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Genetics of Melon yellows virus resistance derived from *Cucumis melo* ssp. *agrestis*

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Abstract

The Melon yellows virus (MYV), a whitefly-transmitted closterovirus, is one of the major pathogens causing crop losses in protected melons in southeastern Spain. An accession of the wild Asiatic *Cucumis melo* ssp. *agrestis* (*Cma*) shows resistance to MYV infection. Results indicate the participation of two resistance mechanisms in this source: firstly, an antixenotic reaction against *Trialeurodes vaporariorum*, the disease vector, and secondly, resistance to the virus. The combined effect of these two mechanisms confers *Cma* a higher level of resistance, expressed as a delayed and milder infection.

The genetics of resistance to the Melon yellows closterovirus have been studied in two families derived from *Cma*. As under natural infection conditions, the effect of antixenosis and virus resistance cannot be distinguished, a biometrical model that permits separation of the two resistance mechanisms operating in the same resistant source, has been proposed to determine genetic control of MYV resistance.

The genetic analysis has been conducted by fitting the disease progress curves of each generation to the biometrical model instead of fitting the final disease ratios. The scoring of disease incidence over time allows for the comparison of data from assays conducted in different conditions (2 years/4 transplanting dates), thus reinforcing the analysis.

The results agree with a simple control of the resistance to MYV derived from *Cma*, with incomplete penetrance of the gene and partial dominance of resistance. The effect of antixenosis on the spread of this plant virus is highly significant in *Cma*, but not in segregant generations.

Since there do not exist crossability barriers between this accession and the cultivated melon, *Cma* could be readily used in breeding programmes to obtain melon varieties resistant to MYV.

Introduction

Yellowing diseases caused by whitefly-transmitted closteroviruses (WTCs) are economically important in many areas of the World (Wisler et al., 1998). Most WTCs belong to the newly proposed genus *Crinivirus* of the Closteroviridae family. This rapidly growing group of viruses includes members transmitted either by the tobacco whitefly *Bemisia tabaci* (Gennadius) (Celix et al., 1996), or by the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood). In a variety of countries, many cucumber and melon disorders have reportedly been caused by *T. vaporariorum*-transmitted clostero-like viruses such as Cucumber yellows virus (Yamashita et al., 1979), Cucumber infectious chlorosis virus (Hristrova and Natskova, 1986), Cucumber chlorotic spot virus (Woudt et al., 1993), or Muskmelon yellows virus (Lot et al., 1982; Jorda et al., 1993).

The Muskmelon yellows virus (MYV) has been causing yellowing disorders in protected and outdoor melon in Southeastern Spain since the early 1980s. This melon yellows disease was found to be associated with the presence of long flexuous (900–950 nm) viral particles, typical of the closterovirus group. Some authors

consider MYV to be very similar or identical to Beet pseudo-yellows virus (BPYV) (Wisler et al., 1998), the first WTC reported (Duffus, 1965), based on virus–vector relationships, host reaction, serological properties (Jorda et al., 1993) and, more recently, in RT-PCR sequence homology studies. However, as both viruses differ in particle length, there is no definitive evidence of MYV to be BPYV.

The symptoms caused by this melon yellows disease appear either as interveinal chlorotic spotting or as a golden-yellow basal stain at the union between the leaf surface and the petiole. As infection progresses, the entire leaf, with the exception of the veins, shows a bright-golden yellowing. The earlier the infection, the more fruit set and development are disturbed, resulting in the inevitable large crop losses.

Unfortunately, cultural practices and vector control by chemical, physical or biological methods are often ineffective in reducing virus incidence, especially when severe attacks occur. Hence, the development of host plant resistance is considered as one of the most effective and sustainable strategies for the management of MYV epidemics. Many efforts have been made to find resistance to this melon yellowing disease. Screening of melons and wild *Cucumis* relatives has been conducted under controlled or natural infection conditions (Esteva, 1989; Soria et al., 1989; Nuez et al., 1991). But, although there is proven resistance to MYV in several wild and exotic, occasionally cultivated, *Cucumis* spp.: *C. africanus* L., *C. anguria* L. var. *anguria*, *C. anguria* L. var. *longipes*, *C. dipsaceus* Ehrenberg ex Spach, *C. figarei* Delile ex Naud., *C. meeusei* C. Jeffrey, *C. metuliferus* E. Meyer ex Naud., *C. myriocarpus* Naud., the strong crossability barriers that exist between these species and the cultivated melon prevent their use in breeding programs (Esquinas-Alcazar and Gulick, 1983).

Tolerance and resistance to MYV have also been reported within *C. melo*, in some wild accessions of Asiatic origin: Nagata Kin Makuwa (NKM), PI 161375, and one accession of *C. melo* L. spp. *agrestis* (*Cma*) Naud. Esteva and Nuez (1992) determined that tolerance to MYV found in NKM and PI 161375 was controlled by single genes, partially dominant in NKM and partially recessive in PI 161375. These tolerance genes show an incomplete penetrance and variable expressivity, highly influenced by environmental factors. Consequently, combined with preventive measures, cultivation of tolerant melon varieties derived from these two sources could lead to a minimization of the present levels of crop losses caused by MYV.

