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Research Application Summary

Transmission pattern of Maize chlorotic mottle virus (Machlomovirus) on maize by *Frankliniella williamsi* and *F. occidentalis* (Thripidae) in Kenya

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Abstract

Maize chlorotic mottle virus (MCMV) infects maize (*Zea mays* L) which is a major staple food crop in sub-Saharan Africa, causing yield losses of between 10-15%. When the virus co-infects with maize infecting potyviruses, they cause Maize lethal necrosis (MLN) disease which reduces yields by 30-100%. Persistence and efficiency transmission of MCMV by *Frankliniella williamsi* (Hood) and *F. occidentalis* (Pergande) was investigated. Transmission efficiency was evaluated at 1, 3, 24 and 48 hour inoculation access periods (IAPs). Virus post acquisition retention period was tested after 1, 2, 3, and 4 days on maize seedlings. Persistence transmission and rates of new re-infections (4, daily cycle) was determined. Plants were covered by nylon-mesh cages inside a greenhouse for symptom observation. Severity was measured and MCMV detected in leaf samples by ELISA and PCR. Means of infected plants at IAPs were log transformed (base 10) and a t-test done. Means of re-infected plants at retention periods were subjected to ANOVA. MCMV transmission rate of *F. occidentalis* rose while that of *F. williamsi* declined after 24 hour IAP. Means of infected plants by both thrips were significantly different ($t = 2.77$, D.F. = 362.97, $P = 0.006$). *F. occidentalis* infected plants had higher symptom severity than those by *F. williamsi* (0.2702, 0.1997), with 1.176 mean difference (was higher by 17.6 %). Plants re-infected by both thrips were significantly different ($F = 10.27$, D.F. = 2, 17, $P = 0.001$). *F. occidentalis* re-infected more plants than *F. williamsi* (0.32 ± 0.04, 0.27 ± 0.04) at 0.125 LSD. Inoculated plants by *F. williamsi* after day 1 post acquisition period were significantly different from those by *F. occidentalis* ($F = 5.66$, D.F. = 2, 17, $P = 0.013$). Both thrips transmitted MCMV in a non-persistent pattern and thus should be targeted in IPM strategies for MCMV control.

Key words: Persistence, retention period, thrips

Résumé

Le virus de la marbrure chlorotique du maïs (MCMV) infecte le maïs (*Zea mays* L), qui est une culture vivrière de base majeure en Afrique sub-saharienne, causant des pertes de rendement de 10 à 15%. Lorsque le virus co-infecte avec le maïs infectant les potyvirus, ils provoquent la nécrose létale du maïs (MLN) qui réduit les rendements de 30 à 100%. La persistance et l'efficacité de la transmission du MCMV par *Frankliniella williamsi* (Hood) et *F. occidentalis* (Pergande) ont été étudiées. L'efficacité

de transmission a été évaluée à des périodes d'accès à l'inoculation (IAP) de 1, 3, 24 et 48 heures. La période de rétention du virus après l'acquisition a été testée après 1, 2, 3 et 4 jours sur des plants de maïs. La transmission de persistance et les taux de nouvelles réinfections (4, cycle quotidien) ont été déterminés. Les plantes étaient couvertes par des cages en filet de nylon à l'intérieur d'une serre pour l'observation des symptômes. La sévérité a été mesurée et le MCMV détecté dans des échantillons de feuilles par ELISA et PCR. Les moyens des plantes infectées aux IAP ont été transformés en log (base 10) et un test t a été effectué. Les moyens des plantes réinfectées pendant les périodes de rétention ont été soumis à une analyse de variance. Le taux de transmission MCMV de *F. occidentalis* a augmenté tandis que celui de *F. williamsi* a diminué après 24 heures de PIA. Les moyennes des plantes infectées par les deux thrips étaient significativement différentes (t = 2,77, D.F. = 362,97, P = 0,006). Les plantes infectées par *F. occidentalis* avaient une sévérité des symptômes plus élevée que celles de *F. williamsi* (0,2702, 0,1997), avec une différence moyenne de 1,176 (était plus élevée de 17,6%). Les plantes réinfectées par les deux thrips étaient significativement différentes (F = 10,27, D.F. = 2, 17, P = 0,001). *F. occidentalis* a réinfecté plus de plantes que *F. williamsi* (0,32 ± 0,04, 0,27 ± 0,04) à 0,125 LSD. Les plantes inoculées par *F. williamsi* après le jour 1 après la période d'acquisition étaient significativement différentes de celles par *F. occidentalis* (F = 5,66, D.F. = 2, 17, P = 0,013). Les deux thrips ont transmis le MCMV selon un schéma non persistant et devraient donc être ciblés dans les stratégies IPM pour le contrôle du MCMV.

