid abundance

Disease incidence and aphid abundance was assessed at monthly intervals from 2 to 37 months. Disease incidence was recorded based on visual symptoms. Plants were inspected carefully for BBTD symptom expression. After sucker emergence, BBTD symptoms were inspected in all the plants of a mat; if a single plant is infected, the whole mat was considered as infected. Aphid abundance was assessed by counting the number of aphids on the lower part of the pseudostem of three internal sampling plants. Aphid assessment was done on the main plant and after flowering and fruit production, counting was shifted to the main sucker of the mat. Additionally, data on number of healthy bunches produced during the 37 months after trial establishment, percentage of BBTD infected suckers and percentage of BBTD mortality were collected.

#### 2.3. Virus incidence by molecular analysis

Leaf samples were collected and virus incidence was evaluated at 37 months after planting by PCR. DNA was isolated from leaf samples following the procedures described by Dellaporta et al. (1983). One pair of primers (Virion sense: BBTV-1: 5'GCGTGAAACGCACAAAAGGCC 3'; Complementary Sense: BBTV-2: 5' GCATACGTTGTCAAACCTTCTCCTC 3') that corresponds to the core region of the DNA-R was used for polymerization reaction resulting in 240 bp (Kumar, 2010; Kumar et al., 2011). PCR products were run on 1.5% agarose gel stained with ethidium bromide and DNA was visualized under UV trans illuminator.

#### 2.4. Statistical analysis

Cumulative aphid-days, a value that provides a measure of aphid abundance over time was calculated for each genotype following this formula (Hanafi et al., 1989)  $\sum_{n=1}^{\infty} = \binom{x_{i-1}+x_i}{2} \times t$  where x is the mean number of aphids on sample day i, xi-1 is the mean number of aphids on the previous sample day, and t is the number of days between samples i - 1 and i. The area under disease progress curve (AUDPC) was calculated following the formula from Shaner and Finney (1977);  $AUDPC = \sum_{i=1}^{n-1} \binom{y_i+y_{i+1}}{2} \times (t_{i+1} - t_i)$  where  $y_i$  the proportion of the infected plant at the *i*th observation,  $t_i$  is time in days at the *i*th observation, and n is the total number of observations. Mortality of BBTD diseased plants was calculated as the ratio

expression to plant death. Statistical analyses were carried out using SAS 9.2. Data from each site were analyzed separately. Parameters recorded were analyzed following analysis of variance (ANOVA) procedure. Means of different treatments were separated using Student Newman

infected plants was estimated as the time taken between symptom

Keuls test at 5%. Correlations were done between aphid abundance and BBTD occurrence, moreover between cumulative aphid-days and cumulative BBTD incidence using the coefficient of correlation of Pearson.

#### 3. Results

#### 3.1. Symptoms expression

Initial symptoms on all the varieties were hooks on leaf lamina, dark green streaks on leaf petioles, followed by marginal chlorosis on leaf and bunchy appearance of the plant. Symptom development on two AAB-local varieties (Essong and Big Essong) was very slow (three to five months from green streaks on leaf petioles to Bunchy appearance) comparing to Williams or Grande Nain (one month from streak to bunchy appearance).

#### 3.2. Disease progress on different genotypes

In Abang Minko'o, BBTD symptomatic plants were observed in all the genotypes at 37 months after planting. The first symptoms appeared earlier at 2 months after planting on Williams (AAA), while Gros Michel (AAA) and Fougamou (ABB) showed the first symptoms respectively at 12 and 23 months after planting. In general, the disease progress curves of BBTD on Williams and PITA 23 appeared exponential at first, but as disease incidence increased and approached 100%, the rate of disease progress gradually slowed to zero; giving curves a somewhat sigmoid shape (Fig. 1). For Gros Michel and Fougamou, the curves of disease progress had a shape of stairs; for others genotypes, the curves appeared to be nearly sigmoid. Regarding the curves of disease progress, three groups of reaction were identified from this study. The highly susceptible group included Williams and PITA 23. The moderately susceptible group included all the AAB local plantains (Essong, Big Essong, Batard, Asung Mbele); the hybrid plantains (PITA 14, PITA 27, FHIA 21); the dessert banana Grande Nain, the hybrid bananas (BITA 3, BITA 8, FHIA 25) while the less susceptible group included Gros Michel and Fougamou. In Kou'ou-si, symptoms were first displayed simultaneously at 5 months after planting on Williams and FHIA 21; while Essong showed the first disease symptoms at 33 months after planting. In this field, curves of disease progress seem more grouped together which indicates small variability in BBTD incidence for all the genotypes (Fig. 2).