The exploitation of the other resistance source, *Cma*, by breeders requires characterizing as widely as possible the mode of inheritance of the trait, and the level of protection that it provides. In order to achieve reproducible disease pressures, inheritance studies on plant disease resistance are usually conducted using artificial inoculation procedures. However, under natural conditions of infection, inheritance studies become more complex as there are many non-controllable factors that can influence disease incidence and evolution. The existence of a mechanism of antixenosis against *T.vaporariorum* in *Cma* has recently been described (Soria et al., 1996). Thereby, breeding programs aimed at exploiting this MYV resistance source should also consider how the virus spread pattern could be influenced by whitefly-resistance traits, that alter vector population size and feeding behavior (Painter, 1951; De Ponti et al., 1990).

The genetic control of plant disease resistance is often simply determined by examining the final mendelian ratios of resistant/susceptible plants in parents and subsequent progenies F1, F2, and backcrosses regardless of whether infection has been conducted under natural or artificial conditions. However, since plant disease is a sequence of processes in time, only recording disease incidence at a particular moment after inoculation could be inadequate, particularly when partial resistance or resistance depending on inoculum pressure exist. In these cases, an additional variable affecting results could be the time at which disease incidence is scored. Consequently, genetic studies should be conducted considering disease progress over time, rather than simply the final disease incidence.

The present work aims to determine the genetic control of MYV resistance found in *Cma*. The appropriate strategy employed for this purpose is the analysis of the mendelian ratios using disease curves by fitting a biometrical model that considers both the effect of antixenosis and virus resistance in the same source.

Materials and methods

Plant material

Two commercial melon varieties of the most popular Spanish melon types, VC-120 (Amarillo type) and VC-21 (Piel de Sapo type), were employed as susceptible parents (P1). An accession of the wild *Cma*,

collected in Northern Korea and maintained in the melon collection at the Genebank of the Polytechnic University of Valencia (UPV), was employed as resistant parent (P2) (Esteva, 1989). For genetic analysis, *Cma* was crossed as male genitor with the two highly susceptible melon varieties (P1) to obtain the two F1 progenies. Each F1 was selfed and backcrossed to the respective parents to produce the F2 (F1 × F1), the BC1 (P1 × F1), and the BC2 (P2 × F1) generations. Two additional melon commercial varieties, one Amarillo type (var Bola de Oro) and the other Piel de Sapo type, were used as susceptible controls.

In all cases, seeds were pregerminated in Petri dishes, then sown in individual cells of 96-cell nursery trays and transplanted to pots at the 2-leaf growth stage. Substrate consisted of a mixture of peat and pearl (50 : 50) enriched with nutrients.

Vector and virus culture, and inoculation procedure

A healthy *T. vaporariorum* population was reared on tomato plants. These plants were grown in mesh-protected cages held in a greenhouse under controlled temperature and relative humidity conditions. Initial MYV isolate was collected from naturally infected plants in Murcia (Southeastern Spain). MYV was detected in these plants by serological DAS-ELISA test essentially as described by Clark and Adams (1977). Specific MYV polyclonal antiserum were obtained, using purified virus extracts of *Nicotiana clevellandii*, by the Department of Plant Pathology of the UPV (Juarez, 1997). MYV culture was maintained on susceptible melon plants by *T. vaporariorum* transmission, renewing stock cultures every 3–4 weeks.

Seedlings at the 2-leaf growth stage were mass-inoculated in large cages (3 × 1 × 1 m) covered with meshes to limit vector dispersal. Ten-leaf susceptible melon plants, previously infected under controlled conditions with the described MYV isolate, served as primary inoculum source. These plants, which held a high *T. vaporariorum* population, were introduced into the cages. Seedlings were then exposed to this high population of viruliferous whiteflies for a period of 10–20 days. After inoculation, seedlings were transplanted to the greenhouse for disease monitoring.

Experimental design and evaluation criteria

Genetic control of MYV resistance was studied in the two families (Family 1: VC-120 × *Cma*; Family 2: VC-21 × *Cma*) for two consecutive years in four assays: Assay 1: family 1/first year; Assay 2: family 2/first year; Assay 3: family 1/second year; Assay 4: family 2/second year. A similar number of plants were employed for each generation in each assay, approximately 25 for parents (P1 and P2), F1, and susceptible controls, 100 for each backcross (BC1 and BC2), and 200 for F2. Plants were grouped in four sets of approximately 100 plants. Each set included parents, susceptible controls, F1 and segregant progenies plants. The plant number of each generation per set was proportional to its sample size in the assay. Each set of plants was cage-inoculated as previously described, and then transplanted to the greenhouse. All 4 assays were conducted during the spring–summer growing season in UPV greenhouses. Planting dates for each assay are indicated in Table 1.

Disease incidence was periodically monitored after first symptoms appeared in susceptible parents and controls. The number of plants showing characteristic MYV symptoms in each generation was periodically recorded. MYV infection was confirmed by serological DAS-ELISA as previously described (Juarez, 1997) considering positive samples with A 405 nm > twice that of the healthy controls. In the case of misleading results, reciprocal inoculation on indicator plants was carried out. Healthy whiteflies were allowed to feed for 48 h in plants to be tested, and then tested in controlled conditions on susceptible *C. melo* and *Nicotiana clevellandii* that developed clear yellowing symptoms after two or three weeks. Observed disease progress curves (ODPC) were constructed for each generation by plotting the percentage of asymptomatic plants against time.

