

Article

Diminishing Farm Diversity of East African Highland Bananas in Banana Bunchy Top Disease Outbreak Areas of Burundi—The Effect of Both Disease and Control Approaches

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Abstract: Disease-driven selection favours evasive, tolerant, and resistant cultivars, changing cultivar diversity significantly. Since its outbreak in Burundi in the late 1980s, Banana Bunchy Top Disease (BBTD) has now spread to 5 out of 18 provinces across the country, principally through informal seed exchanges. Control approaches have focused on using tissue culture clean planting material and eradicating infected mats. This study investigated the impact of BBTD and its control measures on seed selection practices and banana cultivars diversity in Burundi, by comparing two BBTD endemic sites and one where the disease wasn't reported. Results have shown that in addition to agronomic traits used in all sites, some BBTD-typical symptoms were used in seed selection in the endemic areas. Own seed provisioning and formal seed sources networks were more likely to be observed in BBTD-endemic areas, compared with the non-endemic area. Disease control using certified tissue culture planting materials reduced the varietal diversity of local cultivars but enabled the introduction of new cultivars. A general reduction in the diversity of local cultivars grown by farmers in the BBTD endemic zones was observed, with about half of the diversity per farmer compared to the non-endemic zone. Farmer demand for varieties (local and improved) was not different between the two areas. Sustainable conservation of crop genetic diversity in the presence of disease invasions remains a problem to be addressed. Thus, implementing seed system-linked intervention with an explicit and monitored diversity conservation objective would increase the sustainability of agricultural production in such situations.

Keywords: vegetative propagated crops; banana bunchy top disease; banana diversity; planting material; seed systems; Burundi

1. Introduction

Banana (*Musa* spp.) was ranked the world's sixth in production in 2017 (FAO data), with an annual production of about 144.6 million Mg [1]. Less than 10% of the global annual production enters the commercial market, indicating that the crop is more important for local consumption than for export [2,3]. The East African Highlands and the Great Lakes region—Burundi, Kenya, Rwanda, Western Tanzania, Uganda, the Democratic Republic of Congo and the Republic of Congo—are zones

of secondary diversity for bananas, with a large number of locally adapted AAA-EA groups of cultivars and landraces for various uses including cooking, dessert, plantain, and multiple use types [4,5]. Genetic diversity is important for cropping system resilience and sustainability. It enables farmers and plant breeders to adapt to diverse and changing environmental conditions and to provide genes for breeding for disease tolerance [6].

In Burundi, a higher diversity is recorded for beer and cooking banana types compared to dessert and plantain types [7]. Most of these varieties arise from the AAA-EA genome subgroup through clonal variation and farmer selection and are shared locally through informal seed systems. Although not yet contributing significantly to the global banana breeding gene pool, the genetic diversity of banana in Burundi is interesting in its richness, incomplete documentation, and potential as a source of genetic traits for breeding adaptable bananas for production resilience in changing environmental conditions such as water availability, dwindling soil fertility, pests, and diseases. It therefore presents an excellent model for studying such phenomena in seed systems, especially of vegetatively propagated crops, which are little included in regulatory frameworks. With the increased regulation of seeds, this system would be invaluable in assessing the interphase between the formal and informal seed system and the framework for evaluating the consequences of seed degeneration in formal and informal interphases [8]. The conservation of this existing diversity in this area is important to service a diversity of current needs and to mitigate potential future biotic and environmental challenges or to meet social and economic needs [9–11].

It is the informal seed system that ensures the conservation and the distribution of locally adapted varieties with farmer-desired characteristics for many vegetatively propagated crops. Through their networks, farmers multiply and share their preferred varieties including those with traits not produced by formal breeding, such as tolerance of characteristic local stress, or particular organoleptic qualities [6,12]. Acceptable genotypes from formal breeding also enter the informal seed system networks once adopted. In addition, disease invasions present a powerful force of genetic selection favouring crop varieties that are tolerant, resistant, or evasive of infection. This is the case for the Banana Bunchy top disease (BBTD) invasion, which was especially important since small scale farms and informal seed systems were dominant in maintaining and sharing the rich diversity of East African Highland bananas (AAA-EAH group) and because no resistant genotypes are known. The management of such diseases creates room for the introduction of new varieties and trials with cultivars with hitherto non-valued benefits or constraints to the production and seed system on a local scale.

The introduction of Banana Bunchy Top Disease (BBTD) in Burundi in the late 1980s presented a significant, rapidly spreading constraint to local banana production [13]. It is one of the most devastating diseases affecting bananas in the world [14,15], which are among the top 100 of the world's most important invasive alien species [16]. BBTD is presently spreading in Africa and has been reported in at least 18 countries, with five having been invaded in the last ten years alone [17–21]. Once introduced into an area through infected seed, it quickly establishes and spreads locally through the banana aphid, *Pentalonia nigronervosa* (Homoptera; Aphididae), leading to a gradual collapse of production. It is especially severe because it attacks both the production capacity and clean seed systems of bananas [22–24]. The early symptoms of BBTD are usually barely detectable, and often asymptomatic infections exist [25,26]. By the time advanced symptoms begin to appear, a large number of plants are likely infected asymptotically, leading to a lack of clean seeds [8]. No BBTD-resistant cultivars are known [27–29], although there is a wide range of susceptibility and symptom expression among *Musa* genotypes [30]. The outbreak of BBTD may therefore affect the ability of the seed system to preserve, propagate and share existing genetic diversity [31] if control measures are not applied.

The control of BBTD in Burundi has involved the double risk reduction strategy of eliminating affected banana mats, sources of infection and the use of tissue culture clean planting material. Although a level of recovery has been achieved in Burundi [19,28,32], the persistence of parallel formal and informal seed systems meant that the introduced clean plants would eventually enter into informal seed exchange. It is yet unknown how these interactions have influenced farmer seed exchange and

selection. The effect of the BBTD invasion on the seed system may also influence cultivar diversity. This assessment would require a comparison of the varieties on a disease frontier lying in the same agroecological zone, to account for local diversity existence. Since banana is a perennial crop with a low seed replacement rate, such a frontier study is complicated, but a comparison of disease-free and infected areas is more feasible. This study was, therefore, carried out to assess the effect of BBTD on banana seed systems, the disease sweep and recovery on cultivar diversity, and the interaction between formal and informal seed systems in distributing and conserving banana diversity in areas of high secondary diversity (e.g. AAA-EA in the great lakes region), as well as the potential of local seed systems in a high diversity region for the AAA-EA in the East African Highlands in Burundi. We sought to investigate the hypothesis that the effect of disease on seed diversity may be detected in seed selection practices, cultivar preference and seed dissemination channels of different varieties. Understanding this information would inform seed systems linked to such regions and offer a means of engaging vegetative propagated crop seed systems.

