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Transmission efficiency of different non-persistent viruses infecting melon by four aphid species

E. I. Garzo, M. Duque and A. Fereres*

Departamento de Protección Vegetal. Centro de Ciencias Medioambientales (CSIC). Serrano, 115, dpdo. 28006 Madrid. Spain

Abstract

Transmission experiments of different isolates of *Cucumber mosaic virus* (CMV), *Watermelon mosaic virus* (WMV) and *Zucchini yellow mosaic virus* (ZYMV) were conducted using established colonies of *Aphis gossypii* Glover, *Myzus persicae* (Sulzer), *A. fabae* Scopoli and *A. craccivora* Koch as vectors. The transmission procedure used provided an estimate of the virus transmission efficiency for each aphid species. Two different virus isolates/aphid clones were tested for each virus species using the most efficient aphid species as *A. gossypii* was the most efficient vector of CMV (100 ± 0%), while *M. persicae* showed the highest efficiency of transmiting WMV (67.9 ± 28.5%) and ZYMV (96.4 ± 3.6%). Both WMV and ZYMV showed variability in their transmission efficiency by aphids. No significant differences were found in the efficiency of the two CMV isolates tested. No differences were observed in the vector transmission ability between clones of each aphid species tested. Results of our transmission studies together with previously known information on the temporal and spatial patterns of virus epidemics in melon crops in Spain suggest that CMV is mainly transmitted by a colonising aphid such as *A. gossypii*. In contrast, WMV is mainly transmitted by non-colonising transient aphid species such as *M. persicae* that alight on melon crops during late spring.

Key words: *Aphis sp., Myzus persicae,* CMV, WMV, ZYMV, virus-vector relationship, non-persistent transmission.

Resumen

Eficacia de la transmisión de diferentes virus no persistentes de melón por cuatro especies de pulgones

Se realizaron experimentos de transmisión de diferentes aislados de *Cucumber mosaic virus* (CMV), *Watermelon mosaic virus* (WMV) y *Zucchini yellow mosaic* virus (ZYMV) utilizando como vectores colonias establecidas de *Aphis gossypii* Glover, *Myzus persicae* (Sulzer), *A. fabae* Scopoli y *A. craccivora* Koch. El procedimiento de transmisión utilizado proporciona una estimación de la eficacia de transmisión de virus por cada una de las especies de pulgón. Dos diferentes aislados virales/clones de pulgón fueron evaluados para cada especie de virus utilizando como vector la especie de pulgón más eficiente. *A. gossypii* fue el vector más eficiente de CMV (100 ± 0%), mientras que *M. persicae* mostró la mayor eficiencia para transmitir WMV (67,9 ± 28,5%) y ZYMV (96,4 ± 3,6%). Tanto WMV como ZYMV mostraron variabilidad en su eficiencia de transmisión por pulgones. No se encontraron diferencias significativas en la eficiencia de los dos aislados de CMV evaluados. Tampoco se observaron diferencias en la capacidad de transmisión entre los clones de cada especie de pulgón evaluadas. Los resultados de nuestros estudios de transmisión, junto con la información previa de los patrones temporal y espacial de las epidemias de virus en los cultivos de melón en España, sugieren que una especie de pulgón colonizante como *A. gossypii* es la que transmite preferentemente CMV. En contraste, WMV es transmitido preferentemente por especies de pulgones no colonizantes tales como *M. persicae,* que aterrizan en el cultivo de melón a finales de la primavera.

Palabras clave: *Aphis sp., Myzus persicae,* CMV, WMV, ZYMV, relación virus-vector, transmisión no persistente.

