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Epidemics of aphid-transmitted viruses in melon crops in Spain

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Abstract

A survey of mosaic diseases in fields of open air grown melon was done in three provinces of Spain over 3 years. The incidence of the mosaic-inducing viruses *Cucumber mosaic virus* (CMV), *Watermelon mosaic virus*-2 (WMV-2), *Papaya ringspot virus* watermelon strain (PRSV-W) and *Zucchini yellow mosaic virus* (ZYMV) was assessed. The only viruses present in the three provinces during the 3 years studied were CMV and WMV-2, but their incidence differed according to year and region. For each epidemic, disease progress curves (DPC) were obtained, all of them well described by the Gompertz model. With the five descriptive variables of the model, a principal component analysis was done and two principal components, representing a severity factor and a time factor were found. Cluster analyses done with these two principal components, grouped the epidemics into 6 clusters that did not correlate with year, virus or province. Correlation analyses between epidemics caused by WMV-2 and CMV and climatic variables were done. Although the temperature in the spring months was the main factor associated with the severity of the epidemics caused by these two viruses, other differential correlations were found. Spatial evolution of CMV and WMV-2 epidemics was also different suggesting different aphid species acting as vectors for the viruses.

Introduction

Melon (*Cucumis melo*) is an important vegetable crop in Spain, ranking first in acreage and third in production (MAPA, 1997). Almost 75% of Spain's melon production is from open air crops, mainly in the eastern and southern areas of the country. Mosaic diseases caused by aphid-borne viruses are a worldwide problem for melon producers. In field-grown melons in Spain there have been reports of the presence of *Cucumber mosaic virus* (CMV), and the potyviruses *Papaya ringspot virus* watermelon strain (PRSV-W), *Zucchini yellow mosaic virus* (ZYMV) and *Watermelon mosaic virus*-2 (WMV-2) (Luis-Arteaga, 1991; 1994; Luis-Arteaga et al., 1998). Although the relative importance of these viruses varied between the different melon-producing regions of Spain and between years, CMV and WMV-2 seem to be the most prevalent (Luis-Arteaga et al., 1998).

These mosaic-inducing viruses are non-persistently transmitted by different species of aphids (Pirone and Harris, 1970), much of them being efficient vectors of all four viruses (Francki et al., 1979; Lisa and Lecoq, 1984; Purcifull et al., 1984a,b), but the aphids reported as the most efficient vectors for each virus are different (Castel et al., 1992; Palukaitis et al., 1992).

The effect of these viruses on crop production depends on the growth stage at the moment of infection

(Demski and Chalkey, 1974; Blua and Perring, 1989; Alonso-Prados et al., 1997). In spite of the importance of the melon crop in Spain and the obvious symptoms (leaf distortion, mosaic, chlorotic mottle, vein clearing) of virus infections in plants, there is no epidemiological information available to explain the differences in virus incidence between years and supporting efforts to establish efficient disease management strategies. Epidemiological approaches provide information for the management of epidemics caused by different pathogens, including viruses (Madden et al., 1987; Fargette et al., 1994; Gottwald et al., 1996; Kocks et al., 1999; Webb et al., 1999). For viruses, these analyses have been applied mainly to the use of cultural practices such as date of sowing or transplanting (Mora-Aguilera et al., 1996) or vector management (Madden et al., 1987). The efficacy of epidemics management depends on the knowledge of both the virus dispersion patterns and on the vector transmission efficiency.

Epidemics can be compared with several methods (Campbell and Madden, 1990; James and McCulloch, 1990; Workney et al., 1993), including multivariate analysis, which takes into account different biological aspects of disease progress curves (DPC) (Kranz, 1974; Campbell et al., 1980; Anderson et al., 1990). This approach was used to study the epidemics caused by *Papaya ringspot virus* in papaya (Mora-Aguilera et al., 1996), but has not been applied to compare epidemics of different viruses occurring on the same crop.

In this paper, we present data on the temporal and spatial evolution of epidemics produced by mosaic-inducing viruses infecting melon in three different regions of Spain during three consecutive growing seasons (1994–1996). A multivariate analysis has allowed us to correlate DPC for each virus with weather variables and to draw conclusions from their temporal and spatial evolution.