#### 3.3. Disease incidence and aphid abundance

In Abang Minko'o, BBTD incidence showed significant differences among genotypes at 37 months after planting ( $F_{14,30} = 2.50$ , P = 0.017). In this field, BBTD incidence ranged from 8 to 100% (Table 2). PITA 23 (AAB) and Williams (AAA) presented respectively 92 and 100% BBTD incidence. Gros Michel and Fougamou respectively showed 8.33 and 13.33% BBTD incidence. Aphid abundance calculated as cumulative aphid-days varied significantly among genotypes in Abang Minko'o ( $F_{14,30} = 6.39$ , P < 0.0001). Higher aphid-days were observed on local plantains followed by hybrid plantains. Lowest aphid-days were observed on dessert bananas (Williams, Grande Nain).

Meantime in Kou'ou-si, BBTD incidence showed significant differences among genotypes ( $F_{14, 30} = 3.49$ , P = 0.002). At 37 months after planting, BBTD incidence ranged from 0 to 60% (Table 3). The local plantains-AAB (Asung Mbele, Big Essong) and Gros Michel were symptomless at 37 months after planting (0% BBTD incidence) while 60.00% incidence was observed on PITA 23-AAB. Significant differences were observed in aphid abundance among genotypes ( $F_{14, 30} = 2.29$ , P = 0.03). Higher aphid-days was observed on hybrids plantains FHIA 21 (29420.21) and PITA 14 (16765.59) while lowest was observed on Cavendish Williams (3916.435) and Grande Nain (2667.34).

AUPDC showed significant differences in Abang Minko'o ( $F_{14}$ ,  $_{30} = 4.04$ , P = 0.0007), lowest AUDPC ( $44.60 \pm 26.66$ ) was recorded



Fig. 1. Disease progress curves on 15 Musa genotypes in Abang Minko'o during 37 months.



Fig. 2. Disease progress curves on 15 Musa genotypes in Kou'ou-si during 37 months.

#### Table 2

First BBTD symptoms, BBTD incidence, cumulative aphid-days, AUDPC, suckers with BBTD symptoms, mortality, longevity, bunches in Abang Minko'o at 37 months after planting.