Introduction

Non-persistent viruses are widely spread in melon (*Cucumis melo* L.*)* crops grown in Spain with *Cucumber mosaic virus* (CMV, genus *Cucumovirus*) and *Watermelon mosaic virus* (WMV, genus *Potyvirus*) being the most abundant, followed by *Zucchini yellow mosaic virus* (ZYMV, genus *Potyvirus*) and *Papaya ringspot virus* (PRSV, genus *Potyvirus*) (Luis-Arteaga *et al*., 1998). All these viruses are transmitted in a non-persistent manner by many aphid species with variable

* Corresponding author: afereres@ccma.csic.es
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degrees of efficiency. Transmission efficiency has been defined as the probability of infection with a specific virus isolate and vector species biotype under a set of environmental conditions (Sylvester, 1954), and is a measure of the competence or genetic capacity of a given species as a virus vector (Gold, 1979). Vector propensity was a term introduced by Irwin and Ruesink (1986) that refers to the natural ability of a species to inoculate a plant with a virus. The transmission efficiency or propensity of viruses infecting cucurbits by different aphid species has been quantified by several authors, using seedlings as test plants in: zucchini (Castle *et al*., 1992; Yuan and Ullman, 1996), squash (Adlerz, 1987), watermelon (Toba, 1963) and melon (Labonne *et al*., 1982). *Aphis gossypii* has been reported as being the most efficient vector of CMV infecting melon, followed by *A. craccivora*, *A. fabae* and *A. spiraecola* (= *citricola*) (Labonne *et al*., 1982). *M. persicae* and *A. gossypii* are considered to be the most efficient vectors of WMV (Coudriet, 1961). In the case of ZYMV, *A. craccivora* had a higher propensity and efficiency than *A. gossypii* (Yuan and Ulman, 1996).

The capacity of a given virus to be transmitted by an aphid species may vary depending on the virus strain or isolate (Antignus *et al*., 1989). In the genus *Potyvirus*, it is common to find certain strains within the same virus species that have partially or completely lost their ability to be transmitted by aphids (Murant *et al*., 1988). Also, variability between clones of the same aphid species in the transmission ability of a given virus has been previously reported for CMV (Simons, 1959), *Bean yellow mosaic virus* (BYMV, genus *Potyvirus*) (Thottappilly *et al*., 1972) and *Broad bean severe chlorosis virus* (BBSCV, genus C*lostero-virus*) (Thottappilly *et al*., 1977). Information on the role of aphid species as vectors of viruses infecting melon crops grown in Spain is lacking. Therefore, the purpose of this study was to compare the efficiency of four aphid species for transmitting CMV, WMV and ZYMV; the three most widely spread viruses infecting melon crops in Spain. To conduct our study we used two different virus isolates and two aphid clones within each virus/aphid species combination.

Material and Methods

Aphid colonies

The aphid species included in our study had been previously identified as being the most efficient vectors of non-persistent viruses infecting melon fields in south-eastern France (Labonne *et al*., 1982). Laboratory colonies were started from a single viviparous apterae of each selected species and kept in a growth chamber at a temperature of 22:16ºC (day: night) and a photoperiod of 14:10 h (light: dark). The aphid species and clones used for this study were obtained from different localities in Spain; *Aphis gossypii*, clone 93 (collected in 1993 from cucumber at El Ejido, Almería) and *A. gossypii*, clone 98 (collected in 1998 from melon at Aguadulce, Almería) were both maintained on melon plants (cv. Regal). *Myzus persicae*, clone Encín (collected from pepper at «El Encín», Madrid, in 1989) and *M. persicae*, Fuentidueña (collected from peach at Fuentidueña del Tajo, Madrid, in 2000) were both maintained on pepper plants cv. Yolo Wonder. *Aphis fabae* (collected from faba beans at Murcia in 1999) was maintained on faba bean cv. Muchamiel, and *Aphis craccivora* (collected from alfalfa at «El Encín», Madrid in 2000) on lentils cv. Toledo.

Virus isolates

All virus isolates were obtained from samples collected from different melon growing areas in Spain. Two isolates of CMV, M-6 and B-20, were originally obtained from melon plants collected in 1995 at Valencia and Barcelona, respectively. Two isolates of WMV, M-116 and M-486 were isolated from infected melon plants collected in 1995 at Valencia and Murcia, respectively. The two isolates of ZYMV were obtained at Málaga: C-71 (obtained from a zucchini plant in 1996) and M-624 (collected from a melon plant in 1998). All virus isolates were maintained as desiccated tissue at +4ºC and were used for mechanical inoculation of melon plants cv. Regal. Newly infected source plants were periodically generated by aphid inoculation of melon plants when needed.

