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Transmission Efficiency of Two Strains of Moroccan Watermelon Mosaic Virus by Two Clones of Aphis spiraecola (Patch)

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ABSTRACT

Aphis spiraecola (Patch), a ubiquitous aphid in West Africa has been implicated in the transmission of most potyviruses reported from Nigeria. The transmission efficiency of two Nigerian strains of MWMV designated as MWMV-lag and MWMV-cor in this study, by two clones of the aphid was studied. The transmission efficiency was determined based on aphid number per plant, varying Acquisition Access Periods (AAPs) and varying post-acquisition starvation times. Both clones of A. spiraecola were found to be efficient vectors of the two MWMV strains causing 100% transmission rate with as few as 5 individual aphids. The result of this study indicates that transmission efficiencies of MWMV-cor and MWMV-lag strains by the Aphis spiraecola clones declined with prolongation of AAPs. Clone I was more efficient in transmitting MWMV-cor than MWMV-lag. For clone II, both virus strains were transmitted with equal efficiency differing by 6.7% where differences occurred. Both clones transmitted MWMV-lag more efficiently that it did MWMV-cor with respect to the sequestration time regimes employed. Percentage differences ranged from 6.6-40% for MWMV-cor and 11.3-30% for MWMV-lag. On clonal basis, clone I did better in retaining and transmitting MWMV-cor that did clone II while MWMV-lag was retained much longer than MWMV-cor by clone II.

Key words: Aphis spiraecola, clones, Moroccan watermelon mosaic virus, transmission efficiency

INTRODUCTION

Aphis spiraecola Patch commonly called green citrus or Siam weed aphid is pandemic in distribution and has been reported to be a pest of many economically important crops and ornamentals spanning 90 plant families including Cucurbitaceae, Convolvulaceae Fabaceae, Rosaceae and Solanaceae (Blackman and Eastop, 1994). In Nigeria, the natural host is the Siam weed, Chromolaena odorata (L.), a ubiquitous weed that has become a menace in the country.

The aphid has been reported to vector several economically important viruses including *Papaya Ring Spot Virus* (PRSV) (Rivas Platero and Larios, 1994), *Watermelon Mosaic Virus*-2 (WMV-2) (Webb *et al.*, 1994), *Plum Pox Virus* (PPV), (Gildow *et al.*, 2004), *Cucumber Mosaic Virus* (CMV) (Gildow *et al.*, 2008), *Zucchini Yellow Mosaic Virus* (ZYMV) (Katis *et al.*, 2006), *Potato virus* Y^{NTN} (Boukhris-Bouhachem *et al.*, 2011), *Pepper Veinal Mosaic Virus* (PVMV) (Fajinmi *et al.*, 2011) and *Citrus Tristeza Virus* (CTV) (Vidal *et al.*, 2012).

In Nigeria, the aphid has also been implicated in the transmission of most potyviruses reported. These include Cowpea Aphid-borne Mosaic Virus (CAMV) (Atiri et al., 1986), Telfairia Mosaic

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Virus (TeMV) (Shoyinka et al., 1987), Celosia mosaic virus (Owolabi et al., 1998), Senna mosaic virus (Owolabi and Proll, 2001), PRSV (Owolabi et al., 2008), Pepper veinal mottle virus (Fajinmi et al., 2011) and Cucurbita mosaic virus (Owolabi et al., 2011).

The efficiency with which plant viruses are transmitted by specific aphid species has been reported to be influenced by the number of individual aphids and aphid biotypes (Berger *et al.*, 1983; Katis *et al.*, 2006; Moreno *et al.*, 2007; Kalleshwaraswamy and Kumar, 2007), aphid species (Garzo *et al.*, 2004; Gildow *et al.*, 2008) the virus strains (Verbeek *et al.*, 2010; Boukhris-Bouhachem *et al.*, 2011; Mello *et al.*, 2011) and aphid clones (Lucio-Zavaleta *et al.*, 2001; Kanavaki *et al.*, 2006; Symmes and Perring, 2007).