Mots clés : persistance, période de rétention, thrips

Introduction

Maize (*Zea mays* L.) is the most important cereal crop for smallholder farmers in sub-Saharan Africa (SSA), with over 38 million metric tons (MMT) of grain produced on 25 million ha (FAOSTAT, 2016). Maize is critical for food security in SSA where 95% of the produce is used as food, however, maize production has been threatened by Maize lethal necrosis (MLN) disease (Wangai *et al.*, 2012). *Maize chlorotic mottle virus* (MCMV) of the genus *Machlomovirus,* family *Tombusviridae* co-infects maize with *Potyviridae viruses* such as Wheat streak mosaic virus (WSMV), Maize dwarf mosaic virus (MDMV), or Sugarcane mosaic virus (SCMV, formerly MDMV-B) to cause MLN (Uyemoto *et al.*, 1980). In Kenya, MLN was first reported in September 2011 in Longisa Division of Bomet District (Wangai *et al.*, 2012). By 2012, MLN had spread to other districts in Central, Nyanza, Western, and Rift Valley regions of Kenya. The disease spread to Rwanda, Democratic Republic of Congo (DRC), Uganda, Tanzania, South Sudan and Ethiopia (Mahuku *et al.*, 2015). Indeed MLN has threatened maize production in sub Saharan Africa with 30-100% yield losses, but recent findings also indicate that MCMV is a threat on its own and can cause significant yield losses. This virus causes a yield loss ranging between 10 to 15% in natural infections or up to 59% under experimental conditions.

Potyviruses infecting maize especially SCMV have been known in Kenya and Eastern Africa since 1970s but MCMV is new in Eastern Africa (Wangai *et al.*, 2012). Since 1973 when MCMV was first reported in Peru, it had spread into the United States by 1976 and Argentina by 1982. The virus has further spread to China, Taiwan (Deng *et al.*, 2014) and Kenya in Eastern Africa (Wangai *et al.*, 2012). MCMV is the primary problem in MLN disease complex as there exists an overlap between it and MLN occurrence. Recent surveys have also shown that MCMV is widely distributed in Kenya, Tanzania, Uganda and Democratic Republic of Congo (Mahuku *et al.*, 2015).

The causes of rapid spread of MLN from Bomet in Western Kenya and the mechanisms of MCMV spread over a large geographic area within three years has not been understood (Wangai *et al.*, 2012). However, thrips have been suspected to vector MCMV and spread it within maize fields because they occur in almost all MLN affected fields (Mahuku *et al.*, 2015). It was therefore necessary to determine the persistence transmission of MCMV by thrips for effective management of the virus. This study examined the transmission of MCMV by *F. williamsi* and *F. occidentalis* adults, their transmission rates at different IAPs, persistence of transmission through re-infections and post-acquisition retention periods.

Materials and methods

Inoculum preparation and thrips collection. Potted maize seedlings under greenhouse conditions were mechanically inoculated with pure isolates of MCMV at 3 leaf stage. These plants were sap inoculated using standard methods and served as a source of inoculum. *Frankliniella occidentalis* and *F*. *williamsi* adult thrips were collected onto yellow trays from MLN affected maize fields in Bomet and Kisii Counties of western Kenya. The thrips were shipped into the laboratory and identified according to Mound *et al.* (2009). Colonies of *F. occidentalis* and *F*. *williamsi* were raised on French bean pods and maize stalk cuttings, respectively. Modified culture boxes (5 L cylindrical plastic buckets with cloth ventilated 5x5 cm side openings and top cover) were used in rearing the thrips.

