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Research Application Summary

Spatial and temporal spread of maize lethal necrosis disease causing viruses and their vectors within the field

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Abstract

Maize lethal necrosis disease (MLND) is a viral disease of maize resulting from co-infection of *Maize chlorotic mottle virus* (MCMV) and any cereal Potyvirus such as *Sugarcane mosaic virus* (SCMV), *Maize dwarf mosaic virus* (MDMV) or *Wheat streak mosaic virus* (WSMV). It was first reported in Kenya in September 2011 in Bomet County with a yield loss of 90 – 100%. Since its first report, efforts have been directed towards causal viruses, vectors and possible management options. The present study aimed at determining the spatial and temporal distribution of MCMV and SCMV causing MLND and their vectors within the field. Two maize varieties; H614 and Duma 43 were grown and managed in non-replicated plots at the University of Nairobi, Upper Kabete Campus Field Station. The plants were naturally infested with vectors and infected by MLND causing viruses. Vectors abundance on plants was assessed by counting techniques and sticky traps at two weeks interval. Twenty maize leaf samples per variety both asymptomatic and symptomatic were collected at two stages (knee high and prior to flowering) of crop development to confirm the presence or absence of MCMV and SCMV using DAS-ELISA. In the study, vectors began infesting the crop from 3rd – 5th weeks after emergence. Aphids were observed in colonies (on a few plants) in patches while thrips were evenly distributed within the field. In both cases there were no differences in infestation of the two varieties by the vectors. A single peak was observed for aphids while two peaks were observed for thrips during the growing seasons. For maize leaf samples tested, nine samples from H614 and eight samples from Duma 43 tested positive for MCMV, while three samples from H614 and one sample from Duma 43 tested positive for SCMV. Though Kabete is not an MLND hotspot area, the viruses associated with MLND were present.

Key words: Duma 43, H614, Kabete, Kenya, Maize lethal necrosis disease, spatial and temporal spread

Résumé

La maladie de nécrose létale du maïs (MLND) est une maladie virale du maïs résultant de la co-infection du virus de la marbrure chlorotique du maïs (MCMV) et de tout virus potyvirus des céréales tel que le virus de la mosaïque de la canne à sucre (SCMV), le virus de la mosaïque naine du maïs (MDMV) ou la mosaïque des stries du blé virus (WSMV). Il a été signalé pour la première fois au Kenya en septembre 2011 dans le comté de Bomet avec une perte de rendement de 90 à 100%. Depuis

son premier rapport, les efforts ont été dirigés vers les virus causaux, les vecteurs et les options de gestion possibles. La présente étude visait à déterminer la distribution spatiale et temporelle du MCMV et du SCMV causant le MLND et leurs vecteurs dans le champ. Deux variétés de maïs; H614 et Duma 43 ont été cultivés et gérés dans des parcelles non reproduites à l'Université de Nairobi, à la station de terrain du campus Upper Kabete. Les plantes étaient naturellement infestées de vecteurs et infectées par des virus causant le MLND. L'abondance des vecteurs sur les plantes a été évaluée par des techniques de comptage et des pièges collants à deux semaines d'intervalle. Vingt échantillons de feuilles de maïs par variété à la fois asymptomatiques et symptomatiques ont été collectés à deux stades (genou et avant la floraison) du développement de la culture pour confirmer la présence ou l'absence de MCMV et SCMV en utilisant DAS-ELISA. Dans l'étude, les vecteurs ont commencé à infester la culture de la 3eme à la 5eme semaines après la levée. Les pucerons ont été observés en colonies (sur quelques plantes) en parcelles tandis que les thrips étaient uniformément répartis dans le champ. Dans les deux cas, il n'y avait aucune différence d'infestation des deux variétés par les vecteurs. Un seul pic a été observé pour les pucerons tandis que deux pics ont été observés pour les thrips pendant les saisons de croissance. Pour les échantillons de feuilles de maïs testés, neuf échantillons de H614 et huit échantillons de Duma 43 ont été testés positifs pour le MCMV, tandis que trois échantillons de H614 et un échantillon de Duma 43 ont été testés positifs pour SCMV. Bien que Kabete ne soit pas un endroit très infecté par le MLND, les virus associés au MLND étaient présents.

