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Survey of cassava mosaic and brown streak diseases and their vector whiteflies in Malawi

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Abstract

Cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) are the two major threats to cassava production in eastern and southern Africa. There have been several interventions over the years in the fight against the two diseases. Whether these interventions have been effective or not can best be answered by continuous and periodic assessment of the diseases. This study was designed to determine the severity, incidence, and distribution of CMD and CBSD and their vector whiteflies in Malawi. Diagnostic surveys were carried out in 16 districts to assess CMD and CBSD parameters on the leaves in 2018. The magnitude of damage on cassava roots caused by CBSD was assessed in 12 districts. The study was designed also to identify alternative hosts for CMD and CBSD. The national mean CMD severity and incidence were 1.6 and 32%, respectively. The national mean CBSD severity and incidence were 1.3 and 75%, respectively. The highest number of whiteflies were recorded in Mangochi district with about 1.8 adult whiteflies per plant in farmer's fields. There was positive correlation between disease incidence, severity and the number of adult whiteflies for both CMD and CBSD. CMD and CBSD incidence, severity and number of adult whiteflies negatively correlated with altitude. At national level, severity and incidence were higher for CMD than CBSD. CBSD foliar and root incidence and symptom severity were prominent along the shores of lake Malawi. In the contrary, CMD was not confined to the lakeshore areas. Karonga district had the highest CBSD root severity (2.1) and incidence (56%). The national mean percentage of cassava root necrosis was 5.3%. Based on these estimates, Malawi lost about US$ 42.7 million in 2018 due to CBSD root necrosis alone which could be extrapolated to the current cassava production systems. Therefore, it is imperative to improve disease management efforts while also considering the confirmed presence of alternative hosts for CMD in Malawi.

Keywords Cassava mosaic disease · Cassava brown streak disease · Incidence · Severity · Whiteflies

1 Introduction

In Malawian context, cassava can best be described as a reliably drought tolerant crop currently severely affected by cassava mosaic disease (CMD) and cassava brown streak disease (CBSD). Severe symptoms of CMD which go together with reduced vegetative growth is closely related to reduction in root yield [1]. Losses due to CMD in Eastern and Central Africa have been estimated at US$ 1.9–2.7 billion annually [2]. CBSD invaded Malawi prior to the 1950s [3]. Economic losses associated with CBSD in Malawi are estimated at US$ 6–7 million [4]. Combined annual losses of up to US$D 750 million in Malawi, Tanzania, Kenya, and Uganda have been reported [5].

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CMD and CBSD have spread to southern Africa over the course of time [6–8]. CMD is caused by eleven distinct begomoviruses [2, 9] while CBSD is caused by two single stranded RNA (ssRNA) viruses in the family *Potyviridae* and genus *Ipomovirus* which are *Cassava brown streak virus* (CBSV) and *Ugandan cassava brown streak virus* (UCBSV) [6, 10, 11] and they are both simply known as cassava brown streak ipomoviruses (CBSIs) [12]. Both CMD and CBSD are spread through whiteflies vector, *Bemisia tabaci* (Gennadius) [13] while long distance spread is mainly done through movement of infected planting materials [14, 15] which farmers usually share and this makes the control of the diseases difficult.

Epidemiological studies have been carried out in Malawi before to understand the spatial distribution and extent of damage caused by CMD and CBSD. However, studies concerning disease epidemics and vector abundance ought to be carried out at regular intervals to be able to predict outbreaks or upsurges of diseases and vectors. With results from such studies, for instance, CBSD epidemic was predicted to occur between 3 and 12 years after a surge in the number of whiteflies [16]. The previous studies on CMD and CBSD distribution in Malawi were conducted in 2010 [8, 17]. The current study included seven districts of Lilongwe, Mchinji, Mangochi, Ntcheu, Blantyre, Chikwawa and Nsanje which were not included in the previous studies. Government agricultural interventions and human farming behaviors might have altered the status of CMD and CBSD over the course of time. The current study estimated production and economic losses caused by CBSD. The geographical spread of the diseases has been accelerated by the movement of diseased planting materials from one location to another [18]. This study was initiated to determine the geographical distribution and extent of damage of CMD and CBSD in terms of severity, incidence and percentage root necrosis. The study compared the distribution and prominence between the two diseases in Malawi and elucidated the relationship among different disease parameters. The specific objectives of this study included determining the severity, incidence, and distribution of CMD and CBSD, determining the extent of CBSD damage to roots in the form of necrosis, determining whitefly population, and identifying alternative hosts in the of CMD and CBSD in Malawi.

2 Materials and methods

2.1 CMD and CBSD assessment and leaf sampling

Field surveys were conducted in September 2018 in Chitipa, Karonga, Rumphi, Mzimba and Nkhata bay districts in the northern region; Kasungu, Mchinji, Nkhotakota, Salima, Lilongwe and Ntcheu districts in the central region and Balaka, Mangochi, Blantyre, Chikwawa and Nsanje districts in the southern region of Malawi (Fig. 1). These districts were selected due to significant cassava production and history of CMD and CBSD occurrence [8, 17, 18].

In each district, 15 fields were targeted for survey along the main and feeder roads that pass through relatively high cassava production areas. However, in some districts the number was not attainable due to low cassava production activities during the period of the study. The first field in each district was sampled randomly, while consecutive fields were sampled at 5 to 10 km intervals. At each survey site, geo-reference points were taken (latitude, longitude, altitude and name of location). The following were also recorded: name of the cassava cultivars, age in months after planting (MAP), CMD and CBSD severity scores and total number of adult whiteflies.

In each sampled field, 15 cassava plants were selected randomly along an 'X' transect and visually inspected for the presence or absence of CMD and CBSD symptoms. Foliar symptom severity was assessed as the degree of damage expression on the leaves of each sampled plant using a scale of 1–5 where 1 represented no symptoms and 5 represented the most severe symptoms [19, 20] as shown in Table 1. For each field, disease incidence was calculated as a percentage of the number of symptomatic plants out of a total of 15 inspected plants [21]. For foliar disease symptom severity analysis, non-symptomatic plants (those with a score of 1) were included in the dataset. Thus, the average disease severity was computed by including scores of 1.

