Expansion of the cassava brown streak disease epidemic in eastern Democratic Republic of Congo

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Abstract

Cassava plays a key role in assuring food security and generating income for smallholder farmers throughout central Africa, and particularly in the Democratic Republic of Congo (DRC). This status is threatened, however, by cassava brown streak disease (CBSD) which has recently expanded its incidence and range in eastern DRC. The study described here, comprises the first extensive assessment of temporal change in occurrence of CBSD and its causal viruses in DRC, based on surveys conducted during 2016 and 2018. Cassava fields were inspected in Ituri, Nord-Kivu, Sud-Kivu, Tanganyika and Haut-Katanga provinces within eastern DRC, to record foliar incidence and severity of CBSD. Leaf samples were collected for virus detection and species-level identification. New occurrences of CBSD, confirmed by virus diagnostic tests, were recorded in two provinces (Haut-Katanga and Sud-Kivu) and nine previously unaffected territories, covering an area of > 62,000 km^2, and at up to 900 km from locations of previously published reports of CBSD in DRC. Overall, average CBSD incidence within fields was 13.2% in 2016 and 16.1% in 2018. In the new spread zone of Haut-Katanga, incidence increased from 1.7% to 15.9%. CBSD is now present in provinces covering 321,000 km^2 which is approximately 14% of the total area of DRC. This represents a major expansion of the CBSD epidemic, which was only recorded from one province (Nord-Kivu) in 2012. Both cassava brown streak virus (CBSV) and Ugandan cassava brown streak virus (UCBSV) were detected in Ituri, Nord-Kivu and Sud-Kivu, but only CBSV was detected in Haut-Katanga. Considered overall, these results confirm the increasing threat that CBSD poses to cassava production in DRC and describe an important expansion in the African pandemic of CBSD.

Keywords : Cassava, CBSD, epidemiology, surveillance, CBSV, UCBSV.

Introduction

Cassava (*Manihot esculenta* Crantz) is an important staple food crop in sub-Saharan Africa. In central Africa in particular, most people depend on cassava as the main source of carbohydrates (Dunstan and Chuma 2017; Katinila et al. 2002). In the Democratic Republic of Congo (DRC), cassava plays a vital socio-economic role in small-holder farming systems (Masika et al. 2019; Munyahali et al. 2017). Although cassava is adapted to a wide range of agro-ecological conditions (El-Sharkawy 2006), it is vulnerable to infection by several plant viruses (Thresh et al. 1994). In Africa, cassava mosaic virus disease (CMD) and cassava brown streak virus disease (CBSD) are together responsible for losses of more than US$ 1 billion annually (Legg et al. 2006; Ndyetabula et al. 2016). Pandemics of both virus diseases have caused devastating losses to cassava production in East and Central Africa in recent decades. The causal viruses are spread by unusually abundant populations of the whitefly vector - *Bemisia tabaci* (Gennadius) (*Aleyrodidae*) (Legg et al. 2011, 2014). CBSD used to be geographically limited to coastal eastern and southern Africa. New epidemic spread in recent years through Uganda, Burundi, Rwanda, western Kenya, north-western Tanzania and eastern DRC (Alicai et al. 2007; Ntawuruhunga and Legg 2007) has heightened concern about the risk of further spread.

CBSD is caused by two species of cassava brown streak ipomoviruses (CBSIs) (Family *Potyviridae*: Genus *Ipomovirus*): Cassava brown streak virus (CBSV) and Ugandan cassava brown streak virus (UCBSV) (Mbanzibwa et al. 2009; Winter et al. 2010). CBSIs are perpetuated by planting stem cuttings obtained from infected parent stems and through vector transmission by the whitefly *B. tabaci* (Maruthi et al. 2005, 2017; Storey 1936, 1939; Tata-Hangy and Mahungu 2014). Symptoms of CBSD occur on all parts of the cassava plant, but the most important damage is a necrotic root rot which makes them unsuitable for consumption and marketing (Nichols 1950;

Storey 1936). CBSD causes yield loss in susceptible varieties both by reducing the growth as well as through eliciting a dry necrotic rot that develops in storage roots as they mature (Ndyetabula et al. 2016). Symptoms are present on leaves but are much less obvious than those of CMD as they do not distort the leaf shape and occur mainly towards the bottom of the plant (Nichols, 1950). Molecular diagnostic studies have shown that a proportion of plants infected by CBSIs do not show symptoms at the time of sample collection (Adams et al. 2013). This increases the risk that these viruses may be propagated inadvertently through stems used for planting material.

CBSD was reported for the first time from eastern DRC in 2011 (Mulimbi et al. 2012). This first report recorded the presence of UCBSV in Nord-Kivu province. More recently, Casinga et al. (2019) noted the occurrence of both UCBSV and CBSV, as well as mixed infections of the two species in Ituri province, neighboring Nord-Kivu. Reports of CBSD from northern Zambia, in districts bordering DRC (Mulenga et al. 2018), have led to concern about possible CBSD occurrence in south-eastern areas of DRC. There were also unconfirmed reports of CBSD in the Ruzizi Plain and shores of Lake Tanganyika in Sud-Kivu province and there has been concern about further southward and westward spread of CBSD across DRC (FEWSNET 2017). This study aimed to determine the current occurrence of CBSD in eastern DRC through an extensive surveillance of CBSD and CBSIs during two time periods in 2016 and 2018.

Materials and Methods

Survey area

Assessments of CBSD within farmers' fields were conducted during two periods in five provinces of eastern DRC. The first assessment was from November 2015 to September 2016 (hereafter referred to as 2016), and the second from March to August 2018 in Sud-Kivu, Nord-Kivu, Tanganyika and Haut-Katanga provinces in eastern DRC (Figure 1). Ituri province was only

surveyed in 2016. The total area of provinces surveyed covers 461,636 km^2 and is located between 24.75° and 31.66°E longitude and 3.96°N and 13.94°S latitude.

Assessing fields for CBSD occurrence and whitefly abundance

CBSD and whiteflies were assessed within 390 cassava farmers' fields (3 to 6 months old). A total of 201 fields were surveyed in 2016, and 189 in 2018. Geographical coordinates and altitude were recorded for each of the sampled sites with a Garmin Etrex hand-held GPS unit. Cassava fields were inspected at 5 to 10 km intervals along motorable roads, although distance often varied depending on the availability of farmer fields. Variety names were obtained and recorded from farmers. In a small proportion of cases, farmers did not know the variety name, in which case they were recorded as 'Unknown local'. In each sampled field, thirty plants selected randomly along two diagonals (15 plants per diagonal) were assessed for foliar symptoms of CBSD by scoring disease severity using a numbered scale of 1 to 5 where 1 represents no visible symptoms and 5 indicates very severe symptoms (Hillocks et al. 1996). For each diagonal, its distance was estimated, and this value was divided by 15 to give the spacing between sampled plants. All sampled plants were taken from the right-hand side of the transect walk in a systematic way to avoid any bias related to symptom expression. The two diagonals crossed at the center of the field, where care was taken to avoid sampling the same plant twice. Data obtained were eventually used to estimate mean CBSD severity and CBSD incidence values for each of the visited sites. Mean CBSD severity was the average severity score for all plants that were symptomatic (i.e. with score between 2 and 5). CBSD incidence was the percentage of plants that were symptomatic. *Bemisia tabaci* whitefly vector abundance was determined in each visited field. In order to do this, *B. tabaci* adults were counted on the undersides of the top five leaves of the tallest shoot of each of the 30 assessed plants. Total counts for these plants were averaged to produce a mean whitely abundance

value for each field (Sseruwagi et al. 2004). Within each visited field, ten leaf samples were collected for CBSIs RT-PCR testing from every third assessed plant along the two diagonals. The central leaf lobe was detached from the fifth fully expanded leaf of the shoot used for counting whiteflies and attached to a newsprint sheet using masking tape and preserved dry in a herbarium press until required for nucleic acid extraction. Collected leaf samples were transferred to and preserved in the laboratory at IITA-Kalambo, Bukavu, eastern DRC.