| Genotype    | First BBTD<br>symptoms<br>(Months) | BBTD incidence (%)               | Cumulative aphid-days               | AUDPC                         | Suckers displaying<br>BBTD symptoms (%) | Mean mortality<br>) (%)        | Longevity<br>(months)         | Average bunches<br>in uninfected plant<br>per variety |
|-------------|------------------------------------|----------------------------------|-------------------------------------|-------------------------------|---|--------------------------------|-------------------------------|---|
| Asung Mbele | e 6                                | 66.67 ± 33.33 abc                | $40191.69 \pm 14970.24 \ ^{bcd}$    | $454.13 \pm 227.08$ abo       | $0.00 \pm 0.00$ $^a$                    | $53.33 \pm 29.06$ abo          | $14.38 \pm 1.13$ bc           | $4.00\pm2.00^{\rm \ bc}$                              |
| Essong      | 3                                  | $60.00 \pm 11.55$ abc            | 44621.07 ± 14101.28 <sup>abcd</sup> | $424.03 \pm 52.96$ abc        | 16.67 ± 16.67 <sup>a</sup>              | $33.33 \pm 6.67$ abc           | $11.00 \pm 1.61$ bc           | $5.33 \pm 0.88$ bc                                    |
| Big Essong  | 3                                  | $46.67 \pm 24.04 \ ^{abc}$       | 78552.85 ± 16192.75 <sup>a</sup>    | 280.47 ± 154.18 bc            | $0.00 \pm 0.00^{a}$                     | 26.67 ± 13.33 <sup>bc</sup>    | $8.75 \pm 0.75$ <sup>c</sup>  | $4.67 \pm 0.33$ <sup>bc</sup>                         |
| Batard      | 5                                  | $45.00 \pm 16.07$ <sup>abc</sup> | 64305.41 ± 19278.57 <sup>ab</sup>   | 320.73 ± 61.57 <sup>bc</sup>  | $50.00 \pm 50.00^{a}$                   | $45.00 \pm 16.07$ abc          | 14.67 ± 1.01 bc               | $5.33 \pm 0.67$ bc                                    |
| PITA 14     | 6                                  | $60.00 \pm 11.55$ <sup>abc</sup> | 50435.23 ± 9728.03 <sup>abc</sup>   | 376.77 ± 34.70 <sup>abc</sup> | 29.17 ± 15.02 <sup>a</sup>              | 33.33 ± 6.67 <sup>abc</sup>    | 15 ± 1.53 <sup>bc</sup>       | $6.00 \pm 0.00$ <sup>bc</sup>                         |
| PITA 23     | 4                                  | 91.67 ± 8.33 <sup>ab</sup>       | 11643.19 ± 3617.27 <sup>cd</sup>    | 785.83 ± 92.43 <sup>ab</sup>  | $50.00 \pm 50.00^{a}$                   | 78.33 ± 11.67 <sup>ab</sup>    | 19.74 ± 1.75 <sup>b</sup>     | $1.67 \pm 0.88$ <sup>c</sup>                          |
| PITA 27     | 3                                  | $66.67 \pm 24.04$ <sup>abc</sup> | 36679.62 ± 2100.74 bcd              | 460.77 ± 134.40 abc           | 33.33 ± 33.33 <sup>a</sup>              | $40.00 \pm 11.55$ abc          | 17.28 ± 2.86 bc               | $3.67 \pm 0.33$ <sup>bc</sup>                         |
| FHIA 21     | 5                                  | 53.33 ± 13.33 <sup>abc</sup>     | 21989.86 ± 3644.44 <sup>cd</sup>    | 296.03 ± 110.43 bc            | $0.00 \pm 0.00^{a}$                     | $26.67 \pm 6.67$ <sup>bc</sup> | 16.25 ± 1.75 bc               | $2.67 \pm 0.88$ bc                                    |
| FHIA 25     | 6                                  | 66.67 ± 13.33 <sup>abc</sup>     | 10002.07 ± 4988.27 <sup>cd</sup>    | 456.03 ± 106.31 abc           | 25.76 ± 14.45 <sup>a</sup>              | $26.67 \pm 6.67$ <sup>bc</sup> | $27.83 \pm 2.24$ <sup>a</sup> | 5.33 ± 1.33 <sup>bc</sup>                             |
| BITA 3      | 4                                  | $60.00 \pm 11.55$ <sup>abc</sup> | 12576.31 ± 4306.06 <sup>cd</sup>    | 433.37 ± 107.72 abc           | 14.29 ± 14.29 <sup>a</sup>              | 33.33 ± 6.67 <sup>abc</sup>    | $16.00 \pm 2.08$ bc           | $4.67 \pm 1.45$ <sup>bc</sup>                         |
| BITA 8      | 3                                  | $40.00 \pm 11.55$ <sup>abc</sup> | 15236.12 ± 6465.35 <sup>cd</sup>    | 263.8 ± 128.81 <sup>bc</sup>  | $0.00 \pm 0.00^{a}$                     | 33.33 ± 6.67 <sup>abc</sup>    | $9.00 \pm 2.08$ <sup>c</sup>  | $4.67 \pm 1.76$ <sup>bc</sup>                         |
| Fougamou    | 23                                 | 13.33 ± 6.67 <sup>bc</sup>       | 10723.57 ± 6068.54 <sup>cd</sup>    | 44.60 ± 26.66 <sup>c</sup>    | 21.43 ± 12.37 <sup>a</sup>              | $0.00 \pm 0.00$ <sup>c</sup>   | Standing                      | 12.33 ± 1.45 <sup>a</sup>                             |
| Grande Nain | 6                                  | 77.78 ± 11.11 <sup>abc</sup>     | 5269.39 ± 1670.39 <sup>cd</sup>     | 509.33 ± 94.65 <sup>abc</sup> | $100.00 \pm 0.00^{a}$                   | 61.11 ± 5.56 <sup>ab</sup>     | $8.83 \pm 1.92$ <sup>c</sup>  | 1.67 ± 0.33 <sup>c</sup>                              |
| Gros Michel | 11                                 | 8.33 ± 8.33 <sup>c</sup>         | 7973.08 ± 1040.14 <sup>cd</sup>     | 64.54 ± 64.54 <sup>c</sup>    | $0.00 \pm 0.00$ <sup>a</sup>            | $0.00 \pm 0.00$ <sup>c</sup>   | Standing                      | $7.33 \pm 0.67$ <sup>b</sup>                          |
| Williams    | 2                                  | 100.00 $\pm$ 0.00 $^{a}$         | 1839.22 $\pm$ 608.41 $^{\rm d}$     | 856.13 $\pm$ 47.97 $^{\rm a}$ | 100.00 $\pm$ 00.00 $^{\rm a}$           | 86.67 $\pm$ 6.67 $^{\rm a}$    | $12.5 \pm 2.04$ bc            | $1.00 \pm 0.58$ $^{\rm c}$                            |