2. Materials and Methods

Three sites in Burundi were selected for the study. Two BBTD endemic in Collins of Cibitoke Province (Munyika and Gitebe) and one BBTD free zone in Gitega Province (Muremera) (Figure 1). Gitebe is located in Mugina commune with an average altitude of 1375 m above sea level (a.s.l.), Munyika in Rugombo commune with an average of 900 m. a.s.l., and Muremera in Giheta commune with an average of 1800 m. a.s.l. Diversity and seed selection variables were compared between the three zones. Within the BBTD endemic area, farmers actively involved in BBTD control were also compared with those not participating but living in the same locality. This allowed for the investigation of BBTD-linked variables while controlling potential cross site differences.

Quantitative and qualitative data were collected using structured questionnaires (Supplementary Data) in a household survey ($n = 120$) and twelve focus group discussions (FGDs) with eight participants in each. For the household survey, 40 households were selected per site, targeting the principal banana manager for each household. In Cibitoke, the BBTD endemic region, half of participants were randomly selected from 130 banana growers who participated directly in a BBTD control project at the pilot sites, and the rest were selected from their neighbours who grow bananas but did not participate in the project. For the control site Gitega, participants were randomly selected from the banana growers. Structured questionnaires were used for the household survey. For the qualitative study, focus group discussions were used considering sex and age disaggregated groups. Age disaggregation grouped participants as 40 years old and below or above 40 at the time of the study. The FGD guide of open-ended questions was used, led by a facilitator of the same sex as the interviewees. Data and observations were recorded verbatim by a note taker in the same session.

Data were collected on the recall of diversity of bananas planted in 2006, 2010, and currently in 2016; the varieties desired for the next planting; seed usage and preference; seed sourcing standards; and criteria for seed acceptance or rejection linked to biotic stress. Data from focus group discussions were analysed by combining and interpreting the answers to each of the articulated questions [33]. Data from the survey were analysed using Statistical Package for the Social Science (SPSS) version 20 [34]. Descriptive statistics (percentages, frequencies and crosstabs) were performed to assess the trends in banana diversity and preference, the type of seeds used and desired, the sources of seeds used in 2016 and their corresponding source and access conditions, and the criteria and symptoms recognized while selecting planting material. The 4 square methods were used to assess changes in the prevalence of variety groups between 2006 and 2016. Univariate Analysis of Variance (UNIANOVA) was carried out to assess the average number of varieties grown by a farmer in 2006, 2010 and 2016; and the average number of planting material used in 2016. Differences between study areas and farmers' categories were assessed using Pearson's chi-square (χ^2) for categorical variables and an independent t-test for numerical variables.

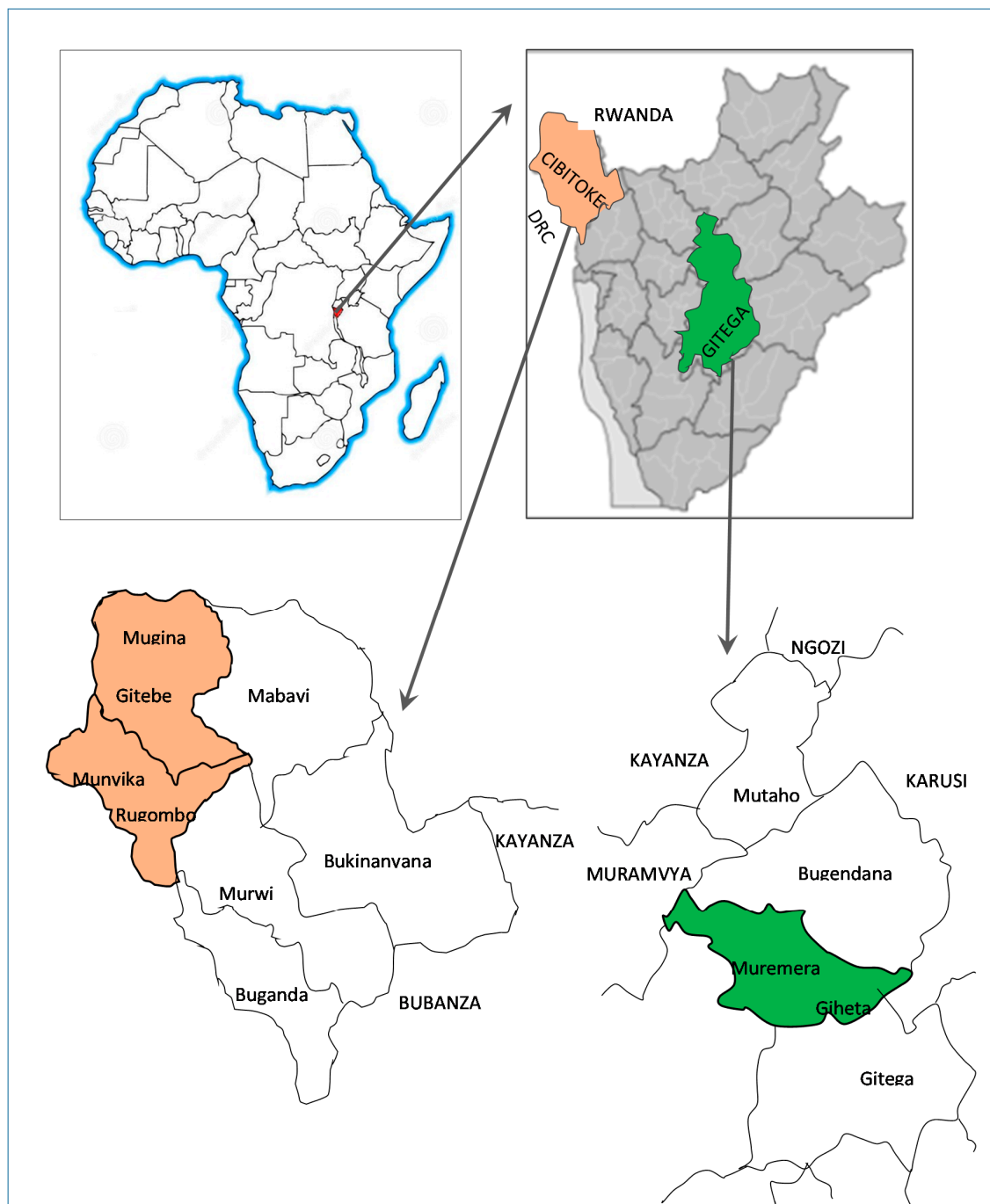


Figure 1. The study included two regions in Burundi (**top panel**). Three regions were compared in a Banana Bunchy Top Disease endemic area (**bottom left**) and a non-endemic area of Gitega Province (**bottom right**).