The number of adult whiteflies was counted on the ventral side of the top most five fully-expanded apical leaves of the tallest shoot of each plant [21]. Counting of both adult whiteflies was done by gently turning the leaf on the underside in order not to disturb the adult whiteflies [21].

Destructive sampling was done in 12 districts of Karonga, Nkhatabay, Nkhotakota, Lilongwe, Rumphi, Blantyre, Zomba, Mangochi, Balaka, Salima, Nsanje and Chikwawa where foliar CBSD symptoms were observed on sampled plants in September 2018. Root necrosis for CBSD was assessed on a maximum of 5 randomly selected plants that showed foliar CBSD symptoms either on leaves, stem or both in each field. The sampled plants were uprooted, the roots were detached and counted. All the harvested roots per plant were weighed and cut longitudinally to check for the presence of CBSD root symptoms. Root CBSD scores were based on the standard five-point scoring scale [22].

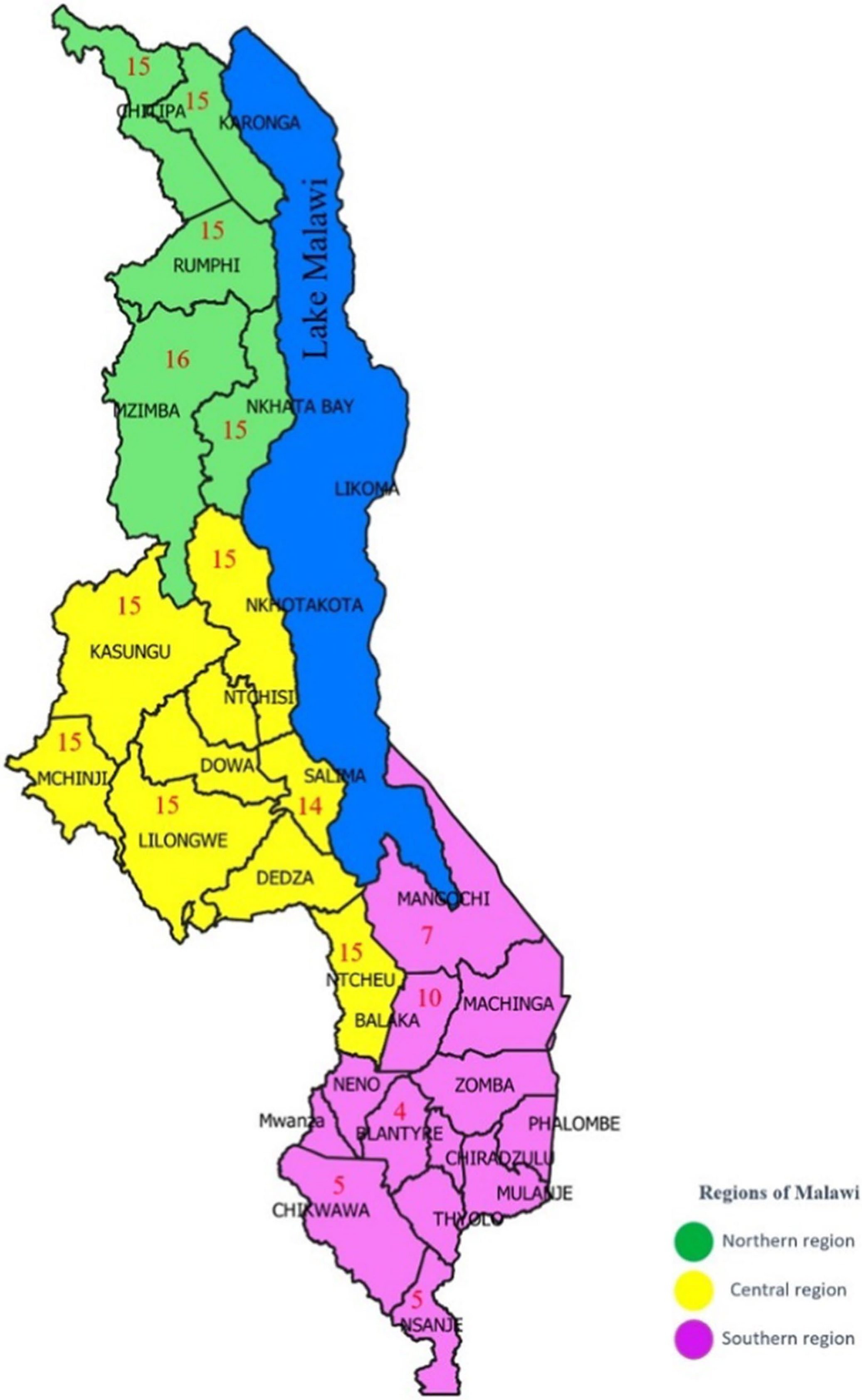

Fig. 1 Map of Malawi showing districts, regions (coloured) and number of fields sampled per district. Numbers in red show the number of fields sampled per district

Table 1 Severity scale for CMD foliar and CBSD foliar and root symptoms

Severity scale	Description		
	Foliar CMD	Foliar CBSD	Root CBSD
1	No symptoms	No symptoms	No necrosis
2	Mild chlorosis and distortions at the base of the leaves with remaining parts of the leaves or leaflets appearing green and healthy	Slight chlorosis on leaves or stems	Trace of necrosis
3	Mosaic patterns on most leaves, narrowing and distortion of lower one-third of the leaflets	Chlorotic spots that are easily observable on leaves or stems. Small lesions are observed on stems	Clearly defined areas of necrosis but necrotic areas can be easily removed
4	Severe mosaic distortion of the thirds of most leaves and general reduction of leaf size and stunting of shoots	Severe chlorotic spots on leaves and severe necrotic lesions enlarged into streaks on stems	Most of root necrotic but may still be possible to remove necrotic areas for home consumption
5	Very severe mosaic symptoms on all leaves, twisting, distortion, misshapen and severe leaf reduction of most leaves accompanied by severe stunting of plants	Very severe chlorotic/necrotic blotches and leaf wilt. Severe necrotic lesions, streaks, withering and die-back on stems	Most or all roots necrotic and unsuitable for human consumption

