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Occurrence and Distribution of Banana Bunchy Top Disease in the Great Lakes Region of Africa

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ABSTRACT

Banana bunchy top disease (BBTD) was first reported in 1958 in sub-Saharan Africa at the INEAC Yangambi research station in the Democratic Republic of Congo (DR Congo). Cases were reported in 1987 in the Rusizi valley encompassing the borders of Burundi, DR Congo and Rwanda. Since then, no study about BBTD had been carried out in this region. A survey was conducted from September to October, 2008 in three provinces (Bujumbura rural, Cibitoke and Bururi) of Burundi, two districts (Kamanyola and Nyangezi) in South Kivu, DR Congo and the Rusizi district in the Western province of Rwanda. A total of 7,830 banana mats, 30 randomly selected per plot, were assessed on 261 farms. A structured questionnaire was used to assess, cultivar diversity, BBTD incidence and severity, presence and occurrence of the aphid vector (*Pentalonia nigronervosa* Coquerel) and farmers' awareness about BBTD management. Leaf samples were randomly collected on symptomatic plants for further PCR analysis to confirm the disease. PCR results of samples collected in the three countries confirmed the presence of BBTV. Similar banana varieties are grown across the three countries, indicating the cross-border movement of planting materials which may have influenced disease spread over the past decennia. The regional average of BBTD incidence and aphid occurrence was 25% and 46%, respectively. However, no significant relationship between aphid occurrence and BBTD incidence (R=0.3, P= 0.623) was observed. Among the interviewed farmers, 90% were able to recognize advanced BBTD symptoms; while 95% of farmers were unaware of disease management options and stated that no locally cultivar is resistant to the disease. This pinpoints the need for farmers' awareness raising and that tolerant cultivars should be part of control option packages.

Keywords: *Banana bunchy top virus* (BBTV), *Musa* spp., *Pentalonia nigronervosa*, survey
Abbreviations: BBTD, banana bunchy top disease; **BBTV**, banana bunchy top virus; **DR Congo**, Democratic Republic of Congo; **GLRA**, Great Lakes Region of Africa; **PCR**, polymerase chain reaction; **masl**, meters above sea level

INTRODUCTION

Banana (*Musa* spp.) is a staple food crop for about 70 millions of people in Africa including Burundi, Democratic Republic of Congo (DR Congo) and Rwanda (Frison and Sharrock 1998; INIBAP 2000). It was ranked as number one crop in terms of earning in Burundi and Rwanda contributing the estimated annual incomes of 263.6 million $ and 576.7 million $ in these two countries respectively. In DR Congo, banana was ranked as number two crop after cassava in terms of importance, contributing about 267.7 million $ in domestic earnings (FAOSTAT, 2009). Unfortunately, banana yields are low due to constraints such as the banana bunchy top disease (BBTD) which is one of the most devastating viral diseases in many banana producing regions of Africa, Asia, and the South Pacific (Dale 1987; Su *et al.* 2003). The occurrence of BBTD represents a serious threat to food security in the regions where banana is one of the main staple crops for small-scale growers.

BBTD is caused by the *Banana bunchy top virus* (BBTV), a complex circular single-stranded DNA virus with multiple genomic components, belonging to the genus *Babuvirus* in the *Nanoviridae* family (Karan *et al*. 1994; Dale *et al.* 2000; Wanitchakorn *et al.* 2000; Horser *et al.* 2001; Su *et al*. 2003; Vishnoi *et al*. 2009). BBTV genetic diversity has been revealed, using pathological and molecular patterns, with the existence of two distinct groups of BBTV isolates (Karan *et al.* 1994; Wanitchakorn *et al.* 2000; Su *et al.* 2003). These two groups were designated as Asian and South Pacific groups. The Asian group comprises of isolates from Asian region such as the Philippines, Taiwan and Vietnam, while the South Pacific group contains isolates from different regions of the South Pacific region of Australia and Fiji as well as isolates from regions located outside this South Pacific region such as Burundi, Egypt and India (Karan *et al.* 1994; Wanitchakorn *et al.* 2000; Horser *et al.* 2001).