Data analysis

The percentage of asymptomatic plants was fitted to a biometrical model by using the least square regression method. Parameters of the model were estimated for each date using data from the parents (P1 and P2), F1, and segregant generations (F2, BC1, and BC2). The expected percentage of asymptomatic plants was then calculated for each generation using the model fitted for each date. By plotting expected data against time, predicted disease progress curves (PDPC) were then constructed for each generation.

The joint fit of data to the proposed model was evaluated in all 4 assays, analyzing the correlation between the observed disease progress curves and the predicted

Table 1. Proportion of asymptomatic plants recorded in parents and segregant generations derived from the cross of a resistant accession of *Cma* with two melon varieties highly susceptible to MYV (Data from 4 assays conducted during two consecutive years)

First year

Assay 1 (VC-120 × *Cma*)
Planting date: 21 May

DPI	33	37	42	47	54	58	63	68	89	108
P1 [1](VC-120)	42	33	24	9	0	0	0	0	0	0
P2 (*Cma*)	100	100	100	100	100	83	83	83	44	0
F1 (P1 × P2)	62	50	29	21	6	6	6	0	0	0
F2 (F1 × F1)	68	63	54	49	43	31	25	12	9	0
BC1 (P1 × F1)	49	45	32	28	17	11	9	2	2	0
BC2 (P2 × F1)	73	70	56	49	41	24	17	14	5	0

Assay 2 (VC-21 × *Cma*)
Planting date: 12 June

DPI	28	33	38	45	59	67
P1 (VC-21)	0	0	0	0	0	0
P2 (*Cma*)	100	80	40	40	27	15
F1 (P1 × P2)	100	47	18	12	0	0
F2 (F1 × F1)	68	35	17	10	7	4
BC1 (P1 × F1)	51	26	9	6	3	0
BC2 (P2 × F1)	92	62	27	12	6	1

Second year

Assay 3 (VC-120 × *Cma*)
Planting date: 8 June

DPI	21	24	26	29	31	33	38	42	49	53	57	80	84
P1[2] (VC-120)	71	36	14	0	0	0	0	0	0	0	0	0	0
P2 (*Cma*)	100	100	89	80	75	70	48	43	35	27	17	0	0
F1 (P1 × P2)	100	72	61	18	16	11	0	0	0	0	0	0	0
F2 (F1 × F1)	63	53	49	17	13	11	7	6	4	3	2	0	0
BC1 (P1 × F1)	53	48	44	16	9	5	1	1	1	0	0	0	0
BC2 (P2 × F1)	82	74	65	20	16	14	7	6	6	2	2	1	1

Assay 4 (VC-21 × *Cma*)
Planting date: 17 July

DPI	14	17	24	26	28	33	42	52	55	65
P1[2](VC-21)	79	29	14	0	0	0	0	0	0	0
P2 (*Cma*)	100	100	100	85	82	70	43	30	2	0
F1 (P1 × P2)	100	88	56	38	34	26	9	0	0	0
F2 (F1 × F1)	92	73	58	41	28	25	10	9	6	0
BC1 (P1 × F1)	91	73	45	28	24	21	7	5	3	0
BC2 (P2 × F1)	100	90	69	51	46	37	9	6	5	0

[1]MYV presence in symptomatic plants was confirmed by serological DAS-ELISA and reciprocal inoculation on indicator plants.
[2]Data of the susceptible control Bola de Oro were used due to cultural problems with the susceptible parent.

disease progress curves for each generation. The coefficient of determination r^2_{op} (The square of the Pearson Product-moment correlation coefficient) (Sokal and Rohlf, 1987) provided a useful measure of the intensity of association between the two variables, ODPC and PDPC.

Results

Disease progress

The first MYV symptoms started to appear between 14 and 30 days postinoculation (DPI) in susceptible parents and controls (Table 1). Disease progressed rapidly to the point two weeks later where nearly all the susceptible plants exhibited severe MYV symptoms. The partial resistance to MYV found in *Cma* was expressed as a delayed infection, starting at 30–60 DPI. In general, symptoms found in the resistant parent, although evident, were less severe than those of the susceptible parent. Only mild symptoms were found in *Cma* infected plants at the end of the assays.

MYV incidence varied between years, being more severe the second year. The effect of the transplanting date on infection levels was also detectable within each year. An earlier and somewhat more severe infection was recorded in later transplantings of each year (assays 2 and 4) (Table 1).

Inheritance of MYV resistance

Relative resistance level among parents (P1 and P2), F1, and segregant generations (F2, BC1, and BC2), was the same in all 4 assays, since disease curves were consistently ordered. The observed disease progress curves for F1 generation were situated between the resistant and susceptible parents curves, therefore indicating a partial dominance of resistance (Table 1).

The genetic model first put forward considered a simple genetic control with partial dominance of resistance. This model included 3 parameters, namely *Prp*, *Pf1*, and *Psp*. *Prp* indicates the probability of an homozygous for the resistance gene plant remaining uninfected. *Pf1* indicates the probability of an heterozygous for the resistance gene plant remaining uninfected.