Sseruwagi et al. [20], Hillocks and Thresh [19]

Using a knife, necrotic regions were removed from the harvested roots and weighed on a scale [23]. CBSD root incidence and % damaged roots was calculated [24]. CBSD root incidence was calculated as in Shirima et al., (2019) by dividing the number of necrotic roots by the total number of roots and then multiplying by 100. Plant root incidence was calculated as the percentage of plants with root necrosis against the total number of plants sampled [24]. Percentage necrosis was calculated according as the percentage of the weighed necrotic part of the roots against the total weight of roots.

2.2 Identification of alternative hosts for CMD and CBSD

Separate diagnostic surveys were carried out in November 2018 and September 2019 in Salima, Nkhotakota, Rumphi, Karonga, Mangochi, Balaka, Machinga, Lilongwe and Chitipa districts where leaf samples of non-cassava plants were collected.

From the first randomly selected sample collection point, the research team stopped after every 15 km along the main and feeder roads. Observations were made on plants in or near cassava fields for viral-like leaf symptoms. Leaf samples collected from those with CMD and/or CBSD-like symptoms. Sampling bottles containing silica gel were used for sample storage during transit from the field to Chitedze Molecular Biology Laboratory for nucleic acid extraction and subsequent detection of EACMV and Cassava brown streak virus (CBSV). Samples collected in November, 2018 were processed at the Natural Resources Institute in the UK. Geo-reference points were taken for each site using a global positioning system (GPS).

2.3 Extraction of total nucleic acid

About 50 mg of each dry leaf sample collected was pulverized in a geno/grinder® (Spex SamplePrep, New Jersy, USA). Total nucleic acid was extracted using Cetyl trimethyl ammonium bromide (CTAB) method modified by Maruthi et al. (2002) except for repeating the phenol: chloroform: isoamylalcohol (25:24:1) for some samples. Quantities and quality of extracted nucleic acid were checked using DU730 Life Science UV/Vis spectrophotometer (Beckman Coulter, California, USA).

2.4 Detection of EACMV and CBSV in non-cassava plants

The EACMV and CBSV were detected in separate uniplex reactions using StepOne real time PCR (Applied Biosystems, California, USA) using TaqMan qPCR protocol. DNA was used as a template in a qPCR reaction for the detection of EACMV. A pair of specific primers CMB Rep/F (CRTCAATGACGTTGTACCA) [7] and Neweac-alt/R (CATGGAGACCGATCA GTATTGTTC) and a probe (FAM-TCTTKGGAGACAGATCCAGGTGTCCACAT-IABkFQ) [25] were used in this study. ROX [25 µM solution of 5-carboxy-X-rhodamine in 10 mM Tris–HCl (pH 8.6), 0.1 mM EDTA, and 0.01% Tween®-20] was used as a passive reference. A total reaction volume of 20 µl was made which contained 10 µl of 2X Express qPCR supermix (Life Technologies, Paisley, UK), 2 µl of DNA template, 1 µl of 500 nM EACMV primers, 0.2 µl of 100 nM probe and 0.5 µl of 375 nM ROX. Samples were incubated for 2 min each at 50 °C and 94 °C which was followed by 40 PCR cycles at 94 °C for 15 s, 54 °C for 20 s and 60 °C for 30 s.

Two step qPCR was used for detection of CBSV with the first step being the synthesis of cDNA using ImProm-II™ Reverse transcription kit (Promega, Southampton, UK) following manufacturer's instructions. The cDNA underwent a 10X dilution before being used for qPCR. Primers CBSVF3 (GGARCCRATGTAYAAATTTGC) and CBSVR4 (GCWGCTTTTATYACAAAMGC) designed by Abarshi et al. (2012) and Otti et al. (2016) respectively and a probe (JOE-TTCCAGCCA/ZEN/AGCAATWYTGAT GTATCAGAATAGTGTGA-IABkFQ) [25] were used in this study. The reaction went as above; however, 1.4 µl of 700 nM CBSV primers and 0.8 µl of 400 nM probe were used in this case.

The extracted nucleic acid from leaf samples that were collected in November, 2018 were quantified using NanoDrop spectrophotometer 2000 (Thermo Fisher Scientific, Wilmington, USA). Samples with DNA 260/280 nanodrop values of at least 1.6 were used for analysis. Analysis for these samples was done using CFX96 Bio-Rad Real-Time PCR detection systems (Bio-Rad laboratories, Inc) without using ROX as passive reference. For detecting both EACMV and CBSV, two technical replicates were used for each sample on a qPCR plate. Each run included a previously PCR tested virus-infected sample as positive control and virus-free sample as negative control.

2.5 Data analysis

Data for CMD and CBSD assessment survey in cassava fields was statistically analysed using R version 3.6.1 [26]. Generalised linear models (glm) were used for analysis of deviance (ANODEV) using MASS package [27]. Negative binomial linear model was used for analysis of all other parameters except severity, while quasibinomial linear model was used for severity. Holm-corrected least significant difference method for grouping the treatment levels was used to carry out multiple comparison in agricolae [28]. Correlations of foliar symptom severity, disease incidence, adult whitefly abundance, age of the crop and altitude using spearman rho correlation analysis were computed. Similarly, CBSD root severity, plant incidence, altitude, age of the plants, root CBSD incidence and percentage necrosis were also correlated using spearman rho correlation analysis. Severity and incidence of CMD were correlated with the corresponding parameters of CBSD using spearman rho correlation to determine the relationships between two diseases. Coefficients of at least 0.8 were considered as strong correlations, those between 0.6 and 0.7 moderate and those less than or equal to 0.5 weak [29]. Coefficients of + 1 or − 1 were considered perfect correlations while 0 represented no relationship. To determine the disease which was more prominent in Malawi, such parameters as incidence and severity for CBSD and CMD were compared using Wilcoxon signed rank test.

