Identification and Distribution of Melon-Infecting Viruses and Their Vectors in Two Provinces of Costa Rica[1]

C. Rivera, W. Villalobos*, M.V. Sánchez*, C. Zumbado**, C.M. Rodriguez**

ABSTRACT

Papaya ringspot virus (PRSV), watermelon mosaic virus II (WMV-II), cucumber mosaic virus (CMV), and zucchini yellow mosaic virus (ZYMV) were identified by enzyme-linked immunosorbent assay (ELISA) in melon in Guanacaste and Puntarenas, the major melon production areas in Costa Rica. These viruses were found, as single and mixed infections in most test samples. Virus disease and aphid surveys were conducted during three growing seasons. PRSV and CMV were the most prevalent and commonly occurring viruses and *Aphis gossypii* (Glover) was the most common aphid vector found. An increase in the *A. gossypii* populations at the end of the growing seasons was followed by an increase in virus incidence.

Key words: Epidemiology, potyviruses, cucumoviruses, aphids.

COMPENDIO

El virus de la mancha anular de la papaya o "papaya ringspot virus" (PRSV), el virus del mosaico de la sandía II o "watermelon mosaic virus II" (WMV-II), el virus del mosaico del pepino o "cucumber mosaic virus" (CMV) y el virus del mosaico amarillo del zucchini o "zucchini yellow mosaic virus" (ZYMV) fueron identificados en melón, mediante el experimento inmunosorbente de enzima ligada (ELISA), en las provincias de Guanacaste y Puntarenas en Costa Rica. Los virus se encontraron tanto en infecciones simples como en las mixtas hasta con los cuatro virus. Mediante experimentos realizados en el campo durante las épocas de siembra de tres años, se determinó que PRSV y CMV fueron los virus que se presentaron con mayor incidencia, y fue *Aphis gossypii* (Glover) el áfido encontrado con mayor frecuencia. Un aumento en las poblaciones de este último se observó al final de las épocas de siembra, seguido de un aumento en la incidencia de virus.

INTRODUCTION

Melon (*Cucumis melo* L.) is one of Costa Rica's most important nontraditional export crop. The commercial production of melon for export began in 1984 with a total area of 50 ha. By 1992, 3000 ha were dedicated to this crop (CINDE 1992). Melon is grown predominantly in Guanacaste and Puntarenas provinces. In most areas, melons are planted consecutively at 1-wk intervals through November to March, under irrigation and often the plantings are near one another.

One of the most important limitations on melon production in Costa Rica are virus diseases. Papaya ringspot virus (PRSV) (Purcifull *et al.* 1984), cucumber mosaic virus (CMV) (Francki *et al.* 1979), watermelon mosaic virus II (WMV-II) (Purcifull *et al.* 1984), zucchini yellow mosaic virus (ZYMV) (Lisa and Lecoq 1984), squash mosaic virus (SqMV) (Campbell 1971), and geminiviruses frequently infect melon and other cucurbits worldwide (Adlerz *et al.* 1983; Avgelis 1989; Brown and Nelson 1986; Davis and Mizuki 1987; Delgadillo *et al.* 1987; Dodds *et al.* 1983; Lastra 1986; Milne *et al.* 1969; Milne 1987; Nameth *et al.* 1985; Nameth *et al.* 1986; Ullman *et al.* 1991). In Costa Rica, PRSV and CMV have been associated with a severe mosaic that affects melons in Guanacaste (Rivera *et al.* 1991). This paper reports the identification, incidence, and

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* Centro de Investigación en Biología Celular y Molecular, Universidad de Costa Rica, C R
** CINDE, San José, C R.

distribution of viruses infecting melon in Costa Rica and the identification and population dynamics of the aphid vectors.

