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Role of maize residues in transmission of *maize chlorotic mottle virus* and effect on yield

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Abstract

Maize chlorotic mottle virus (MCMV) is the only established member of the genus *Machlomovirus* and it is imperative in the development of maize lethal necrosis (MLN) disease. Infection of maize plants with MCMV can cause loss of 10 to 59% in grain yield, while up to 100% in co-infection with cereal infecting potyviruses. The study was carried out to determine the role of MLN disease infected maize residues in transmission of MCMV in the soil and effect on yield. Sowing of commercial hybrid varieties, H614 and WE1101 was done at 0, 15, 30, 45, 60 and 90 days after incorporation of MLN infected maize residues in the soil. Data collected consisted of virus titre, number of plants with disease symptoms and severity score, plant height and grain yield. Area under disease progress curve (AUDPC) was calculated using the MLN severity data. The highest MCMV titre of 0.2 was detected in H614 sown in freshly incorporated MLN infected residues. Highest disease incidence at 31.9 and 100% was noted in the field and screen house respectively. Maximum disease severity at 21.3 was record in H614 plant sown immediately after incorporating the residues. The highest reduction in plant height at and grain yield at 3.6% and 44.8% respectively was attained in plants established in media incorporated with freshly MLN infected residues. The study confirmed that MCMV was transmitted through MLN infected maize residues in the soil with notable reduction in grain yield. Farmers should be encouraged to practice proper disposal of MLN diseased infected materials practice crop rotation with noncereal crops

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Introduction

Maize is the most constituent of Kenyan meal with 35.2% of calories coming from it while the per capital consumption level is at 78 kilogrammes per person per year (Ranum *et al.* 2014). Among the viruses infecting maize, *Maize chlorotic mottle virus* was first detected infecting maize in Kenya in 2012 (Adams *et al.*, 2012, Wangai *et al.*, 2012). *Maize chlorotic mottle virus* is a crucial pathogen in that it is the only established member of the genus *Machlomovirus* and when it co-infects maize with cereal potyviruses it causes MLN disease (Wang *et al.*, 2017; Mwathi *et al.,* 2018). This virus complex causes severe disease more than the additive symptoms of either MCMV or potyviruses which can result in plant death especially when infection occurs at an early stage (Uyemoto *et al.*, 1980; Nibblet and Claflin *et al.*, 1978). Yield losses of 10-15% due to infection with MCMV were reported in flourly and sweet maize varieties in Peru while in experimental plots it was up to 59% (Castillo and Hebert, 1974).

Maize chlorotic mottle virus is transmitted through seeds, vectors and infectedmlN maize residues and soil (Uyemoto, 1983; Jensen *et al.*, 1991; Cabanas *et al.*, 2013). A study conducted by Scheets (2008) revealed that MCMV-KI strain had a soil and water connection implying that the pathogen could be soil and water borne. The virus has been found to overwinter in infected maize residue and can became the source of infection for maize seedling in the following season (Bockelman *et al.*, 1982; Uyemoto 1983; Hilker *et al.*, 2017). *Maize chlorotic mottle virus* is transmitted by adult and larval of (Jiang *et al.*, 1992). The larva of chrysomelid beetle acquires the virus through feeding on infected maize plant residues that had have remained in the soil (Jensen *et al.*, 1991, Jiang *et al.*, 1992). It is also assumed that the soil borne viruses enter the plants through the young roots of plants which normally lack the protective sheath or at the tips of roots that ooze out chemicals that attract vectors and in the process transmit them (Hiruki and Teakle, 1987; Montenegro and Castillo, 1996). The pathogen has to be within the plant rooting zone within the soil profile for it to be taken up to the plant system (Veena *et al.*, 2014).

The soil viruses travel long distances upward through vascular vessels for them to induce symptoms in the aerial part of the plant (Andika *et al.*, 2016).