Data are means $\pm$  standard errors. Means followed by the same letter in each column are not significantly different from each other according to Student Newman Keuls at p < 0.05. BBTD incidence was calculated as follow: (number of diseased plants of variety x)/(total number of plants of variety)x100. Percentage of suckers displaying BBTD symptoms was calculated as the ratio of suckers displaying BBTD symptoms. Mean mortality was calculated as the ratio of plants which collapsed from BBTD infection. Average bunches was calculated as the number of bunches produced per genotypes. PITA 24 and Batard were overlapping; Batard was planted in Abang Minko'o while PITA 24 was planted in Kou'ou-si.

on Fougamou while greatest (856.13) was observed on Williams (Table 2). In Kou'ou-si significant differences were observed among genotypes ( $F_{14,30} = 4.19$ , P = 0.0005); AUDPC varied from 0.00 on Asung Mbele, Big Essong and Grande Nain to 422.63 on PITA 23 (Table 3).

#### 3.4. Mortality and longevity

Mortality of BBTD diseased plants varied significantly among varieties in Abang Minko'o ( $F_{14, 30} = 4.63$ , p = 0.0002); 78.33% of Williams and 86.67% of PITA 23 collapsed while none of infected

Fougamou died (Table 2). In Kou'ou-si, mortality showed significant differences ( $F_{14, 30} = 4.71$ , p = 0.0002); 40.00% of BITA 8 collapsed while none of infected Essong, or PITA 27 died (Table 3).

Longevity was calculated as the time between symptom apparition and the death of the plant; significant differences were observed among genotypes ( $F_{12, 23} = 7.52$ , P < 0.0001) in Abang Minko'o (Table 2). The highest longevity was observed on FHIA 25 (27.83 months) while the shortest was observed on Big Essong (8.75 months) and Grande Nain (8.83 months). In Kou'ou-si, no significant difference was observed among genotypes regarding longevity of BBTD diseased plants ( $F_{7,9} = 0.84$ , P = 0.58) (Table 3).

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First BBTD symptoms, BBTD incidence, cumulative aphid-days, AUDPC, suckers with BBTD symptoms, mortality, longevity, bunches in Kou'ou-si at 37 months after planting.