3. Results

3.1. Banana Diversity

3.1.1. Banana Diversity and Preference

A total of 36 varieties were cited by farmers as grown and desired for next planting (Table 1). In a few cases, some varieties represented variety groups (e.g. Honduras Foundation for Agricultural Research FHIAs, Pro-Vitamin A). Thus, banana varieties could not be directly compared between

sites. The varieties were grouped by origin (local cultivars/landraces, Hybrids and Pro-Vitamin A cultivars) to enable analysis linked to the approximate introduction of cultivars and the seed system channel (formal vs. informal). The varieties therefore covered local seed systems (local cultivars), recent introductions in response to BBTD (FHIAs), and very recent introductions (Pro-Vitamin A).

Table 1. Banana varieties names, their corresponding type, and the number of farmers growing (2016) and desiring them (next planting) at the Gitebe, Munyika and Muremera sites.

Variety Names	Type	Gitebe (n = 40)		Munyika (n = 40)		Muremera (n = 40)	
		2016	Next Planting	2016	Next Planting	2016	Next Planting
Gros Michel	Local	0	1	0	0	0	0
Igihanda	Local	3	5	1	5	0	0
Igihobe	Local	0	1	1	1	0	0
Igihonyi	Local	0	0	0	0	6	0
Igikashi	Local	0	0	0	0	1	0
Igipaca	Local	0	0	0	0	12	0
Igisahira	Local	0	9	2	5	40	0
Igisenyi	Local	10	0	9	0	0	0
Igisubi	Local	8	6	8	2	1	0
Igitsiri	Local	0	0	0	0	37	0
Ikgurube	Local	0	5	0	1	8	1
Ikimalaya	Local	1	1	0	0	0	0
Ikivuvu	Local	2	0	4	1	0	0
Ikiyove	Local	0	0	0	0	1	0
Incakara	Local	0	0	0	0	6	0
Indarama	Local	0	0	0	0	5	1
Inkira	Local	0	0	0	0	0	1
Ingoromoka	Local	0	0	1	0	0	0
Kamaramasenge	Local	1	14	4	8	21	2
Kampala	Local	1	0	0	0	0	0
Yangambi Km5	Local	40	0	37	1	8	0
Umuzuzu	Local	0	5	1	2	1	13
Umuhonyo	Local	0	1	0	0	0	0
Umuzirampiza	Local	0	2	0	0	0	0
Poyo	Local	0	0	0	0	20	0
Sohokunkorere	Local	0	2	1	4	0	3
Mabereyinkumi	Local	0	0	1	0	0	0
FHIAs (various)	Hybrid	30	20	21	21	8	18
Apantu	ProVit A	0	0	2	1	1	0
Bira	ProVit A	0	0	1	0	3	2
Provitamin A	ProVit A	0	0	3	9	1	5
Lai	ProVit A	0	0	0	0	1	0
Muracho	ProVitA	0	0	1	0	0	0
Pelipita	ProVitA	0	0	4	1	3	0
Pisang Papan	ProVit A	0	0	1	0	0	0
Too	ProVit A	0	0	1	1	0	0
Total		96	72	104	63	184	46

Different cultivar groups dominated sites reflecting dominant seed supply routes among the producers (Table 1). In all sites, local cultivars (AAA-EA—East African Highland bananas group) were the most dominant, while the Hybrids and the more recently introduced Pro-Vitamin A cultivars were less dominant. These local varieties were planted at least six times more in 2006 and were twice more desired by farmers in 2016 compared with Hybrids and Pro-Vitamin A. Local cultivars seem to rapidly decline in proportion to the number of farmers growing them, compared with the Hybrid and Pro-Vitamin A. A significantly higher increase in the demand for local cultivars, especially in BBTD endemic areas, was also observed. The farmers in BBTD endemic areas described these varieties, saying that they “have been grown in the past” but are “no longer available” despite being “productive in their farming system” and “tasty”.

“These disappeared varieties should come back! We want to see the varieties that were grown by our ancestors. Igisubi, a disappeared beer banana type, was more productive and gave the beer of a good taste. We have not yet tasted these local varieties (Igihonyi and Igisukari) grown by our parents.

If they come back, they will be loved to test their flavour because among us and our generation, no one has tasted these varieties" (Young Women's FGD, Munyika).

In contrast, farmers in Muremera (the non-BBTD endemic area) expressed significantly higher demand for hybrid varieties that were less common in their area than those in the BBTD endemic zone (ANOVA, $P = 0.000$) (Table A1 in Appendix A). In fact, compared with 2016, Muremera farmers stated desire to plant six times more than the present holding of these varieties, compared with farmers in the BBTD endemic zone who desire significantly less than the present holding of these varieties ($P = 0.000$). Compared to planting in 2016, new plantings of local varieties changed very significantly, but this was not the case for Hybrids (FHIA) and Pro-Vitamin A varieties ($P = 0.000$). In all three areas, farmers wish to change the diversity relative to the plants currently grown. Expansion of diversity is a key consideration for those who want to plant. Expansion in Muremera trends towards high yielding Hybrids and Vitamin A, while it trends towards local varieties in the BBTD affected areas ($P = 0.000$). The evaluation of cultivars was an ongoing process modified after the experience of growing new cultivars. The reduced preference for Hybrids was also linked to their agronomic properties and fit with the farming system. Also, cultivars introduced during a disease control project could have been expected to be resistant to the biotic challenge while maintaining the desired characteristics of local cultivars, but farmers experienced the opposite.

