Population Dynamics of *Cucumber mosaic virus* in Melon Crops and in Weeds in Central Spain

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ABSTRACT

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Understanding epidemiology and evolution of plant viruses requires knowledge of their ecology: obtaining information about the dynamics and genetic structure of viral populations in their various hosts is necessary to analyze host specialization and to identify reservoirs and inoculum sources. We present here a 3-year analysis of the population dynamics of *Cucumber mosaic virus* (CMV) in Central Spain in weed hosts and melon. CMV infection in weed hosts was detected throughout the year and showed a clear seasonality, with maximum incidence of 20 to 30% in summer and autumn. The dynamics of CMV incidence were different in

various weed habitats (i.e., fallow fields, edges, and wastelands), which differed in stability due to the degree of human intervention. CMV incidence in weed habitats was correlated with the amount of vegetation, estimated both as biomass and as soil cover by plants. CMV population dynamics in melon crops was unrelated to that in weed habitats. Genetic characterization by ribonuclease protection assays and biological characterization on differential hosts showed no significant differences in the frequency of genotypes or biotypes for CMV isolates from weeds or melon. Hence, different population dynamics in various hosts and habitats has not resulted in population differentiation, suggesting that migration between hosts and habitats prevents fragmentation of the population.

Additional keywords: virus ecology.

Control of plant viral diseases depends on the elimination of inoculum sources and on the limitation of inoculum multiplication and dispersal. Hence, knowledge of plant virus ecology, particularly inoculum sources and survival in the absence of the crop, is essential for implementation of efficient control strategies. For annual crops of temperate regions with no seed transmission, inoculum sources will be external to the crop. Inoculum survival between crops will occur in alternative hosts, either in their seed and other propagation organs or in actively growing plants. For nonpersistently transmitted viruses, inoculum reservoirs and sources would be in close proximity to the crop.

Wild plants growing within the same fields as crops or in nearby areas (i.e., weeds *sensu lato*) can be inoculum sources and reservoirs of viruses for crops (8), and the literature abounds with lists of wild hosts of plant viruses (5). However, the ecology of plant viruses in wild hosts has not been analyzed often, and data on virus incidence (6,25,33,35), and, even more so, on the dynamics and impact of virus infection in wild hosts (14,24,43) are scarce. Also, few studies have addressed host adaptation in determining genetic structure of plant virus populations. Multi-host parasites can adapt to different hosts, and adaptation to one host may involve trade-offs on other hosts, as shown in experiments involving host changes (9,10). Trade-offs associated with host adaptation will result in population differentiation according to host plant species, which should be immediately relevant to virus epidemiology in a crop. The few available data (e.g., on crops and weed isolates of *Beet western yellow virus* [8] and *Beet curly top virus* [7,42]) suggest that this may be the case for plant viruses with broad host ranges.

We have analyzed the dynamics and structure of *Cucumber mosaic virus* (CMV) populations infecting weeds and melon crops in

Central Spain. CMV, the type member of the genus *Cucumovirus*, is an isometric virus with a tripartite genome of messenger-sense, single-stranded RNA. CMV is an outstanding example of a multi-host parasite, capable of infecting over 1,200 species in more than 100 plant families. CMV is transmitted efficiently in a nonpersistent manner by more than 80 aphid species and, with different efficiencies, via seed of more than 20 host species (28,29). The role of weeds as sources of CMV inoculum for crops, and as survival hosts, has been addressed (4,12,31,34,40). However, the dynamics and structure of CMV populations in weeds, compared with crops, have not been investigated. CMV is endemic in Spain, causing significant losses in field-grown vegetable crops (15). Melon is the major field-grown vegetable in Central Spain, grown from May to September. CMV epidemics in melon differ in severity from year to year, but often reach high incidence (over 70%), reducing both yield and quality of melon (2,3).

Here we report on the dynamics and structure of CMV populations infecting melon and weeds growing in habitats with different degrees of human intervention to assess whether the virus population in a given area is fragmented due to host and habitat effects.

MATERIALS AND METHODS

Field surveys and sample collection. Crops and weeds were sampled in the Tagus river valley south of Madrid. Weeds were surveyed in three different habitats with different degrees of human intervention: (i) agricultural fields between crops (i.e., fallow fields, hereafter “fields”), (ii) edges between horticultural fields (hereafter “edges”), and (iii) abandoned fields that have not been tilled for two or more years (hereafter “wastelands”).