Aphid transmission procedure

The virus source plants used in the transmission procedure were obtained by aphid inoculation of melon plants 30 days before starting the experiment. We used adult apterae aphids of the same age (7-9 days old). Aphids were subjected to a 1 h pre-acquisition starvation period and were then placed on the last expanded

leaf of an infected source plant for a 5 min acquisition access period. Groups of 5 aphids were transferred to test plants (20 days old) for a 2 h-inoculation period. Then, all plants were sprayed with Confidor 20 SL (a.i. Imidacloprid) and placed in an aphid-free chamber for 3 weeks at 26:20ºC (D:N) and a photoperiod of 16:8 h (L:D). All source and test plants were melon cv. Regal. Test plants were checked for virus infection by symptom expression and by ELISA (Clark and Adams, 1977) using a polyclonal antibody ELISA kit for CMV and a general anti-potyvirus kit for the detection of WMV and ZYMV (Elkhart, Indiana, USA). In all cases, the antibodies were conjugated with alkaline phosphatase and p-nitrophenyl phosphate was used as substrate. Absorbance was recorded at 405 nm using an ELISA plate photometer (SLT Lab Instruments Model 340 ATC, A-5082 Grödig/Salzburg, Austria) 1 and 2 h after the addition of the substrate. Plants were considered infected when absorbance values reached three times the mean value of the healthy controls.

Transmission efficiency between virus isolates

Transmission tests were conducted with two different virus isolates from each of the selected virus species (CMV, WMV and ZYMV) to detect if there was any variability in their transmission rate. The comparison between isolates was conducted using *A. gossypii* (clone 93) as the vector. Those isolates that were best transmitted by *A. gossypii* (clone 93) were then used for further experiments.

Transmission efficiency of selected CMV, WMV and ZYMV isolates by different aphid species and clones

The virus isolates that were transmitted with highest efficiency were selected for another set of transmission tests to compare their transmission rate between different aphid species. *A. gossypii* (clone 98)*, M. persicae* (Encín) and *A. fabae* were used as vectors in a first set of tests. A second set of experiments was run to compare the transmission efficiency of *A. craccivora* with that of the aphid species that was previously found to be the most efficient vector of each of the viruses tested.

Two different clones of *A. gossypii* (clone 93 and clone 98) were used to compare their efficiency to transmit CMV. In the case of WMV and ZYMV, two different clones of *M. persicae* (Encín and Fuentidueña) were used for comparison purposes in the transmission tests.

Statistical analysis

Each transmission assay was repeated twice for each virus/vector combination. The number of test plants used for each assay was 28 (n = 28). Transmission rate calculated as a percentage was compared among the different treatment groups using a Chi-square test and, if the expected values were lower than 5, we used Fisher's Exact Test (Statview II, Abacus concepts, 1987).

Results

Variability in the transmission between virus isolates

In the case of CMV, for both replicates, the transmission rate of M-6 (mean = 93%) was higher than that of B-20 (mean = 78%), although the differences observed were not statistically significant ($P > 0.05$) (Table 1). The transmission rate of the M-116 isolate of WMV was significantly higher ($P \leq 0.05$) than that obtained for isolate M-486 in both of the tests conducted. In the case of ZYMV, the transmission rate of isolate C-71 was significantly higher ($P \leq 0.05$) than for isolate M-624 in one of the two replicates tested. In the second test, no significant differences between the transmission rates of either isolate could be found (Table 1).