| Genotype    | First BBTD<br>symptoms<br>(Months) | BBTD incidence (%)               | Cumulative aphid-days               | AUDPC                           | Suckers displaying<br>BBTD symptoms<br>(%) | Mean mortality<br>(%)           | Longevity<br>(months)         | Average bunches<br>in uninfected<br>plant per variety |
|-------------|------------------------------------|----------------------------------|-------------------------------------|---------------------------------|--|---------------------------------|-------------------------------|---|
| Asung Mbele | Asymptomatic                       | $0.00 \pm 0.00$ <sup>c</sup>     | 26064.58 ± 10781.51 <sup>a</sup>    | $0.00 \pm 0.00$ <sup>c</sup>    | $0.00 \pm 0.00$ <sup>a</sup>               | Asymptomatic                    | Asymptomatic                  | $4.67 \pm 0.33$ <sup>a</sup>                          |
| Essong      | 33                                 | $6.67 \pm 6.67$ <sup>bc</sup>    | 25721.43 ± 7320.30 <sup>a</sup>     | 10.93 ± 10.93 <sup>c</sup>      | $0.00 \pm 0.00$ <sup>a</sup>               | $0.00 \pm 0.00$ <sup>b</sup>    | Standing                      | $7.67 \pm 1.45^{a}$                                   |
| Big Essong  | Asymptomatic                       | $0.00 \pm 0.00$ <sup>c</sup>     | 22332.59 ± 4310.93 <sup>a</sup>     | $0.00 \pm 0.00$ <sup>c</sup>    | $0.00 \pm 0.00$ <sup>a</sup>               | Asymptomatic                    | Asymptomatic                  | $5.00 \pm 0.00^{a}$                                   |
| PITA 24     | 6                                  | 13.33 ± 6.67 <sup>abc</sup>      | 4329.259 ± 1587.25 <sup>a</sup>     | 127.43 ± 63.83 <sup>abc</sup>   | $0.00$ $\pm$ 0.00 $^{\rm a}$               | 13.33 ± 6.67 <sup>ab</sup>      | $19.00 \pm 10.00^{a}$         | $6.67 \pm 1.46$ <sup>a</sup>                          |
| PITA 14     | 10                                 | 20.00 ± 11.55 <sup>abc</sup>     | $26362.58 \pm 6649.98$ <sup>a</sup> | 158.33 ± 94.32 <sup>abc</sup>   | $0.00$ $\pm$ 0.00 $^{\rm a}$               | $20.00 \pm 11.56$ <sup>ab</sup> | $12.75 \pm 1.25$ <sup>a</sup> | $6.67 \pm 0.88$ $^{a}$                                |
| PITA 23     | 7                                  | $60.00 \pm 11.55$ <sup>a</sup>   | 10028.31 ± 1819.56 <sup>a</sup>     | $422.63 \pm 88.79$ <sup>a</sup> | $20.83 \pm 11.02$ <sup>a</sup>             | $33.33 \pm 6.67$ <sup>ab</sup>  | $17.17 \pm 4.35$ <sup>a</sup> | $5.00 \pm 0.58$ $^{a}$                                |
| PITA 27     | 10                                 | $20.00 \pm 20.00$ <sup>abc</sup> | $16765.59 \pm 5108.03$ <sup>a</sup> | 118.8 ± 118.8 <sup>abc</sup>    | $0.00$ $\pm$ 0.00 $^{\rm a}$               | $0.00 \pm 0.00$ <sup>b</sup>    | 17 <sup>a</sup>               | $8.00 \pm 2.00$ $^{a}$                                |
| FHIA 21     | 5                                  | 26.67 ± 13.33 <sup>abc</sup>     | 29420.21 $\pm$ 16592.31 $^{\rm a}$  | 208.53 ± 109.85 abc             | $0.00$ $\pm$ 0.00 $^{a}$                   | 26.6713.33 <sup>ab</sup>        | 10.5 $\pm$ 1.50 $^{\rm a}$    | $6.33 \pm 0.88$ <sup>a</sup>                          |
| FHIA 25     | 9                                  | $20.00 \pm 0.00^{\text{ abc}}$   | 4397.08 ± 902.75 <sup>a</sup>       | 102.53 ± 30.04 <sup>abc</sup>   | $20.00 \pm 20.00$ <sup>a</sup>             | $0.00 \pm 0.00$ <sup>b</sup>    | Standing                      | $6.00 \pm 1.00^{a}$                                   |
| BITA 3      | 11                                 | 26.67 ± 13.33 <sup>abc</sup>     | 10319.22 ± 2377.39 <sup>a</sup>     | $102.2 \pm 60.69^{\text{ abc}}$ | $0.00 \pm 0.00$ <sup>a</sup>               | $6.67 \pm 6.67$ <sup>b</sup>    | Standing                      | 6.67 ± 1.33 <sup>a</sup>                              |
| BITA 8      | 6                                  | 53.33 ± 6.67 <sup>ab</sup>       | $11442.38 \pm 5416.66$ <sup>a</sup> | 355.17 ± 36.73 <sup>ab</sup>    | $0.00$ $\pm$ 0.00 $^{\rm a}$               | 40.00 $\pm$ 0.00 $^{a}$         | $9.75 \pm 1.25$ <sup>a</sup>  | 5.33 ± 1.15 <sup>a</sup>                              |
| Fougamou    | 19                                 | $6.67 \pm 6.67$ <sup>bc</sup>    | 6698.593 ± 993.65 <sup>a</sup>      | 36.47 ± 36.47 bc                | $0.00$ $\pm$ 0.00 $^{\rm a}$               | $6.67 \pm 6.67$ <sup>b</sup>    | Standing                      | $9.00 \pm 2.08$ $^{a}$                                |
| Grande Nain | 9                                  | 31.11 ± 17.36 <sup>abc</sup>     | $2667.34 \pm 655.65$ <sup>a</sup>   | 195.61 ± 98.16 <sup>abc</sup>   |  | $11.11 \pm 11.11$ <sup>ab</sup> | 8.75 $\pm$ 0.25 $^{\rm a}$    | $3.00 \pm 1.53$ <sup>a</sup>                          |
| Gros Michel | Asymptomatic                       | $0.00 \pm 0.00$ <sup>c</sup>     | $8498.194 \pm 4899.82$ <sup>a</sup> | $0.00 \pm 0.00$ <sup>c</sup>    | $0.00 \pm 0.00$ <sup>a</sup>               | Asymptomatic                    | Asymptomatic                  | $8.00 \pm 1.53^{a}$                                   |
| Williams    | 4                                  | $40.00 \pm 0.00$ <sup>abc</sup>  | $3916.435 \pm 487.55$ <sup>a</sup>  | 331.67 ± 50.30 <sup>abc</sup>   | $16.67 \pm 16.67$ <sup>a</sup>             | $33.33 \pm 6.67$ <sup>ab</sup>  | $8.67 \pm 3.66$ <sup>a</sup>  | $5.33 \pm 0.67$ <sup>a</sup>                          |

Data are means $\pm$  standard errors. Means followed by the same letter in each column are not significantly different from each other according to Student Newman Keuls at p < 0.05. BBTD incidence was calculated as follow: (number of diseased plants of variety x)/(total number of plants of variety)x100. Percentage of suckers displaying BBTD symptoms was calculated as the ratio of suckers displaying BBTD symptoms. Mean mortality was calculated as the ratio of plants which collapsed from BBTD infection. Average bunches was calculated as the number of bunches produced per genotypes. PITA 24 and Batard were overlapping; Batard was planted in Abang Minko'o while PITA 24 was planted in Kou'ou-si.