3 Results

3.1 Foliar CMD incidence and severity

A total of 2940 plants from 196 fields were assessed against foliar CMD symptoms across the 16 target districts in the three regions of Malawi. The survey showed a country-wide occurrence of CMD with high incidences in the southern parts. Typical symptoms of CMD in the field were as seen in Fig. 2.

Analysis of deviance showed significant differences in both CMD incidence (Chi-square = 95.4, df = 15, $p < 0.001$) and severity among some districts (Chi-square = 20.4, df = 15, $p < 0.001$). A lake shore district of Mangochi in the southern region, registered the highest mean disease incidence (78%), while Mchinji, a district in the central region, had the lowest mean incidence of approximately 0% (Fig. 3a). Blantyre in the southern region had the highest mean CMD severity scores of 2.6 (Fig. 3b). Mchinji district had fields with healthy cassava as the mean CMD severity score was 1.0. CMD severity was generally lower in high altitude areas compared to low altitude areas (particularly the lakeshore whose average altitude ranged from 490 to 658 masl). The national mean CMD incidence and severity were 32% and 1.6 respectively.

3.2 Foliar CBSD incidence and severity

CBSD occurrence was more concentrated in the lake shore districts of Karonga, Rumphi, Nkhatabay, Salima, Nkhotakota and Mangochi (Fig. 3c). Incidence (Chi-square = 209.1, df = 15, $p < 0.001$) and severity (Chi-square = 26.1, df = 15, $p < 0.001$) of CBSD were significantly different among the districts. Karonga district had highest mean foliar CBSD incidence of 75% followed by Mangochi (61%). Foliar CBSD severity for these districts on the other hand was 2.2 and

Fig. 2 CMD symptoms observed in a field in Salima district

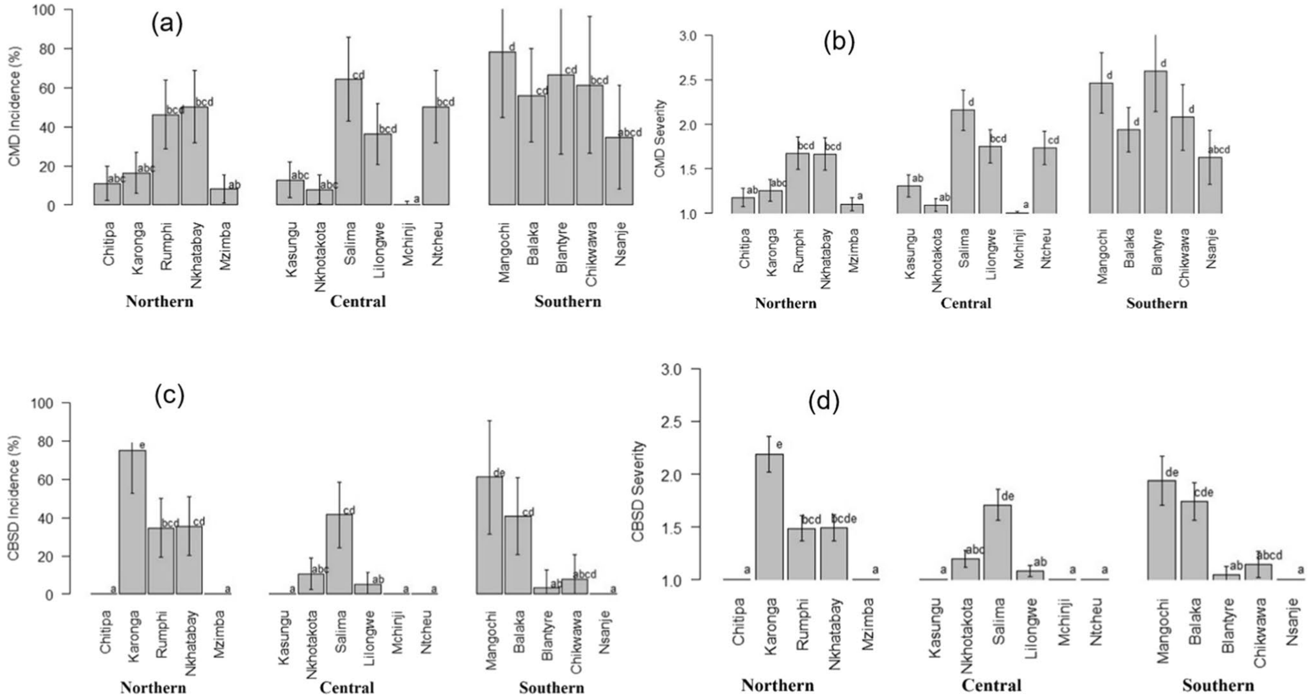

Fig. 3 CMD and CBSD incidence and severity. **a** Mean CMD incidence for various districts in Malawi. **b** Mean CMD severity for various districts in Malawi. **c** Mean CBSD incidence for various districts in Malawi. **d** Mean CBSD severity for various districts in Malawi. Bars with the same letters were not significantly different. The error bar represents standard error of the mean

1.9, respectively (Fig. 3d). The mean foliar CBSD incidence ranged between 0 and 75% with a national mean of 20% while the mean severity ranged between 1.0 and 2.2 (Fig. 3c and d). The national mean severity was 1.3.