The disease was reported for the first time in Fiji Islands in 1889 (Magee 1927) and has been recorded in 33 countries of Africa, Asia, Australia and the South Pacific Islands but does not occur in Central and South America (Diekmann and Putter 1996; Ferreira *et al.* 1997; Amin *et al.* 2008; Kumar and Hanna 2008; Kumar *et al.* 2011). In Africa, BBTD was first reported in Egypt in 1901 and subsequently in 1958 in sub-Saharan Africa at the INEAC Yangambi agricultural research station in central DR Congo (Wardlaw 1961; Fouré and Manser 1982). It was also reported in 1964 in Eritrea (Saverio 1964). Cases of the disease were reported in 1987 in the Rusizi valley encompassing parts of Burundi and Rwanda (Sebasigari and Stover 1987). In 1982, BBTD was reported in Gabon, Congo-Brazaville and Equatorial Guinea (Fouré and Manser 1982). In the early 1990s, it was described in Malawi and Angola (Kumar and Hanna 2008). Currently, BBTD has been reported in 13 countries in Africa including Cameroon, Central African Republic and Zambia (Saverio 1964; IITA 2009).

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BBTD spread with exchange of infected propagules from place to place and through banana aphid, *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae) from plant to plant (Magee 1927; Ferreira *et al.* 1997; Robson *et al.* 2006). *P. nigronervosa* was also reported to occur on members of Zingiberaceae and Araceae, but studies based on morphological and morphometric analysis confirmed aphids on these non-*Musa* hosts as a separate species, *Pentalonia caladii* van der Goot (Foottit *et al*. 2010; Kumar *et al.* 2011). *P. nigronervosa* Coquerel has been, thereby, confirmed to have high host specificity to *Musa spp.* for propagation and BBTV transmission, and has been found in almost all the banana growing countries around the world (Dale 1987; Hu *et al*. 1996; Yasmin *et al.* 2001; Kumar *et al*. 2011).

The aphid acquires the virus after at least 4 h of feeding on an infected plant and retains BBTV throughout its adult life (i.e. 15-20 days) but does not transmit it to its progeny (Nelson, 2004). The winged aphids which often develop after 7 to 10 generations of wingless individuals are most likely responsible for the spread of the virus (Nelson 2004; Young and Wright 2005). These winged aphids can transmit the virus to a healthy banana plant by feeding on it for as little as 15 min to about 2 h (Dale 1987; Hu *et al*. 1996; Ferreira *et al*. 1997). On the other hand, long distance spread of the disease occurs through the exchange of planting materials obtained from BBTV-infected plantations (Dale 1987; Kumar *et al.* 2011). The virus is not transmitted mechanically and therefore the implements of the routine management of the plantation are not a potential source of contamination (Wardlaw 1961; Ferreira *et al.* 1997; Kumar *et al.* 2011).

The history of BBTD spread has mainly been attributed to the exchange of planting materials, and this has been the case with the spread of the disease in sub-Saharan Africa which include the Great Lakes Region (Kumar and hanna, 2008, Kumar et al. 2011). For instance, the presence of a BBTD susceptible cultivar 'Yangambi Km5' (AAA) as its name suggests, originates from Yangambi in DR Congo and spread across the GLRA.

In the field, characteristic BBTD symptoms are easily observable and different from other known banana virus. These symptoms include development of morse code streaking of variable length in the leaf veins, midribs and petioles; progressive dwarfing of leaves and development of marginal leaf chlorosis, upright and crowded leaves at the apex of the plant, hence the name bunchy top disease (Magee 1927; Fry 1982; Dale 1987; Ferreira *et al.* 1997). The plants infected by the BBTV at an early growth stage are unable to produce bunches whereas those infected at a later stage of growth produce small bunches often of poor quality (Dale 1987; Ariyatne and Liyanage 2002; Su *et al*. 2003).

In most regions, such as GLRA, where banana is produced for local consumption, research on BBTD has been limited. Indeed in most of African countries where there an occurance of BBTD there are little or no resources for virus diagnosis (Dale 1987), resulting in few works on BBTV-isolates prevailing in Africa, with the first publication on large, but not exostive BBTV isolates of sub-Saharan Africa, in 2011 by Kumar *et al.* (2011).