As the susceptible parents (VC-21 and VC-120) have no resistance genes, the percentage of non-infected plants found in this parents in the first stages of infection should correspond with plants that escape inoculation. Assuming the hypothesis that the escape is an environmental effect independent of the genotype, we can apply it to all progenies by using a third parameter. *Psp* indicates the probability of a plant (parental, F1, F2, BC1 or BC2) remaining uninfected due to an environmental effect i.e. the risk of escape. Considering this model, the probability of a plant of any generation remaining uninfected could be due to an environmental effect (*Psp*), or otherwise, if it is not an escaped plant $(1 - Psp)$, to the resistance conferred by the presence of the resistance gene in homozygous (*Prp*) or heterozygous (*Pf1*). The estimates of the three parameters provides the expected proportions of uninfected plants in each generation ($P1 = Psp$; $P2 = Psp + (1 - Psp)\ Prp$; $F1 = Psp + (1 - Psp)Pf1$). These estimates were also used to predict the response of segregant generations, taking into consideration the genotypes and the proportion they represent in each segregant generation. For example, assuming that resistance is controlled by a single gene, the F2 population is expected to be composed of 25% of homozygous for the resistance gene plants, 50% of heterozygous for the resistance gene plants and 25% of plants without the resistance gene. Expected proportion of uninfected plants would be then calculated in F2 as follows:

$$F_2 = Psp + (1 - Psp)(0.5 \times Pf1 + 0.25 \times Prp).$$

After fitting the described 3-parameter model, PDPC were constructed. A similar evolution pattern was observed for PDPC and ODPC in each generation. However, this model was clearly inadequate in explaining these data. The expected proportion of uninfected plants in *Cma* was always lower than that observed (PP2 $<$ OP2 in Figure 1). Contrarily, the expected level of resistance of the BC2 generation was higher than that observed in all 4 assays (PBC2 $>$ OBC2 in Figure 1). This effect was not clearly observed in any other generation, where both over and underestimated values were found (curves not shown).

The unexpectedly higher resistance level to MYV found in *Cma* could be explained if we take into account that the antixenosis mechanism against *T. vaporariorum* described in *Cma* acts by increasing the level of resistance to MYV in *Cma* against the expected level only considering the resistance gene. It could be that this mechanism also acts in other generations. In this case, the most important effect would be evident in the BC2 generation. In order to prove this

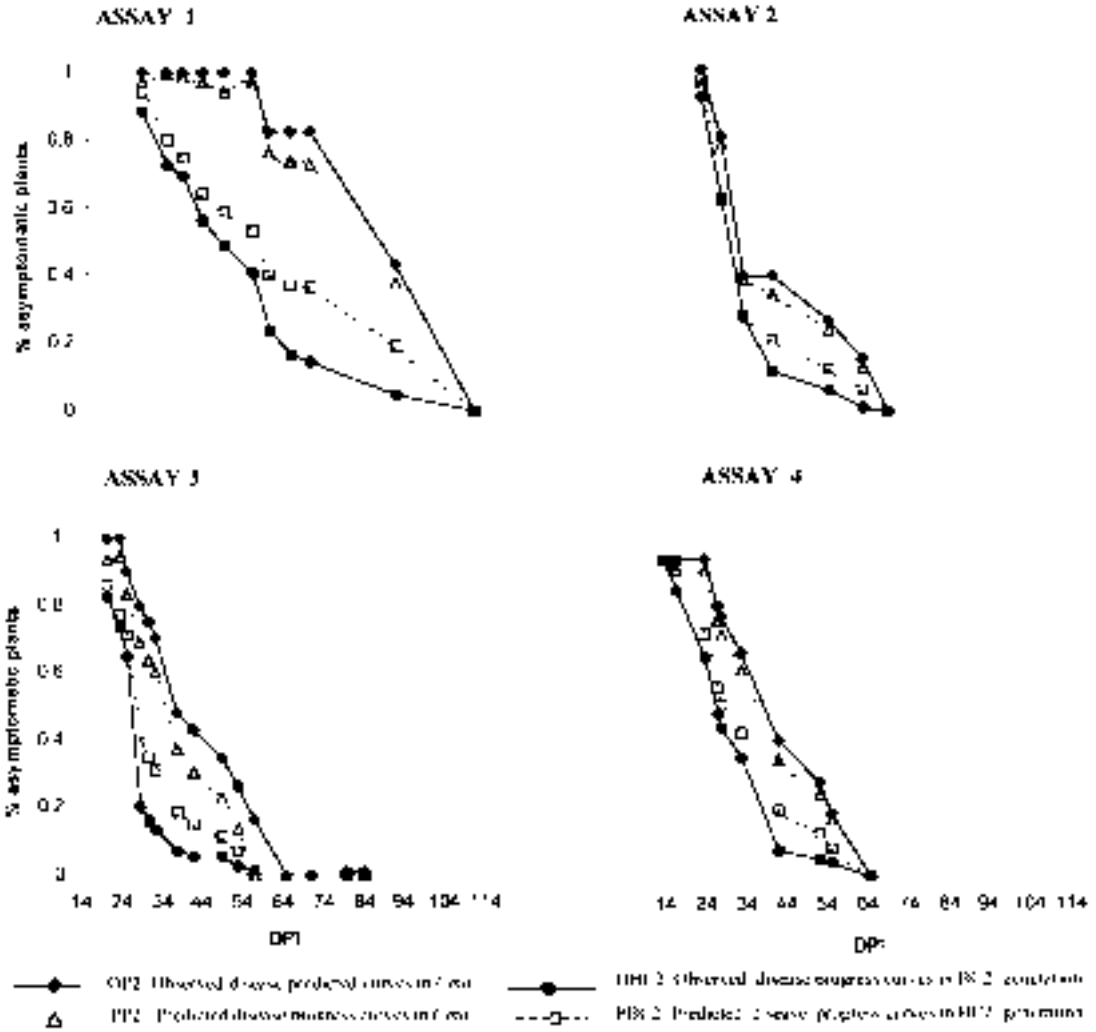

Figure 1. Derivation between observed disease progress curves and predicted disease progress curves of resistant parent *Cma* and BC2 generations obtained fitting the 3-parameter model (*Psp*, *Prp* and *Pf1*) that considers a simple genetic control with incomplete penetrance of the resistance and partial dominance of resistance.