Retention and efficiency transmission of MCMV by thrips. The retention period of MCMV Kenyan isolate by *F. occidentalis* and F. *williamsi* adults was tested on maize cultivar Duma 43 raised on 2 kg plastic pots covered with insect proof cages (1x1x2 M) in a greenhouse. Thrips transmission efficiency of MCMV (proportion of plants successfully infected by these thrips) was evaluated at 1, 3, 24, and 48 hour inoculation access periods (IAPs). Ten adult thrips were starved for 24 hours inside modified 50 ml plastic laboratory universal bottles (clothe ventilated 2x5 cm side openings and cotton wool top cover). Wet leaf discs (5x2cm) from symptomatic leaves were introduced into the universal bottles for the thrips to feed for 48 hours to acquire the virus. The leaf discs were removed from the bottles using a pair of forceps and thrips starved for 4 hours. Leaf tips of healthy 14 day old maize seedlings were inserted into the universal bottles for the thrips to feed and inoculate the virus. The same procedure was used for testing post-acquisition retention period (number of days thrips remain viruliferous) at 1, 2, 3, and 4 day sequence after virus acquisition. Thrips were maintained on honey and pollen after starvation. Water was supplied by wet cotton wool used as top cover. Re-infection by MCMV (number of plants re-infected in days) using thrips was evaluated in 1, 2, 3, and 4 day cycles using the same procedure. Negative controls were exposed to thrips fed on healthy leaf discs while positive controls were mechanically sap inoculated (5g of leaf crushed in 0.05 M phosphate buffer, pH 7.2; sap directly rubbed using carborundum of maize leaves at the 3 leaf stage). Sixty four plants arranged in a complete randomized design (CRD) were inoculated for each experiment. The plants were covered in insect proof cages inside a greenhouse and observed for symptoms development. RT-PCR and ELISA were used to detect MCMV in leaf samples after inoculation by thrips. Variations in MCMV prevalence on maize at different post acquisition and retention periods was tested by ANOVA and means separated by Fisher's protected least significant difference (LSD) at 0.05 significance level. Transmission rates were fitted to linear regressions approximation using least squares approach and plotted against time using the statistical software GenStat, 15th Edition.

Results

Frankliniella occidentalis and *F. williamsi* transmitted MCMV in 1, 3, 24, and 24 IAPs with initial symptoms developing in week 5 against those of positive controls that appeared in week 2 after inoculation). Plants inoculated by *F. occidentalis* had their highest severity at 3 hours IAP and those of *F. williamsi* at 1 hour. MCMV severity on plants inoculated by *F. williamsi* was significantly different from that of *F. occidentalis* (t = 2.77, D.F. = 362.97, P = 0.006). Plants inoculated by *F.* williamsi had higher symptom severity than those by F. occidentalis (0.270, 0.199) with a mean difference of 1.176. The disease severity for *F. williamsi* inoculated plants was 17.6 % higher than plants inoculated by *F. occidentalis*.

MCMV transmission rate by *F. williamsi* was relatively higher than that of *F. occidentalis* and after 24 hours IAP it increased while that of *F. occidentalis* declined (Fig. 1). There was significant mean differences (F = 5.66, D.F. = 2, 17, P = 0.013) between proportion of plants inoculated by *F. williamsi* and *F. occidentalis* at 1, 2, 3, and 4 days post acquisition retention periods. Number of plants transmitted after day 2 of acquisition were not significantly different from those infected after day 1 but was different from day 3 (0.26 ± 0.044, 0.402 ± 0.043, 0.497 ± 0. 061) at 0.149 LSD. *Frankliniella williamsi* and *F. occidentalis* transmitted MCMV in a similar pattern over the four days of post-acquisition.

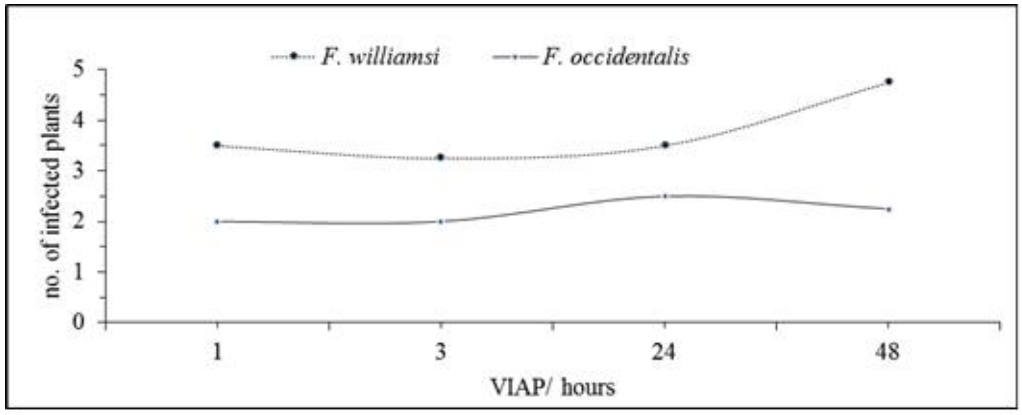

Figure 1. Prevalence of MCMV transmission on maize plants by ten thrips of *Frankliniella williamsi* and F. *occidentalis* adults. Thrips were fed on infected leaf discs for 48 hours to acquire the virus followed by transfers to healthy maize seedlings in 1, 2, 24 and 48 hour VIAPs.