Designing of management techniques is complicated since soil borne viruses are hard to detect, difficult to eradicate due to complex situation in the soil whereas they can exist in the soil for very many years (Robert, 2014; Andika *et al.,* 2017; Koh *et al.,* 2017). However, minimal reduction of virus inoculum can be achieved through crop rotation with non-cereal crops, tillage with sufficient period of fallow to allow residues to decompose (Uyemoto, 1983). However, long term management of the virus is through development of resistant varieties to the viruses (Gowda *et al.*, 2015). Some genotypes have shown tolerance MCMV that would form the basis of selection for MCMV- and MLN-tolerant maize hybrids (Jones *et al.*, 2018). In Kenya, after harvesting of maize, some farmers leave maize stovers in the fields, which are later on ploughed back into the soil while in other instance, maize residues are decomposed for use as soil ammendments as manure (Berazneva, 2013). Maize which may have been infected with viruses causing MLN disease could be a source of virions to be transmitted to the newly planted maize crop. However, duration the infected maize residues remain infective after incorporation into the soil has not been confirmed. The aim of the present study was therefore to determine the role of infected MLN diseased maize residues play in transmission of MCMV and assess effect on the yield.

Materials and methods

Preparation of maize lethal necrosis infected maize residue

The source of infected residue was maize which had have been planted in a separate screen house during different periods to coincide with the timing when they were harvested to be incorporated into the soil. The maize seedlings were inoculated with SCMV and MCMV while additional maize having MLN disease was sourced from farmers' fields in Sagana irrigation scheme in Nyeri County where the disease was endemic.

The harvested MLN infected maize plants were chopped using a chaff cutter into small pieces before incorporation into the soil.

Field and screenhouse experimental design and layout

Experiments were conducted in the field and screen house over two crop cycles in 2016 and 2017 long rains using two maize varieties, H614 and WE1101. Hybrid 614 is an old variety that has been in production for over 20 years and it is susceptible to MLN disease while WE1101 is drought tolerant and has been in production for at least 5 years. In the field, plots of 2.5 x 1.5M and separated by 1.5M with the spacing between blocks at 2.5M were used. Sowing of the two maize varieties was done in the furrows filled with chopped infected residues at intra- and inter-spacing of 25cm and 75cm respectively. During planting NPK, (23:23:0) was applied at 200 kg per hectare while top dressing with Calcium Ammonium Nitrate (26%N) at of 250 kg per hectare was done on the 4th and 7th week post emergence. Hand weeding was done on the 3rd and 6th week after seedling emergence. The maize stalk borers were controlled on the fourth week after germination by applying one brief shake of the applicator of Tremor® granules, a.i. 0.5gm/kg of Beta-cyfluthrin into the funnel of each maize plant. Control of other pests was done using Robust® a.i 480gm/litre chlorpyriphos and Karate®, 50gm/litre lambda-cyhalothrin interchangeably, with the first application done three weeks after germination. The residues were incorporation into the furrows at 5.3MT/ha at 0, 15, 30, 45, 60 and 90 days before the sowing of seeds for the two maize varieties. The experiment was laid out as a randomized complete block design with split plot arrangement each having three replications. The maize variety was the main plot while duration (days) the infected MLN disease residue remained in the soil was the sub-plot treatment. Control plot has no MLN infected residues incorporated

In the screen house, 60 x 45cm polythene bags were filled half way with medium comprising of loam soil, sand and manure in a ratio of 2:2:1, respectively. The second portion was mixed with 250 gms/bag MLN disease infected maize residues at 0, 15, 30, 45, 60 and 90 days before sowing (Yang *et al*., 2015). During planting, 25 gms of N.P.K (23:23:0) was added in each bags and then five seeds were sown in each bag and later thinned to three plants.

Four weeks after sowing, plants were top dressed with Calcium Ammonium Nitrate (CAN); (26% N) at a rate of 15g per bag. The experiment was set up as completely randomized design, with split plot arrangement each having three replications. The main plot, subplot and control are as explained in field experiment. Data collected in the two experiments included virus titre, number of plants with disease symptoms, disease severity score, plant height and grain yield.