"We have found that they do not last long. Even the new varieties FHIA are infected by BBTD, they give one or two productions and they disappear" (Old women's FGD, Gitebe)

3.1.2. BBTD and Banana Diversity

An analysis of farmer diversity revealed significant shifts before and after the BBTD invasion in Burundi. A decline in local cultivars was associated with disease invasion while the introduction of, and increase in, new varieties (mainly FHIA hybrids and more recently Pro-Vitamin A varieties) were associated with control efforts (Figure 2) ($\chi^2 = 121.502$, $P = 0.000$). Between 6 and 8 local varieties grown in BBTD areas in 2006 were no longer detected in farmers' fields in 2016. This has been proven by the significant decrease in the number of local varieties grown per farmer in Gitebe between 2006 and 2010 (UNIANOVA; $df = 2$, $F = 3.309$, $P = 0.04$) and in Munyika between 2010 and 2016 (UNIANOVA; $df = 2$, $F = 3.309$, $P = 0.02$). However, the number of local varieties per farmer in Muremera was still increasing (Figure 3). At the same time, the proportion of farmers growing hybrid varieties (FHIA) increased more in BBTD endemic regions than in Muremera (Figure 2). Thus, farmers in BBTD areas appeared to replace local cultivars with new varieties. This happened much less in the non-BBTD site. These changes in the popularity of variety groups grown between 2010 and 2016 were significantly higher in Gitebe ($\chi^2 = 38.905$, $P = 0.000$), Munyika ($\chi^2 = 27.725$, $P = 0.000$) and Muremera ($\chi^2 = 60.47$, $P = 0.000$). Pro-Vitamin A varieties were only observed among project farmers, and so this comparison was not associated with BBTD incidence.

While analysing diversity for the next planting season, there was a little difference between villages in terms of the diversity of their planting material needs ($P = 0.02$). The number of local varieties requested by farmers was higher in Gitebe (2.2 ± 1.31) and Munyika (1.80 ± 1.27) compared with Muremera (1.63 ± 1.15). Pairwise, comparison only showed differences in terms of the varieties needed between Muremera and Gitebe ($P = 0.01$).

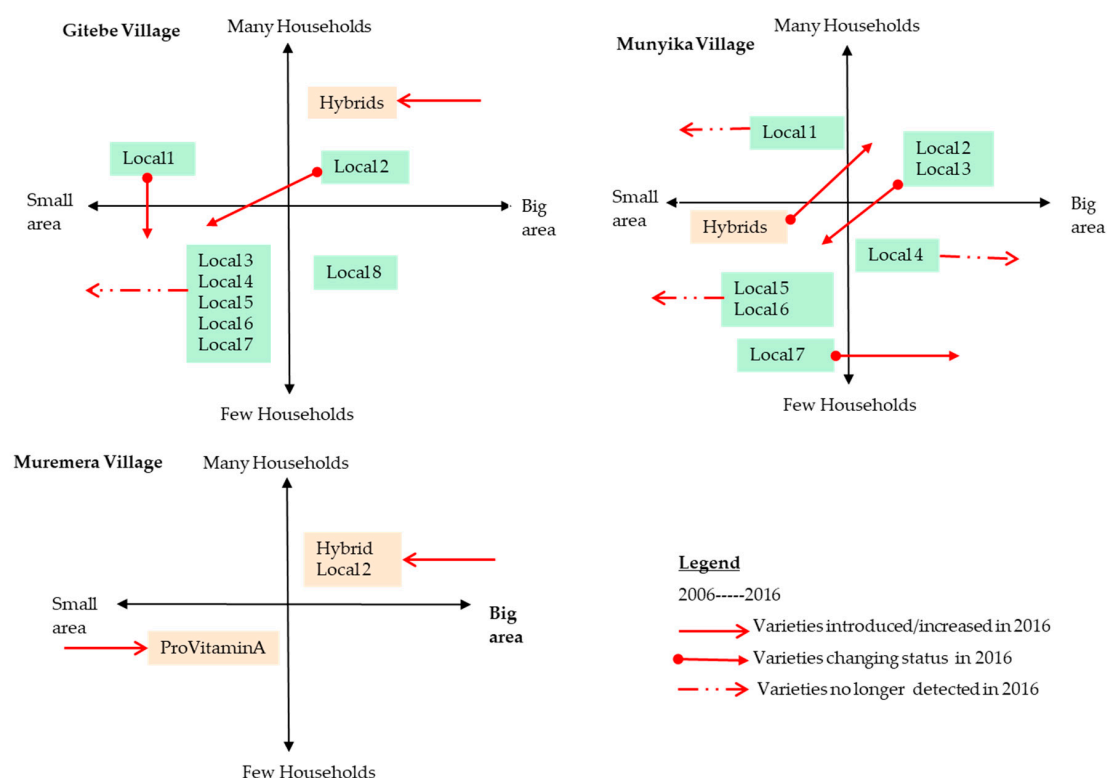


Figure 2. Changes in the prevalence of local and Hybrid banana varieties between 2006 and 2016 for surveyed farmers in Gitebe, Munyika and Muremera villages.

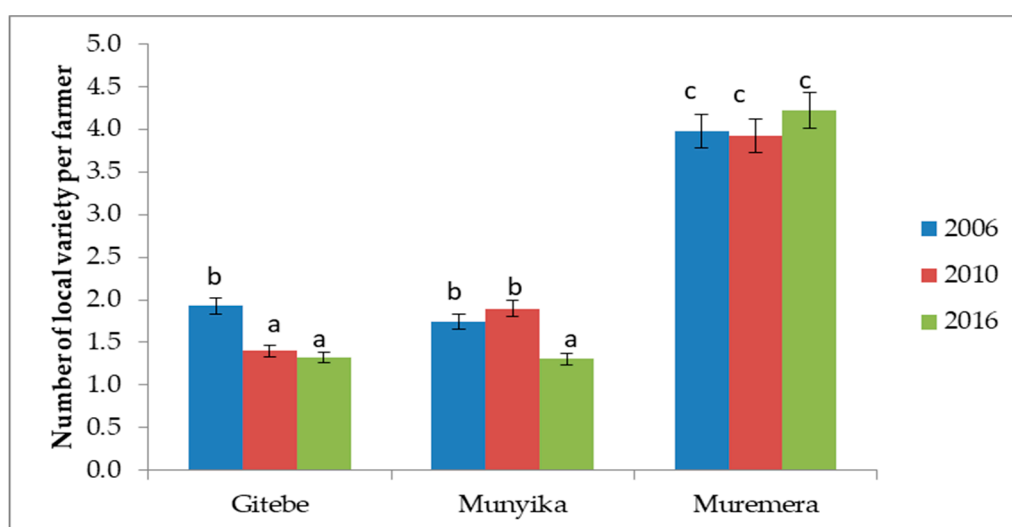


Figure 3. Average Number of varieties grown per farmer (men and women) in Gitebe, Munyika and Muremera in 2006, 2010 and 2016. The bars above the columns are error bars. In the same category of farmer at a site, the columns with the same letter do not differ significantly at $P = 0.05$.

