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Further observations on variation of lettuce mosaic virus in relation to lettuce (*Lactuca sativa*), and a discussion of resistance terminology

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Abstract. Two potyvirus isolates from endive, originating from southern France (Ls252) and from the Netherlands (Ls265), that were highly and poorly pathogenic on lettuce, respectively, were compared with a common isolate (Ls1) of lettuce mosaic virus (LMV) and with two highly deviant Greek isolates from *Helminthia (Picris) echioides* (Gr4) and endive (Gr5), earlier recognized as LMV. The isolates could not be distinguished by particle morphology and serology, and were all identified as LMV. Leaf curling, plant stunting and necrosis were more characteristic of the virus than mosaic. The isolates studied varied considerably on differential host species and a range of lettuce cultivars including pathotype differentials of Pink et al. [1992b]. Ls1 and Ls265 reacted largely as pathotype II, including the 'common strain' of the virus, but Ls265 was least pathogenic on lettuce. Ls252 fitted pathotype IV and was very similar to LMV-E (the 'Spanish strain'). The Greek isolates were very similar to each other in causing very severe symptoms on some non-lettuce hosts and a number of lettuce cultivars. In lettuce varietal reaction Gr4 resembled pathotype I, but Gr5 severely affected 'Salinas 88', resistant to pathotypes I, II and III, and it appears to be a novel pathotype. Genetic interaction between lettuce and LMV is not following a simple yes-or-no pattern, and it is not a mere matter of resistance versus susceptibility. Adoption of a more realistic resistance terminology is proposed. None of the lettuce cultivars tested was resistant to the most pathogenic isolate Ls252, but resistance to it was found in 2 out of 12 wild *Lactuca* species tested (*Lactuca perennis* and *L. tatarica*) while the symptomless plants of *L. perennis* clearly reacted in ELISA.

Introduction

Lettuce mosaic potyvirus (LMV) has long been considered an important constraint to the growing of lettuce (*Lactuca sativa*) because of its damaging, mainly growth reducing effect, its rapid spread in a nonpersistent manner by many aphid species, and transmission via seed [Tomlinson, 1970; Dinant and Lot, 1992]. Infected seed is regarded as the major source of infection [Broadbent et al., 1951; Grogan et al., 1952]. At least 21 wild plant species from 19 genera and 9 families [Horváth, 1991] and the recently discovered highway daisy (*Osteospermum fruticosum*) [Opgenorth et al., 1991] have been found naturally infected, but spread of nonpersis-

tently transmitted viruses by aphids is not over long distances. Therefore, these species are probably less important as sources of inoculum for epidemic outbreaks of the disease. The virus occurs world-wide because of seed transmission [Dinant and Lot, 1992], but it has caused major concern where aphid population densities are high, such as in California [Patterson et al., 1986; Zink et al., 1956], and is increasingly doing so in Mediterranean countries [Dinant and Lot, 1992] with rapidly expanding horticulture.

Seed certification – requiring infestation rates below 0.1% in Europe [Tomlinson, 1962], or virtually virus-free seed in California [Grogan, 1980, 1983] – and genetic resistance to the virus have helped in reducing disease incidence and damage to acceptable proportions. In California, control emphasis has been on stringent seed testing involving samples of 30,000 seeds that had to test negative [Kimble et al., 1975; Grogan, 1980]. Elsewhere, resistance was a more important measure of control.

In lettuce, resistance to LMV, originally derived from the Argentinian lettuce cultivar 'Gallega de Invierno' [Von der Pahlen and Crnko, 1965], was ascribed to a single recessive gene *g* [Bannerot et al., 1969]. That from three Egyptian wild *L. sativa* lines (among others PI251245, Pink et al. [1992b]) and from two Spanish cultivars [Ryder, 1976], was ascribed to the recessive gene *mo* [Ryder, 1968, 1970]. The genes *g* and *mo* were long thought to be identical, until Lot and Deogratias [1991] and Pink et al. [1992b] have shown that they are different alleles or closely linked genes. They are now denoted mo^1_1 and mo^2_1 [Pink et al., 1992b]. Most resistant cultivars contain either the *g* (European cultivars) or the *mo* gene (American crisphead and cos cultivars] [Pink et al., 1992b] and no seed transmission was thought to occur in cultivars possessing the *g/mo* gene [Ryder, 1973]. A dominant gene Mo_2, found in the cv. Ithaca, has also been shown to be involved in resistance [Pink et al., 1992b].

Resistance of lettuce to LMV was believed to be an outstanding example of stable or durable resistance despite its supposedly monogenic nature. However, variants of the virus interacting diversely with lettuce cultivars were already found in California in the early 1960s, and one of them, variant 4, killed most cultivars [McLean and Kinsey, 1962, 1963]. Zink et al. [1973] thereafter detected a new virulent isolate (LMV-L) in California in the weed species bristly oxtongue, *Picris echioides*, and in lettuce. It was lethal on many lettuce cultivars and overcame resistance of 'Gallega'. In Europe, Merchat [1978] was the first to isolate more aggressive LMV strains from endive in France, and Bem and Kyriakopoulou [1984] and Kyriakopoulou [1985] did so from lettuce, endive, and *Helminthia* (*Picris*) *echioides* in Greece. Aggressive strains, as from endive [Merchat, 1978; Lot and Maury-Chovelon, 1985] and from lettuce in Spain, have recently become prevalent in France [Dinant and Lot, 1992]. Another highly pathogenic isolate (LMV-YAR) was found in Yemen [Walkey et al., 1990]. Pink et al. [1992a,b] have just discussed the breakdown of genetic resistance of lettuce to LMV and have distinguished four pathotypes of the

virus. The more severe isolates were generally assumed not to be seed transmissible in lettuce [Zink et al., 1973], but a strain (LMV-13), that is able to produce very severe symptoms on resistant lettuce cultivars, was recently proved to be seed transmissible [Dinant and Lot, 1992].

Our long-term research at IPO-DLO in support to resistance breeding, yielded apparently differing isolates of LMV, particularly in recent years. Results of our differentiation studies largely corroborate those just published by Pink et al. [1992a,b], but provide information on further variation of the virus. For a preliminininary note, see Bos and Huijberts [1992].