MATERIALS AND METHODS

Data collection

Surveys were conducted in lowland areas of the Pacific coast (Table 1) during the growing seasons (November to March) of 1988-1989, 1989-1990 and 1990-1991 (hereafter referred to as the 1989, 1990 and 1991 growing seasons). A total of 15 farms growing melons were sampled: twelve growing melons were located in Guanacaste, and three in Puntarenas. At the beginning of the growing season, an early planting was selected on those farms (Table 1), and it was evaluated for eight weeks. During the 1989 growing season, only one plot was studied on all farms. During 1990 and 1991, a second and a third plot were also studied in some farms (Table 1), corresponding to mid-season and late plantings. The number of plots evaluated was determined by the duration of the growing season, up to a maximum of three plots per farm. Two sampling areas of 30 x 30 m were established, located at the northern and southern corners of each plot. Data for three types of surveys were collected in these sampling areas. First, 100 plants were randomly selected and examined for mosaic symptoms each week for six to eight weeks. This procedure gave an estimate of the overall virus incidence based on symptomatic plants at the last reading date

Table 1. Number of melon plots evaluated in each farm during the 1989, 1990, and 1991 growing seasons.

Farm	Province	Number of plots		
		1989	1990	1991
A	Guanacaste	1	2	
B	Guanacaste	1		
C	Guanacaste	2		
D	Guanacaste	2		
E	Guanacaste	2	3	
F	Guanacaste	1	1	
G	Guanacaste	1	3	3
H	Puntarenas	2	2	
I	Puntarenas	1		
J	Guanacaste		3	
K	Puntarenas			1
L	Guanacaste	1		
M	Guanacaste	1		
N	Guanacaste	1		
O	Guanacaste	1		

The second type of survey involved the serological assay of some symptomatic plants found in the first survey by enzyme-linked immunosorbent assay (ELISA) (Voller *et al.* 1977). A maximum of 10 symptomatic plants per sampling area per week was collected at random during the 1989 and 1990 seasons, up to eight weeks. During the 1991 season, samples were only collected at three and six weeks after crop emergence. A total of 116, 714, and 228 samples were tested by ELISA during 1989, 1990 and 1991, respectively. Coating antibodies, enzyme-conjugated antibodies against PRSV, CMV-vi, WMV-II and ZYMV, as well as ELISA protocols from Agdia, Inc., Indiana, were used.

ELISA reactions were measured spectrophotometrically at 405 nm using a MR 700 Microplate Reader. Positive controls consisted of *Cucurbita pepo* leaf tissue extracts from plants infected separately with each of the four viruses tested. Negative controls consisted of healthy *C. pepo* leaf tissue extracts. Positive and negative controls were maintained in a greenhouse. Samples were considered positive if the $A_{405\ nm}$ values were equal or greater than the mean healthy plant controls (n=4), plus three standard deviations.

The third type of survey was the collection and identification of alate aphids. Aphids were collected weekly in 225 cm² yellow-pan water traps containing a 50% solution of ethylene glycol and water. One trap per sampling area was located above the plant canopy. Aphids were stored in 70% ethanol and later identified with taxonomic keys (Holman 1974; Medler and Ghost 1969).

RESULTS

Virus incidence

During the three growing seasons, the highest virus incidence in the early plots was 4.5%, 18.5%, and 16.5% respectively (Table 2). In both growing

seasons when mid-and late-planted plots were studied (1990 and 1991) an increase of virus incidence was observed in the latest plantings for most farms, reaching highest values of 52.5% and 73.5% for the 1990 and 1991 growing seasons, respectively. No incidence increase was observed on farm J in the last plot (Table 2).