Diagnostics and sequencing of CBSIs samples

Samples were tested from 131 of the 201 surveyed fields in 2016, and from 134 of 189 fields in 2018. These included representative fields from all provinces surveyed. Testing focused more strongly on northern provinces in 2016 and southern provinces in 2018. Individual samples were tested for all fields in which symptoms of CBSD were observed. Where these symptoms were not observed, nucleic acid extracts of the ten samples were pooled for testing. Total RNA extraction was performed using a standardized cetyltrimethyl ammonium bromide (CTAB) method (Lodhi et al. 1994; Xu et al. 2010). For 2016, samples were analyzed through a single-step RT-PCR with the primers CBSV10 (5'-ATCAGAATAGTGTGACTGCTGG-3') and CBSV11 (5'-CCACATTATTATCGTCACCAGG-3') (Monger et al. 2001), which are used to provide a generic test of the presence of CBSIs. A 25 µL single-step RT-PCR assay was setup containing 1x M-MLV RT buffer (New England Biolabs, NEB), 200 mM dNTPs, 200 µM each primer, 1 unit of Taq DNA polymerase (NEB), 10 units of Moloney Murine Leukemia Virus Reverse Transcriptase (NEB) and 60 ng total RNA. Suitable controls were included for each batch of RT-PCR testing where positive controls were previously identified RNA samples and PCR grade water was used as a negative control. Additionally, a cytochrome oxidase I gene assay was included as an internal control to check for RNA quality (Adams et al. 2013). For species level confirmation, ten samples

selected from territories in Ituri, Sud-Kivu and Nord-Kivu provinces were tested using TaqMan real-time qRT-PCR (Adams et al. 2013; Shirima et al. 2017) at the IITA Molecular Biology Laboratory in Dar es Salaam, Tanzania. For 2018, samples were tested for CBSIs from 134 sites (both symptomatic and asymptomatic) using TaqMan real-time qRT-PCR at IITA-Kalambo, Bukavu, DRC. For the 2016 sample set, 18 CBSIs-positive samples were selected for direct PCR product sequencing. These comprised ten sequences from Aru and Mahagi territories in Ituri province, five from Rutshuru territory in Nord-Kivu province and three from Uvira territory in Sud-Kivu province. Amplicons for this purpose were generated using a two-step RT-PCR method where 826 bp (CBSV) and 732 bp (UCBSV) RT-PCR products were generated employing primer pairs: CBSV-CP-Fer2 [3'-GAAGGGATTGGAYTRGAAGGA-5']/CBSV-CP-R1-1 [3'-GAACGCGGTATCCACACATA-5'] and UCBSV-CP-F1-1 [3'-AGAGATCTGGAAAGGAAGT-5']/UCBSV-CP-R1-1 [3'-CTCGCCAYGACTTCTCATT-5'] (Shirima et al. 2017). The amplicon target for CBSV spanned a portion of the coat protein gene (CP) from nucleotide positions 7390-8216 (GenBank Accession number GQ329864) while that for UCBSV targeted the CP region of UCBSV between nucleotide positions 7689-8421 (GenBank Accession number FN434109). Sequences were generated using the standard Sanger dideoxy method at Macrogen Inc. (Rockville, Maryland, USA), edited and assembled with CLC Main Workbench v7 (Qiagen, Venlo, Netherlands).

Data analysis

QGIS 2.6.1 (https://qgis.org/en/site/) software was used to map the survey locations and maps illustrating CBSD data were produced using ArcGIS 2.16 software (ESRI, Redlands, CA). Variation among sampled variables between provinces and years was investigated using generalized linear mixed model (GLMM) ANOVA, which was run using SAS (SAS Institute,

Cary, NC, USA). The two-way ANOVA examining both year and province was run excluding the Ituri data since these were only collected in 2016. One-way ANOVA to assess variation amongst whitefly abundance as well as CBSD incidence was run separately for years 2016 and 2018 using a generalized linear mixed model approach in SAS. GLMM ANOVA tests for CBSD incidence and whitefly abundance used the Gaussian and Poisson distributions, respectively. Mean severity scores for CBSD-affected fields in different provinces were compared using the non-parametric Kruskal-Wallis one-way ANOVA on ranks procedure in which Dunn's Method was used for pairwise comparisons (SigmaStat, Systat Software Inc., San Jose, CA, USA). A phylogenetic tree was generated for CBSI sequence data using the Maximum Likelihood procedure with the Tamura-Nei model (Tamura and Nei 1993) in Mega 7.0 with default settings and 1000 bootstraps (Kumar et al. 2016). Based on the phylogenetic relationship results for sequences, the generated clades were selected to estimate similarities between the group means (Tamura et al. 2007). Eight of these sequences were published in a Disease Note (Casinga et al. 2019).

Results

Cassava brown streak disease symptoms, incidence and severity

Leaf, stem, and root symptoms (Figure 2), typical of CBSD, were observed during field inspections in eleven territories during the two survey periods. Considering only the four provinces sampled in both 2016 and 2018, 13 territories were sampled in both years, of which five (39%) had fields with CBSD in 2016, increasing to eight (62%) in 2018. There was no significant change in the incidence of CBSD from 2016 to 2018, but there were significant differences in incidence between the provinces based on an analysis of the whole dataset for both years (Table 1) ($F = 9.58$, $df = 3$, $P < 0.0001$). Considering 2016 data independently, there were significant differences in CBSD

incidence between provinces (F = 11.63, df = 4, $P < 0.0001$). Sud-Kivu and Ituri had higher incidences than both Haut-Katanga and Tanganyika (Table 1). In 2018, there were no significant differences in incidence between provinces (F = 2.36, df = 3, $P = 0.073$). CBSD was completely absent during both surveys in Tanganyika province (Figures 3 and 4). In 2016, the highest incidence was recorded in the most northerly province of Ituri (Figure 3). CBSD was only present in the northernmost part of Haut-Katanga province, in Pweto territory (Figure 3), and overall incidence in this province was very low (1.7%). The most notable change between the two survey periods was the increase in incidence in Haut-Katanga province, as it rose to 15.9% in 2018 (Table 1). Associated with this was a large increase in prevalence (percentage of fields with at least one sampled symptomatic plant) from 6/60 (10%) in 2016 to 11/36 (31%) in 2018. Significant differences were observed in CBSD foliar severity between provinces in 2016 (Table 1) (H = 22.5, df = 3, $P < 0.001$), but not in 2018. Symptoms were more severe in Ituri, Nord-Kivu and Sud-Kivu provinces than they were in Haut-Katanga (Table 1).

Varieties and responses to CBSD

Eighty-three cassava varieties were recorded during the two survey periods, of which 73 were local and 10 improved varieties released by the national research system. The 21 most frequently encountered varieties (≥ 5 fields of each) made up 66.4% of all fields (Supplementary Table 1). Only three of the improved varieties were frequently encountered, although improved Sawasawa (MM96/3920) was the second most frequent overall, after Ndelekulwa. All provinces had a similarly high degree of varietal diversity and most of the varieties encountered were confined to one or two territories within a province. The widely varying frequency with which different varieties occurred precluded an analysis of varietal interactions with whitefly abundance, CBSD incidence and CBSD severity. However, there was no clear difference in the pattern of CBSD

infection in improved versus local varieties (Supplementary Table 1). The highest incidence of CBSD was recorded for variety Nyagota, in Ituri province (77.2%). This variety also had the most severe CBSD symptoms (3.62; Supplementary Table 1). Two commonly grown varieties - Mwabayilo and Nyamwalirwa - had no CBSD, despite being cultivated in Sud-Kivu province in which CBSD was otherwise widespread.