Detection of Maize chlorotic mottle virus in maize leaf tissues

Young leaf samples were cut from the upper most leaves of each plant per treatment at 8th week post emergence and they were store at -20^{o}C. Samples of healthy asymptomatic plants were included while known diseased samples were obtained from ICIPE. The samples were detected using DAS-ELISA as described by Clark and Adams (1977). The MCMV antisera kit was purchased from German Collection of Microorganisms and Cell Cultures (Leibniz Institute DSMZ), while the buffers were from Agdia Biofords in Grigny, France. All the chemicals were used according to manufacturer's instructions and all the samples were assayed for MCMV.

Preparation of the samples was done by crushing 0.5g maize leaf samples in 2.0ml of the extraction buffer (4.0g PVP-40000, 2.0g egg albumin) in crushing bags. Each microtitre plate was coated with 200µl of coating buffer (0.318µg Na_2CO_3, 0.586µg $NaHCO_3$, 0.06µg NaN_3, and 18.0ml distilled water) and the plates were covered tightly and incubated at 37^{o}C for three hours. The plates were emptied then dried using an absorbent paper and washed thrice using Phosphate buffer saline-tween (8.0g NaCl, 0.2 g KH_2PO_4, 1.15 g Na_2HPO_4, 0.2g KCL, 0.195g NaN_3, 1.0 litre distilled water, 0.5ml tween). The wells were loaded with 200µl of the extracted samples and incubated in refrigerator at 4^{o}C overnight. Three controls were used in the ELISA, the healthy sample which acted as negative control from healthy plants, positive control plants while the third control consisted of no sample where the wells in the pates were left blank. Thereafter, the plates were then washed thrice as explained above.

Thereafter a conjugate solution was prepared by mixing 35µl of conjugate antiserum (IgG-A'p) with 10ml of conjugate buffer (0.4g PVP-40000, 0.04gm egg albumin). Two hundred microliter of enzyme conjugate was added to each well and then incubated at 37°C for 3 hours followed by washing. Finally a substrate solution was prepared through dissolving substrate tablet (17.46ml Diethanolamine, 9.6ml distilled water, 2.4ml HCL (37%) in 10ml of substrate buffer and thereafter 200 µl of the substrate solution was added to each well of the plate and the plates left for 30-60 minutes at room temperature for reaction to take place. The results were assessed by observing visually and by spectrometric ELISA reader measurement of absorbance at 405nm.

A positive reaction was indicated by development of a yellow colour. Colour intensity was determined by spectrophotometer at 405nm wavelength. A sample was considered positive when the readings at 405nm was twice the sum of mean and standard deviation absorbance values of healthy maize control at 405nm while those below were grouped negative according to the relationship $x \geq \bar{\imath} * (2+0.5)$, where x = positive sample, ī = average value of healthy controls and 0.5 is the standard deviation.

Determination of incidence and severity of maize chlorotic mottle disease

Determination of incidence and severity of maize chlorotic mottle disease (MCMD) commenced on the 5th week after seed emergence in the field and screen house. The number of plant with disease symptoms were counted and on the 5th week after emergence both in the screen house and in the field and it was carried out on a weekly basis until 50% of the crop had tasseled. The percentage disease incidence was calculated using equation 1,

Equation 1

$$\% \text{ disease incidence} = \frac{\text{number of plant expressing the disease symptoms}}{\text{Total number of plants in each plot/polythene bag}} * 100$$

Visual scoring of MLN severity for maize plants in each plot and polythene bag was done using modified Horsfall-Barrat scale (Horsfall and Barrat, 1945) and scoring was done as per the 12 classes/category. Disease severity was calculated using the equation 2,

Equation 2,

$$\text{Percent severity} = \frac{n*v*\ 100\%}{V*N}$$

Where;
n= Number of plants in each category
v= Numerical value of symptoms category/code
N= Total number of plants
V=maximum numerical value of symptoms category

Data on percent severity for each plot and polythene bag was used to compute area under disease progress curve (AUDPC) using the formula by Simko and Piepho (2012) given in equation 3,

$$AUDPC = \sum[(Yi + 1 + Yi) * (0.5) * (Ti + 1 - Ti)]$$

where Y = disease severity at time T,
and i = the time of the assessment (in days numbered sequentially beginning with the initial assessment).