CBSD foliar symptoms appeared with different magnitude. However, typical symptoms of CBSD on leaves observed in the study were as shown in Fig. 4.

3.3 Adult whiteflies abundance

Statistically significant differences were observed in mean whitefly numbers per plant among districts with Mangochi having the highest (1.8) while Nsanje and Blantyre had the lowest (0.00) (Chi-square = 57.8, df = 15, $p < 0.00001$) (Fig. 5). Some fields, especially in the south did not register adult whitefly. Karonga district had the second highest (1.6) mean number of adult whiteflies per plant followed by Nkhatabay (1.2).

Fig. 4 CBSD symptoms observed in a field in Salima district

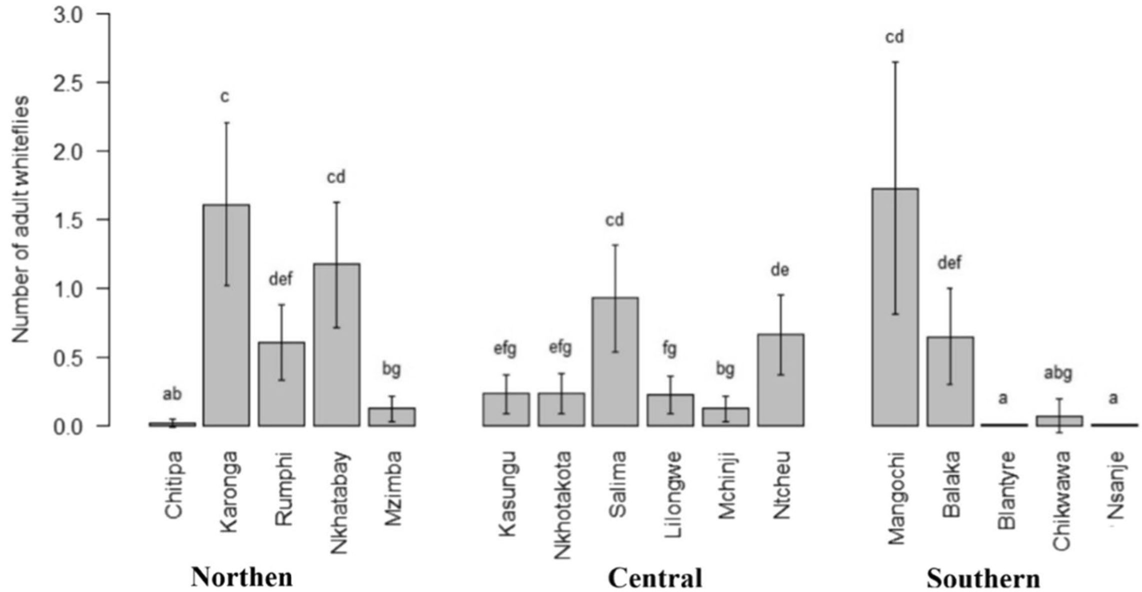

Fig. 5 Number of adult whiteflies in various districts. Bars with the same letters were not significantly different. The error bar represents standard error of the mean

3.4 Correlation of disease and insect parameters

Spearman rank correlation analysis was done to determine the correlation among various parameters related to CMD, CBSD and whiteflies. For CMD, the greatest positive correlation was observed between incidence and severity ($r = 0.99$, $p < 0.001$; Table 2). CMD incidence was positively correlated to total number of adult whiteflies ($r = 0.33$, $p = 0.001$). CMD incidence was however, negatively correlated to altitude ($r = -0.31$, $p = 0.001$). Just like incidence, CMD severity was positively correlated to total number of adult whiteflies ($r = 0.32$, $p = 0.001$). CMD severity was negatively correlated to altitude ($r = -0.52$, $p < 0.001$). The number of adult whiteflies was negatively correlated to altitude ($r = -0.31$, $p < 0.001$).

Based on foliar scores, correlation of CBSD parameters followed almost the same trend as CMD parameters. CBSD incidence was highly positively correlated with severity of symptoms ($r = 0.99$, $p < 0.001$; Table 3). Incidence was positively correlated with the total number of adult whiteflies ($r = 0.38$, $p < 0.001$) but the correlation was weak. The incidence was moderately negatively correlated with altitude ($r = -0.56$, $p < 0.001$). Severity was weakly correlated with total number of adult whiteflies ($r = 0.38$, $p < 0.001$) but moderately negatively correlated with altitude ($r = -0.56$, $p < 0.001$). The total number of adult whiteflies was weakly negatively correlated with altitude ($r = -0.31$, $p < 0.001$).

Wilcoxon signed rank test showed that CMD incidence (32%) and severity (1.6) were significantly higher than CBSD incidence (20%) and severity (1.3) (Table 4).

CBSD and CMD incidences were positively but weakly correlated ($r = 0.25$, $p < 0.001$; Table 4). CBSD severity versus CMD severity were positively correlated ($r = 0.24$, $p < 0.001$, respectively).

Table 2 Correlation between CMD incidence, symptom severity, number of adult whiteflies, altitude, and age of cassava plants

	Severity	Number of adult whiteflies	Altitude (masl)	Age (MAP)
Incidence (%)	0.99***	0.33***	−0.31***	−0.17*
Severity		0.32***	−0.31***	−0.16*
Number of adult whiteflies			−0.31***	−0.10
Altitude				0.20**

***p < 0.001; **p < 0.01; *p < 0.05; masl: metres above the sea level; MAP: months after planting

Table 3 Correlation between CBSD incidence, foliar symptom severity, number of adult whiteflies, altitude and age of cassava plants

	Severity	Number of adult whiteflies	Altitude (masl)	Age (map)
Incidence (%)	0.99***	0.38***	−0.56***	−0.08
Severity		0.38***	−0.56***	−0.08
Number of adult whiteflies			−0.31***	−0.10
Altitude				0.20**