Material and methods

Disease assessment. Commercial fields of open air grown melon were selected in the provinces of Madrid (Mad), Barcelona (Bar) and Zaragoza (Zar) during the years 1994–1996. These provinces were representing different crop management systems and weather conditions, and hence are representative of the different conditions of open air melon cultivation in Spain. In each province, one field was arbitrarily selected every year (in 1995 and 1996, two different fields were sampled in Barcelona) in the main melon growing area, and disease progress was assessed by monitoring each of 500 melon plants of a plot (25 rows $\times$ 20 plants) selected within each melon field avoiding field borders. Melon varieties used in the three provinces were *Cucumis melo* var. *saccharinus* Naud. (cv. Piel de Sapo in Madrid and Zaragoza; and cvs Pinyonet or Roget in Barcelona), the date of planting differed for each province and year but, in all the cases, melon was planted during the month of May. The melon fields selected in the three provinces were surrounded either by other melon fields, or by tomato, maize and sunflower fields.

Plots were surveyed every 7–10 days for viral symptoms from transplanting to the end of the crop, and all symptomatic plants were sampled by collecting 3–4 young leaves per plant. In this way, plots were visited a minimum of 7 (i.e. Zaragoza 1996) and a maximum of 17 (i.e. Madrid 1994) times. The presence of CMV, WMV-2, PRSV-W and ZYMV was assayed in the collected samples by DAS-ELISA (Clark and Adams, 1977), using commercial antisera. A sample was considered as infected when absorbance at 405 nm (Multiskan MCC/340, Labsystem, Helsinki, Finland) was at least twice the absorbance of the negative controls. When one plant was positive for any of the four analysed viruses, it was assumed to remain infected by this virus in subsequent visits. Each symptomatic plant was sampled until all four viruses were detected, or until the end of the crop. On each sampling date, incidence of each virus was evaluated by ELISA and calculated as the frequency of plants infected (%).

Temporal analyses. The incidence of each virus was calculated for every plot and survey date and DPC was obtained for each virus. The appropriateness of the linearized forms of the monomolecular, logistic, Gompertz and Weibull models (Campbell and Madden, 1990) to describe the resulting DPCs was examined by linear regression analysis of disease incidence data. The independent variable was 'days after transplanting' (dat), the dependent one was 'frequency of infected plants'. In these analyses, the Weibull model was used as a two-parameter model because parameter *a* (days between transplanting and appearance of the first symptoms) was known for all cases (Pennypacker et al., 1980). The best model to describe each epidemic was chosen by comparing the observed and expected DPCs, examining the coefficients of regression and the plots of residual errors *versus* predicted values (Campbell and Madden, 1990).

Each epidemic was identified with name of the virus, year of occurrence and province (i.e. ZYMV/Mad/1996 means the epidemic of ZYMV in Madrid in 1996). For 1995 and 1996 in Barcelona, the name of the province (Bar) is followed by a numeral that indicates the two different sites sampled.

Multivariate comparisons. DPCs were characterized by the variables associated with the Gompertz distribution function (Berger, 1981; Campbell and Madden, 1990). The variables were: AUDPCs: area under the DPC standardized for the duration of the epidemic in days (AUDPCs = AUDPC/T_t); y_f: final disease incidence; T_{50}: time in days to reach a disease incidence of 50%; T_t: time, in days, from the observation of the first symptom to the end of the crop; and r: the rate parameter of the Gompertz model. The variables that were not normally distributed (r, y_f, T_t and AUDPCs) were transformed with the logarithmic transformation (r and y_f), or the square root transformation (AUDPCs and T_t).

The five variables associated to the Gompertz distribution function of the 30 epidemics were analysed by a principal component analysis (PCA; Madden and Pennypacker, 1979) with varimax rotation, extracting those factors with an eigenvalue above 0.7. The epidemiological variables that had the higher correlation coefficient with the chosen factors were associated with them for biological interpretation. The PCA were done with the STATISTICA for Windows V. 4.5 software program of StatSoft (Tulsa, OK, USA).