Mots clés : Duma 43, H614, Kabete, Kenya, Nécrose létale du maïs, propagation spatiale et temporelle

Background

Maize (*Zea mays* L.) is regarded as the main staple food for more than 1.2 billion people in both Latin America and Sub-Saharan Africa (Iken and Amusa, 2014). It is ranked as the third most important cereal crop after wheat and rice (Khalili *et al*., 2013). Maize provides food and income to over 300 million resource poor small-scale holders in sub-Saharan Africa (Tefera *et al*., 2011). In Kenya, over 1.6 million hectares of land is currently under maize production (Schroeder *et al*., 2013). Maize as for both human and animal nutrition and is a raw material for manufacture of many industrial products such as maize oil, maize syrup, maize starch, maltodextrins, and products of fermentation and distillation industries. Maize has also been utilized for the production of biofuel (Romains, 2001). Kenya leads in maize consumption in Eastern Africa region with an estimated average per capita consumption of between 98 to 100 kgs, which translates to roughly 30 to 34 million bags (2.7 to 3.1 million metric tons) per year (Jayne *et al*., 2001; Muiru, 2008).

According to MoALF (2012), only 25,000 tons of maize is produced in the country against the national demand of 35,000 tons. This low production is caused by many biotic and abiotic factors (Fakorede *et al*., 2003; Vivek *et al*., 2010), among them *Maize lethal necrosis* (MLN). *Maize lethal necrosis* is a viral disease caused by *Maize chlorotic mottle virus* (MCMV), a single virus infection or in a combination with any cereal infecting *Potyviridae* family like S*ugarcane mosaic virus* (SCMV), Wheat streak mosaic virus (WSMV) or *Maize dwarf mosaic virus* (MDMV) (Uyemote *et al*., 1981; Bockelman *et al*., 1982). The co-infection of two viruses, MCMV and SCMV, is more severe than single infection resulting to a condition known as *Maize lethal necrosis disease* (MLND) or *Corn lethal necrosis* (CLN) (Niblett and Claflin, 1978; Uyemoto *et al*., 1980 , 1981; Scheets, 1998).

Infection of maize by MLND and lack of proper management practices is a major challenge to maize production in Kenya resulting in huge losses. The severity of MLND has placed Kenya as a country

dependent on maize at risk of food shortage due to high losses. The disease causes yield loss of 90 – 100% in highly affected areas greatly affecting maize production in the country (Ajala *et al*., 2010; Morais *et al*., 2012; Ochieng *et al*., 2012; Wangai *et al*., 2012; Adams *et al*., 2013). This poses a threat to about 90% of the Kenyan population that depend largely on maize directly or indirectly in terms of food, labour and income (Ochieng *et al*., 2012).

Recorded evidence indicate that MLND was first reported in East Africa Region (EAR), specifically Kenya in September 2011 (Wangai *et al*., 2012a, b) along Rift Valley regions of Bomet, Narok, Chepalungu, Naivasha, Transmara, Sotik, Bureti, Konoin, Nakuru, South Narok and Mathira East, Iment South and Nyeri districts of Central Kenya (Wangai *et al*., 2012c). From the time MLND was reported, a lot of information has been and is still being generated on causal viruses, their vectors, and possible management options. Efforts have been directed towards cultural means of disease management, use of pesticides to control vectors and use of certified seeds for production, but there is still limited information on the epidemiology of the disease which is critical in the management of the disease and the vectors of the causal pathogens. Although, maize thrips and aphids are known to transmit the disease, it is not clear how the disease, MLND causing viruses and the vectors occur and spread within maize fields. This study, therefore, determined the spatial and temporal distribution of MCMV, and SCMV causing MLND and their vectors within the field.