However, PITA 24 survived 19.00 months with BBTD while Grande Nain survived only 8.75 months with BBTD.

## 3.5. Percentage of suckers displaying BBTD symptoms and bunch production

The percentage of suckers displaying BBTD symptoms did not differ significantly among genotypes either in Abang Minko'o ( $F_{14}$ ,  $_{22} = 4.71$ , p = 0.09) (Table 2) or Kou'ou-si ( $F_{13}$ ,  $_{23} = 0.93$ , p = 0.54) (Table 3). In Abang Minko'o, average of healthy bunches varied significantly among genotypes, ( $F_{14}$ ,  $_{30} = 66.67$ , p < 0.0001) (Table 2); Williams produced 1.00 bunch while Fougamou produced 12.33 bunches. In Kou'ou-si, no significant difference was observed among genotypes ( $F_{14}$ ,  $_{30} = 1.72$ , p = 0.10); however average of healthy bunches ranged from 3.00 on Grande Nain to 9.00 on Fougamou (Table 3).

# 3.6. Correlation between aphid abundance, BBTD occurrence, cumulative aphid abundance and cumulative BBTD incidence

In general in Abang Minko'o, the number of new BBTD infections was positively and significantly correlated with aphid abundance on PITA 23 (R = 0.44, p = 0.02) and Williams (R = 0.48, p = 0.01) while in Kou'ou-si, the number of new BBTD infections was positively correlated with aphid abundance for PITA 14 (R = 0.52, p = 0.005) and PITA 23 (R = 0.40, p = 0.04) (Table 4). Others genotypes did not show any significant correlation between new infections and aphid abundance. However, in both fields, cumulative BBTD incidence were highly and positively correlated with cumulative aphid-days in all the genotypes, except on Essong, in Kou'ou-si where this correlation was marginally not significant (R = 0.34, p = 0.07).

#### 3.7. Virus incidence

Virus incidence at 37 months after planting is presented in Table 5. In Abang Minko'o, six varieties (Asung Mbele, Essong, PITA 14, FHIA 25, Fougamou, Gros Michel) were infected and symptomless while the others genotypes (Batard, PITA 23, BITA 3, BITA 8, FHIA 21, PITA 27, Big Essong) were virus free. In Kou'ou-si, only Grande Nain was BBTV infected while other genotypes were BBTV free.

#### 4. Discussion

Aphid abundance varied with genotypes in both fields, plantains (locals and hybrids) were the most suitable host for *P. nigronervosa* followed by cooking bananas then dessert bananas. These results are similar to those reported by Hanna and Kumar (2009) who found during a field survey that large colonies of banana aphid were found on plantains. Study of population dynamics of banana aphid in a field experiment showed that high magnitude of aphid population was observed on plantains (Ngatat, unpublished data). This is similar to previous studies conducted on plantlets in screen house which showed that aphid growth is faster on plantains than bananas (Ngatat, 2010). But these results are contrasting with Jose (1981) who reported that *P. nigronervosa* is not normally found on plantains, suggesting that aphids may not prefer plantains.

In Abang Minko'o, regarding to the curves of disease progress, three different reactions were sorted out from this study; the highly susceptible to BBTD (Williams, PITA 23), the moderately susceptible include all the AAB plantains and the least susceptible (Fougamou and Gros Michel). In Kou'ou-si, PITA 23 was the most susceptible variety while Asung Mbele, Big Essong and Gros Michel were virus free. The difference in BBTD spread and incidence between the two sites could be explained by the distance to the nearest banana plantation with BBTD infection (about 10 m in Abang Minko'o and 100 m in Kou'ou-si); indicating that source of infection was also from outside field. Besides, survey was done around field to estimate the prevalence of BBTD in each area. BBTD prevalence in Abang Minko'o was 37.27% whereas in Kou'ou-si, BBTD prevalence was 1.76% which would mean that inoculums level was lower in Kou'ou-si than Abang Minko'o. Our results indicated that in Abang Minko'o, where the inoculum level is high, all the genotypes were infected but in Kou'ou-si where the inoculum level is low, some genotypes (Asung Mbele, Big Essong and Gros Michel) escaped the infection. This is confirmed by molecular analysis which revealed that amongst all the samples collected at 37 months after planting from non-symptomatic plants in Kou'ou-si and tested for BBTV, none was infected excepted Grande Nain. These results are convergent with Magee (1948) who found that the partial resistance of some genotypes to BBTV is owing to their ability to escape infection in case of low inoculums level. In the same vein, Waterhouse (1987) underlined the fact that, under high aphid population pressures; all commercial cultivars of bananas were