Materials and methods

Table 1 lists the LMV isolates we have investigated (Column 1), and those of which literature data [Pink et al., 1992a,b] were used for reference

Table 1. Virus isolates investigated (Column 1), and those of which resistance data published by Pink et al. [1992a, b] (Column 2, lower part) were used for comparison.

Isolate investigated, IPO code	Other code, or other isolate	Origin crop/location	Reference
Ls1[1]	common isolate	lettuce/1974, Netherlands	IPO collection
Ls 252	severe isolate	endive/1990, France	this publication
Ls 265	endive isolate	endive/1992, Netherlands	this publication
Gr1	Lm-V (LMV-V)	lettuce/1982, Volos, Greece	Bem [1983], Bem and Kyriakopoulou [1984]
Gr2	LMV 1–1075	lettuce/1987, Attica, Greece	Kyriakopoulou [1990][2]
Gr3	Lm-lethal (LMV-lethal)	*H. echioides*/1983, Kiphissia Attica, Greece	Bem and Kyriakopoulou [1984]
Gr4	LMV-lethal	*H. echioides*/1990, Kiphissia Attica, Greece	Kyriakopoulou [1990]
Gr5	LMV-endive	endive/1983, Megara Attica, Greece	Kyriakopoulou [1990]
Gr6	LMV-chicory	endive/1983, Megara Attica, Greece	Kyriakopoulou [1990]
	LMV-E ('Spanish strain')	Dr H. Lot, Montfavet, France	Pink et al. [1992a]
	LMV-F ('Firestone strain')	Dr J.E. Duffus, Salinas, CA, USA	Pink et al. [1992a]
	LMV-YAR	lettuce/Yemen, Arab Republic	Pink et al. [1992a]
	LMV-W	lettuce seed/ Wellesbourne UK	Walkey et al. [1985], Pink et al. [1992a]
	LMV-9	endive/1983, Bouche du Rhone, France	Pink et al. [1992b]

[1] Codes printed in italic letters are of isolates studied in detail and/or used for comparison.
[2] Dr P.E. Kyriakopoulou, Athens, Greece [personal communication Jan. 8, 1990].

(Column 2, lower part). Ls1 is the lettuce isolate from the IPO-collection and commonly used at IPO-DLO since 1974. Ls252 is a 1990 isolate from endive obtained from France via a seed company. Ls265 derives from field-grown endive in the Netherlands in 1992. A number of Greek isolates (Gr1–6), identified as LMV [Bem, 1983; Bem and Kyriakopoulou, 1984], were kindly provided by Dr P.E. Kyriakopoulou, then at the Benaki Phytopathological Institute, Kiphissia, Athens, Greece [personal communication Dr P.E. Kyriakopoulou, Jan. 8, 1990]. Gr1 and 2 were common isolates, Gr3 and 4 were lethal isolates obtained from *H. echioides*, and Gr5 and 6 were similar isolates from endive. The Greek isolates (except Gr4) were received in leaf material dried and stored over $CaCl_2$.

During our experiments, the isolates were maintained in lettuce 'Patty' and in *Chenopodium quinoa*, and were regularly transferred by sap inoculation of carborundum-dusted leaves using plant sap obtained by grinding virus-containing leaf material 1:5 (w:v) in 0.03 M potassium-phosphate buffer solution containing 0.25% $Na_2S_2O_5$, 0.5% Dieca, and 7.5% activated charcoal (Norit SX1). Preservation was in leaf material dried and stored over $CaCl_2$ at 4 °C, or in plant sap freeze-dried and stored in vacuum. Presence and concentration of virus were assessed by ELISA.

Seed of lettuce cultivars was provided by commercial companies, and seed of wild *Lactuca* species by the Netherlands Centre for Genetic Resources (GCN) of the DLO Centre for Plant Breeding and Reproduction Research (CPRO-DLO), Wageningen. Lettuce genotypes were usually inoculated twice with a time interval of two days to ensure optimal infection. Inoculated plants of lettuce cultivars (three plants per pot) were kept in the greenhouse, mostly for one week at 22 °C and thereafter at 15 °C for optimal symptom expression [Tjallingii and Huijberts, 1973]. Observations were usually made at weekly intervals from 1–5 weeks after the first inoculation.

ELISA was according to Clark and Adams [1977], and crude sap (undiluted or diluted 1:1 in conjugation buffer) from leaf material (second and third reasonably developed tip leaves) harvested 3–4 weeks after inoculation. Incubation coating was for 24 h at 4 °C, and incubation with plant sap for 16–24 h at 4 °C, with enzyme conjugate for 5 h at 30 °C, and with substrate for 1 h at 20 °C. Absorbance (optical density) was measured with an EL312e Bio-Kinetics ELISA reader at 405 nm, 1 h after addition of the substrate. One antiserum was to Ls1, provided by Mr D.Z. Maat of IPO-DLO, with a homologous titre of 215, and another one was to the Greek LMV-lethal Gr4, with a homologous titre of 512 used at a concentration of 1 µg/ml IgG, provided by Dr P.E. Kyriakopoulou, Athens, Greece.

Results and discussion

Virus identification

In preliminary biological experiments, the isolates Gr1 and 2 appeared virtually identical to each other and to our common isolate Ls1. Isolates Gr3 and 4, and Gr5 and 6 were also mutually identical. We have therefore chosen Ls1 from lettuce, Gr4 from *H. echioides*, and Gr5 from endive for comparison with, and further detailed strain identification of our unidentified isolates from endive from France (Ls252) and the Netherlands (Ls265). Bem [1983], Bem and Kyriakopoulou [1984], and Kyriakopoulou [1985] earlier identified Lm-V (or LMV-V) and Lm-Lethal (or LMV-Lethal), most likely identical to Gr1 and Gr3 and 4, respectively, as LMV.

The reactions we obtained with all isolates on lettuce (see below) and their particle morphology and size suggested all to be isolates of LMV, or some of them of a closely related potyvirus since the lettuce varietal reactions were very diverse and the Greek isolates differed largely on non-lettuce species. With a view to considerable variation of, and biological and serological overlap between viruses within the potyvirus group [Bos, 1970, 1992], further identification of the isolates became necessary to establish their species affiliation.