Transmission efficiency of Spanish isolates of CMV, WMV and ZYMV by different aphid species and clones

Table 2 shows that the transmission rate of CMV by *A. gossypii* (clone 98) (mean = 100%) was significantly higher ($P \leq 0.05$) than for *M. persicae* (mean = 63.5%) and *A. fabae* (mean = 8.9%) in both of the assays conducted. Conversely, the rate of transmission of WMV was highest for *M. persicae* in both assays 3 and 4 (mean = 67.8%), although statistically significant ($P > 0.05$) differences were not observed when compared to the transmission rate by *A. gossypii* (mean =

Table 1. Transmission efficiency by *A. gossypii* (clone 93) of CMV, WMV and ZYMV infecting melon plants

Virus	Isolate	Assay	% transmission[1]	Test	
				χ^2	P
CMV	M-6	1	93 (26/28) a	2.33	0.1513
	B-20		78 (22/28) a		
	M-6	2	93 (26/28) a	2.33	0.1513
	B-20		78 (22/28) a		
WMV	M-486	3	60.7 (17/28) a	6.09	0.0136
	M-116		92.6 (25/27) b		
	M-486	4	59.2 (16/27) a	4.14	0.0419
	M-116		82.1 (23/28) b		
ZYMV	C-71	5	75 (21/28) a	23.62	< 0.0001
	M-624		11 (3/28) b		
	C-71	6	46.4 (13/28) a	1.81	0.1789
	M-624		64.3 (18/28) a		

[1] Percentage of infected plants. Number of plants infected per total number of plants tested is in parentheses. Transmission (%) followed by different letters within each assay indicates significant differences ($P \leq 0.05$) according to a chi-square test and to a Fisher exact test when the expected values were lower than 5 (Statview, Abacus Concepts, 1987).

57.1%). In the case of ZYMV, *M. persicae* was again the most efficient vector in both assays (mean = 96.4%), and its transmission rate was significantly higher (χ^2=12.60, $P < 0.0004$) than that for *A. gossypii* in one of the two assays (assay 6). In the second set of transmission assays we found that *A. craccivora* transmitted CMV with significantly ($P < 0.05$) less efficiency than *A. gossypii* (Table 3). Also, *A. craccivora* was less efficient in the transmission of WMV and ZYMV than *M. persicae*. Both *A. fabae* and *A. craccivora* were the least efficient vectors among all the aphid species for the viruses tested (Tables 2 and 3).

Another set of transmission assays was made to compare if there were interclonal differences in the transmission ability of CMV by *A. gossypii*, the most efficient vector of the virus. In the case of WMV and ZYMV, different clones of *M. persicae* were tested. No significant ($P > 0.05$) differences were found in the vector transmission ability between clones of the same aphid species in any of the virus/vector systems tested (Table 4).

Discussion

Non-persistent viruses such as CMV, WMV and ZYMV can be transmitted by several aphid species, even those that do not colonise the crop (Raccah *et al*., 1985; Yuan and Ullman, 1996). Our work reported in the present paper has shown that *A. gossypii* is the most efficient vector of CMV while *M. persicae* is the most efficient vector of both WMV and ZYMV in melon. These results are consistent with the findings of Ng and Perry (1999) who reported that *A. gossypii* transmits CMV with a higher efficiency than *M. persicae* under laboratory conditions. The other two species that we tested, *A. fabae* and *A. craccivora*, transmitted CMV, WMV and ZYMV much less efficiently than *A. gossypii* and *M. persicae*, although when alighting in high numbers they may also contribute to the spread of the disease. Our results agree with those reported by Labonne *et al*. (1982) where *A. gossypii* was identified as the major vector of CMV infecting melon in the laboratory and under field conditions in France. Our results also agree with those reported by Castle *et al.* (1992), who found *M. persicae* to be the most efficient vector of both WMV and ZYMV in field and laboratory tests carried out in California. Furthermore, Adlerz (1974) reported that *M. persicae* is one of the most important vectors of WMV in watermelon crops grown in Florida. Yuan and Ullman (1996) concluded that *A. craccivora* had a significantly higher efficiency and propensity of transmiting ZYMV to zucchini plants than *A. gossypii*. However, they excluded *M. persicae* in the comparison. *M. persicae* was the most efficient vector of ZYMV in our transmission tests and in previously published studies (Lecoq *et al.*, 1981; Lisa *et al.*, 1981; Castle *et al.*, 1992).