| Table 4  |                  |                  |               |                   |                  |             |         |
|--|------------------|------------------|---------------|-------------------|------------------|-------------|---------|
| Correlation coefficient between aphid abundance. | BBTD occurrence. | cumulative aphid | davs and BBTD | ) incidence in Al | bang and Kou'ou- | si after 37 | months. |

|              | Abang Minko'o  |      |  |          | Kou'ou-si   |      |  |          |  |
|--------------|--|------|--|----------|---|------|--|----------|--|
|              | Correlation between aphid<br>abundance and BBTD occurrence |      | Correlation between cumulative aphid-days and BBTD incidence |          | Correlation between aphid abundance and BBTD occurrence |      | Correlation between cumulative aphid-days and BBTD incidence |          |  |
| variety      | R  | р    | R  | р        | R   | р    | R  | р        |  |
| Asung Mbele  | 0.17   | 0.37 | 0.95   | < 0.0001 | NA  | NA   | NA   | NA       |  |
| Essong       | -0.19  | 0.34 | 0.95   | < 0.0001 | 0.02  | 0.91 | 0.34   | 0.07     |  |
| Big Essong   | 0.14   | 0.47 | 0.85   | < 0.0001 | NA  | NA   | NA   | NA       |  |
| Batard       | -0.07  | 0.74 | 0.73   | < 0.0001 | Np  | Np   | Np   | Np       |  |
| PITA 24      | Np   | Np   | Np   | Np       | 0.02  | 0.93 | 0.62   | 0        |  |
| PITA 14      | 0.04   | 0.84 | 0.95   | < 0.0001 | 0.51  | 0.01 | 0.91   | < 0.0001 |  |
| PITA 23      | 0.44   | 0.02 | 0.93   | < 0.0001 | 0.4   | 0.04 | 0.98   | < 0.0001 |  |
| PITA 27      | 0.07   | 0.72 | 0.93   | < 0.0001 | -0.05   | 0.79 | 0.92   | < 0.0001 |  |
| FHIA 21      | -0.15  | 0.45 | 0.92   | < 0.0001 | 0.11  | 0.57 | 0.94   | < 0.0001 |  |
| FHIA 25      | 0.15   | 0.45 | 0.92   | < 0.0001 | -0.21   | 0.28 | 0.83   | < 0.0001 |  |
| BITA 8       | 0.23   | 0.23 | 0.91   | < 0.0001 | 0.09  | 0.65 | 0.77   | < 0.0001 |  |
| BITA 8       | -0.03  | 0.88 | 0.93   | < 0.0001 | -0.15   | 0.44 | 0.84   | < 0.0001 |  |
| Fougamou     | 0.01   | 0.95 | 0.85   | < 0.0001 | -0.17   | 0.39 | 0.72   | < 0.0001 |  |
| Grande Naine | 0.15   | 0.45 | 0.93   | < 0.0001 | 0.23  | 0.24 | 0.88   | < 0.0001 |  |
| Gros Michel  | -0.04  | 0.83 | 0.73   | < 0.0001 | NA  | NA   | NA   | NA       |  |
| William      | 0.48   | 0.01 | 0.96   | <0.0001  | 0.03  | 0.88 | 0.88   | <0.0001  |  |

R: Pearson coefficient of correlation, P probability. NP = not planted; PITA 24 and Batard were overlapping; Batard was planted in Abang Minko'o while PITA 24 was planted in Kou'ou-si. NA: not applicable; Asung Mbele, Big Essong and Gros Michel were symptomless after 37 months.

 Table 5

 Virus incidence at 37 months after planting in Abang Minko'o and Kou'ou-si.