Materials and methods

The study was conducted in Upper Kabete Campus Field Station of the University of Nairobi. Kabete stands at an altitude of 1820m, latitude 10 15‘S and longitude 360 44‘E, characterized by well drained, very dark reddish brown to dark red, friable clay with acid humic top soils (humic nitisols) developed from Limuru Trachyte. The area receives an average annual rainfall of about 1000mm with a mean monthly maximum temperature of 23^0 C and a minimum of 12^0 C (Michieka, 1979).

Maize crop of the variety Hybrid 614 and Duma 43 were grown and managed with normal agronomic practices in the open field for two seasons from May 2015 to October 2016. The total area under study was 50 m by 20 m (1000 m^2). The area was divided into two for the two varieties of maize each having 500 m^2 (25 m by 20 m). Each 500 m^2 area was divided into 100 plots/quadrats each measuring 2.5 m by 2 m, which were non-replicated. Each plot had thirty plants sown at the rate of two seeds per hill making a plant population of 3,000 for each variety and a total of 6,000 (two varieties) plants for the whole experiment. Abundance of virus vectors present on plants were quantified using counting techniques, namely; visual observations (three plants per plot) and use of sticky traps over different crop stages under field conditions. Insect pests already identified as possible MLND virus vectors; maize leaf aphid (*Rhopalosiphum maidis*) and maize thrips (*Franklinniella williamsi*) were quantified in this study. Quantification of virus vectors was done at interval of two weeks through destructive sampling where the sampled plants were cut down and leaves removed one by one. Three plants per plot were sampled from each of the 100 plots per variety. Observations for the presence of both maize leaf aphids and thrips were done by the aid of a hand lens on all internal plant parts; in whorls, in sheaths, under cob husks, on silk and on tassels. All the collected aphids and thrips were counted per plant and recorded. Additionally, monitoring of virus vectors was conducted using blue sticky traps (HORIVER-TR, size; 25 x 10 cm) obtained from Koppert Biological Systems (K) Limited. Ten traps per variety were set and retrieved after every two weeks (14 days). Traps were equidistance placed each covering 50 m^2 (equivalent to 10 plots each measuring 2.5 x 2.0 m). Traps were placed 30 – 50 cm above the crop canopy level according to manufacturer’s instructions. Traps were adjusted with increasing crop height. Adult insect vectors were continuously trapped on two varieties of maize, H614 and Duma 43, during the two seasons of the study. Trapped insect vectors were taken to Entomology Laboratory of the University of

Nairobi Field Station for counting and identification. Vectors were morphologically identified using taxonomic keys with the help of entomologists in the University.

Leaf samples of maize plants showing typical characteristic of MLND symptoms and asymptomatic ones were collected from all maize varieties. Leaf samples were collected at different stage of maize growth; knee high and middle vegetative prior to flowering. Plant leaves were packed in sealed plastic bags and transported to the molecular laboratory, Upper Kabete Campus of the University of Nairobi for storage before analysis. Virus present or absent in the samples was determined using double antibody Sandwich enzyme linked immuno-sorbent assay (DAS-ELISA) as described by Clark and Adams (1977).

The data collected for distribution and dynamics of virus vectors within the field were transformed before analysis. The data were then subjected to analysis of variance (ANOVA), without blocking to derive the means of vectors at different time-points of developmental stages of maize crop. Statistical analysis was performed using GenStat – PC v.14.1, 14th Edition (Payne *et al*., 2011).

Results

The abundance of vectors on maize plants from May to October, 2015 is shown in Table 1. The first incidence of aphids and thrips occurred on maize plants in the month of June when plants were three Weeks after Emergence (WAE). At the early stages of maize development (3rd and 4th WAE) the highest number of aphids (more than 2 aphids per plant) was found on the test plants. A general decrease in aphids number was observed with increase in age of the maize plants reaching as low as 1 aphid per plant when the plants were at 20th WAE and cobs were drying. On other hand, thrips number increased from 3rd WAE to its peak (more than 4 thrips per plant) between 9th and 11th WAE. The number dropped and again increased in the early stage of tasseling and cob formation – indicating second generation infestation of thrips on maize plants. At the stage of tassel development and cob formation, a maximum of 2 – 3 thirips per cob were recorded.