Table 2. Transmission efficiency by different species of aphids of CMV, WMV and ZYMV infecting melon plants

Virus (isolate)	Assay	Vector (clone name)	% transmisión[1]
CMV (M-6)	1	*A. gossypii* (clone 98)	100 (28/28) a
		M. persicae (Encín)	71.4 (20/28) b
		A. fabae	10.7 (3/28) c
	2	*A. gossypii* (clone 98)	100 (28/28) a
		M. persicae (Encín)	55.6 (15/28) b
		A. fabae	7.1 (2/28) c
WMV (M-116)	3	*A. gossypii* (clone 98)	85.7 (24/28) a
		M. persicae (Encín)	96.4 (27/28) a
		A. fabae	29.6 (8/27) b
	4	*A. gossypii* (clone 98)	28.6 (8/28) a
		M. persicae (Encín)	39.3 (11/28) a
		A. fabae	7.1 (2/28) b
ZYMV (C-71)	5	*A. gossypii* (clone 98)	85.7 (24/28) a
		M. persicae (Encín)	100 (28/28) a
		A. fabae	42.8 (12/28) b
	6	*A. gossypii* (clone 98)	50.0 (14/28) a
		M. persicae (Encín)	92.8 (26/28) b
		A. fabae	10.7 (3/28) c

[1] Percentage of infected plants. Number of plants infected per total number of plants tested is in parentheses. Transmission (%) followed by different letters within each assay indicates significant differences ($P \leq 0.05$) according to a chi-square test and to a Fisher exact test when the expected values were lower than 5 (Statview, Abacus Concepts, 1987).

A. gossypii, which was found to be the most efficient vector of CMV in our study, is also the main aphid species that colonises melon crops in Spain (Nieto-Nafria *et al*., 1984). This finding is in agreement with the type of spatial distribution of CMV and WMV in field studies conducted in Spain by Alonso-Prados *et al*. (2003). They found that CMV was preferentially spread along the rows between adjacent plants following a rectangular pattern, while the spread of WMV rarely occurred between adjacent plants. The observed spatial pattern of CMV suggests the involvement of an aphid species that colonises melon, which tends to disperse within rows in a contagious pattern, in the secondary spread of the virus. Movement of a non-colonising aphid species is unlikely to occur between adjacent plants, since they tend to fly away and search for another host. Other species capable of colonising melon crops are *M. persicae* and *A. craccivora* (Blackman and Eastop, 1984), but these two species have not been cited as colonisers of melon crops in Spain (Nieto-Nafria *et al*., 1984). The work by Alonso-Prados *et al*. (2003) showed a pattern of infection of WMV in which infected plants were not adjacent and formed less compact foci than in CMV. This pattern indicates that aphid species that do not usually colonise the crop are acting as vectors of this virus. Our transmission experiments (Table 2) showed that *M. persicae*, a species that does not colonise melon, was the most efficient vector of WMV.

The two WMV and ZYMV isolates tested differed in their ability to be transmitted by *A. gossypii*. This variability in the transmission ability is commonly found among potyviruses and has been previously reported by other authors (Antignus *et al*., 1989). It is interesting to note that such differences were not found when testing the two isolates of CMV using the same procedure, which is consistent with the fact that variability in the transmission ability by their vectors is more frequently found in *Potyviridae* than in *Cucumoviridae*. The latter group lacks a helper component protein acting as a bridge during the transmission process, and therefore variability in the ability to transmit is restricted to changes in the coat protein (CP) of the virus (Perry, 2001). A variation in the transmissibility of potyviruses can thus result from mutations in either the CP or the helper component protein (HC-PRO) (Atreya *et al*., 1991; Granier *et al.*, 1993; Blanc *et al*., 1998; Llave *et al*., 1999; López-Moya *et al*., 1999).