***p < 0.001; **p < 0.01 and *p < 0.05; masl: metres above the sea level; MAP: months after planting

Table 4 Correlation between parameters of CBSD with corresponding parameters of CMD

Parameter 1 (CBSD)	Parameter 2 (CMD)	Rho	P value
Incidence	Incidence	0.25	0.001***
Severity	Severity	0.24	0.001***

***p < 0.001

3.5 CBSD root necrosis

In some areas, although foliar symptoms were observed, the plants were too young for harvest, hence the root CBSD status for such plants was not assessed. Similarly, no foliar CBSD symptoms were observed in some districts which led to root CBSD assessment not being performed. It was generally observed that most cultivars that were assessed were local cultivars. Out of the thirty cultivars that were assessed for root CBSD, only 2 were improved cultivars (Sauti and Sagonja) and one local recommended cultivar (Mbundumali also called Manyokola). Higher CBSD root incidence and symptom severity were prominent along the shore of lake Malawi.

Districts differed significantly in percentage of plants with necrotic roots (Chi-square = 8795.3, df = 11, $p < 0.001$) with Karonga district having the highest (56%) while Lilongwe and Nsanje districts had the least (0% each). The national mean percentage of plants with necrotic roots was 18.8. CBSD root incidence ranged between 0 and 26.8% with a national average of 8% and was significantly different among districts (Chi-square = 70.1, df = 11, $p < 0.001$; Table 5). Like percentage of plants with necrotic roots, Karonga had the highest CBSD root incidence (23.6%) while Lilongwe and Nsanje had the least score (0%) each. Root symptom severity varied significantly among districts and Karonga district had the highest severity (2.1) followed by Rumphi (1.97) while Lilongwe and Nsanje had the lowest with 1.0 (Chi-square = 48.4, df = 11, $p < 0.001$; Table 5).

3.6 Percentage root necrosis

The percentage of root necrosis due to CBSD for various districts was estimated. Significant differences were observed among districts (Chi-square = 29.0, df = 11, $p < 0.001$; Fig. 6). Rumphi district had the highest percentage of necrosis (16.4%) followed by Karonga (15.2%) while no necrosis was recorded in Lilongwe and Nsanje (Fig. 6). The national mean percentage necrosis was 5.3%.

3.7 Cassava production loss estimates due to CBSD root necrosis

Total national cassava production for 2018 in Malawi was 5,410,506 metric tonnes (https://www.fao.org/faostat/en/#data/QC). The farmgate price for cassava per kilogramme was MK 115.8 (Kanyamuka et al. 2018). In monetary value the production was equal to MK 626,536, 594,800 (~ US$ 806,789,506.30) using an exchange rate of MK776.58 to 1 US dollar (https://www.exchange-rates.org/Rate/MWK/USD/12-31-2018). Production loss due to percentage necrosis based on 2018 production was obtained by multiplying the national mean percentage necrosis (5.3%) by

Table 5 CBSD root severity, incidence and percentage of plants with necrotic roots in major cassava producing districts of Malawi

District	Mean altitude (masl)	Mean age of plants (map)	Percentage of plants with necrotic roots	CBSD root incidence	mean severity score of roots with CBSD (1–5)
Karonga	494.7 d	7.7 c	56 a	23.6 ab	2.10 a
Rumphi	477.9 d	8.1 bc	52 a	26.8 a	1.97 ab
Nkhatabay	542.1 d	9.6 bc	29 b	11.9 bc	1.53 bc
Nkhotakota	509.0 d	8.4 bc	33 ab	8.8 bc	1.46 abc
Salima	512.8 d	7.5 c	9 cd	3.6 c	1.13 c
Lilongwe	1145.7 a	11.5 ab	0 d	0.0 c	1.00 c
Balaka	660.9 c	9.2 bc	12 cd	5.8 c	1.22 c
Blantyre	666.6 c	9.8 abc	15 bcd	4.9 c	1.16 c
Chikwawa	515.4 d	8.8 bc	5 cd	1.0 c	1.07 c
Mangochi	628.5 c	7.8 c	17 bc	10.0 bc	1.35 c
Zomba	799.1 b	11.6 ab	8 cd	2.5 c	1.00 c
Nsanje	543.6 d	14.0 a	0 d	0.0 c	1.05 c
P value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
df	11	11	11	11	11
SEM	10.399	0.222	1.264	0.977	0.008

Districts with the same letters were not significantly different

Masl: metres above the sea level; MAP: months after planting; df: degrees of freedom; SEM: standard error of the mean

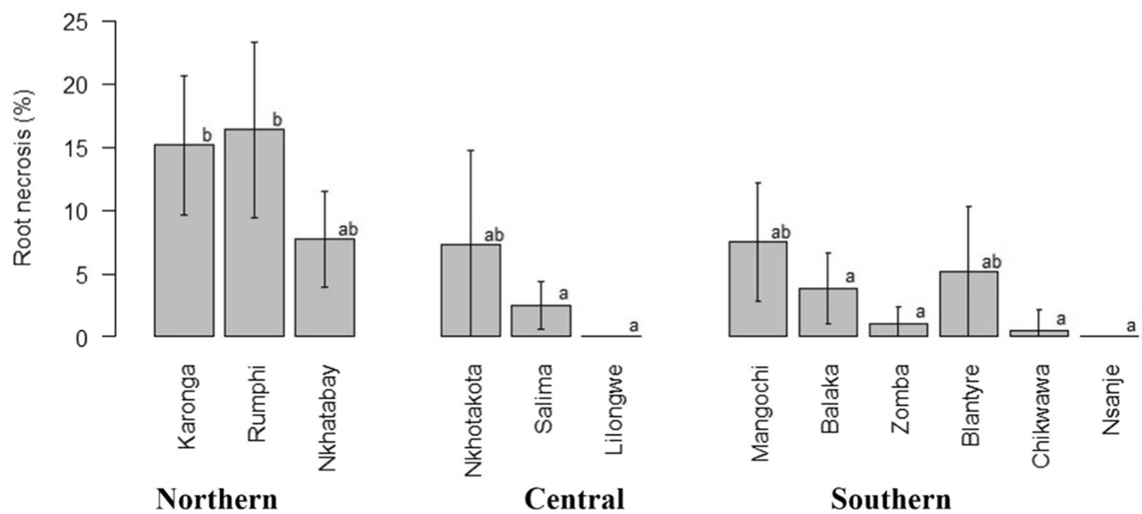

Fig. 6 Percentage necrosis of CBSD. Bars with the same letters were not significantly different. The error bar represents standard error of the mean

the total production and the result was 286,756.82 tonnes which translated to approximately MK 33,206,439,524 (US$ 42,759,844) loss in monetary value.