Table 2. Summary of reactions of some plant species other than lettuce

	Virus isolates				
Plant species	Ls1	Ls252	Gr4	Gr5	Ls265
Chenopodium					
amaranticolor	$L^{-}-$[1]	L –	L S^{s}	L S^{s}	$L^{-}-$
quinoa	L S	L S^{-}	L $S^{c***(n)}$	L $S^{c***(n)}$	L S
Cichorium					
intybus (endive)	l s	L S^{m}	L –	L S^{s*}	L S^{s**}
Nicotiana					
benthamiana	l S^{m*}	l S^{m*}	l S^{m**}	l $S^{(m)s***}$	l $S^{vc**(m)}$
glutinosa	– –	– –	– –	– –	– –
tabacum					
'White Burley'	– –	– –	– –	L –	– –
Pisum sativum					
'Rondo'	l $S^{m*(s*)}$	l $S^{m*(s**)}$	L $S^{(m*)s*}$	L $S^{(m*)s**}$	l $S^{(m)(s*)}$

[1] L = local symptoms, l = symptomless local infection, S = systemic symptoms consisting of ^{c} = chlorosis, ^{m} = mosaic, ^{n} = necrosis, ^{s} = stunting, ^{vc} = vein chlorosis, or $^{-}$ = very mild; – = no symptoms, but not tested for infection; number of asterisks indicate symptom severity; codes in brackets indicate erratic occurrence.

Symptoms. Table 2 summarizes the reactions of a number of important diagnostic species. It indicates close biological relationships of all isolates with our standard LMV isolate Ls1. However, there were clear differences between isolates in severity of symptoms.

Chenopodium amaranticolor reacted with many small chlorotic local lesions, which later in yellowing leaves turned into dark green to necrotic rings. Systemic infection with Gr4 and 5 led to plant stunting, and to curling and slight malformation of non-inoculated leaves. *C. quinoa* invariably reacted with similar local lesions, and all isolates showed systemic chlorotic spotting and more or less severe leaf malformation and curling. Systemic symptoms were weak with Ls252 and very severe with Gr4 and 5, involving severe stunting and chlorosis (Fig. 1).

Nicotiana benthamiana reacted with a mild mosaic or mottle to all isolates, although brightest with Gr4, dominated by severe stunting with Gr5, and more of a vein-mosaic or vein-clearing type of variegation with Ls265. *N. glutinosa* reacted similarly to all isolates. *N. tabacum* 'White Burley' reacted to Gr5 only, and did so with several large diffuse clorotic local rings.

Cichorium intybus (endive) cv. Nummer Vijf 2 reacted late with few large diffuse chlorotic rings extending along the veins (especially Ls252 and Gr4), and with a diffuse mosaic or mottle in non-inoculated leaves and plant stunting, notably with the endive isolates Ls252 and Gr5, but especially with Ls265 (Fig. 2).

Pisum sativum (pea) 'Rondo' reacted with discrete necrotic local lesions to Gr4 and 5 only. Systemic mosaic or mottle, preceded by vein clearing, and growth reduction, were rather erratic and most severe with Ls252 and Gr5.

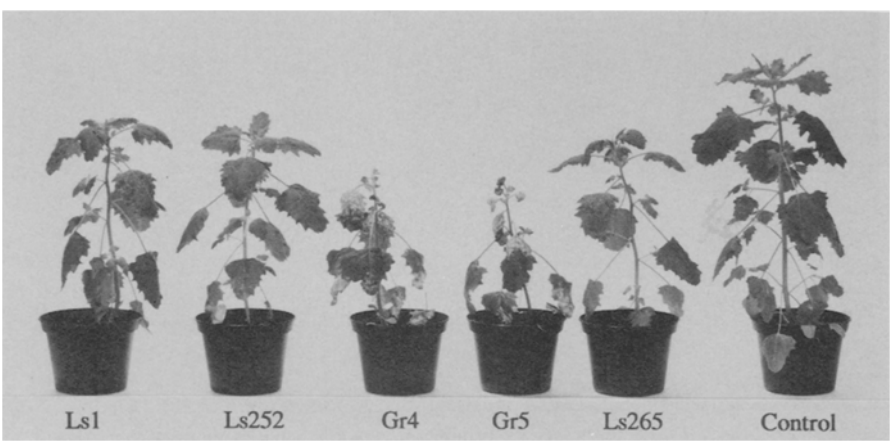

Fig. 1. Symptoms of LMV isolates in *Chenopodium quinoa*, 15 days after inoculation; right, non-inoculated control.

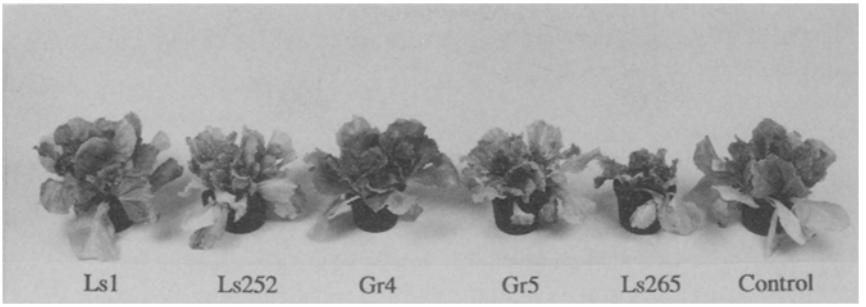

Fig. 2. Symptoms of LMV isolates in *Cichorium intybus* (endive), 64 days after inoculation; right, non-inoculated control.

Serology. Results of ELISA in some of the differential host species are given in Table 3. High virus concentrations were encountered in *N. benthamiana* and pea, and low concentrations in endive. Ls265 was especially deviant in occurring in relatively high concentrations in endive and in very low concentrations or entirely absent in *N. benthamiana*. Between the other four isolates in this species and pea there was no striking overall quantitative difference in reaction to antiserum to Ls1.