For 3 years (from October 1999 to September 2002), monthly surveys were conducted at four to five sites in a radius of ≈ 3 km: two edges, one wasteland, and one to two fields. The edges and wasteland were always the same, but the fields varied according to horticultural use. In the fields and wasteland, a 10-by-10-m

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plot was chosen randomly at each survey, and plants were collected every 3 m following fixed itineraries (≈ 25 samples per plot). In the edges, areas of 20 by 1 m were randomly chosen at each survey, and plants were collected every 1.5 m. Collected plants were taken to the laboratory for taxonomic determination and analyses of CMV infection (see below). At each survey and site, the plant cover of the soil was estimated visually as percent area covered by plants, and the average height of vegetation was estimated from measurements taken at 10 random points. Plant biomass was estimated for each survey and site as the product of average height, coverage, and average plant density. Average plant density was considered a constant, the result of dividing the fresh weight by the volume (measures of the canopy diameter and height) of 500 plants randomly sampled from those collected in every survey at each site. For calculations, the relative areas of fields, wasteland, and edges (1:1:0.2) were taken into account. Grasses were not considered in these analyses, because our previous unpublished results had shown that they were rarely infected by CMV.

During the same 3 years, two melon fields were surveyed monthly from transplant (mid-May) to harvest (early to mid-September). Fields were not the same from year to year, because melon is not planted in the same field consecutively due to soil-borne diseases. Melon plants (cv. Piel de sapo) were spaced 1.3 by 1.6 m apart and drip irrigated. At each field, 200 plants regularly spaced along fixed pathways were inspected for CMV symptoms, and young leaves were collected from symptomatic plants for analyses of CMV infection. During the central period of crop growth (July to August), weeds growing within the melon fields also were sampled randomly. Meteorological data (data not shown) were obtained from a station 2 km from the analyzed sites.

Analysis of CMV infection and multiplication of CMV isolates. CMV infection in plant samples was detected by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) using commercial antisera (Bio-Rad, Marnes-La-Coquette, France) according to the manufacturer's instructions. Initially, other detection methods also were employed, including transplantation of whole plants and delaying ELISA analyses until after 2 weeks of plant growth in a greenhouse, or inoculation to other hosts (*Chenopodium quinoa* Willd. and *Nicotiana tabacum* L. Xanthi-nc). These assays did not improve detection over DAS-ELISA on field samples, and were discontinued after the first 6 months.

CMV isolates from field samples were multiplied in Xanthi-nc tobacco after inoculation of leaf extracts ground in 0.01 M phosphate buffer, pH 7.0, 2% diethyldithiocarbamate. Plants were kept in a greenhouse (20 to 30°C, 16 h of light) and, 2 to 3 weeks after inoculation, virions were purified as in Lot et al. (23) and virion RNA extracted as described (16).

Characterization of CMV isolates. Some CMV isolates, randomly sampled from those infecting melon and weeds over the period of this work, were characterized. Genetic characterization of CMV isolates was by ribonuclease protection assay (RPA) of four RNA probes complementary to nucleotides 851 to 1,694 in RNA1, 1,210 to 2,139 in RNA2, 250 to 706 in RNA3, and 1,389 to 1,840 in RNA3 of Fny-CMV, as in Fraile et al. (13). RPA yielded patterns that enabled differentiation of isolates belonging to subgroups IA, IB, and II of CMV (36).

For biological characterization, CMV isolates were inoculated onto four plants each of six differential hosts (*N. tabacum* Xanthi-nc [tobacco], *Vigna unguiculata* (L.) Walp. cv. Black Eye [cowpea], *C. quinoa*, *Ocimum basilicum* L. [basil], *Diplotaxis erucoides* (L.) DC., and *Cucumis melo* L. cv. Piel de Sapo [melon]). Infection was confirmed by ELISA and symptoms recorded over a 2-week period.