Whitefly abundance

Bemisia tabaci whitefly adults occurred throughout sampled regions at moderate to low abundances but there was no consistent regional pattern of abundance (Table 1). Statistical analysis of the data revealed significant differences between provinces ($F = 9.86$, $df = 3$, $P < 0.0001$) as well as between years ($F = 122.1$, $df = 1$, $P < 0.0001$); there were more whiteflies in 2016 than in 2018. In 2016, *B. tabaci* was most abundant in Ituri and Haut-Katanga provinces and least abundant in Tanganyika. In 2018, abundance was greatest in Nord-Kivu and least in Haut-Katanga. Abundance of *B. tabaci* whiteflies varied greatly from field to field. 66.2% of fields had mean abundance values of less than 1, while 5.1% had abundance greater than 20 adults per top five leaves. The only region that provided an exception to this pattern was the far south-eastern portion of Haut-Katanga province, in which all fields sampled had low abundance of *B. tabaci* whiteflies.

Occurrence and phylogenetic relationship of CBSIs in eastern DR Congo

PCR testing confirmed the presence of CBSIs for virtually all territories in which CBSD symptoms were observed in both 2016 and 2018 (Table 2). The only exceptions to this were a single field in Djugu territory, Ituri province in 2016 where CBSIs were detected in the absence of clear CBSD symptoms, as well as two fields in Kalehe territory, Sud-Kivu province where CBSIs were not detected when CBSD symptoms were observed.

In 2016, both CBSV and UCBSV were detected in Ituri province (Mahagi territory), while only UCBSV was present in Aru territory (Ituri province), in Sud-Kivu province (Uvira territory) and in Rutshuru territory in Nord-Kivu province (Figure 5). UCBSV was detected in all 10 fields (100%) from which qPCR results were obtained while CBSV was only recorded from two fields (20%) in Mahagi territory. CBSV occurred only as mixed infections with UCBSV in both fields, and not singly. In 2018, single infections of UCBSV were detected in three territories of Nord-Kivu (Masisi, Rutshuru and Nyiragongo) (Figure 6). Only single infections of CBSV were detected in Pweto territory in Haut-Katanga and Kabare territory in Sud-Kivu, while both viruses occurred in Walungu, Uvira and Fizi territories in Sud-Kivu (Figure 6). Neither CBSV nor UCBSV were detected in either year in Tanganyika province. Twenty-eight of the 38 detections in 2018 were CBSV (74%) compared to 13 for UCBSV (34%). There was therefore a large increase overall in the proportion of CBSV infected fields in 2018 (74%) compared to 2016 (20%), although the number of qPCR tests was much less in 2016. In three fields in Walungu, Uvira and Fizi, both viruses were detected, but in each case different virus species were present in different plants (i.e. there were no dual infections).

Phylogenetic analysis of 12 CBSV and UCBSV coat protein sequences obtained from Ituri, Nord-Kivu and Sud-Kivu confirmed that all were closely related to UCBSV or CBSV sequences from locations in East and Southern Africa (Figure 7). UCBSV sequences from Nord-Kivu (Rutshuru) and Ituri fell within a single large clade that also includes sequences from Uganda, coastal Kenya, coastal Tanzania and Malawi. All of the UCBSV sequences from the current study shared greater than 99% homology with nearest relatives in GenBank (Figure 7) with the exception of Uvira553 (MF511057) which was 98.4% identical to a sequence obtained from Mtwapa, coastal Kenya. The two CBSV sequences from this study were identical and fitted clearly within the major CBSV

246 species clade. They were most closely related to a sequence from Namulonge, south-central
247 Uganda (KJ606250) with which they share 97.3% homology. Previously unpublished sequences
248 described in this study have been deposited in GenBank with the accession codes (MF511057-
249 MF511060). Sequences presented in Figure 7 with their GenBank codes are: MF511057
250 (Uvira553), MF511058 (Rutshuru586), MF511059 (Rutshuru27), MF511060 (Rutshuru9),
251 MF511061 (Rutshuru4), MF511062 (Ituri574), MF511063 (Ituri573), MF511064 (Ituri572),
252 MF511065 (Ituri568), MF511066 (Ituri562u), MF511067 (Ituri562c), MF511068 (Ituri557).

253

254 ## Discussion

255 Cassava brown streak disease is a growing problem affecting cassava production in eastern,
256 southern and central Africa. Although it was confined to coastal eastern Africa for several decades
257 following its first report from coastal Tanzania (Storey 1936), since 2004 it has spread widely
258 through mid-altitude zones of East and Central Africa (Alicai et al. 2007; Legg et al. 2011, 2015).
259 The first confirmation of the occurrence of CBSD in DRC, supported by molecular diagnostic
260 tests, was made from cassava leaf samples collected in Lubero territory, Nord-Kivu province
261 (Mulimbi et al. 2012). Our study reports the results of the first comprehensive region-wide survey
262 of CBSD in eastern DRC. Monitoring the pattern of development of CBSD in this part of Africa
263 is particularly critical, since cassava is the most important staple food crop in central Africa,
264 including DRC. Additionally, there is a high risk that CBSD will spread from central to West
265 Africa through DRC. The country straddles the center of the continent, and spread of CBSD from
266 the East to the West of the country would pose a major threat to the important cassava-producing
267 countries of West Africa, notably Nigeria, which is the world's largest cassava producer.

Symptoms of CBSD observed during the surveys that ran from late 2015 to 2018 were typical of those associated with the disease in other parts of East and Central Africa, and as originally described from Tanzania by Nichols (1950). Symptoms resembling those caused by CBSD have been reported widely from different parts of Central and West Africa, including western DRC (Mahungu et al. 2003). However, in all such cases, symptoms have been confined to the tuberous roots, and characteristic foliar and stem symptoms have not been described. In the study reported here, all of the three main foliar symptoms (veinal chlorosis or chlorotic blotching on lower leaves, necrotic lesions on green stems, and stem die-back in severe infections) were observed in all territories where the presence of CBSD was also confirmed through virus diagnostic tests. New reports made here confirm the expansion of the area known to be affected by CBSD to two additional provinces (Sud-Kivu and Haut-Katanga) and nine additional territories. Administrative zones reported as affected for the first time cover a total area of more than 62,000 km^2. Additionally, affected areas of Pweto territory, in south-eastern DRC, are more than 900 km away from the locations of previously published reports of CBSD in the country. As such, the prevalence of CBSD reported here represents a major expansion of the CBSD pandemic in Africa. It is significant that Pweto territory shares a border with northern Zambia, another country/region that has reported a recent outbreak of CBSD (Mulenga et al. 2018). The surveys reported here represent the first geographically extensive record of CBSD in DRC. As such, the data obtained provide an important baseline against which future assessments of the disease and its epidemiology can be measured. They also provide an important complement to country or region-wide assessments of CBSD, including Tanzania (Jeremiah et al. 2015; Legg and Raya 1998), Mozambique (Hillocks et al. 2002), Rwanda (Munganyinka et al. 2018) and Uganda (Alicai et al. 2019), which contribute to building a continental picture of the development of the CBSD pandemic.

The lower severity of CBSD symptoms in Haut-Katanga province may be a consequence of the more recent infection of many of the fields in this region, as evidenced by the large increase in incidence from 2016 to 2018. CBSD is known to become more severe over repeated cropping cycles, resulting in 'degeneration' of cassava varieties (Shirima et al. 2019). It is also possible, however, that this effect is related to varietal interactions or virus strain variation (Mohammed et al. 2012). Varieties differ greatly in their responses to CBSIs (Kaweesi et al. 2014; Shirima et al. 2019), and patterns of distribution of varieties can influence CBSD incidence and severity in the regions in which they occur (Ndyetabula et al. 2016). Also, strains of CBSIs are known to vary in the severity of symptoms that they induce (Mohammed et al. 2012) and Haut-Katanga was unique as a province in which only CBSV was detected. Further investigation would be required to determine which of these possible causes are responsible for the symptom severity differences observed.