hypothesis and to establish the genetic model of the resistance, it was necessary to modify the previously mentioned model (3-parameter). The proposed model was extended to include two more parameters, *Pa1* and *Pa2*. In this new model, *Psp*, *Prp*, and *Pf1* have the same meaning as previously described, and *Pa1* and *Pa2* indicate the probability of a plant of the resistant parent, and the BC2 respectively remaining uninfected due to the effect of the antixenosis mechanisms on virus transmission. The data were once more subjected to a multiregression analysis. The fit of this model confirmed that the effect of antixenosis was not important in the BC2 generation, obtaining low *Pa2* values, in most cases not being significantly higher than 0 (data not shown), while *Pa1* was highly significant in most cases.

The observed fact that the plants from the segregant generations derived from the cross *C. melo* × *Cma*, including BC2, have leaves which are more like the susceptible parent (low pubescence) than the resistant parent, *Cma* (high pubescence), could be linked to this behaviour.

Previous results lead to a final 4-parameter (*Psp*, *Prp*, *Pf1*, *Pa1*) model shown in Table 2. The model-fitting

Table 2. Goodness of fit of the general model (4-parameters) of the inheritance of monogenic resistance to MYV. The model considers partial resistance, partial dominance of resistance, and an antixenosis mechanisms in the resistant parent *Cma*

Generations	Model	$r^{2\ 1}_{op}$			
		First year		Second year	
		Assay 1 VC-120 × *Cma*	Assay 2 VC-21 × *Cma*	Assay 3 VC-120 × *Cma*	Assay 4 VC-21 × *Cma*
P1	R[2] Psp[4] S[3] $1 - Psp$	0.96	—	0.99	0.99
P2	R $Psp + (1 - Psp)(Prp^5 + Pa1^6)$ S $1 - [Psp + (1 - Psp)(Prp + Pa1)]$	0.99	0.99	0.99	0.99
F1 (P1 × P2)	R $Psp + (1 - Psp)\ Pf1^7$ S $1 - [Psp + (1 - Psp)Pf1]$	0.99	0.99	0.99	0.99
F2 (F1 × F1)	R $Psp + (1 - Psp)(0.5Pf1 + 0.25Prp)$ S $1 - [Psp + (1 - Psp)(0.5Pf1 + 0.25Prp)]$	0.96	0.99	0.99	0.99
BC1 (P1 × F1)	R $Psp + (1 - Psp)(0.5Pf1)$ S $1 - [Psp + (1 - Psp)(0.5Pf1)]$	0.96	0.99	0.93	0.98
BC2 (P2 × F1)	R $Psp + (1 - Psp)(0.5Pf1 + 0.5Prp)$ S $1 - [Psp + (1 - Psp)(0.5Pf1 + 0.5Prp)]$	0.99	0.99	0.99	0.99

[1]Coefficient of determination; [2]R: Proportion of asymptomatic plants; [3]S: 1 − R; [4]*Psp*: Probability of a plant of any generation (parent, F1, F2, BC1, BC2) remaining uninfected due to an environmental effect. Risk of escape; [5]*Prp*: Probability of a plant homozygous for the resistance gene remaining uninfected; [6]*Pa1*: Probability of a plant of the resistant parent remaining uninfected due to the effect of antixenosis; [7]*Pf1*: Probability of a plant heterozygous for the resistance gene remaining uninfected.

results that include the model parameter estimates for each date and the significance level for each parameter are shown in Table 3.

After fitting the model, ODPC and PDPC were constructed for each generation (Figures 2 and 3) and r^2_{op} for each pair of curves was calculated. The high values of r^2_{op} (0.93–0.99) (Table 2) obtained for all generations demonstrate the good fit to the model during the whole disease progress, thereby indicating that this model adequately explains the data.

The values estimated for the parameters $Psp \lll Pf1 < Prp$ (Table 3) confirm that partial dominance of resistance exists. The effect of antixenosis on virus spread has been found to be variable both between and within the years. The effect of the transplanting date is also observed in *Pa1*, which reaches higher values in earlier transplantings (assays 1 and 3).

Discussion

The high resistance level found in *Cma* makes it a promising source for breeding MYV resistant melons. However, the response of this accession to MYV varies depending on the inoculation conditions i.e. greenhouse and field trials or controlled whitefly-mediated inoculation (Soria et al., 1996). This variable response suggests the existence of two types of resistance mechanisms operating successively in *Cma*: resistance to the MYV, and antixenosis against the disease vector *T. vaporariorum*.

Antixenosis against *T. vaporariorum* (sensu Kogan and Ortman, 1978) prevents this whitefly from selecting *Cma* for feeding and oviposition when *Cma* is cultivated together with other *C. melo* accessions or cultivars (Soria et al., 1996). The nature of the antixenosis mechanism has been studied in other host–whitefly systems. Colour has been reported as one of the most important factors in host–plant selection by whiteflies from a distance. After landing and internal probing, non-preference seems to be based on chemical or physical plant properties (Van Lenteren and Noldus, 1990; Chu et al., 1995; Yee and Toscano, 1996). The fact that *Cma* exhibited a more dense leaf pubescence to that of commercial melons and derived generations, even BC2, could contribute to antixenosis, but further studies are required to study the nature of the mechanisms.