3.8 Correlations between CBSD parameters

Strong correlations were observed between root severity and percentage necrosis ($r = 0.96$, $p < 0.001$), severity and CBSD root incidence ($r = 0.99$, $p < 0.001$) and percentage necrosis and CBSD root incidence ($r = 0.96$, $p < 0.001$; Table 6). Strong correlations were observed between root severity and percentage necrosis ($r = 0.96$, $p < 0.001$), severity and CBSD root incidence ($r = 0.99$, $p < 0.001$) and yield losses and CBSD root incidence ($r = 0.96$, $p < 0.001$; Table 6).

Significant correlation exists between CBSD foliar incidence with root incidence ($r = 0.65$, $p = 0.031$; Table 7) and root severity ($r = 0.64$, $p < 0.035$; Table 7). Another positive correlation was observed between CBSD foliar severity and root

Table 6 Correlation between CBSD root incidence, root severity, percentage necrosis, altitude and age of cassava plants

	CBSD root severity	Percentage necrosis	CBSD root incidence	Altitude (masl)	Age of cassava plants (map)
Percentage of plants with necrotic roots	0.69***	0.67***	0.68***	−0.40***	−0.19***
CBSD root severity		0.96***	0.99***	−0.32***	−0.33***
Percentage necrosis			0.96***	−0.30***	−0.15***
CBSD root incidence				−0.32***	−0.18***
Altitude (masl)					0.37***

***p < 0.001; **p < 0.01 and *p < 0.05; masl: metres above the sea level; MAP: months after planting

Table 7 Correlation between CBSD foliar and root parameters

Parameter 1 (Foliar)	Parameter 2 (Root)	rho	P value
Incidence	Percentage of plants with necrotic roots	0.57	0.067
Incidence	CBSD root incidence	0.65	0.031*
Incidence	Percentage necrosis	0.59	0.589
Incidence	Severity	0.64	0.035*
Severity	Severity	0.65	0.030*
Severity	Percentage necrosis	0.60	0.05*
Severity	Incidence	0.59	0.059

*p ≤ 0.05

severity ($r = 0.65$, $p = 0.030$; Table 7). Foliar CBSD severity was positively correlated with percentage necrosis ($r = 0.60$, $p = 0.05$; Table 7). All the significant correlations were moderate.

3.9 Alternative hosts of CMD and CBSD

A total of 67 leaf samples from 44 species were collected in different districts of all the three regions of Malawi. Of these, 62 plants showed CMD-like symptoms in the field. While two plants of *Vigna anguiculata* had CBSD-like symptoms. Majority of CMD/CBSD-like-symptomatic samples were collected along Lake Malawi shores.

Five plants from two plant species, *Solanum lycoperscum* and *Solanum incanum* (*S. incanum*), had both CMD-like symptoms and the presence of whiteflies during the time of sampling. Laboratory analysis using qPCR, however, showed the presence of EACMV in *Senna occidentalis,* which was collected from the lakeshore district of Rumphi and showed virus-like leaf symptoms.

4 Discussion

The current study has shown a country-wide occurrence of CMD with high incidences in the southern parts of Malawi. Blantyre district trailed Mangochi in CMD incidence but superseded Salima. Furthermore, this district had the highest CMD severity scores of 2.6. Blantyre is not in the shores of lake Malawi which gives an indication that although lakeshore areas might be hotspots for CMD, the disease is present in other areas that are not along lake Malawi. The highest CMD incidence in the southern parts could be attributed to, among other factors, the type of cultivars grown by the farmers. Mbundumali, a sweet cassava variety highly favored for fresh market, is among the widely grown varieties in the region. The variety, however, is known to be highly susceptible to CMD [30].

Complementary to the recent reports of CBSD in Malawi, the disease is localised to the low-lying lakeshore areas [8] where temperatures are usually high confirming the observation of Jeremiah et al. (2015) on the influence of temperature on CBSD incidence. This is not surprising considering that high temperature promotes virus accumulation in plants as reported in *Nicotiana benthamiana* against *Peanut stunt virus* [31]. The incidence and foliar symptom severity of CBSD vary geographically. In this study, incidence and severity were significantly different among districts with Karonga, having the highest foliar CBSD incidence. Similar findings were also reported in previous studies [4, 8]. High incidence in Karonga, is more likely to do with the biophysical factors such as cultivars, environment (temperature and rainfall) and vector activities. On average, temperature in Karonga ranges between 31.7 and 27.8 ^{0}C in the hottest and coolest months of the year. Shirima et al. (2020) associated CBSD spread to disease pressure and abundance of whiteflies that are influenced by environmental factors such as temperature and relative humidity [32–34].