Identification and distribution of viruses

From a total of 116 samples analyzed by ELISA, 106 were positive to CMV and/or PRSV in 1989. Both viruses were observed in single and mixed infections, with single infections being higher than 60%. During the 1990 and 1991 growing seasons, WMV-II and ZYMV were also detected, in addition to CMV and PRSV (Figs. 1 and Fig. 2). Two exceptions were observed during the last two growing seasons: ZYMV was not detected on farm H during 1990, but it was present during the 1991 growing season; and CMV and WMV-II were not detected in farm K during 1991 (Figs. 1 and 2). During the 1990 season, 624 samples tested positive by ELISA from a total of 714; and 204 from a total of 228 during 1991. The four viruses were found infecting the crop in single and mixed infections, with mixtures of two, three, or four viruses in all possible combinations. The percentage of single infections was higher than that of the mixed infections for most farms (B, C, E, F, H and I) during the 1990 growing season (Table 3). During the 1991 season, the percentage of single and mixed infections was similar for farms E, G, and J, close to 50%, with a variation of 20% for farms E and G. Farms H and K had predominantly single infections, 65% and 100% respectively (Table 3).

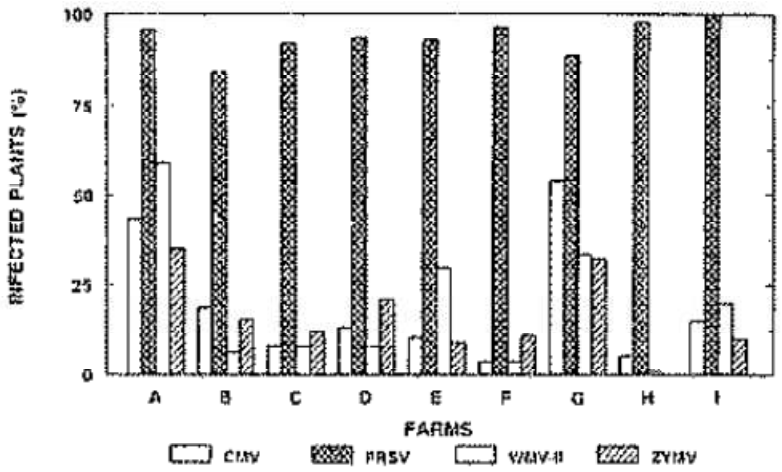

Fig. 1. Percentage of infected plants (tested by ELISA) for each farm during the 1990 growing season.

CMV = cucumber mosaic virus, PRSV = papaya ringspot virus, WMVII = watermelon mosaic virus II, and ZYMV = zucchini yellow mosaic virus.

Table 2. Highest virus incidence observed in each plot during the three growing seasons.

Farms	Highest incidence per growing season in each plot						
	1989	1990			1991		
	Plots						
	1	1	2	3	1	2	3
A	4.0	3.5	13.5				
B		8.5					
C		0.5	19.5				
D		18.5	52.5				
E		6.0	52.0		3.5	3.0	73.5
F	4.5	5.0					
G	0.5	1.5	13.5	44.5	16.5	12.5	42.5
H		4.0	25.0		11.0	15.0	
I			8.5				
J					7.5	1.0	5.5
K					7.5		
L	1.0						
M	3.5						
N	1.5						
O	2.5						

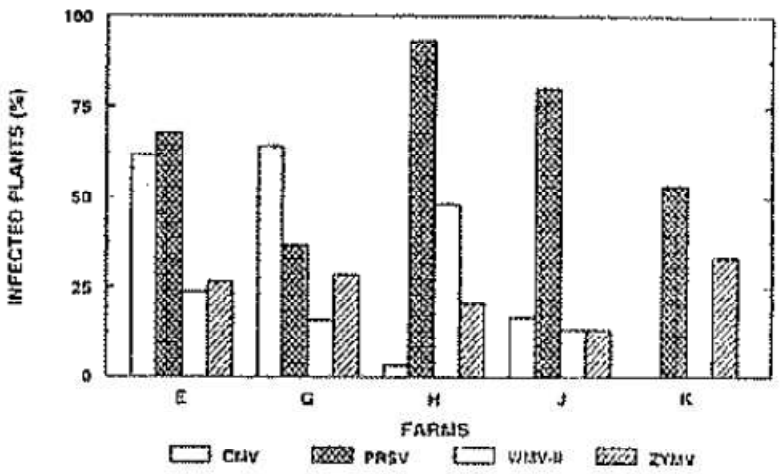

Fig. 2. Percentage of infected plants (tested by ELISA) for each farm during the 1991 growing season.