Antixenosis has often been suggested as a successful strategy to combat whitefly-borne virus diseases (Vetten and Allen, 1983; De Ponti et al., 1990).

Table 3. Estimates of the proportion of asymptomatic plants in homozygous (*Prp*) and heterozygous (*Pf1*) for the gene of resistance to MYV. Asymptomatic plants in *Cma* due to the effect of the antixenosis mechanism (*Pa1*) and percentage of escape (*Psp)* are also indicated

	VC-120 × *Cma*					VC-21 × *Cma*				
	DPI	*Psp*	*Pa1*	*Prp*	*Pf1*	DPI	*Psp*	*Pa1*	*Prp*	*Pf1*
First	37	0.3**	0.1	0.9**	0.3**	33	0	0.1	0.7**	0.5**
year	42	0.2**	0.1	0.8**	0.2*	38	0	0.1**	0.4**	0.2**
	47	0.1	0.2	0.8**	0.2**	45	0	0.3**	0.1**	0.1**
	54	0.1	0.2	0.8**	0.1	59	0	0.1**	0.1**	0
	58	0	0.3*	0.5**	0.1	67	0	0.1**	0	0
	63	0	0.3**	0.5**	0.1					
	68	0	0.5**	0.3**	0.1					
	89	0	0.3**	0.1*	0					
	108	0	0.3**	0.1*	0					
Second	24	0.4**	0.4**	0.6**	0.5**	17	0.3**	0.1	0.9**	0.8**
year	26	0.1**	0.3**	0.6**	0.5**	24	0.1*	0.1	0.9**	0.5**
	29	0	0.6**	0.2**	0.2**	26	0	0.2*	0.6**	0.4**
	31	0	0.6**	0.2**	0.2**	28	0	0.2**	0.5**	0.4**
	33	0	0.5**	0.2**	0.1**	33	0	0.3**	0.5**	0.3**
	38	0	0.3**	0.2**	0	42	0	0.3**	0.1**	0.1**
	42	0	0.3**	0.1**	0	52	0	0.2*	0.1*	0
	49	0	0.2**	0.1**	0	55	0	0.1*	0.1*	0
	53	0	0.2**	0.1**	0					
	57	0	0.1**	0.1**	0					

Significant at 5% (*) or at 1% (**).

However, the sole existence of antixenosis in *Cma* does not explain the high degree of MYV resistance found in this accession. Additional resistance mechanisms to MYV, operating after *T. vaporariorum* has reached the plant and transmitted MYV, delay infection and minimize symptom severity. The nature of this resistance to MYV still remains unknown, and it could be related to mechanisms preventing viral replication or short/long distance viral movement in the plant.

Results show how the antixenosis mechanism acting in *Cma* affects the response of this accession to MYV infection. The effect of antixenosis on virus transmission has been studied in other vector–virus–host systems. It is a complex effect that depends both on the level of resistance to the vector, and on the virus transmission mode i.e. virus persistence in the insect, length of acquisition, latency, and inoculation periods, etc. It has been suggested that epidemics caused by non-persistent-transmitted virus could be more rapidly spread in varieties with resistance to the vector, because the latter may be restless and therefore increase probing (Kennedy, 1976). However, most studies on aphid and whitefly-transmitted viruses report an increased field resistance to virus infection due to these mechanisms (Al-Musa, 1982; Roberts and Foster, 1983; Yoshida and Kohyama, 1986; Gunasinghe et al., 1988; De Ponti et al., 1990; McCreight, 1993). We have also found a similar response in the system *T. vaporariorum*-MYV-*Cma*. The increased resistance level to MYV found in *Cma* could be a consequence of an initially reduced number of colonizers in *Cma* due to antixenosis. In our assays, controlled inoculation before transplanting is also subjected to the antixenosis mechanism since it has not been individually conducted, and the whiteflies have a free choice of plants on which to feed and reproduce. Studies on *T. vaporariorum* feeding behaviour indicate that test probing, the main process in host selection, is significantly longer on preferred leaves than on non-preferred leaves. In the latter case, rejection of the host plant occurs after probing for a few minutes, reducing the chance of virus transmission (Xu et al., 1994). After probing, *T. vaporariorum* would select another more attractive host for feeding, and the infestation level would remain lower in this accession. It only becomes important when the whitefly population increases.

The effect of antixenosis also makes genetic analysis of virus resistance more difficult. The 3-parameter