In comparison, H614 was more attacked by vectors than Duma 43 but no difference in aphid and thrip infestation was observed at the vegetative and flowering stage of the cultivars used. More thrips were collected than aphids.

In 2016, the first incidence of vectors appeared in May when maize plants were at 5 WAE. Generally, plots where H614 variety of maize was grown were more infested compared to plots where Duma 43 variety of maize was grown. The general peak of thrips number (3 – 4 thrips per plant) was in the month of August between 14th and 17th WAE during tasseling and cob formation stage of the crop. The highest number of aphids (2.03 aphids per plant) was recorded in July when crop was at the 11th WAE in H614 plots during vegetative stage, while a maximum number of aphids (1.57 aphids per plant) was recorded in Duma 43 plots in the month of August when crop was at 15th WAE during tasseling and cob formation stage of crop development. The last incidence of both vectors was recorded in September when the crop was at 19th WAE and the cobs formed were drying up (Table 2).

Table 1. Mean population of aphids and thrips per plant over sampling period in season one (May – October, 2015)

Pest		Sampling period (WAE)										
	Stage of crop	Vegetative phase							Tasseling and cob formation			
	WAE	3rd	4th	6th	9th	11th	14th	16th	17th	18th	19th	20th
	Month	June		July		August			September			October
	Variety											
Aphids	H614	2.39	2.58	1.64	1.12	1.77	1.28	1.31	1.36	1.69	1.48	1.45
	Duma 43	2.38	2.45	1.49	1.09	1.09	1.06	1.24	1.35	1.59	1.42	1.2
LSD							0.07					0.08
%CV							65.8					75.2
Thrips	H614	1.1	1.07	2.84	4.72	4.29	1.26	3.3	3.64	2.88	2.18	1.51
	Duma 43	1.16	1.41	2.67	4.48	3.44	1.14	2.15	2.46	1.85	2.23	1.74
LSD							0.06					0.06
%CV							31.7					36.7

Data are means of ANOVA (no blocking) at $p < 0.05$. Transformation Formula is: T = Square root of (X+1), where T is transformed data, X is the data to be transformed and 1 is the constant. CV – Coefficient of variation, LSD – Least significant difference, = WAE – Weeks After Emergence

The first incidence of vectors trapped was when the crop was at 5th and 6th WEA in 2015 and 2016, respectively. The highest mean number of aphids trapped in 2015 was 7.08 and 9.80 aphids per trap for H614 and Duma 43 variety of maize, respectively. This was mid-vegetative stage of crop development in the late month of July when the crop was at 7th WAE, then followed by a gradual decrease in aphids number captured from month of July (9th WAE) up to tasseling phase in late September (17th WAE). Thrips abundance increased from late June, i.e., early periods of crop development reaching the peak in the early dry month of August with mean of 29.58 and 27.66 for H614 and Duma 43, respectively. This was followed by a gradual decrease in thrips abundance towards tasseling phase stage of the crop in the month of September (Table 3). In 2016 (season two), the behaviour of these insect vectors was slightly different. There were no aphids trapped. However, thrips seemed to exhibit same trend experienced in season one. The general picture for thrips was that more thrips were trapped in the dry early month of July (10th WAE) at 20.30 and 21.55 per trap for H614 and Duma 43 variety, respectively. The thrips numbers then dropped in late July (12th WAE) before increasing to 21.73 thrips per for H614 and 23.39 thrips per trap for Duman 43 variety in the early month of August (12th WAE). Thrips trapped then decreased towards tasseling stage of crop development in the late month of August (Table 4).

A total of 40 maize leaf samples were tested for both MCMV and SCMV using DAS-ELISA, 20 for each variety. Of these 10 out of 20 samples per variety were collected at different stage of crop development. Seven out of ten were asymptomatic samples while the remaining three samples were symptomatic. First sampling of leaves was done at knee high while second sampling was done at prior to flowering stage of crop development. Nine (9) out of twenty (20) samples were tested positive for

MCMV while three out of 20 samples tested positive for SCMV for H614 variety. On other hand, eight out of 20 samples were tested positive for MCMV while one out of 20 samples tested positive for SCMV for Duma 43 variety (Table 5).