Despite viral disease presenting the major threat of banana production in GLRA, no specific in-depth investigations have been undertaken to generate sufficient information for the development of suitable disease management strategies. Therefore, this study was conducted in six localities of the GLRA, known to be BBTV-infected. The study aimed at assessing the incidence of banana bunchy top disease, its severity and the importance of its vector *P. nigronervosa*. Farmers' awareness on the disease, management options and *Musa* cultivars grown were also recorded.

MATERIALS AND METHODS

Study area

The study was conducted in three countries bordering the Rusizi valley namely Burundi, DR Congo and Rwanda. Altitudes at the surveyed localities in Burundi range from 1,297 to 2,096 masl in Bujumbura rural province, 906 to 1,330 masl in Cibitoke province, and from 769 to 934masl in Rumonge of Bururi province. The surveyed localities in the Eastern South-Kivu Province of DR Congo have an altitude ranging from 895 to 972 masl at Kamanyola district, and from 1,254 to 1,937 masl at Nyangezi district. The surveyed sites in Rwanda are located in the Rusizi district of the Western province with altitudes of between 964 and 1,652 masl.

Process

The survey for the current study was carried out from September to October 2008. A structured questionnaire was used to obtain information on the incidence and the severity of BBTD, first observation of BBTD symptoms and the occurrence of *P. nigronervosa*. Other types of information obtained and analysed for this study include the *s*ource of planting material, *Musa* cultivars grown, possible existence of resistant varieties, seasonal influences on the expression of BBTD symptoms and farmers' knowledge and perceptions on the disease and its management in the surveyed areas were also assessed. The number of banana plantations, ranging from 10 to 75 per locality were visited. The distance between two farms within the same village was at least 100 m, and the distance between one and another surveyed village was 5 km. In each plantation, 30 mats were randomly selected for data collection. Mats containing one or more plants with moderate to severe observable BBTD symptoms were considered as infected. Subsequently, the disease severity was assessed using a scale ranging from 0 to 5 based on BBTD characteristics symptoms where 0 is no BBTD symptoms; 1 is dark green streaks on the leaf veins; 2 is dark green streaks on leaf midribs and petioles; 3 is marginal leaves chlorosis, 4 is dwarfing of leaves; and 5 is "bunchy top" aspect of the plant showing upright, crowded, and brittle leaves. The presence of *P. nigronervosa* was also assessed on all 30 mats and the occurrence of *P. nigronervosa* was scored using a scale ranging from 0 to 5 where 0 is no aphid, 1 is a simple colony (no winged individuals), 2 is several simple colonies (no winged individuals), 3 is a large colony with one or more winged individuals, 4 is several colonies with winged individuals, and 5 is generalized colonies at the level of the leaves and the pseudostem of a banana plant with numerous winged individuals.

The geographical coordinates of each surveyed location were recorded using a Global Positioning System (GPS) receiver (Magellan® Sport Trak Pro 2003).

The molecular detection using PCR (polymerase chain reaction) was conducted on 110 banana leaf samples collected during the survey. The sampling strategy consisted of taking at least three samples on symptomatic leaves per village in the six localities across the three countries. Hence, 60 samples were collected in Burundi (21 in Bujumbura rural province, 21 in Cibitoke Province and 18 in Rumonge of Bururi province), 26 in DR Congo (6 in Kamanyola and 20 in Nyangezi districts) and 24 in the Rusizi district of Rwanda. Sampling was carried out using the PhytoPass system, with a single abrasive membrane used per BBTD symptomatic banana plant. The plant tissue fragments were recovered by putting the abrasive membrane into a Falcon tube containing 1 ml of extraction buffer with subsequent vortexing (Busogoro *et al.* 2009). For each sample, 500 µl of crude extract was recovered and stored at -20°C pending PCR analysis. PCR was performed using primer pair BBT1 (5′-CTCGTCATGTGCAAGGTTATGTCG-3′) and BBT2 (5′-GAAGTTCTCCAGCTATTCATCGCC-3′) designed to amplify a 349-bp fragment of the BBTV putative replicase gene (Thomson and Dietzgen 1995).