Assessment of plant height and grain yield

In the field experiment, ten plants were randomly selected and then tagged for plant height measurement while in the screen house, height for the three plants in each polythene bags was recorded. Initial measurement of plants height in the field and screen house was done on the 4th week after emergence and carried out on a weekly basis until the 9th week. When the maize cobs reached physiological maturity, harvesting was done from plants that had been tagged in the field and three plants from each bag. Five cobs were randomly selected from each sample harvested from field. Cobs from the field and screen house were hand shelled and then dried to moisture content of 15%. The shelled grain for each sample was weighed.

Data analysis

Data collected from virus titre, disease incidence, severity, AUDPC, plant height and grain weight for screen house and in the field was subjected to analysis of variance using GenSat computer software package (Lawes Agricultural Trust Rothamsted Experimental Station, 2016). Separation of means of the treatments was by the Fisher's protected Least Significant Difference (LSD) test at 5% confidence interval.

Results and discussion

Virus titre in maize leaf tissues

There was a decline in the virus titre with reduction of days the MLN maize residues remained in the soil (Table 1). *Maize chlorotic mottle virus* (MCMV) was detected in maize planted in furrows and polythene bags incorporated with MLN infected maize residues up to 90 days and 60 days respectively. The duration the MLN infected maize residues had been incorporated in the soil before sowing significantly affected MCMV titre in maize planted in the field and screen house during the two seasons. The highest MCMV titre at 0.2 was detected in H614 variety which was sown in the field during 2017 long rains season. Comparatively more virus titre was detected in H614 plants sown in plots/polythene bags incorporated with MLN infected maize residues as compared to WE1101 plants in both sites during the two seasons.

Table 1. *Maize chlorotic mottle virus* titre in two maize varieties grown in media incorporated with maize lethal necrosis infected maize residues.

Days after residue incorporation	Variety H614			Variety WE1101		
	2016 long rains	2017 long rains	Mean	2016 long rains	2017 long rains	Mean
Field experiment						
0	0.17	0.20	0.18	0.18	0.14	0.16
15	0.15	0.17	0.16	0.12	0.11	0.12
30	0.09	0.13	0.11	0.12	0.08	0.10
45	0.06	0.08	0.07	0.08	0.06	0.07
60	0.03	0.06	0.05	0.03	0.05	0.04
90	0.01	0.01	0.01	0	0	0
Non-incorporated	0	0	0	0	0	0
LSD $_{(p\leq0.05)}$ (V)	NS	NS		NS	NS	
LSD $_{(p\leq0.05)}$ (D)	0.04	0.03		0.04	0.03	
LSD $_{(p\leq0.05)}$ (VxD)	NS	NS		NS	NS	
CV (%)	25.5	21.5		25.5	21.5	
Screen house experiment						
0	0.16	0.17	0.17	0.18	0.04	0.11
15	0.14	0.12	0.13	0.10	0.04	0.07
30	0.10	0.10	0.10	0.09	0.04	0.07
45	0.10	0.08	0.09	0.05	0.06	0.06
60	0.08	0.04	0.06	0.01	0.02	0.02
90	0	0	0	0	0	0
Non-incorporated	0	0	0	0	0	0
LSD $_{(p\leq0.05)}$ (V)	NS	NS		NS	NS	
LSD $_{(p\leq0.05)}$ (D)	0.04	0.04		0.04	0.04	
LSD $_{(p\leq0.05)}$ (VxD)	NS	NS		NS	NS	

Lsd= Least significant difference; CV= coefficient of variation; V= Variety; D= Duration ;VxD= interaction between variety and severity.

The findings are in agreement with the result by Jensen (1985) that infectivity of dried infected maize through mechanical inoculation was lost rapidly and at 3 months only trace of virus was found while the virus could not be recovered after 4 months. Mekureyaw (2017) reported that *Maize chlorotic*