Statistical analyses. Data on frequency distribution were compared by contingency tables, from which the χ^2 statistic was

calculated. The probability of rejecting the null hypothesis of equal distribution was derived from the simulation of 1,000 tables in which marginal totals were fixed according to the data (model III). The same method was used for the significance of the differences between percentages. Differences in proportions were analyzed by the test of homogeneity of odds ratios (Mantel-Hanszel procedure). Means were compared by nonparametric test: Wilcoxon for one-way analysis of variance (ANOVA) and Friedman for two-way ANOVA. Correlation between data series was analyzed by the nonparametric Spearman rank correlation test and, when significant, the proportion of explained variation was estimated by the square of the product-moment correlation coefficient (r). All these methods are described in Sokal and Rohlf (38). The seasonality of temporal series data was analyzed with the Friedman test, as in Kendall and Ord (20).

RESULTS

Temporal variation of weed vegetation. The total variation of soil cover by weeds is shown in Figure 1, either for the three habitats as a whole (Fig. 1A) or for each of them separately (Fig. 1B to D). Similarly, Figure 1 presents the variation of biomass for weed vegetation. The average density of weeds was estimated as 37.6 ± 7.9 kg (fresh weight)/m³ from the analysis of 500 plants collected at all three habitats over the whole 3 years. Data for the autumn are the mean of the data of October, November, and December, data for the winter are the mean of January, February, and March, data for the spring are the mean of April, May, and June, and data for the summer are the mean of July, August, and September (Fig. 1).

Cover and biomass varied over the analyzed period. Mean cover did not differ for the three studied agricultural years (i.e., October to September), being 30% for 1999–2000, 21% for 2000–01, and 38% for 2001–02 ($P \leq 0.76$, Wilcoxon nonparametric test), but variation between seasons was not the same ($P = 0.53$, Friedman test of seasonality): in 1999–2000 and 2001–02, cover was maximal in winter and minimal in summer, and in 2000–01 cover was minimal in winter and maximal in spring and summer. The average biomass in the horticultural year 1999–2000 (2.43 kg/m³) was higher than for years 2000–01 and 2001–02 (1.18 and 2.07 kg/m³, respectively, $P \leq 0.05$, Wilcoxon test). The seasonal variation of biomass was not the same for the 3 years ($P = 0.21$, Friedman), with minima in the summer for 1999–2000, and in the fall and winter for 2000–01 and 2001–02. Hence, the seasonal variation in biomass and cover followed similar patterns in 1999–2000 and 2000–01 but not in 2001–02. Note that an increase of biomass when the cover diminishes is due to an increase in plant height with a decrease in plant number, whereas an increase in cover with a decrease of biomass is due to the appearance of (small) young plants and the death of older (and larger) plants.

Variation in cover and biomass was significantly different for the three habitats ($P \geq 0.11$, Friedman test in a two-way ANOVA). In the edges (Fig. 1C), cover was constant, and biomass had two maxima (winter to spring 2000, spring to summer 2001). In fields (Fig. 1B) and in the wasteland (Fig. 1D), the pattern of seasonal variation of cover and biomass was the same (except for fields in the winter of 2002). Changes of cover and biomass were not explained by temperature or rainfall over the seasons and years (data not shown). For example, the summer of 2000 and the winter of 2001 were unusually wet, but cover and biomass were minimal. Most possibly, human intervention is a major factor in the variation of the weed vegetation in the area.

Incidence of CMV infection in weeds. A total of 2,286 weed samples were analyzed for CMV infection over 3 years. CMV infection was detected in 40 species belonging to 16 families of dicotyledons and 1 family of monocotyledons (data not shown). The mean incidence of CMV infection for 3 years in the more

frequent weed species, arbitrarily defined as those that had at least 1% of mean cover, is shown in Table 1. CMV infection was detected in all the more frequent weeds, with the exception of *Anacyclus clavatus* (Desf.) Pers., *Anthemis arvensis* L., *Capsella bursa-pastoris* (L.) Medicus, *Papaver rhoeas* L., and *Xanthium strumarium* Moretti. CMV incidence did not correlate with weed frequency ($P = 0.12$, χ^2), and was highest in *Portulaca oleracea* L., *Cirsium arvense* (L.) Scop., and *Taraxacum* spp. (Table 1). The relatively high incidence of CMV in *Diplotaxis erucoides* (27%) (Table 1) is to be noted, because this species was by far the most frequent in the three habitats.