These results clearly showed all isolates to be LMV, serologically very similar, but biologically rather different. The Greek isolates from *H. echioides* (Gr4) and endive (Gr5) were the most pathogenic ones, especially on *C. quinoa* and *N. benthamiana*, and the three endive isolates (Gr5, Ls252 and Ls265) were higly pathogenic on the endive genotype tested. Gr5 and Ls252 were also severest on pea. The following tests with a

Table 3. Summary of two ELISAs in some plant species of Table 2; readings (OD at 405 nm × 100) with antiserum to Ls1, four weeks after inoculation

Cultivars	Virus isolates				
	Ls1	Ls252	Gr4	Gr5	Ls265
Cichorium intybus (endive)	34/136	11/89	–/–[1]	15/36	181/123
Nicotiana benthamiana	300/356	326/319	71/143	187/195	6/144
glutinosa	–	–	–	–	–
tabacum 'White Burley'	–/–	–/–	–/–	–/–	–/–
P. sativum 'Rondo'	313/316	292/311	185/–	159/–	nt[2]/129

[1] Readings up to and including 5 were considered negative and are not recorded here.
[2] nt = not tested.

number of lettuce genotypes were meant to further differentiate the isolates, possibly as distinct strains of LMV.

Isolate differentiation on lettuce genotypes

Symptoms. First symptom on many lettuce genotypes was a more or less severe, but usually transient diffuse vein clearing, appearing some 1–2 weeks after inoculation. 'Vanguard 75' reacted to most isolates with chlorotic local lesions, and 'Vanguard 4880' produced many chlorotic local rings with Ls265. The local reaction of both Vanguard cvs. was more severe when plants were older at the time of inoculation. Only a few cultivars, such as 'Patty' with Ls1, Gr4 and Ls265 and especially 'Ithaca' with Ls265, reacted with a clear mosaic pattern. Most genotypes, when reacting, did so with leaf curling and especially plant stunting dominating other symptoms and rapidly leading to retarded plant development, reduced hearting and final yield reduction (Fig. 6, Table 4). Various types and grades of systemic necrotic spotting developed in several genotypes, including 'Gallega' (Fig. 3: Gr5), and with different isolates, as in 'Patty' (Fig. 5). In some cultivars, extensive necrosis developed along and in the veins, as with Ls252 in 'Soraya' (Fig. 4) and in 'Patty' (Fig. 5). Necrosis often contributed or led to leaf malformation and distortion and to premature plant death as in 'Patty' with Gr5 (Fig. 6).

Comparison of virus isolates. For comparing the effect of the isolates, a number of lettuce genotypes, earlier found to more or less differentiate between isolates, were used. Some of these were independently also used by Pink et al. [1992a,b] for pathotype differentation, and during later

Table 4. Summary of reactions of lettuce cultivars, including LMV differentials of Pink et al. [1992a,b] (names printed italics)

Cultivars	Virus isolates				
	Ls1	Ls252	Gr4	Gr5	Ls265
Saladin (= Salinas)	m s**[1]	s*** n*	s*** n*	s**** n**	s*
Patty	s** n*	s*** n*	s*** n*	s*** n*	s**
Ithaca	s** n*	s*** n**	(s*)	–	s*
Vanguard 4880	vc** s** n*	s**** n**	–	–	s***
Soraya	(s*)	s*** n*	s**	s**	–
Salinas 88	(s*)	s*** n*	s*	s*** (n*)	–
Gallega	(sp)	s** (n*)	–	s** (m*)	–
Elvira	–	s*	(s*)	–	(s*)
Vanguard 75	(s*)	s** n***	–	–	–

[1] m = mosaic, n = necrosis, s = stunting, sp = spotting, vc = vein clearing, – = no symptoms; number of asterisks indicate severity, brackets indicate erratic reaction.

Fig. 3. Systemic chlorotic and necrotic spotting in lettuce cv. Gallega, 50 days after inoculation with LMV isolate Gr5.

experiments some of their other differentials were added. The butterhead (Roman) lettuce cvs. Patty and Gallega were used as susceptible and resistant controls, respectively. In later experiments, the crisphead cultivars Saladin (= Salinas) and Vanguard 75 were added for the same purpose. Six plants of each cultivar (three plants per pot) were tested per experiment; all cultivars were tested twice and some, four times. In the earliest experiments, symptoms were scored for type and severity according to a scale of 0–6. Since the effect of different symptoms was not merely additive, one single figure would suggest non-existing precision. Therefore, in the later experiments to be recorded here, the main yield-depressing symptoms, particularly stunting and necrosis, were separately coded and quantified for severity. The results of symptom scoring are summarized in Table 4. An overview of the effect on plant growth in the butterhead lettuce genotypes is given in Fig. 6. In two of the four experiments, all genotypes were tested in ELISA for virus concentration using the Ls1 antiserum, and in one of these the Greek antiserum was also used (Table 5).

Fig. 4. Systemic veinal necrosis and ensuing chlorosis and leaf malformation in lettuce cv. Soraya, 21 days after inoculation with LMV isolate Ls252.

In lettuce varietal spectrum and in severity of symptoms, there were striking differences between isolates, and these were in line with those observed on differential plant species as recorded in Table 2. The overall effect of Ls1 was mild, and the effect was slight or absent on the cultivars of Table 4 from and including 'Soraya' downward. This was corroborated by measurements of the virus concentrations by ELISA (Table 5). Per isolate, symptom severity was usually correlated with virus concentration. The endive isolate Ls252 was by far the most pathogenic isolate and was so on all lettuce genotypes, also reaching high virus concentrations in most of them. It differed considerably from the endive isolate Ls265, which was poorly pathogenic like Ls1 on the commonly resistant lettuce cultivars, but it was severe on endive. Ls252 was weakest on *C. quinoa*. On non-lettuce plant species, especially *C. quinoa* and *N. benthamiana,* the Greek isolates Gr4 and 5 occupied a highly deviant position, and this was also true for their lettuce varietal reaction. They infected a number of commonly resistant lettuce cultivars including 'Gallega', causing severe symptoms on them, but they did not infect the commonly susceptible cultivars Ithaca and