mottle virus was found to survive on decomposing maize residues and this has been revealed as one method of transmission. A study undertaken by Nyakundi (2017) revealed that maize genotypes planted in infected soil with MCMV had characteristic symptoms of the virus which gave positive results during detection of the virus. In another study done by Fillhart *et al.* (1998) detected *Tomato mosaic tobamovirus* (ToMV) and *Tobacco mosaic virus* (TMV) in forest soils which were later transmitted to *Chenopodium quinoa* plants. Infectivity of dried leaf tissues with *Yellow tailflower mild mottle virus* was maintained for at least a year when incubated at -80 or 22°C, or at fluctuating ambient temperatures of 0.8 to 44.4C (Koh *et al.*, 2017). A study by Uyemoto (1983) found out that there was an increase in MCMV incidence from 1.6 to 12.2 in plots that were continuously cropped with maize. The activities of microorganisms in the soil may create wounds in maize which would be avenue for entry of viruses within soil (Hull, 1989; Katan, 2017). During cultural practices such as weeding, wounds inflicted on the roots could also be avenues for virus transmission (Uyemoto, 1983).

The decline in amount of virus may be as a result of decomposition of plant residues therefore reducing the number of infective virions (Katan, 2017). With increased precipitation or irrigation over time it is possible to increase the dilution level of the virions and they can also be washed off or leached beyond the rooting zones (Veena *et al.*, 2014). The number of vectors involved in the transmission of the viruses in the soil would reduce due to lack of suitable host with prolonged duration of incorporation of infected material before sowing of maize seeds (Hiruki and Teakle, 1987; Roberts, 2014).

Effect on incidence and severity of maize chlorotic mottle disease

The duration the MLN infected residues remained in the soil had a significant effect on the percentage incidence of maize chlorotic mottle disease **(**MCMD) in crop planted both sites during the two seasons (Table 2). The highest percentage incidence of MCMD at 100 was recorded in H614 and WE1101 plants from plots incorporated with MLN infected maize residues up to 45 days in the screen house.

Table 2. Incidence % of maize chlorotic mottle disease in two maize varieties grown in media incorporated with maize lethal necrosis infected maize residues.

Days after residue incorporation	Variety H614			Variety WE1101		
	2016 long rains	2017 long rains	Mean	2016 long rains	2017 long rains	Mean
Field experiment						
0	31.9	20.3	24.0	19.4	17.4	18.4
15	19.1	16.1	19.7	14.4	13.8	14.1
30	19.1	13.7	16.4	13.3	11.5	12.4
45	18.0	11.6	14.8	12.2	10.2	11.2
60	12.6	6.2	9.4	11.4	4.9	8.2
90	3.9	1.0	2.5	3.5	1.0	2.3
Non-incorporated	0	0	0.0	0	0	0.0
LSD $_{(p\leq0.05)}$ (V)	NS	NS		NS	NS	
LSD $_{(p\leq0.05)}$ (D)	5.9	2.5		5.9	2.5	
LSD $_{(p\leq0.05)}$ (VxD)	NS	NS		NS	NS	
CV (%)	29.5	5.5		29.5	5.5	
Screen house experiment						
0	100	100	100	100	100	100
15	100	100	100	100	100	100
30	100	100	100	100	100	100
45	100	100	100	100	100	100
60	88.9	77.9	83.4	88.9	66.7	77.8
90	66.7	33.3	50	55.6	0	27.8
Non-incorporated	0	0	0	0	0	0
LSD $_{(p\leq0.05)}$ (V)	NS	NS		NS	NS	

LSD $_{(p\leq0.05)}$ (D)	10.9	23.7	10.9	23.7
LSD $_{(p\leq0.05)}$ (VxD)	NS	NS	NS	NS
CV (%)	2.5	16.9	2.5	16.9

Lsd= Least significant difference; CV= coefficient of variation; V= Variety; D= Duration ;VxD= interaction between variety and severity.

The percentage severity of MCMD was significantly affected by the duration the mlN infected maize residues remained in the soil for the crop which was planted in the screen house during both seasons (Fig. 1 and 2). Similar observation was made in the crop that was planted in the field during 2016 long rains.