3.2. Banana Seeds Systems

3.2.1. Planting Material Used in 2016 and Desired for the Next Season

Significant differences were observed in the use of different types of planting materials in the three communes studied. Three type of planting material (tissue culture, suckers and macropropagation) were recorded, but macropropagation was used less and was not subject to comparison in BBTD areas. Tissue culture plantlets were most used and preferred in the BBTD endemic areas of Munyika and

Gitebe ($\chi^2 = 19.006$, $P = 0.000$ and $\chi^2 = 27.388$, $P = 0.000$), while suckers were still preferred in the BBTD-free commune ($\chi^2 = 18.895$, $P = 0.000$) (Figure 4). In BBTD areas, suckers were mainly preferred by older farmers, and young farmers tended to prefer plantlets ($\chi^2 = 8.302$, $P = 0.016$). However, at the BBTD endemic site Munyika, farmers associated with the pilot site had better access to tissue culture plantlets than those that were not ($\chi^2 = 13.85$, $P = 0.001$), even though non-participating farmers still significantly preferred plantlets when compared with non-endemic sites. The preference for plantlets in BBTD areas was associated with the expectation that they would be cleaner and healthier compared to suckers of unknown sources. This is expressed in the reported rapid reinfection of mat established by such suckers becoming immediately diseased, while those from tissue culture had a window before reinfection. None of the interviews revealed recognition of latent or asymptomatic infections, but this is consistent with the following observation from an FDG:

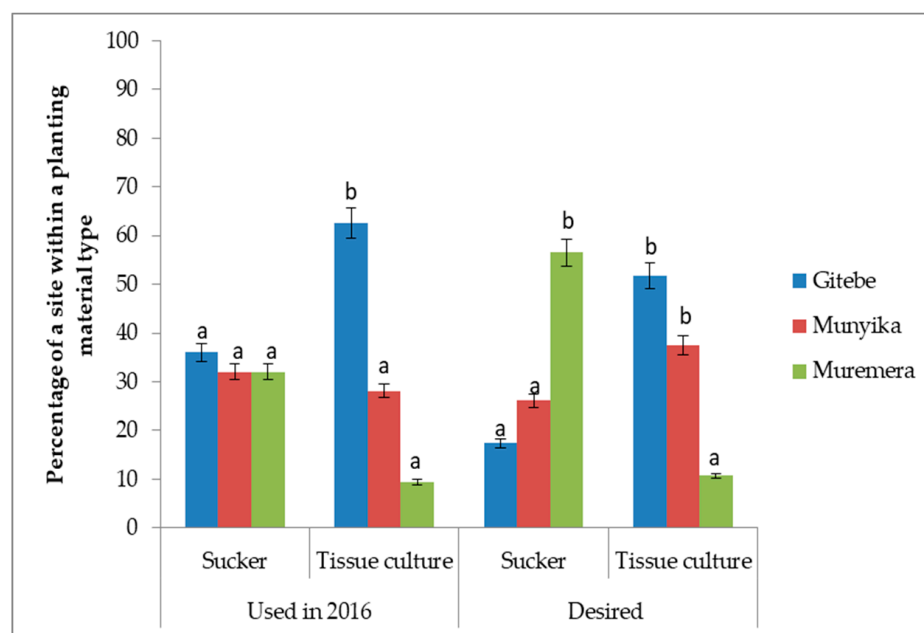


Figure 4. The proportion of farmers at the sites Gitebe, Munyika, Muremera who have used and desired suckers and tissue culture. The bars above the columns are error bars. For the same type of planting material within a growing period site, the columns with the same letter do not differ significantly at $P = 0.05$.

“The method of multiplication of the plantlets is so complicated. Therefore we are just assured that whatever we receive is clean. Banana mats established with plantlets could be infected after 2 or 3 productions while a sucker from our banana fields showed symptoms within a month only after plantation” (Younger women’s FGD, Munyika).

In terms of the quantity of planting material, farmers in the BBTD endemic areas were more than twice as likely to plant new fields and plants than those in the non-endemic area (Table 2; UNIANOVA = 12.25, $P = 0.000$). However, seed demand per farmer for the next planting was not significantly different between the endemic and non-endemic regions (UNIANOVA = 2.82, $P = 0.062$). Within BBTD areas, there were differences in the quantity of seed demand linked to age and sex (Table 2). Older farmers’ seed demand was more than twice that of younger farmers in Gitebe ($t = 2.387$, $P = 0.019$), while in Munyika, younger farmers desired at least twice many seeds as older farmers. In Muremera, age-linked differences were recorded in terms of the seed used, and younger farmers used almost twice the quantity used by older farmers ($P = 0.047$). Men in BBTD areas planted more and demanded more plantings than women, but this differed significantly in Munyika ($t = 1.989$, $P = 0.047$). These differences were related to project interventions because between the non-project farmers of Gitebe and Munyika, the quantity of seed used was almost the same across age and sex groups.

Table 2. Means of the quantity of planting materials used in 2016 and desired per farmer in Gitebe, Munyika and Muremera. Figures in parenthesis () are standard deviations.