Classification of epidemics and correlation with climatic variables. In the vectorial space defined by the two factors obtained by the PCA described above, the epidemics were classified by cluster analysis, using the square of the Euclidean distance between them.

Linear correlation coefficients between the variables of the Gompetz model and different climatic variables were calculated. The climatic variables used were:

1. The average of the mean temperatures of January, February and March (winter, T_{medW}), or April, May and June (spring, T_{medS}).
2. The average of the minimum temperatures of January, February and March (winter, T_{minW}), or April, May and June (spring, T_{minS}).
3. The average of the maximum temperatures of January, February and March (winter, T_{maxW}), or April, May and June (spring, T_{maxS}).
4. The average of the total precipitation of January, February and March (winter, P_{medW}), or April, May and June (spring, P_{medS}).

These analyses were done with the STATISTICA for Windows V. 4.5 software program of StatSoft.

Spatial analyses. Melon plots were considered as a plant distribution lattice in which infected plants were recorded in each sampling date; the cropping rows being the columns of the lattice. Spatial analyses were done for each sampling date, by determining number of foci, focus size, shape, orientation and compactness measured as proximity index (PI) (Nelson, 1996). These analyses were done by using the program FOCI (Nelson, 1996), kindly supplied by Dr. S. Nelson (Hawaii University).

This kind of analysis was done for those CMV and WMV-2 epidemics with more observation dates and a high disease incidence at the end of the crop.

Results and discussion

Temporal analyses of epidemics

The cucumovirus CMV and the potyviruses WMV-2, PRSV-W and ZYMV have been reported as the most frequent viruses infecting melon crops world wide (Lovisolo et al., 1982; Lecoq and Pitat, 1983; 1984; Nameth et al., 1986; Lecoq et al., 1988; Grafton-Cardwell et al., 1996; Luis-Arteaga et al., 1998). The epidemics caused by these viruses were analysed in three provinces that represent different conditions of open air melon cultivation in Spain. Barcelona was chosen to represent the mild conditions of the Mediterranean coastal areas. Madrid represented the more continental conditions high plateau (600 m) of Central Spain. Zaragoza represented conditions of milder winters than in Madrid but colder than in the Mediterranean coast.

The relative importance of each virus varied depending on the year and the province (Table 1). Madrid was the only site in which PRSV-W and ZYMV were found every year whereas they were never detected in Zaragoza. In Barcelona PRSV-W was only found in 1994 and ZYMV in 1994 and 1996 (Table 1). Both CMV and WMV-2 were prevalent throughout the 3 year period in three provinces, but their incidence at the end of the cropping season varied largely with region and year (Table 1). The incidence of these viruses was

Table 1. Incidence of CMV, WMV-2, PRSV-W and ZYMV at the end of the melon cropping season in different Spanish provinces and in different years[1]

Region	Year	CMV	WMV-2	PRSV-W	ZYMV
Madrid	1994	70.4	48.2	16.0	24.8
	1995	43.2	57.8	18.8	21.8
	1996	42.4	57.8	1.6	8.0
Zaragoza	1994	83.3	86.2	0.0	0.0
	1995	95.0	58.8	0.0	0.0
	1996	85.0	73.1	0.0	0.0
Barcelona	1994	24.0	35.6	11.4	6.6
	1995				
	A[2]	12.2	10.8	0.0	0.0
	B[2]	25.7	3.0	0.0	0.0
	1996				
	A[2]	4.0	12.7	0.0	5.6
	B[2]	6.8	11.6	0.0	3.2

[1]Data are percentages of infected plants.
[2]For 1995 and 1996 in Barcelona two different melon fields were sampled in the same area.

higher in Zaragoza ($p \leq 10^{-4}$) than in the other regions for the period studied, reaching values of 95% infected plants (CMV-1995). Virus incidence was lower in Barcelona, with a maximum of 36% (WMV-2 1995). The variation in incidence found for these viruses agrees with other reports from Spain (Luis-Arteaga et al., 1998), and other countries (Lovisolo et al., 1982; Sammons et al., 1989; Grafton-Cardwell et al., 1996).