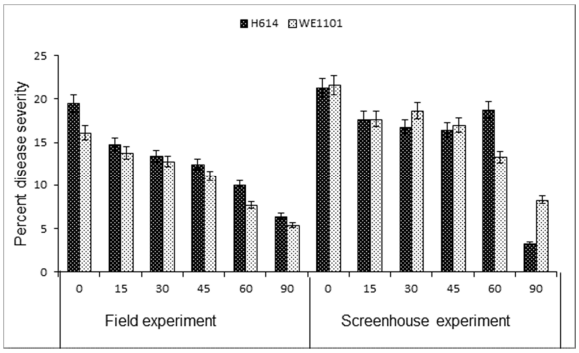

Fig. 1. Severity % of maize chlorotic mottle disease in two maize varieties grown in media incorporated with maize lethal necrosis infected maize residues during 2016 long rains.

The variety which was sown had a significant effect on the severity of MCMD in the crop which was planted in the field during 2016 and 2017 long rains. It was noted that, the interaction between the variety which was sown and the duration the MLN infected maize residues remained in the soil had significant effect on the severity of MCMD in the crop that was planted in the field during 2017 long rains. Highest severity of MCMD at 27.3 was recorded in H614 plants germinated from seeds sown immediately after incorporation of MLN infected maize residues in the screen house during 2017 long rains season.

The variety sown had a significant effect on the AUDPC in the crop that was planted in the field during 2016 long rains (Table 3). The duration the MLN infected maize residues was incorporated in the soil had a significant effect on the AUDPC in the crop that was planted in both sites during the two seasons.

The highest AUDPC at 628 was noted in the H614 crop which was planted in the screen house during 2016 long rains. The interaction between the variety sown and the duration the MLN infected maize residues remained in the soil had significant effect on the AUDPC in the crop that was planted in the screen house during 2016 long rains.

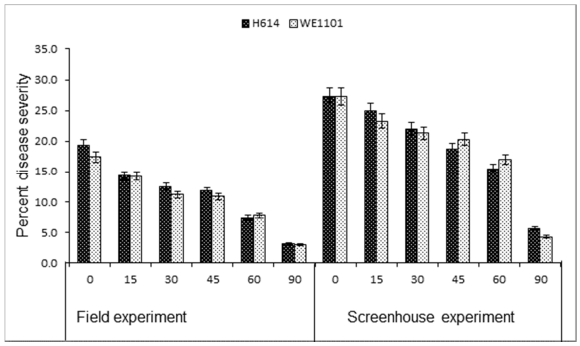

Fig. 2. Percentage severity of maize chlorotic mottle disease in two maize varieties grown in media incorporated with maize lethal necrosis infected maize residues during 2017 long rains.

Findings by Allen (1981) showed that the amount of *Tobacco mosaic virus* present in the soil was directly related to the number of plants and leaves that were contaminated and the lesion counts. Ammara *et al.* (2017) also found out that the virus titre of tomato yellow leaf curl disease decreased with the relative decline in symptom severity scale. A study conducted by Asare-Bediako *et al.* (2018) to assess cowpeas genotypes resistance to *Cucumber mosaic virus*, *Cowpea severe mottle virus* and *Cowpea mosaic virus* revealed that the viral disease severity was positively related to AUDPC.

It is possible that the freshly incorporated infected maize residues have a higher concentration of MCMV and under the same environmental condition and varieties assessed; there would be more disease intensity as compared to other treatments (Jensen (1985). The amount of inoculum can decrease with increased period of incorporation of infected maize residues due to decomposition taking place.

Hence the disease development is negatively correlated to duration of incorporation of infected material into the soil and subsequently the amount of disease over time as indicated by AUDPC.

Effect on plant height and grain yield

The variety which was sown had a significant effect on maize plant height for the crop which was grown in the field during 2017 long rains (Table 4). Height of maize plant was significantly affected by the duration the MLN infected maize residues remained in the soil in the field the two seasons and in screen house during 2017 short rains.

The highest reduction in plant height at 33.6% was realized in WE1101 variety which was sown in media immediately after incorporation MLN infected maize residues (Table 4). However, interaction between the variety and the duration the MLN disease infected maize residues remained in the soil before sowing had no significant effect on the height of maize height for the crop which was sown in the field and screen house during the two seasons. The variety which was sown had a significant effect on maize grain yield harvested from crop plants grown in the field during 2016 long rains.

Table 3. Area under maize chlorotic mottle disease progress curve of two varieties grown in media incorporated with maize lethal necrosis infected maize residues.