Moroccan Watermelon Mosaic Virus (MWMV), a potyvirus which was first reported by Fischer and Lockhart (1974) from southern Europe including Spain, Italy and France (Quiot-Douine et al., 1990; Roggero et al., 1998; Lecoq et al., 2007) and in East, West, Central and Southern Africa (Van der Meer and Garnett, 1987; Lecoq et al., 2001; Arocha et al., 2008; Yakoubi et al., 2008). The virus which is now considered an emerging threat to cucurbit production (Lecoq et al., 2001; Lecoq, 2004) induces severe mosaic and leaf malformation in susceptible cucurbit hosts (Owolabi et al., 2012; Malandraki et al., 2014).

Recently, two strains of MWMV isolated from Lagenaria breviflora Robert and Coccinia barteri (Hook. f.) Keay were reported from Nigeria (Owolabi et al., 2012). The Lagenaria strain was particularly found to be more virulent than the Coccinia strain, causing severe stunting of cucumber and Cucumeropsis mannii Naudin. Both strains were transmitted in a fore-gut (non-persistent) manner by Myzus persicae (Sulzer) and by a few other aphid species. Preliminary aphid transmission tests in our screenhouse have also shown A. spiraecola to be a veritable vector of the two virus strains. Researches on the economic significance of the virus strains in our screen-house (unpublished data) showed that both portent serious danger to cucurbit production. This study was undertaken to investigate the transmission efficiency of the Nigerian strains of MWMV by two clones of the A. spiraecola. The epidemiological significance of the results is discussed.

MATERIALS AND METHODS

Virus strains: The virus strains used in the study were two strains of MWMV isolated from Lagenaria breviflora and Coccinia barteri described by Owolabi et al. (2012) herein designated MWMV-lag and MWMV-cor, respectively. They were propagated and maintained in Cucumeropsis manni in the screenhouse at 25±3°C.

Sourcing and rearing of aphid cultures: The aphid species used was *A. spiraecola*. One aphid culture was obtained in Calabar herein referred to as clone 1. The second culture was sourced from Idundu, 30 km north of Calabar herein called clone II. Both locations are in different Local Government Areas of Cross River State, Nigeria.

The insects were dislodged from the natural host by breathing heavily on them. They were carefully transferred by using the tip of moistened artist brush to young seedlings of *C. mannii*. Rearing was performed by period transfer of apterous individuals of the aphid cultures to young seedlings of the plant every other week in insect proof cages.

Influence of aphid number on per plant on transmission efficiency: To determine the effect of aphid number on transmission efficiency of the insect, the aphid clones were obtained, starved

for 1 h and subjected to 1 min Acquisition Access Period (AAP) on symptomatic leaves of *C. mannii*, the source plant. Groups of 1, 3, 5 and 7 viruliferous aphids were then transferred to each of five seedlings of *Cucumis sativus* L. (the test plant) for 3 min Inoculation Access Period (IAP). The aphids were considered to have fed when they became still on the source plant. The inoculated plants were left in insect proof cages in the screen-house for two weeks for symptom development.

Influence of varying Acquisition Access Periods (AAP) on transmission efficiency: For the determination of varied acquisition access period on transmission efficiency, individual aphids from both clones were allowed AAPs of 1, 3, 5, 7 min time regimes. Seven aphids were allowed to feed but 5 viruliferous individuals were transferred immediately for the transmission tests. For the 1 min AAP, aphids were transferred singly from the source plant to the test plants to ensure time precision while for the other time regimes the aphids were transferred in groups.

Influence of post-acquisition starvation (sequestration time) on transmission efficiency: For this experiment, groups of aphids of both clones were subjected to starvation periods of 1, 10, 15 and 20 min after they had been allowed 1-3 min AAP from the source plant. Five viruliferous aphids were then transferred to each of five test plants.

In all the experiments, the aphids were starved in petri dishes with lids made of gauze (to ensure aeration), tightly held in place at the rims with transparent tapes. Transferring of viruliferous aphids from source plant to the test plants was carried out using moistened paint brush, after a gentle teasing of the insects from their posterior ends to avoid damage to the stylets. Insect-inoculated plants were sprayed with Pirimor 50-DF (2-Dimethylamino-5,6-dimethylpyrimidin-4-yl dimethyl-carbamate) and left in insect-screened cages in the screenhouse with ambient temperature of 25±2°C. Whether test insects were inoculative was determined on the basis of symptom development in indicator assay plants.