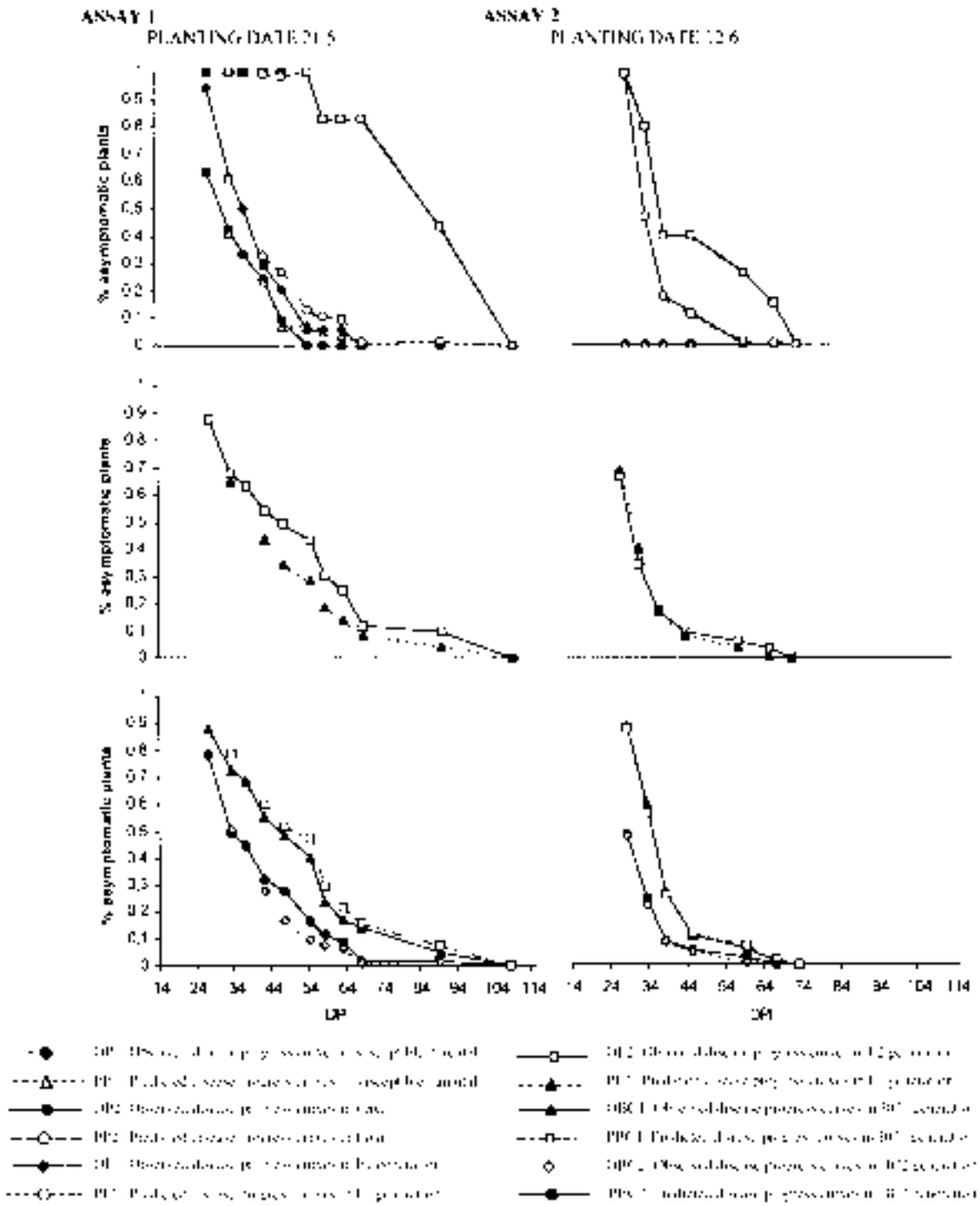

Figure 2. Deviation between observed disease progress curves and predicted disease progress curves obtained when fitting the 4-parameters model (*Psp*, *Prp*, *Pf1* and *Pa1*) that considers a simple genetic control with incomplete penetrance of the resistance, partial dominance of resistance and an antixenosis mechanism in *Cma* (first year: assays 1 and 2).