Days after residue incorporation	Variety: H614			Variety: WE1101		
	2016 long rains	2017 long rains	Mean	2016 long rains	2017 long rains	Mean
Field experiment						
0	529	481	505	457	481	469
15	419	409	414	388	409	399
30	336	333	334	327	333	330
45	288	298	293	279	298	288
60	248	196	222	193	196	195
90	163	110	137	99	110	105
Non-incorporated	0	0	0	0	0	0
LSD $_{(p\leq 0.05)}$(V)	28.0	NS		28.0	NS	
LSD $_{(p\leq 0.05)}$ (D)	30.7	23.4		30.7	23.4	
LSD $_{(p\leq 0.05)}$ (VxD)	NS	NS		NS	NS	
CV (%)	3.0	2.5		3.0	2.5	
Screen house experiment						
0	628	416	522.0	541	395	468.0
15	554	336	445.0	425	326	375.5
30	590	301	445.5	478	323	400.5
45	609	291	450.0	436	281	358.5
60	520	319	419.5	402	249	325.5
90	350	61	205.5	173	103	138.0
Non-incorporated	0	0	0.0	0	0	0.0
LSD $_{(p\leq 0.05)}$(V)	NS	NS		NS	NS	
LSD $_{(p\leq 0.05)}$ (D)	22.8	91.2		22.8	91.2	
LSD $_{(p\leq 0.05)}$ (VxD)	6.5	NS		6.5	NS	

Table 4. Plant height (cm) of two maize varieties grown in media incorporated with maize lethal necrosis infected maize residues.

Days after residue incorporation	Variety: H614			Variety: WE1101		
	2016 long rains	2017 long rains	Mean	2016 long rain	2017 long rains	Mean
Field experiment						
0	125.8	125.8	125.8	113.4	113.4	113.4
15	133.1	136.0	134.6	119.4	119.4	119.4
30	136.0	133.1	134.6	123.7	127.7	125.7
45	146.1	146.1	146.1	127.7	123.7	125.7
60	150.0	150.0	150.0	138.7	137.8	138.3

Days after residue incorporation	Variety: H614			Variety: WE1101		
	2016 long rains	2017 long rains	Mean	2016 long rain	2017 long rains	Mean
90	162.6	162.6	162.6	141.2	138.7	140.0
Non-incorporated	170.2	166.9	168.6	169.4	169.4	169.4
LSD $_{(p\leq0.05)}$ (V)	NS	26.5		NS	26.5	
LSD $_{(p\leq0.05)}$ (D)	6.9	7.3		6.9	7.3	
LSD $_{(p\leq0.05)}$ (VxD)	NS	NS		NS	NS	
CV (%)	5.4	5.4		5.4	5.4	
Screen house experiment						
0	137.3	118.5	133.7	131.9	115.9	123.9
15	147.9	126.8	137.4	133.5	121.6	127.6
30	148.8	140.3	152.6	143.6	140.7	142.2
45	156.2	141.0	152.7	150.0	144.9	147.5
60	160.6	141.4	148.8	165.3	140.4	152.9
90	164.9	148.5	142.9	166.2	143.8	155.0
Non-incorporated	164.3	158.0	159.3	166.5	152.0	159.3
LSD $_{(p\leq0.05)}$(V)	NS	NS		NS	NS	
LSD $_{(p\leq0.05)}$ (D)	NS	10.8		NS	10.8	
LSD $_{(p\leq0.05)}$ (VxD)	NS	NS		NS	NS	
CV (%)	8.5	3.7		8.5	3.7	

Lsd= Least significant difference; CV= coefficient of variation; V= Variety; D= Duration; VxD= interaction between variety and severity.

Duration the MLN infected maize residues remained in the soil before sowing had significant effect on maize grain yield harvested from plants grown in the field and screen house during both seasons (Fig. 3 and 4). Highest reduction in maize grain weight at 3.8 to 44.8% was recorded in grains harvested from plants established in media with freshly incorporated MLN infected maize residues during 2016 long rains. The interaction between the variety sown and duration the MLN infected maize residues remained in the soil before sowing had no significant effect on maize grain yield realized from plants grown in the two sites during both seasons.