DPC for each virus/year/province combination, i.e., for each epidemic, was obtained by plotting virus incidence (% infected plants) against time [days after transplanting (dat)] (Figure 1). DPC were different for each epidemic, but usually epidemics of CMV and WMV-2 begun earlier and progressed faster than those of ZYMV and PRSV-W, suggesting that at least some epidemiological factor(s) similarly affected the evolution of the epidemics of these two viruses (see below). Considering only the epidemics of CMV and WMV-2 (that occurred in all the provinces and years) their behaviour in the three provinces was very different. In Zaragoza, epidemics of CMV were always the earliest and the fastest ones, in Barcelona and Madrid the DPC of each epidemic depended on the year, but epidemics never developed as fast as in Zaragoza. In Madrid epidemics begun later (dat $\geq$ 48) and progressed fast, but final virus incidences were not above 70%. DPC for Barcelona presented an intermediate situation with epidemics that begun early and had low final disease incidences (i.e. CMV/Bar2/1996; dat = 24), or begun later and progressed more or less fast (i.e. WMV-2/Bar/1994, dat = 41; or WMV-2/Bar1/1995, dat = 66). Incidence data for each epidemic are available upon request from afp@bit.etsia.upm.es.

Comparison of epidemics: progress curve analyses

In an attempt to understand the factors that determine the observed differences among the epidemics, their temporal evolution was compared. The linearized forms of the monomolecular, the logistic, the Gompertz and the Weibull models were evaluated for goodness of fit to the data of the temporal evolution of the viral incidence for each epidemic by examining the coefficient of regression, the standard error of the regression coefficient, and the plots of residual errors. None of the models could describe the PRSV/Mad/1996 epidemic because its highest incidence was 1.6% 105 days after transplanting. Therefore, it was not included in the analyses. Of all models Gompertz model described well all the epidemics ($R^2 \geq 0.84$), and was consequently used for comparative analysis. Each one of the 30 DPCs was characterized by the descriptive variables associated with the Gompertz model as has been suggested by Kranz (1974) (see Material and methods, and Table 2). With the values of these variables, transformed for normality if necessary, a PCA was done and two principal components were obtained (eigenvalue > 1.3) that accounted for 85.56% of the total variance (Table 3). The first principal component, which accounted for 59.20% of the total variance, was associated with the variables r, T_{50}, AUDPCs and y_f, and represented a severity factor. The second principal component accounting for 26.36% of the variance, was associated to the variable T_t, and represented a time factor.

Comparison of epidemics: cluster analyses

In the space defined by the above described principal components, a cluster analysis of the 30 epidemics was done using the squared Euclidean distance between them. ANOVA of the variables using the clusters as groups showed that the first clustering in which all five epidemiological variables were significantly different for all groups was the one obtained by cutting at an squared Euclidean distance of 0.44 (Figure 2). This cutoff resulted into 6 clusters that did not group the epidemics according to year, virus or province. The only

observed relationship with site was that epidemics of Barcelona and Zaragoza never appeared in the same cluster, except for WMV/Zar/1995 that clustered with epidemics form Madrid and Barcelona (Figure 2). The cluster based on the Gompertz model (Figure 2) had the following common characteristics: Group I: epidemics with the onset 40 or more days after transplanting, and a high r value. Group II: epidemics with an early onset (dat $\leq$ 28) and with a high r value, which lead to a final incidence near 100%. Group III: epidemics that began at an intermediate time (dat $\geq$ 30) and/or had a low r. Group IV: epidemics that began late (dat $\geq$ 40) and had a low r. Group V and Group VI differed only in the time of the onset of the epidemics (dat = 15 and 28, respectively), both had a high r value but reached final incidences lower than those of Group II.

To analyse if there was any relationship between the epidemics caused by the same virus, or between the epidemics that occurred in the same site, independent analyses for each virus, or province, were done. For these analyses, only the epidemics caused by CMV and WMV-2 were considered, because these were the only viruses present every year at every site. When the analyses were done for each province separately, results showed that the epidemics did not cluster according to either virus or year (data not shown). When the analyses were done separately for each virus, the epidemics of Barcelona did not cluster with those of Zaragoza, whilst the epidemics of Madrid were related to some of Barcelona and some of Zaragoza. These results suggested that factors (climatic, biological, etc.) different from virus or province determined the progress of each epidemic.