In common with most important cassava-growing regions of sub-Saharan Africa, there was a high level of varietal diversity in the eastern provinces of DRC, although about a quarter of these accounted for two-thirds of the sampled fields. Both local and improved varieties in farmers' inspected fields were infected and susceptible to CBSIs. Two varieties (Mwabayilo and Nyamwalirwa) had no symptoms of infection even though they were widely cultivated in Sud-Kivu where CBSD was otherwise widespread. This provides a suggestion that they may be relatively more resistant to CBSD than other varieties being grown in the same area, although varietal comparison experiments planted at a single location with known inoculum pressure conditions would be required to verify this possibility. An important practical implication of the increased geographical coverage and incidence of CBSD is that cassava varieties possessing both whitefly and disease resistances are urgently needed. Previous initiatives to facilitate the

314 introduction and mass dissemination of CMD-resistant varieties in eastern DRC were undertaken
315 as part of the Great Lakes Cassava Initiative (GLCI), which ran from 2007 to 2012 (CRS, 2012).
316 More than 17 million stems of these varieties were distributed in Burundi, DRC, Kenya, Rwanda,
317 Tanzania and Uganda as planting material during this time, reaching more than 800,000 farmer
318 beneficiaries. Six of the ten improved CMD-resistant varieties recorded from the current survey
319 were distributed during this initiative (Sawasawa (MM96/3920), Sukisa (MM96/1666), Liyayi
320 (MM96/0287), Mayombe (MM96/7752) and Nabana (MM96/4653)) and one more Obama (TME
321 419) was evaluated in participatory varietal selection trials. These seven were the primary varieties
322 being cultivated at 49 of the surveyed sites, equivalent to 12.6% of the total, and one or more were
323 present in all of the provinces. Although these have clearly had a significant impact in sustaining
324 cassava production in eastern DRC over the last decade, it has been widely recognized in recent
325 years that varieties resistant to CMD are mostly susceptible to CBSD (Alicai et al. 2007; Legg et
326 al. 2006). This phenomenon was supported by our data, although there was no evidence to suggest
327 that the improved CMD-resistant varieties were more or less susceptible to CBSD than local
328 varieties; the two varieties with highest CBSD incidences and most severe symptoms were both
329 local varieties. Although there were several widely cultivated varieties without any incidence of
330 CBSD (Supplementary Table 1), it is not possible to conclude from these survey results that any
331 of these had resistance to CBSD. Controlled experiments would be required to verify this
332 possibility. Studies of this type have been conducted in coastal Tanzania and used to identify
333 several local varieties with promising levels of resistance to CBSD (Masinde et al. 2018). Most of
334 the current cassava improvement focus in Africa, however, is aimed at combining sources of
335 resistance to both CMD and CBSD (Kawuki et al. 2016), as well as using transgenic approaches

to engineer CBSD resistance into varieties that already carry resistance to CMD (Wagaba et al. 2017).

Although overall abundance of *B. tabaci* was moderate to low during the periods of the surveys in 2016 and 2018, 15% of fields had 'abundant' whitefly populations (*sensu* Legg et al. 2011), and these fields occurred throughout the surveyed area. In addition, a substantial proportion (55%) of the sampling was conducted during cool dry seasons (May-September), which may have contributed to the overall moderate to low whitefly abundances: cassava-colonizing *B. tabaci* is known to thrive during periods of hot, moist weather (Dengel 1981; Fargette et al. 1993). Increased abundance of *B. tabaci* whiteflies has been widely recognized as a major factor driving CBSD spread in East and Central Africa (Hillocks et al. 1999; Legg et al. 2011).

UCBSV as the causal virus of CBSD was first reported from two territories of Nord-Kivu in eastern DRC from surveys conducted in late 2011 (Mulimbi et al. 2012). CBSD was clearly very rare at that time as UCBSV was only detected from two out of 100 farmer fields surveyed in Beni and Lubero territories. More recently, the occurrence of both CBSV and UCBSV was reported further north from Ituri province (Casinga et al. 2019). Results presented in our study greatly expand the known distribution of CBSIs in DRC. This includes the new reporting of UCBSV from Sud-Kivu, the expanded presence of UCBSV in Nord-Kivu (including Rutshuru, Masisi and Nyiragongo territories), as well as the new occurrence of CBSIs in Haut-Katanga province (CBSV). This information on patterns of occurrence of these two viruses reflects the general situation elsewhere from Kenya, Tanzania and Uganda, in which both species are reported from most regions affected by CBSD. Both viruses are reported from Kenya (Adams et al. 2013), Malawi (Mbewe et al. 2015), Tanzania (Mbanzibwa et al. 2010) and Uganda (Kaweesi et al. 2014), although exceptions to this pattern are Mozambique from which only CBSV has been detected (Amisse et al. 2019;

359 Mohammed et al. 2012) and Burundi where only UCBSV has been identified (Bigirimana et al.
360 2011). However, given the occurrence of CBSV in countries surrounding Burundi, including
361 Rwanda, Tanzania and DRC, it seems highly likely that CBSV will also spread there in the near
362 future.

363 Sequence data revealed that UCBSV sequences from eastern DRC were most closely related to
364 UCBSV isolates from central, southern Uganda and that there was a high degree of homology
365 within the DRC sequences themselves. The generally high level of homology amongst UCBSV
366 sequences from diverse locations throughout East and Central Africa, however, means that it
367 cannot be assumed that these isolates, now in DRC, originated directly from Uganda. However,
368 the cryptic nature of CBSD symptoms means that infected planting material can be inadvertently
369 moved from place to place, and there have been both unofficial and official large-scale movements
370 of cassava stems between countries in East and Central Africa that may have unintentionally
371 resulted in the spread of CBSIs within the region. CBSV sequences were more divergent from
372 nearest relatives in the GenBank database. These findings are unsurprising, as an analysis of a
373 large group of sequences from East and Southern Africa, revealed a generally higher level of
374 variability amongst CBSV isolates than UCBSV (Mbanzibwa et al. 2011). Future studies will be
375 required, however, to examine a larger set of both UCBSV and CBSV isolates from eastern and
376 south-eastern DRC in order to improve the resolution in the understanding of CBSI diversity in
377 DRC.

378 The CBSD surveillance study described here has provided an important update on the status of
379 this highly damaging disease of cassava. Evidence was obtained for an increase in the occurrence
380 of the disease from 2016 to 2018, particularly in the southern part of the sampling domain, in Haut-
381 Katanga. Central DRC is a sparsely populated forest, which will impede the westwards spread of

382 the disease. However, regions to the west of Haut-Katanga, including Haut-Lomami and Lualaba
383 are important savanna-based cassava-growing regions, and may provide a route through which
384 CBSD can be carried further westwards. CBSD management strategies for East Africa have
385 comprised both the development/deployment of resistant varieties (Jennings 1960; Kawuki et al.
386 2016) as well as the application of phytosanitary measures (Hillocks and Jennings 2003; Legg et
387 al. 2017). The implementation of such strategies is urgently required for the provinces of eastern
388 DRC currently affected. Arguably more important for the mitigation of the CBSD pandemic,
389 however, will be the prevention of spread to provinces further to the west, as this increases the
390 threat that the disease poses to important production centers feeding the metropolis of Kinshasa,
391 as well as other key cassava-producing zones in Central/West Africa. To achieve this, government
392 institutions, NGOs and international partners will need to put in place systems to raise awareness
393 of the threat posed by CBSD, monitor its spread, prevent movements of planting material from
394 east to west, and to identify, multiply and disseminate CBSD-resistant varieties.

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402

403 # Figure legends

404

405 **Figure 1.** Geographical location of survey sampling sites in eastern Democratic Republic of Congo
406 (DRC). The dark shaded area corresponds to the group of provinces in eastern DRC that were
407 included in the surveys. 201 fields were sampled in 2016 (yellow markers) and 189 in 2018 (blue
408 markers).