Site	Factors	Groups	Mean (SD) of planting material	UNIANOVA/T test	df	P
Gitebe Munyika Muremera	Used in 2016		26.3 (30.6) 11.4 (13.5) 8.6 (10.3)	12.25	2	0.000
Gitebe Munyika Muremera	Next planting		42.3 (41.9) 51.8 (174.5) 11.43 (15.7)	2.82	2	0.062
Gitebe	Used in 2016	<40 Years (<i>n</i> = 19)	20.9 (20.4)	0.958	52	0.342
		>40 Years (<i>n</i> = 35)	29.3 (34.8)			
		Female (<i>n</i> = 17)	20.6 (26.2)	0.970	52	0.336
		Male (<i>n</i> = 37)	29.1 (32.4)			
		Projects (<i>n</i> = 20)	25.5 (27.9)	0.140	52	0.890
		Non-Projects (<i>n</i> = 34)	26.8 (32.5)			
	Next planting	<40 Years (<i>n</i> = 23)	24.9 (14.4)	2.387	83	0.019
		>40 Years (<i>n</i> = 62)	48.7 (46.8)			
		Female (<i>n</i> = 29)	37.6 (33.4)	0.737	83	0.463
		Male (<i>n</i> = 56)	44.7 (45.8)			
		Projects (<i>n</i> = 20)	51.8 (48.6)	2.234	83	0.028
		Non-Projects (<i>n</i> = 34)	32.0 (30.8)			
Munyika	Used in 2016	<40 Years (<i>n</i> = 28)	10.3 (12.3)	0.607	51	0.546
		>40 Years (<i>n</i> = 25)	12.6 (14.9)			
		Female (<i>n</i> = 36)	10.3 (12.2)	0.829	51	0.411
		Male (<i>n</i> = 17)	13.7(16.3)			
		Projects (<i>n</i> = 33)	10.3 (12.6)	0.597	51	0.443
		Non-Projects (<i>n</i> = 20)	13.3 (15.1)			
	Next planting	<40 Years (<i>n</i> = 33)	69.15 (257.7)	0.758	73	0.451
		>40 Years (<i>n</i> = 42)	38.3(52.2)			
		Female (<i>n</i> = 51)	24.9(27.7)	1.989	73	0.047
		Male (<i>n</i> = 24)	109.1 (302.1)			
		Projects (<i>n</i> = 36)	37.4 (55.4)	0.688	73	0.493
		Non-projects (<i>n</i> 3ws2= 39)	65.3 (236.9)			
Muremera	Used in 2016	<40 Years (<i>n</i> = 15)	13.1 (18.5)	2.00	55	0.047
		>40 Years (<i>n</i> = 42)	7.0 (4.2)			
		Female (<i>n</i> = 47)	9.0 (11.3)	0.458	55	0.501
		Male (<i>n</i> = 10)	6.6 (2.7)			
	Next planting	<40 Years (<i>n</i> = 22)	11.9 (10.7)	0.158	63	0.875
		>40 Years (<i>n</i> = 43)	11.2 (17.8)			
		Female (<i>n</i> = 48)	13.4 (17.7)	1.703	63	0.09
		Male (<i>n</i> = 17)	5.94 (4.19)			

3.2.2. BBTD and Banana Seed Sourcing

The occurrence of BBTD has had little effect on seed sourcing and opened up little space for formal seed systems in Burundi. In all three sites, farmers sourced seeds mainly from formal seeds support and own seeds, representing respectively 36.5% and 31.5% of seed used (Table 3). Farmers in the BBTD endemic area sourced planting materials from informal seeds (from self, neighbours and relatives) with 15% less than those in the BBTD non-endemic area. Local nurseries existed only in BBTD areas, while macropropagation nurseries and seed mother gardens were not observed. There were differences in the BBTD endemic areas in terms of seed sourcing. Project farmers were significantly more likely to source seeds from their own plantation in Gitebe, while seed support, e.g. from non-Governmental organisations NGOs, was more common in Munyika. Non-project farmers mainly sourced seeds from the local nursery in Gitebe, while their own farms dominated in Munyika. The difference between project and non-project farmers in terms of seed sourcing was significantly higher in Munyika ($\chi^2 = 12.06$, $P = 0.007$).

Table 3. Percentages of farmers of Gitebe, Munyika and Muremera who used planting material from own their fields, seeds support, neighbours and local nursery in 2016. In the same source of planting material, percentages followed by the same letter do not differ significantly from each other at $P = 0.05$.

		Own Fields (%)	Seeds Support (%)	Neighbors (%)	Local Nursery (%)	χ^2	df	P
Gitebe		33.3a	29.6a	14.8a	22.2a			
Munyika	Used in 2016	20.8a	39.6a	20.8a	18.5a	16.97	6	0.009
Muremera		40.4a	40.4a	19.2a	0.0b			
Gitebe	<40 Years ($n = 19$)	26.3a	42.1a	15.8a	15.8a	2.538	3	0.469
	>40 Years ($n = 35$)	37.1a	22.9a	14.3a	25.7a			
	Female ($n = 17$)	35.3a	35.3a	23.5a	5.9a	4.550	3	0.208
	Male ($n = 37$)	32.4a	27.0a	10.8a	29.7a			
	Project ($n = 20$)	45.0a	35.0a	10.0a	10.0a	4.239	3	0.237
	Non-Project ($n = 34$)	26.5a	26.5a	17.6a	29.4a			
Munyika	<40 Years ($n = 28$)	21.4a	42.9a	17.9a	17.9a	0.44	3	0.931
	>40 Years ($n = 25$)	20.0a	36.0a	24.0a	20.0a			
	Female ($n = 36$)	16.7a	47.2a	19.4a	16.7a	2.9	3	0.40
	Male ($n = 17$)	29.4a	23.5a	23.5a	23.5a			
	Project ($n = 33$)	9.1a	48.5a	15.2a	27.3a	12.06	3	0.00
	Non-project ($n = 20$)	40.0b	25.0a	30.0a	5.0b			
Muremera	<40 Years ($n = 15$)	33.3a	26.7a	40.0a	0	5.714	2	0.048
	>40 Years ($n = 42$)	42.9a	45.2a	11.9b	0			
	Female ($n = 47$)	40.4a	38.3a	21.3a	0	0.82	2	0.662
	Male ($n = 10$)	40.0a	50.0a	10.0a	0			

The banana seeds in all three villages were mostly acquired for free, even when they were obtained from the formal seed sector (Table 4). However, farmers in BBTD endemic areas were up to five times more likely to purchase planting materials, compared with those in the non-endemic area ($\chi^2 = 9.46$, $P = 0.009$). Within the BBTD endemic area, the non-project farmers were more willing than the project farmers to purchase seeds from nurseries, where free seed support was not available ($\chi^2 = 4.195$, $P = 0.041$). This underlines an increased demand (and low supply) of clean planting materials in BBTD endemic areas. Farmers of both sexes cited the low supply/availability of clean seed and suggested the need to train more farmers (groups) or production practices for tissue culture plantlets (clean seed). This demand for clean seed was more notable in BBTD endemic area.

Table 4. Percentages of farmers who used free and purchased planting material in 2016. In the same column, percentages followed by the same letter do not differ significantly from each other at $P = 0.05$.