Hence climatic variables, mainly temperature and precipitation, might influence the course of epidemics induced by vector-borne pathogens by conditioning the abundance and/or behaviour of the vectors.

Comparison of epidemics: factors that determine the disease progress curve

To determine if climatic factors were responsible for the observed associations between epidemics, correlation analyses were done between the epidemiological variables and some climatic variables, described in Material and methods. This analysis was done only with the epidemics caused by CMV and WMV-2 and, for both viruses, the severity of the epidemics, as measured by y_f, was positively correlated with spring temperatures, both with the average of the maximum temperature (T_{maxS}, $r > 0.69$, $p \leq 0.018$) and, particularly, with the average of the mean temperature (T_{medS}, $r > 0.74$, $p \leq 0.009$). These correlations could explain, at least in part, the positive correlation between the epidemiological variables describing the epidemics of both viruses.

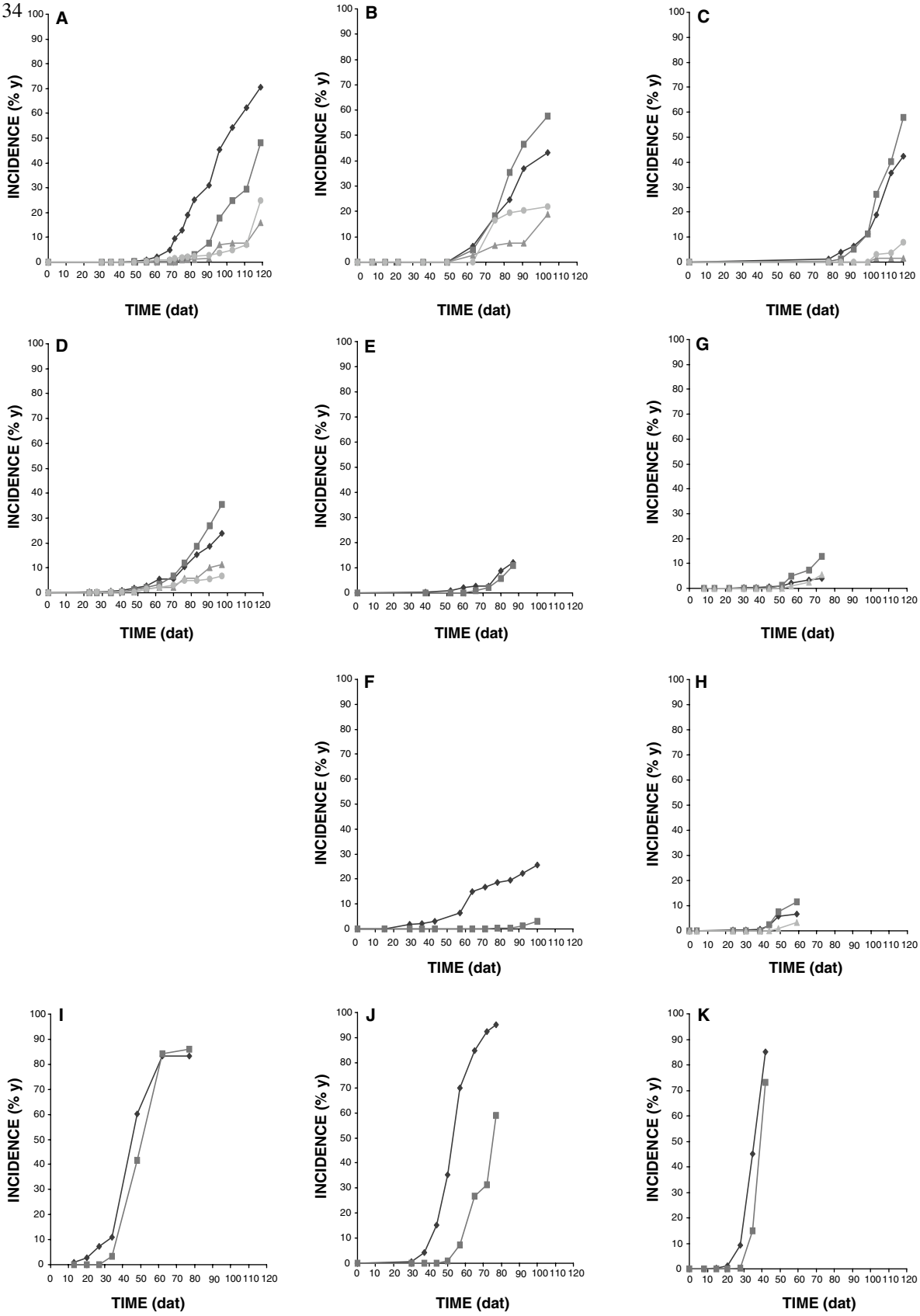
For CMV epidemics, AUDPCs was correlated with the average of the maximum temperature ($r = 0.77$, $p = 0.004$) and the average of the mean temperature ($r = 0.80$, $p = 0.003$) in spring, and AUDPCs and y_f were negatively correlated with the precipitation during spring (P_S, $r = -0.68$ and -0.71, for AUDPCs and y_f respectively, $p \leq 0.036$). This means that the epidemic progressed slower with the rainfall in spring.