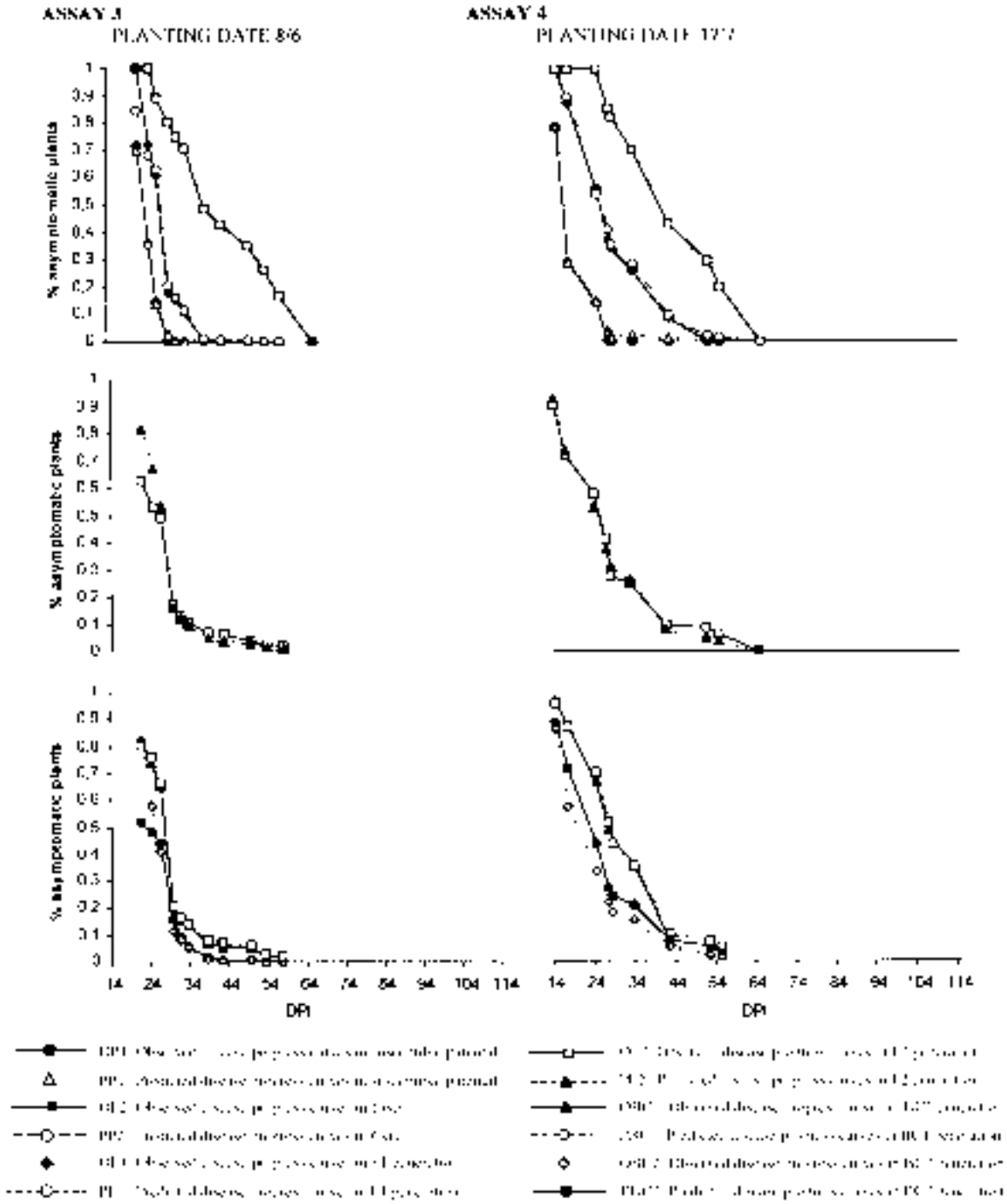

Figure 3. Deviation between observed disease progress curves and predicted disease progress curves obtained when fitting the 4-parameters model (*Psp, Prp, Pf1* and *Pa1*) that considers a simple genetic control with incomplete penetrance of the resistance, partial dominance of resistance and an antixenosis mechanism in *Cma* (second year: assays 3 and 4).

model that only considers the effect of the resistance gene, underestimates the actual level of resistance to MYV observed in *Cma*. In this model the estimate of *Prp* by multiple regression analysis includes two effects. Firstly, the resistance conferred by the gene in homozygous condition, and secondly, part of the excess resistance due to antixenosis. The use of the *Prp* parameter for the estimation of the percentage of uninfected plants in the BC2 generation leads to an overvaluation of the resistance in this generation, the most affected by Prp (BC2 $= (Psp + (1 - Psp)(0.5Prp + 0.5Pf1))$ (Figure 1). These deviations could erroneously lead to the conclusion that MYV resistance has not a monogenic but a more complex genetic control.

The fit including *Pa* parameters allow us to separate the effect of the resistance to the vector from the virus resistance effect found in the same source. The significant values found for *Pa1* indicates that antixenotic effect increases virus resistance in *Cma*. The existence of this antixenosis effect in any other generation could have also been detected by adding more parameters to the model, as it has 6 degrees of freedom. The low values, which are not significantly higher than 0, obtained for *Pa2* confirms that the antixenotic effect on virus resistance level is not important in BC2, where it could most be expected.

However, the fact that the model fits including the antixenosis parameter *Pa1* only in *Cma* does not imply the non-existence of antixenosis in any other generation. It only means that antixenosis effects are not so important in altering virus spread. The high effectiveness of *T. vaporariorum* in transmitting MYV (Soria et al., 1991) could explain the fact that virus transmission is not affected in the segregant generations even when antixenosis mechanisms act. Therefore, in this study we cannot determine in detail the effect of antixenosis on virus transmission. We show that antixenosis in *Cma* causes an increase in the level of MYV resistance in this accession, in our assays conditions. Further studies on correlation on antixenosis with resistance to virus transmission in segregant populations must be conducted under controlled conditions with specific laboratory tests to lead to a full understanding of the process (Khush and Brar, 1991; Nemoto et al., 1994; Panda and Kush, 1995).

From the results of this fitted model we can conclude that there is a monogenic control of MYV resistance in *Cma*. Effects such as incomplete penetrance, giving rise to partial overcoming of resistance, and a gene dosage effect, which result in incomplete dominance ($Pf1 < Prp$), are also quantified by the parameters of the model (Table 2), allowing a wider study of the genetics of resistance.

It is also important to underline that the study of disease progress curves allows for the contrast of data from trials performed in variable conditions (2 years/4 transplanting dates). In these assay conditions, results are likely to be altered by environmental factors influencing the three components of the system (vector–virus–host). The earlier and more severe infection observed in later assays (Assays 2 and 4) could be an effect of temperature. Higher temperatures could favor vector population increases and a more rapid disease progress. These increases in vector population could also explain the lower antixenosis values found in these later assays (Table 3). Comparison of these assays would have been highly erratic if studying segregation ratios at punctual moments. Adjusting a model considering time series instead of concrete moments reinforced the analysis, eliminating random selection of a moment after infection to contrast the genetic model.

The simple genetic control of partial resistance to MYV found in *Cma* makes this useful for breeding programs aimed at developing commercial melon varieties resistant to this virus.

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