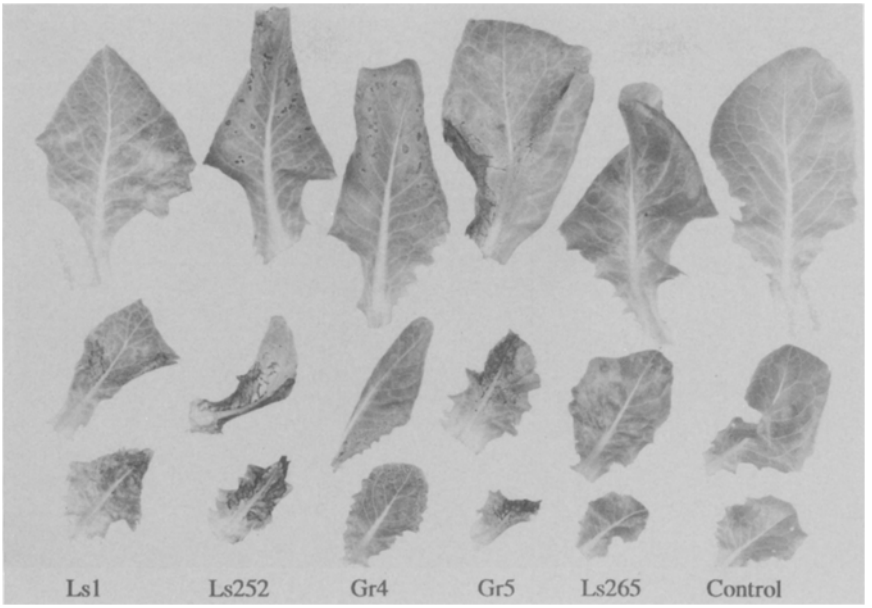

Fig. 5. Systemic chlorotic spotting, necrosis, and leaf malformation in lettuce cv. Patty, 16 days after inoculation with LMV isolates; right, non-inculated control; youngest leaves at bottom.

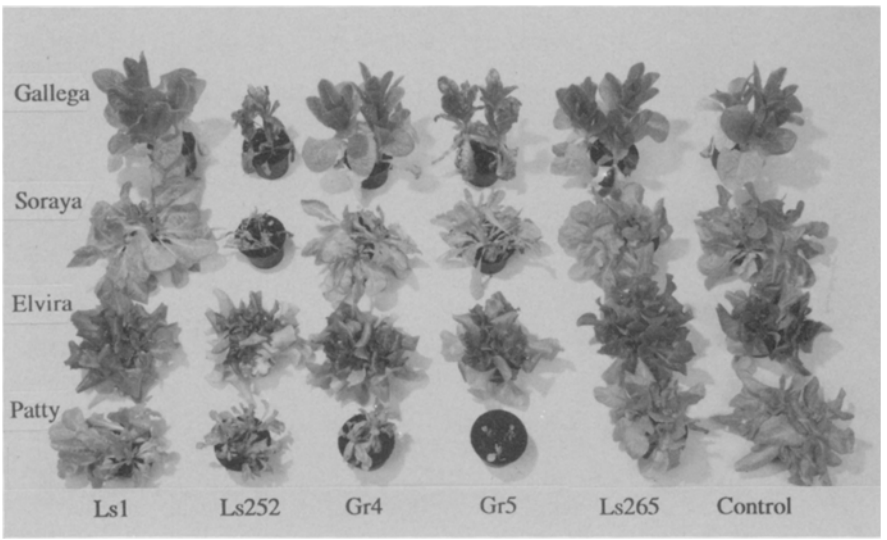

Fig. 6. Survey of the effect of the LMV isolates on butterhead lettuce cultivars, 50 days after inoculation; right, non-inoculated controls.

Table 5. Summary of ELISA readings (OD at 405 nm × 100) in two partially overlapping experiments I and II with antisera to Ls1 (Exp. I and II, figures in roman) and to isolate Gr4 (Exp. II, figures in italics) in lettuce cultivars 3–4 weeks after inoculation

Cultivars[1]	Virus isolates									
	Ls1		Ls252		Gr4		Gr5		Ls265	
	I	II	I	II	I	II	I	II	I	II
Saladin (= Salinas)		315/*109*		285/*77*		9/–[2]		21/*8*		285/*77*
Patty	322	298/*83*	289	275/*70*	24	43/*9*	101	50/*21*	190	253/*75*
Ithaca		320/*107*		193/*50*		–/–		–/–		291/*81*
Vanguard 4880		299/*109*		277/*71*		–/–		–/–		297/*87*
Soraya	128 (–)[3]		273		24		13 (68)		–	
Salinas 88		23/*7*		266/*76*		–/–		123/*49*		125/*44*
Gallega	–		275		84 (–)			40		–
Elvira	–		159		–		–		–	
Vanguard 75		21/*14*		42/*10*		–/–		–/–		–/–

[1] Cultivar names in italics are of LMV differentials of Pink et al. [1992a,b].
[2] Readings up to and including 5 were considered negative, and are not recorded here.
[3] Readings in brackets are of a few tests that were repeated.

Vanguard 4880. Peculiarly, most of the cultivars that reacted with symptoms to Gr4 and 5 did so severely, but reacted poorly in ELISA when first tested with antiserum to Ls1. This then seemed to corroborate biological differences found between these Greek isolates and the other ones, and to prove the existence of a distinct potyvirus. However, similar differences in virus titre concentrations were found when using the Greek antiserum to the lethal strain (Gr4) (Table 5). Our earlier decoration electron miscroscope tests, using Ls1 antiserum suggested slight differences between isolates in decoration [Bos and Huijberts, 1992], but this could not be confirmed later when also extensively using the Greek antiserum. For example, particles of Ls1 were very strongly decorated with the Greek antiserum. This indicated that the low virus titres obtained with ELISA in lettuce cultivars with the Greek isolates are due to poor multiplication in lettuce.