		Free (%)	Purchased (%)	χ^2	df	P
Gitebe		74.1a	25.9a			
Munyika		77.4a	22.6a	9.46	2	0.009
Muremera		94.7b	5.3b			
Gitebe	<40 Years ($n = 19$)	84.2a	15.8a	1.568	1	0.210
	>40 Years ($n = 35$)	68.6a	31.4a			
	Female ($n = 17$)	88.2a	11.8a	2.591	1	0.107
	Male ($n = 37$)	67.6a	32.4a			
	Project Farmers ($n = 20$)	90.0a	10.0a	4.195	1	0.041
	Non-Project Farmers ($n = 34$)	64.7b	35.7b			
Munyika	<40 Years ($n = 28$)	75.0a	25.0a	0.189	1	0.664
	>40 Years ($n = 25$)	80.0a	20.3a			
	Female ($n = 36$)	83.3a	16.7a	2.288	1	0.130
	Male ($n = 17$)	64.7a	35.3a			
	Projects ($n = 33$)	72.7a	27.3a	1.071	1	0.301
	Non-Projects ($n = 20$)	85.0a	15.0a			
Muremera	<40 Years ($n = 15$)	100.0a	0.0a	1.131	1	0.288
	>40 Years ($n = 42$)	92.9a	7.1a			
	Female ($n = 47$)	95.7a	4.3a	0.546	1	0.460
	Male ($n = 10$)	90.0a	10.0a			

“The nurseries are few and the seedlings produced are not sufficient! The best would be to train other seedling nursery producers so that . . . We will request those who can to train us on the techniques of production (hardening) of vitro plants so that we can have them in sufficient quantities to plant our fields and give to others” (Men and Women’s FGDs combined project and non-project farmers of Cibitoke).

In the non-BBTD endemic area of Muremera, however, suckers of common varieties were largely available in old plantations, diminishing willingness to incur extra cost for their acquisition. However farmers expressed willingness to pay a premium for a new seed if it had some desired characteristics and varieties. We did not find a direct mention of a premium for clean planting material.

“Suckers are available for all varieties. All farmers use suckers . . . For all varieties, we ask for suckers from neighbours or we take them from our fields. With us, the suckers of old varieties cannot be bought or sold . . . but for the new varieties, a suckers was bought at 1500 Burundian Francs (BIF) in 2015 and now it is only 1000 BIF” (Men and Women’s FGDs, Muremera)

3.2.3. Criteria Used to Select Good Planting Material

The evaluation of banana planting materials involved the consideration of agronomic attributes and avoidance of pest and disease symptoms. Thus, sucker type (sword sucker versus water sucker) and “Leaf symptoms” (disease symptoms and damage) were the common criteria used to select good banana planting material in the three villages (Figure 5). The modalities ‘absence of leaf symptoms’ and ‘sword sucker (narrow leaves and thick stem/corm)’ were the most dominant criteria of good planting material at Cibitoke sites ($\chi^2 = 25.974$, $P = 0.000$) and Muremera ($\chi^2 = 21.978$, $P = 0.000$), respectively. Two criteria not recognized in all three sites were the ‘absence of aphid’ (in Gitebe only with 6%) and the ‘corm appearance’, which was used less in Gitebe (5%) than in Muremera (15%). Leaf chlorosis and corm/root symptoms were the symptoms recognized by surveyed farmers when selecting planting material (Figure 6). Corm and root symptoms (blackening and holes) were more important in terms of seed selection in Muremera than in Cibitoke ($\chi^2 = 24.172$, $P = 0.000$). Within the BBTD endemic zone, additional BBTD-linked symptoms (bunchy appearance of leaves in mother mat (17.2%) and stunting of plant or fruit (5.6%)) were found, as opposed to general health indicators found in the non-endemic zone. However, no differences were observed in terms of the seed selection criteria used between farmers in direct contact with BBTD control activities and non-participants in this zone.

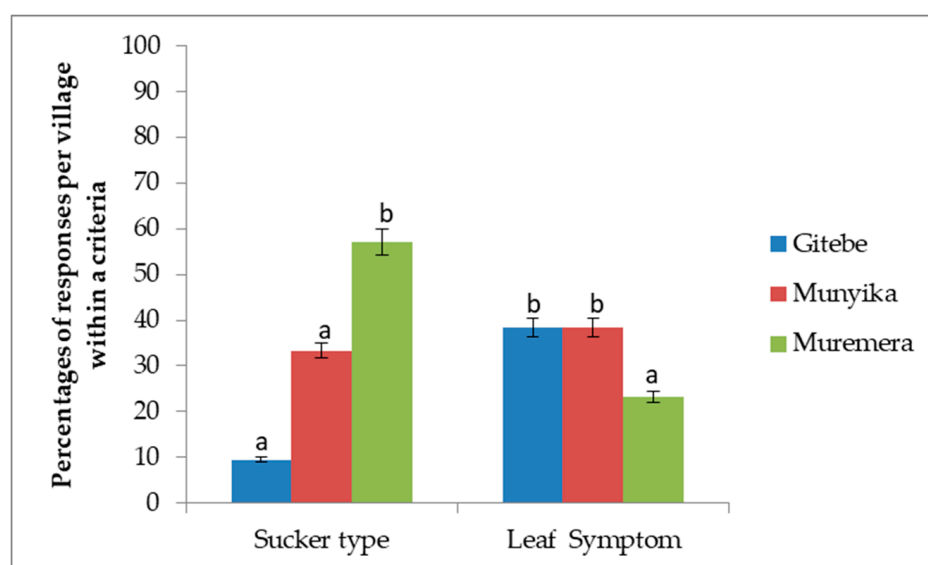


Figure 5. Percentage of farmers per site who considered the sucker type and leaf symptoms when selecting banana planting material in Gitebe ($n = 40$), Munyika ($n = 40$) and Muremera ($n = 40$). Within a criterion, the same letters above columns denote non-difference in percentages between sites.

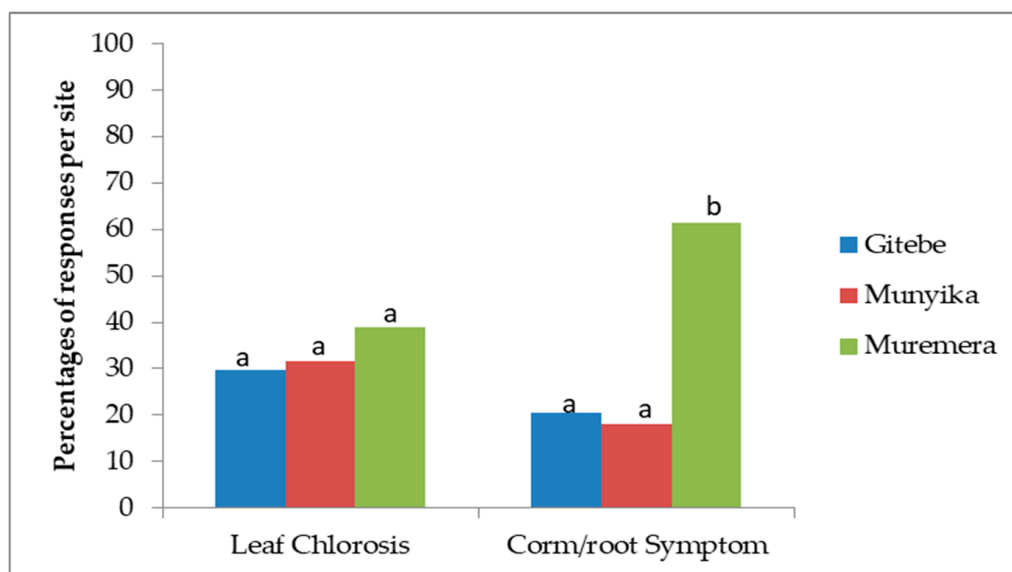


Figure 6. Percentage of site responses per symptom of infection (chlorosis of leaves and corm/root symptoms) recognised in banana plants when selecting planting material in Gitebe ($n = 40$), Munyika ($n = 40$) and Muremera ($n = 40$). Within a symptom, the same letters denote the non-difference in percentage between sites.