For WMV-2, minimum temperatures in winter and spring were negatively correlated with the duration (T_t) of the epidemics (T_{minW} $r = -0.66$, $p = 0.027$; T_{minS} $r = -0.76$, $p = 0.007$). No other correlation was found to be significant.

The fact that spring temperatures were positively correlated with epidemics of both viruses could be explained by the build up of aphid populations vectoring both viruses (Francki et al., 1979; Purcifull et al., 1984a). Nevertheless, epidemiological parameters were different for CMV and WMV-2 epidemics, notably minimum winter temperatures had an effect on the duration of WMV-2 but not CMV epidemics (the lower the temperatures, the later the epidemic would start), and spring rain had a negative effect on the severity of CMV, but not on WMV-2, epidemics. This suggests that overwintering reservoirs and/or vectors of WMV-2 are more cold-sensitive than those of CMV, and that spring dispersion of both viruses could depend, at least in part, on different vectors. Overwintering reservoirs of these viruses could be weeds or volunteers present in the cropping area and, at least in the melon area of Madrid, many weeds present in winter could be reservoir of both viruses (Sacristán et al., 2001), suggesting that differences may be due to different vectors.

Comparison of epidemics: spatial analysis

To determine if correlations between epidemiological and climatic variables had an influence on the spatial distribution of CMV and WMV-2, maps of CMV and WMV-2 incidence were drawn for all sampling dates (data not shown). Values for number of foci, PI, maximum row and column distances and focus size were calculated and plotted in relation to disease incidence. This allowed a differential evaluation of each individual


A
B
C
D
E
G
F
H
I
J
K
INCIDENCE (% y)
TIME (dat)
0 10 20 30 40 50 60 70 80 90 100 110 120

virus contributing to the disease of a specific location. Virus epidemics in Mad/1994, Mad/1995, Mad/1996, Zar/1994, Zar/1995 and Bar/1994 with final incidences up to 20% for the two viruses were evaluated.

In CMV epidemics the number of foci increased rapidly after first appearance of virus, reaching a high number of foci of small size and high PI at low incidences (no more than 24%). In WMV-2 epidemics, the number of foci increased slowly with virus incidence, and the maximum number of foci was never as high as for CMV. The foci in WMV-2 epidemics were larger than for CMV, but less compact as estimated by the PI.

Table 2. Mean, standard deviation and range for the epidemiological variables associated with the Gompertz model calculated for 30 disease progress curves in melon crops, induced by CMV, WMV-2, PRSV-W and ZYMV

Variable (units)*	Mean	Standard deviation	Range
r (days^{-1})	0.0436	0.0427	0.2145–0.0053
AUDPCs (% days/days)	0.1976	0.1881	0.7159–0.0020
y_f (% infected plants)	37.6707	30.2488	95.09–2.8993
T_{50} (days)	127.30	51.90	256.78–31.39
T_t (days)	42.667	19.245	74–14

*r: rate parameter of the Gompertz model, AUDPCs: area under the disease progress curve standarized for the duration of the epidemic in days (AUDPCs = AUDPC/T_t); y_f: final disease incidence, T_{50}: time to reach 50% disease incidence, T_t: time from the observation of symptoms to the end of the crop.