Testing of wild Lactuca *species*

Since Ls252 appeared to be the most pathogenic isolate of LMV, breaking all resistances available so far, this isolate was used in comparison with Ls1 to screen a number of wild *Lactuca* species for possible resistance. The results are summarized in Table 6. No symptoms developed in both entries

Table 6. Vulnerability of wild *Lactuca* species and accessions to the two virus isolates with lowest (Ls1) and highest (Ls252) pathogenicity

Species	Gen Accession Nr.	Virus isolate		Species	Gen Accession Nr.	Virus isolate	
		Ls1	Ls252			Ls1	Ls252
L. aculeata	9357	4[1]	4	*L. virosa*	4678	6	6
L. altaica	4664	5	5		4681	6	6
L. dregeana	4790	4	5		4683	3	4
L. muralis	5005	4	4		4950	6	6
	51164	4	4		954	6	6
L. perennis	9317	0	0		4964	6	6
	9318	0	0		4970	6	6
L. saligna	4662	4	5		5020	6	6
	9311	4	5		5145	4	4
L. serriola	4953	4	4		5148	4	4
	4973	4	5		5266	4	4
	5113	4	5		5331	6	6
	5917	4	4		5794	3	4
L. tatarica	9390	0	0		5941	6	6
L. tenerrima	9386	0	4		9316	3	4
L. viminea	9326	0	4		9365	3	4

[1] Average of six plants; symptoms recorded according to severity from 0–6: 0 = no symptoms; 3 = diffuse vein spotting; 4–5 = mosaic; 6 = necrosis.

of *L. perennis* tested and in the only tested entry of *L. tatarica*. Symptomless plants of both accessions of *L. perennis* were tested in ELISA and contained reasonable amounts of virus (absorption of nr. 9317 with Ls1: 58 and with Ls252: 95, and of nr. 9318: 71 and over 200, respectively). This indicates that absence of symptoms in these accessions is more a matter of tolerance than of resistance to infection. Likewise, the symptomless plants with Ls1 of the only lines of *L. tenerrima* and of *L. viminea* tested, contained high concentrations of virus.

General discussion

The Greek isolates (Gr4 and Gr5) were indeed highly deviant in their reaction, especially on non-lettuce differential host species. Although they reached lower virus concentrations in lettuce cultivars when tested with antiserum to Ls1, a similar difference was obtained with the Greek antiserum to Gr4 (Table 4). The absence of clear serological differences between the isolates is further supported by the apparent lack of differences in virus concentrations in the non-lettuce hosts when using the Ls1 antiserum. Pink et al. [1992a] found that some 'severe' isolates did not react very well with antiserum to LMV-W (a common Wellesbourne isolate), but relatively high titres were obtained with most isolates, including a Greek one, with an antiserum to LMV-YAR. Our earlier slight differences between Gr4 and 5 and other isolates in decoration electron microscopy, using Ls1 antiserum [Bos and Huijberts, 1992], could later not be confirmed when also using the Greek antiserum, and, e.g., the intensive decoration of particles of Ls1 with the Greek antiserum demonstrates a close relationship of the Greek isolates with common LMV. It is, therefore, now concluded that all five isolates tested belong to a single virus species: LMV. This virus appears to be much more variable than long supposed. Leaf curling, plant stunting and even necrosis are more characteristic symptoms of the virus than mosaic in spite of the name of the virus.

When comparing our isolates mutually and with data from the literature, similarities between certain isolates and differences among others are striking. In our tentative experiments, Ls1, Gr1 and Gr2 were nearly identical, and as rather common isolates they may well be considered isolates of a 'common strain' of the virus. Other isolates or groups of isolates differed considerably from these. They showed mutual similarity in certain aspects, but differed in others. For example, Gr4 and Gr5 were very similar to each other on non-lettuce hosts (Table 2, Fig. 1) and in symptom severity on most of the lettuce cultivars they infected (Fig. 6), but they still differed appreciably on certain cultivars (Table 4). Our endive isolate Ls265 largely resembled Gr4 in the range of lettuce cultivars infected (Table 4), but was much less pathogenic on lettuce (Fig. 6) and on non-lettuce host

species (Fig. 1). Ls252 is unique in its omnipathogenicity on the lettuce cvs. tested. So, these are all different *isolates*, but are they also different *strains*, or do some, such as the Greek isolates, still belong to one and the same strain?

No taxonomic definition of strain exists in plant virology. For the sake of reproducibility and for recognition purposes, major criteria usually are biological criteria – that can be standardized, particularly varietal interaction with a major natural host (*pathotype*) – or serological specificity (*serotype*). These two, unfortunately, often do not coincide because pathological and serological specificity may, and often do reside in completely different parts of the viral genome. Thus the terms strain, serotype and pathotype are not synonymous. Single pathotypes may be further differentiated serologically, and the reverse. Strain identification is especially important for plant breeders in order to specify resistances found. It has sometimes achieved high precision when the genetic background is well-known and yes-or-no criteria are available, as for the interaction between *Phaseolus* bean and bean common mosaic virus, where for most of the factors a gene-for-gene type of relationship has been detected [Drijfhout, 1978]. More insight into the genetics of LMV resistance has allowed a beginning of order in the classification of strains, or more specifically: of pathotypes, of LMV [Pink et al., 1992a,b].

In Table 7, we have tried to combine our results of Tables 4 and 5 with those of Pink et al. [1992b], using their terminology and pathotype classification (see Table 7, note 3). This leads to some interesting conclusions.