409

410 **Figure 2.** Cassava brown streak disease (CBSD) symptom expression on leaves and tuberous roots
411 in eastern Democratic Republic of Congo with a) Healthy cassava plant leaves, b) CBSD foliar
412 symptoms, c) CBSD root constriction and necrotic rot symptoms, d) CBSD stem lesions, e) CBSD
413 shoot dieback.

414

415 **Figure 3.** Cassava brown streak disease incidence (%) in eastern Democratic Republic of Congo
416 in 2016.

417

418 **Figure 4.** Cassava brown streak disease incidence (%) in eastern Democratic Republic of Congo
419 in 2018.

420

421 **Figure 5.** Occurrence of Cassava brown streak ipomoviruses (*Cassava brown streak virus* and
422 *Ugandan cassava brown streak virus*) in eastern Democratic Republic of Congo in 2016.

423

424 **Figure 6.** Occurrence of Cassava brown streak ipomoviruses (*Cassava brown streak virus* and
425 *Ugandan cassava brown streak virus*) in eastern Democratic Republic of Congo in 2018.

426

427 **Figure 7.** Phylogenetic tree of Cassava brown streak virus (CBSV) and Ugandan cassava brown
428 streak virus (UCBSV) sequences generated through a maximum likelihood analysis method using
429 the Tamura-Nei model with 1000 bootstraps (Tamura and Nei 1993). CBSV and UCBSV coat
430 protein specific primers were used to produce amplicons and nucleotide sequences were obtained
431 by direct sequencing of PCR products. DRC sequences from this study are shown in blue (CBSV)
432 and black (UCBSV).

433

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621 **Table 1**. Incidence and severity of cassava brown streak disease and whitefly abundance in surveyed

622 fields of cassava in the provinces of eastern Democratic Republic of Congo: 2016 and 2018

	2016				2018			
	Number of fields	**Whitefly Abundance[y, z]**	**CBSD Incidence[z]**	**CBSD Severity[z]**	**Number of fields**	**Whitefly Abundance[z]**	**CBSD Incidence[z]**	**CBSD Severity**
Haut-Katanga	60	7.41 a	1.67 b	2.03 b	36	0.87 c	15.93 ab	2.97
Ituri	36	8.15 a	30.09 a	3.24 a	NS	NS	NS	NS
Nord-Kivu	12	4.17 b	16.11 ab	3.32 a	29	3.32 a	24.02 a	2.78
Sud-Kivu	52	5.04 b	24.88 a	2.92 a	106	1.75 b	16.76 ab	2.93
Tanganyika	41	2.24 c	0.00 b	ND	18	1.41 bc	0.00 b	ND
***Total field no.* or average**	***201***	**5.68**	**13.29**	**2.92**	***189***	**1.79**	**16.12**	**2.90**
Four province average[x]		**4.72**	**10.67**	**2.76**		**1.84**	**14.18**	**2.89**

623 [x] Average value of the province means for the four provinces that were surveyed in both 2016 and 2018

624 [y] Whitefly abundance is the mean number of adult whiteflies per plant counted on 30 plants in each surveyed field.

625 For each sampled plant, whiteflies were counted on the undersides of the top five leaves of the tallest shoot.

626 [z] Mean values for provinces with different letters were different at the $P < 0.05$ level

627 ND – No disease

628 NS – Not surveyed

629

630

631 **Supplementary Table 1**. CBSD incidence (%), CBSD severity and whitefly abundance recorded in the
632 most frequently occurring (≥ 5 fields) local and improved (I) varieties in eastern Democratic Republic of
633 Congo during surveys conducted in 2016 and 2018

Province [y]	Variety name	Number of fields	Whitefly abundance	CBSD incidence (%)	CBSD severity (1-5)
Haut-Katanga (18/99)	Local unknown	6	0.28	25.5	2.90
	Ndelekulwa[z2]	38	5.01	0.6	2.53
	Shilefasi[z2]	26	5.44	22.6	2.82
	Mwakamoya	9	0.80	0	1.00
	Njongo	6	0.07	0	1.00
Ituri (17/36)	Nyagota	6	4.80	77.2	3.62
	Olamu	6	12.40	30.8	3.10
Nord-Kivu (10/41)	Ndakuoweya[z2]	16	3.58	28.3	2.77
Sud-Kivu (41/157)	Liyayi (I)[z2]	6	0.72	45.6	2.97
	Kumigulu	6	3.32	11.7	3.00
	Mushikuzi[z2]	18	0.42	6.5	3.02
	Musikilo	5	2.47	21.3	2.80
	Mvuama (I)	13	1.57	19.8	2.95
	Mwabayilo	10	1.11	0	1.00
	Nambiyombiyo	15	0.30	0.7	2.67
	Nyamwalirwa	7	1.80	0	1.00
	Sawasawa (I)[z2]	28	5.29	21.2	2.95

	Wamilo	6	0.57	74.4	3.02
Tanganyika (12/57)	Kibibi	5	0.15	0	1.00
	Ngululu[z2]	8	0.33	0	1.00
	Wilalanga[z3]	17	1.51	0	1.00

[y]Numbers in parentheses indicate the number of different varieties over the total number of fields for each of the provinces.

[z]Several of the varieties occurred in more than one province. For each of these cases, average results for all fields of that variety are recorded under the province in which the variety occurred most frequently. The z number indicates the number of provinces in which the variety occurred.

639 **Table 2.** Frequency of fields surveyed in 2016 and 2018 in eastern Democratic Republic of Congo in which CBSD symptoms were present or
640 absent, and for which positive or negative test results were obtained from single step RT-PCR and qRT-PCR diagnostic tests.

Province	Territory	CBSD affected	CBSD free	PCR positive	PCR negative	Not tested	CBSD affected	CBSD free	Not tested	qPCR positive	qPCR negative
		2016					2018				
Ituri	Mambasa	0	6	0	6	0	NS	NS	NS	NS	NS
	Irumu	0	7	0	7	0	NS	NS	NS	NS	NS
	Mahagi	7	8	8	7	0	NS	NS	NS	NS	NS
	Aru	5	2	7	0	0	NS	NS	NS	NS	NS
	Djugu	0	1	1	0	0	NS	NS	NS	NS	NS
Nord-Kivu	Masisi	0	7	0	7	0	5	12	16	1	0
	Rutshuru	4	0	4	0	0	7	0	1	6	0
	Nyiragongo	0	1	0	1	0	1	4	2	2	1
Sud-Kivu	Kabare	0	7	0	2	5	1	18	4	1	14
	Walungu	2	3	2	1	2	7	16	12	5	6
	Uvira	9	1	1	0	9	8	1	5	4	0
	Fizi	21	9	7	10	13	12	10	13	9	0

	Kalehe	NS	NS	NS	NS	NS	2	13	0	0	15
	Idjwi	NS	NS	NS	NS	NS	0	18	0	0	18
Tanganyika	Kalemie	0	19	0	19	0	0	18	0	0	18
	Moba	0	22	0	22	0	NS	NS	NS	NS	NS
Haut-Katanga	Pweto	5	27	1	18	13	12	6	2	10	6
	Kasenga	0	17	NT	NT	17	0	5	0	0	5
	Kipushi	0	5	NT	NT	5	0	9	0	0	9
	Sakania	0	2	NT	NT	2	0	3	0	0	3
	Kambove	0	4	NT	NT	4	0	1	0	0	1
TOTAL		**53**	**148**	**31**	**100**	**70**	**55**	**134**	**55**	**38**	**96**

641 PCR positive – Fields in which positive test results were obtained with single step RT-PCR diagnostic tests

642 PCR negative – Fields in which only negative test results were obtained with single step RT-PCR diagnostic tests

643 qPCR positive – Fields in which positive test results were obtained with qRT-PCR diagnostic tests

644 qPCR negative – Fields in which only negative test results were obtained with qRT-PCR diagnostic tests