Statistical analysis: Each transmission experiment was repeated trice for each virus/vector combination. The number of test plants for each was five (N = 5). Data were collected and analyzed using Statistical Package for Social Science (SPSS) version 14. Means were separated using the Least Significant Difference (LSD).

RESULTS

Effect of aphid number on transmission efficiency of the virus strains by the aphid clones: The results of the transmission of MWMV-cor and MWMV-lag strains by the two aphid biotypes based on aphid number per plant are presented in Table 1. Generally, the two strains were readily transmitted by both clones of A. spiraecola. Also, percentage transmission of both virus strains by the aphid clones increased as the number of aphids employed per plant increased. Five aphids of both clones were enough to cause 100% transmission of the virus strains. There was a 20% increase in the transmission of MWMV-lag compared to that of MWMV-cor when single aphid of clone I was employed per plant. Conversely, the same margin of increase was observed for the transmission of MWMV-cor in comparison to that of MWMV-lag when three aphids were used. Employing 7 aphids per plant produced a marginal 6.7% increase for MWMV-cor over the figure obtained for MWMC-lag.

Table 1: Transmission efficiency of MWMV-cor and MWMV-lag strains by two clones of Aphis spiraecola based on aphid number/plant

	MWMV-cor		MWMV-lag				
	Percentage			Percentage			
Aphid/plant	Infected plant/Total palnt	transmission	Infected plant/Total palnt	transmission	Difference (%)		
Clone 1							
1	$3/15^{a}$	20.0^{b}	6/15ª	40.0^{b}	20.0°		
3	12/15	80.0	9/15	60.0	20.0		
5	15/15	100.0	15/15	100.0	-		
7	15/15	100.0	14/15	93.3	6.7		
Clone 2							
1	6/15ª	40.0^{b}	6/15ª	40.0^{b}	-c		
3	12/15	80.0	12/15	80.0	-		
5	15/15	100.0	15/15	100.0	-		
7	15/15	100.0	15/15	33.3	20.0		

^aNominator is the number of plants infected while the denominator is the total number of plants in 3 trials (5 plants per trial),

Table 2: Influence of aphid number on the transmission efficiency of Coccinia and Lagenaria strains of Morocco watermelon mosaic virus by two clones of Aphis spiraecola

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Aphid clone	MWMV-ce	or		MWMV-lag					
	AL 1	AL 3	AL 5	AL 7	AL 1	AL 3	AL 5	AL 7	
Clone I	1.00a	4.00a	5.00ª	5.00ª	2.00a	3.00 ^a	5.00ª	4.67ª	
Clone II	2.00^{b}	4.00^{a}	5.00 ^a	5.00 ^a	2.00a	4.00^{b}	5.00 ^a	5.00ª	
LSD 0.29									

Means (obtained by dividing the number of plants infected/the number of trials) followed by the same letter in each column are not significantly different (p = 0.05) using Least Significant Difference (LSD), AL 1: One aphid/plant, AL 3: Three aphids/plant, AL 5: Five aphids/plant, AL 7: Seven aphids/plant

For clone II, both strains of virus were transmitted with equal efficiency by the clones when 1, 3 and 5 aphids were employed per plant. However, MWMV-cor was transmitted more efficiently with a 20% increase over the figure obtained for MWMV-lag when 7 aphids were used.

Comparing the transmission of MWMV-cor by both clones, the virus strain was transmitted with equal efficiency by the two clones of the aphid species (columns 3 and 8), except when 1 aphid was employed for the transmission test when clone II was found to be twice efficient in transmitting the virus strain with 40% infection compared to clone I which produced 20% infection.

With regard to the transmission of MWMV-lag by the two clones, clone II caused higher percentage infection (80%) using 3 aphids compared to 60% for clone I. The strain was transmitted with equal efficiency when 1 and 5 aphids were employed (columns 5 and 10). Statistically, the transmission efficiency of MWMV-cor by clone II was only significantly higher (p<0.05) with single aphid inoculation compared to the value for clone I (Table 2). For the MWMV-lag strain, using three and seven aphids led to significantly higher transmission efficiency than recorded for clone I.