1. Ls1 clearly classifies in pathotype II, comprising other 'common' isolates such as LMV-W from Wellesbourne, considered to closely resemble the one described by Tomlinson [1970] in the 'Descriptions of Plant Viruses' as some sort of neotype isolate of LMV. It could be regarded as 'common lettuce mosaic virus' or 'common strain' LMV. Our endive isolate Ls265, mild on most of the lettuce cultivars it infected, also seems to fit pathotype II.
2. Our endive isolate Ls252 is extremely pathogenic to lettuce. It closely resembles LMV-E, the 'Spanish strain' and also originates from the south (southern France). Both clearly belong to pathotype IV, overcoming all resistances available so far. Ls252 even causes severe disease in crisphead 'Vanguard 75', and, to a lesser extent, in the highly resistant butterhead 'Elvira'.
3. The highly pathogenic *H. echioides* isolate Gr4 and the endive isolate Gr5 are similar on non-lettuce differential host species (Table 1, Fig. 1) and on several lettuce cultivars (Table 4, Fig. 6). Resistance (if not immunity) to Gr4 and Gr5 was found in 'Elvira' and especially 'Vanguard 75'. The two isolates differed on 'Salinas 88', which was resistant to Gr4 but very vulnerable to Gr5. This isolate was also quite

Table 7. Summary of lettuce varietal reactions to LMV isolates studied here (codes printed in italic letters), in comparison with results of Pink et al. [1992a,b] and using their host reaction codes. Cultivars arranged from top to bottom according to increasing resistance

Lettuce cultivars[1]	Virus isolates/strains								
	I[2]		*II*			*?*	*III*	*IV*	
	LMV-YAR	*Gr4*	LMV-W, LMV-F	*Ls1*	*Ls265*	*(Gr5)*	(LMV-9)	LMV-E	*Ls252*
Saladin[1] (= Salinas)	*S*[3]	S	*S*	S	S	S	*S*	*S*	S
Patty		S		S	S	S			S
Soraya		S		R/S[4]	R	S			S
Ithaca	*R*	R	*S*	S	S	R	*S*	*S*	S
Vanguard 4880		R		S	S	R			S
Malika	*R*		*R*				*S*	*S*	
Salinas 88	*R*	R	*R*	R	R/S	S	*R*	*S*	S
Gallega		R/S		R	R	S			S
Elvira		R		R	R	R			S
Vanguard 75	*R*	R	*R*	R	R	R	*R*	*S*	S

[1] Names printed in italic letters are of cultivars used by Pink et al. [1992a,b] for pathotype differentiation.

[2] Roman figures denote pathotype groups of Pink et al. [1992b].

[3] R (resistant) and S (susceptible) in terminology of Pink et al. [1992a,b], referring to the non-expression or expression, respectively, of disease (and thus of clear symptoms; see Discussion). Reaction codes printed in italic letters are according to Pink et al. [1992a,b].

pathogenic on 'Gallega'. Gr4 therefore seems to fit pathotype I. In fact, Pink et al. [1992a] did classify their Greek isolate there. However, Gr5 does not fit in the scheme of Pink et al. [1992b] and appears to be a novel pathotype. It seems intermediate between pathotypes II and III because of the resistance of cvs. Ithaca and Vanguard 4880, both possessing the Mo_2 gene.

4. There is no well-defined single 'lethal strain' of LMV, although both, Gr4 and Gr5, had been designated LMV-lethal. Ls252, belonging to a completely different pathotype, is even more lethal in its effect on many lettuce cultivars than the Greek isolates. Likewise, there are no well-defined single '*Helminthia* strain' and 'endive strain'. The Greek isolate from *H. echioides* (Gr4) is different from the Greek isolate from endive (Gr5), and both are considerably different from our two endive isolates (Ls252 and Ls265). The Greek endive isolate might have originated from *H. echioides*. It looks similar to the lethal or virulent LMV-L strain found in California on lettuce and nearby *H. echioides*. The latter wild species was considered a reservoir of infection in lettuce crops [Zink et al., 1973]. Costa and Duffus [1958] had earlier isolated a common strain of LMV from this weed species in California. In the coastal areas of Dalmatia (Yugoslavia) a common isolate of the virus

was found to be common in the species growing naturally in non-cultivated areas and grass plots [Pleše and Besić, 1987].

Isolate pathotyping with a set of host genotypes may be blurred by the fact that not only host genes are involved but those of the virus as well, and both, the effect of the host and of the virus on symptom severity and their extent may be hard to distinguish. Various factors determining *host vulnerability* versus *disease resistance* (= *susceptibility/virus resistance* + *sensitivity/tolerance*) and *virus pathogenicity* (= *aggressiveness* + *virulence*) must be taken into account [Bos, 1983]. They may well all be governed by different genes and must therefore be carefully analysed for their contribution. In the past, breeders have judged host/virus interaction mainly by the presence or absence of symptoms. Host reactions were simply recorded as susceptibility and resistance, respectively, and this custom persists to date (see Pink et al. [1992a,b] and Table 7). To analyse the share of the various factors, we must therefore clearly distinguish between the fate of the virus and that of the host. ELISA provides valuable information on the multiplication of the virus, and thus on the aggressiveness of the virus and on the suceptibility/resistance of the genotype to infection. Comparison of data on virus multiplication with those on (severity of) symptom expression will indicate whether and to which extent virus virulence and host sensitivity/tolerance may also be involved. Consensus and clarity in resistance terminology are long overdue.

An important problem further complicating the quantitative assessment of host vulnerability/disease resistance is the definition of *severity* of the effect on the host, and whether there are simple parameters of severity. For example, in the case of LMV is it plant stunting or necrosis, or the cumulative effect of both? The degree of stunting might reflect the final effect on yield, but other phenomena may be involved in determining, for example, head weight of lettuce, and economic yield is largely determined by quality and thus by, for example, discoloration and necrosis. In assessing resistance of lettuce genotypes to LMV, Walkey et al. [1985] found that the correlation between cultivars for symptom expression and yield reduction values was low. In their experiments, the large reduction in yield (head weight) caused by LMV in some resistant cultivars was striking, and this indicates that yield is indeed co-determined by factors other than leaf yellowing or mosaic discoloration. Yield may even be reduced in the absence of visible symptoms and of appraisable disease, as in latent infections. This strictly limits the usefulness of severity of virus symptoms as indicators of yield [Bos, 1982]. That is why in Table 4 we have refrained from simply recording vulnerability of lettuce genotypes in a single figure, as done for the wild *Lactuca* genotypes in Table 6.

A few examples from Tables 4 and 5 clearly illustrate the terminological confusion. Conclusions on host resistance, as of 'Gallega' to LMV, were for long exclusively based on examination for absence of symptoms.