Variation of CMV incidence in weeds is shown in Figure 1, compared with cover and biomass of the weed vegetation. CMV incidence significantly varied between years and between seasons ($P = 0.008$ and 0.01 , respectively, in a two-factor ANOVA), being maximal in 2001–02. Variation of CMV incidence for each year showed a marked seasonality ($P = 0.04$, Friedman), with maxima in summer and minima in winter. Figure 1A suggests that there is a correlation between CMV incidence and cover or biomass, in the same season or with a time lag. However, no significant correlation was found between biomass and CMV incidence in either the same season or with any introduced lag ($P \geq 0.18$). Also, no significant correlation was found between cover and CMV incidence in the same or next season ($P \geq 0.26$), but there was a sig-

nificant correlation between cover in one season and CMV incidence two seasons later ($P = 0.06$, $r_s = 0.54$, Spearman rank correlation) and between cover in one season and average CMV incidence in that season and the next two ($P = 0.06$, $r_s = 0.53$) that explained 38% of incidence variation ($r = 0.62$). Hence, the amount of weeds had an effect on CMV incidence over two seasons.

CMV incidence was significantly higher ($P \leq 0.001$, χ^2) in fields (21% average for the 3-year period) than in edges or wasteland (12% in both). Incidence variation for each habitat is presented in panels B, C, and D of Figure 1, compared with biomass and cover. CMV incidence followed different dynamics in the three habitats: variation in incidence in the wasteland was weakly correlated with that in edges and fields ($r_s = 0.3$ to 0.52 , $P \leq 0.05$, Spearman rank correlation), but variation of incidence in edges and fields was not correlated ($P = 0.42$). Incidence variation in edges was not correlated with plant cover or biomass (Fig. 1C) ($P \geq 0.14$). In the wasteland, both cover and biomass were correlated with CMV incidence two seasons later (Fig. 1D). Cover variation explained 53% of the variation in CMV incidence ($r_s = 0.78$, $P = 0.005$, $r = 0.73$) in the wasteland. However, correlation with biomass was lower ($r_s = 0.5$, $P = 0.07$). Similarly, in fields, variation in cover explained 69% of variation in CMV incidence in that season ($r_s = 0.78$, $P \leq 0.002$, $r = 0.83$), and 86% of the variation in CMV incidence in that season and the next ($r_s = 0.93$, $P \leq 0.001$, $r = 0.93$). Correlation between field biomass and CMV incidence was lower ($r_s = 0.63$, $P = 0.02$) (Fig. 1B).

Incidence of CMV infection in melon fields. CMV incidence in the melon crop is shown in Figure 2, compared with incidence in weeds in the three habitats. Variation of CMV incidence differed for the 3 years in melon. In 2000 and 2002, epidemics started later than in 2001, with no CMV infection detected 40 days after transplantation. In 2001, CMV infection was detected 30 days after transplantation (data not shown). Also, final incidence did not differ in 2000 and 2002 (11 and 9%, respectively, $P = 0.7$, χ^2), and was lower than in 2001 (70%, $P \leq 0.001$). Final CMV incidence in melon was not related to incidence in weeds at the same period for each of the three habitats, nor with the incidence in the months previous to the crop (October to May) according to analyses following the procedure of Mantel-Hanszel (data not shown).

CMV incidence also was analyzed in weeds growing within melon fields (Table 1). CMV summer incidence was not significantly different for any year for weeds growing within the melon fields compared with those growing in edges, fields, or wasteland ($P \geq 0.10$, χ^2). CMV incidence in weeds growing in melon fields did not differ from CMV incidence in the melon crop for 2000 and 2001 (9 and 25%, respectively), but was significantly higher for 2002 (22%, $P \leq 0.001$, χ^2).

Genetic structure of CMV population in melon and in weeds. A random sample of CMV isolates was characterized genetically and biologically. RPA analyses with RNA probes complementary to regions in RNA1 and RNA2 yielded two different patterns, corresponding to those named A and II (13). RPA analyses with the two probes complementary to regions in the two open reading frames (ORFs) of RNA3 (for the 3a protein and the coat protein [CP], respectively) yielded two different patterns, named A and B (13). For each probe, patterns A and B characterize sequences typical of isolates in subgroups IA and IB, respectively, and pattern II sequences of isolates in subgroup II (13; unpublished data). The pattern obtained with all four probes defined a genetic type (haplotype).