645 NT – Not tested

646 NS – Not surveyed

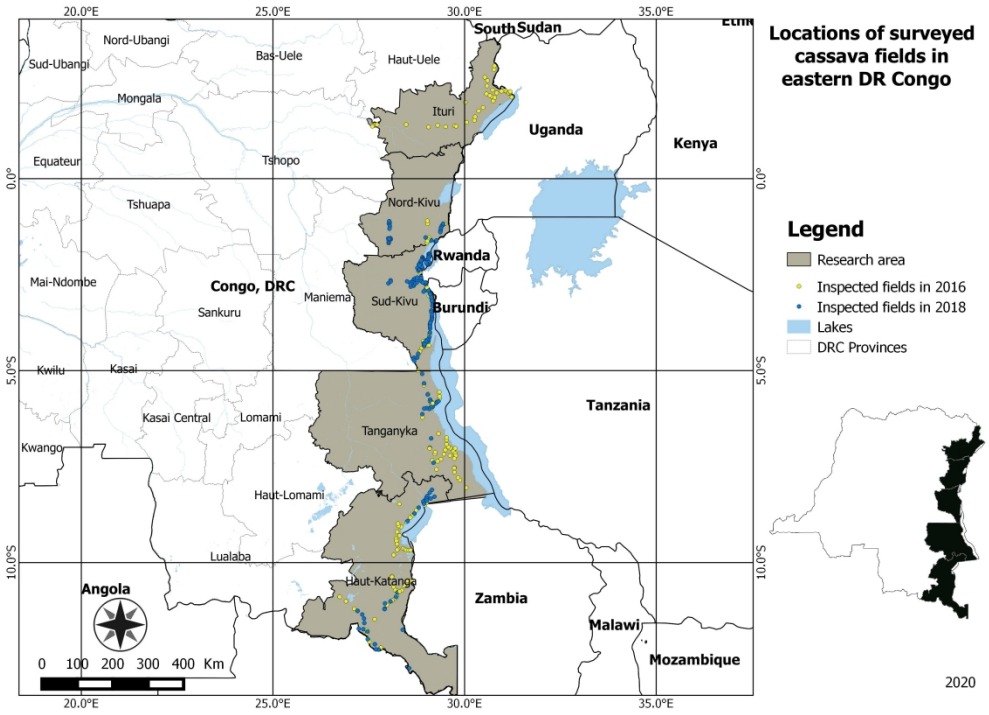


Figure 1. Geographical location of survey sampling sites in eastern Democratic Republic of Congo (DRC). The dark shaded area corresponds to the group of provinces in eastern DRC that were included in the surveys. 201 fields were sampled in 2016 (yellow markers) and 189 in 2018 (blue markers).

296x209mm (300 x 300 DPI)



Figure 2. Cassava brown streak disease (CBSD) symptom expression on leaves and tuberous roots in eastern Democratic Republic of Congo with a) Healthy cassava plant leaves, b) CBSD foliar symptoms, c) CBSD root constriction and necrotic rot symptoms, d) CBSD stem lesions, e) CBSD shoot dieback.

338x89mm (120 x 120 DPI)

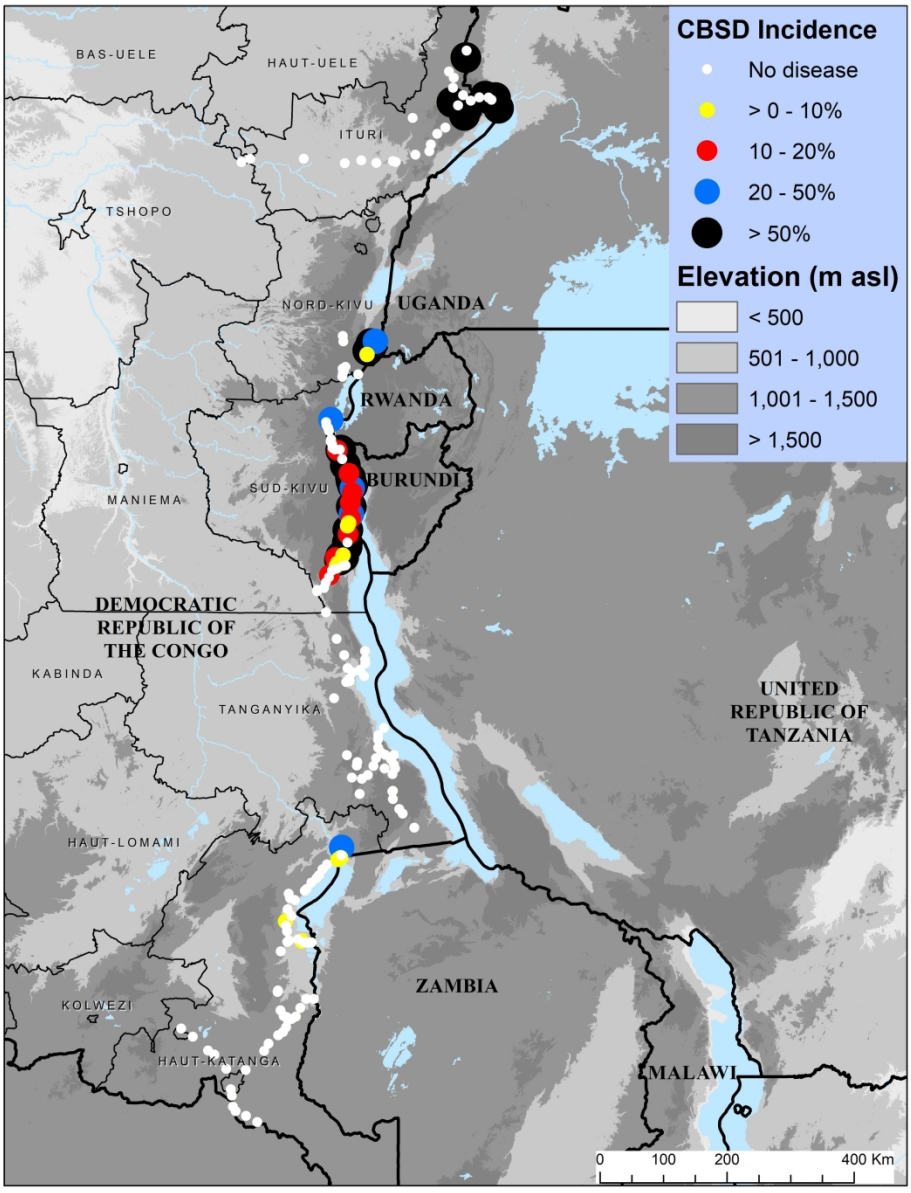


Figure 3. Cassava brown streak disease incidence (%) in eastern Democratic Republic of Congo in 2016.