The occurrence of the CMD and CBSD in Malawi might have been prolonged over the years due to limited knowledge of the two diseases by farmers (Dual cassava annual report for 2019, unpublished). According to the report, 30.2% of farmers interviewed in a field survey were able to recognize CMD symptoms and only 2.8% were able to identify CBSD symptoms. Meanwhile, Chipeta et al. (2016) observed a slightly higher percentage (10.1) of farmers who were able to recognize foliar CBSD symptoms in Salima, Nkhotakota and Nkhatabay districts. These districts are notably high cassava producing districts and have high CBSD incidences. The ability to recognize CMD than CBSD might be due to the prominence of CMD in Malawi than CBSD as observed in this study. In any case, these studies have shown that farmers are less likely to recognise CBSD symptoms as compared to CMD symptoms. Recognising disease symptoms is of particular importance to farmers when they intend to select materials for planting or to remove diseased plants to minimise disease spread. There is therefore need for all stakeholders in the agriculture sector to develop and disseminate extension messages suitable for the management of CMD and CBSD. Lack of knowledge on the two diseases and use of local susceptible cultivars is also a potential reason for high, prolonged and widespread occurrence of CMD and CBSD in Malawi. Nevertheless, farmers sometimes might be aware and have the knowledge of the good cultivars and management practices of crops but fail to cultivate the recommended cultivars due to the unavailability or high prices of the planting materials [35–37]. As such, deliberate effort in seed systems that ensure increased access to adequate planting materials of cassava is of paramount importance.

Lack of harmonious approaches in integrating crop variety development by crop breeding institutions; seed production, certification and quality control by seed companies and regulatory institution; and technology dissemination by government might be other reasons for prolonged occurrence of the two diseases. A strategy that would integrate plant disease resistance, clean seed supply and management of disease vectors would help manage the diseases [37]. Malawi gazetted the new seed regulations in 2018 that incorporated the production, certification and quality control of vegetatively propagated crops such as cassava in order to control the spread of diseases and ensure that farmers have access to high quality and clean planting materials. Previously, the government has been having countrywide programmes such as the Agricultural Sector Wide Approach project (ASWAp) that involved distribution of cassava planting materials, that were taken from one district to the other, to farmers for drought resilience (Government of Malawi 2011, unpublished). Such movement of planting materials may have exacerbated spread of the two diseases, particularly, if materials were sourced from areas with high disease incidences to low disease incidence.

Necrosis of cassava root was high in the districts that are along the shore of lake Malawi. This is not surprising considering the geolocational pattern of CBSD spread. However, Salima district, despite being along the lake shore, had low percentage of necrosis in cassava roots. This concurs with root severity and root incidence levels which were also low in this district. This would be due to cassava varieties grown by farmers in Salima as some varieties although exhibit high foliar symptoms do show low or no symptoms in roots [38]. The most predominant varieties in Salima were Mbundumali together with two improved varieties Sagonja and Sauti.

A total loss of US$ 42–43 million might have been incurred in 2018/19 cassava production season in Malawi. The loss in monetary terms estimated in this study was higher than the US$ 6–7 million estimated by Gondwe et al. (2003). The difference could be due to low production levels in 2001 when the study was conducted which was less than 2.4 million tonnes [39]. However, methods employed for estimation of production loss between this study and [4] are different which could also be a source of further variation between the results of the two studies.

Several parameters enable researchers to measure the extent of a disease in a geographical area or among cultivars under investigation. This study has shown that while all other parameters (severity, incidence and number of adult whiteflies) for CMD and CBSD may correlate positively with each other, altitude and age showed a negative correlation with the parameters mentioned. It can be deduced from findings of this study that in areas with high incidences, the fields are likely to have plants with high disease severity which is similar to an observation by Jeremiah et al. (2015). Furthermore, the study has shown that the number of adult whiteflies increases with decreasing altitude. Higher altitudes usually have lower temperatures that are not favourable for whitefly development/multiplication which leads to reduced disease transmission [34, 40, 41].

Due to a positive correlation between foliar incidence and CBSD root incidence, in a place of high foliar incidences of the disease, there is likely to be an increase in CBSD root incidence. Contrary to the expectation, we observed no correlation between foliar incidence and the percentage of plants with necrotic roots [22]. The observation might be as a result of suppression mechanisms of virus replication in roots of plants than leaves [42]. In addition, foliar incidence of CBSD and percentage of plants with necrotic roots have been reported to have a linear relationship [43]. This is not the case in this study; however, CBSD foliar incidence was positively correlated with the CBSD root incidence. The significance levels in this study were low and this might explain the differences in observation between this study and previous ones. Such relationships might be partly dependent on cultivars encountered during the study. Interestingly, only two cultivars (Sagonja and Sauti) encountered in this study were improved varieties showing that most farmers in all regions cultivate local varieties. These improved varieties were reported in Salima and Chitipa.

Cassava viruses have been reported in other non-cassava plants [44, 45]. *East African cassava mosaic Cameroon virus* (EACMCV), for instance, was first reported in *S. occidentalis* [44]. In this study *S. occidentalis* was diagnosed with EACMV representing first report of cassava virus infection in the non-cassava plant species in Malawi. *S. occidentalis* has been reported to host viruses that infect another crop of agronomic importance, *Glycine max* [46]. This renders it an important plant species to consider in the fight against cassava viruses as it is distributed in all the three regions of Malawi.

5 Conclusion

Both CMD and CBSD are still prevalent in Malawi despite previous breeding work, research efforts and government financial investments that have been employed in their fight over the years. CBSD is more concentrated along Lake Malawi while CMD is widespread across the country. Of the two diseases, CMD is more prominent in Malawi than CBSD in severity and incidence. The incidence and symptom severity of CBSD and CMD vary geographically with low altitude areas especially the lake shores having the highest incidence and severity scores. Altitude correlates negatively with disease incidence, severity and number of adult whiteflies. Therefore, CMD and CBSD incidence, and severity decrease with increasing altitude. *S. occidentalis* was identified as an alternative host of CMD in Malawi. Any control measures developed for CMD must consider the presence of this alternative host in farmers' fields.

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Data availability Apart from collecting data in farmers' fields, the study involved capturing personal information of the farmers. Therefore, data generated during this study is available from the corresponding author upon request.

Declarations

Competing interests The authors declare no competing interests.

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