In all, 60 isolates were analyzed by RPA: 33 from melon and 27 from weeds. Three genetic types were identified. Type 1 had a II pattern for RNAs 1 and 2, and an A pattern for both ORFs in RNA3 (haplotype II, II, A-A). Type 2 had an A pattern for all four probes (haplotype A, A, A-A), and type 3 had an A pattern for RNAs 1 and 2 and for the CP ORF and a B pattern for the

Fig. 1. Variation of cover (■), biomass (Δ), and *Cucurbit mosaic virus* (CMV) incidence (◆) in weed vegetation during the autumn (Au), winter (W), spring (Sp), and summer (Su) from 1999 to 2002. Data are presented for A, all three habitats or for B, fallow fields, C, edges, and D, wastelands separately.

3a ORF (haplotype A, A, B-A). The frequency of each type according to the original host of the isolate is shown in Table 2. The frequency of genetic types did not differ significantly according to host origin (melon or weeds) of the isolates ($P = 0.43$, χ^2).

Eighteen isolates randomly sampled from those 60 were characterized by the symptoms induced in six indicator species. All isolates infected melon, causing a systemic mosaic. For the other five hosts, different reactions were observed: in tobacco, systemic vein clearing and mosaic, or etch in the inoculated leaves with systemic mosaic; in cowpea, small or large necrotic local lesions, or no symptoms, in the inoculated leaves, infection becoming systemic or not; in *C. quinoa*, necrotic local lesions with or without systemic mosaic; in *D. erucoides*, no infection or asymptomatic systemic infection; in basil, no infection or systemic mosaic. The combination of the reactions in the five differential hosts defined 10 different biotypes, one of which (biotype I), was found in eight isolates (44% of the isolates); another biotype (III) was found in two isolates. The remaining biotypes each occurred only once (Table 3). Isolates in biotype I had haplotypes of types 2 and 3 regardless of the host of origin (data not shown). The distribution of these biotypes according to the isolate host of origin is shown in Table 3. Biological type of the isolates was not dependent on host of origin ($P = 1$, χ^2) or on RPA haplotype ($P = 0.55$, χ^2).

DISCUSSION

The ecology of plant viruses in alternative hosts other than crops has been recognized as relevant to understanding and predicting virus epidemics in crops (8). More recently, the biology of plant viruses in wild hosts is the object of renewed interest as related to risk assessment of virus resistant transgenic crops: escape of transgenes to wild relatives of the crop could confer resistance to a virus and increase the competitive ability of that genotype (39). However, analyses of the plant-virus population dynamics in weed reservoirs are scarce and, with few exceptions (6,33), mostly refer to weeds growing within the crop or in immediate association with it (18,22,34).

Fig. 2. Monthly variation of *Cucurbit mosaic virus* (CMV) incidence in melon crops (■) and in weed habitats (◆) A, from October 1999 to September 2000, B, from October 2000 to September 2001, and C, from October 2001 to September 2002. Data for weeds correspond to the three habitats (fallow fields, edges, and wasteland).

TABLE 1. *Cucurbit mosaic virus* (CMV) infection in the more frequent weed species^a

Species	Life cycle ^b	Growth period ^c	Habitat ^d	Mean cover (%)	Mean CMV incidence (%) in	
					Habitats F, E, and W	Melon fields ^e
<i>Amaranthus</i> spp.	A	Sp-Au	F	5	36	16
<i>Anacyclus clavatus</i> (Desf.) Pers. or <i>Anthemis arvensis</i> L.	A	Sp	E	1	0	...
<i>Capsella bursa-pastoris</i> (L.) Medicus	A	W-Sp	H	1	0	...
<i>Chenopodium album</i> L.	A	Sp-Au	F	3	5	29
<i>Cirsium arvense</i> (L.) Scop.	P	Sp-Su	F	3	69	...
<i>Convolvulus arvensis</i> L.	P	Sp-Su	F	5	8	...
<i>Conyza</i> spp.	A	Y	W	3	7	...
<i>Datura stramonium</i> L.	A	Su-Au	F	2	7	5
<i>Diploaxis erucoides</i> (L.) DC.	A	Y	H	32	27	100
<i>Lactuca serriola</i> L.	A,B	W-Su	H	4	20	...
<i>Lamium amplexicaule</i> L.	A	W-Sp	F	2	14	0
<i>Malva sylvestris</i> L.	B,P	Y	E	1	13	...
<i>Medicago sativa</i> L.	P	Y	E	2	11	...
<i>Papaver rhoeas</i> L.	A	Y	H	4	0	...
<i>Plantago</i> spp.	A,P	Y	W	6	12	...
<i>Portulaca oleracea</i> L.	A	Su-Au	F	1	71	0
<i>Silybum marianum</i> (L.) Gaertner	B,P	Au-Sp	W	2	6	...
<i>Solanum nigrum</i> L.	A	Su-Au	F	1	17	0
<i>Sonchus oleraceus</i> L.	A	W-V	H	6	8	...
<i>Taraxacum</i> spp.	B,P	Au, Sp	H	2	43	...
<i>Trifolium pratense</i> L.	P	Y	E	1	3	...
<i>Xanthium strumarium</i> Moretti.	A	Sp-Su	F	1	0	...