Discussion

In 2015, the mean number of maize leaf aphids ranged from 1.06 – 2.58 individuals per plant, while number of maize thrips recorded on plant ranged from 1.07 – 4.72 individuals per plant (Table 1). In 2016, the number of maize leaf aphids and maize thrips that were recorded maize plants ranged from 1.08 – 2.03 and 1.07 – 4.79 individuals per plant, respectively (Table 2). Maize leaf aphids that were trapped in 2015 ranged from 1.41 – 9.14 individuals per trap, while maize thrips ranged from 1.65 – 29.58 individuals per trap (Table 3). In 2016, the number of maize thrips trapped ranged from 5.17 – 23.39 individuals per trap. There were no activities of maize leaf aphids in traps in 2016 (Table 4).

Table 2. Mean population of aphids and thrips per plant during the sampling period in season two (April – September, 2016)

Pest		Sampling period (WAE)										
	Stage of crop	Vegetative phase							Tasseling and cob formation			
	WAE	5th	7th	9th	11th	13th	14th	15th	16th	17th	18th	19th
	Month	May		June		July			August		September	
	Variety											
Aphids	H614	1.12	1.27	1.37	2.03	1.82	1.32	1.13	1.06	1.23	1.15	1.18
	Duma 43	1.10	1.16	1.19	1.39	1.43	1.27	1.57	1.55	1.14	1.37	1.08
LSD						0.08						0.05
%CV						83.3						67.0
Thrips	H614	1.07	2.06	2.72	3.37	1.87	3.06	4.31	4.79	4.72	3.58	2.91
	Duma 43	1.22	2.02	2.88	1.86	2.16	2.95	4.32	4.46	3.80	3.24	2.40
LSD						0.08						0.07
%CV						51.4						30.7

Data are means of ANOVA (no blocking) at $p < 0.05$. Transformation Formula is; T = Square root of (X+1), where T is transformed data, X is the data to be transformed and 1 is the constant. CV – Coefficient of variation, LSD – Least significant difference, = WAE – Weeks After Emergence

Individual aboveground plant parts along the changing developmental stages of maize including leaves, whorls, sheaths, cob husks and tassels were examined during the entire period of the study. Maize whorls, sheaths and inside cob husks were observed to habour more vectors compared to open leaves and tassels. Similar results were found by Pawe *et al*. (2013) in Poland. In another study carried out by Obsrist *et al.* (2005; 2006) in Spain *Frankliniella tenuicornis* were mainly found on leaves and stems, while *Frankliniella occidentalis* infested mainly flowers. With regard to time of infestation, the current study showed that the first incidence of individual vectors on maize plants occurred in early June when maize plants were at 3 WAE and in late May when crops were at 5 WAE in 2015 and 2016, respectively (Tables 1 and 2). The findings in this study agree with those of Lisowicz (1996) and Kucharczyk *et al.* (2011) who reported similar trends. After years of study on fodder maize in southeastern Poland, Lisowicz (1996) found out that the first thrips on maize plants occurred in the third week of May or in early June (4-5 WAE), while Kucharczyk *et al*. (2011)

Table 3. Mean population of aphids and thrips per trap over trapping season one (May – October, 2015)

Pest		Sampling period (WAE)						
Stage of crop		Vegetative phase					Tasseling and cob formation	
WAE		5th	7th	9th	11th	13th	15th	17th
Month		June			July	August	September	
Variety								
Aphids	H614	6.06	7.08	5.35	3.49	3.48	2.06	1.54
	Duma 43	6.14	9.8	5.23	3.5	4.07	1.41	1.71
LSD								0.38
%CV								25.8
Thrips	H614	1.65	13.8	22.2	29.58	14.68	18.13	9.41
	Duma 43	2.33	13.92	20.16	27.66	19.54	10.46	
LSD							0.99	
%CV							19.1	