CMV = cucumber mosaic virus, PRSV = papaya ringspot virus, WMVII = watermelon mosaic virus II, and ZYMV = zucchini yellow mosaic virus.

Table 3. Percentage of single and mixed infections for each farm for the 1990 and 1991 growing seasons. Data based on ELISA test.

	Type of infection per growing season			
	1990		1991	
Farms	Single	Mixed	Single	MIXED
A	38 0	62 0	—/a	—
B	73 3	26 7	—	—
C	92.0	8.0	—	—
D	12.5	87.5	—	—
E	82.6	17 4	40.6	59 4
F	81 5	18 5	—	—
G	38 9	61.1	59 6	40.4
H	95.8	4.2	65 5	34 5
I	84.6	15.4	—	—
J	—	—	50.0	50 0
K	—	—	100 0	0

/a These farms were not sampled in this season.

PRSV was the most prevalent virus in all farms. More than 90% of tested samples were PRSV positive by ELISA in the 1990 growing season CMV, WMV-II and ZYMV incidence was lower than 30% for most of the farms, the exceptions being farms A and G. During the 1991 season, the PRSV incidence decreased and that of the other viruses generally increased, as compared to the 1990 growing season. CMV incidence was higher than 60% in farms E and G, but lower than 30% in farms H and J The incidence for the other two viruses ranged between 15% and 45% for WMV-II and between 15% and 30% for ZYMV (Fig 2).

Aphid population dynamics

A. gossyppi was the most common aphid trapped in melon fields in Costa Rica (Table 4). Winged and wingless (the wingless were observed colonizing the crop) forms were observed during the three growing seasons on all farms For farm G, the alate *A. gossypii* population in the earliest plot was small, but increased rapidly by the last planting (Fig 3). This situation was also observed for many other farms (data not shown). Those farms which had a considerable increase in aphid populations also showed a significant increase in virus incidence a few days later as observed for farm G (Fig. 3). Those plots where the aphid population was small generally had low virus incidence.

The other aphid species (Table 4) were present at far lower levels than *A. gossypii*, and their presence was erratic.

DISCUSSION

Our results show that the melon virus epidemic composition in commercial fields in Costa Rica has changed drastically in a short time. During the 1989 growing season, a low virus incidence prevailed (Table 2), and only PRSV and CMV were detected in all farms studied (data not shown) During the 1990 and 1991 growing seasons, a progresive increase in virus incidence was observed, and ZYMV and WMV-II were also detected, in addition to CMV and PRSV (Table 2, Figs. 1 and 2). The observed increase in virus diversity and disease incidence over time could be related to the number of seasons the crop has been grown on each farm. Farms with a short farming record (one or two seasons) usually had a lower virus diversity and lower disease incidence than those with a long record (three or more seasons).

The high virus incidence observed in early plantings on farms G and H during the third growing season (Table 2) could be due to an increase in virus infection of susceptible weed hosts, as well as of aphid-vector host plants, near the melon fields The

progressive increase in virus incidence observed during the last plantings in the 1990 and 1991 growing seasons for most farms (Table 2) could be a consequence of the intensive melon farming system used in Costa Rica, with weekly plantings each season. These conditions could allow the colonization of the melon by *A. gossypii*, the establishment of a large population of alate aphid forms, and a rapid spread of virus from older to younger plants.