^a Arbitrarily defined as those having $\geq 1\%$ of mean cover.

^b A = annual, B = biennial, P = perennial.

^c Au = autumn, W = winter, Sp = spring, Su = summer, Y = all year.

^d F = fields, E = edges, W = wasteland, H = all three habitats.

^e ... Indicates species not present in the random samples.

Here we have analyzed the dynamics of CMV infection in weeds growing in three different habitats in Central Spain. Weeds are plants in some way associated with human activity (27), and the three analyzed habitats (fallow fields, edges between fields, and pastured wastelands) represent decreasing degrees of human intervention. Fields are irrigated and fertilized during the crop, and tilled between the crops; hence, weed populations cannot establish permanently in this habitat but are colonizers that benefit from nutrients, water, and soil preparation from horticulture. Edges are not tilled, but weeds in this habitat suffer herbicide and fire treatments; however, perennials persist for years and benefit from irrigation and fertilization of the nearby crop. Wastelands are not tilled, fertilized, or irrigated, and no form of weed control is exerted. The only human activity in this habitat is occasional pasturing by sheep.

Our results show that CMV incidence in weeds in all three habitats is common, because infection was detected at every survey except for the winter of 2000. CMV incidence reached 30%, with much higher incidences in certain species. Similar incidences have been reported for other viruses in wild hosts (32, 33,35,43) and for CMV in horticultural areas of southern France and the northeastern United States (31,34). CMV incidence in weeds varied and exhibited clear seasonality, with maxima in summer and fall. In spite of the limitations of a 3-year analysis, our data of variation in weed vegetation and of CMV incidence uncovered some important trends: (i) CMV incidence follows different dynamics in the three weed habitats and (ii) CMV incidence is positively correlated with the amount of weed vegetation as a whole, although there is no correlation between incidence and frequency for each particular weed species.

The varied population dynamics of CMV in the three habitats could be related to stability of weed populations. In the edges and wasteland, weeds (including biennials and perennials) grow during the whole year, maintaining populations of CMV and, possibly, of its aphid vectors, during the whole year (Table 1). Still,

weed populations are more stable in the edges than in the wasteland, which receive water only from rain (Fig. 1B and C). Winter hosts for CMV were found in these two habitats (*Conyza* spp., *D. erucoides*, *Lactuca serriola*, *Malva sylvestris*, *Medicago sativa*, *Plantago* spp., *Silybum marianum*, *Sonchus oleraceus*, and *Trifolium pratense*) (Table 1). Hence, edges and wastelands could have an important role for CMV survival and inoculum sources for the third habitat (fields) and for crops. Weeds in fallow fields grow only for certain periods (between tillage operations) and often do not complete their biological cycle (*unpublished data*), such that CMV and its aphid vectors will be mostly migrants from the other habitats. Accordingly, seasonal variation of CMV incidence was less in edges and wasteland than in fields (Fig. 1). CMV infection could occur during the whole year, as shown by CMV incidence in the fields in January 2002. Winter infection can be due to both seed transmission, described in several weed species (11), and aphid activity. The only study on aphid activity in horticultural crops in this area did not include the winter (30), but the presence of active alatae morphs of aphids during the winter was documented in the higher and colder plateau surrounding the Tagus valley (26).