Table 3. Eigenvectors and eigenvalues of the principal components deduced from the epidemiological variables associated with the Gompertz model calculated for 30 disease progress curves in melon crops, induced by CMV, WMV-2, PRSV-W and ZYMV[1]

Variables[2]	Principal components	
	PC1	PC2
% variance	59.20	26.36
r	**−0.893**	−0.349
y_f	**−0.864**	0.451
AUDPCs	**−0.781**	0.369
T_t	0.043	**0.919**
T_{50}	**0.886**	0.174
Eigenvalues	2.959	1.318

[1]Data are coefficients of the eigenvector associated with each principal component. Bold numbers represent the highest coefficient values associated with each principal component.
[2]Epidemiological variables as in Table 2.

As an example, Mad/1994 for CMV, the number of foci had a maximum ($N = 41$) for 19% incidence, and afterwards declined until the end of the crop (incidence 62%, $N = 3$) (Figure 3A). PI diminished from the onset of the epidemic until 54% virus incidence (PI = 0.76), increasing afterwards (PI = 0.87, incidence = 62%). During all the epidemic, the maximum column distance was higher than the maximum row distance spanned by a focus. This means that foci were enlarging along cropping rows (Figure 3C).

For the 1994 WMV-2 epidemic in Madrid, the number of foci was maximum for an incidence of 18% ($N = 37$), declining afterwards until the end of the crop (incidence 48%, $N = 7$) (Figure 3B). PI varied more than for CMV, reaching lower values (PI = 0.73, incidence 48%). This means that foci were less compact than in CMV epidemics. The analysis of maximum column and row distance spanned by a focus showed that both had similar values (Figure 3D).

The hypothesis that different aphid species could be the main vectors for CMV and WMV-2 is corroborated by this spatial analysis: for CMV, high numbers of small compact foci were present at low disease incidences, with foci that enlarged mainly along the cropping rows. This distribution is probably reached by the arrival of viruliferous aphids from outside the crop, possibly from long distance crops as the evolution of CMV epidemics was not influenced by winter temperatures. These aphid species seemed to be melon-colonizers as foci were compact and enlarged slowly and mainly along the cropping rows. The most abundant aphid in melon crops in Spain, *Aphis gossypii* (Anonymous, 1968; García-Marí et al., 1994) is a very efficient vector of CMV (Fereres, personal communication; Labonne et al., 1982). For WMV-2, the presence of fewer and less compact foci than with CMV, suggests that the main aphid vector(s) for this virus are not colonizing on melon (less foci), but accidentally enter the melon crops without colonising contiguous plants (foci less compact).

The results presented suggested that WMV-2 vectors overwinter in the area, on their hosts and enter melon crops later than CMV-vectors (Figure 1) probably only when their hosts are senescent or highly infested.

Figure 1. Disease progress curves in melon crops, in epidemics induced by *Cucumber mosaic virus* (—♦— CMV), *Watermelon mosaic virus*-2 (—■— WMV-2), *Papaya ringspot virus* watermelon strain (—▲— PRSV-W) and *Zucchini yellow mosaic virus* (—●— ZYMV) in Madrid (A–C), Barcelona (D–H) and Zaragoza (I–K) in 1994 (A, D and I), 1995 (B, E, F and J) and 1996 (C, G, H and K).