Transmission of the virus strains by the aphid clones based on AAPs: The result of the transmission efficiency of MWMV-cor and MWMV-lag strains by the two aphids clones of *A. spiraecola* based on AAPs are presented in Table 3. Generally, percentage transmission of both MWMV strains by the two aphid clones decreased as AAPs increased.

^bPercentage transmission, ^cDifference in percentage transmission between the two strains

Table 3: Transmission efficiency of MWMV-cor and MWMV-lag by two clones of *Aphis spiraecola* based on acquisition access period/inoculation access period

	MWMV-cor		MWMV-lag		
AAP/IAPa(min)	Infected plant/Total palnt	Percentage transmission	Infected plant/Total palnt	Percentage transmission	Diff
Clone 1	miecteu piant/Totai pami	transmission	miected plant/Total paint	transmission	Difference (%)
1/1	14/15 ^b	93.3°	12/15 ^b	80.0°	13.3 ^d
3/3	12/15	80.0	9/15	60.0	20.0
7/7	9/15	60.0	8/15	53.3	6.7
10/10	6/15	40.0	5/15	33.3	6.7
Clone 2					
1/1	$15/15^{b}$	100.0°	$15/15^{\rm b}$	100.0°	_d
3/3	12/15	80.0	11/15	73.3	6.7
7/7	9/15	60.0	9/15	60.0	-
10/10	5/15	33.3	6/15	40.0	6.7

^aAAP/IAP: Acquisition access period/inoculation access period, ^bNominator is the number of plants infected while the denominator is the total number of plants in 3 trials (5 plants per trial), ^cPercentage transmission, ^dDifference in percentage transmission between the two strains

Comparing the transmission of MWMV-lag and MWMV-cor by clone I, the clone was more efficient in transmitting the latter than the former. Percentage transmission figures were higher for all the time regimes employed (columns 3 and 5). While percentage transmission values of 93.3, 80, 60 and 40 were obtained for MWMV-cor, the values recorded for MWMV-lag were 80, 60, 53.3 and 33.3 at 1, 3, 7 and 10 AAPs, respectively.

There was no definite trend observed comparing the transmission of MWMV-lag with MWMV-cor by clone II. Transmission was only higher by 6.7% at 10 APP but lower than that of MWMV-cor by the same margin at 3/3 AAP (columns 8, 10).

Comparing the transmission of MWMV-cor by both clones (columns 3 and 8), differences in transmission efficiency were only observed between both strains when allowed 1 and 10 AAPs with a marginal 6.7% difference in both cases. While clone II achieved 100% transmission of the virus strain when given 1 AAP, the percentage transmission value for clone I was 93.3%. On the other hand, when given 10 AAP clone I achieved 40% transmission efficiency compared to 33.3% for clone II.

Clone II transmitted the MWMV-lag strain more efficiently than observed for clone I at all the AAP time regimes employed. While the percentage transmission by clone II was 100% at 1, the value obtained for clone I was 80%. At 3, 7 and 10 APPs, percentage transmission values for clone II were 73.3, 60 and 40%, respectively compared to 60, 53.3 and 33.3% for clone I (columns 5 and 10). Percentage differences ranged from 6.7-20 (column 6).

The values obtained for both clones with respect to MWMV-cor transmission were statistically insignificantly (p<0.05) irrespective of the AAPs. For MWMV-lag transmission, clone II only engendered statistically significant higher value at AIT 1 than clone I (Table 4).

Effect of post-acquisition on transmission of the virus strains by the aphid clones: The results of the transmission of MWMV-lag and MWMV-cor based on retention capability after virus acquisition and transmission (post acquisition starvation or sequestration period) by the two clones of A. spiraecola are presented in Table 5. Generally, percentage transmission of both strains decreased as post-acquisition time increased for both clones.