The second important aspect of CMV ecology shown by our results is the relationship between CMV incidence and the amount of weed vegetation in previous seasons. This relationship is stronger with plant cover than with biomass (i.e., with the number of plants rather than with the size of the plants). The relationship between cover and CMV incidence could be due to two nonexcluding causes. First, a large plant cover could be associated with high aphid populations because conditions favoring high cover also favor aphid population growth or because high cover directly contributes to maintenance of high aphid populations (8). Second, an increase in cover could be due to an increase in the number of plants and, hence, of those infected via seed, which would be inoculum sources. Other studies have reported a negative correlation between plant cover and CMV incidence (1,31,41), which was attributed to migratory aphids landing preferentially on isolated plants (17). This is not in contradiction with our results, because even if high cover would not favor landing of migrant aphids, larger aphid populations may be sustained. CMV incidence for each weed species was not related to species frequency in the weed population. Whether this is due to some species being better hosts for CMV or being more often visited by aphid vectors remains to be established.

The severity of CMV epidemics in melon crops varied largely for the 3 years analyzed. The comparative analyses of 11 CMV epidemics in melon indicated that CMV incidence at harvest was positively correlated with mean temperatures and negatively correlated with rainfall in spring (3). In agreement with this prediction, CMV incidence was highest for 2001, when spring temperature was higher and rainfall less (data not shown), than in 2000 and 2002. That relationship between environmental conditions and CMV incidence also was observed for weeds in fields between crops. In these fields, as in the melon crop, primary inocu-

TABLE 2. Distribution of *Cucumber mosaic virus* haplotypes according to host plant species^a

Host plant species	N ^b	Haplotypes ^c		
		1	2	3
Melon	33	0	19	14
<i>Datura stramonium</i>	5	0	2	3
<i>Solanum nigrum</i>	3	0	3	0
<i>Amaranthus</i> spp.	5	0	1	4
<i>Diploaxis erucoides</i>	13	0	7	6
<i>Medicago sativa</i>	1	1	0	0
Total weeds	27	1	13	13
Total	60	1	32	27

^a Data are number of isolates.

^b N = number of characterized isolates.

^c Haplotypes 1 (ribonuclease protection assay [RPA] pattern II, II, A-A), 2 (RPA pattern A, A, A-A), and 3 (RPA pattern (A, A, B-A) are as defined in text.

TABLE 3. Distribution of *Cucumber mosaic virus* biotypes according to host plant species^a

Host plant species	N ^b	Biotype ^c									
		I	II	III	IV	V	VI	VII	VIII	IX	X
Melon	7	4	1	1	...	...	...	...	1	...	...
<i>Datura stramonium</i>	1	...	...	...	...	1	...	...	...	...	...
<i>Amaranthus</i> spp.	3	1	...	...	1	...	1	...	...	...	...
<i>Diploaxis erucoides</i>	6	3	...	1	...	...	...	1	...	1	...
<i>Medicago sativa</i>	1	...	...	...	...	...	...	...	...	...	1
Total	18	8	1	2	1	1	1	1	1	1	1

^a Data are number of isolates.

^b N = number of characterized isolates.

^c The most frequent biotype, I, caused vein clearing and mosaic in tobacco, small necrotic local lesions (nll) without systemic infection in cowpea, nll without systemic infection in *Chenopodium quinoa*, asymptomatic infection in *D. erucoides*, and did not infect basil.

lum comes mostly from outside sources, and spring temperature and rain could affect both population size and migratory activity of aphid vectors (19,21,37). No relationship between spring temperature or rainfall on CMV incidence was observed for the other two habitats, where the population dynamics of aphids need not depend on migrations. Indeed, the dynamics of CMV incidence in weed habitats and melon crops was otherwise unrelated. Notably, severity of the epidemics on melon was unrelated to incidence in the weed habitats (i.e., to the availability of inoculum). Rist and Lorbeer (34) found a correlation between CMV incidence in lettuce and in weeds 10 m but not 20 to 40 m distant from the lettuce fields. These distances are much smaller than those between the three weed habitats and melon crops (≈ 1 km). Hence, our results do not mean that weed habitats are not inoculum sources for epidemics on crops, but suggest that actions directed to diminish inoculum reservoirs in weeds might not be effective for the control of CMV in crops.

In spite of the different ecology of the four analyzed habitats (melon fields, fallow fields, edges, and wastelands) and the different population dynamics of CMV in them, analyses of population structure show a single CMV population, not differentiated according to host plant species. This is so even when CMV isolates are classified according to pathogenicity in different hosts, a method that reveals a higher degree of genetic variation than RPA (Table 3). This indicates that, in spite of different population dynamics in the different weed and crop habitats, migration between hosts and between habitats apparently is sufficient to prevent population differentiation.

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