Data analysis

The data were analyzed using the Statistical Package for Social Scientists (SPSS) version 16.0. The relationship between BBTD and *P. nigronervosa* populations was determined by correlation

Table 1 *Musa* cultivars commonly grown in the six surveyed localities in Burundi, DR Congo and Rwanda.

Location	Country	Use of different cultivars		
		Brewing	**Cooking**	**Dessert**
Bujumbura rural province	Burundi	1.'Indarama'(AAA,EA) 2.'Kayinja'(ABB) 3. 'Inkira'(AAA,EA) 4. 'Igitsiri' (AAA, EA)	5. 'Igisahira' (AAA,EA) 6. 'Ibiganda' (AAA, EA)	7. 'Ikimaraya'(AAA) 8. 'Gros Michel'(AAA) 9. 'Ibigurube'(AAA)
Cibitoke province	Burundi	1. 'Yangambi Km5' (AAA) 2. 'Kayinja'(ABB) 3. 'Indarama'(AAA,EA)	4. 'Ibihanda'(AAA,EA) 5. 'Ibiganda'(AAA,EA)	6. 'Ikimaraya' (AAA, EA)
Rumonge in Bururi province	Burundi	1. 'Kayinja'(ABB) 2. 'Indarama' (AAA, EA) 3. 'Yangambi Km5' (AAA)	4. 'Igisahira' (AAA, EA)	5.'Ikimaramasenge' (AAB) 6.'Ibigurube'(AAA)
Kamanyola district	DR Congo	1. 'Yangambi Km5' (AAA) 2. 'Kayinja'(ABB)	3. 'Igisahira' (AAA, EA)	4. 'Kampala' (AAA, EA)
Nyangezi district	DR Congo	1. 'Yangambi Km5' (AAA) 2. 'Kayinja'(ABB) 3. 'Magizi' (AAA, EA)	4. 'Igisahira' (AAA, EA) 5.'Imizuzu' (AAB, plantain) 6. 'Rufufu' (AAA, EA) 7.'Naruvu' (AAA, EA)	8. 'Musheba'(AAB) 9.'Ikimaramasenge' (AAB) 10. 'Gros Michel'(AAA)
Rusizi district	Rwanda	1. 'Yangambi Km5' (AAA) 2. 'Kayinja'(ABB) 3.'Igitsiri' (AAA, EA)	4.'Igisahira' (AAA, EA) 5.'FHIA17'(AAAA) 6.'Barabesha' (AAA, EA)	7. 'Kampala' (AAA, EA) 8. 'Gros Michel' (AAA)

Table 2 BBTD incidence and occurrence of *P. nigronervosa* in six surveyed localities in Burundi, DR Congo and Rwanda (across 261 fields with 30 mats per surveyed field).

Location	Country	Number of mats assessed	BBTD incidence (%)	Occurrence of *P. nigronervosa* (%)
Bujumbura rural province	Burundi	1830	26	26
Cibitoke province	Burundi	2250	30	56
Rumonge in Bururi province	Burundi	1800	14	42
Kamanyola district	DR Congo	300	23	40
Nyangezi district	DR Congo	720	29	41
Rusizi district	Rwanda	930	28	64
Total/ Mean		7830	25	46

analysis using GenStat 12th Edn VSN Int. The disease incidence as well as aphid occurrence were expressed as percentage, calculated as the proportion of plants showing BBTD symptoms or containing aphids on the total number of plants assessed (Saghir *et al*. 2002).

RESULTS

Common *Musa* varieties in six localities surveyed

A total of 7830 banana mats were assessed in this study of which 73% were brewing varieties, 11% were cooking, 4% plantains and 12% consisted of dessert varieties. Farmers (72%) reported that the establishment of plantations dates back to more than a decade ago. Intercropping was practiced on 83% of surveryed farms.