Out of 64 plants tested in four replicates, 7 (43%) of *F. occidentalis* and 5 (31%) of *F. williamsi* re-transmitted MCMV in series one after feeding for 48 hours on infected leaf discs. Only *F. williamisi* re-transmitted 3 (18%) in the second series of re-infection after which there was no re-infection of maize plants in 3 and 4 series. The severity for *F. occidentalis* infected plants was highest (2.05 ± 0.43) and 0.95 ± 0.35 for *F. williamsi* plants against 3.10 ± 0.48 for mmechanically inoculated positive controls. Reverse transcriptase PCR showed a decrease in the rate of MCMV transmission.

Discussion

Plants inoculated by *F. occidentalis* had their highest severity at 3 hours IAP and those of *F. williamsi* at 1 hour. Thrips spend a lot of time exploring their food substrates rather than first attempting to

feed on new introduced food sources (Cabanas *et al.*, 2013). Thus 3 and 1 hour IAPs were the optimum time for MCMV transmission by *F. occidentalis* and *F. williamsi*, respectively. Symptoms developed five weeks after inoculation in all the IAPs which could be due to low viral titres and translocation mechanisms (Nagata *et al.*, 2002). Infected mild symptomatic plants could serve as reservoirs for new virus infections within maize fields since they are often overlooked. Mechanically inoculated controls had higher severity than thrips inoculated plants which could be attributed to the level of virus titres in extract leaf sap and thrips. New MCMV infections by *F. occidentalis* can induce low severities or asymptomatic maize plants which facilitates virus spread. The mode of MCMV transmission by the *F. williamsi* thrips was similar to those for the same virus by *F. occidentalis*. *Frankliniella williamsi* thrips transmit MCMV soon after acquisition and retained the virus for short periods with no evidence of a latent period. *Frankliniella williamsi* transmitted MCMV after an acquisition periods of 3 h, with no evidence for a latent period for foul beetles (Mahuku *et al.*, 2015).

Transmission rate of MCMV by *F. williamsi* was relatively higher than that of *F. occidentalis* and it increased with longer IAPs for both thrips. The capacity of virus transmission by thrips depends on compatibility among the thrips species, MCMV isolate and plants used for acquisition and inoculation. The difference in transmission competence could be related to MCMV translocation mechanisms and virus migration from vectors to plants. A poor migration of the virus through the ligament to the salivary glands may translate into low transmission rates as was observed in *T. tabaci* which acquired TSWV but could not transmit it (Nagata *et al.*, 2002).

The variations in MCMV prevalence at different post acquisition and retention periods between *F. williamsi* and *F*. *occidentalis* was due to a decrease in viral titers and eventual depletion after two days as was demonstrated by Cabanas *et al.* (2013). *Frankliniella williamsi* and *F*. *occidentalis* transmitted MCMV in a similar pattern over the four days post-acquisition periods as was also indicated by Ng and Falk (2006) that plant viruses of the same genus are transmitted by specific taxa of vectors.

Both thrips did not re-transmit MCMV in series 3, and 4 sets of plants. The inability of *F. occidentalis* to re-transmit the virus indicated that MCMV was non-persistently transmitted. However *F. williamsi* was able to re-transmit the virus in a semi-persistent manner which agrees with Cabanas *et al.* (2013) that the virus could be semi-persistently transmitted by maize thrips. This pattern of MCMV transmission indicates that the virus did not enter the hemocoel or any vector cell membrane as in persistent virus transmissions. Non-persistent viruses are retained by their vectors for less than a few hours whereas semi-persistent viruses are retained for days, weeks, or even years (Link and Fuchs, 2005). Real time RT-PCR also showed a decrease in the rate of MCMV transmission with an increase in inoculation cycle.

Conclusion

The study confirms that MCMV is transmitted soon after acquisition by both *F. williamsi* and *F. occidentalis. Frankliniella williamsi* appears to retain MCMV for longer periods than *F. occidentalis,* presenting a unique feature among these thrips since they belong to the same genus. MCMV translocation mechanisms and determinants for virus migration from vectors to plants need to be established. This can present a valuable model to clarify the viral determinants that are responsible for variations in transmission rates of a virus by arthropod vectors within a genus.

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