215x279mm (300 x 300 DPI)

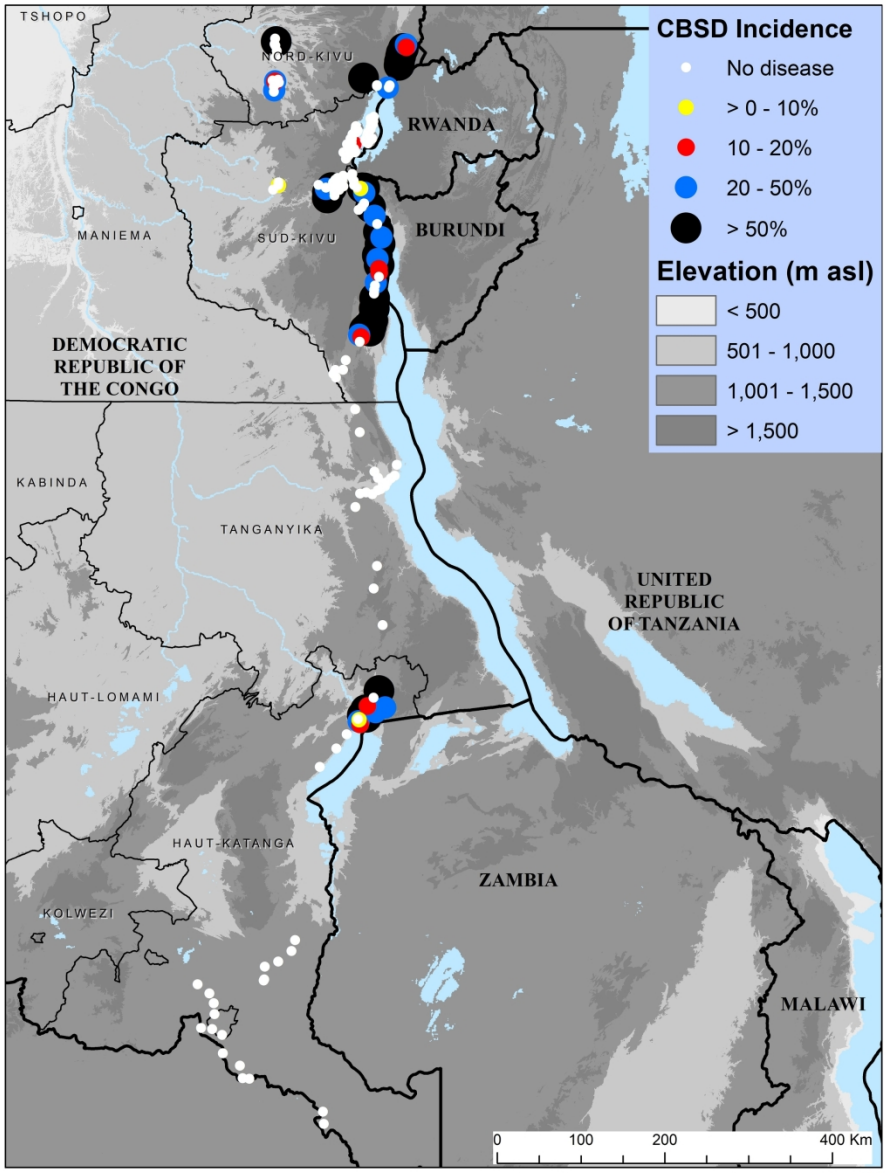


Figure 4. Cassava brown streak disease incidence (%) in eastern Democratic Republic of Congo in 2018.

215x279mm (300 x 300 DPI)

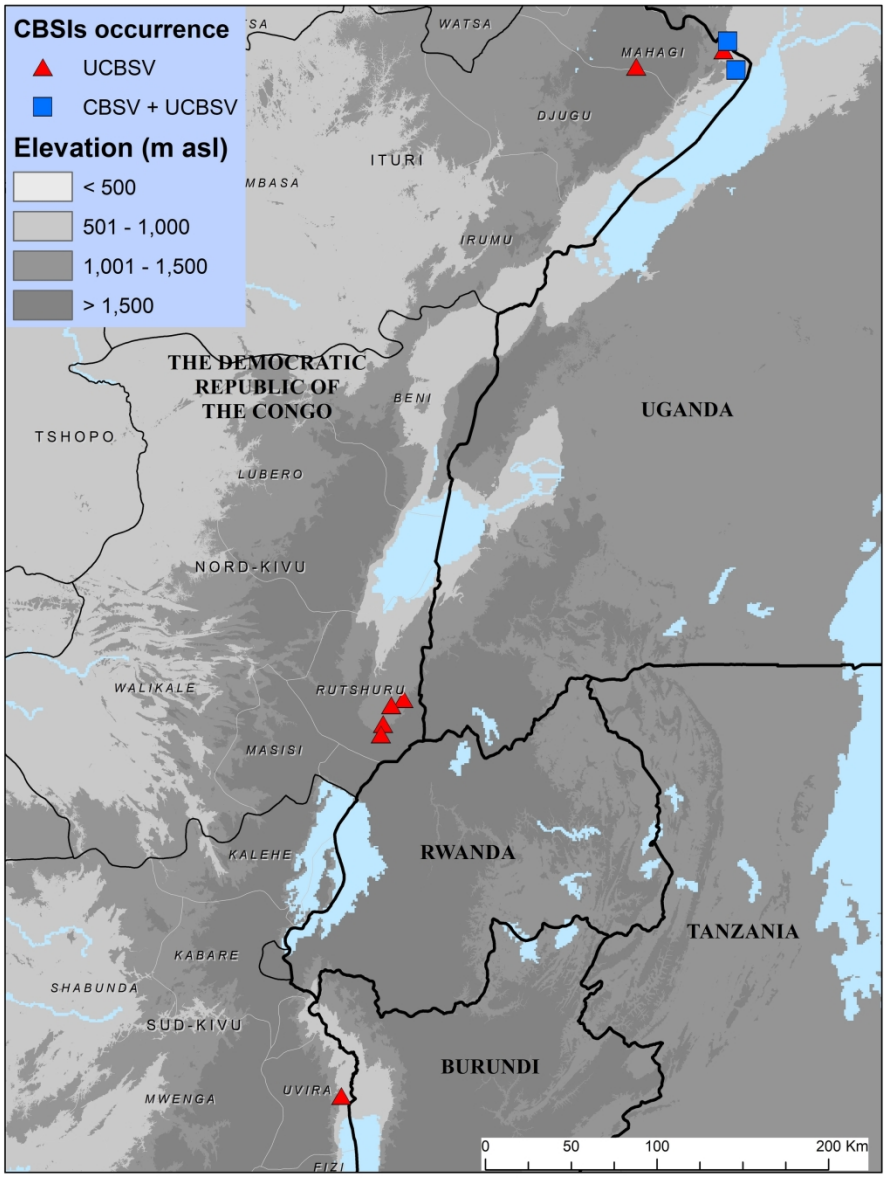


Figure 5. Occurrence of Cassava brown streak ipomoviruses (Cassava brown streak virus and Ugandan cassava brown streak virus) in eastern Democratic Republic of Congo in 2016.

215x279mm (300 x 300 DPI)