Data are means of ANOVA (no blocking) at $p < 0.05$. Transformation Formula is; T = Square root of (X+1), where T is transformed data, X is the data to be transformed and 1 is the constant. CV – Coefficient of variation, LSD – Least significant difference, = WAE – Weeks After Emergence

Table 4. Mean population of aphids and thrips per trap over trapping season two (April – September, 2016)

Pest	Sampling period (WAE)						
	Stage of crop	Vegetative phase				Tasseling	
	WAE	6th	8th	10th	12th	14th	16th
	Month	June		July		August	
	Variety						
Aphids	H614	1.15	1.00	1.00	1.00	1.00	1.00
	Duma 43	1.00	1.00	1.00	1.00	1.00	1.00
LSD							0.05
%CV							13.1
Thrips	H614	5.78	10.76	20.3	18.87	21.73	16.28
	Duma 43	5.17	10.15	21.55	18.98	23.39	14.28
LSD							1.36
%CV							23.9

Data are means of ANOVA (no blocking) at $p < 0.05$. Transformation Formula is; T = Square root of (X+1), where T is transformed data, X is the data to be transformed and 1 is the constant. CV – Coefficient of variation, LSD – Least significant difference, = WAE – Weeks After Emergence

recorded them in the second week of May and in the second week of June. Based on many years of observations both Lisowicz (1996) and Kucharczyk *et al*. (2011) identified a single peak in the development of these insects, which is consistent with this study results for aphids but not for thrips where two peaks were observed during vegetative phase and tasseling and cob formation phase of the crop. However, this pattern is likely not the same for all areas of the world, and likely also varies depending on the host plant (Reynolds and Wilson, 1989; Kuo *et al*., 2006; van Emden and Harrington, 2007; Hesler and Dagel, 2010).

Table 5. DAS-ELISA results for MCMV and SCMV in maize leaves of H614 and Duma 43 variety Season two (April – September, 2016)

	First sampling (knee high)				Second sampling (prior to flowering)			
Variety	H614		Duma		H614		Duma 43	
Sample no.	MCMV	SCMV	MCMV	SCMV	MCMV	SCMV	MCMV	SCMV
1	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-
3	-	+	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-
5	+	-	+	-	-	-	+	-
6	+	-	-	-	+	-	-	-
7	-	-	-	-	-	-	-	-
8	+	+	+	-	+	-	+	-
9	+	-	+	+	+	-	+	-
10	+	-	+	-	+	+	+	-

Sample 1 – 7 = Asymptomatic maize leaves; Sample 8 – 10 = Symptomatic maize leaves; + = Virus present - = Virus absent

The difference between the time-points of peak numbers of the vectors on maize plants in this study may have been associated with the type of maize variety, H614 and Duma 43, in terms of the earliness or lateness of the variety to mature. However, both previous and present studies have demonstrated that regardless of the maize type, thrips usually end up feeding in the second, or in the third decade of September, when the plants began to dry out (Lisowicz, 1996; Kucharczyk *et al*., 2011). In addition, the late vegetative phase of crop development resulted in low attack of virus vectors because of lack of the most vulnerable stage of plants corresponding with the period between two generations of virus vectors – early attack (on young plants) and late attack (start of cob formation). The second infestation on cobs did not last long as the plants reached the phenological phase of cropping when the plant leaves and cob husks began to dry thus probably becoming more tolerant to virus vectors infestation. In this study, MCMV and SCMV were detected by DAS-ELISA in some of the maize leaf samples tested though the samples were not mechanically inoculated. This indicates that the disease (MLND) could be occurring naturally within the field, (Cabanas *et al*., 2013; Lanoiselete *et al*., 2008). The presence of MCMV and SCMV in maize samples also tallies with previous reports occured of MLND in Kenya (Wangai *et al*., 2012).

Conclusion

The high number and pattern of distribution of virus vectors on maize crops could impact on crop heath and consequently on spread of MLND viruses within the field. Therefore, timely management of these vectors before they reach their peak will be of help to reduce the spread and damage by MLND.

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