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Table 4: Influence of acquisition access period/inoculation access periods on the transmission efficiency of Coccinia and Lagenaria strains of Morocco watermelon mosaic virus by two clones of Aphis spiraecola

		· ·						
	MWMV-co	or			MWMV-lag			
Aphid clone	AIT 1	AIT 3	AIT 7	AIT 10	AIT 1	АІТЗ	AIT 7	AIT 10
Clone I	4.67ª	4.00ª	3.00ª	2.00ª	4.00a	3.00ª	2.67ª	1.67ª
Clone II	5.00 ^a	4.00 ^a	3.00 ^a	1.67ª	5.00^{b}	3.67ª	3.00^{b}	2.00^{a}
LSD 0.83								

Means (obtained by dividing the number of plants infected/the number of trials) followed by the same letter in each column are not significantly different (p = 0.05) using Least Significant Difference (LSD). AIT 1: 1 min acquisition/inoculation access time, AIT 3: 3 min acquisition/inoculation access time, AIT 7: 7 min acquisition/inoculation access time, AIT 10: 10 min acquisition/inoculation access time

Table 5: Retention capability of the MWMV-cor and MWMV-lag by two clones of Aphis spiraecola

	MWMV-cor		MWMV-lag			
		Percentage		Percentage		
Retention time (min)	Infected plant/Total palnt	transmission	Infected plant/Total palnt	transmission	Difference (%)	
Clone 1						
1	$14/15^a$	93.3^{b}	14/14 ^a	$100.0^{\rm b}$	6.7°	
10	9/15	60.0	15/15	100.0	40.0	
15	6/15	40.0	7/14	50.0	10.0	
20	3/15	20.0	4/15	26.6	6.6	
Clone 11						
1	$12/15^{a}$	80.0^{b}	15/15 ^a	100.0^{b}	20.0°	
10	9/15	60.0	11/15	73.3	13.3	
15	3/15	20.0	7/14	50.0	30.0	
20	3/15	20.0	5/15	33.3	11.3	

a Nominator is the number of plants infected while the denominator is the total number of plants in 3 trials (5 plants per trial),

Comparing the transmission of MWMV-cor and MWMV-lag by clone I, the result showed that MWMV-lag was transmitted more efficiently than MWMV-cor as percentage transmission values were higher at all the time regimes used (columns 3 and 5). The percentage transmission values were 100% after 1 and 10 min, 50 and 26.6% after 15 and 20 min sequestration periods for MWMV-lag while the corresponding values for MWMV-cor were 93.9, 60, 40 and 20%.

Clone II appeared to be more efficient in retaining MWMV-lag and consequently produced higher percentage transmission values than MWMV-cor. Percentage transmission values were also higher for the former than the latter at all the time regimes employed (columns 8 and 10).

Comparing the transmission of MWMV-cor by both clones, the data showed that clone I appeared more efficient in retaining the virus strain than clone II for shorter sequestration times of between 1-15 min. But further increase in the sequestration time produced similar effect (columns 3 and 8).

For MWMV-lag, except at 20 min post acquisition starvation time when clone I produced 26.6% percentage transmission compared to 33.3% for clone II, the former appeared more efficient than the latter in retaining and consequently transmitting the virus strain (columns 5 and 10). Transmission efficiency of MWMV-cor by clone I was significantly higher (p<0.05) at ST I and ST 15 in comparison to the values obtained for clone II (Table 6). For MWMV-lag transmission efficiency was only significantly higher at ST 10 compared to that of clone II.

^bPercentage transmission, ^cDifference in percentage transmission between the two strains

Table 6: Influence of post-acquisition starvation time on the transmission efficiency of Coccinia and Lagenaria strains of Morocco watermelon mosaic virus by two clones of Aphis spiraecola

		·							
	MWMV-co	or			MWMV-la	MWMV-lag			
Aphid clone	ST 1	ST 10	ST 15	ST 20	ST 1	ST 10	ST 15	ST 20	
Clone I	4.67ª	3.00ª	2.00ª	1.00ª	4.67ª	5.00ª	2.33ª	1.33ª	
Clone II	4.00^{b}	3.00 ^a	1.00^{b}	1.00 ^a	5.00^{b}	$3.67^{\rm b}$	2.33ª	1.67^{a}	
LSD 0.44									

Means (obtained by dividing the number of plants infected/the number of trials) followed by the same letter in each column are not significantly different (p = 0.05) using Least Significant Difference (LSD), ST 1: 1 min post-acquisition starvation time, ST 10: 10 min post-acquisition starvation time, ST 15: 15 min post-acquisition starvation time, ST 20: 20 min post-acquisition starvation time

DISCUSSION

A. spiraecola is ubiquitous in Nigeria and has been implicated as a vector of most potyviruses reported from the country. Moroccan watermelon mosaic virus on the other hand, is regarded as an emerging threat to cucurbit production wherever it occurs. In this study, the transmission efficiency of two strains of MWMV virus by two clones of A. spiraecola was investigated. Transmission efficiency was determined based on aphid number per plant, varying acquisition access periods inoculation access periods and ability of the clones to retain and transmit the viruses after varying post-acquisition starvation times.