The number of banana varieties per location varied from 6 to 10 with similar varieties being found in different localities surveyed across the three countries. The frequently reported varieties differently named in each locality were 'Igisubi'/'Kayinja'/'Pisang awak' (ABB, brewing), 'Yangambi Km5'/'Ibota' (AAA, brewing), 'Igisahira'/ 'Barabesha'/ 'Gisamunyu' (AAA-EA, cooking), 'Igitsiri'/ 'Intuntu' (AAA-EA, brewing), 'Indarama' (AAA-EA, brewing) and 'Imizuzu' (AAB, plantain). The 'Yangambi Km5' and 'Kayinja' varieties were the most reported across Rwanda, Burundi and DR Congo (**Table 1**).

BBTD incidence and occurrence of *P. nigronervosa* in the GLRA

Banana bunchy top disease was reported in all the six surveyed localities. BBTD incidences ranging from 23 to 30% were observed in five of the six localities. On the other hand, a relatively lower incidence of 14% was reported in Burundi at Rumonge. The occurrence of aphids in Bujumbura rural province, located at relatively higher altitude, was 26% whereas in the other surveyed localities it was more than 40% (**Table 2**).

BBTD severity was assessed on infected mats using a scale range of 1 to 5. The scores of 3 to 5, which are characterized by marginal leaves chlorosis to a bunchy top appearance, were more frequent than the scores of 1 or 2 which represent the initial symptoms manifested by dark green streaks. The average severity of BBTD which was 18% (i.e. 3-5) was higher than that of BBTV-infected plants at early stage which was 7.1% (i.e., 1-2) for the GLRA. The percentage of surveyed mats per location with higher disease severity (i.e. 3-5) was 16, 22, 9, 21.5, 21 and 24% in Bujumbura rural, Cibitoke, Rumonge, Kamanyola, Nyangezi and in the Rusizi district, respectively (**Table 3**).

The *P. nigronervosa* colonies were found on both symptomatic and asymptomatic mats. Apterous (i.e. wingless) *P. nigronervosa* in simple colonies (scoring 1 to 2) were most frequently observed (36%) while winged individuals (scoring 3 to 5) were observed on an average of 9% of the surveyed mats. The mats containing winged aphids (scoring 3 to 5), potential vectors transmitting BBTV from plant to plant, varied according to each location with 4, 8, 7, 16, 17 and 18% of mats surveyed in Bujumbura rural, Cibitoke, Rumonge, Kamanyola, Nyangezi, and in the Rusizi district, respectively (**Table 4**).

The aphids' typologies (i.e. scores ranging from 0 to 5) across the six localities were not significantly correlated ($R = 0.2$, $P = 0.16$) with BBTD severity (i.e. scores 0-5). Moreover, the occurrence of *P. nigronervosa* colonies and BBTD incidence were not significantly correlated ($R = 0.3$, $P = 0.623$) across the 3 countries.

Ninety five percent of farmers indicated that no BBTD-resistant *Musa* varieties were present in their plantations. Based on the data collected during the survey, BBTD incidences, ranging from 25 to 48%, were observed on 'Gros Michel' (AAA, dessert), 'Kamaramasenge' (AAB, dessert), 'Yangambi Km5' (AAA, brewing) and 'Indarama' (AAA-EA, brewing), while 13 to 17% were reported on 'Igitsiri' (AAA-EA, brewing), 'Kayinja' (ABB, brewing) and 'Igisahira' (AAA-EA, cooking) varieties. A relative low incidence of 9% was reported on 'Imizuzu' (AAB, plantain) cultivars.

Table 3 BBTD severity ranging from 0 to 5 scores (%) for the six surveyed localities.

Locations	Country	Scores ranging from 0 to 5					
		0	**1**	**2**	**3**	**4**	**5**
Bujumbura rural province	Burundi	74	7	3	7	5	4
Cibitoke province	Burundi	70	5	3	9	7	6
Rumonge in Bururi province	Burundi	86	3	2	3	4	2
Kamanyola district	DR Congo	76.5	2	0	7	7	7.5
Nyangezi district	DR Congo	71	5	2	5	8	9
Rusizi district	Rwanda	72	3	1	9	8	7
Average		74.8	4.5	2.6	6.8	6.0	5.2

Table 4 Typology of *P. nigronervosa* colonies (scores ranging from 0 to 5) in the six surveyed localities.