Resistance of 'Gallega' has been also called tolerance [Marrou, 1969] or mosaic resistance [Ryder, 1968, 1970]. Ryder first pointed to a reduced virus multiplication and spread within inoculated resistant lettuce plants [Ryder, 1970]; in them, symptom appearance was delayed, symptoms were milder or neglegible, stunting less obvious, and a greater proportion of plants escaped infection [Ryder, 1976]. We found 'Gallega' to be highly resistant to the common isolate Ls1 (that is, resistant to multiplication of the virus), but not immune, and systemic spotting on its leaves is erratic. 'Ithaca' and 'Vanguard 4880', highly susceptible to three isolates (that is, allowing their extensive multiplication), were found to be immune to the highly pathogenic Gr4 and 5. Virus isolates themselves also influence their multiplication. The 'severe' Greek isolates seem to have a low degree of aggressiveness as judged by low virus concentrations in infected genotypes, and this may explain why a limited number of genotypes were infected. The high severity of symptoms in the lettuce genotypes infected and in other suceptible species in spite of low aggressiveness, indicates very high virulence of these isolates. Their final pathogenicity thus is mainly due to high virulence. In contrast, Ls252 is highly aggressive, as expressed in high virus concentration and high number of lettuce genotypes infected, that is, all cultivars tested. Symptoms in them are also severe, but this seems to be more a matter of aggressiveness than of virulence of the isolate. The more aggressive a virus or virus isolate, the higher usually also the number of plants infected out of the number of plants inoculated. When referring to their Table 1, Pink et al. [1992a] claim that their isolates differ in aggressiveness on the universally susceptible cv. Saladin, but from that table they could only have concluded the existence of differences in pathogenicity. In their discussion they are using the term virulent where only pathogenic would have been justified. Often, symptom severity is correlated with degree of virus muliplication (and thus with aggressiveness), but not necessarily so. For example, symptom development in 'Saladin' with Ls265 was poor, although the cultivar is highly susceptible to the virus. Thus it is tolerant to infection by Ls265.

Table 8, adapting Table 7 to updated terminology, although it may seem complicated to the breeder, provides a more realistic approach, better representing natural complexity. Further research will undoubtedly reveal more resistance and/or susceptibility genes of lettuce, and will provide information on pathogenicity genes of LMV to better explain the genetic interaction between host and virus. More than four pathotypes of LMV appear to occur, and more than the three resistance genes identified so far may be involved in resistance of lettuce to LMV. Walkey et al. [1985] have already indicated that 'background' genes or environmental factors may greatly effect the level of virus multiplication and symptom expression. Pink et al. [1992a] admit that they have omitted butterhead cultivars from their pathotyping studies, because these were more variable in their response to inoculation than crisp cultivars.

Table 8. Summary of lettuce varietal reactions to isolates of lettuce mosaic virus studied here (codes printed in italic letters) in comparison with results of Pink et al. [1992a,b] and using terminology as discussed in this publication. Cultivars arranged from top to bottom according to increasing resistance

Lettuce cultivars[1]	Virus isolates/strains								
	I[2]		*II*			*?*	*III*	*IV*	
	LMV-YAR	*Gr4*	LMV-W, LMV-F	*Ls1*	*Ls265*	*Gr5*	LMV-9	LMV-E	*Ls252*
Saladin (= Salinas)	*V**[4]	V*	*V**	V*	V	V*	*V*	*V**	V*
Patty		V*		V*	V*	V*			V*
Soraya		V		V(T)	V	V			V*
Ithaca	*I*	R(I)	*V**	V	T	I	*V*	*V**	V
Vanguard 4880		I		V	V*	I			V*
Malika	*R*		*R*				*V*	*V*	
Salinas 88	*R*	R	*R(T)*	R*	R(T)	V*	*R*	*V*	V*
Gallega		T		R	I	V			V
Elvira		R(I)		R(I)	R(I)	R(I)			V
Vanguard 75	*R(I)*	(R)I	*R*	R(I)		(R)I	*R*	*V**	V*

[1] Names printed in italic letters are of cultivars used by Pink et al. [1992a,b] for pathotype differentiation.

[2] Roman figures denote pathotype groups of Pink et al. [1992b].

[3] I (immune = insusceptible), R (resistant to infection), V (vulnerable = susceptible + sensitive: i.e., subject to damage and loss of yield), T (tolerant = susceptible but poorly sensitive).

[4] Asterisk indicates high degree of vulnerability or resistance. Reaction codes printed in italic letters are according to Pink et al. [1992a,b].

The recently coming to the fore of highly pathogenic and resistance-breaking 'strains' of LMV is alarming. This is the more so since such a 'strain' (LMV-13), that is severe on resistant cultivars, was recently found also to be seed transmissible in genotypes with resistance genes *g* or *mo* [Lot and Deogratias, 1991; Dinant and Lot, 1992]. In our experiments, the Greek isolates Gr4 and 5 were very severe and often necrotic on most of the lettuce cultivars they infected. According to Dr Kyriakopoulou (pers. comm. 10 June 1994) in experiments performed in Greece during 1984 and 1985, Gr4 was even more severe than reported here. It killed several Roman type varieties and one butterhead variety within 20–30 days without previous mosaic or any other symptom, but conditions may have been different in Greece. Pathotype-IV isolates (LMV-E and our Ls252) are extremely pathogenic on lettuce, and all lettuce genotypes tested so far were vulnerable to infection. Out of 32 entries of wild *Lactuca* species screened for resistance to Ls252, three entries belonging to two species did not develop symptoms, but those of *L. perennis*, when tested serologically, did contain much virus. In France, some accession numbers of *L. saligna* and *L. virosa* were recently found resistant to individual or all strains of

LMV [Dinant and Lot, 1992]. Further search for resistance in wild species and other plant introductions seems worthwhile. For example, of pea (*Pisum sativum*) initially all commercially available cultivars were found to be susceptible to the LMV-related pea seedborne mosaic potyvirus. Very extensive screening finally revealed resistance in 16 out of 1835 PI lines tested [Hampton and Braverman, 1979]. New 'strains' of LMV seem to increasingly attract attention on endive. The more aggressive strains isolated from endive have recently become prevalent in France [Dinant and Lot, 1992]. They deserve further investigation also in their genetic interaction with endive.

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