A similar situation was reported by Ullman *et al.* (1991), and Davis and Mizuki (1987) on the Hawaiian Islands and New Jersey, respectively. Even though melon plantings are spatially and temporally isolated by plantings with nonsusceptible crops, melon viruses and *A. gossypii* have wide host ranges. The diversity of the weed community observed nearby the melon fields in Costa Rica (Sanchez *et al.*, personal communication) provides the conditions and opportunities for many viruses and aphid species to survive during the melon-crop-free periods. The type and quantity of alternative host species will determine the primary aphid and virus inoculum for the next growing season. The four viruses detected infecting melon in Costa Rica have often been reported for this crop and for other cucurbits all over the world. Mixed infections like those found in Costa Rica (Table 3) have also been reported for melon in other countries (Adlerz *et al.* 1983; Delgadillo *et al.* 1987; Lastra 1968; Milne *et al.* 1969; Ullman *et al.* 1991).

Our results indicate that *A. gossypii* is the most prevalent vector of these viruses in Costa Rica (Table 4). It is often reported as an efficient vector of the melon viruses found in this research (Castle *et al.* 1992; Eastop 1983; Francki *et al.* 1979; Lisa and Lecoq 1984; Purcifull *et al.* 1984; Purcifull *et al.* 1984; Wang *et al.* 1992). Nelson and Tuttle (1969) and Adlerz (1973) did not find a relationship between the increase in *A. gossypii* population and the increase in disease incidence, as it was observed in farm G (Fig. 3) and other farms in this study (data not shown). The other aphid species captured in our study had been reported as vectors of some melon viruses (Francki *et al.* 1979; Lisa and Lecoq 1984; Purcifull *et al.* 1984; Purcifull *et al.* 1984), but their low incidence and erratic appearance (Table 4) make them insignificant factors in the spread of melon viruses in Costa Rica, and therefore they can be excluded as major vectors of the viruses.

Table 4. Total number of aphid species collected during the three growing seasons.

Aphid species	Aphids per growing season (no.)		
	1989	1990	1991
Aphis gossypii	3 894	2 810	2 921
Myzus persicae	0	9	2
Rhopalosiphum maidis	1	0	1
Rhopalosiphum padi	1	0	0
Aphis spiricola	42	9	15
Pentalonia nigronervosa	0	9	12
Others species[a]	39	50	153

[a] None of these species has been reported as a virus vector for any of the viruses found infecting melon in Costa Rica.

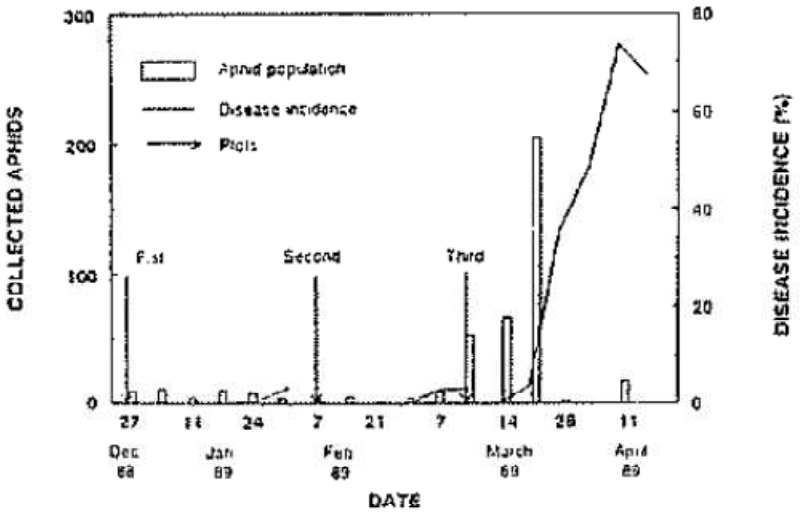

Fig. 3. Total virus incidence and aphid population dynamics for farm G during the 1989 growing season. Three planting plots were studied during this season.

Additional epidemiological studies on alternative plant hosts and the aphid vectors of the viruses infecting melon are being conducted. The knowledge acquired in studies of melon virus epidemics will help establish integrated conventional control measures, and in the near future will permit exploration of non-conventional protection, such as coat protein-mediated insulation.

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