Locations	Country	Scores ranging from 0 to 5 (%)					
		0	**1**	**2**	**3**	**4**	**5**
Bujumbura rural province	Burundi	74	18	5	3	0.5	0.5
Cibitoke province	Burundi	44	36	12	6	1.5	0.5
Rumonge in Bururi province	Burundi	58	28	7	5.5	1	0.5
Kamanyola district	DR Congo	60	15	9	15	0.5	0.5
Nyangezi districts	DR Congo	59	16	8	15	1	1
Rusizi district	Rwanda	36	29	17	16	1	1
Average		55.1	26.8	9.1	7.5	0.9	0.5

Table 5 Occurrence of *P. nigronervosa* and BBTD incidence for the major banana varieties cultivated in the Great Lakes region of Africa.

Cultivars (genome)	Number of assessed mats	Occurrence of *P. nigronervosa* (%)	BBTD Incidence (%)
'Yangambi Km5' (AAA-brewing)	2168	61	36
'Kayinja' (ABB-brewing)	2045	41	16
'Igisahira' (AAA-EA-cooking)	1061	36	17
'Igitsiri' (AAA-EA-brewing)	815	34	13
'Indarama' (AAA-EA-brewing)	695	43	48
Dessert varieties:'Gros Michel' (AAA) and 'Kamaramasenge'(AAB)	832	33	25
'Imizuzu' (AAB-plantain)	107	59	9
Tissue culture derived plantlets (FHIA01(AAAB), FHIA17 (AAAA) and FHIA25 (AAB)	19	58	5
Total of surveyed mats/averages	7830	46	21

The BBTD incidence on banana varieties, which were established using tissue culture derived plants, was lowest (5%), although high aphid populations were reported on their mats.

The *P. nigronervosa* occurrence was superior to the regional average (46%) on 'Yangambi Km5' (AAA, brewing), 'Imizuzu' (AAB, plantain) and in the established varieties using tissue culture derived plants. About 40% of aphids occurrence were reported on 'Indarama' (AAA-EA, brewing) and 'Kayinja' (ABB, brewing), while about 35% were reported on the highland varieties 'Igisahira'(AAA-EA, cooking),'Igitsiri' (AAA-EA, brewing) and the dessert varieties (**Table 5**).

Farmers' knowledge and management strategies against BBTD

Most of the 261 interviewed farmers (90%) were able to recognize advanced BBTD symptoms in their fields, while the remaining 10% did not follow the disease symptoms. Of these farmers, 67% used a local names [e.g. 'Kagazi'= leaves appearance of BBTV-infected plant are compared to those of palm tree, 'Saturubwato'=means no necessary to keep brewing-material once banana plantations are BBTV-infected, 'Sindika'= means that a BBTV-infected plant become stunted] to identify the disease. All of the interviewed farmers reported that the disease is systemic and, overtime, it spreads to all plants within a mat. Seventy two percent of farmers reported that banana production ceases within a year after infection. Nine percent indicated that production could continue for up to two years after a mat gets infected, while five percent reported three years of post-infection production. A small proportion of the farmers (11%) was not aware of the effect of BBTV infection on banana production.

Among the interviewed farmers, 83% reported to have used their own suckers in the establishment of new plantations, 65% obtained suckers from nearby (<10 Km) plantations, while 31% obtained suckers far away (>10 Km) from their home villages. The use of *in vitro* derived plants was reported by 11% of the interviewed farmers. The tissue culture derived plants were mainly distributed by government institutions and non-governmental organizations.

Regarding the occurrence of the disease in their fields, 56% of the interviewees attributed the origin of the disease either to their own or to neighbouring fields, 39% attributed the source to remote (>10 km) villages, while 10% suspected that the contamination was coming from bordering countries.

In the new plantations established with suckers either from own or from neighbours' fields, four percent noticed the disease after about three weeks, 36% of the interviewees noticed BBTD symptoms after about three months, 25% identified the symptoms after more than one year whereas, 31% did not pay attention to the disease development after planting. Among the 11% of farmers who had established banana fields using tissue culture derived plantlets, two percent observed BBTD symptoms about three months after planting.