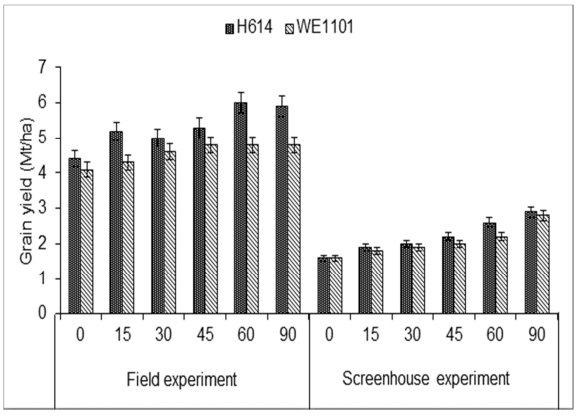

Fig. 3. Grain yield (Mt/ha) of two maize varieties grown in media incorporated with maize lethal necrosis infected maize residues 2016 long rains.

Study by Viswanathan and Balamuralikrishnan (2005) revealed that infection of *Co 740* and *CoC 671* sugarcane varieties had their growth significantly reduced due to infection with *Sugarcane mosaic virus* as compared to virus free canes. It is possible that the virus inoculum may have reduced with time due as the maize residues decomposing and therefore resulting in variation in plant height. Reports by Castillo and Hebert (1974) indicated that yield losses due to infection with MCMV of flourly and sweet maize varieties in Peru maize at 10-59%. Maize plants that are infected with the virus are stunted and have short internodes (Goldberg and Brakke, 1987; Wang *et al.*, 2017).

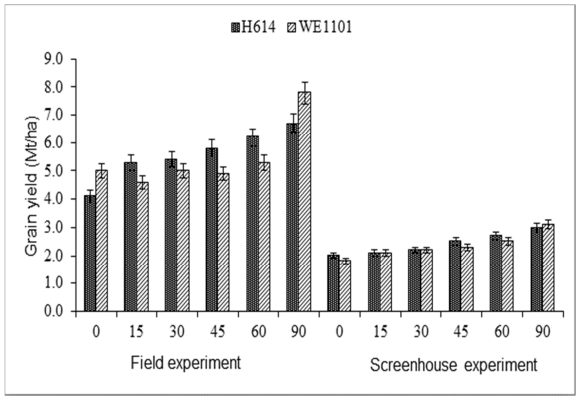

Fig. 4. Grain yield (Mt/ha) of two maize varieties grown in media incorporated with maize lethal necrosis infected maize residues 2017 long rains.

It has been reported that infection maize with MCMV caused abnormalities in ears and hence reduced yields (Deng *et al*., 2014). However, the highest yield losses is anticipated when by coincidence the plants are also infected with any cereal poty viruses resulting in synergistic reaction to give rise tom lN disease (Adams *et al*., 2012; Wangai *et al*., 2012). The reduction in weight may be caused by the virus interfering with the physiological processes of the plant. With increase with the leaf that has symptoms are mottled would mean that there is limited chlorophyll and hence decline in food manufactured (Wangai *et al*., 2012; Mahuku *et al.,* 2015).

Conclusion

Plants that were sown at 90 days after incorporation of residues gave zero to traces of virus during detection. Virus titre of MCMV, disease severity, AUDPC declined with increase in the number of days MLN infected residues remained in the soil. However plant height and grain yield was positively correlated to the period the MLN infected materials was incorporated into the soil before sowing took place. The study revealed that MLN infected maize residue incorporated into the soil during or before sowing seeds was the source of MCMV which infected maize plants.

Recommendations

Farmers should continuously be trained on proper handling of maize residues to avoid infecting subsequent maize crop from the infected residues and soil. They are also advised to leave land fallow for not less than three months and if possible rotated cereal crops with non-host of MCMV plants before replanting with cereals to reduce disease transmission through infected soil. The Government of Kenya should allocate adequate resources for comprehensive research on development of resistance/tolerant varieties to MCMV and viruses causing MLN for sustainable management of the disease.

Conflict of interests

The authors have not declared any conflict of interests.

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