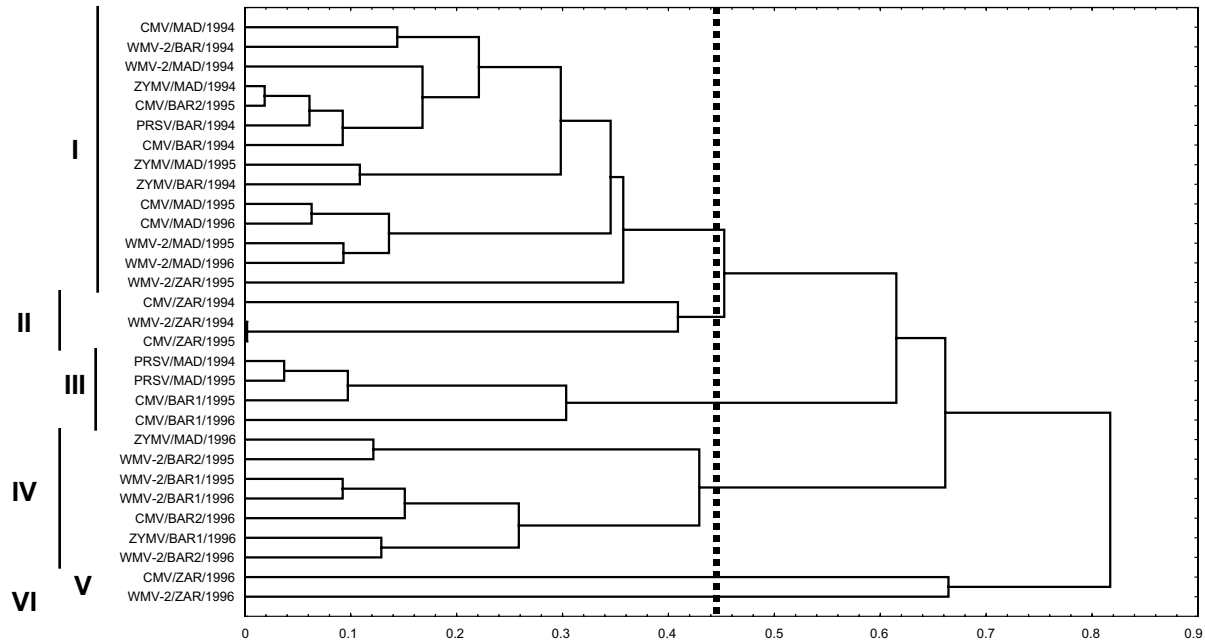


Figure 2. Dendrogram representing relative similarities among 30 epidemics caused by different viruses in three Spanish localities during three years. Epidemics were identified by virus/locality/year.

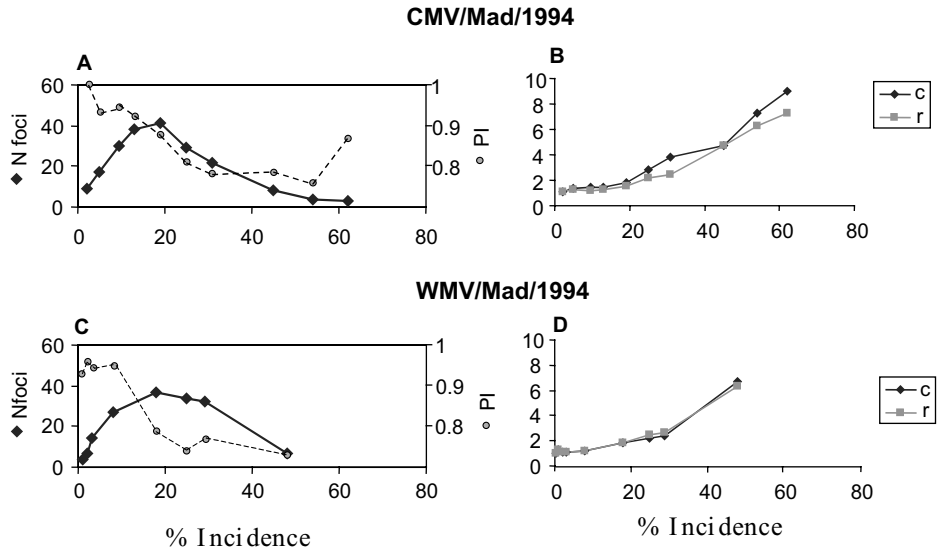


Figure 3. Development of number of foci ♦—♦ and proximity index ●—● (A and C), and column —♦— and row —■— distances of foci (B and D) in relation to disease incidence for CMV (A and B) and WMV-2 (C and D) in Madrid 1994.

CMV vectors arrive from long distance crops (probably melon sown on more warm areas), and they colonize melon crops. Proof for this hypothesis is pending the identification of aphid species vectoring the respective viruses in these crop cultivation areas.

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