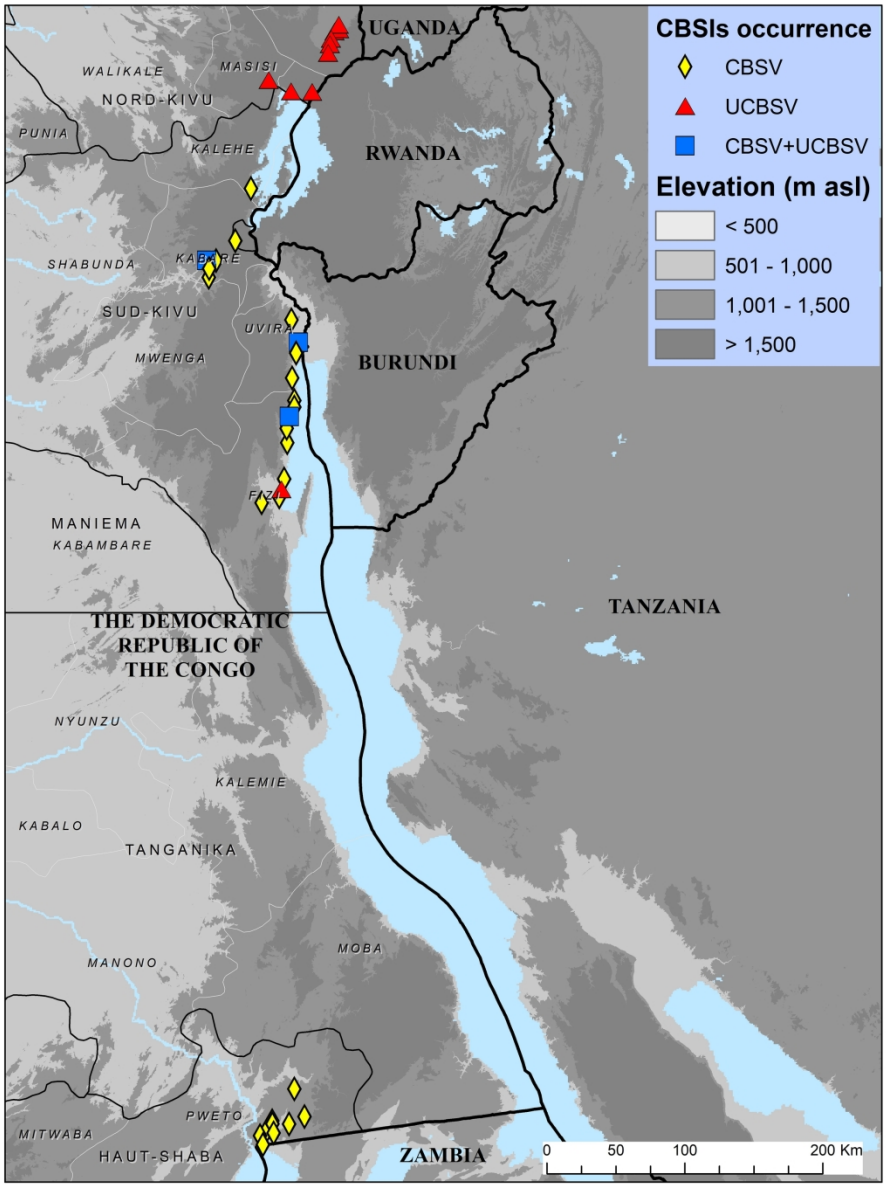


Figure 6. Occurrence of Cassava brown streak ipomoviruses (Cassava brown streak virus and Ugandan cassava brown streak virus) in eastern Democratic Republic of Congo in 2018.

215x279mm (300 x 300 DPI)

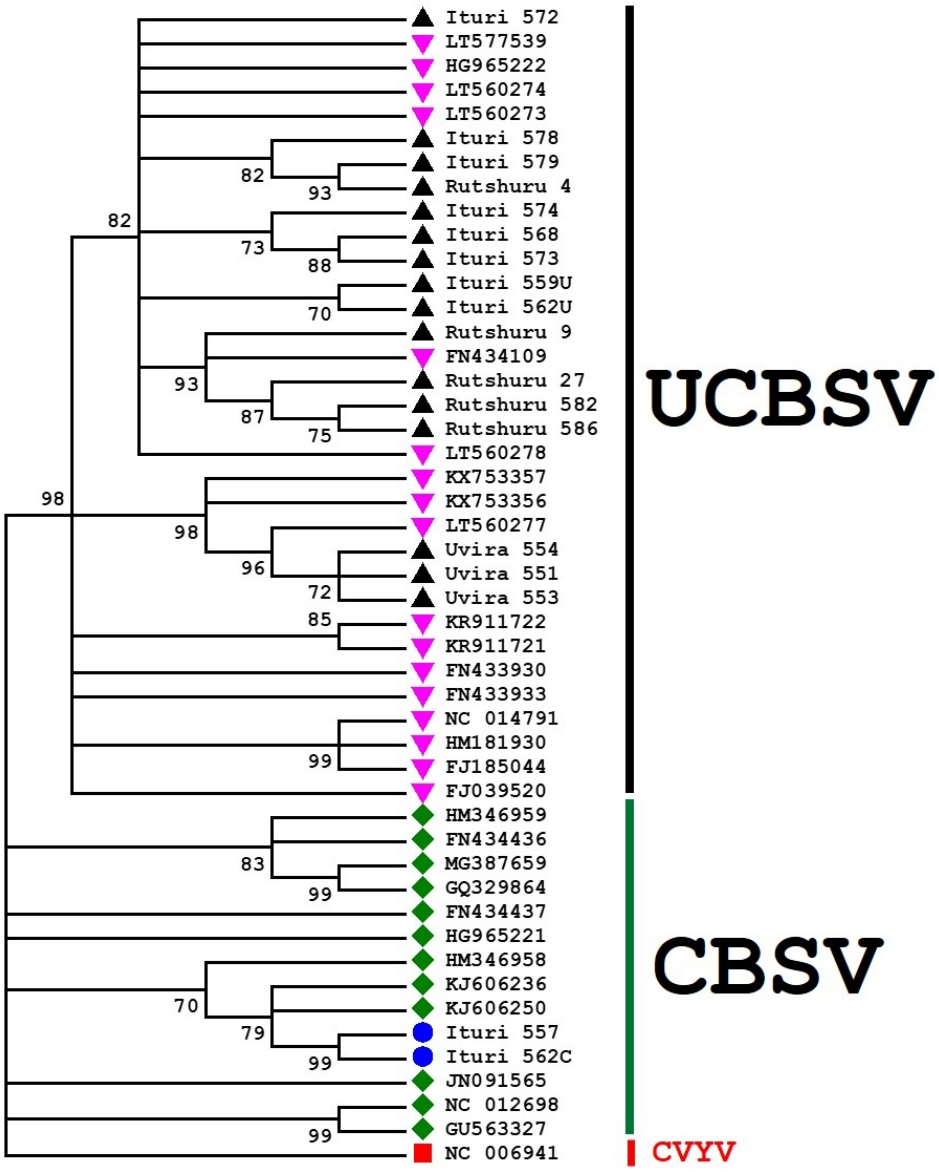


Figure 7. Phylogenetic tree of Cassava brown streak virus (CBSV) and Ugandan cassava brown streak virus (UCBSV) sequences generated through a maximum likelihood analysis method using the Tamura-Nei model with 1000 bootstraps (Tamura and Nei 1993). CBSV and UCBSV coat protein specific primers were used to produce amplicons and nucleotide sequences were obtained by direct sequencing of PCR products. DRC sequences from this study are shown in blue (CBSV) and black (UCBSV).

161x193mm (150 x 150 DPI)

Supplementary Table 1. CBSD incidence (%), CBSD severity and whitefly abundance recorded in the most frequently occurring (≥ 5 fields) local and improved (I) varieties in eastern Democratic Republic of Congo during surveys conducted in 2016 and 2018

Province [y]	Variety name	Number of fields	Whitefly abundance	CBSD incidence (%)	CBSD severity (1-5)
Haut-Katanga (18/99)	Local unknown	6	0.28	25.5	2.90
	Ndelekulwa[z2]	38	5.01	0.6	2.53
	Shilefasi[z2]	26	5.44	22.6	2.82
	Mwakamoya	9	0.80	0	1.00
	Njongo	6	0.07	0	1.00
Ituri (17/36)	Nyagota	6	4.80	77.2	3.62
	Olamu	6	12.40	30.8	3.10
Nord-Kivu (10/41)	Ndakuoweya[z2]	16	3.58	28.3	2.77
Sud-Kivu (41/157)	Liyayi (I)[z2]	6	0.72	45.6	2.97
	Kumigulu	6	3.32	11.7	3.00
	Mushikuzi[z2]	18	0.42	6.5	3.02
	Musikilo	5	2.47	21.3	2.80
	Mvuama (I)	13	1.57	19.8	2.95
	Mwabayilo	10	1.11	0	1.00
	Nambiyombiyo	15	0.30	0.7	2.67
	Nyamwalirwa	7	1.80	0	1.00
	Sawasawa (I)[z2]	28	5.29	21.2	2.95

	Wamilo	6	0.57	74.4	3.02
Tanganyika (12/57)	Kibibi	5	0.15	0	1.00
	Ngululu[z2]	8	0.33	0	1.00
	Wilalanga[z3]	17	1.51	0	1.00

[y]Numbers in parentheses indicate the number of different varieties over the total number of fields for each of the provinces.

[z]Several of the varieties occurred in more than one province. For each of these cases, average results for all fields of that variety are recorded under the province in which the variety occurred most frequently. The z number indicates the number of provinces in which the variety occurred.