Forty eight percent of the farmers said that the disease becomes more severe during dry season, although the disease is omnipresent during the whole year.

In terms of BBTD management efforts, 5% of growers particularly in Rwanda reported having attended training on how to manage the disease. Seventy eight percent of farmers believed that selecting asymptomatic suckers would reduce disease incidence. An estimated 43% of the farmers were aware that the disease can be transmitted with infected planting material, while only 13% of the farmers reported having informed that the disease can be transmitted by insect vectors. Thirty six percent of farmers were not aware about disease transmission and believed that the disease can be spread through the soil, while eight percent attributed new infections to the use of contaminated garden tools.

Table 6 PCR results of samples collected using PhytoPass kits during the survey.

Countries	Number of locations	Number of plants sampled	Sample preservation (days)	Tested positive	Proportion (%)
Burundi	3	15	10	11	73
		45	37	6	13
DR Congo	2	26	14	10	39
Rwanda	1	5	12	3	60
		19	15	9	47
Total/average	6	110	18	39	46.4

Seventy seven percent of farmers reported to have been cutting single diseased plants at the pseudostem base without removing the whole mat, while only 15% stated to have been uprooting the entire mat when at least one plant showed typical BBTD symptoms. Lack of measures to quarantine infected from non infected areas was reported by 98% of farmers. In all of the surveyed localities, the use of chemicals to control *P. nigronervosa* was not reported by farmers.

BBTV status of collected samples

BBTV was confirmed in the samples collected on, BBTD symptomatic plants, in the three countries of the GLRA. The PCR results on 110 sampled PhytoPass kits varied with respect to the period between sampling and extraction of crud extract. The highest positives rates of 73% and 60% were reported on samples extracted 10 - 12 days after sampling, while the least PCR positives results were observed with 13% of samples extracted after 37 days of PhytoPass kits conservation prior to PCR tests (**Table 6**).

DISCUSSION

'Yangambi Km5' (AAA,,brewing) and 'Kayinja' (ABB, brewing) varieties were reported to have been widely grown across large parts of the Rusizi valley encompassing Burundi, Eastern DR Congo and Western province of Rwanda (**Table 1**). These two banana brewing varieties are easy to multiply and this could be the main reason for their preference by farmers in the Rusizi valley region (Nsabimana *et al*. 2008).

The disease incidence of more than 23% was observed in five surveyed localities. However, the lowest BBTD incidence of 14% was reported at Rumonge in Burundi (**Table 2**). This implies that Rumonge was later infected with the introduction of infected plants in around 2,000 unlike in the other five high-BBTD incident areas. In addition, the difference in the severity (**Table 3**) of the disease per location can be attributed to the seemingly poor maintenance of banana plantations (Smith *et al*. 1998).

P. nigronervosa is the only vector known to be transmitting BBTV and reproducing efficiently on banana (Ferreira *et al.* 1997; Yasmin *et al*. 2001; Foottit *et al.* 2010). The BBTV vector was found in all the surveyed banana fields at the six localities. The occurrence of aphids was observed to be high in the region with simple colonies and harbouring winged aphids which contribute to the spread of the disease (**Table 4**). Winged aphids were observed to range from 4 to 18% across the six localities. The lowest numbers of winged aphids (4%) observed in Bujumbura Rural may be attributed to lower temperatures at the higher altitude locations (Ferreira *et al.* 1997).

Poor maintenance of the perennial banana crop and its dense canopy might also help to increase the aphid vector population. The dense canopy also partially prevents rainfall from reaching the leaves and pseudostem of banana suckers, and thereby favouring the aphids' multiplication (Young and Wright 2005).

Among *Musa* varieties, the BBTD incidence was observed to be varying from 5% to 48% (**Table 5**) with the highest incidence being observed in 'Indarama' (AAA-EA, brewing) and 'Yangambi Km5' (AAA, brewing); and the lowest incidence being observed in plants sourced from tissue culture and 'imizuzu' (AAB, plantain). Aphid populations were higher on 'Yangambi Km5' (AAA, brewing) and 'imizuzu' (AAB, plantain) unlike in the other banana cultivars. However, the aphid population did not have a direct correlation with BBTD incidence.