The results of this study showed a linear relationship between aphid number per plant and the number of infected plants as percentage transmission for both virus strains by the aphid clones increased as the number of aphids employed per plant increased. For both virus strains, five aphids were enough to cause 100% transmission by the two clones. This result lends credence to earlier reports by Katis *et al.* (2006) and Kalleshwaraswamy and Kumar (2007) when they showed that multiple aphid inoculations led to higher transmission efficiencies of ZYMV and PRSV by their aphid vectors than single aphid inoculations.

Aphid transmission of fore-gut borne (non-persistent or stylet-borne) viruses, according to Hull (2002) is characterized by short acquisition and inoculation access feeding times often a matter of seconds or minutes while long acquisition and retention times reduced efficiency of transmission. The result of this study indicates that transmission efficiencies of MWMV-cor and MWMV-lag strains by the two A. spiraecola clones declined with prolongation of AAPs. However, clone I consistently demonstrated greater efficiency in transmitting MWMV-cor than it did the MWMV-lag. No such consistency was observed for clone II in the transmission of MWMV-cor compared to MWMV-lag. Singh and Singh (2010) reported a decline in the transmission efficiency of PRSV by M. persicae while increasing the acquisition feeding and starvation times. Kostiw and Trojanowska (2011) have also shown that continuous prolongation of the feeding time caused a slow decrease in the effectiveness of transmission of PVY^{NTN} and PVY^N by M. persicae.

Regarding post-acquisition periods and transmission efficiencies of the virus strains, both clones showed greater efficiencies in transmitting MWMV-lag than they did MWMV-cor. With longer post-acquisition time employed in this study, clone II was more efficient than clone I in transmitting MWMV-lag while there was parity in the transmission of MWMV-cor by the two clones. Aphid clones have been shown to differ in the efficiency with which they transmit fore-gut viruses depending on the virus strain-vector combinations. Garzo et al. (2004) found no significant difference between clones of Aphid fabae, Aphid gossypii, Myzus persicae and A craccivora in the transmission of different isolates of CMV and ZYMV. Symmes and Perring (2007) also found no

significant difference in the transmission efficiencies of ZYMV by two clones of *M. persicae* obtained and reared on brassicaceous plants. On the other hand, Kanavaki *et al.* (2006) found that *M. persicae nicotianae* was more efficient than *M. persicae* in transmitting PVY^N by 12 clonal lineages of both aphids. In their study, Berger *et al.* (1983) also demonstrated that clones of biotype E was generally more efficient in transmitting two strains of MDMV (MDMV-A and MDMV-B) than biotype C. Verbeek *et al.* (2010) reported comparable transmission efficiencies for PVY^N, PVY^{NTN} and PVYN-^{Wi} by biotype Mp2 of *M. persicae*. Mello *et al.* (2011) have also reported no apparent differences in the ability of a clone of *M. persicae* in transmitting five isolates of PVY^O and PVY^{NW-i}.

CONCLUSION

From the result of this study, both clones of A. spiraecola could be said to be efficient vectors of MWMV strains causing 100% transmission rate with as few as 5 individual aphids. Both clones transmitted MWMV-lag, the more virulent of the two strains, more efficiently than MWMV-cor and Clone II appeared to be more efficient than clone I in transmitting both virus strains in several of the tests. The aphid clones could also retain the virus strains for as long as 20 min post acquisition. The implication of both clones retaining the virus strains for this length of time is the prospect of epiphytotics and possible crop losses should the A. spiraecola clones carrying MWMV strains be brought into susceptible cucurbit crops in the fields.

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