4. Discussion

Overall, this study revealed that the BBTD outbreak and control in Burundi was associated with changes in banana cultivar diversity, seed systems and seed selection standards. These changes involved the replacement of some varieties with others and a greater penetration of formal seeds in local plantings. Changes in seed selection indicators were observed and linked to a greater acceptance of formal seeds in BBTD areas compared with non-outbreak area. This study also reveals the susceptibility of the informal seed system to disease invasions. The seed-system-linked interventions weakened the informal seed sector creating a potentially unintended variety turnover. Thus, a lower inflow of these cultivars coupled with the removal of infected plants gradually diminishes their availability, leading to reduced overall diversity and an unintended variety turnover.

The invasion of BBTD created an opportunity for rapid cultivar turnover associated with both disease outbreak and management. Varietal susceptibility and resistance has been associated with such changes in other systems. For example, worldwide, *Fusarium* race 1 was responsible for the replacement of Gros Michel by Cavendish, though preferred to the commercial dessert banana. In Burundi, Kayinja (Pisang' Awak) has been replaced by Yangambi Km5 as the principal beverage banana in the Great lakes region due to the *Fusarium* invasion. Although all banana varieties are assumed to be susceptible to BBTD, significant variation in disease susceptibility has been reported [30]. In this study, reduction of the local East African Highland banana varieties was observed in the endemic area, which could contribute to the decline of provisioning, regulating, and cultural ecosystem services as reported before [35]. A large number of these varieties exist in Burundi in not yet affected areas, principally maintained as perennial plantations with seed exchange occurring through the informal seed sharing system. These present an increased relative risk to BBTD due to minimal quality assurance and the general longevity of plantations. Indeed, although farmers recognized BBTD symptoms and mentioned considering them in seed selection, symptomatic seed selection misses asymptomatic plants in the incubation period, which is common in BBTD [8]. Varietal changes could also arise due to the limited availability of diverse local cultivars in the tissue culture system and to the available varieties with other beneficial characteristics (e.g. high yield and adaptability) introduced during the disease mitigation projects. Typically, projects attempt to distribute preferred varieties, or comparable ones, in disease mitigation efforts, often leading to the unavailability of some preferred varieties, replacement

with ‘comparable’ hybrids, or non-consideration of peripheral local cultivars. This replacement is a concern over the loss of germplasm diversity [6,36]. Indeed, in this study, we observed both an introduction of different varieties (hybrids FHIA and Pro-Vitamin A) but also an increased demand from local cultivars, where these varieties were most dominant.

Changes in the sourcing of seed, supply channels and standards were also observed in this study. An increase in the acceptance of tissue culture plantlets observed in BBTD endemic areas was related to them being perceived as disease free but also to their desirable high yielding varieties. Farmers in this area were also more likely to pay for planting materials. In contrast, the non-BBTD endemic area considered agronomic potential, varietal identity and observable symptoms as key selection qualities, although old plantations served as preferred sources of planting material. They were relatively more trusted as sources of adaptable planting materials, lower in cost and favoured for the potential to observe the garden and mother plant in seed selection. Interestingly, farmer demand for new planting varieties contrasted with their existing stand, demonstrating an ongoing desire for crop improvement. Farmers in the BBTD endemic region desired less available local varieties, citing organoleptic properties, adaptability and agronomic suitability in the local production system; while those in the non-endemic area desired hybrids citing reported high yield levels. This confirms the previous findings [37,38]. Together, these observations show the dynamic considerations related to varieties and underline the possibility of seed-based interventions achieving unintended outcomes or collapsing altogether. For example, the trends of seed demand in BBTD areas might push farmers towards unconfirmed seed sources, increasing the risk of BBTD reinvasion.

We concluded that BBTD has triggered a reduction of diversity in the informal seed system and affected the potential of local seeds systems to produce clean planting material in BBTD endemic areas. It has influenced the farmers’ choice of banana varieties and the planting material type, motivating actors in the seeds systems to introduce improved varieties while revealing more preferred banana varieties per site. This information on specific variety demand should guide the choice of banana varieties to be introduced into these areas. To ensure the sustainability of banana diversity, it is necessary and urgent to identify and promote more tolerant, or BBTD-tolerant, varieties in infested areas while managing the disease before the reintroduction of susceptible varieties, which may quickly disappear again.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2071-1050/12/18/7467/s1>.

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Conflicts of Interest: Authors declare that no conflict of interest exists

Appendix A

Table A1. Interactions between variety grown per type, per sex and per locality.

	Df	Deviance	Resid. Df	Resid. Dev	Pr (>Chi)
NULL			325583	7265.6	
Growing	1	75.54	325582	7190.0	$<2.2 \times 10^{-16}$ ***
variety_type	2	394.53	325580	6795.5	$<2.2 \times 10^{-16}$ ***
Sex	1	18.75	325579	6776.8	9.346×10^{-6} ***
locality	2	12.50	325577	6764.3	0.001432 **
Growing:variety_type	2	28.67	325575	6735.6	3.000×10^{-7} ***
Growing:Sex	1	0.40	325574	6735.2	0.516381
Growing: locality	2	27.01	325570	6708.0	7.154×10^{-7} ***
Growing:variety_type:Sex	2	1.42	325562	6586.8	0.476452
Growing:variety_type:locality	4	28.48	325558	6558.3	5.296×10^{-6} ***
Growing:Sex:locality	2	1.87	325556	6556.5	0.375840
Growing:variety_type:Sex:locality	4	0.96	325548	6550.3	0.908722

Significance codes: 0 '***' 0.001 '**' 0.01.

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