The high aphid populations on 'Imizuzu' (AAB, plantain) could be due to green yellow pseudostem colour that may attract aphids (Simmonds 1966; Kumar and Hanna 2008). The dense leaf canopy of 'Yangambi Km5' (AAA, brewing) mats may also favour a build up of aphids' population. On the other hand, the reason that some banana cultivars were observed to have lower aphid numbers but higher BBTD incidence, such as 'Indarama' (AAA-EA, brewing), could be attributed to the fact that such banana cultivars are highly susceptible to the disease. Previous studies have also indicated that banana cultivars whith at least one B genome are more tolerant to BBTD as opposed to those with only A genomes (Espino *et al.* 1993, Hooks *et al*. 2009). This could be the reason for the relatively lower BBTD incidence in 'Kayinja' (ABB, brewing) and the 'Imizuzu' (AAB, plantain). In contrast, the lower BBTD incidence reported for the highland genotypes 'Igisahira' (AAA-EA, cooking) and 'Igitsiri' (AAA-EA, brewing) can be attributed to their occurrence at higher altitudes where fewer winged aphids, which transmit BBTV from plant to plant (Nelson 2004), were reported resulting in subsequent less BBTV-infection.

Although farmers could recognize and had local names for BBTD-symptoms, they were not well informed about how the disease can be managed. Several factors played an important role in the disease spread; these include lack of knowledge of using virus-free planting materials and long periods of insecurity in many parts of the GLRA which resulted into mass movement of people and planting materials. The most recent appearance of BBTD in Rumonge could have resulted from mass movements of people across Burundi during the political crisis of 1993 to 2003. The infected planting materials of 'Yangambi Km5' (AAA, brewing) and 'Indarama' (AAA-EA, brewing) may have been brought to Rumonge during that period.

Unfortunately, once established BBTD has never been eradicated from countries where it occurred (Jones 2009), though in some areas in Australia, BBTD was managed by early detection of the diseased stools and immediate removal. This required partnership between communities and government working together for a common purpose (Magee 1938; Brooks 1999; Robson *et al.* 2006; Daniells 2009). Furthermore, in American Samoa, the BBTD incidence was reduced to about 5% using a public information campaign on rapid removal of infected mats as a method of disease control (Brooks 1999). These examples may be adapted to small-scale agriculture of the GLRA and contribute in reduction of BBTD incidence.

The BBTD was confirmed in the samples from the three countries with PCR results ranging from 13 to 73% of positives (**Table 6**) among the samples collected on symptommatic plants using the PhytoPass system. Although it was reported that the PhytoPass kit may be conserved in ambient conditions 138 days pending molecular tests (Busogoro *et al.* 2009), the period between sampling and extraction affected the success rates for diagnostic tests. Such results can be attributed to the banana extract quantity on PhytoPass membrane which may influence the success of PCR tests due to the likelihood of inhibition of phenolic compounds contained in the banana leaves (Dale 1987; Wu and Su 1989).

CONCLUSIONS

The BBTD incidence was generally high in the infected regions of the GLRA while *P. nigronervosa* was reported within all the surveyed locations. However, the BBTD incidence and aphid occurrence were not significantly correlated. The fluctuations of winged *P. nigronervosa* may explain the subsequent no significant correlation between occurrence of *P. nigronervosa* colonies and BBTD incidence across the 3 countries.

From the interviewed farmers, different factors such as the lack of awareness about BBTD management, lower use of *in vitro* plantlets and exchange of planting materials should have influenced the widespread of the disease in GLRA. In context of small scale growers of the GLRA, previous successful experiences of other countries on BBTD management strategy should be encouraged. This entails raising awareness among stakeholders at all levels (policy makers, extension services, NGOs and farmers), promoting the use of disease-free planting materials, reduction of inocula by collective eradication of BBTV-infected mats, controlling aphid populations and implementing quarantine measures in order to prevent the spread of the disease in areas of the Great Lakes region of Africa which are not yet infested with BBTV.

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