|             | Abang Minko'o |               |               |                 | Kou'ou-si    |               |               |                 |  |
|-------------|---------------|---------------|---------------|-----------------|--------------|---------------|---------------|-----------------|--|
|             | Total sample  | BBTV negative | BBTV positive | % BBTV positive | Total sample | BBTV negative | BBTV positive | % BBTV positive |  |
| Asung Mbele | 3             | 2             | 1             | 33.33           | 3            | 3             | 0             | 0               |  |
| Essong      | 4             | 3             | 1             | 25              | 3            | 3             | 0             | 0               |  |
| Big Essong  | 5             | 5             | 0             | 0               | 2            | 2             | 0             | 0               |  |
| Batard      | 2             | 2             | 0             | 0               | NP           | NP            | NP            | NP              |  |
| PITA 24     | NP            | NP            | NP            | NP              | 4            | 4             | 0             | 0               |  |
| PITA 14     | 5             | 4             | 1             | 20              | 2            | 2             | 0             | 0               |  |
| PITA 23     | 1             | 0             | 0             | 0               | 2            | 2             | 0             | 0               |  |
| PITA 27     | 3             | 3             | 0             | 0               | 4            | 4             | 0             | 0               |  |
| FHIA 21     | 3             | 3             | 0             | 0               | 4            | 4             | 0             | 0               |  |
| FHIA 25     | 5             | 4             | 1             | 20              | 6            | 6             | 0             | 0               |  |
| BITA 3      | 4             | 4             | 0             | 0               | 4            | 4             | 0             | 0               |  |
| BITA 8      | 3             | 3             | 0             | 0               | 3            | 3             | 0             | 0               |  |
| Fougamou    | 7             | 6             | 1             | 14.29           | 5            | 5             | 0             | 0               |  |
| Grande Nain | /             | 1             | /             | /               | 1            | 0             | 1             | 100             |  |
| Gros Michel | 6             | 5             | 1             | 16.67           | 7            | 7             | 0             | 0               |  |
| Williams    | 1             | 1             | 1             | 1               | 3            | 3             | 0             | 0               |  |

PITA 24 and Batard were overlapping; Batard was planted in Abang Minko'o while PITA 24 was planted in Kou'ou-si.

susceptible to bunchy top infection, but some exhibit resistance when aphid populations are lower. Consequently, this author suggested lowering the aphid populations as a way to allow the expression of some degree of BBTD resistance in some genotypes.

In Abang Minko'o, BBTD symptoms appeared on Gros Michel at 23 months after planting while in Kou'ou-si, Gros Michel did not exhibit any symptom; which is an indication of some degree of resistance or tolerance to BBTD. Magee (1948) reported the tolerance of Gros Michel to BBTV and the susceptibility of Cavendish cultivars, corroborating with our observations in both fields. Generally, cultivars in the AA and AAA genomic groups are known to be highly susceptible with the exception of Gros Michel, whereas cultivars containing B genome are regarded as less susceptible (Espino et al., 1993; Hooks et al., 2009; Niyongere et al., 2011) or tolerant (Ariyaratne and Liyanage, 2002). Likewise, our data also suggest that overall the B genome in local plantains, cooking bananas, improved plantains (except PITA 23-AAB) could explain their lower BBTD incidence, especially in fields with relatively low inoculums level. Additionally, based on our observations, symptom development on local plantains Essong and Big Essong was very

slow (3-4 months from streaks to bunchy appearance) compared to genotypes with only A genome. Otherwise, in the cultivars containing A and B genome, Espino et al. (1993) reported 100% infection on 2 genotypes. The late infection of Fougamou-ABB in both fields (at 19 and 23 months after planting respectively in Kou'ou-si and in Abang Minko'o), confirms the observations of Gondwe et al. (2007) in Mozambique, who observed that in BBTV hot spot, AAA and AAB were first wiped out before the ABBs as Bluggoe and Fougamou. Even though there is difference in disease susceptibility amongst genotypes, symptom expression in all genotypes was similar. In Abang Minko'o, infected Grande Nain and BITA 8 collapsed after surviving for less than 12 months while PITA 23 and FHIA 25 survived respectively for 19.74 and 27 months with BBTD. In Kou'ou-si, Grande Nain, Williams and BITA 8 collapsed with less than 10 months survival after symptom expression. To be able to acquire the virus, banana aphid should feed for at least 4 h on the plant (Hu et al., 1996) and at least 15 min to transmit BBTV. Magee (1948) showed that the resistance of Gros Michel can be overcome by increasing the number of aphid vector. This implies that there is a positive correlation between infection and aphid

number. In our results, positive and significant correlation between BBTD occurrence and aphid abundance was only found on PITA 23 and Williams in Abang Minko'o and PITA 14 and PITA 23 in Kou'ousi. Otherwise, that correlation for other genotypes in both fields was non-significant. In both fields, cumulative BBTD incidence was highly and positively correlated with cumulative aphid abundance in each genotype excepting Essong in Kou'ou-si where the correlation was not significant; this could be explained by the fact that, aphids were abundant on this genotype in all sampling dates but the first infected plant was recorded at 33 months after planting. Interestingly, BBTD expression on the same genotype planted in different area reveals that BBTD expression is not only related to the varietal trait but also to the epidemiological situation of the area. This is the first attempt of BBTD research in Cameroon.

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