Biologically distinct clones of *A. gossypii*, may differ in their ability to transmit CMV (Simons, 1959). This author reported that the clone collected and raised in *Hibiscus cannabinus* (kenaf) was a much less efficient vector of CMV than another clone, which was collected and raised in pepper. However, our work reported in this paper shows that the two clones tested of *A. gossypii* did not differ in their transmission ability of CMV (Table 4). Similar results were obtained by Lupoli *et al.* (1992), who found only one clone to be more efficient than others from a sample of 72 *A. gossypii* clones collected from south-eastern France. One possible explanation for these divergent results is that Simons worked with clones collected in Florida (USA), where *A. gossypii* has been reported to be holocyclic (sexual life cycle). Conversely, our aphid clones and those used in Lupoli's work were collected

Table 3. Comparison of the transmission efficiency of CMV, WMV and ZYMV by *Aphis craccivora* and the most efficient aphid vector of each virus

Virus (isolate)	Assay	Vector (clone)	% transmission[1]	Test	
				χ^2	P
CMV (M-6)	7	*A. gossypii* (clone 98) *A. craccivora*	85.7 (24/28) a 10.7 (3/28) b	31.54	< 0.0001
	8	*A. gossypii* (clone 98) *A. craccivora*	96.4 (27/28) a 35.7 (10/28) b	23.02	< 0.0001
WMV (M-116)	9	*M. persicae* (Encín) *A. craccivora*	96.4 (27/28) a 32.1 (9/28) b	27.15	< 0.0001
	10	*M. persicae* (Encín) *A. craccivora*	92.8 (26/28) a 28.6 (8/28) b	24.26	< 0.0001
ZYMV (C-71)	11	*M. persicae* (Encín) *A. craccivora*	82.1 (23/28) a 57.1 (16/28) b	4.14	0.0419
	12	*M. persicae* (Encín) *A. craccivora*	96.4 (27/28) a 53.6 (15/28) b	13.71	0.0002

[1] Percentage of infected plants. Number of plants infected per total number of plants tested is in parentheses. Transmission (%) followed by different letters within each assay indicates significant differences ($P \leq 0.05$) according to a chi-square test and to a Fisher exact test when the expected values were lower than 5 (Statview, Abacus Concepts, 1987).

in Europe, where *A. gossypii´*s life cycle is thought to be entirely anholocyclic (Blackman and Eastop, 1984). However, the number of clones used in our study is too low to reach any general conclusion. Another explanation for this discrepancy could be due to the number of aphids used in the experiment. We used five aphids per plant and Simons (1959) only used one aphid. Lupoli *et al.* (1992) used the same number of aphids as Simons and only observed a difference in one of the 72 clones evaluated. Therefore the number of aphids used per plant does not explain these differences in transmission efficiency.

In summary, our results on transmission are consistent with the results obtained by Alonso Prados *et al.* (2003), suggesting that CMV is mainly transmitted to melon crops in Spain by an aphid species

Table 4. Transmission efficiency of CMV, WMV and ZYMV infecting melon plants by different aphid clones of *A. gossypii* and *M. persicae*

Virus (isolate)	Species	Clone	Assay	% transmission[1]	Test	
					χ^2	P
CMV (M-6)	*A. gossypii*	93 98	1	93.6 (26/28) a 96.4 (27/28) a	0.35	0.618
		93 98	2	93.6 (26/28) a 93.6 (26/28) a	—	>0.999
WMV (M-116)	*M. persicae*	Encín Fuentidueña	3	39.3 (11/28) a 42.8 (12/28) a	0.07	0.785
		Encín Fuentidueña	4	46.4 (13/28) a 25.0 (7/28) a	2.80	0.094
ZYMV (C-71)	*M. persicae*	Encín Fuentidueña	5	100 (28/28) a 100 (28/28) a	—	>0.999
		Encín Fuentidueña	6	100 (28/28) a 100 (28/28) a	—	>0.999

[1] Percentage of infected plants. Number of plants infected per total number of plants tested is in parentheses. Transmission (%) followed by different letters within each assay indicates significant differences ($P \leq 0.05$) according to a chi-square test and to a Fisher exact test when the expected values were lower than 5 (Statview, Abacus Concepts, 1987).

such as *A. gossypii* that tends to colonise the crop and transmit the virus with high efficiency. In contrast WMV is mainly transmitted by non-colonising transient aphid species such as *M. persicae.* Labonne *et al.* (1982) also pointed out that the *A. gossypii* complex was the most important vector of CMV under field conditions in southern France. Nevertheless, further field work on the activity and timing of aphids landing on melon crops will give very valuable information in confirming the real contribution of each species to melon virus epidemics.

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